Liste des abréviations

THM	Trihalométhanes	**FT-ICR/MS**	*Fourrier Transform-Ion Cyclotron Resonance/Mass Spectrometry*
SPD	Sous-produits de désinfection	**ESI**	*Electrospray Ionization*, ionisation par nébulisation
SPOX	Sous-produits organohalogénés	**AHA**	Acide haloacétiques
EDF	Electricité De France	**Hk**	Halocétones
CRF	Circuit de refroidissement	**HAcAm**	Haloacétamides
CNPE	Centre nucléaire de production d'électricité	**HP**	Halophénols
MCA	Monochloramine	**HAL**	Haloacétaldéhydes
AOX	*Adsorbable organic halogen compounds*, composés organohalogénés adsorbables sur charbon actif	**HAN**	Haloacétonitriles
BF	Bassin froid	**HNM**	Halonitrométhanes
ASN	Autorité de Sûreté Nucléaire	**CRT**	Chlore résiduel total
REACH	*Registration, Evaluation, Authorization and Restriction of CHemicals*	**CRL**	Chlore résiduel libre
AFSSET	Agence Française de Sécurité Sanitaire de l'Environnement et du Travail	**SDB**	Styrène divinyl benzène
DCE	Directive cadre sur l'eau	**UF**	Ultrafiltration
SL	Saint-Laurent	**COD**	Carbon organique dissous
CAT	Cattenom	**C-IC**	*Combusion-Ion Chromatography*
CHO	Chooz	**GC-MS**	*Gas Chromatography-Mass Spectrometry*
NCI	National Cancer Institute		
US EPA	United States Environmental Protection Agency		
OMS	Organisation mondiale de la santé		
TAR	Tours aéroréfrigérantes		
EDA	Effect-Direct Analysis		

Table des matières

INTRODUCTION GENERALE

Les biocides oxydants à base de chlore tels que le chlore gazeux (Cl_2), l'eau de Javel (NaClO), le dioxyde de chlore (ClO_2) ou la monochloramine (NH_2Cl) sont largement utilisés pour le traitement des eaux. Ils sont utilisés en tant que « désinfectants » pour prévenir le développement des pathogènes d'origine hydrique et/ou comme produits « *antifouling* » pour limiter l'encrassement biologique des installations industrielles en contact avec de l'eau. Le traitement des eaux aux biocides concerne un nombre important de secteurs industriels. Citons, à titre d'exemple, la potabilisation des eaux (désinfection), la production d'électricité (désinfection des eaux de refroidissement), le transport maritime (traitement des eaux de ballast pour empêcher le développement de pathogènes et le transport d'espèces envahissantes), les eaux de piscines (désinfection), l'aquaculture (désinfection), la gazéification du gaz naturel liquifié (*antifouling*), les stations d'épuration des eaux usées (désinfection), etc.

A la fin du XIX^{ème} siècle, les épisodes épidémiques (ex. fièvre typhoïde, choléra) liés à l'exposition à une eau impropre à la consommation faisaient encore des ravages dans les pays occidentaux, aux Etats-Unis comme en Europe. L'utilisation du chlore pour la désinfection des eaux a permis des progrès considérables en termes de santé publique. Son emploi à cette fin s'est généralisé dans les pays industrialisés au cours du XX^{ème} siècle. De nos jours, le chlore, principalement sous forme d'hypochlorite de sodium, est l'oxydant le plus utilisé au monde pour le traitement des eaux. Il doit sa grande popularité à son faible rapport coût/efficacité et à sa facilité d'utilisation.

Cependant, l'usage du chlore pour le traitement des eaux n'est pas dépourvu d'effets secondaires. En effet, les années 1970 ont marqué un tournant important dans l'histoire de la désinfection des eaux, en révélant le revers de cette technique : la formation, lors du processus de chloration de l'eau, de produits chimiques potentiellement dangereux pour la santé. Ces sous-produits, appelés usuellement sous-produits de désinfection (SPD), résultent de la réaction entre le chlore et la matière organique naturelle (acides humiques et fulviques, par exemple) présente dans toute eau de surface naturelle [1]. Rook (1974) et Bellar *et al* (1974) furent les premiers à relater, d'une part la formation de SPD dans l'eau potable traitée au chlore et, d'autre part, l'identification des trihalométhanes (THM) en tant que SPD [2,3]. Une étude réalisée par l'US EPA (*United States Environmental Protection Agency*) sur l'ensemble du territoire des USA en 1976 a révélé l'omniprésence des THM dans les réseaux de distribution de l'eau potable traitée au chlore [4]. Peu après, le National Cancer Institute (NCI) a publié les résultats d'un essai biologique de cancérogenèse du chloroforme, composé faisant partie de la famille des THM, révélant ainsi un problème de santé publique émergent lié à l'exposition aux SPD. Les tumeurs du rein et du foie observées chez le rat et la souris ont conduit à classer le chloroforme parmi les substances présumées cancérogènes pour l'Homme [5]. Ces premiers résultats ont conduit la communauté scientifique et les décideurs de santé publique à formuler l'hypothèse selon laquelle

d'autres SPOX potentiellement toxiques pourraient être formés lors de la désinfection des eaux. Dès lors, un nombre important d'études ont été menées afin de mieux caractériser les sous-produits formés et d'en évaluer les risques sanitaires et environnementaux. Les études ont principalement concerné le domaine de l'eau potable traitée au chlore, comparativement aux autres types d'eaux et produits biocides. A l'heure actuelle, environ sept-cents sous-produits de chloration ont été identifiés. Connaissant les éventuels risques pouvant être associés aux SPD, des recommandations et des normes ont été établies par les autorités pour certains SPD traceurs (ex. THM). Dans les eaux de robinet, les THM réglementés au niveau national par le code de la santé publique (arrêté du 11 janvier 2007) sont le chloroforme, le bromodichlorométhane, le dibromochlorométhane et le bromoforme.

Depuis plus de quatre décennies, la réduction des concentrations en SPD formés par chloration des eaux représente un enjeu scientifique et industriel majeur. Les efforts ont porté sur l'optimisation des traitements en place en vue de réduire à la source la quantité des biocides utilisée. D'autres recherches ont porté sur la recherche de biocides alternatifs engendrant moins de SPD tout en conservant une bonne efficacité de traitement [6-8]. Du fait de sa forte efficacité et de son faible potentiel générateur de SPD, la monochloramine (MCA : NH_2Cl) constitue une alternative très intéressante à l'utilisation du chlore [9]. La monochloramine pénètrerait également mieux dans les biofilms que le chlore [10]. La désinfection des eaux par la monochloramine est aujourd'hui une technologie bien établie et même recommandée par l'organisation mondiale de la santé (OMS) [11-12]. Elle est actuellement mise en place pour désinfecter l'eau destinée à la consommation humaine par de nombreux pays comme le Canada, le Japon, les Etats-Unis, le Royaume-Uni et l'Australie. Environ 25% des stations de potabilisation aux USA utilisent la MCA ; 8 à 12% supplémentaires envisageraient le recours à ce mode de traitement au cours des prochaines années [13].

En France, l'utilisation de MCA demeure prohibée pour la désinfection des eaux de distribution. Son usage est cependant autorisé pour le traitement des eaux industrielles. Le traitement à la monochloramine est mis en œuvre par la Société Electricité de France (EDF) pour désinfecter les eaux de refroidissement des circuits tertiaires semi-fermés (CRF) de ses centres nucléaires de production d'électricité (CNPE) alimentés en eau brute de rivières. EDF a opté pour ce traitement pour son efficacité sur les germes pathogènes thermophiles *Legionella Pneumophila* (légionelles) et *Naegleria Fowleri* (amibes) [14-15]. Ces derniers, naturellement présents dans les eaux douces (lacs et rivières), trouvent dans les réseaux d'eau chaude ou dans les CRF un environnement privilégié de développement : la température de l'eau, les conditions hydrauliques, le contact avec l'air et la présence de biofilms (milieux de réplication et de protection vis-à-vis de l'action de certains biocides) favorisent leur prolifération. L'exposition à *Naegleria fowleri* se fait par contact avec la muqueuse nasale, celle à *Legionella Pneumophila* par l'inhalation d'aérosols fins contenant cette bactérie. Sur les

CNPE, le traitement préventif consiste à injecter la monochloramine préformée (produite *in situ* par un mélange d'ammoniaque et d'eau de Javel), à l'amont des condenseurs (figure I-1), pendant la période à risque (période estivale). L'effet biocide escompté est assuré par le maintien d'une concentration résiduelle en sortie condenseur autour d'une valeur cible entre 0,2 et 0,30 mg éq. $Cl_2.L^{-1}$, selon le CNPE concerné.

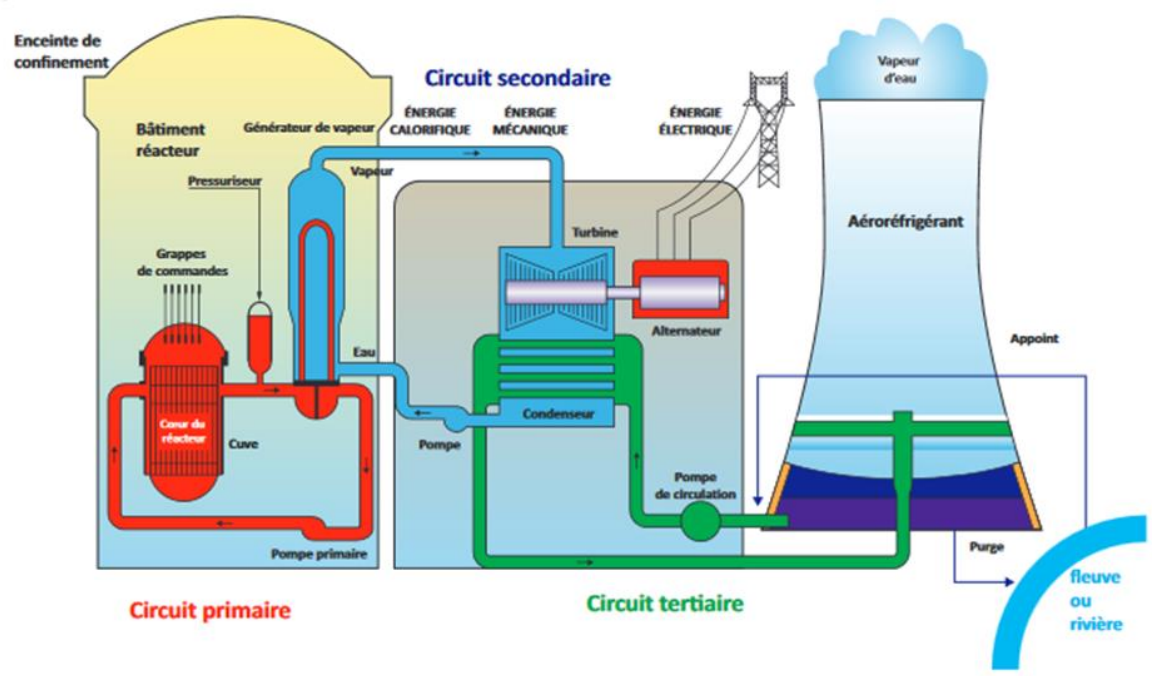

Figure I-1. Schéma représentatif du fonctionnement d'une CNPE et du point d'injection de la monochloramine (en amont du condenseur, juste avant la pompe de circulation – circuit de refroidissement tertiaire en couleur verte).

La maîtrise du risque microbiologique lié à l'exploitation des CNPE constitue donc un enjeu de santé publique. Les modalités de surveillance et les seuils limites à ne pas dépasser dans les eaux des CRF sont encadrés par une réglementation très stricte. Afin de renforcer davantage la prévention de ce risque, l'Autorité de Sûreté Nucléaire (ASN) a adopté très récemment une décision (N° 2016-DC-0578, 2016) imposant des modalités de surveillance et des seuils limites encore plus drastiques. L'enjeu pour l'exploitant est de trouver un meilleur compromis entre la gestion du risque sanitaire microbiologique et la gestion du risque environnemental lié aux rejets des SPD de la monochloramine *via* les purges de déconcentration des CRF. Bien que la monochloramine produise beaucoup moins de sous-produits organohalogénés que le chlore, les flux de SPD formés et libérés dans le milieu

aquatique peuvent être importants, compte tenu des débits d'eau monochloraminée restituée au milieu naturel (environ 2 $m^3.s^{-1}$ par tranche).

Le traitement à la monochloramine fait l'objet depuis sa mise en place en 1999 d'interrogations récurrentes des administrations réglementaires quant à la nature des SPD formés et leur impact environnemental. Au cours des dix dernières années, plusieurs travaux visant à identifier et à quantifier les SPD de la monochloramine ont été menés par EDF R&D. Un effort particulier a été porté sur le suivi des composés organohalogénés (SPOX) via le paramètre analytique dénommé AOX (pour composés organohalogénés adsorbables sur charbon actif). Ce paramètre réglementaire revêt une importance toute particulière puisqu'il permet d'estimer quantitativement la charge d'une eau en composés organohalogénés. Préalablement à ce travail de thèse, l'état des lieux réalisé par EDF R&D a montré que, en dépit du nombre important de composés organohalogénés recherchés spécifiquement lors des campagnes de mesures sur échantillons « réels », le bilan massique associé aux sous-produits organohalogénés identifiés imputables au traitement à la monochloramine reste très faible. A titre d'exemple, ces bilans avoisinent les 50% dans le cas de la chloration des eaux potables et atteignent rarement 10% pour les eaux de rivières [16]. Ce constat met en lumière le besoin d'intensifier la recherche scientifique sur la nature des SPOX « inconnus » ainsi que sur les niveaux d'occurrence (exposition) et les effets potentiels (danger) de ces derniers sur l'écosystème aquatique.

Le travail entrepris dans le cadre de cette thèse de doctorat s'inscrit dans la continuité des travaux menés par EDF R&D sur la problématique des sous-produits de désinfection. **Il ambitionne de progresser sensiblement dans la spéciation chimique des SPOX constitutifs des AOX via une démarche analytique intégrée.** Celle-ci se décline au travers de trois grands axes :

1. Réduction des incertitudes entourant la méthode d'établissement des bilans matière par :

- la mise en place de protocoles efficaces pour l'extraction et l'enrichissement des SPOX sur phase solide (SPE),
- le développement de méthodes d'analyse plus sensibles et permettant de doser simultanément plusieurs SPOX ou familles de SPOX connus et,
- la fiabilisation de la méthode de mesure des AOX.

2. Mise en place d'approches analytiques permettant d'améliorer les connaissances sur la nature chimique des composés constitutifs des AOX par :

- le développement d'une méthode d'analyse permettant d'établir le taux d'incorporation des différents halogénures (Cl, Br, I) dans les AOX,
- la mise en place d'une méthode de fractionnement des SPOX basée sur l'ultrafiltration sur membranes de différents seuils de coupure,

– la réalisation d'essais de « *screening* » non ciblé par GC-MS et LC-MS couplés à des expériences de fragmentométrie avec interprétation des spectres de masse pour tenter d'identifier les SPOX qui échapperaient à l'analyse ciblée de substances spécifiques,
– l'évaluation du potentiel d'un spectromètre de masse à ultra haute résolution, le FT-ICR/MS (pour, *Fourier Transform Ion Cyclotron Resonance Mass Spectrometer*), pour l'identification des SPOX de haut poids moléculaire par comparaison de données issues d'analyse pré et post-traitement.

3. Evaluation d'une approche analytique dirigée par les effets (*Effect-Direct Analysis, EDA*) pour la recherche ciblée de SPOX potentiellement toxiques

La figure I-2 illustre l'ensemble des actions de recherche développées dans le cadre de ce travail de thèse et leurs articulations.

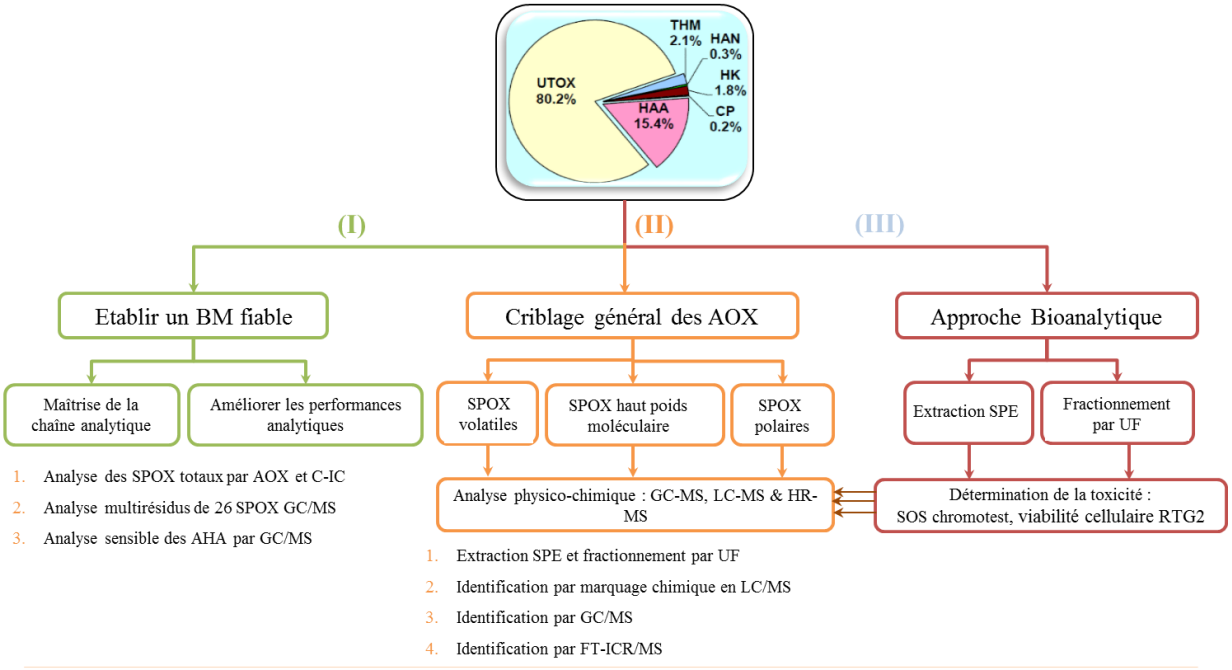

Figure I-2. Présentation générale des actions de recherche développées et leurs articulations

Ce manuscrit de thèse est organisé en trois grands chapitres ; certains résultats y sont présentés sous forme d'articles acceptés ou soumis.

Le premier chapitre est une synthèse bibliographique portant sur l'état de l'art des connaissances sur les SPOX. Il est constitué d'une série de trois articles publiés dans la revue « *Trends in Analytical Chemistry* ». Le premier définit les SPOX et donne un large aperçu de leurs mécanismes de formation et des facteurs influençant cette dernière. Les différentes méthodes analytiques

communément utilisées pour leur caractérisation sont présentées en détails dans les deux articles suivants.

Le second chapitre présente le volet expérimental de l'étude et vise à justifier et argumenter les choix méthodologiques. Il se dévise en deux parties. La première présente les sites d'étude choisis et détaille la conduite des essais et l'ensemble des analyses réalisées. La seconde décrit les matériels et méthodes mis en place pour répondre aux objectifs de cette thèse.

Le troisième chapitre est consacré à la description des résultats obtenus et à leur interprétation.

Une première partie expose les développements méthodologiques effectués pendant ces travaux de thèse. Parmi les méthodes analytiques développées, trois sont présentées sous forme d'articles scientifiques prêts à être soumis pour publication dans les revues scientifiques à comité de lecture suivantes : « *Journal of Chromatography A* », « *Talanta* » et « *Rapid Communications in Mass Spectrometry* ». Les deux premières publications décrivent le développement de méthodes d'extraction par SPE et d'analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse en tandem pour la quantification de 11 acides haloacétiques (AHA) et de 26 autres SPOX. La troisième publication présente le développement et l'optimisation d'une méthode de spéciation des AOX en fractions « chlorés », « bromés » et « iodés ». Ces trois articles sont présentés dans l'ordre chronologique du développement des travaux de recherche. Des rappels de notions, méthodes ou problématiques sont présentés dans chacun des trois articles afin que ceux-ci puissent être lus séparément. Une partie des résultats issus de l'application de ces méthodes à l'analyse d'échantillons d'eau issus des sites d'étude est également exposée.

La seconde partie est dédiée à la caractérisation des échantillons d'eau issus des sites d'étude (CNPE de Saint-Laurent, Chooz et Cattenom). Elle présente et discute les résultats obtenus et s'attache à les comparer à des résultats antérieurs.

Le manuscrit s'achève par une conclusion générale qui synthétise les résultats obtenus, pose un regard sur la démarche employée et aborde les perspectives de recherche ouverte par ces travaux.

Références bibliographiques

[1] C. Li, D. Wang, X. Xua, Z. Wang, Formation of known and unknown disinfection by-products from natural organic matter fractions during chlorination, chloramination, and ozonation. Science of the Total Environment 587 (2017) 177-184

[2] J.J. Rook, Formation of haloforms during chlorination of natural waters, Water Treat. Exam. 23 (1974) 234-243.

[3] T.A. Bellar, J.J. Lichtenbert, R.C. Kroner, The occurrence of organohalides in chlorinated drinking waters, J. Am. Water Works Assoc. 66 (1974) 703-706.

[4] F.C. Kopfler, R.G. Melton, R.D. Lingg, W.E. Coleman, GC/MS determination of volatiles for the national organics reconnaissance survey (NORS) of drinking water. L.H. Keith (Ed.), Identification and Analysis of Organic Pollutants in Water, Ann Arbor Science, Ann Arbor, MI (1976) 87-104.

[5] J.C. Loper, Mutagenic effects of organic compounds in drinking water, Mutat. Res. 76 (1980) 241-268.

[6] L. Duvivier, La monochloramine, un désinfectant alternatif pour le conditionnement des circuits de réfrigération industriels. Partie 1 : Aspects théoriques. La Tribune de l'eau, 54(610) (2001) 3-11.

[7] G. Hua, D.A. Reckhow, Comparison of disinfection byproduct formation from chlorine and alternative disinfectants, Water Res. 41(8) (2007) 1667-1678.

[8] C.M. Bougeard, E.H. Goslan, B. Jefferson, S.A. Parsons, Comparison of the disinfection by-product formation potential of treated waters exposed to chlorine and monochloramine, Water Res. 44(3) (2010), 729-740.

[9] J. Hu, H. Song, T. Karanfil, Comparative analysis of halonitromethane and trihalomethane formation and speciation in drinking water: the effects of disinfectants, pH, bromide, and nitrite. Environ. Sci. Technol, 44(2) (2009), 794-799.

[10] M.W. Lechevallier, C.D. Cawthon, R. G. Lee, Inactivation of biofilm bacteria. Appl. Environ. Microbio, 54(10)(1988), 2492-2499.

[11] WHO (2004). Monochloramine in Drinking-water : Background document for development of WHO Guidelines for Drinking-water Quality. Geneva, World Health Organization: 13.

[12] WHO (2008). Guidelines for Drinking-water Quality - Third Edition Incorporating The First And Second Addenda, Volume 1 - Recommendations. Geneva, WHO: 668

[13] J. L. Kool, J. C. Carpenter, B. S. Fields, Effect of monochloramine disinfection of municipal drinking water on risk of nosocomial Legionnaires' disease. The Lancet, 353(9149)(1999), 272-277.

[14] M. DUPUY, Association légionelles-amibes et traitements biocides pour les circuits de refroidissement, Doctoral dissertation, École nationale supérieure d'ingénieurs (Poitiers), 2013.

[15] D. Blangis, Identification et quantification des sous-produits organohalogénés formés par réaction de la monochloramine avec les matières organiques naturelles d'eaux de surface, Doctoral dissertation, École nationale supérieure d'ingénieurs (Poitiers), 2004.

[16] A. Kinani, S. Kinani, B. Richard, M. Lorthioy, S. Bouchonnet, Formation and determination of organohalogen by-products in water. Part I. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method, Trends in Anal. Chem., 85 (2016) 273-286.

CHAPITRE I – SYNTHESE BIBLIOGRAPHIQUE

Cette première partie a pour but de présenter un état de l'art critique des connaissances actuelles sur les SPD organohalogénés. Elle est constituée d'une série de trois articles publiés dans la revue internationale à comité de lecture « *Trends in Analytical Chemistry* ».

*Formation and determination of organohalogen by-products in water – **Part I.** Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method. Trends in Analytical Chemistry, 85 (2016) 273-280.*

Cette première publication traite des sous-produits organohalogénés (SPOX) au sens large et donne un large aperçu des différents facteurs influençant leur formation. Ce travail de synthèse est réalisé en considérant l'ensemble des biocides oxydants communément employés pour le traitement des eaux (ex. monochloramine, chlore, dioxyde de chlore, etc.) ainsi que les différents types d'eaux traitées (ex. eaux douces, eaux salines, eaux souterraines, eaux usées, etc. La proportion de SPOX inconnus est discutée en fonction de la nature du biocide utilisé. L'article propose également une analyse critique des principales méthodes analytiques intégratives permettant une estimation quantitative des SPOX présents dans un échantillon d'eau.

*Formation and determination of organohalogen by-products in water – **Part II.** Sample preparation techniques for analytical approaches. Trends in Analytical Chemistry, 85 (2016) 281-294.*

Cette publication dresse un état des lieux des pratiques relatives à une étape capitale dans le domaine de l'analyse ciblée et non ciblée des SPOX : la préparation d'échantillon. Cette dernière inclut principalement deux phases : premièrement, un conditionnement chimique destiné à maintenir l'intégrité de l'échantillon entre le moment de son prélèvement et l'analyse ; deuxièmement, une extraction dédiée à la purification et à l'enrichissement des SPOX. Les différentes techniques rapportées dans la littérature sont présentées ; leurs avantages et limites sont discutés.

*Formation and determination of organohalogen by-products in water. **Part III.** Characterization and quantitative approaches. Trends in Analytical Chemistry, 85 (2016) 295-305.*

Cette dernière publication est consacrée à l'analyse physico-chimique des sous-produits organohalogénés. Elle fournit une synthèse critique des différentes techniques d'analyse actuellement employées pour l'analyse des SPOX en fonction des polarités et poids moléculaires de ces derniers. Les approches peuvent être réparties en trois catégories : celles visant à identifier les SPOX inconnus sans aucune information *a priori* sur leur structure chimiques, dites « non-ciblées », celles de confirmation de la présence effective de SPOX connus, dites *« suspected-target screenings »*, et celles de quantification destinées à mesurer la concentration en composés connus, dites « ciblées ».

Formation and determination of organohalogen by-products in water - Part I. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method

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Abstract

Halogen-based oxidants are widely used in water treatment processes in order to inactivate pathogenic microorganisms. They react with naturally occurring organic matter as well as with other contaminants, leading to the formation of a wide range of unwanted by-products including organohalogen by-products (OXBPs), which have shown toxic properties. The quantity of OXBPs in a water sample can be estimated by a parameter called AOX (halogenated organic compounds adsorbable on activated carbon). Despite the growing number of quantified OXBPs, the material balance (percentage of known OXBPs measured individually divided by the AOX value) indicates that a significant portion of OXBPs remains unknown. This lack of knowledge is a barrier in the assessment of the effects of water treatments on ecosystems and human health. This manuscript is the first part of a review trio on OXBPs; the 2^{d} and 3rd articles are devoted to OXBPs extraction processes and analytical techniques, respectively.

Keywords: Organohalogen; Halogen-based oxidants; By-products; Water; AOX parameter; Material balance

I.1 Introduction

Halogen-based oxidants, such as chlorine (gaseous form: Cl_2, liquid bleach: NaOCl or calcium hypochlorite ($Ca(OCl)_2$), chlorine dioxide (ClO_2), and monochloramine (NH_2Cl)), are widely used in water treatment processes in order to inactivate pathogenic microorganisms, control biofouling and reduce the risk of introducing invasive species into local aquatic ecosystems. The first applications were for disinfection of wastewater and drinking water in order to eradicate waterborne bacterial diseases, such as typhoid, cholera and dysentery [1]. Chlorine is the most widely used biocide in industry today due to its low cost, ease of use, its high efficiency and wide range of applications. A "side-effect" of water treatment by halogen-based oxidants is that they react with naturally occurring organic matter as well as with bromide and iodide ions, microorganisms, biofilms, anthropogenic contaminants, etc., leading to the formation of a wide range of unwanted chemical by-products, commonly called disinfection by-products (DBPs) [2]. Among the chemical compounds formed, organohalogen by-products (OXBPs) have received particular attention from the scientific community and public authorities, since the discovery in the mid-1970s of the carcinogenic effect of trihalomethanes (THMs), following the discovery of these chlorination by-products in drinking water [3]. OXBPs can be quantitatively estimated using the AOX group parameter (Adsorbable Organic Halides), which represents the sum of the organically bound chlorine, bromine and iodine. However, these indicator parameters may not provide a final answer because the toxicological significance of the obtained values cannot be established without knowledge of the chemical structures of the measured compounds [4]. To date, more than 600 DBPs have been identified. The main OXBPs identified with regular frequency in water are THMs, haloacetic acids (HAAs), haloacetonitriles (HANs), cyanogen halides (CX), haloketones (HKs), haloaldehydes (HALs), halonitromethanes (HNMs), haloacetamides (HAcAms), halophenols (HPs), haloaldehydes, halofuranones, halopyrroles, and haloquinones [5]. Some of these substances have shown toxic effect [6]. Nitrogenous and iodinated organohalogen by-products have recently received increased attention, since toxicological studies suggest that they have higher toxicity than the currently regulated carbonaceous by-products [7]. THMs and HAAs are still considered to be the dominant OXBPs groups on a weight basis in drinking water. Their formation mechanism is now well understood and their concentration levels are regulated in various countries [8]. Despite the growing number of quantified OXBPs and the increasing sensitivity of analytical methods, the material balance (MB), defined as the percentage of known OXBPs, indicate that a significant portion of OXBPs remains unknown: only 50% of the AOX can be attributed to individual known OXBPs under typical chlorination conditions. In the case of monochloramine and chlorine dioxide, the MB is even lower (<20%) [9]. More than 40 years after the discovery of THMs, the determination of UOXBPs identity and toxicity remains a major objective and a continuously evolving challenge. This highlights the need for development of new analytical methodologies and alternative approaches to identify UOXBPs or to monitor OXBPs previously identified.

This manuscript is the first part (part 1) of a literature review on OXBPs generated through water treatment by halogen-based oxidants, which is divided into three sections. The first section summarizes the state of knowledge concerning OXBPs formation, including an overview of the different factors influencing their concentration and speciation. The second section presents the main OXBP groups commonly produced in water and a selection of regulatory and guidelines values. In this section, we also discuss the percentage of unknown organohalogen by-products. The last section presents a detailed description and critical analysis of the methods used to estimate the total quantity of OXBPs presents in a water sample (group parameters).

I.2 Origin of organohalogen by-products in water

There is considerable evidence that organic matter constituents act as precursors in the formation of organohalogen by-products during water treatment. Incorporation of halogen into organic compounds takes place through three possible reaction pathways namely oxidation, substitution and addition. Oxidation may be the dominant reaction occurring between oxidant and organic matter in water [10]. However, addition and substitution are the reactions that lead to the formation of more organohalogen by-products [11]. The formation of low molecular weight organohalogen by-products involves successive isomerization, rearrangement, hydrolysis, and elimination reactions. It is important to note that only a very small amount of the oxidant introduced for water treatment turns into organohalogen by-products [12]. The OXBP concentration and speciation is influenced by the water quality and treatment modalities. These two parameters encompass many influencing factors, including the nature and form of the organic matter, the presence of amino-nitrogen, bromide and iodide, the nature and amount of the halogen-based oxidant, the removal of organic matter prior to introducing the oxidant, the contact time, temperature and pH [13] and [14]. The effect of each parameter on the potential generation and speciation of OXBPs is summarized in the following sections.

I.2.1 Influence of the nature of organic matter

Organic matter in aquatic environments is a heterogeneous complex mixture of organic compounds with different molecular weight, elemental composition, hydrophobicity and functional groups. Moreover, its composition varies considerably, depending on the water source, season, local geology, water flow, weather and pollution events, etc. According to its origin, organic matter can be divided into four categories: (1) natural organic matter (NOM), (2) organic matter from algal blooms, (3) organic matter from biological sources such as microorganisms and biofilms, and (4) anthropogenic organic chemicals. Substantial studies have been conducted to evaluate the effect of NOM on the formation of OXBPs and to understand the chemical mechanisms involved in their formation [15].

NOM is a major contributor to dissolved organic carbon (DOC) in natural waters (river and lake waters). It consists predominantly of humic substances (50 to 80%), mainly composed of humic and fulvic acids. They are natural biopolymers with heterogeneous functional groups and a wide molecular weight distribution. The contribution of natural organic matter is dominant in the formation of several families of OXBPs, particularly THMs, HAAs, HANs and HKs [16]. The NOM characteristics (e.g. relative proportions of fractions of hydrophilic/hydrophobic, aromatic/aliphatic, low/high molecular weight and dissolved organic carbon/dissolved organic nitrogen compounds) have strong influences on the OXBPs concentrations and speciation.

OXBPs are not only formed from reactions between halogen-based oxidants and dissolved NOM in the treated water. The treated water also contains particulate organic matter (POM) including bacteria, algae, protozoa, etc. [17]. The reactivity of these biological sources of organic matter with halogen-based oxidants has not been thoroughly studied. Recently, Huang et al. showed that chlorination of POM significantly contributes to the formation of OXBPs [18]. The chlorination tests performed on the effluents from secondary domestic wastewater showed that POM accounts for 26 to 46% of the total amount of dichloroacetonitrile (DCAN) and dichloroacetamide (DCAcAm). Chlorination tests carried out on two model bacteria, *Escherichia coli* and *Escherichia faecalis*, also revealed the formation of DCAN and DCAcAm, confirming the precursor role of microorganisms in the formation of OXBPs. A study by Wang et al. reported that chlorination and monochloramination of *Escherichia coli* leads to the formation of the main OXBPs usually considered as resulting from chlorination of NOM [19].The authors also observed a positive correlation between the amount of generated OXBPs and bacterial decay, suggesting that the destruction of microorganisms by chlorinated oxidants results in the formation of OXBPs and their precursors. Several recent studies have reported the role of both algal cells and their excreted metabolic substances in OXBPs formation. It has been shown that organic matter from algae contains hydrophilic low molecular weight molecules that can act as effective precursors of OXBPs, especially THMs and HAAs [20]. The role played by anthropogenic organic chemicals in OXPBs formation is presented in Supplementary material 1.

I.2.2 Influence of the nature and dose of the halogen-based oxidant

The nature and dose of the oxidant (or combination of oxidants) greatly influences the amount and speciation of the OXBPs formed. Numerous studies report a strong correlation between the oxidant dose and the potential formation of OXBPs. In general, increasing the oxidant dose will result in increasing the formation of OXBPs [21]. Compared to chlorine, chloramines and chlorine dioxide are less reactive towards the organic matter and therefore produce less AOX and regulated by-products (THMs and HAAs). Compared to chlorine, monochloramine reduces the formation of THMs and HAAs by 88 and 68%, respectively [22]. Although reduced, the generation of dihalogenated HAAs remains high compared to other HAAs. However, monochloramination of iodide-containing waters as

well as chlorination in the presence of ammonia favors the formation of iodinated organic compounds such as I-THMs and iodinated acetic acids. Chloramination can also generate nitrogenous organohalogen by-products such as HNMs, chloral hydrate and HAcAms at levels sometimes exceeding those obtained by chlorination [23]. To explain this result, Yang et al. investigated the sources of nitrogen in trichloronitromethane and DCAN formed as by-products during chloramination of 31 organic nitrogen compounds, including amino acids, amines, dipeptides, purines, pyrimidones and pyrroles using isotopically-labelled monochloramine ($^{15}NH_2Cl$) [24]. They found that the nitrogen in these two by-products might originate from both monochloramine and organic nitrogen compounds, and that the nitrogen partition percentages depends on the nature of the reactants and pH. Yang et al. reported that switching from chlorination to chloramination sometimes led to an increase in the formation of unregulated dihalomethanes, particularly dichloromethane that became the main HMs species in the final drinking water treatment plants [25]. Johnson and Jensen studied the AOX produced from monochloramination and chlorination of isolated fulvic acid and found that AOX resulting from chloramination was more hydrophilic and had a higher molecular weight than AOX produced by chlorine. Hua et al. studied OXBPs precursors based on hydrophobicity and molecular size and showed that monochloramine produces higher molecular weight OXBPs than chlorine [26]. Unlike chlorine, chlorine dioxide does not react with ammonia to form chloramines but it reacts with water constituents to form inorganic by-products, with chlorite and chlorate representing the major part [27].

I.2.3 Influence of the nature and concentration of halide ion

Several studies have been carried out to characterize and understand the influence of bromide (Br^-) and iodide (I^-) ions on OXBPs speciation [28] and [29]. The particular case of fluoride ions is discussed in Supplementary material 2. In general, the presence of bromide and iodide ions in water at significant concentrations increases the formation of AOBr and AOI at the expense of their corresponding AOCl analogues [30]. However, the effect of these ions must be considered in conjunction with other key influencing parameters that may act concomitantly, such as contact time, pH, oxidant dose, temperature, ammonia concentration, and chemical characteristics of organic matter. Usually, bromide and iodide ions are present at low levels in freshwater but can be present at higher levels in certain waters depending on geology, notably proximity to salt deposits, and in marine and estuarine waters as well as in urban and industrial wastewater discharges. Iodide ions can be oxidized instantaneously by many oxidants such as chlorine, chlorine dioxide, and chloramines to form active iodine (I_2/HOI/IO^-), while ozone and chlorine rapidly oxidize bromide to form active bromine (Br_2/HOBr/BrO^-) [31]. Literature reported that monochloramine oxidizes only a small amount of bromide to bromine. However, it can react rapidly with either bromide and/or bromine to form inorganic bromamines which were found to be responsible of most of the AOBr formed. Unlike chlorine, bromine, ozone and

chlorine dioxide, chloramines do not quickly oxidize active iodine to iodate (IO_3^-), allowing for a greater contact time between active iodine and organic matter [32]. The main reaction equations involved in the reduction of bromide and iodide ions are presented in Fig. 1. The presence of bromide ions in high concentration significantly modifies the chlorination reactions. The injected chlorine instantaneously oxidizes bromide ions into hypobromous acid (HOBr) [28]. Hypobromous acid can also be formed as a by-product when seawater and saltwater are treated with ozone [33]. It is known that hypochlorous acid is a more effective oxidant than hypobromous acid ($E_{HOCl/Cl^-} = +1.63$ V; $E_{HOBr/Br^-} = +1.33$ V), whereas hypobromous acid is a more efficient halogen substitution agent. Uyak and Toroz reported that bromine is approximately 20 times more reactive with organic matter than chlorine [34]. Heeb et al. reported in their review that the reactions of HOBr with phenolate and halophenolates are on average around 3,000 times greater than the analog reactions with HOCl [31]. Consequently, the presence of bromide ions increases the overall mass concentration of OXBPs and produces more AOBr at the expense of AOCl analogues. THMs, HHAS and HNMS yields were observed to increase at increasing levels of bromide in water, under both disinfection processes, chlorination and chloramination [35]. Ged and Boyer studied the effect of seawater intrusion on the formation of bromine-containing THMs and HAAs during chlorination [36]. They found that when the bromide concentration increased from 38 µg/L in fresh groundwater to 974 µg/L in 2% intrusion seawater, the concentration of THMs increased from 43 to 206 µg/L and the concentration of HAAs increased from 39 to 75 µg/L. This increase was attributed both to the increased formation of AOBr species as well as to a shift from chlorinated species to their brominated analogues, with as a consequence a decrease in the concentration of regulated HAAs and THMs. In chlorinated seawater, the OXBPs generated are almost exclusively brominated ones [37]. The oxidation of bromide by chlorine dioxide is a very slow process that can be neglected in freshwaters where typical concentrations of bromide ions are low. However, in the presence of high bromide levels such as those found in seawater, chlorine dioxide produces OXBPs with a shift from chlorinated to more brominated OXBPs [38]. However, chlorine still forms more TOCl and TOBr than chloramines and chlorine dioxide in the presence of bromide. In freshwater, AOI can be formed by reaction between active iodine and organic matter. According to Krasner, the formation yield of AOI depends on the nature of the oxidant used and generally follows the order: monochloramine > chlorine dioxide > chlorine [39]. Ye et al. explored the formation of triiodomethane, iodoacetic acid (IAA) and triiodoacetic acid when iodide containing synthesized waters and raw waters were treated with chlorine dioxide [29]. The authors showed that among the monitored compounds, triiodomethane was the major by-product formed.

$$HOCl + Br^- \rightarrow HOBr + Cl^- \quad (1)$$

$$HOCl + I^- \rightarrow HOI + Cl^- \quad (2)$$

$$NH_2Cl + I^- + 2H_2O \rightarrow HOI + NH_4Cl + {}^-OH \quad (3)$$

$$O_3 + Br^- \rightarrow BrO_3^- \quad (4)$$

$$2ClO_2 + 2I^- \rightarrow I_2 + 2ClO_2^- \quad (5)$$

$$ClO_2^- + 4I^- + 4H^+ \rightarrow 2I_2 + Cl^- + 2H_2O \quad (6)$$

Fig. 1. Main reaction equations involved in the reduction of bromide and iodide ions during water treatment with oxidant agents.

During chlorination, the AOX, AOCl and AOI concentrations decreased substantially with increasing initial iodide concentrations due to the very fast oxidation of iodine to iodate. This reaction plays an important part in limiting the amount of active iodine that will subsequently be incorporated into organic matter. Fig. 2 displays the reactions of iodide with halogen-based oxidants and organic matter to form iodate and iodinated organohalogen by-products.

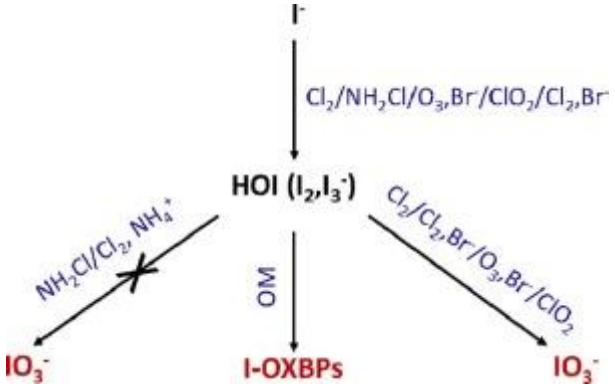


Fig. 2. Schematic illustration of the reactions of iodide with halogen-based oxidants and organic matter (OM) to form iodate and iodinated organohalogen by-products.

I.2.4 Influence of the contact time between water and halogen-based oxidants

The contact time between water and oxidant plays a very important role in both treatment efficiency and OXBPs formation and speciation. This parameter also determines the nature and amount of OXBPs to which human and aquatic systems may be exposed. In numerous studies, the speciation of OXBPs was related to contact time, mainly regarding THMs and HAAs formation. The common observation is a rapid initial formation of THMs and HAAs during chlorination, followed by a continued formation over time with a slower rate of increase [40]. Increasing contact time can lead to the decomposition of other by-products. The experiments of Zhang et al. on chlorinated ultrapure water spiked with commercial humic acid concluded that increasing contact time decreases the concentration of DCAN, chloropicrin, 1,1-dichloropropanone and 1,1,1-TCPN [40]. The decrease of

HAN and HK concentrations has been attributed to hydrolysis of these compounds and/or their reactions with residual chlorine. However, the contact time may show different trends depending on the quality of the treated water and the nature of the oxidant used. THMs; HHAS and HNMS yields were observed to increase at increasing levels of bromide in water, under both disinfection processes, chlorination and chloramination [35].

I.2.5 Influence of the temperature and pH value

A number of authors have discussed the effect of temperature on OXBPs formation. Increasing water temperature increases both reaction rates and formation levels of numerous OXBPs. In a study devoted to chlorination and monochloramination of ultrapure water spiked with NOM extracted from river water, Kristiana et al. showed that an increase in temperature from 20 to 50°C increases the amount of total OXBPs generated [41]. However, they did not observe any significant effect of temperature on the incorporation rate of halogen atoms in the NOM. It has been reported that the temperature influence varies significantly depending on the nature of the OXBPs. For example, Liu and Reckhow studied the effect of water temperature on the formation of THMs, HAAs, DHANs, chloropicrin, 1,1,1-TCPN and AOX during chlorination of drinking water and found that THMs, DCAA and chloropicrin concentrations were substantially higher in hot tap water than in cold tap water [42].

In almost all common uses of halogen-based oxidants in water treatments, operations take place at neutral to alkaline pH. The pH value is a critical parameter that can affect both treatment efficiency as well as OXBPs formation in different manners. For example, chlorine exhibits a pH-dependent equilibrium between the hypochlorous acid (HOCl) and the hypochlorite ion (OCl-). The pKa of $HOCl/ClO^-$ is approximately 7.5 (at 25°C). Therefore, in water at pH 8, about 30% of the free chlorine is present as HOCl, and at pH 6.5, 90% is present as HOCl. Of the two forms of free chlorine, hypochlorous acid is the most effective biocide and the form that generates the most OXBPs. In contrast to chlorine, the biocidal efficiency of chlorine dioxide does not depend on pH. The pH value also determines the speciation and stability of the chloramines. Generally, monochloramine is produced by adding chlorine to water containing ammonia (chlorine/nitrogen ratio ≤ 1) at a pH value around 8; below this value other forms of chloramines will predominate, thereby decreasing the biocidal effect and modifying the by-products formation potential in water. Monochloramine is generally the desired form. It is important to note that the decrease of concentrations of several by-products depending on pH is not only the result of deactivation of the mechanisms leading to their formation, but is also due to decomposition reactions. It has been reported that many OXBPs tend to be hydrolyzed under alkaline pH conditions ($pH > 8$) [2]. Hua and Reckhow examined the effect of pH on the potential generation of THMs and HAAs as well as on the stability of AOX in the absence of chlorine [43]. They found that hydrolysis of unstable THMs and HAAs intermediates at a basic pH

could be an important pathway leading to increased concentrations of THMs and HAAs. The stability of TOCl, TOBr, and TOI under alkaline pH conditions was in the following order: TOCl > TOBr > TOI. The UAOX-to-AOX ratio decreased with increasing pH value and contact time.

I.3 Main organohalogen by-product families and some regulatory limits for drinking and surface waters

Since the discovery in the mid-1970s of the carcinogenic effect of THMs, toxicological assessments in experimental animals have shown that other OXBPs may cause multiple adverse effects, including several types of cancer and disruption of the endocrine system [44]. Improvements in analytical techniques over recent years have revealed the formation of a large number of OXBPs. The main OXBPs detected with a regular frequency are: THMs, HAAs, HANs, HKs, CH, CX, HALs, HNMs, and HPs. It has been demonstrated that brominated OXBPs are generally more carcinogenic than their chlorinated analogues and the results of recent in vitro studies have demonstrated that the presence of iodine atoms in OXBPs increases the cytotoxicity and genotoxicity of the by-products as compared to their brominated and chlorinated analogues [45]. However, one should note that the tests carried out on animals and/or in vitro are performed at high concentrations; it is therefore difficult to estimate how these results can be extrapolated to human populations concerned mainly by chronic low-level exposure. Epidemiological investigations have indicated an increased risk of cancers in individuals who consume tap water treated with chlorine as well as an association between DBPs in drinking water and adverse reproductive or developmental health effects [46]. The occurrence of OXBPs in drinking water and the discharges of these contaminants from different industries into the aquatic environment is an issue of interest to policy makers, drinking water providers and engineers, epidemiologists, biologists and risk assessors. In order to minimize the ecological and health risks associated with exposure to OXBPs, the levels of some of these chemicals are regulated in various countries around the world, mainly in drinking water. A selection of regulatory limits and guideline values is provided in Supplementary material 3. Regulatory limits were recently reviewed by Richardson and Postigo [5]. Up till now, only about 50% of the AOX can be attributed to individual known OXBPs under typical chlorination conditions. In the case of monochloramine and chlorine dioxide the MB is even lower and he sum of the known halogenated by-products often accounts for less than 20% of AOX (see Table 1).

Table 1. Examples of percentages of UAOX depending on the oxidant used and the type of treated water in different experimental studies

Halogen-based oxidant	Water type	OXBPs monitored	Method used to estimate total organic halogen	UAOX
HOCl	Tap water	THMs, HAAs	C-IC off-line	***
HOCl	Cold & Hot Tap water	THMs, HAAs, HANs, Hks	C-IC off-line	Cold water 42% Hot water 30%
HOCl	Ultrapure water spiked with fulvic acid	***	C-IC on-line	***
HOCl & MCA	Raw water	THMs & I-THMs, HAAs, HANs, Hks	C-MC & C-IC off-line	HOCl 55-66% MCA 74-85%
HOCl & MCA	Raw water	THMs & I-THMs, HAAs, HANs, Hks	C-MC & C-IC off-line	HOCl 40-60% MCA 60-80%
HOCl & MCA	Raw water	THMs	C-IC off-line	HOCl 53% MCA 93%
HOCl & MCA	Raw water	THMs, HNMs, Hks	C-IC off-line	***
HOCl & MCA	Simulated drinking water	***	C-IC on-line	***
HOCl	Raw water	THMs, HAAs, MX, MCcA	C-MC	34-60%
HOCl & MCA	Raw water	THMs, HANs, HNMs, Hks	C-IC off-line	***
HOCl	Pool water	***	C-IC off-line	***
Cl_2, Cl_2-NH_2Cl, NH_2Cl	Raw water	THMs, HANs	C-MC	Cl_2 60-72% Cl_2-NH_2Cl 67-73% NH_2Cl 73-87%
HOCl	Tap Water	THMs, HAAs, HANs, HALs, HNMs	C-MC	41%
HOCl	River water concentrated, lyophilized, and reconstituted	THMs, HAAs, HANs, Hks	C-MC	***
HOCl	Raw water	THMs, HAAs, HANs, Hks	C-MC & C-IC	60-80%
HOCl	Raw water	THMs	C-IC on-line	***
HOCl	Raw water	THMs, HAAs, HANs, Hks	C-MC & C-IC	28-48.5%
HOCl	Swimming pool water	THMs, HAAs, CH, HAN	C-MC	53.7%
HOCl	Swimming pool water	THMs, HAAs, CH, HAN	C-MC	62%

Abbreviations: C-MC: adsorption, oxidative combustion followed by microcoulometric titration; C-IC: adsorption, oxidative combustion followed by ion chromatography titration; UOXBPs: unknown organohalogen byproducts; MCcA: mucochloric acid; MX: halogenated furanones; ***: Missing Information

Great uncertainty remains about the chemical nature of UOXBPs. This lack of knowledge is a barrier in the assessment of the effects of water treatments on ecosystems and human health. Therefore, more than 40 years after the discovery of THMs, the identification and toxicity assessment of UOXBPs remains a major goal and a continuously evolving challenge. This highlights the need to adopt a multidisciplinary comprehensive approach in order to significantly advance the knowledge about OXBPs. In some cases, this may reflect the need for development of new methods and alternative approaches for identifying missing OXBPs or quantifying already identified OXBPs, and in others, a need for improvement of the sensitivity of existing methods.

I.4 Quantitative analysis of OXBPs

Methodologies for quantitation of OXBPs are of two distinct types: those aiming to quantify individual known by-products and those referred to as "integrative" or "global" analysis used to measure the total concentration of OXBPs regardless of the chemical identity of the compounds measured. Quantitative and qualitative analysis of a wide range of known OXBPs would not only be prohibitively expensive but simply impossible due to limits in the available analytical methods for many chemicals. The target chemical analysis approach inevitably misses not only unidentified OXBPs, but also a major part of those whose chemical structure is already known. This raises the need to use analytical techniques capable of providing a comprehensive picture of the overall OXBPs formed. The following sections present a summary of the different organic halogen group parameters, with a description of their measurement principles, advantages, and limitations.

I.4.1 Integrative analysis - the AOX parameter

AOX is an analytically defined parameter that provides the concentration of overall OXBPs present in water samples regardless of the individual chemical identity and amounts of the compounds measured. In other words, AOX is defined as the amount of chlorine, bromine and iodine chemically bonded to organic compounds that is determined under specified analytical conditions ($AOX = AOI + AOBr + AOCl$). It detects all organic halides that are adsorbed by granular activated carbon under the conditions of the adsorption procedure. Generally, methods for determination of AOX involves four steps:

(1) Extraction of OXBPs from water samples by adsorption onto activated carbon;

(2) Washing of the filter cake with a nitrate solution to remove any trapped inorganic halides;

(3) Oxidative pyrolysis of the activated carbon to convert the adsorbed OXBPs to hydrogen halides (HX) and;

(4) On-line titration of X released with silver ion and measurement by microcoulometry.

A schematic diagram of an AOX apparatus is presented in Supplementary material 4, which also includes a description of its principle of operation and limitations. Since the introduction of the concept of AOX parameter in 1970s, many studies have been conducted in order to better characterize this technique and to improve its performance. The determination of AOX parameters is now the subject of numerous national and international standard methods, e.g. DIN 38414, EN ISO 9562, EPA 9020, ASTM D 4744, AWWA 5320 B. It has been reported that the major interferences when determining the AOX of a water sample are high concentrations of inorganic halides and dissolved organic carbon (DOC). The inorganic halides may tend to overestimate the measured concentrations while a high level of DOC tends to reduce the available surface area of activated carbon and thus underestimate the measured concentrations. Therefore, these standards are restricted to water samples whose levels of inorganic halides and DOC do not exceed 1 g/L and 100 mg/L, respectively. Regarding adsorption of organic halogen compounds onto activated carbon, the most described methods in the literature are the adsorption column method and the batch shaker procedure. For the batch method, activated carbon, in suspended form, is introduced into the water sample in a volumetric flask and the mixture is shaken for at least one hour. After the adsorption step, the activated carbon is separated from the water by filtration using usually a quartz frit. The column method consists in passing a water sample through a pair of mini-columns mounted in series. The sample passes through the columns with an inert gas under a flow rate of 3 ml/min approximately. The major concern when using the AOX method as a base to estimate the share of unknown OXBPs remains the precise and accurate determination of AOX values. Any overestimation of this measure inevitably leads to a high percentage of the part attributed to UAOX.

I.4.2 Integrative analysis - the EOX and POX parameters

Other parameters deriving from the AOX measurement have been introduced to differentiate OXBPs according to their polarity and volatility into two categories: EOX (Extractable Organic Halides) and POX (Purgeable Organic Halides). Compared to the conventional AOX procedure, the extraction on activated carbon is replaced by liquid-liquid extraction for EOX. This parameter represents the total amount of OXBPs which can be extracted by organic solvents (e.g. n-hexane, pentane, heptane, petroleum-ether or MTBE) especially from solid samples (sediments, organisms). For the POX parameter, it provides information on the volatile fraction of OXBPs in a water sample. The measurement consists of purging 10-ml aliquot of the sample using a stream of CO_2 at a temperature of approximately 45°C and sending the gas thus obtained directly to the pyrolysis furnace for online measurement of HX formed from volatile OXBPs by microcoulometry. These two parameters are especially useful because they allow having some idea on the physicochemical properties of the OXBPs. There are a few studies dedicated to the quantification of OXBPs into POX and EOX in water samples. In the study by Shukairy et al., the formation kinetics and the impact of chlorine and

monochloramine dose were investigated [61]. It was shown that chloramination produces significantly less POX, compared to chlorination. In addition, for both disinfectants and for both sources of organic matter, the EOX formation rate was found to be much faster than that of POX.

I.4.3 Integrative analysis - the AOCl, AOBr and AOI parameters

Halogen speciation of AOX into adsorbable organic chlorine, bromine and iodine, respectively AOCl, AOBr and AOI, is extremely important since it has been shown that iodinated and brominated organic by-products tend to be more toxic than their chlorinated analogues [45] and [62]. Several methods have been developed to quantify specifically halide ions formed by pyrolysis of AOX. The neutron activation analysis is one of the first methods to be used. Its implementation involves irradiation of the sample by a neutron flux to identify and quantify the radioactive halogen isotopes created [63]. Although sensitive and specific, it cannot be applied for routine analysis due to its high cost and skill level required. Ion chromatography (IC) has been reported in several studies as a method of choice for specific halide measurement. It associates the coupling of an off-line or online combustion oven with IC separation and conductivity or UV detection (Table 2). This method is not yet standardized and requires several development and optimization studies. Brandt and Kettrup developed a system to measure AOCl and AOBr with organic chlorine and bromine model compounds [64]. The system uses pure oxygen as combustion gas, hydrogen peroxide (H_2O_2) as absorption solution to convert the halogen species that can be formed in the combustion oven to halide ions (X^-), and separation by off-line IC and detection by conductivity. In another study, Echigo et al. added carbon dioxide as an auxiliary gas with the oxygen as a carrier gas during the combustion step and combustion gas collection directly into ultra-pure water [65]. This system was compared by Hua et al. with that developed by Oleksy-Frenzel et al. using pure oxygen and sulfuric acid to remove water vapor before gas collection into sodium sulfide solution and off-line IC [66]. This later achieved complete recovery of AOCl, AOBr and AOI from the OXBP model compounds, which was not the case for Echigo et al.'s method due to condensation of halide ions before the absorption and difficulty in their flushing out. Other problems encountered in the development of specific halogen analysis are the choice of absorption solution and detection mode after IC separation. It has been shown that the use of hydrogen peroxide is not suitable for AOI quantification. It converts I^- to other iodine species and consequently provides an underestimation of AOI recovery [67]. In addition, the use of ultra-pure water only as absorption solution cannot reduce dihalogens formed into halide ions before IC separation. To avoid this problem, Oleksy-Frenzel et al. used sodium sulfide to reduce other halogen forms into halide ions. For the detection mode, it has been shown that some chromatographic columns, such as IonPac AS14A, do not produce chromatograms with clear and quantifiable iodide peaks using conductivity detection at low levels [66]. The reason for this is that the quantification of Cl^- and Br^- are carried out by conductivity detection and I^- by UV-visible detection with two separate columns in two different

analyses. Oleksy-Frenzel et al. used conductivity and UV-visible double-channel detection for the analysis of Cl-, Br- and I- simultaneously at low levels [66]. Recently, Kristiana et al. performed a study where they analyzed Cl^-, Br^- and I^- simultaneously using separation by IonPac AS19 column and conductivity detection with detection limits lower than 3 µg/L for Cl^-, Br^- and I^- [68]. Developments in IC have produced a sensitive method for halide detection with the possibility to measure their incorporation rate in a water sample. However, there are still few studies addressing the development, optimization and halogen-specific analysis regarding OXBPs in water [47] and [64]. In addition, the lack of a universal protocol leads to varying degrees of OXBP recoveries.

Table 3. Main studies on specific halogen quantification using C-IC coupling

Sample	Online vs. off-line	Combustion temperature (°C)	Combustion time (min)	Combustion gas & flow (ml/min)	Absorption solution	IC column	Eluent & flow (ml/min)	Target ions & detection mode
Hydrophobic fraction of NOM from river water after chlorination and chloramination	Off-line	1000	10	O_2 150	EUP	AS9-HC Dionex	Na_2CO_3 1 ml/min	Cl^- & Br^- Conductivity
						AS11 Dionex	NaOH 1 ml/min	I^- UV-visible
Chlorinated drinking water	Off-line	1000	10	O_2 150	EUP	AS14A Dionex	Na_2CO_3 / $NaHCO_3$ 1 ml/min	Cl^- & Br^- Conductivity
						AS16A with AG16A Dionex	NaOH 1 ml/min	I^- UV-visible
Analytical standards of THMs and HAAs	Off-line	1000	10	O_2 150	EUP	AS14A with AG14A Dionex	Na_2CO_3 / $NaHCO_3$ 1 ml/min	Cl^- & Br^- Conductivity
						AS16A with AG16A Dionex	NaOH 1 ml/min	I^- UV-visible
Sample of peat	Off-line	800	10	O_2 150	EUP with Na_2SO_3	AS9-SC Dionex	Na_2CO_3 / $NaHCO_3$ / H_3BO_3 2 ml/min	Cl^- by Conductivity
								Br^- by UV_{210} nm I^- by UV_{226} nm
Chlorinated and brominated organic model compounds	Off-line	1000	10	O_2 150	EUP with Na_2SO_3	AS9-SC Dionex	Na_2CO_3 / $NaHCO_3$ / H_3BO_3 2 ml/min	Cl^- by Conductivity
								Br^- by UV_{210} nm I^- by UV_{226} nm
Simulated drinking water	Off-line	1000	10	O_2 150	EUP	ICS-90 Dionex	Na_2CO_3 / $NaHCO_3$ 1 ml/min	Cl^- & Br^- Conductivity
Sample of carbon	On-line	1050	Automatic + 3 min post-combustion	O_2 300	H_2O_2	Metrosep A supp 5 Metrohm	Na_2CO_3 / $NaHCO_3$ 0.7 ml/min	Cl^-, Br^- & I^- Conductivity
groundwater and surface water	On-line	1050	Automatic	O_2 400	H_2O	IonPac AS19 with IonPac AG1, Dionex	Na_2CO_3 / $NaHCO_3$ 1 ml/min	Cl^-, Br^- & I^- Conductivity

I.5 Conclusion

Since the discovery of THMs as water treatment by-products, research has focused on OXBPs formation mechanisms and on characterizing NOM or testing parameters (pH, temperature and water quality) that can explain and reduce OXBP formation, with a particular attention to the "regulated OXBPs", THMs and HAAs. Alternative disinfectants to chlorine, such as monochloramine, ozone or chlorine dioxide continue to be used in place of, or in combination with, chlorine to produce less THMs and HAAs. However, it has been shown that these alternative disinfectants may also generate higher concentrations of "unregulated OXBPs" some of which being more toxic than regulated OXBPs.

AOX is a key parameter for estimating the total amount of OXBPs in a water sample. Nevertheless, its significance for OXBP studies should not be overestimated. Several factors can affect the AOX value, such as the volatility and stability of some OXBPs, the removal of inorganic halides by the nitrate washing solution used in the AOX measurement procedure and the reduction of some OXBPs adsorbed onto activated carbon. These factors raise some questions about the reliability of the MB between unidentified and total OXBPs and trigger the need to take into account the amount of OXBP not recovered through the different stages of the AOX measurement method in the MB calculation.

The speciation of AOX into AOCl, AOBr and AOI is extremely important since iodinated and brominated organic by-products have been shown to be more toxic than their chlorinated analogues. Several methods have been developed to quantify specifically halide ions formed by pyrolysis of AOX and the most used and reported method is ion chromatography. However, there are still only a few studies addressing the development, optimization and halogen-specific analysis of OXBPs in water samples and there is a need for studies comparing the total amount of OXBPs given by the AOX parameter and the halogen specific-AOX analysis.

References

[1] R.D. Morris, R. Levin, Estimating the incidence of waterborne infectious disease related to drinking water in the United States, IAHS Publications-Series of Proceedings and Reports-Intern Assoc, Hydrol. Sci. 233 (1995) 75-88.
[2] P.C. Singer, Control of disinfection by-products in drinking water, J. Environ. Eng. (New York) 120 (1994) 727-744.
[3] J.J. Rook, Formation of haloforms during chlorination of natural waters, Water Treat. Exam. 23 (1974) 234-243.
[4] J.G. Pressman, S.D. Richardson, T.F. Speth, R.J. Miltner, M.G. Narotsky, I.I.I.E.S. Hunter, et al., Concentration, chlorination, and chemical analysis of drinking water for disinfection byproduct mixtures health effects research: U.S. EPA's Four Lab Study, Environ. Sci. Technol. 44 (2010) 7184-7192.
[5] S.D. Richardson, C. Postigo, Drinking water disinfection by-products, Chapter 4, in: The Handbook of Environmental Chemistry: Emerging Organic Contaminants and Human Health, vol. 20, Springer, New York, NY, 2012, pp. 93-137.
[6] S. Chowdhury, P. Champagne, P.J. McLellan, Models for predicting disinfection byproduct (DBP) formation in drinking waters: a chronological review, Sci. Total Environ. 407 (2009) 4189-4206.
[7] M.J. Plewa, E.D. Wagner, Quantitative Comparative Mammalian Cell Cytotoxicity and Genotoxicity of Selected Classes of Drinking Water Disinfection By-Products, Illinois Water Conference, 2008.
[8] M. Deborde, U. Von Gunten, Reactions of chlorine with inorganic and organic compounds during water treatment - kinetics and mechanisms: a critical review, Water Res. 42 (2008) 13-51.
[9] G. Hua, D.A. Reckhow, Comparison of disinfection byproduct formation from chlorine and alternative disinfectants, Water Res. 41 (2007) 1667-1678.
[10] R.L. Jolley, J.H. Carpenter, Aqueous Chemistry of Chlorine: Chemistry, Analysis, and Environmental Fate of Reactive Oxidant Species, Oak Ridge National Lab., TN (USA), 1982.
[11] E.E. Lavonen, M. Gonsior, L.J. Tranvik, P. Schmitt-Kopplin, S.J. Köhler, Selective chlorination of natural organic matter: identification of previously unknown disinfection byproducts, Environ. Sci. Technol. 47 (2013) 2264-2271.
[12] P. Singer, H. Weinberg, S. Krasner, H. Arora, I. Najm, Relative Dominance of HAAs and THMs in Treated Drinking Water, AWWA Research Foundation and American Water Works Association, 2002. ISBN 1-58321-117-9.
[13] K. Doederer, W. Gernjak, H.S. Weinberg, M.J. Farré, Factors affecting the formation of disinfection by-products during chlorination and chloramination of secondary effluent for the production of high quality recycled water, Water Res. 48 (2014) 218-228.
[14] S.D. Richardson, C. Postigo, Formation of DBPs: state of the science, Chapter 11, in: Recent Advances in Disinfection By-Products, vol. 1190, ACS Symposium Table 2 Main studies on specific halogen quantification using C-IC coupling Series, 2015, pp. 189-214.
[15] W.-T. Li, S.-Y. Chen, Z.-X. Xu, Y. Li, C.-D. Shuang, A.-M. Li, Characterization of dissolved organic matter in municipal wastewater using fluorescence PARAFAC analysis and chromatography multi-excitation/emission scan: a comparative study, Environ. Sci. Technol. 48 (2014) 2603-2609.
[16] A.C. Diehl, G.E. Speitel, J.M. Symons, S.W. Krasner, C.J. Hwang, S.E. Barrett, DBP formation during chloramination, J. Am. Water Works Assoc. 92 (4) (2000) 76-90.
[17] H.K. Shon, S. Vigneswaran, S.A. Snyder, Effluent organic Matter (EfOM) in wastewater: constituents, effects, and treatment, Crit. Rev. Environ. Sci. and Tech. 36 (2006) 327-374.
[18] H. Huang, Q.-Y. Wu, X. Tang, R. Jiang, H.-Y. Hu, Formation of haloacetonitriles and haloacetamides during chlorination of pure culture bacteria, Chemosphere 92 (2013) 375-381.
[19] J.-J. Wang, X. Liu, T.W. Ng, J.-W. Xiao, A.T. Chow, P.K. Wong, Disinfection byproduct formation from chlorination of pure bacterial cells and pipeline biofilms, Water Res. 47 (2013) 2701-2709.
[20] K. Zhang, T.F. Lin, T. Zhang, C. Li, N. Gao, Characterization of typical taste and odor compounds formed by Microcystis aeruginosa, J. Environ. Sci. 25 (2013) 1539-1548.
[21] J. Hu, H. Song, T. Karanfil, Comparative analysis of halonitromethane and trihalomethane formation and speciation in drinking water: the effects of disinfectants, pH, bromide, and nitrite, Environ. Sci. Technol. 44 (2010) 794-799.
[22] C. Tian, R. Liu, H. Liu, J. Qu, Disinfection by-products formation and precursors transformation during chlorination and chloramination of highly-polluted source water: significance of ammonia, Water Res. 47 (2013) 5901-5910.

[23] T. Bond, N.H.M. Kamal, T. Bonnisseau, M.R. Templeton, Disinfection by-product formation from the chlorination and chloramination of amines, J. Hazard. Mater. 278 (2014) 288-296.
[24] X. Yang, Q. Shen, W. Guo, J. Peng, Y. Liang, Precursors and nitrogen origins of trichloronitromethane and dichloroacetonitrile during chlorination/ chloramination, Chemosphere 88 (2012) 25-32.
[25] F. Yang, J. Zhang, W. Chu, D. Yin, M.R. Templeton, Haloactamides versus halomethanes formation and toxicity in chloraminated drinking water, J. Hazard. Mater. 274 (2014) 156-163.
[26] G. Hua, D.A. Reckhow, Characterization of disinfection byproduct precursors based on hydrophobicity and molecular size, Environ. Sci. Technol. 41 (2007) 3309-3315.
[27] S.D. Richardson, A.D. Thruston, C. Rav-Acha, L. Groisman, I. Popilevsky, O. Juraev, et al., Tribromopyrrole, brominated acids, and other disinfection byproducts produced by disinfection of drinking water rich in bromide, Environ. Sci. Technol. 37 (2003) 3782-3793.
[28] G. Ding, X. Zhang, M. Yang, Y. Pan, Formation of new brominated disinfection byproducts during chlorination of saline sewage effluents, Water Res. 47 (2013) 2710-2718. [29] T. Ye, B. Xu, Y.-L. Lin, C.-Y. Hu, L. Lin, T.-Y. Zhang, et al., Formation of iodinated disinfection by-products during oxidation of iodide-containing waters with chlorine dioxide, Water Res. 47 (2013) 3006-3014.
[30] K. Watson, M.J. Farré, N. Knight, Strategies for the removal of halides from drinking water sources, and their applicability in disinfection by-product minimisation: a critical review, J. Environ. Manage. 110 (2012) 276-298.
[31] M.B. Heeb, J. Criquet, S.G. Zimmermann-Steffens, U. Von Gunten, Oxidative treatment of bromide-containing waters: formation of bromine and its reactions with inorganic and organic compounds - a critical review, Water Res. 48 (2014) 15-42.
[32] Y. Bichsel, U. Von Gunten, Formation of iodo-trihalomethanes during disinfection and oxidation of iodide-containing waters, Environ. Sci. Technol. 34 (2000) 2784-2791.
[33] U. Von Gunten, Ozonation of drinking water: part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine, Water Res. 37 (2003) 1469-1487.
[34] V. Uyak, I. Toroz, Investigation of bromide ion effects on disinfection by-products formation and speciation in an Istanbul water supply, J. Hazard. Mater. 149 (2007) 445-451.
[35] H. Hong, Y. Xiong, M. Ruan, F. Liao, H. Lin, Y. Liang, Factors affecting THMs, HAAs and HNMs formation of Jin Lan Reservoir water exposed to chlorine and monochloramine, Sci. Total Environ. 444 (2013) 196-204.
[36] E.C. Ged, T.H. Boyer, Effect of seawater intrusion on formation of bromine containing trihalomethanes and haloacetic acids during chlorination, Desalination 345 (2014) 85-93.
[37] H. Shi, Z. Qiang, C. Adams, Formation of haloacetic acids, halonitromethanes, bromate and iodate during chlorination and ozonation of seawater and saltwater of marine aquaria systems, Chemosphere 90 (2013) 2485-2492.
[38] X. Yang, W. Guo, Q. Shen, Formation of disinfection byproducts from chlor(am)ination of algal organic matter, J. Hazard. Mater. 197 (2011) 378-388.
[39] S.W. Krasner, The formation and control of emerging disinfection by-products of health concern, Philos. Trans. A Math. Phys. Eng. Sci. 367 (2009) 4077-4095.
[40] X. Zhang, H.W. Yang, X.M. Wang, J. Fu, Y.F. Xie, Formation of disinfection by-products: effect of temperature and kinetic modeling, Chemosphere 90 (2013) 634-639.
[41] I. Kristiana, H. Gallard, C. Joll, J.-P. Croué, The formation of halogen-specific TOX from chlorination and chloramination of natural organic matter isolates, Water Res. 43 (2009) 4177-4186.
[42] B. Liu, D.A. Reckhow, Disparity in disinfection byproducts concentration between hot and cold tap water, Water Res. 70 (2015) 196-204.
[43] G. Hua, D.A. Reckhow, DBP formation during chlorination and chloramination: effect of reaction time, pH, dosage, and temperature, J. Am. Water Works Assoc. 100 (2008).
[44] Y. Deng, Y. Zhang, R. Zhang, B. Wu, L. Ding, K. Xu, et al., Mice in vivo toxicity studies for monohaloacetamides emerging disinfection byproducts based on metabolomic methods, Environ. Sci. Technol. 48 (2014) 8212-8218.
[45] M.S. Attene-Ramos, E.D. Wagner, M.J. Plewa, Comparative human cell toxicogenomic analysis of monohaloacetic acid drinking water disinfection byproducts, Environ. Sci. Technol. 44 (2010) 7206-7212.
[46] J. Colman, G.E. Rice, J.M. Wright, E.S. Hunter, L.K. Teuschler, J.C. Lipscomb, et al., Identification of developmentally toxic drinking water disinfection byproducts and evaluation of data relevant to mode of action, Toxicol. Appl. Pharmacol. 254 (2011) 100-126.
[47] G. Hua, D.A. Reckhow, Determination of TOCl, TOBr and TOI in drinking water by pyrolysis and off-line ion chromatography, Anal. Bioanal. Chem. 384 (2006) 495-504.
[48] Y. Li, X. Zhang, C. Shang, Effect of reductive property of activated carbon on total organic halogen analysis, Environ. Sci. Technol. 44 (2010) 2105-2111.

[49] P.A. Neale, A. Antony, M.E. Bartkow, M.J. Farré, A. Heitz, I. Kristiana, et al., Bioanalytical assessment of the formation of disinfection byproducts in a drinking water treatment plant, Environ. Sci. Technol. 46 (2012) 10317-10325.
[50] H. Zhai, X. Zhang, X. Zhu, J. Liu, M. Ji, Formation of brominated disinfection byproducts during chloramination of drinking water: new polar species and overall kinetics, Environ. Sci. Technol. 48 (2014) 2579-2588.
[51] D. Zheng, R.C. Andrews, S.A. Andrews, L. Taylor-Edmonds, Effects of coagulation on the removal of natural organic matter, genotoxicity, and precursors to halogenated furanones, Water Res. 70 (2015) 118-129.
[52] M.J. Farré, S. Day, P.A. Neale, D. Stalter, J.Y.M. Tang, B.I. Escher, Bioanalytical and chemical assessment of the disinfection by-product formation potential: role of organic matter, Water Res. 47 (2013) 5409-5421.
[53] F. Xiao, X. Zhang, H. Zhai, I.M.C. Lo, G.L. Tipoe, M. Yang, et al., New halogenated disinfection byproducts in swimming pool water and their permeability across skin, Environ. Sci. Technol. 46 (2012) 7112-7119.
[54] C. Tian, R. Liu, T. Guo, H. Liu, Q. Luo, J. Qu, Chlorination and chloramination of high-bromide natural water: DBPs species transformation, Separ. Sci. Technol. 102 (2013) 86-93.
[55] J.G. Pressman, D.L. McCurry, S. Parvez, G.E. Rice, L.K. Teuschler, R.J. Miltner, et al., Disinfection byproduct formation in reverse-osmosis concentrated and lyophilized natural organic matter from a drinking water source, Water Res. 46 (2012) 5343-5354.
[56] S. Liu, Z. Zhu, Y. Qiu, J. Zhao, Effect of ferric and bromide ions on the formation and speciation of disinfection byproducts during chlorination, J. Environ. Sci. 23 (2011) 765-772.
[57] E.M. Smith, M.J. Plewa, C.L. Lindell, S.D. Richardson, W.A. Mitch, Comparison of byproduct formation in waters treated with chlorine and iodine: relevance to point-of-use treatment, Environ. Sci. Technol. 44 (2010) 8446-8452.
[58] G. Hua, D.A. Reckhow, Effect of alkaline pH on the stability of halogenated DBPs, J. Am. Water Works Assoc. 104 (2012) 49-50.
[59] D.A. Freyfer, Sous-produits de chloration dans les eaux de piscine-Effet de l'ozonation (Ph.D. thesis), Institut des milieux et matériaux, Université de Poitiers., 2012 (accessed 05.04.16).
[60] J. De Laat, F. Berne, R. Brunet, C. Hue, Sous-produits de chloration formés lors de la désinfection des eaux de piscines. Etude bibliographique, Eur. J. Water Qual. 40 (2009) 109-128.
[61] H.M. Shukairy, R. Scott Summers, The impact of preozonation and biodegradation on disinfection by-product formation, Water Res. 26 (1992) 1217-1227.
[62] Y. Yang, Y. Komaki, S.Y. Kimura, H.-Y.B.J. Mariñas, Toxic impact of bromide and iodide on drinking water disinfected with chlorine or chloramines, Environ. Sci. Technol. 48 (2014) 12362-12369.
[63] P. Mello, J. Barin, F. Duarte, C. Bizzi, L. Diehl, E. Muller, et al., Analytical methods for the determination of halogens in bioanalytical sciences: a review, Anal. Bioanal. Chem. 405 (2013) 7615-7642.
[64] G. Brandt, A. Kettrup, Determination of organic group parameters (AOCl, AOBr, AOs) in water by means of ion-chromatographic detection, Fresen. J. Anal. Chem. 327 (1987) 213-219.
[65] S. Echigo, X. Zhang, A. Minear Roger, J. Plewa Michael, Differentiation of total organic brominated and chlorinated compounds in total organic halide measurement: A new approach with an ion-chromatographic technique, natural organic matter and disinfection by-products, American Chemical Society, Washinton D.C., 2000, pp. 330-342.
[66] J. Oleksy-Frenzel, S. Wischnack, M. Jekel, Application of ion-chromatography for the determination of the organic-group parameters AOCl, AOBr and AOI in water, Fresen. J. Anal. Chem. 366 (2000) 89-94.
[67] H. Narayanan, Trace level halides in coal by metrohm combustion IC, in: Application Work AW IC US6-0167-012013, Metrohm. 2013 (accessed 05.04.16).
[68] I. Kristiana, S. McDonald, J. Tan, C. Joll, A. Heitz, Analysis of halogen-specific TOX revisited: method improvement and application, Talanta 139 (2015) 104-110.
[69] A. Putschew, M. Mania, M. Jekel, Occurrence and source of brominated organic compounds in surface waters, Chemosphere 52 (2003) 399-407.

Supplementary Materials (SM)

SM 1. Role played by anthropogenic organic chemicals in OXPBs formation

Anthropogenic organic chemicals are compounds introduced into the aquatic environment primarily through human activities. They include chemicals of widely differing classes and properties such as pesticides, polynuclear aromatic hydrocarbons (PAHs), natural hormones, phtalic acid diesters, prescription and therapeutic drugs, veterinary drugs, personal care products, several alkanes, organic solvents, detergents, and surfactants. These contaminants are frequently detected in ground water, streams, rivers, and drinking water sources. Although present at trace concentrations compared to NOM, their low concentration may be counterbalanced by their very high reactivity. Therefore, research on this category of chemicals is currently accelerating, with most studies focusing on chlorination. For example, Hu et al. showed that chlorination of bensulfuron-methyl, which is used as a herbicide, forms trichloromethane (TCM), DCAN, 1,1,1-TCP and chloropicrin [1]. Li et al. found that in drinking water bisphenol-A (BPA) reacts quickly with chlorine sequentially producing chlorinated and brominated BPA congeners, halogenated phenols, and finally THMs and HAAs [2]. Xie et al. investigated the nature of the OXBPs formed during filtration of chlorinated water by polyvinyl pyrrolidone-polysulfone (PVP-PSF) membranes [3]. They identified five OXBPs, namely TCM, CH, 1,1-dichloro-2-propanone, 1,1,1-TCP and chloropicrin, formed under drinking water treatment conditions. Brominated THMs were also detected and correlated to the presence of bromide ions. Zhou et al. (2014) investigated the OXBPs formed during chlorination and monochloramination of tetracycline antibiotics (TCs) [4]. They observed the formation of TCM, carbon tetrachloride, DCAN and dichloroacetone (DCAce). Duirk et al. showed that chlorination and monochloramination of iopamidol, a chemical used in radiology as iodinated X-ray contrast medium, yielded only trace amounts of iodinated trihalomethanes (I-THMs) and iodinated acetic acids (I-HAAs), whereas the levels of I-THMs and I-HAAs were enhanced substantially in raw water samples (containing NOM) [5]. Kumkum, Ye et al., Wang et al. and Postigo and Richardson reported similar results. It has been demonstrated that pharmaceuticals containing iodine could be a significant source of iodide ions and thus a significant contributor to the formation of total organic iodide [6-8].

References

[1] C.-Y. Hu, M. Cheng, Y.-L. Lin, Chlorination of bensulfuron-methyl: Kinetics, reaction factors and disinfection by-product formation, J. Taiwan Inst. Chem. E. 53 (2015) 46-51.

[2] C. Li, Z. Wang, Y.J. Yang, J. Liu, X. Mao, Y. Zhang, Transformation of bisphenol A in water distribution systems: A pilot-scale study, Chemosphere 125 (2015) 86-93.

[3] P. Xie, C.-F. de Lannoy, J. Ma, M.R. Wiesner, Chlorination of polyvinyl pyrrolidone-polysulfone membranes: Organic compound release, byproduct formation, and changes in membrane properties, Ecotox. Environ. Safe. 489 (2015) 28-35.

[4] S. Zhou, Y. Shao, N. Gao, S. Zhu, Y. Ma, J. Deng, Chlorination and chloramination of tetracycline antibiotics: Disinfection by-products formation and influential factors, Ecotox. Environ. Safe. 107 (2014) 30-35.

[5] S.E. Duirk, C. Lindell, C.C. Cornelison, J. Kormos, T.A. Ternes, M. Attene-Ramos, J. Osiol, E.D. Wagner, M.J. Plewa, S.D. Richardson, Formation of toxic iodinated disinfection by-products from compounds used in medical imaging, Environ. Sci. Technol. 45 (2011) 6845-6854.

[6] P. Kumkum, Iodinated Pharmaceuticals as Precursors to Total Organic Halogen Formation in the Presence of Chlorinated Oxidants and Absence of Natural Organic Matter, Master of Science in Engineering, University of Akron, USA, 2013.

[7] T. Ye, B. Xu, Z. Wang, T.-Y. Zhang, C.-Y. Hu, L. Lin, S.-J. Xia, N.-Y. Gao, Comparison of iodinated trihalomethanes formation during aqueous chlor (am) ination of different iodinated X-ray contrast media compounds in the presence of natural organic matter, Water Res. 66 (2014) 390-398.

[8] Z. Wang, B. Xu, Y.-L. Lin, C.-Y. Hu, F.-X. Tian, T.-Y. Zhang, N.-Y. Gao, A comparison of iodinated trihalomethane formation from iodide and iopamidol in the presence of organic precursors during monochloramination, Chem. Eng. J. 257 (2014) 292-298.

[9] C. Postigo, S.D. Richardson, Transformation of pharmaceuticals during oxidation/disinfection processes in drinking water treatment, J. Hazard. Mat. 279 (2014) 461-475.

SM 2. Discussing the case of fluorine containing byproducts

Fluoride is a ubiquitous constituent of natural waters, with concentrations from less than 0.01 ppm to 50 ppm. However, the complexation of fluoride ions (F^-) by calcium ions limits the maximum amount of this element in solution to approximately 6 ppm, except in water containing an abnormally low calcium ion concentration. It is important to stress that the fluoride ion concentrations in natural waters exceeds those of iodide, for example.

The values of standard reduction potentials of fluorine, chlorine, bromine and iodine are 2.78, 1.36, 1.07 and 0.53, respectively. This explains why when waters containing bromide and iodide are treated with free chlorine; these are oxidized to their corresponding halogen. Organobromines and organoiodines are then produced from the reaction of organic matter with iodine and bromine. Given its reduction potential, fluorine (F2) cannot be obtained from fluoride by chemical oxidation, even with a very strong oxidant such as ozone (E = 2.07 V). Furthermore, fluorine cannot be prepared in aqueous solution, following its reaction with water ($F_2 + 2\ H_2O \Rightarrow O_2 + 2\ HF$; $E°(H_2O/O_2) = 1.23$ V).

Fluorinated chemicals can be found in naturel water. They can be issued from natural or anthropogenic sources (pharmaceuticals, pesticides, surfactants, refrigerants). The known biologically produced organofluorines contain only one fluorine atom, while synthetic ones contain more fluorine atoms. For example, the trifluoromethyl group or the fluorophenyl group are often incorporated into drug molecules to make them more resistant to being metabolized. The water treatment by halogen-based oxidants may possibly change the chemical nature of initial organofluorines and lead to others chemicals.

The C-X bond strength decreases in the order F > Cl > Br > I. Thus, the organofluorines are very stable and therefore tend to persist in the environment. There is a lack of data on the toxicity of these chemicals. Data from animal studies of polyfluorinated compounds, like perfluorooctanoic acid and perfluorooctane sulfonate, indicate that these compounds can cause several types of tumors and neonatal death and may have toxic effects on the nervous, digestive and immune systems.

A response on the formation of fluorinated products is a little dated by Bunn et al in 1975 [1]. They confirmed one of the suspicious of Rook that chlorinated river water in the presence of added fluoride, bromide and iodide ions lead to the formation of all possible chlorine, bromine and iodine compounds and not fluorine compounds [2]. Recently, all studies mention the formation and evolution of chlorine, bromine and iodine containing compounds but only a few ones mention the presence of fluorinated contaminants in a number of tap waters [3-6].

Organofluorine compounds cannot be assessed by the conventional AOX method with microcoulometric detection. The microcoulometric detection is based on the low aqueous solubilities of AgCl, AgBr, and AgI. Due to the good solubility of AgF (1.8 kg/L at 25 °C) this sensitive method fails for the detection of organofluorine compounds.

References

[1] W.W. Bunn, B.B Hass, E.R. Deane, R.D. Kleopfer, Formation of trihalomethanes by chlorination of surface water, Environ. Lett. 10 (1975) 205-213.

[2] J.J. Rook, Formation of haloforms during chlorination of natural waters, J. Water Treat. Exam. 23 (1974) 234-243.

[3] P.C. Rumsby, C.L. McLaughlin, T. Hall, Perfluorooctane sulphonate and perfluorooctanoic acid in drinking and environmental waters, Phil. Trans. R. Soc. A 367 (2009) 4119-4136.

[4] F.T. Lang, M. Wenz, C.K. Schmidt, H.J. Brauch, Occurrence of perfluoroalkyl sulfonates and carboxylates in German drinking water sources compared to other countries, Water Sci. Technol. 56 (2007) 151-158.

[5] C. Eschauzier, J. Haftka, P.J. Stuyfzand, P. de Voogt, Perfluorinated compounds in infiltrated river Rhine water and infiltrated rainwater in coastal dunes, Environ. Sci. Technol. 44 (2010) 7450-7455.

[6] A. Wagner, B. Raue, H.J. Brauch, E. Worch, F.T. Lange, Determination of adsorbable organic fluorine from aqueous environmental samples by adsorption to polystyrene-divinylbenzene based activated carbon and combustion ion chromatography, J. Chrom. A 1295 (2013) 82-89.

SM 3. List of the main OXBP groups commonly produced in water depending on the halogen-based oxidant used and a selection of regulatory and guideline values for OXBPs. Synthetized from Mouly et al. (2008), Hrudey (2009), Chowdhury et al. (2009), and Richardson (2011) [1-4].

Main group	Compound	Halogen-based oxidant				Regulations µg/L			
		Chlorine	ClO_2	Chloramines	O_3	WHO	U.S. EPA	EU	WFD
Trihalomethanes THMs	Chloroform	+	-	+	-	300			2,5
	Bromodichloromethane	+	-	+	-	60			
	Dibromochloromethane	+	-	+	-	100			
	Dibromochloramine	+	-	+	-				
	Bromoform	+	-	+	+(c)	100			
	TTHMs	+	-	+	-		80	100	
Iodo-Trihalomethanes I-THMs	Dichloroiodomethane	-	-	+	+(b)				
	Chlorodiiodomethane	-	-	+	+(b)				
	Bromodiiodomethane	-	-	+	+(b)				
	Bromochloroiodomethane	-	-	+	+(b)				
	Dibromoiodomethane	-	-	+	+(b)				
	Iodoform	-	-	+	+(b)				
Haloacetic acids HAAs	Monochloroacetic acid	+	-	+	-	20	ΣHAA5 = 60		
	Dichloroacetic acid	+	-	+	-	50			
	Trichloroacetic acid	+	-	+	-	200			
	Monobromoacetic acid	+	-	+	-				
	Dibromoacetic acid	+	-	+	-				
	Tribromoacetic acid	+	-	+	-				
	Bromochloroacetic acid	+	-	+	-				
	Bromodichloroacetic acid	+	-	+	-				

	Dibromochloroacetic acid	+	-	+	-				
Iodo-Haloacetic acids I-HAAs	Iodoacetic acid	-	-	+	-				
	Bromoiodoacetic acid	-	-	+	-				
Haloacetonitriles HANs	Chloroacetonitrile	+	+	+	+				
	Dichloroacetonitrile	+	+	+	+	20			
	Trichloroacetonitrile	+	+	+	+				
	Bromoacetonitrile	+	+	+	+				
	Dibromoacetonitrile	+	+	+	+	70			
	Tribromoacetonitrile	+	+	+	+				
	Bromochloroacetonitrile	+	+	+	+				
	Bromodichloroacetonitrile	+	+	+	+				
	Chlorodibromoacetonitrile	+	+	+	+				
Iodo-Haloacetonitriles	Iodoacetonitrile	-	-	+	-				
Halonitromethanes HNMs	Chloronitromethane	+	-	+	+ (a)				
	Dichloronitromethane	+	-	+	+ (a)				
	Trichloronitromethane	+	-	+	+ (a)				
	Bromonitromethane	+	-	+	+ (a)				
	Dibromonitromethane	+	-	+	+ (a)				
	Tribromonitromethane	+	-	+	+ (a)				
	Bromochloronitromethane	+	-	+	+ (a)				
	Bromodichloronitromethane	+	-	+	+ (a)				
	Chlorodibromonitromethane	+	-	+	+ (a)				
Haloacetamides HAcAms	Chloroacetamide	+	-	+	-				
	Dichloroacetamide	+	-	+	-				
	Trichloroacetamide	+	-	+	-				

	Bromoacetamide	+	-	+	-				
	Dibromoacetamide	+	-	+	-				
	Tribromoacetamide	+	-	+	-				
	Bromochloroacetamide	+	-	+	-				
	Bromodichloroacetamide	+	-	+	-				
	Chlorodibromoacetamide	+	-	+	-				
Iodo-Haloacetamides I-HAcAms	Iodoacetamide	-	-	+	-				
	Diiodoacetamide	-	-	+	-				
	Chloroiodoacetamide	-	-	+	-				
	Bromoiodoacetamide	-	-	+	-				
Haloketones HKs	Chloropropanone	+	-	-	-				
	1,1-Dichloropropanone	+	-	-	-				
	1,3-Dichloropropanone	+	-	-	-				
	1,1-Dibromopropanone	+	-	-	-				
	1,1,1-Trichloropropanone	+	-	-	-				
	1,1,3-Trichloropropanone	+	-	-	-				
	1-Bromo-1,1-dichloropropanone	+	-	-	-				
	1,1,1-Tribromopropanone	+	-	-	-				
	1,1,3-Tribromopropanone	+	-	-	-				
	1,1,3,3-Tetrachloropropanone	+	-	-	-				
	1,1,1,3-Tetrachloropropanone	+	-	-	-				
	1,1,3,3-Tetrabromopropanone	+	-	-	-				
Haloaldehydes HALs	Chloroacetaldehyde	+	+	-	+ (b)				
	Dichloroacetaldehyde	+	+	-	+ (b)				
	Trichloroacetaldehyde	+	+	-	+ (b)				

	Bromochloroacetaldehyde	+	+	-	+ (b)				
	Bromoacetaldehyde	+	+	-	+ (b)				
	Dibromoacetaldehyde	+	+	-	+ (b)				
	Tribromoacetaldehyde	+	+	-	+ (b)				
Halofuranones MX compounds	3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone	+	-	-	-				
	3-Chloro-4-(dichloromethyl)-2(5H)-furanone	+	-	-	-				
	(E)-2-Chloro-3-(dichloromethyl)-butenedioic acid	+	-	-	-				
	(E)-2-Chloro-3-(dichloromethyl)-4-oxobutenoic acid	+	-	-	-				
	2,3-Dichloro-4-oxobutenoic acid	+	-	-	-				
	3-Chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone	+	-	-	-				
	3-Chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone	+	-	-	-				
	3-Bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone	+	-	-	-				
	(E)-2-Chloro-3-(bromochloromethyl)-4-oxobutenoic acid	+	-	-	-				
	Chloro-3-(dibromomethyl)-4-oxobutenoic acid	+	-	-	-				
	(E)-2-Bromo-3-(dibromomethyl)-4-oxobutenoic acid	+	-	-	-				
Halobenzoquinones HBQs	2,6-Dichloro-1,4-benzoquinone	+	-	+	-				
	2,6-Dichloro-3-methyl-1,4-benzoquinone	+	-	+	-				
	2,3,6-Trichloro-1,4-benzoquinone	+	-	+	-				
	2,6-Dibromo-1,4-benzoquinone	+	-	+	-				

References

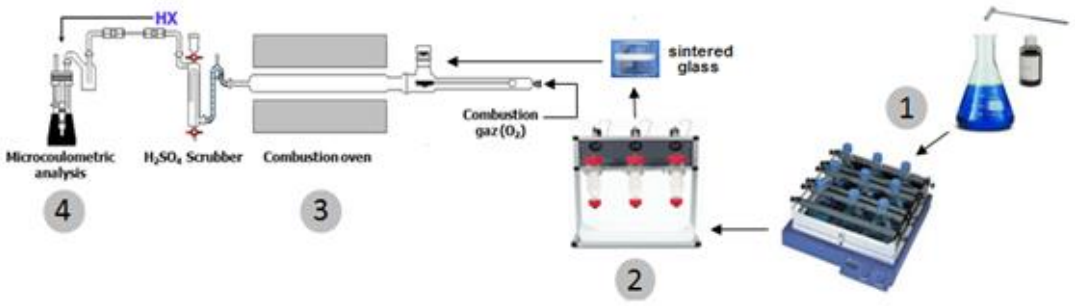
[1] D. Mouly, E. Joulin, C. Rosin, P. Beaudeau, A. Zeghnoun, A. Olszewski-Ortar, J.-F. Munoz, Les sous-produits de chloration dans l'eau destinée à la consommation humaine en France. Campagnes d'analyses dans quatre systèmes de distribution d'eau et modélisation de l'évolution des trihalométhanes, Institut de Veille Sanitaire (InVS), 2008.

[2] S.E. Hrudey, Chlorination disinfection by-products, public health risk tradeoffs and me, Water Res. 43 (2009) 2057-2092.

[3] S.D. Richardson, Disinfection by-products: formation and occurence in drinking water, J.O. Nriagu (Ed.), Encyclopedia of Environmental Health. Elsevier Science Inc., Burlington, 2011, pp. 110-136.

[4] S. Chowdhury, P. Champagne, P.J. McLellan, Models for predicting disinfection byproduct (DBP) formation in drinking waters: a chronological review, Sci. Tot. Environ. 407 (2009) 4189-4206.

SM 4. Schematic diagram of AOX apparatus (batch shaker procedure) - principle of operation and limitations



Several OXBPs are present under their ionic form under typical water treatment conditions. For example, HAAs are moderately strong and therefore dissociated nearly completely (> 99.9%) to the haloacetate ions at pH > 6. Thus, to improve the adsorption of this class of OXBPs on activated carbon, it is recommended to acidify the samples at pH < 2 with concentrated nitric acid. The detection principle is based on the reaction between generated halide ions and silver ions maintained at constant concentration in a titration cell. This concentration is managed by a potential between two electrodes: an indicator electrode and a reference electrode. When hydrogen halides produced from the combustion of organic halogen compounds enter into the cell, they rapidly react with the silver ions to form silver halide complexes, AgX, which are insoluble in acetic acid electrolyte and will precipitate. This will cause a change in the concentration of the silver ions and give rise to a change in the potential of reference. To restore the silver ions consumed, a potential voltage is applied to a solid silver electrode (anode) to produce silver ions in the cell. The intensity of the electrical current produced during the combustion is proportional to the number of moles of halides captured in the cell. Unlike the other silver halides, which are insoluble in water like silver fluoride complex (AgF) (solubility value approximately 1720 g/L in water) due to the high solvation enthalpy of fluoride ions, organofluorine compounds cannot be measured by the argentometric method and therefore do not fit into the AOX classification. On the other hand, microcoulometric titration is incapable of distinguishing between halides and therefore the AOX method cannot differentiate between AOCl, AOBr, and AOI; the AOX content is commonly expressed as micrograms equivalent chloride per litre (µg eq Cl-/L). In order to establish the percentage of known AOX, the concentration of each measured OXBPs must be converted to chloride ion. The mass concentrations of OXBPs mixture must be converted first to their chlorinated analogues, and then to chloride ions (e.g. DCBM, DBCM and TBM converted to TCA). The MB is determined according to the following equation:

$$\%MB\ (part\ of\ known\ AOX) = 100\,.\frac{\frac{n\Sigma(OXnBPi).M(Cl)}{M(OXnBPi)}}{AOX}$$

Formation and determination of organohalogen by-products in water - Part II. Sample preparation techniques for analytical approaches

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Abstract

There is a crucial need of analytical methods allowing the characterization and/or the quantitation of known and unknown organohalogen by-products (OXPBs) in water. This article is the second one (Part II) of a review trio dedicated to the formation and determination of organohalogen by-products in water; it presents an overview of the sample preparation techniques generally used prior to OXPBs analysis and details their advantages and drawbacks. After a comparison of the water samples generally analyzed (natural water versus deionized water spiked with organic matter), this section deals with sample preservation, OXPBs enrichment and selective extraction.

Keywords: Organohalogen; By-products; Water; Sample preparation; Extraction; Analysis

Abbreviations

AC	Activated charcoal
ACN	Acetonitrile
AOBr	Adsorbable organic bromine
AOCl	Adsorbable organic chlorine
AOI	Adsorbable organic iodine
AOX	Adsorbable organic halide
CAR	Carboxen
CF	Continuous flow
CLSA	Closed-loop stripping analysis
CWX	Carbowax
DAD	Diode array detector
DAI	Direct aqueous injection
DBA	Dibutylamine
DBP	Disinfection by-product
DELCD	Dry electrolytic conductivity detector
DHS	Dynamic headspace
DI-SPME	Direct immersed solid phase microextraction
DLLME	Dispersive liquid-liquid microextraction
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DVB	Divinylbenzene
ECD	Electron capture detector
ESI	Electrospray ionization
GC	Gas chromatography
HAAs	Haloacetic acids
HAcAms	Haloacetamides
HALs	Haloacetaldehydes
HANs	Haloacetonitriles
HBQs	Halobenzoquinones
HF-LPME	Hollow-fiber based LPME
HKs	Haloketones
HNMs	Halonitromethanes
HS	Headspace
HSs	Humic substances
I-THMs	Iodinated trihalomethanes
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LOD	Limit of detection
LPME	Liquid-phase microextraction
MB	Mass balance
MEPS	Microextraction by packed sorbent
MIP	Molecularly imprinted polymers
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MtBE	Methyl tert-butyl ether
MWCO	Molecular weight cut-of
NOM	Natural organic matter
NRD	Non reported data
OXBPs	Organohalogen by-products
P&T	Purge and trap
PDMS	Polydimethylsiloxane
PS-DVB	Styrene divinylbenzene copolymers
PTV	Programmable temperature vaporizing
RO	Reverse osmosis
RSD	Residual standard deviation
SA-DLLME	Salt-assisted dispersive liquid-liquid microextraction
SBSE	Stir-bar sorptive extraction
SDME	Single-drop microextraction
SHS	Static headspace
SPE	Solid phase extraction
SPME	Solid phase microextraction
TCM	Trichloromethane, Chloroform
TD	Thermal desorption
THMs	Trihalomethanes
TPH	Transphilic compounds
UAOX	Unknown AOX
UF	Ultrafiltration
VALLME	Vortex assisted liquid-liquid microextraction

II.1 Introduction

The discovery of chloroform (TCM) as a disinfection by-product (DBP) in chlorinated water in 1974 and evidence of its carcinogenicity have led to the hypothesis that other potentially toxic organohalogen DBPs could also be generated [1]. Since then, extensive research has been directed toward developing a better understanding of the formation of organohalogen DBPs as a first step in the evaluation of their toxic effects on humans and aquatic ecosystems. For this purpose, various strategies coupled with modern analytical techniques have been developed and applied to the molecular identification of "unknown" organohalogen by-products (OXBPs), in both laboratory-scale experiments and studies of samples from full-scale water treatment processes. Despite the increase in the number of quantified OXBPs and the improved sensitivity of analytical methods, the material balance (MB), defined as the percentage of known halides attributed to quantified known OXBPs divided by the adsorbable organic halide (AOX) values, remains significantly below 100%. It is estimated that 50% of the AOX in chlorinated drinking water has been identified [2]. The MB is particularly low in the case of drinking water treated with monochloramine and chlorine dioxide, where the value is around 20% [3]. In the case of raw river water, the MB is even lower. The limited portion of OXBPs identified in the case of monochloramine is not surprising, considering its low reactivity with organic substances, due to a lower oxidation potential compared with free chlorine and the molecular complexity of the NOM. Many studies have shown that chloramination produces highly polar and high molecular mass AOX compounds [4]. Low MB values might also be partly attributed to the loss and/or transformation of target OXBPs during sample treatment procedures and/or the insufficient sensitivity of the analytical methods used [5]. As mentioned previously, the identity and the biological effect of a significant portion of OXBPs formed during the chemical oxidation of water with halogen-based oxidants are still unknown. Hence, the toxicological and ecotoxicological effects due to exposure to a mixture of these chemicals cannot be assessed precisely. It is, therefore, crucial to continue to identify new OXBPs and to increase the scope and sensitivity of the analytical techniques employed. Determining OXBPs in a water sample generally involves an appropriate sample preparation stage to preserve and enrich the analytes. This is then followed by an instrumental analysis, mainly using chromatographic techniques coupled with mass spectrometry. The differences in the chemical structures, polarities and volatility levels of OXBPs require a combination of different instrumental techniques and analytical methodologies. A diagram of the analytical process for determining OXBPs in water is presented in Fig. 1. This article is the second one (Part II) of a review trio dedicated to the formation and determination of organohalogen by-products in water; it deals with the sample preparation techniques available for OXPBs analysis and compares their advantages and drawbacks. The first article of the trio (Part I) is devoted to the formation of OXPBs by-products and to the relevance of the AOX parameter usually used to estimate their concentrations in different types

of waters [6]. The third and last article (Part III) is devoted to the analytical techniques currently used for OXPBs structural elucidation and quantitation [7]. After discussing the water samples generally analyzed (natural water versus deionized water spiked with organic matter), this article deals with sample preservation, OXPBs enrichment and selective extraction.

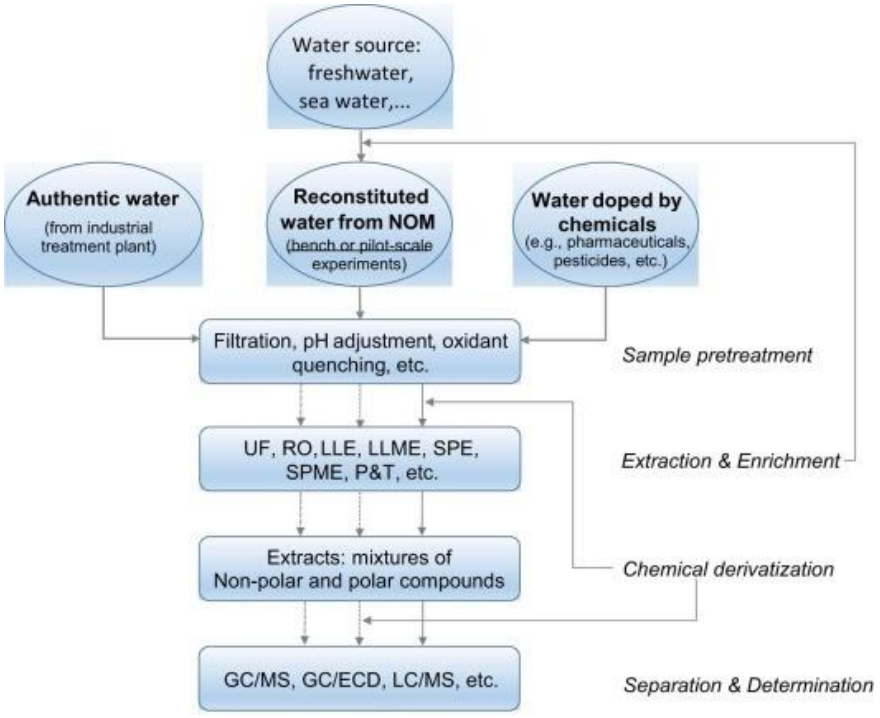

Fig. 1. Flow chart showing the common steps used in sample preparation and DBP analysis.

UF: UltraFiltration, RO: Reverse Osmosis, LLE: Liquid-Liquid Extraction, LLME: Liquid-Liquid MicroExtraction, SPE: Solid Phase Extraction, SPME: Solid Phase MicroExtraction, P&T: Purge and Trap, GC: Gas Chromatography, MS: Mass Spectrometry, ECD: Electron Capture Detection, LC: Liquid Chromatography.

II.2 Water sample types: natural vs synthetic (reconstituted) water

Various strategies coupled with modern analytical techniques have been developed and applied to the molecular identification of "unknown" organohalogen by-products, in both laboratory-scale experiments and studies based on samples from full-scale water treatment processes. These can be divided into three different approaches: (i) those based on the analysis of samples from actual water treatment facilities, (ii) those based on the analysis of treated ultrapure water doped by fractions of natural organic matter isolated from real water, and (iii) those based on the analysis of treated ultrapure water, fortified by commercial organic compounds considered as potential or model precursors to OXBPs, such as pharmaceuticals, pesticides, NOM models, humic acid, etc. In the first approach, more commonly used in current practice, treated water is sampled from a variety of sources, such as rivers, seas, swimming pools or water reprocessing cycles. The use of real water samples

eliminates any need to change the processing conditions, which could affect the quantity or quality of the OXBPs actually formed. However, the use of these kinds of samples is not without drawbacks. The spatial and temporal variations in the water quality associated with ad hoc sampling mean that identified OXBPs will not be representative of those likely to be formed in other water sources. On the other hand, the presence of NOM, anthropogenic organic chemicals and inorganic compounds like bromide, induces competitive reactions and consequently leads to the formation of a wider range of OXBPs [6]. The detection and tentative structural elucidation of unknown OXBPs formed in complex matrices at very low concentration levels are extremely difficult tasks. Moreover, the OXBPs formed may be unstable, potentially leading to the formation of other organohalogen by-product families during the time between sample collection and analysis in the laboratory. To overcome these limitations, the use of synthetic water samples, prepared by diluting OXBP precursors in ultrapure water (purified, deionized or distilled water), followed by treatment under controlled laboratory conditions (e.g. prolonged contact times with high oxidant levels), is widely reported in literature [8], [9], [10] and [11]. The aim of this approach is to produce relatively "clean water matrices" with high concentrations of OXBPs to facilitate their structural identification. However, it is important to address the question of whether identified OXBPs obtained under simple conditions can be representative of those likely to be formed in a more complex medium. Obviously, the occurrence of identified chemicals must be verified a posteriori in real water. Two kinds of samples are commonly used: (i) ultrapure water doped with commercially available organic compounds considered as potential precursors to OXBPs, and (ii) ultrapure water doped with NOM fractions previously extracted and concentrated from raw water. In some cases, NOM is first extracted from water and then used to dope water at high concentration to investigate DBP formation under these particular conditions [12]. Many studies have focused on the isolation, concentration and fractionation of NOM, according to its molecular weight and its physicochemical properties. Among NOM isolation and fractionation techniques, reverse osmosis (RO), ultrafiltration (UF), size exclusion chromatography, and resin adsorption chromatography are the most widely used. NOM isolation and characterization can have two main objectives: (i) to investigate relationships between the formation of DBPs and various NOM characteristics (e.g. hydrophobicity, specific ultraviolet absorbance at 254 nm, etc.), and (ii) to separate and structurally characterize OXBPs [13]. These techniques will be discussed in section II.

Most recent research on the identification of OXBPs has been conducted on laboratory water samples, rather than on actual water samples. However, all of these approaches are still complementary and each one has its advantages and drawbacks. Their application to different types of water has enabled the identification of various OXBPs, such as trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs) and halonitromethanes (HNMs) in drinking water, and THMs, HAAs, HANs, HNMs, halobenzoquinones (HBQs) and halokenones (HKs) in swimming pool water,

secondary effluent for the production of high quality recycled water, and urban wastewater [13], [14] and [15]. The characterization of emerging OXBPs derived from reactions between halogen-based oxidants and environmental contaminants is a matter of increasing concern, given the toxicity of the parent compounds [16] and [17]. These contaminants include pesticides, personal care products, estrogens, bisphenol A, alkylphenol surfactants and algal toxins. However, given the very low levels of water contaminants, the organohalogen by-products formed from these chemicals account for only a minor portion of AOX.

II.3 Sample preparation

Sample preparation for OXBP identification and quantification may consist of multiple steps including: sample preservation, enrichment and selective extraction and fractionation to isolate the targeted chemicals. Sample preparation serves several purposes, such as to stabilize the organohalogen by-products prior to their analysis, to eliminate or reduce potential interference, to enhance the sensitivity of the analytical procedure by increasing the analyte concentration, and to convert the analytes into a more suitable form that can be easily characterized by the techniques used. We review these different sample preparation steps in the following subsections.

II.3.1 Sample preservation

At the time of sample collection, steps must be taken to prevent further OXBPs from forming, while minimizing any loss. Sample preservation involves three main aspects: (i) residual oxidant quenching, (ii) pH adjustment to minimize chemical hydrolysis, and (iii) protection from microbial degradation. These steps are crucial, especially if the water samples are not analyzed immediately after they have been collected.

II.3.1.1 Quenching agents effect

Water treated with halogen-based oxidants often contains a residual oxidant that continues to react with organic matter until the water samples are extracted. To prevent any change, or the continued formation of OXBPs during the holding time between sample collection and analysis, the residual oxidant must be neutralized immediately after sampling. This is carried out by reducing it to a non-reactive form through the action of a quenching agent, based on the chemical reactions summarized in Fig. 2. The ideal reagent should not affect the OXBP concentrations during the residual oxidant quenching process or during sample storage. Thus, several reducing agents have been tested for both their efficacy in reducing residual halogen-based oxidants and their ability to preserve the targeted OXBPs. The more popular ones are: ammonium salts (ammonium chloride (NH_4Cl) and ammonium sulfate ($(NH_4)_2SO_4$), sulfur derivatives (sodium sulfite (Na_2SO_3) and sodium thiosulfate ($Na_2S_2O_3$)),

ascorbic acid ((2R)-2-[(1S)-1,2-dihydroxyethyl]-4,5-dihydroxyfuran-3-one), and sodium arsenite ($NaAsO_2$) [18] and [19]. Other quenching agents, such as oxalic acid, borohydride ($NaBH_4$) and amines like tris(hydroxymethyl)aminomethane, are less frequently used than those quoted above [20], [21] and [22]. In order to determine AOX in surface and waste water samples, the ISO 9562:2004 standard method recommends using sodium sulfite (N_2SO_3) [23]. This method does not provide precise details about the appropriate quenching time. However, since chlorine, inorganic chloramines and chlorine dioxide react rapidly with sulfur derivatives, a contact time of one to five minutes is considered to be sufficient to completely reduce the residual oxidant. On a weight-to-weight basis, approximately 1.77, 3.14 and 1.20 parts of sodium sulfite are required to remove one part of chlorine, monochloramine and chlorine dioxide respectively. Generally, quenching agents are added in excess relative to the amount of residual oxidant. However, numerous studies have demonstrated that sulfur derivatives cause the decomposition of a fraction of AOX [24]. The effects of quenching agents on the preservation of regulated OXBPs have been widely investigated and discussed, but their effects on unregulated OXBPs have only been partially addressed. Examples of studies devoted to quenching agents for sample preservation are detailed in Supplementary Material S1. The reviewed performance levels of the different quenching agents are listed in Table 1. Please note that, to avoid more than 200 references in the manuscript, the references corresponding to Table 1, Table 2, Table 3, Table 4 and Table 5 are given in Supplementary Material S2. Particular attention must be paid to the choice of quenching agent used for storing water samples prior to their analysis. In studies seeking to identify a wide range of known OXBPs, different quenching agents must be used. More research is still needed on the effects of quenching agents, especially on the stability of emerging organohalogen by-products (such as iodinated and brominated OXBPs) and unknown OXBPs.

Reactions with chlorine:

$$Na_2SO_3 + HOCl \rightarrow Na_2SO_4 + HCl \quad (1)$$

$$NH_4Cl + HOCl \rightarrow NH_2Cl + HCl + H_2O \quad (2)$$

$$Na_3AsO_3 + HOCl \rightarrow Na_3AsO_4 + HCl \quad (3)$$

$$C_5H_5O_5CH_2OH + HOCl \rightarrow HCl + H_2O + C_5H_3O_5CH_2OH \quad (4)$$

Reactions with monochloramine:

$$Na_2SO_3 + NH_2Cl + H_2O \rightarrow Na_2SO_4 + NH_4Cl \quad (5)$$

$$Na_3AsO_3 + NH_2Cl + H_2O \rightarrow Na_3AsO_4 + NH_4Cl \quad (6)$$

$$C_5H_5O_5CH_2OH + NH_2Cl \rightarrow NH_4Cl + C_5H_3O_5CH_2OH \quad (7)$$

Reactions with chlorine dioxide:

$$Na_2SO_3 + 2\,ClO_2 + H_2O \rightarrow Na_2SO_4 + 2\,HClO_2 \quad (8)$$

$$NH_4Cl + 2\,ClO_2 \rightarrow NH_2Cl + 2\,HClO_2 \quad (9)$$

$$Na_3AsO_3 + 2\,ClO_2 + H_2O \rightarrow Na_3AsO_4 + 2\,HClO_2 \quad (10)$$

$$C_5H_5O_5CH_2OH + 2\,ClO_2 \rightarrow 2\,HClO_2 + C_5H_3O_5CH_2OH \quad (11)$$

Fig. 2. Main reactions involved in the quenching process for chlorine, monochloramine and chlorine dioxide.

Table 1. Most frequently used quenching agents, depending on the OXBP family, the pH and the type of chlorinated oxidant. (the corresponding references are given in Supplementary Material 2)

OXBP family	Cl_2		MCA		ClO_2	
	Quenching agent	pH	Quenching agent	pH	Quenching agent	pH
THMs	$C_6H_8O_6$	< 7	$C_6H_8O_6$	< 7	NH_4Cl	7
		7-8		7		
		7		7-8		
	Ag^+/H_2O_2	7	Na_2SO_3	< 6		
				5		
				7		
				7-8		
	$NaAsO_2$	7-8	$NaAsO_2$	7-8		
	$NaBH_4$	7-8	$NaBH_4$			
	NH_4Cl	5	$Na_2S_2O_3$	7		
		7				
		7-8				
	$Na_2S_2O_3$	6				
	Na_2SO_3	7				
		7-8				
HAAs	$C_6H_8O_6$	< 7	$C_6H_8O_6$	< 7	NH_4Cl	0.5
		7-8		7-8		
	NH_4Cl	5	Na_2SO_3	< 6		
		7-8	Na_2SO_3	7-8		
		7	$NaAsO_2$	7-8		
	Ag^+/H_2O_2	7	$NaBH_4$	7-8		
	Na_2SO_3	7-8	NH_4Cl	5		7
	$NaAsO_2$			7		
	$NaBH_4$	7-8				
			$Na_2S_2O_3$	7		
HANs	$(NH_4)_2SO_4$	5	$C_6H_8O_6$	7	NH_4Cl	7
	NH_4Cl	< 7	NH_4Cl	5		
		7	$Na_2S_2O_3$	7.5		
		5		7		
	$Na_2S_2O_3$	6				
HKs	$C_6H_8O_6$	1.5	$C_6H_8O_6$	< 7	NH_4Cl	7
		< 7		7		
	NH_4Cl	7	$Na_2S_2O_3$	7.5	-	-
				7		
	$Na_2S_2O_3$	6				
HALs	$C_6H_8O_6$	4.5	$C_6H_8O_6$	< 7	-	-
		< 7		7-8		
		7-8	$NaAsO_2$	7-8	-	-
	$NaAsO_2$	7-8	$NaBH_4$	7-8		

	$NaBH_4$	7-8				
HNMs	$(NH_4)_2SO_4$	5	$C_6H_8O_6$	< 7	-	-
	NH_4Cl	< 7				
	$C_6H_8O_6$	< 7			-	-
HAcAms	NH_4Cl	5	$C_6H_8O_6$	< 7	-	-
		< 7				
	$C_6H_8O_6$	< 7		7.5		
		7		7		
		7.5				
AOX	$NaAsO_2$	2	Na_2SO_3	2	Na_2SO_3	2
	Na_2SO_3	2	$Na_2S_2O_3$	2		

II.3.1.2 pH effect

Extensive studies have been conducted to investigate the effect of pH on the stability of OXBPs during the storage of water samples. In general, increasing the water pH increases the hydrolysis reaction rate of many OXBPs. Hua and Reckhow showed that THM and dihaloacetic acid concentrations increased substantially under alkaline pH conditions, even in the absence of chlorine residuals [25]. They found that: (i) the hydrolysis of unstable THM and dihaloacetic acid intermediates accounted for approximately 20% of the total quantity of these two compounds, (ii) hydrolysable intermediates accounted for approximately 20% of the unknown AOX (UAOX), and (iii) the UAOX-to-AOX ratio decreased as the pH value increased and also over time. In addition to quenching the residual oxidant, the ISO 9562 standard method recommends acidifying the water sample with nitric acid ($pH < 2$) to preserve the AOX. Hydrolysis reaction rates depend on the functional group, the number of halogens and the halogen type. These reactions can lead to the loss of organic halides, resulting in the formation of fewer halogenated organic by-products or none at all. Chen reviewed the hydrolysis rate constants (kH) for six groups of OXBPs; they found that the kHvalues were ordered as follows (for chlorinated and brominated species): haloketones ($6.4\ 10^{-8}$ & $38.2\ 10^{-1}\ h^{-1}$) > haloacetonitriles ($8.1\ 10^{-4}$ & $5.4\ 10^{-1}\ h^{-1}$) > haloacetaldehydes ($2.1\ 10^{-4}$ & $2.4\ 10^{-1}\ h^{-1}$) > haloacetic acids ($6.6\ 10^{-12}$ & $5.4\ 10^{-3}\ h^{-1}$) > trihalomethanes ($1.3\ 10^{-8}$ & $4.3\ 10^{-7}\ h^{-1}$). Within each OXBP class, chlorinated organic by-products were found to undergo a slower hydrolysis process than their brominated counterparts; at a later stage, these may be hydrolyzed more slowly than their iodinated counterparts [26]. In terms of stability, adsorbable organic chlorine (AOCl), adsorbable organic bromine (AOBr) and adsorbable organic iodine (AOI) should be ordered as follows: AOCl > AOBr > AOI [25]. Glezer et al. studied the hydrolysis rates of haloacetonitriles in aqueous buffer solutions [26]. They found that the stability of HANs decreased with an increasing pH value and an increasing number of halogen atoms in the molecule. They observed the highest hydrolysis rates for the trihaloacetonitriles, followed by the

dihaloacetonitriles and, finally, the monohalogenated forms. They suggested preserving HANs in weak acid solutions between sampling and analysis. The hydrolysis of OXBPs may result in the formation of other forms of OXBPs. For example, it has been reported that the hydrolysis of haloketones, trihalogenated acetaldehydes and acetic acids at a pH > 6 will lead to the formation of THMs [27, 28] and [29]. The hydrolysis of haloacetonitrile compounds under basic conditions yields the corresponding haloacetamides which can be further hydrolyzed into the corresponding haloacetic acids [26]. Chu et al. reported that haloacetamides could be preserved for seven days by adjusting the pH value of the samples to 5 [30]. A pH of 4.5 was recommended by Koudjonou and LeBel for HAL analysis [29]. According to Serrano et al., to ensure the integrity of HKs for at least one week, samples should be acidified to a pH of 1.5 [31]. The hydrolysis of OXBPs significantly affects their speciation. Water samples should be analyzed as soon as possible after sampling and, if they need to be stored, a low pH condition is generally recommended. The pH of water samples may be adjusted to within the desired range by adding an adequate quantity of a strong mineral acid, typically hydrochloric acid (HCl), sulfuric acid (H2SO4) or nitric acid (HNO3). Although the three above-mentioned acids are reported in literature, no study has been carried out to date on the impact of the type of acid on the stability of OXBPs. Nonetheless, care should be taken when selecting the type and quality of the acid to be used. Some sources of hydrochloric acid may contain residual chlorinated compounds that can interfere with the determination of targeted OXBPs and may even lead to erroneous identification results, whereas nitric acid is a powerful oxidant that can oxidize the analytes of interest.

II.3.1.3 Antimicrobial agent effect

Organohalogen by-products can be subject to microbiological degradation during the transportation and storage of water samples. For example, it has been reported that HAAs are biodegradable over short periods of time in the absence of appropriate levels of disinfectants. In contrast to the biodegradation of HAA species, THMs do not biodegrade. The order of biodegradability has been reported as monochloroacetic acid > dichloroacetic acid > trichloroacetic acid, with the corresponding brominated species undergoing better degradation than chlorinated species [32]. Water samples containing appropriate levels of free chlorine can be protected against microbial degradation, even after quenching, using ammonium salts, as chloramines formed in situ have biocidal properties. In the absence of a residual disinfectant, a microbial inhibitor must be added to protect the OXBPs from biodegradation, especially if the samples are to be stored prior to analysis. The most frequently used chemical preservatives are mercuric chloride ($HgCl_2$), sodium azide (NaN_3), phosphoric acid/copper sulfate (H_3PO_4/$CuSO_4$), 2-mercaptopyridine-1-oxide sodium salt, hydrazoic acid and formaldehyde. Only a very small number of studies have reported data on the influence of preservatives on OXBPs in natural water. Singer et al. examined two biocides, mercuric chloride ($HgCl_2$) and sodium azide (NaN3), in relation to HAA stability at pH levels of 7 and 10 [33]. They observed that $HgCl_2$ had a

slightly negative impact on some HAA species, whereas NaN3 appeared to maintain the stability of all HAA species for up to two weeks at either pH value. Despite the lack of information regarding the biotic stability of OXBPs in water samples, it is well known that most bacteria grow optimally within a pH range between 5 and 9. Therefore, it is expected that the stability of OXBPs could be improved at low pH values and that the acidification of water samples would minimize the biodegradation of some OXBPs. A low pH would also prevent the adsorption of some OXBPs onto organic suspended matter and sample containers. There is currently no study dealing with the effect of the nature of containers on OXBPs conservation. Amber glass - to avoid sunlight exposure - or high density polyethylene are the sample containers most used in literature.

II.3.2 Enrichment and selective extraction of organohalogen by-products

Conducting laboratory experiments under well-defined and controlled conditions is the most frequently used approach for identifying unknown DBPs. As concentrations of DBPs and DBP precursors are usually lower in real conditions, laboratory experiments are carried out with high concentrations of oxidant and/or OXBP precursors. Batch experiments can be performed with samples from real water, ultrapure water doped with OXBP precursors, or ultrapure water fortified with natural organic matter isolated from real water.

II.3.2.1 Isolation and fractionation of natural organic matter and OXBPs

Natural organic matter has been recognized as the most significant source of DBP precursors. The isolation and fractionation of NOM is necessary in order to obtain more uniform material for further characterization and to gain a better understanding of its reactivity with halogen-based oxidants. This reactivity depends on the NOM's chemical composition and its structural characteristics. Isolated fractions of NOM are also required to evaluate the potential formation of organohalogen by-products and to obtain high levels of OXBPs, which are usually formed in low concentrations in conventional treatment processes. Various approaches and fractionation methods have been developed in the past four decades. Among them, the most applied are reverse osmosis (RO), ultrafiltration, and resin adsorption chromatography [34].

II.3.2.1.1 Reverse osmosis

RO is a membrane separation process widely used for NOM concentration from natural water [35] and [36]. The reverse osmosis membrane acts as a semi-permeable barrier allowing the selective transfer of water molecules while retaining the NOM. Pressure is exerted on the side containing natural water to force the water molecules to move through the membrane to the fresh water side. Water that passes through the RO membrane is released from the system as permeate. NOM molecules that do not pass

through the membrane are recirculated and concentrated in the system as retentate. The advantages of the RO method include: (i) the absence of harsh chemical conditions such as extreme pH values or contact with chemical solvents during isolation, (ii) the recovery of very large amounts of dissolved organic matter (DOM) with minimal fractionation (yield higher than 80%, quantified as dissolved organic carbon), (iii) high sample concentration values, and (iv) the ability to process large volumes of water in a relatively short period of time. Nevertheless some disadvantages should be mentioned, especially: (i) the concentration of inorganic ions along with the DOM, which may need to be removed prior to subsequent characterization and reactivity studies, and (ii) the loss of some DOM components due to sorption onto the membrane, leakage via permeate, or precipitation within the concentrate [37]. Dissolved organic carbon (DOC) yields are calculated using the following equations:

$$\%\,\mathrm{Recovery} = \frac{C(\mathrm{mf})\,\mathrm{V(mf)} + \mathrm{C(mr)}\,\mathrm{V(mr)}}{C(\mathrm{sw})\,\mathrm{V(sw)}} \times 100 \qquad \text{(eq. 1)}$$

$$\%\,\mathrm{Loss} = \frac{C(\mathrm{mp})\,\mathrm{V(mp)}}{C(\mathrm{sw})\,\mathrm{V(sw)}} \times 100 \qquad \text{(eq. 2)}$$

$$\%\,\mathrm{Rejection} = \frac{C(\mathrm{mr})\,\mathrm{V(mr)}}{\mathrm{C(mr)}\,\mathrm{V(mr)} + C(\mathrm{mp})\,\mathrm{V(mp)}} \times 100 \qquad \text{(eq. 3)}$$

where V and C are the volumes and DOC concentrations of the water flush (mf), retentate (mr), permeate (mp) and source water (sw), respectively. Many studies do not specify whether the membrane cleaning step was performed, concentration mass balances are rarely provided, and only a few studies report the percentage of loss or rejection [38]. It is important to specify these parameters for a better evaluation of the RO method. According to Hu et al., the RO method recovers more acid and neutral fractions than alkaline fractions due to the repulsion induced by the negative charge of the RO membrane. Also, high molecular weight hydrophobic compounds are recovered with higher yields than low molecular weight hydrophilic ones. These findings raise many questions about the OXBPs that may result from the basic and polar low molecular weight compounds of NOM and their potential effects on the MB. Kitis et al. reported that RO isolation preserves NOM reactivity with regard to trihalomethane and haloacetic acid formation upon reaction with chlorine [37]. Recently, Pressman et al. found that the chlorination of NOM from lyophilized RO isolates produced similar concentrations of halogenated DBPs to those produced during the chlorination of drinking water. However, bromine incorporation appeared to be slightly lower in RO concentrates, probably due to the formation of unknown brominated compounds 39]. In the study by Speth et al., RO was used to concentrate 2,400 L of drinking water to approximately 18 L, corresponding to a volume concentration factor of 133 [40]. Several studies have examined the effectiveness of RO membranes in concentrating various known DBPs in retentate. It has been shown that some of these by-products are not concentrated, due to the same rejection mechanisms described above.

II.3.2.1.2 Ultrafiltration

Ultrafiltration is a simple fractionation technique used to separate NOM according to molecular size, with apparent molecular weight cut-off (MWCO) values generally ranging from 0.5 to 30 kiloDaltons (kDa) [41] and [42]. A major advantage of this technique is its ability to process a large volume of water without denaturing the chemical composition of the NOM. MWCO values such as 0.5, 1, 3, 5, 10 and 30 kDa are commonly used. However, only a few studies have focused on OXBPs with a MW above 5 kDa; these species are dismissed as being of little toxicological significance but would seem to warrant more attention. In spite of its simplicity, this technique has certain disadvantages that can affect OXBP characterization. Among these, the non-uniformity of membrane pores can lead to the loss of 10% of the molecules expected to be retained using a MWCO method. In addition, humic substances (HSs) interact with the surface of the membrane and create a repulsive load related to organic anions, thus requiring a better choice of membrane based on its zeta potential. Decreasing the volume of water in the ultrafiltration cell leads to the recovery of OXBPs with MWs substantially below the membrane cut-offs, as a result of the formation of aggregates with HSs [42]. Neglecting this effect can lead to an overestimation of the molecular sizes of OXBPs and may yield non-reproducible results. Many investigators have studied NOM molecular weight distribution and its impact on DBP formation and it has been reported that small MW fractions (<3 kDa) play an important role here [43]. However, it has been shown that some emerging OXBPs, such as iodinated compounds, come from MW fractions greater than 10 kDa [44]. On the other hand, a significant portion of unidentified DBPs can be attributed to high molecular weight OXBPs. Thus, knowledge of the molecular weight distribution of OXBPs may contribute to the development of methodologies more suited to the physicochemical properties of this category of compounds. The MW distribution is determined by combining UF experiments with the detection of radiolabeled chlorine (^{36}Cl) or by AOX parameter analysis [45].

II.3.2.1.2 Adsorption chromatography

Adsorption chromatography based on Amberlite XAD-type resins is the most frequently used technique to separate NOM into groups according to behavior (e.g. hydrophobic, transphilic and hydrophilic). XAD adsorption resins were first utilized by Leenheer and Huffman for NOM fractionation [46]. Since then, several separation schemas, combining XAD resins with different polarities, have been tested. Using two columns in series packed with XAD-8 and XAD-4 resins remains the more widely used protocol [43]. With this configuration, three distinct fractions may be obtained: hydrophobic compounds are adsorbed on the XAD-8 resin and transphilic compounds (TPHs) on the XAD-4 resin, while hydrophilic compounds are not adsorbed on any resin. XAD resins can fractionate NOM with relatively high mass recoveries using a large quantity of water. In addition,

they present a low affinity for inorganic salts and a high elution efficiency. Another advantage of this fractionation technique is that the same effluent can be used for both XAD-8 and XAD-4 columns. However, fractionation using XAD resins presents some limitations. It has been shown that some hydrophilic and volatile compounds can be lost during XAD fractionation [39] and that fractions can be easily contaminated if the resin is not carefully cleaned. In addition, the absence of a universal fractionation and concentration protocol may lead to diverging results. XAD adsorption resins can be used to identify the effect of each fraction of NOM on DBP production, making it possible, in the case of drinking water treatment, for example, to concentrate NOM removal efforts on the most problematic fractions. Many studies have been performed for this purpose [47]. It is important to note that the relative mass of each NOM fraction differs from source to source, as does the distribution of compounds within each fraction. This leads to variations in the reactivity and OXBPs formed in each fraction from source to source, making it difficult to generalize the results of one study to different water sources.

II.3.2.2 OXBP extraction methods

When developing analytical methods to determine OXBPs, both the complex matrix composition and the typically very low concentration at which these compounds can present themselves in water samples should be taken into account. In this regard, a preliminary extraction step is required for three main reasons: to remove interferences that would otherwise affect the determination of the analytes (such as humic substances, salts, etc.), to enrich the target compounds to detectable concentrations, and to perform solvent switching to achieve the desired solvent conditions for instrumental detection. The fact that OXBPs have different physicochemical properties (e.g. chemical structures, polarity, and volatility) makes their extraction through a one-step enrichment and isolation procedure a difficult task. Thus, several extraction techniques have been explored. The relative advantages and limitations of each technique are discussed below.

II.3.2.2.1 Liquid-liquid extraction

Liquid-liquid extraction (LLE) is the most frequently employed technique for the extraction of volatile and semi-volatile OXBPs from treated water. The extraction efficiency mainly depends on the structure of the analyte and its affinity towards the extractant. Non-polar solvents such as n-hexane and n-pentane were the first organic solvents used [5]. However, non-polar organic solvents extract only non-polar or weakly polar compounds. Methyl tert-butyl ether (MtBE) has been examined for its volatility and ability to extract a wide range of OXBPs [5] and [48]. In addition to the Van Der Waals

interactions, MtBE develops polar-polar and hydrogen bonding interactions. It is possible to selectively extract acidic, neutral or basic OXBPs by changing the pH of the water samples. For example, when adjusting the pH of the water samples to a value less than 2, basic OXBPs become fully ionized and are not extracted by the MtBE, allowing the selective extraction of acidic (e.g. HAAs) and neutral compounds (e.g. THMs). Obviously, this practice supposes that the analytes of interest are stable within the extraction pH range. MtBE is relatively soluble in water because of its ability to act as a hydrogen bond donor and a hydrogen bond acceptor, causing no clear biphasic separation.

Generally, the addition of an inert salt increases the ionic strength of the water and, therefore, improves extraction efficiency, especially with more polar analytes, and enhances separation between the organic and aqueous phases. The effect of salt, which is analyte-specific, has been discussed widely. Some researchers have reported that ionic strength exerts a negative effect on extraction efficiency. For example, Montesinos et al. examined the influence of NaCl, KCl, Na_2SO_4 and $MgSO_4$ on the extraction of halonitromethanes. They found that the best conditions for the simultaneous extraction of nine HNMs were obtained using Na_2SO_4, and that KCl and NaCl led to very low yields even at high concentrations [49]. This result can be explained by an antagonistic effect due to a change in the physicochemical properties of the Nernst diffusion film, thus reducing the diffusion rate of the analyte into the organic phase [50], [51] and [52]. Likewise, KCl and NaCl may contain sufficient bromide, which has been shown to promote the formation of brominated by-products, particularly if the extraction time is long. Serrano et al. investigated the effect of sodium sulfate (Na_2SO_4), magnesium sulfate ($MgSO_4$) and ammonium sulfate $(NH_4)_2SO_4$ on the extraction yield of haloacetamides. They found that $MgSO_4$ partially degrades the brominated acetaldehydes in their corresponding THMs [53]. This result was attributed to the fact that the anhydrous $MgSO_4$ heated the aqueous solution (exothermic hydration), inducing the degradation of thermally unstable haloacetamides. The Na_2SO_4 displayed a high extraction efficiency without degrading the aldehydes, whereas yields obtained using the $(NH_4)_2SO_4$ were weak due to the lower hydration ability of the ammonium ion. Accordingly, Na_2SO_4 appears to be the best choice. Sodium sulfate is also recommended in EPA Method 551.1 for determining halogenated volatile organic compounds [54].

Traditional LLE is generally time-consuming and requires a large volume of organic solvents. Furthermore, it often requires a drying step, by rotary evaporation or under a gas stream, to concentrate the analytes before analysis. Drying can be a source of sample contamination or OXBP loss through volatilization. In 2002, Weinberg et al. reported a methodology for the GC/ECD analysis of DBPs using LLE without further rotary evaporation [55]. In accordance with the principles of "Green Chemistry", a recent trend in sample preparation involves substantially reducing the quantities of organic solvents used. This has led to the emergence of a new extraction technique termed Liquid-

Phase MicroExtraction (LPME). Three main categories of LPME have been developed, namely single-drop microextraction (SDME), hollow-fiber based LPME (HF-LPME) and dispersive liquid-liquid microextraction (DLLME). SDME is based on the extraction of analytes in a drop of solvent (~3 µL) which is suspended in the needle of a microsyringe that can be directly immersed in the aqueous sample (DI-SDME) or in its headspace (HS-SDME). Several aspects should be considered in the choice of solvent. It should have sufficient viscosity to prevent loss of the drop and should not be too volatile to prevent its evaporation in the headspace application. Moreover, the solvent should not interfere with the target analytes. Among the solvents explored to extract OXBPs (including o-xylene, ethyl acetate, 1-hexanol, 1-octanol, 1-undecanol, pentane, decane and methylene chloride), 1-octanol and ethyl acetate are the most frequently used. Problems of drop instability and poor precision have often been reported for SDME. The uncertainty associated with drop volume variation may be corrected by using an internal standard. In HF-LPME, a hydrophobic membrane made of polypropylene separates the sample water (donor phase) and the organic phase (acceptor phase). Typically, the volume of organic solvent inside the HF ranges between 5 and 30 µL. The small pore size of the HF provides good selectivity and very clean extracts. The major limitation of HF-LPME is the slow exchange kinetics between the donor and acceptor phases, leading to relatively long extraction times. Usually, equilibrium extraction times range between 15 and 45 minutes for sample volumes below 2 mL. The second disadvantage concerns membrane handling, which requires the pre-conditioning and removal of excess organic solvent, as this can affect recovery and extraction precision. The DLLME procedure involves the introduction into the water samples of a binary mixture of a water-immiscible organic solvent (extractant in the µL range) and a dispersant miscible with both the extractant and water. High and low density organic solvents can be used. The disperser solvent represents approximately 90% of the total volume of the extracting mixture. Benefits of the dispersion of fine extractant droplets in the aqueous phase include increased extraction rates and a considerable reduction in solvent consumption. Typical dispersers are polar solvents such as acetone, ethanol, methanol, acetonitrile and tetrahydrofuran. The most frequently used disperser solvent is acetone [56]. The dispersant is removed after centrifugation and the extracting solvent containing enriched analytes, deposited at the bottom, is collected for analysis using a microsyringe. The extraction time for DLLME ranges from a few seconds to five minutes, which is much shorter than for other LPME techniques. However, DLLME suffers from some limitations, mostly arising from the solvents used in this technique. These include (i) hazardous chlorinated solvents with a higher density than water, and (ii) dispersive solvents, resulting in decreased analyte partition coefficients in the extraction solvent. Other limitations of DLLME are the extra time required for the centrifugation step and the difficulty associated with automating the method. In order to overcome the drawbacks of the DLLME technique, a new version, termed vortex-assisted liquid-liquid microextraction (VALLME), was introduced by Yiantzi et al. In this method, an organic solvent with a density lower than water is introduced to the

sample (without the addition of a dispersive solvent) and the mixture is vigorously shaken with a vortex agitator [57]. Due to the shorter diffusion distance and the larger specific surface area, the fine droplets formed can extract the target analytes more quickly. Centrifugation is sometimes needed in order to speed up the separation of the aqueous and organic phases. Surfactants and salts are also used to enhance the extraction efficiency and improve the separation between the aqueous and organic phases. The use of VALLME to extract organohalogen by-products from water samples is still very limited. Several analytical procedures using LPME coupled with gas chromatography (GC) have been developed and successfully employed for the extraction and determination of numerous classes of OXBPs in water samples, including THMs, iodinated trihalomethanes (I-THMs), HNMs, HAAs and HALs. However, so far, these applications have remained limited and have not progressed beyond the research and development stage. In studies to identify unknown OXBPs, the application of LPME approaches has not yet been reported, probably due to the small quantities of extracts obtained by these methods. A brief summary of various LPME techniques, together with their performance levels, is provided in Table 2.

Table 2. A brief summary of various liquid-phase microextraction methods and their performance levels (references are given in Supplementary Material 2)

LPME technique	Matrix	Extractant	Compounds	Determination technique	Recovery (%)
DLLME	Tap and swimming pool water	1-Undecanol (50 µL)	THMs	GC-µECD	79-113
DLLME	Drinking water	Carbon disulfide (20 µL)	THMs	GC-MS	92-108
DLLME	Water	2-Dodecanol (10 µL)	Organohalogen compounds	GC-ECD or GC-MS	-
DLLME	Drinking and swimming pool water	n-Pentane	HAAs	HS-GC/MS	93-98
DLLME	Water	Chlorobenzene (10 µL)	Chlorophenols	GC-ECD	83-118
SA-DLLME	Drinking water	DCME	HANs	PTV-GC-MS	79-105
SA-DLLME	Drinking and swimming pool water	Ethyl acetate	HAcAms	IPS-GC-MS	76-94%
HF-LPME	Drinking water	n-Octanol	THMs, I-THMs	GC-µECD	96-105
HF-LPME	Drinking and tap water	n-Octanol	THMs	GC-ECD	98-105
HF-LPME	Water	n-Octanol	HAAs	GC-ECD	97-109
DI-SDME	Water	n-Hexane	THMs	GC-ECD	73-78
HS-SDME	Drinking water	n-Octanol	THMs	GC-ECD	101-112
HS-SDME	Tape and swimming poll water	n-Hexanol (2 µL)	7 HNMs	GC-MS	3-20
SDME	River and tap water	n-Octanol	6 HAAs	GC-MS	82-98
LPME	Treated water	Ethyl acetate (30 µL)	HALs	PTV-GC-MS	-
LPME	Tap water	Ethyl acetate (200 µL)	THMs, HNMs	PTV-GC-MS	85
LPME	Tap and swimming pool water	n-Hexane (200 µL)	9 HAAs, 4 I-HAAs	PTV-GC-MS	91-97
VALLME	Water	Isopropyl ether (600 µL)	9 HAAs	LC-DAD	78-89

Abbreviations: SA-DLLME: Salt-Assisted Dispersive Liquid-Liquid MicroExtraction, ECD: Electron Capture Detector, IPS-GC-MS: Injection-Port Silylation Gas Chromatography-Mass Spectrometry, HAcAms: Haloacetamides, LC-DAD: Liquid Chromatography-Diode Array Detector, PTV: Programmable temperature vaporizing

II.3.2.2.2 Solid phase extraction (SPE)

Solid phase extraction (SPE) is gradually replacing LLE and is becoming the most common procedure for sample clean up, class fractionation and the pre-concentration of analytes at trace level in many areas of analysis. Compared to conventional LLE, SPE has many advantages, including: (i) higher recoveries with concentration factors for target analytes of up to three orders of magnitude, achieving nanogram per liter detection thresholds, (ii) improved selectivity, specificity and reproducibility, (iii) the diversity of stationary phases currently available or under development, (iv) less organic solvent usage, and (v) easier operation and the possibility of automation [5]. Another advantage of SPE is that sorbed analytes can be prevented from decomposition and can thus be stored for a certain period of time without any change in their concentration or identity. The selection of an appropriate SPE sorbent depends mainly on the physicochemical properties of the OXBPs, such as the pKa and the log Kow, as well as the pH of the water. This choice also requires an understanding of the interaction mechanism(s) between the sorbent and the analytes of interest. Typical interactions are hydrophobic (nonpolar-nonpolar and van der Waals forces), hydrophilic (polar-polar, hydrogen bonding, dipole-dipole and dipole-inducted dipole), cationic-anionic and selective antigen-antibody. OXBPs are complex mixtures of organic compounds that cover a wide range of polarity. For example, the log Kow values of some known OXBPs range between -0.43 (monobromoacetic acid) and 2.95 (monoiodoacetic acid). In addition, several OXBPs can have acidic and/or basic functionalities; their ionization rate depends on acidic dissociation constants (or pKa values) and is controlled by the pH of the water. The deprotonation of acidic compounds and the protonation of basic compounds should be suppressed to ensure the sufficient hydrophobicity of the analytes and thus improve their extraction yield using SPE with a non-polar stationary phase. For example, HAAs ($pKa < 2.86$) are in ionic form in the pH range of 6 to 9, whereas they become predominantly neutral when the pH of the water sample is adjusted to a value < 1.

The loss of semi-volatile substances has been reported during the loading of samples and the drying of solid sorbents. In addition, many organic and inorganic interferents, which may affect the sensitivity and selectivity of analytical techniques, may also be enriched during the extraction of the molecules of interest. Some authors have pointed out that a major drawback of the SPE methodology is that it shows an outstanding lack of selectivity. To support this argument, Table 3 highlights the wide diversity of compounds that can be simultaneously extracted by the same type of cartridges and eluted using similar elution protocols. The structural diversity and physical properties of OXBPs suggest the use of different types of interaction to extract all of these compounds. Thus, a combination of different solid sorbents and pH values is suggested for the sequential extraction of different classes of OXBPs. The most challenging topic in the area of SPE remains the extraction of polar compounds. Some of the

most recent applications of SPE for the extraction of OXBPs from water samples are described in Supplementary Material S2 and summarized in Table 3.

Table 3. Survey of SPE methods for the extraction of OXBPs from water samples (the corresponding references are given in Supplementary Material 2)

Compounds	Matrix	Sorbent type	SPE parameters	Recovery (%)
29 OXBPs including: THMs, I-THMs, HNMs, HANs, HALs	River water	Oasis HLB (500 mg/6 mL)	Conditioning: 10 mL MtBE, 10 mL MeOH, 10 mL H_2O Loading: 1 L, 5 mL/min Elution: 10 MtBE	65-103
35 OXBPs including: THMs, I-THMs, HNMs, HANs, HALs	Water	Elut-PPL (200 mg/ 3 mL)	Conditioning: 8 mL MeOH, Loading: 36 mL, 5 mL/min Elution: 1.6 ml MtBE	-
THMs	Pure water	Supelclean ENVI-18 (1 g/6 mL) HyperSepTM C-18 (1 g/6 mL)	Conditioning: 4 mL ACN, 4 ml H_2O *vs* 50 µL MeOH, 50µL H_2O Loading: 100 mL, 0.8 mL/min vs 100 µL Washing: 100 µL Elution: 1 mL MeOH *vs* 100 µL MeOH	33-74 30-82
THMs	Drinking water	Supelclean ENVI-18 (2 g/12 mL)	Conditioning: 2 mL ACN, 2 ml H_2O Loading: 1 L, 15 mL/min Elution: 5 mL pentane	96.5
HAAs	Aqueous environments	LiChrolut EN (200 mg/ 6 mL)	Conditioning: 5 mL MeOH, 3 ml H_2O (pH < 2.5) Loading: 50 mL, 5 mL/min Washing: 1 mL H_2O (pH < 2.5) Elution: mix 0.5 mL H_2O + 3.5 mL MeOH-Acetone (v/v)	35-69
HAAs	Drinking water	Silicabond SAX (300 mg/3 mL)	Conditioning: 2x10 mL MeOH, 10 mL H_2O Loading: 50 mL, 1.5 mL/min Washing: 10 mL MeOH Elution: MeOH (10% H_2SO_4)	70-115
HAAs	Tap water	LiChrolut EN (200 mg/ 6 mL)	Conditioning: 5 mL MeOH, 5 ml H_2O (pH < 2.5) Loading: 500 mL, 5 mL/min Washing: 0.5 mL H_2O Elution: 2 mL MeOH	37-85
HAAs	Drinking water	LiChrolut EN (500 mg/ 6 mL)	Conditioning: 5 mL MeOH, 5 ACN, 5 mL H_2O (pH < 2.5) Loading: 100 mL, 5 mL/min Washing: 1 mL H_2O (pH < 2.5) Elution: 4 x 2 mL of mix. DBA/ACN (95:5, v/v)	60-102

HAAs	Drinking water	LiChrolut EN (200 mg/ 3 mL)	Conditioning: 2x3 mL MeOH, 3 mL H_2O (pH < 0.3) Loading: 50 mL, 1 mL/min Washing: 0.5 mL H_2O Elution: 3 mL H_2O (NaOH: 10 mM)	62-88
HAAs	Hospital wastewater	LiChrolut EN (200 mg/ 3 mL)	Conditioning: 2x3 mL MeOH, 3 mL H_2O (pH < 0.3) Loading: 50 mL, 2 mL/min Washing: 1mL H_2O Elution: 1 mL H_2O (NaOH: 1 mM)	64-91
HAAs	Drinking water	Silicabond SAX (300 mg/3 mL)	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 50 mL, 2 mL/min Elution: 3 mL H_2O (10% H_2SO_4)	-
HAAs	-	MEPS: Silica-C18 (4 mg)	Conditioning: 100 µL MtBE, 100 µL H_2O (pH = 4). Loading: 25x100 µL, 5 µL/s Washing: 100 µL H_2O Elution: 20 µL MtBE	83-117
HAcAms	Drinking water	Oasis HLB	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 500 mL, 3-5 mL/min Washing: 5 mL H_2O: MeOH (95:5, v/v) Elution: 5mL MeOH	78
Elucidation of unknown OXBPs - HBQs	Drinking water	Oasis HLB (200 mg/6 mL)	Conditioning: 6 mL MeOH (0.25% FA), 6 mL H_2O (0.25% FA) Loading: 500 mL, 2 mL/min Washing: 6 mL H_2O (0.25% FA) 6 mL H_2O: MeOH (50:0, v/v with 0.25% FA) Elution: 6 mL MeOH (0.25% FA)	-
Toxic and bioactive disinfection by-products	Wastewater effluents	Oasis HLB	Conditioning: 6 mL MeOH, 6 mL H_2O Loading: 900 mL, 10 mL/min Washing: 5 mL H_2O Elution: 8 mL MeOH	-
Toxic and bioactive disinfection by-products (e.g. AOX, AOCl, AOBr)	Swimming pool water	Oasis HLB (1 g/20 mL)	Conditioning: 10 mL MtBE, 20 mL MeOH, 10 mL H_2O (pH=1) Loading: 2 L (pH = 1) Elution: 20 mL MeOH + 10 mL MtBE under gravity	-
Toxic and bioactive	Drinking water	Oasis HLB (1 g/20 ml)	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 2 L (pH = 3)	60

disinfection by-products			Elution: 10 mL MeOH, 10 ml hexane: acetone (1:1, v/v)	
Toxic and bioactive disinfection by-products	Source waters	HyperSep ™	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 100 mL (pH = 2) Elution: 4 mL MeOH, 4 mL acetone, 4 mL ACN	-

Abbreviations: DBA: Dibutylamine, ACN: Acetonitrile, MtBE: Methyl tert-butyl ether, HBQs: Halobenzoquinones, HANs: Haloacetonitriles, HKs: Haloketones, HNMs: Halonitromethanes, HAcAms: Haloacetamides, HALs : Haloacetaldehydes

II.3.2.2.3 Solid phase microextraction (SPME)

Solid phase microextraction (SPME) is a variant of SPE. This technique emerged in response to a need for a simple, rapid and automatic sample preparation method that would incorporate numerous analytical steps, such as sampling, isolation, enrichment and, in the case of gas chromatography applications, sample introduction for instrumental analysis, into one process. SPME is available based on different configurations, solid phases and devices. In its most known configuration, the SPME device consists of an extraction phase coated onto a fused-silica rod. SPME can operate in two different extraction modes, depending on the nature of the analytes and the matrix: direct immersed solid phase microextraction (DI-SPME) and headspace solid-phase microextraction (HS-SPME). In HS-SPME, which is the most commonly used SPME extraction mode, the fiber is positioned in the head space above the sample. The main advantage of HS-SPME is its better selectivity due to the discrimination of non-volatile and high-molecular-weight molecules. In DI-SPME, the fiber is directly immersed into the aqueous sample. The SPME principle is based on the equilibrium of analytes throughout the two or three phases of the system (sample-headspace-coating in HS mode, and sample-coating in DI mode). Various parameters can affect the extraction efficacy in SPME, namely the configuration and nature of the SPME method, the surface contact area, the extraction mode (DI vs HS), the addition of salt, the sample volume, the agitation rate, the extraction temperature and the duration, as well as the intrinsic characteristics of the water samples. The choice of the type of SPME fiber coating is a key factor for achieving optimal extraction efficiency. Currently, several SPME fibers with different polarities for sampling a wide range of analytes are commercially available. They comprise single-phase absorbents, such as polydimethylsiloxane (PDMS), polyacrylate and polyethylene glycol, also known as Carbowax (CWX), and mixed-phase sorbents, such as Carboxen/polydimethylsiloxane (CAR/PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and Carbopack Z/PDMS. Theoretically, non-polar PDMS fiber coatings are more suitable for extracting volatile and semi-volatile analytes with low or no polarity, whereas PA and CWX are the coatings of choice for relatively semi-polar and polar compounds. Thus, mixed phase sorbents were developed to extract a wide range of organic analytes, from non-polar to polar, at the same time. Many authors agree that SPME is mainly governed by the physical retention of the analytes in the pores of the sorbents and not by chemical retention processes such as polar or non-polar interactions [58]. It should be noted that only neutral species are extracted using SPME. Thus, low pH values promote the extraction of acidic compounds, whereas high pH ranges are suitable for the extraction of basic compounds. Since its introduction in the 1990s, SPME has been widely studied and successfully applied to the determination of volatile analytes in aqueous matrices, including some families of OXBPs in water samples. More recent studies are summarized in Table 4 and detailed in Supplementary Material S3. Among the known OXBPs, regulated THMs are the most studied compounds to date. Numerous

methods for their quantification have been reported in literature, with the majority accomplished through the use of gas chromatography coupled with an electron capture detector (ECD), which is widely used due to its very high sensitivity, or mass spectrometry (MS). The first investigations were conducted using PDMS as the sorbent. However, mixed-phase sorbents have been shown to offer better extraction efficiency. One limitation of SPME is that the rates of extraction are low for polar analytes. To improve the extractability of polar compounds, a first approach is to convert the polar functions into less polar derivatives. This approach is often used to determine HAAs in aqueous matrices. For example, in the method developed by Hammami et al., the HAAs were first extracted by LLE, the solvent was evaporated to dryness, the HAAs were converted to their ethyl esters with acidic methanol, and the esters were then extracted from the headspace by SPME using PDMS fiber [59]. However, in some cases the addition of salts could reduce the extraction yield. This depends on the nature of the target analyte and the salt concentration. Thus, the effect of the addition of salt should be determined experimentally for each category of OXBPs. Another limitation of conventional SPME is the low capacity of the fiber, mainly when sampling complex liquid matrices, which leads to low levels of enrichment in comparison with SPE and LLE [58]. Another configuration of SPME, which is more recent and offers increased capability, is stir-bar sorptive extraction (SBSE). This variant uses a thick extraction phase film, most often PDMS, on a glass-coated magnetic stirring bar for microextraction from samples. The theoretical aspects of SBSE are the same as for SPME. After a period of extraction, the extracted analytes may be recovered from the stir bar device by thermal desorption directly into a GC system, or back-extracted with an organic solvent for large volume GC injection or for an LC application. Due to the increased volume of adsorbed phase, SBSE (24-126 µL) has much greater sensitivity than SPME (maximum 0.5 µL) and has minimum saturation effects. Despite its high extraction capacity, this technique has been explored very little for the extraction of OXBPs from water. The few studies reported in literature concern the analysis of THMs [60] and chlorophenols [61].

Table 4. Recent SPME and SBSE-based methods for OXBP analysis (the corresponding references are given in Supplementary Material 2)

Matrix	Analytes	Sorbent nature	Configuration	Time / temperature /salt	Method	Recoveries LQ
Drinking water	THMs	PDMS	HS-SPME	20 min / 35 °C / NaCl (25%, w/v)	GC-ECD	- 4.5-18 ng/L
Soft drink	THMs	CAR/PDMS	HS-SPME	15 min / 30 °C / NaCl (20%, w/v)	GC-ECD	>90% 0.6-1.4 µg/L
Drinking water	THMs	CAR/PDMS	HS-SPME	30 min / 35 °C / NaCl (25%, w/v)	GC-ECD	- 15-30 ng/L
Drinking water	THMs	CAR/PDMS	HS-SPME	30 min / 37.5 °C / NaCl (25%, w/v)	GC-ECD	-
Swimming pool water	THMs	PDMS	HS-SPME	25 min / 45 °C / NaCl (25%, w/v)	GC-ECD	- 14-40 µg/L
Drinking water	THMs	PDMS/DVB	HS-SPME	20 min / 40 °C / NaCl (35%, w/v)	GC-ECD	88-109% 0.2-1.6 µg/L
Drinking water	THMs	DVB/CAR/PDMS	DI-SPME	30 min / Room temperature -	GC-MS	80-119% 0.06-2.1µg/L
Tap water and swimming pool water	HAAs	CAR/PDMS	HS-SPME	60 min / - / 5 g of Na_2SO4	GC-MS	- -
Tap water	HAAs	PDMS	HS-SPME	10 min / 25 °C / -	GC-ECD	- 0.06-2.1µg/L
Drinking water	I-THMs	CWX/DVB	HS-SPME	30 min / room temp. / NaCl (25%, w/v)	GC-ECD	- 3.6-9 ng/L

Water	THMs, I-THMs	CAR/PDMS/DVB	HS-SPME	15 min / 70 °C / NaCl (25%, w/v)	GC-MS	84-103% 3-60 ng/L
Water	ClCN, BrCN	CAR/PDMS/DVB	HS-SPME	15 min / Room temp. / NaCl (25%, w/v)	GC-ECD	- 0.6-2.5µg/L
Drinking water	HNs	DVB/CAR/PDMS	HS-SPME	15 min / 40 °C / $NaSO_4$ (30%, w/v)	GC-MS	- 3-240 ng/L
Drinking water	HKs	DVB/CAR/PDMS	HS-SPME	15 min / 40 °C / 6 g of Na_2SO_4	GC-MS	- 35-1200 ng/L
Drinking water	HANs, HKs, chloropicrin	DVB/CAR/PDMS	HS-SPME	- / - / -	GC-MS	- 2-180 ng/L
Drinking water	I-THMS, HNMs, HANs	DVB/CAR/PDMS	HS-SPME	30 min / 40 °C / $(NH_4)SO_4$ (25%, w/v)	GC-MS	- 3-165 ng/L
Drinking water	Unknown OXBPs	CAR/PDMS	HS-SPME	30 min / - / -	GC-MS	- -
Water	Unknown OXBPs	CAR/PDMS	HS-SPME	30 min / - / -	GC-MS	- -
Water	Unknown OXBPs	CAR/PDMS	HS-SPME	- / Room temperature / NaCl (20%, w/v)	GC-MS	- -
Water	THMs	PDMS	DI-SBSE	30 min / - / -	TD-GC-HRMS	- -
Tap and river water	CPs	PDMS	DI-SBSE	- / Room temperature / -	TD-GC-MS	95% 3-6 ng/L

Abbreviations: HANs: Haloacetonitriles, HKs: Haloketones, TD: Thermal Desorption, CPs: Chlorophenols, HNMs: Halonitromethanes.

II.3.2.2.4 Headspace (HS)

Headspace (HS) sampling is one of the most commonly used techniques for the analysis of volatile and semi-volatile compounds from different matrices. Its main advantage compared to conventional pre-concentration techniques (i.e. SPE and LLE) is that it largely avoids the problem of the co-extraction of interfering species. Table 5 summarizes the main works published in recent years based on the HS pre-concentration technique applied to the determination of volatile and semi-volatile OXBPs in water samples. HS sampling can be performed in a static (SHS) or a dynamic (DHS) mode. In static mode, the water sample is placed in a sealed vial and is heated to a specific temperature for a sufficient time to obtain a thermodynamic equilibrium between the analytes in the liquid and vapor phases. Once this equilibrium is reached, a known volume of the headspace vapor is sampled with a gas-tight syringe and transferred to a GC for analysis. The SHS technique offers many advantages, such as simple sample preparation, inexpensive equipment, automated extraction and analysis, and the use of small sample volumes. Applications of SHS for the extraction of OXBPs from water samples are described in Supplementary Material S4. Despite the ability to inject large volumes, the SHS technique suffers from poor sensitivity, particularly for semi-volatile and non-volatile analytes. The limitations of SHS sampling can be overcome by recourse to dynamic headspace mode, also commonly known as the purge and trap (P&T) method. With P&T, greater amounts of volatile and semi-volatile compounds are transferred from the aqueous to the gaseous phase by bubbling an inert gas through the water samples. Thus, the extraction efficiency is significantly improved because the equilibrium is constantly displaced by purging the headspace above the sample. The purged compounds are firstly trapped by cryogenic condensation (cold trap) and/or adsorbed on solid phases and, secondly, thermally desorbed and transferred into the analytical system by the rapid heating of the trap. The choice of adsorbent governs the range of analytes which can be effectively trapped. TENAX is the most widely used resin for trapping non-polar compounds like THMs. Several adsorbents must be combined (multiple layer adsorption) to enable the sampling of a wide range of volatile compounds with satisfactory efficiency. Adsorption on the solid phases is often performed at low temperature and P&T systems are usually coupled on-line with the gas chromatograph. P&T-GC has become an accepted method for the analysis of volatile organic compounds in water and has been adopted as a standard by the EPA (method 524-2) and other standardization bodies worldwide for the determination of THMs and other DBPs in water. The extraction efficiency of the DHS technique can be improved by increasing the volume of water purged and/or the amount of analytes introduced into the GC system. However, water molecules that are extracted together with the analytes will saturate the detector and distort the chromatographic peaks. In recent years, much work has gone into developing hydrophobic adsorbents that minimize water collection on the trap. Extensive studies recommend incorporating a dry purge cycle to remove water from the trap prior to desorption. It has been reported that drying the vapor stream prior to entry into the trap can compromise the measurement of some

chemicals (e.g. oxygenated species). In addition, the use of different cryogenic and adsorbent traps, as well as improvements to the purge process itself, can lead to an increase in detection limits. Thus, some authors have focused their research on improving the efficiency of the purge process. An excellent overview of purge efficiency in relation to the determination of THMs in water was provided by Ruiz-Bevia et al. [74]. Applications of SHS for the extraction of OXBPs from water samples are detailed in Supplementary Material S4.

Table 5. Published applications using the headspace sampling technique for determining halogenated volatile organic compounds in water samples

Type of water	Analyte	HS mode	Sample volume (salt), purge temperature and time	Trap	Desorption	Determination technique	Recovery LQ
Beverages	THMs, I-THMs	HS	10 mL (4g NaCl) / 80 °C / 15 min	-	-	GC/MS	- 90-900 ng/L
Tap and swimming pool water	HNMs	HS	12 mL + 250 µL MtBE / 80 °C / 20 min	-	-	GC/MS	- 90-1800 ng/L
Tap water	THMs, HNMs, HANs	HS	12 mL + 250 µL MtBE (6 g Na_2SO_4) / 80 °C / 20 min	-	-	GC/MS	- 30-650 ng/L
Water	THMs	DHS	5 mL / 90 °C / 30 min	Tenax at 5 °C	TD (250°C)	PTV-GC/MS	- 2-8 ng/L
Water	I-THMs	DHS	5 mL / 90 °C / 30 min	Tenax		GC/MS	- 3-6 ng/L
Drinking water	THMs		5 mL / 25 °C / 11 min	Vocarb 3000	TD (250°C)	GC/MS	100-111% 120-600 ng/L
Drinking water	THMs	DHS	On-line (2.5 mL/min) / 65 °C / 15 min	Tenax at 40 °C	TD (220°C)	GC/DELCD	110-128% < 3 µg/L
Drinking water	THMs	DHS	NRD / NR / 13 min	NRD	TD (180°C)	GC/DELCD	> 89 % 0.27-0.74 µg/L
Drinking water	THMs, HANs, HKs	DHS	NRD / 30 °C / 11 min	Vocarb 3000	TD (250°C)	GC/MS	- 0.03-30 µg/L
Drinking water	THMs	P&T-CF	On-line (4.6 mL/min) 25 °C / 0.5 min	Tenax at - 165 °C	TD (200°C)	GC/MS	- 30-150 ng/L
Drinking water	THMs, HANs, HKs, HAAs, chloral hydrate, chloropicrin	CLSA	900 mL (70 g Na_2SO_4) 35 °C / 120 min	AC (1.5 mg)	CS_2 (30 µL)	GC/MS	5-94% 0.15-210 ng/L
Drinking water	THMs, HANs, HKs, HAAs, chloral hydrate, chloropicrin	CLSA	900 mL (70 g Na_2SO_4) NRD / 120 min	AC (1.5 mg)	CS_2 (30 µL)	GC/MS and GC/ECD	NRD NRD
Drinking water	Unknown OXBPs	CLSA	100 mL / 60 °C / NRD	AC at 65 °C	DCM (30 µL)	GC/MS	NRD NRD

Abbreviations: AC: Activated Charcoal, TD: Thermal Desorption, NRD: Non-Reported Data, DELCD: Dry Electrolytic Conductivity Detector, P&T-CF: Purge and Trap Continuous Flow system, CLSA: Closed-Loop Stripping Analysis

II.3.2.2.5 Direct aqueous injection (DAI)

Analytical methods involving direct aqueous injection (DAI) into an analytical system for determining OXBPs have been described in various papers. With the DAI technique, no analyte isolation or enrichment is needed, thus minimizing the risk of analyte loss or sample contamination during sample preparation. Many papers have been published in which DAI coupled with gas chromatography has been used for the determination of THMs as well as other volatile OXBPs in water samples [75] and [76]. Use of the DAI-GC/ECD method was reported for the analysis of THMs in drinking water by Carpi et al. By injecting 1 µL of a water sample into an injector heated to a temperature of 93°C, they were able to obtain LODs lower than 1 µg/L [75]. The authors also compared DAI-GC/ECD with Standard Method 6232, which is based on solvent extraction. This comparison indicated no significant differences between the results from both methods in terms of precision and accuracy, although Standard Method 6232 remains more sensitive (LODs < 0.1 µg/L). On-column and programmable temperature vaporizing (PTV) injection techniques have been developed to allow the injection of tens to hundreds of microliters of organic solvent. To avoid the negative effects of water, various methodologies have been developed to separate analytes from water before entry into the GC column. These include the use of either pre-column sorbents or a PTV injector [77]. Otherwise, it has been found that non-polar liquid film columns with immobilized coatings are sufficiently resistant towards water injected as solvent. Recently a large-volume DAI-GC method was developed for the trace analysis of 27 high boiling point volatile organic compounds, belonging to seven subclasses (i.e. halogenated aliphatic hydrocarbons, chlorobenzenes, nitrobenzenes, anilines, phenols, polycyclic aromatic hydrocarbons and organic sulfides), in water [77]. Limits of quantitation ranging from 0.01 to 3 µg/L were obtained. To date, the use of the DAI-GC technique for analyzing OXBPs has been limited to THMs and, as far as we know, there have been no reports on the application of this technique for the structural identification of unknown OXBPs.

The combination of the sensitivity of modern LC-MS instruments and the selectivity of mass spectrometry has led to the development of direct injection methods, many of which are described in literature. Until now, most studies have mainly focused on HAAs with regard to the analysis of OXBPs. Before injection, water samples are often filtered or centrifuged to remove particulate matter that might potentially clog the columns and other LC components. The DAI-LC methodology has been used in several recent studies for the structural identification of unknown OXBPs. For example, DAI (10 µL) coupled with LC(C_{18})-ESI(+)-MS/MS was used to study the reactivity of tamoxifen and its major active metabolites during water chlorination, as well as to identify their by-products [78]. This methodology resulted in the identification of seven chlorinated by-products. It should be noted, however, that DAI-LC may only be effective for the structural elucidation of unknown by-products if the tests are carried out in clean waters and with high concentrations of oxidants and DBP precursors.

II.4 Conclusion

It is clear that we now need to acquire a deeper knowledge of the nature of unidentified OXBPs. AOX measurement provides a window into these compounds, as the most direct estimate of unknown organohalogen by-products is obtained by subtracting the major known organohalogen by-products from the measured AOX. Several approaches based on real and laboratory water samples have been devised over the last three decades to identify the majority of missed OXBPs (80% in the case of monochloramination treatment). The most analytical methods used in these approaches are those based on gas or liquid chromatography coupled with mass spectrometry, due to their sensitivity and their potential to scan many or most products from water samples. Prior to GC-MS or LC-MS analysis, the preparation of water samples represents a key step, which is clearly responsible for the loss of a large percentage of unknown organohalogen by-products before analysis. In addition, the large quantity of NOM and interferents can affect the sensitivity of analytical methods and, consequently, the identification and quantitation of OXBPs at trace level.

References

[1] S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research, Mutat. Res.-Rev. Mutat. 636 (2007) 178-242.

[2] D. Brown, J. Bridgeman, J.R. West, Predicting chlorine decay and THM formation in water supply systems, Rev. Environ. Sci. Biotechnol. 10 (2011) 79-99.

[3] G. Hua, D.A. Reckhow, Comparison of disinfection byproduct formation from chlorine and alternative disinfectants, Water Res. 41 (2007) 1667-1678.

[4] E.E. Lavonen, M. Gonsior, L.J. Tranvik, P. Schmitt-Kopplin, S.J. Kohler, Selective chlorination of natural organic matter: identification of previously unknown disinfection byproducts, Environ. Sci. Technol. 47 (2013) 2264-2271.

[5] H.S. Weinberg, Modern approaches to the analysis of disinfection by-products in drinking water, Philos. Trans. A Math. Phys. Eng. Sci. 367 (2009) 4097-4118.

[6] A. Kinani, S. Kinani, B. Richard, M. Lorthioy, S. Bouchonnet, Formation and determination of organohalogen by-products in water. Part 1. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method, accepted for publication in TrAC.

[7] A. Kinani, S. Kinani, S. Bouchonnet, Formation and determination of organohalogen by-products in water. Part III. Characterization and analytical approaches, submitted for publication in TrAC.

[8] P.C. Singer, Humic substances as precursors for potentially harmful disinfection by-products, Water Sci. Technol. 40 (1999) 25-30.

[9] P. Westerhoff, P. Chao, H. Mash, Reactivity of natural organic matter with aqueous chlorine and bromine, Water Res. 38 (2004) 1502-1513.

[10] I. Kristiana, H. Gallard, C. Joll, J.-P. Croué, The formation of halogen-specific TOX from chlorination and chloramination of natural organic matter isolates, Water Res. 43 (2009) 4177-4186.

[11] X. Yang, W. Guo, W. Lee, Formation of disinfection byproducts upon chlorine dioxide preoxidation followed by chlorination or chloramination of natural organic matter, Chemosphere 91 (2013) 1477-1485.

[12] G.A. De Vera, D. Stalter, W. Gernjak, H.S. Weinberg, J. Keller, M.J. Farré, Towards reducing DBP formation potential of drinking water by favouring direct ozone over hydroxyl radical reactions during ozonation, Water Res. 87 (2015) 49-58.

[13] I. Montesinos, M. Gallego, Speciation of common volatile halogenated disinfection by-products in tap water under different oxidising agents, J. Chrom. A 1310 (2013) 113-120. [14] W. Wang, Y. Qian, J.M. Boyd, M. Wu, S.E. Hrudey, X.-F. Li, Halobenzoquinones in swimming pool waters and their formation from personal care products, Environ. Sci. Technol. 47 (2013) 3275-3282.

[15] Y.-X. Sun, Q.-Y. Wu, H.-Y. Hu, J. Tian, Effect of bromide on the formation of disinfection by-products during wastewater chlorination, Water Res. 43 (2009) 2391-2398.

[16] R.O. Pereira, C. Postigo, M.L. de Alda, L.A. Daniel, D. Barceló, Removal of estrogens through water disinfection processes and formation of by-products, Chemosphere 82 (2011) 789-799.

[17] C. Postigo, S.D. Richardson, Transformation of pharmaceuticals during oxidation/ disinfection processes in drinking water treatment, J. Hazard. Mater. 279 (2014) 461-475.

[18] G.D. Onstad, H.S. Weinberg, S.W. Krasner, Occurrence of halogenated furanones in U.S. drinking waters, Environ. Sci. Technol. 42 (2008) 3341-3348.
[19] H.S. Weinberg, W.H. Glaze, A unified approach to the analysis of polar organic by-products of oxidation in aqueous matrices, Water Res. 31 (1997) 1555-1572.
[20] D. Liew, K.L. Linge, C.A. Joll, A. Heitz, J.W. Charrois, Determination of halonitromethanes and haloacetamides: an evaluation of sample preservation and analyte stability in drinking water, J. Chrom. A 1241 (2012) 117-122.
[21] Y. Li, J.S. Whitaker, C.L. McCarty, Analysis of iodinated haloacetic acids in drinking water by reversed-phase liquid chromatography/electrospray ionization/tandem mass spectrometry with large volume direct aqueous injection, J. Chrom. A 1245 (2012) 75-82.
[22] I. Kristiana, A. Lethorn, C. Joll, A. Heitz, To add or not to add: the use of quenching agents for the analysis of disinfection by-products in water samples, Water Res. 59 (2014) 90-98.
[23] ISO, 9562:2004. Water Quality - Determination of Adsorbable Organically Bound Halogens (AOX), 2004.
[24] J. Liu, X. Zhang, Effect of quenching time and quenching agent dose on total organic halogen measurement, Int. J. Environ. Anal. Chem. 93 (2013) 1146-1158.
[25] G. Hua, D.A. Reckhow, Effect of alkaline pH on the stability of halogenated DBPs, J. Am. Water Works Assoc. 104 (2012) 49.
[26] V. Glezer, B. Harris, N. Tal, B. Iosefzon, O. Lev, Hydrolysis of haloacetonitriles: linear free energy relationship, kinetics and products, Water Res. 33 (1999) 1938-1948.
[27] Y. Takahashi, S. Onodera, M. Morita, Y. Terao, A problem in the determination of trihalomethane by headspace-gas chromatography/mass spectrometry, J. Health Sci. 49 (2003) 1-7.
[28] X. Zhang, R.A. Minear, Decomposition of trihaloacetic acids and formation of the corresponding trihalomethanes in drinking water, Water Res. 36 (2002) 3665-3673.
[29] B.K. Koudjonou, G.L. LeBel, Halogenated acetaldehydes: analysis, stability and fate in drinking water, Chemosphere 64 (2006) 795-802.
[30] W. Chu, N. Gao, Y. Deng, Stability of newfound nitrogenous disinfection by-products haloacetamides in drinking water, Chin. J. Org. Chem. 29 (2009) 1569-1574.
[31] M. Serrano, M. Silva, M. Gallego, Fast and “green” method for the analytical monitoring of haloketones in treated water, J. Chrom. A 1358 (2014) 232- 239.
[32] S. Hashimoto, T. Azuma, A. Otsuki, Distribution, sources, and stability of haloacetic acids in Tokyo Bay, Japan, Environ. Toxicol. Chem. 17 (1998) 798-805.
[33] P.C. Singer, H.S. Weinberg, K. Brophy, Relative Dominance of Haloacetic Acids and Trihalomethanes in Treated Drinking Water, Awwa Research Foundation/ American Water Works Association, Denver, 2002.
[34] Y. Zhang, W. Huang, Y. Ran, J. Mao, Compositions and constituents of freshwater dissolved organic matter isolated by reverse osmosis, Mar. Pollut. Bull. 85 (2014) 60-66.
[35] L. Sun, E. Perdue, J. McCarthy, Using reverse osmosis to obtain organic matter from surface and ground waters, Water Res. 29 (1995) 1471-1477.
[36] J.E. Kilduff, S. Mattaraj, A. Wigton, M. Kitis, T. Karanfil, Effects of reverse osmosis isolation on reactivity of naturally occurring dissolved organic matter in physicochemical processes, Water Res. 38 (2004) 1026-1036.

[37] M. Kitis, J.E. Kilduff, T. Karanfil, Isolation of dissolved organic matter (DOM) from surface waters using reverse osmosis and its impact on the reactivity of DOM to formation and speciation of disinfection by-products, Water Res. 35 (2001) 2225-2234.
[38] O. Orr, The characterization, isolation, and fractionation of natural organic matter from three surface waters (PhD Thesis), Clemson University, 2009.
[39] J.G. Pressman, S.D. Richardson, T.F. Speth, R.J. Miltner, M.G. Narotsky, I. Hunter, et al., Concentration, chlorination, and chemical analysis of drinking water for disinfection byproduct mixtures health effects research: US EPA's four lab study, Environ. Sci. Technol. 44 (2010) 7184-7192.
[40] T.F. Speth, R.J. Miltner, S.D. Richardson, J.E. Simmons, Integrated disinfection by-products mixtures research: concentration by reverse osmosis membrane techniques of disinfection by-products from water disinfected by chlorination and ozonation/postchlorination, J. Toxicol. Env. Heal. A 71 (2008) 1149-1164.
[41] C.J. Tadanier, D.F. Berry, W.R. Knocke, Dissolved organic matter apparent molecular weight distribution and number-average apparent molecular weight by batch ultrafiltration, Environ. Sci. Technol. 34 (2000) 2348-2353.
[42] Y. Cai, Size distribution measurements of dissolved organic carbon in natural waters using ultrafiltration technique, Water Res. 33 (1999) 3056-3060.
[43] G. Hua, D.A. Reckhow, Characterization of disinfection byproduct precursors based on hydrophobicity and molecular size, Environ. Sci. Technol. 41 (2007) 3309-3315.
[44] L. Lin, B. Xu, Y.-L. Lin, C.-Y. Hu, T. Ye, T.-Y. Zhang, et al., A comparison of carbonaceous, nitrogenous and iodinated disinfection by-products formation potential in different dissolved organic fractions and their reduction in drinking water treatment processes, Sep. Purif. Technol. 133 (2014) 82-90.
[45] X. Zhang, R.A. Minear, Formation, adsorption and separation of high molecular weight disinfection byproducts resulting from chlorination of aquatic humic substances, Water Res. 40 (2006) 221-230.
[46] J.A. Leenheer, E. Huffman, Classification of organic solutes in water by using macroreticular resins, J. R. Stat. Soc. 4 (1976) 737-751.
[47] H. Zhang, J. Qu, H. Liu, X. Zhao, Characterization of isolated fractions of dissolved organic matter from sewage treatment plant and the related disinfection by-products formation potential, J. Hazard. Mater. 164 (2009) 1433-1438.
[48] S.C. Wendelken, M.V. Bassett, T.A. Dattilio, B.V. Pepich, D.J. Munch, Method 515.4-1 Determination of chlorinated acids in drinking water by liquid-liquid extraction, derivatization and gas chromatography with electron capture detection, Technical support center office of ground water and drinking water U. S. Environmental Protection Agency, Cincinnati, OH, 2000 (accessed May 2016).
[49] I. Montesinos, M. Cardador, M. Gallego, Determination of halonitromethanes in treated water, J. Chrom. A 1218 (2011) 2497-2504.
[50] M. Saraji, M. Ghani, Hollow fiber liquid-liquid-liquid microextraction followed by solid-phase microextraction and in situ derivatization for the determination of chlorophenols by gas chromatography-electron capture detection, J. Chrom. A 1418 (2015) 45-53.
[51] Y. Yamini, A. Esrafili, M. Ghambarian, Online injection-based hollow fiber liquid-phase microextraction-high-performance liquid chromatography as a fully automatic sample processing for phthalate esters analysis, Food Anal. Method. 9 (2015) 1-9.

[52] B. Ebrahimpour, Y. Yamini, M. Rezazadeh, A sensitive emulsification liquid phase microextraction coupled with on-line phase separation followed by HPLC for trace determination of sulfonamides in water samples, Environ. Monit. Assess. 187 (2015) 1-13.
[53] M. Serrano, M. Silva, M. Gallego, Micro liquid-liquid extraction combined with large-volume injection gas chromatography-mass spectrometry for the determination of haloacetaldehydes in treated water, J. Chrom. A 1218 (2011) 8295-8302.
[54] EPA. 1995. Method 551.1-1 Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection, Revision 1.0, DOI.
[55] H.S. Weinberg, S.W. Krasner, S.D. Richardson, A.D. Thruston Jr., The Occurrence of Disinfection By-Products (DBPs) of Health Concern in Drinking Water: Results of a Nationwide DBP Occurrence Study, National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Athens, GA EPA/600/R-02/068, September 2002, (accessed July 2016).
[56] T. Rodríguez-Cabo, M. Ramil, I. Rodríguez, R. Cela, Dispersive liquid-liquid microextraction with non-halogenated extractants-7 for trihalomethanes determination in tap and swimming pool water, Talanta 99 (2012) 846-852.
[57] E. Yiantzi, E. Psillakis, K. Tyrovola, N. Kalogerakis, Vortex-assisted liquid-liquid microextraction of octylphenol, nonylphenol and bisphenol-A, Talanta 80 (2010) 2057-2062.
[58] S. Allard, J.W. Charrois, C.A. Joll, A. Heitz, Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid-phase microextraction and gas chromatography mass-spectrometry, J. Chrom. A 1238 (2012) 15-21.
[59] B. Hammami, M. Driss, Development of dry derivatization and headspace solid-phase microextraction technique for the GC-ECD determination of haloacetic acids in tap water, J. Anal. Chem. 68 (2013) 671-679.
[60] N. Jakubowska, B. Henkelmann, K.-W. Schramm, J. Namiesnik, Optimization of a novel procedure for determination of VOCs in water and human urine samples based on SBSE coupled with TD-GC-HRMS, J. Chromatogr. Sci. 47 (2009) 689-693.
[61] M. Kawaguchi, Y. Ishii, N. Sakui, N. Okanouchi, R. Ito, K. Saito, et al., Stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography-mass spectrometry for determination of chlorophenols in water and body fluid samples, Anal. Chim. Acta 533 (2005) 57-65.
[62] I. Montesinos, M. Gallego, How the inclusion of treated water in Beverages influences the appearance of halogenated volatile organic compounds, J. Agric. Food Chem. 62 (2014) 10240-10247.
[63] I. Montesinos, M. Gallego, Headspace gas chromatography-mass spectrometry for rapid determination of halonitromethanes in tap and swimming pool water, Anal. Bioanal. Chem. 402 (2012) 2315-2323.
[64] J.L.P. Pavón, S.H. Martín, C.G. Pinto, B.M. Cordero, Headspace-programmed temperature vaporizer-fast gas chromatography-mass spectrometry coupling for the determination of trihalomethanes in water, J. Chrom. A 1194 (2008) 103-110.
[65] Y. Xiao, R. Fan, L. Zhang, J. Yue, R.D. Webster, T.-T. Lim, Photodegradation of iodinated trihalomethanes in aqueous solution by UV 254 irradiation, Water Res. 49 (2014) 275-285.

[66] A. Lara-Gonzalo, J.E. Sánchez-Uría, E. Segovia-García, A. Sanz-Medel, Critical comparison of automated purge and trap and solid-phase microextraction for routine determination of volatile organic compounds in drinking waters by GC-MS, Talanta 74 (2008) 1455-1462.
[67] M.A. Brown, S. Miller, G.L. Emmert, On-line purge and trap gas chromatography for monitoring of trihalomethanes in drinking water distribution systems, Anal. Chim. Acta 592 (2007) 154-161.
[68] M.Y. Tominaga, A.F. Mídio, Comparison of two validated gas-chromatographic methods for the determination of trihalomethanes in drinking water, Rev. Bras. Cienc. Farm. 39 (2003) 195-202.
[69] A.D. Nikolaou, T.D. Lekkas, S.K. Golfinopoulos, M.N. Kostopoulou, Application of different analytical methods for determination of volatile chlorination by-products in drinking water, Talanta 56 (2002) 717-726.
[70] T.-C. Chen, G.-R. Her, On-line monitoring of trihalomethanes in drinking water using continuous-flow purge and cryofocusing gas chromatography-mass spectrometry, J. Chrom. A 927 (2001) 229-235.
[71] A.A. Kampioti, E.G. Stephanou, Simultaneous determination of halogenated neutral and acidic disinfection by-products in drinking water by closed-loop stripping extraction and capillary gas chromatography, J. Chrom. A 857 (1999) 217-229.
[72] A.A. Kampioti, E.G. Stephanou, An inclusive study of the presence of the disinfection by-products in Greek drinking water. 9th International Conference on Environmental Science and Technology, Rhodes Island, Greece, September 2005.
[73] C.A. Joll, M.J. Alessandrino, A. Heitz, Disinfection by-products from halogenation of aqueous solutions of terpenoids, Water Res. 44 (2010) 232-242.
[74] F. Ruiz-Bevia, M.J. Fernandez-Torres, M.P. Blasco-Alemany, Purge efficiency in the determination of trihalomethanes in water by purge-and-trap gas chromatography, Anal. Chim. Acta 632 (2009) 304-314.
[75] M. Carpi, C. Zufall, GC analysis of trihalomethanes in drinking water-A rapid and direct quantitative method, LC GC N. Am. 21 (2003) 88-91.
[76] M. Tobiszewski, J. Namies´nik, Determination of chlorinated solvents in industrial water and wastewater by DAI-GC-ECD, Anal. Bioanal. Chem. 399 (2011) 3565-3572.
[77] B. Yu, Y. Song, L. Han, H. Yu, Y. Liu, H. Liu, Optimizations of packed sorbent and inlet temperature for large volume-direct aqueous injection-gas chromatography to determine high boiling volatile organic compounds in water, J. Chrom. A 1356 (2014) 221-229.
[78] N. Negreira, J. Regueiro, M.L. de Alda, D. Barceló, Transformation of tamoxifen and its major metabolites during water chlorination: identification and in silico toxicity assessment of their disinfection byproducts, Water Res. 85 (2015) 199-207.

Supplementary Materials (SM)

SM 1. Some examples of effects of quenching agents on the preservation of regulated OXBPs

The effects of quenching agents on the preservation of regulated OXBPs have been widely investigated and discussed. For example, a study reported by Bauman and Stenstrom demonstrated that treating chlorinated water with sulfite leads to a significant reduction in AOX levels [1]. Fam and Stenstrom showed that the most reactive group of compounds with sulfite ions was unsaturated halogenated aliphatics [2]. Liu and Zhang also observed a significant reduction in AOX levels using sodium arsenite ($NaAsO_2$) [3]. They found that an excessive amount of $NaAsO_2$ coupled with a prolonged quenching time may cause halogen atom loss through the reduction or decomposition of organohalogen byproducts. The stoichiometric sodium arsenite to chlorine residual ratio was demonstrated to be an appropriate choice. The effects of quenching agents on the preservation of regulated OXBPs have been widely investigated and discussed, but their effects on unregulated OXBPs have only been partially addressed. For example, Singer et al. tested the impact of five different chlorine quenching agents on the stability of haloacetic acids [4]. The tested chemicals were: NH_4Cl, $(NH_4)_2SO_4$, Na_2SO_3, $Na_2S_2O_3$ and $NaAsO_2$. When the sample is stored for one to two weeks, with pH values ranging from 7 to 10, Na_2SO_3 and $Na_2S_2O_3$ destroy a significant part of some species of HAAs, particularly monochloracetic acid and monobromoacetic acid, while $NaAsO_2$ slightly degrades HAAs. Under the same conditions, Na_2SO_3 also degrades bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid and tribromoacetic acid. When samples were quenched with ammonium salts, they were found to remain stable even after two weeks. The authors suggest ammonium sulfate as the optimal quenching agent because amounts of bromide present in analytical-grade ammonium chloride can contribute to the formation of organobromine by-products when the reagent is added to water samples containing free chlorine. Ammonium salts do not quench chloramines formed in situ during chlorine quenching or when chloramines are used as a biocide. A number of investigators have demonstrated that HAAs continue to be produced when ammonium salts are used to quench residual chlorine [5]. However, Pepich et al. showed that quantities of HAAs formed after over two weeks of sample storage remained very low [5]. They reported an increase in the total HAA concentration of less than 2 $\mu g.L^{-1}$ in ammonium chloridequenched samples, and 41 $\mu g.L^{-1}$ in unquenched chlorinated samples during the same period. Many other OXBPs are also known to be formed as a result of reactions between chloramines and NOM, including haloacetonitriles, haloacetamides and THMs [6]. Sulfur derivative agents are commonly used to quench residual oxidants in THM samples [7]. To the best of our knowledge, no study has reported a negative effect of these chemicals on the stability of THMs. Various undesirable interactions have been reported between quenching agents and a number of unregulated and emerging OXBPs. Xie and Reckhow reported that sodium sulfite, the most widely used dechlorinating agent, can destroy cyanogen halides in a few hours [8]. Recently, Chu et al. investigated the influence of five dechlorinating agents,

including Na_2SO_3, $Na_2S_2O_3$, ascorbic acid and NH_4Cl, on the stability of 13 haloacetamides (HAcAms) [9]. They reported that the first three reagents degraded the HAcAm compounds to some degree, especially the brominated and iodinated ones. In contrast, ammonium chloride had little influence on the stability of the 13 HAcAms over a 24-hour period during sample storage. In another study, Liew et al. examined the stability of six HNMs and five HAcAms in the presence of different quenching agents [10]. Stability tests showed that the tri-HNMs immediately degraded in the presence of ascorbic acid, sodium sulfite and sodium borohydride, and were also reduced in samples treated with NH_4Cl or with no form of preservation. While ammonium chloride does not have an impact on the stability of HNMs and HAcAms, it does not quench chloramines. An alternative quenching agent for water from chloraminated systems that does not affect HNMs has yet to be proposed. Ascorbic acid is appropriate for chloraminated samples, but for preserving HAcAms only. To preserve both HNM and HAcAm concentrations, it is recommended to analyze the water sample as soon as possible after collection [10,11]. Most recently, Kristiana et al. studied the effects of five quenching agents on the stability of seven different classes of DBPs commonly found in drinking waters. Ascorbic acid was found to be suitable for the analysis of HANs and HKs, while sodium arsenite, sodium borohydride and ascorbic acid were all deemed acceptable for the analysis of haloacetaldehydes (HALs) [7]. The measured concentrations of HAAs and HKs in both the quenched and non-quenched samples decreased throughout the experiment, confirming their inherent instability in aqueous solution. At the exception of trichloroacetonitrile, the concentrations of HANs were also stable over time in the nonquenched samples. According to Zhang and Minear, some HAA species (bromodichloroacetic acid, chlorodibromoacetic acid and tribromoacetic acid) are decomposed by decarboxylation at ambient temperature and neutral pH, forming their corresponding THMs [12]. Reckhow and Singer reported that 1,1,1-trichloropropanone hydrolyses to chloroform at alkaline pH [13].

References

[1] L. Bauman, M.K. Stenstrom, Observations of the reaction between organohalides and sulfite, Environ. Sci. Technol. 23 (1989) 232-236.

[2] S. Fam, M.K. Stenstrom, The reaction of dechlorinating agents with some non-volatile halogenated organics, Environ. Technol. 9 (1988) 833-846.

[3] J. Liu, X. Zhang, Effect of quenching time and quenching agent dose on total organic halogen measurement, Int. J. An. Ch. 93 (2013) 1146-1158.

[4] P.C. Singer, H.S. Weinberg, K. Brophy, Relative dominance of haloacetic acids and trihalomethanes in treated drinking water, Awwa Research Foundation/American Water Works Association, Denver, 2002.

[5] B.V. Pepich, M.M. Domino, T.A. Dattilio, P.S. Fair, D.J. Munch, Validating sample preservation techniques and holding times for the approved compliance monitoring methods for haloacetic acids under the US EPA's stage 1 D/DBP rule, Water Res. 38 (2004) 895-902.

[6] W. Chu, X. Li, T. Bond, N. Gao, D. Yin, The formation of haloacetamides and other disinfection by-products from non-nitrogenous low-molecular weight organic acids during chloramination, Chem. Eng. J. 285 (2016) 164- 171.

[7] I. Kristiana, A. Lethorn, C. Joll, A. Heitz, To add or not to add: The use of quenching agents for the analysis of disinfection by-products in water samples, Water Res. 59 (2014) 90-98.

[8] Y. Xie, D.A. Reckhow, A rapid and simple analytical method for cyanogen chloride and cyanogen bromide in drinking water, Water Res. 27 (1993) 507-511.

[9] W. Chu, N. Gao, D. Yin, S.W. Krasner, M.R. Templeton, Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization, J. Chrom. A 1235 (2012) 178-181.

[10] D. Liew, K.L. Linge, C.A. Joll, A. Heitz, J.W. Charrois, Determination of halonitromethanes and haloacetamides: An evaluation of sample preservation and analyte stability in drinking water, J. Chrom. A 1241 (2012) 117-122.

[12] X. Zhang, R.A. Minear, Decomposition of trihaloacetic acids and formation of the corresponding trihalomethanes in drinking water, Water Res. 36 (2002) 3665-3673.

[13] D.A. Reckhow, P.C. Singer, Mechanisms of organic halide formation during fulvic acid chlorination and implications with respect to pre-ozonation, in Water Chlorination: Environmental Impact and Health Effects, Vol. 5, Lewis Publishers Inc., Chelsea,1985, pp. 1229-1257.

SM 2. Effect of a quenching agent on a given OXBP

OXBPs Family	OXBPs	$NaSO_3$ or $Na_2S_2O_3$	$NaBH_4$	$NaAsO_2$ or $NaAsO_3$	NH_4Cl	$C_6H_8O_6$	H_2O_2	Reference
Trihalomethanes (THMs)	Chloroform	S	S	S	S	S	S	[9-11]
	Bromodichloromethane	S	S	S	S	S	S	
	Chlorodibromomethane	S	S	S	S	S	S	
	Bromoform	S	S	S	S	S	S	
Haloacetic acids (HAAs)	Monochloroacetic acid	S	S	S	S	S	S	[9, 11]
	Dichloroacetic acid	S	S	S	S	S	S	
	Trichloroacetic acid	S	S	S	S	S	S	
	Monobromoacetic acid	S	S	S	S	S	S	
	Dibromoacetic acid	S	S	S	S	S	S	
	Tribromoacetic acid	S	S	S	S	S	S	
	Bromochloroacetic acid	S	S	S	S	S	S	
	Bromodichloroacetic acid	S	S	S	S	S	S	
	Chlorodibromoacetic acid	S	S	S	S	S	S	
Haloacetonitriles (HANs)	Monochloroacetonitrile	END	END	END	END	S	END	[9, 10, 12]
	Monobromoacetonitrile	D	END	END	END	S	END	
	Dichloroacetonitrile	END	END	END	END	S	END	
	Dibromoacetonitrile	D	END	END	END	S	END	
	Trichloroacetonitrile	D	END	END	END	S	END	
Haloketones (HKs)	Monochloropropanone	END	END	D	END	S	END	[9, 10, 12]
	1,1-Dichloropropanone	D	END	D	END	S	END	
	1,1,1-Trichloropropanone	D	END	D	END	S	END	
	1,3-Dichloropropanone	D	END	D	END	S	END	
	1,1,3-Trichloropropanone	END	D	D	END	S	END	
Haloacetaldehydes (HALs)	Bromochloroacetaldehyde	D	S	S	D	S	END	[9]
	Dibromoacetaldehyde	D	S	S	END	S	END	
	Bromodichloroacetaldehyde	D	S	S	D	S	END	
	Chlorodibromoacetaldehyde	D	S	S	END	S	END	

	Trichloroacetaldehyde	D	S	S	END	S	END	
	Tribromoacetaldehyde	D	S	S	END	S	END	
Halonitromethanes (HNMs)	Bromochloronitromethane	END	END	END	S	S	END	[9, 10, 12, 13]
	Dichloronitromethane	END	END	END	S	S	END	
	Bromodichloronitromethane	D	D	END	S	D	END	
	Dibromochloronitromethane	D	D	END	S	D	END	
	Chloropicrin	D	D	D	S	D	END	
	Bromopicrin	D	D	D	D	D	END	
Haloacetamides (HAcAms)	Monochloroacetamide	D	D	D	S	S	END	[13]
	Monobromoacetamide	D	D	D	S	S	END	
	Dichloroacetamide	D	D	D	S	S	END	
	Dibromoacetamide	D	D	D	S	S	END	
	Trichloroacetamide	D	D	D	S	S	END	

References

[1] D. Liew, K.L. Linge, C.A. Joll, A. Heitz, J.W. Charrois, Determination of halonitromethanes and haloacetamides: An evaluation of sample preservation and analyte stability in drinking water, Journal of Chromatography A, 1241 (2012) 117-122.

[2] S. Batterman, L. Zhang, S. Wang, Quenching of chlorination disinfection by-product formation in drinking water by hydrogen peroxide, Water Research, 34 (2000) 1652-1658.

[3] I. Kristiana, A. Lethorn, C. Joll, A. Heitz, To add or not to add: The use of quenching agents for the analysis of disinfection by-products in water samples, Water research, 59 (2014) 90-98.

[4] A. Serrano, M. Gallego, Rapid determination of total trihalomethanes index in drinking water, Journal of Chromatography A, 1154 (2007) 26-33.

[5] D. Munch, D. Hautman, Determination of chlorinated disinfection byproducts, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid-liquid extraction and gas chromatography with electron-capture detection, EPA method, 551 (1995).

[6] R. Chinn, T. Lee, S. Krasner, M. Dale, S. Richardson, J. Pressman, T. Speth, R. Miltner, J. Simmons, Solid-phase extraction of 35 DBPs with analysis by GC/ECD and GC/MS, Proceedings of the American Water Works Association Water Quality Technology Conference, Denver, CO, 2007.

[7] W.-H. Chu, N.-Y. Gao, Y. Deng, Formation of haloacetamides during chlorination of dissolved organic nitrogen aspartic acid, J. Hazard. Mater. 173 (2010) 82-86.

SM 3. Most frequently used quenching agents, depending on the OXBP family, the pH and the type of chlorinated oxidant.

OXBP family	Cl_2			MCA			ClO_2		
	Quenching agent	pH	Ref	Quenching agent	pH	Ref	Quenching agent	pH	Ref
THMs	$C_6H_8O_6$	< 7	[1, 2, 3]	$C_6H_8O_6$	< 7	[1, 3]	NH_4Cl	7	[4, 5, 6] [11]
		7	[7]		7-8	[9]			
	Ag^+/H_2O_2	7	[8]	Na_2SO_3	< 6	[9]			
					5	[10]			
					7	[11]			
	$NaAsO_2$ $NaBH_4$ NH_4Cl Na_2SO_3 $C_6H_8O_6$	7-8	[12]	$NaAsO_2$ $NaBH_4$ Na_2SO_3 $C_6H_8O_6$	7-8	[12]			
	NH_4Cl	5	[10, 13]	$Na_2S_2O_3$	7	[14, 15]			
		7	[11]						
	$Na_2S_2O_3$	6	[16]	Na_2SO_3		[17]			
	Na_2SO_3	7	[17]						
HAAs	$C_6H_8O_6$	< 7	[1]	$C_6H_8O_6$	7	[1]	NH_4Cl	0.5	[4, 5, 6]
	NH_4Cl	7	[11] [18]						
				Na_2SO_3	<6	[9]			
	Ag^+/H_2O_2	7	[8]	Na_2SO_3 $NaAsO_2$ $C_6H_8O_6$ $NaBH_4$	7-8	[12]			
	Na_2SO_3 $NaAsO_2$ $C_6H_8O_6$ NH_4Cl $NaBH_4$	7-8	[12]	NH_4Cl	5	[3, 10]			
					7	[11, 17]		7	[10]
	$C_6H_8O_6$	<7	[19]						
	NH_4Cl	5-7	[3, 10, 13, 17]	$Na_2S_2O_3$	7	[15]			
HANs	$(NH_4)_2SO_4$	5	[20]	$C_6H_8O_6$	7	[3, 12]	NH_4Cl	7	[5]
	NH_4Cl	< 7	[1]	NH_4Cl	5	[10]			
		7	[12]	$Na_2S_2O_3$	7.5	[14]			
		5	[10, 13]		7	[15]			
	$Na_2S_2O_3$	6	[21]						
HKs	$C_6H_8O_6$	1.5	[22]	$C_6H_8O_6$	< 7	[1, 3]	NH_4Cl	7	[5]
		< 7	[1, 3]		7	[12]			
	NH_4Cl	7	[12]	$Na_2S_2O_3$	7.5	[14]			

					7	[15]			
	$Na_2S_2O_3$	6	[16]						
HALs	$C_6H_8O_6$	4.5	[23]	$C_6H_8O_6$	<7	[1]	-	-	-
		< 7	[1, 24]						
	$NaAsO_2$ $C_6H_8O_6$ $NaBH_4$	7-8	[12]	$NaAsO_2$ $C_6H_8O_6$ $NaBH_4$	7-8	[12]			
HNMs	$(NH_4)_2SO_4$	5	[20]	$C_6H_8O_6$	<7	[1, 3]	-	-	-
	NH_4Cl	< 7	[40]						
	$C_6H_8O_6$	< 7	[1, 3]						
HAcAms	NH_4Cl	5	[14]	$C_6H_8O_6$	< 7	[1, 40]	-	-	-
		< 7	[13]						
	$C_6H_8O_6$	< 7	[1]						
		7	[25, 26]		7.5	[27]			
					7	[7]			
		7.5	[27]						
AOX	$NaAsO_2$	< 2	[28]	Na_2SO_3	2	[11]	Na_2SO_3	2	[11]
	Na_2SO_3	2	[11]	$Na_2S_2O_3$	2	[29]			

References

[1] R. Chinn, T. Lee, S. Krasner, M. Dale, S. Richardson, J. Pressman, T. Speth, R. Miltner, J. Simmons, Solid-phase extraction of 35 DBPs with analysis by GC/ECD and GC/MS, Proceedings of the American Water Works Association Water Quality Technology Conference, Denver, CO, 2007.
[2] A. Serrano, M. Gallego, Rapid determination of total trihalomethanes index in drinking water, J. Chrom. A 1154 (2007) 26-33.
[3] C.M. Bougeard, E.H. Goslan, B. Jefferson, S.A. Parsons, Comparison of the disinfection by-product formation potential of treated waters exposed to chlorine and monochloramine, Water Res. 44 (2010) 729-740.
[4] C.-Y. Chang, Y.-H. Hsieh, S.-S. Hsu, P.-Y. Hu, K.-H. Wang, The formation of disinfection by-products in water treated with chlorine dioxide, J. Hazard. Mater. 79 (2000) 89-102.
[5] C.-Y. Chang, Y.-H. Hsieh, Y.-M. Lin, P.-Y. Hu, C.-C. Liu, K.-H. Wang, The organic precursors affecting the formation of disinfection by-products with chlorine dioxide, Chemosphere 44 (2001) 1153-1158.
[6] C.-Y. Chang, Y.-H. Hsieh, I.-C. Shih, S.-S. Hsu, K.-H. Wang, The formation and control of disinfection by-products using chlorine dioxide, Chemosphere 41 (2000) 1181- 1186.
[7] T. Ye, B. Xu, Z. Wang, T.-Y. Zhang, C.-Y. Hu, L. Lin, S.-J. Xia, N.-Y. Gao, Comparison of iodinated trihalomethanes formation during aqueous chlor(am)ination of different iodinated X-ray contrast media compounds in the presence of natural organic matter, Water Res. 66 (2014) 390-398.
[8] S. Batterman, L. Zhang, S. Wang, Quenching of chlorination disinfection by-product formation in drinking water by hydrogen peroxide, Water Res. 34 (2000) 1652-1658.
[9] Y. Hong, S. Liu, T. Karanfil, Understanding DBP formation during chloramination, Florida Water Resource Journal 60 (2008) 51-53.
[10] E.H. Goslan, S.W. Krasner, M. Bower, S.A. Rocks, P. Holmes, L.S. Levy, S.A. Parsons, A comparison of disinfection by-products found in chlorinated and chloraminated drinking waters in Scotland, Water Res. 43 (2009) 4698-4706.
[11] G. Hua, D.A. Reckhow, Comparison of disinfection byproduct formation from chlorine and alternative disinfectants, Water Res. 41 (2007) 1667-1678.
[12] I. Kristiana, A. Lethorn, C. Joll, A. Heitz, To add or not to add: The use of quenching agents for the analysis of disinfection by-products in water samples, Water Res. 59 (2014) 90-98.
[13] K.M. Hansen, S. Willach, M.G. Antoniou, H. Mosbæk, H.-J. Albrechtsen, H.R. Andersen, Effect of pH on the formation of disinfection byproducts in swimming pool water-Is less THM better?, Water Res. 46 (2012) 6399-6409.
[14] X. Yang, C. Shang, P. Westerhoff, Factors affecting formation of haloacetonitriles, haloketones, chloropicrin and cyanogen halides during chloramination, Water Res. 41 (2007) 1193-1200.
[15] X. Yang, C. Shang, W. Lee, P. Westerhoff, C. Fan, Correlations between organic matter properties and DBP formation during chloramination, Water Res. 42 (2008) 2329- 2339.
[16] C.V. Antoniou, E.E. Koukouraki, E. Diamadopoulos, Determination of chlorinated volatile organic compounds in water and municipal wastewater using headspace-solid phase microextraction-gas chromatography, J. Chrom. A 1132 (2006) 310-314.
[17] J. Lu, T. Zhang, J. Ma, Z. Chen, Evaluation of disinfection by-products formation during chlorination and chloramination of dissolved natural organic matter fractions isolated from a filtered river water, J. Hazard. Mater. 162 (2009) 140-145.
[18] B.V. Pepich, M.M. Domino, T.A. Dattilio, P.S. Fair, D.J. Munch, Validating sample preservation techniques and holding times for the approved compliance monitoring methods for haloacetic acids under the US EPA's stage 1 D/DBP rule, Water Res. 38 (2004) 895-902.
[19] L. Meng, S. Wu, F. Ma, A. Jia, J. Hu, Trace determination of nine haloacetic acids in drinking water by liquid chromatography-electrospray tandem mass spectrometry, J. Chrom. A 1217 (2010) 4873-4876.
[20] Q. Luo, X. Chen, Z. Wei, X. Xu, D. Wang, Z. Wang, Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation, J. Chrom. A 1365 (2014) 45-53.
[21] P. Westerhoff, P. Chao, H. Mash, Reactivity of natural organic matter with aqueous chlorine and bromine, Water Res. 38 (2004) 1502-1513.
[22] M. Serrano, M. Silva, M. Gallego, Fast and "green" method for the analytical monitoring of haloketones in treated water, J. Chrom. A 1358 (2014) 232-239.
[23] B.K. Koudjonou, G.L. LeBel, Halogenated acetaldehydes: Analysis, stability and fate in drinking water, Chemosphere 64 (2006) 795-802.
[24] S. Krasner, S. Pastor, R. Chinn, M. Sclimenti, H. Weinberg, S. Richardson, A. Thruston Jr, The occurrence of a new generation of DBPs (beyond the ICR), Proceedings of American Water Works Association Water Quality Technology Conference, Denver, CO, USA, 2001.

[25] H. Huang, Q.-Y. Wu, H.-Y. Hu, W.A. Mitch, Dichloroacetonitrile and dichloroacetamide can form independently during chlorination and chloramination of drinking waters, model organic matters, and wastewater effluents, Environ. Sci. Technol. 46 (2012) 10624-10631.
[26] W.-H. Chu, N.-Y. Gao, Y. Deng, Formation of haloacetamides during chlorination of dissolved organic nitrogen aspartic acid, J. Hazard. Mater. 173 (2010) 82-86.
[27] W. Chu, N. Gao, D. Yin, S.W. Krasner, Formation and speciation of nine haloacetamides, an emerging class of nitrogenous DBPs, during chlorination or chloramination, J. Hazard. Mater. 260 (2013) 806-812.
[28] J. Liu, X. Zhang, Effect of quenching time and quenching agent dose on total organic halogen measurement, Int. J. An. Ch. 93 (2013) 1146-1158.
[29] D. Brown, J. Bridgeman, J.R. West, Predicting chlorine decay and THM formation in water supply systems, Rev. Environ. Sci. Biotechnol. 10 (2011) 79-99.

SM 4. A brief summary of various liquid-phase microextraction methods and their performance levels

LPME technique	Matrix	Sample volume (mL)	Extractant	Compounds	Determination technique	Recovery (%)	Ref
DLLME	Tap and swimming pool water	18	1-Undecanol (50 µL)	THMs	GC-µECD	79-113	[1]
DLLME	Drinking water	5	Carbon disulfide (20 µL)	THMs	GC-MS	92-108	[2]
DLLME	Water	5	2-Dodecanol (10 µL)	Organohalogen compounds	GC-ECD or GC-MS	-	[3]
DLLME	Drinking and swimming pool water	10	n-Pentane	HAAs	HS-GC/MS	93-98	[4]
DLLME	Water	5	Chlorobenzene (10 µL)	Chlorophenols	GC-ECD	83-118	[5]
SA-DLLME	Drinking water	8	DCM	HANs	PTV-GC-MS	79-105	[6]
SA-DLLME	Drinking and swimming pool water	10	Ethyl acetate	HAcAms	IPS-GC-MS	76-94	[7]
HF-LPME	Drinking water	20	n-Octanol	THMs, I-THMs	GC-µECD	96-105	[8]
HF-LPME	Drinking and tap water	20	n-Octanol	THMs	GC-ECD	98-105	[9]
HF-LPME	Water	10	n-Octanol	HAAs	GC-ECD	97-109	[10]
DI-SDME	Tap water	5	n-Hexane	THMs	GC-ECD	73-78	[11]
HS-SDME	Drinking water	25	n-Octanol	THMs	GC-ECD	101-112	[12]
HS-SDME	Tape and swimming pool water	10	n-Hexanol (2 µL)	7 HNMs	GC-MS	3-20	[13]
SDME	River and tap water	3	n-Octanol	6 HAAs	GC-MS	82-98	[14]
LPME	Treated water	9	Ethyl acetate (30 µL)	HALs	PTV-GC-MS	-	[15]
LPME	Tap water	9	Ethyl acetate (200 µL)	THMs, HNMs	PTV-GC-MS	85	[16]
LPME	Tap and swimming pool water	10	n-Hexane (200 µL)	9 HAAs, 4 I-HAAs	PTV-GC-MS	91-97	[17]
VALLME	Water	5	Isopropyl ether (600 µL)	9 HAAs	LC-DAD	78-89	[18]

Abbreviations: SA-DLLME: Salt-Assisted Dispersive Liquid-Liquid MicroExtraction, ECD: Electron Capture Detector, IPS-GC-MS: Injection-Port Silylation Gas Chromatography-Mass Spectrometry, HAcAms: Haloacetamides, LC-DAD: Liquid Chromatography-Diode Array Detector, PTV: Programmable temperature vaporizing.

References

[1] T. Rodríguez-Cabo, M. Ramil, I. Rodríguez, R. Cela, Dispersive liquid-liquid microextraction with non-halogenated extractants-7 for trihalomethanes determination in tap and swimming pool water, Talanta 99 (2012) 846-852.
[2] R.R. Kozani, Y. Assadi, F. Shemirani, M.M. Hosseini, M. Jamali, Determination of trihalomethanes in drinking water by dispersive liquid-liquid microextraction then gas chromatography with electron-capture detection, Chromatographia 66 (2007) 81-86.
[3] M.-I. Leong, S.-D. Huang, Dispersive liquid-liquid microextraction method based on solidification of floating organic drop combined with gas chromatography with electroncapture or mass spectrometry detection, J. Chrom. A 1211 (2008) 8-12.
[4] M. Cardador, A. Serrano, M. Gallego, Simultaneous liquid-liquid microextraction/methylation for the determination of haloacetic acids in drinking waters by headspace gas chromatography, J. Chrom. A 1209 (2008) 61-69.
[5] N. Fattahi, Y. Assadi, M.R.M. Hosseini, E.Z. Jahromi, Determination of chlorophenols in water samples using simultaneous dispersive liquid-liquid microextraction and derivatization followed by gas chromatography-electron-capture detection, J. Chrom. A 1157 (2007) 23-29.
[6] H. Ma, Y. Li, H. Zhang, S.M. Shah, J. Chen, Salt-assisted dispersive liquid-liquid microextraction coupled with programmed temperature vaporization gas chromatographymass spectrometry for the determination of haloacetonitriles in drinking water, J. Chrom. A 1358 (2014) 14-19.
[7] T.-L. Chen, S.-H. Tzing, W.-H. Ding, Rapid screening of haloacetamides in water using salt-assisted liquid-liquid extraction coupled injection-port silylation gas chromatography-mass spectrometry, J. Chrom. A 1422 (2015) 340-344.
[8] A. Domínguez-Tello, A. Arias-Borrego, T. García-Barrera, J. Gómez-Ariza, Application of hollow fiber liquid phase microextraction for simultaneous determination of regulated and emerging iodinated trihalomethanes in drinking water, J. Chrom. A 1402 (2015) 8-16.
[9] N. Vora-adisak, P. Varanusupakul, A simple supported liquid hollow fiber membrane microextraction for sample preparation of trihalomethanes in water samples, J. Chrom. A 1121 (2006) 236-241.
[10] P. Varanusupakul, N. Vora-Adisak, B. Pulpoka, In situ derivatization and hollow fiber membrane microextraction for gas chromatographic determination of haloacetic acids in water, Anal. Chim. Acta 598 (2007) 82-86.
[11] A. Tor, M.E. Aydin, Application of liquid-phase microextraction to the analysis of trihalomethanes in water, Anal. Chim. Acta 575 (2006) 138-143.
[12] R.-S. Zhao, W.-J. Lao, X.-B. Xu, Headspace liquid-phase microextraction of trihalomethanes in drinking water and their gas chromatographic determination, Talanta 62 (2004) 751-756.
[13] I. Montesinos, M. Cardador, M. Gallego, Determination of halonitromethanes in treated water, J. Chrom. A 1218 (2011) 2497-2504.
[14] M. Saraji, A.A.H. Bidgoli, Single-drop microextraction with in-microvial derivatization for the determination of haloacetic acids in water sample by gas chromatographymass spectrometry, J. Chrom. A 1216 (2009) 1059-1066.
[15] M. Serrano, M. Silva, M. Gallego, Micro liquid-liquid extraction combined with large-volume injection gas chromatography-mass spectrometry for the determination of haloacetaldehydes in treated water, J. Chrom. A 1218 (2011) 8295-8302.
[16] I. Montesinos, M. Gallego, Solvent-minimized extraction for determining halonitromethanes and trihalomethanes in water, J. Chrom. A 1248 (2012) 1-8.
[17] M.J. Cardador, M. Gallego, Eco-friendly microextraction method for the quantitative speciation of 13 haloacetic acids in water, J. Chrom. A 1340 (2014) 15-23.
[18] P. Rocío-Bautista, V. Pino, B. Delgado, A.M. Afonso, J.H. Ayala, Vortex-assisted emulsification microextraction followed by in-syringe ultrasound-assisted backmicroextraction to determine haloacetic acids in waters, Anal. Methods 6 (2014) 4115-4123.

SM 5. Survey of SPE methods for the extraction of OXBPs from water samples

Compounds	Matrix	Sorbent type	SPE parameters	Recovery (%)	Ref
29 OXBPs including: THMs, I-THMs, HNMs, HANs, HALs	River water	Oasis HLB (500 mg/6 mL)	Conditioning: 10 mL MtBE, 10 mL MeOH, 10 mL H_2O Loading: 1 L, 5 mL/min Elution: 10 MtBE	65-103	[1]
35 OXBPs including: THMs, I-THMs, HNMs, HANs, HALs	Water	Elut-PPL (200 mg/3 mL)	Conditioning: 8 mL MeOH, Loading: 36 mL, 5 mL/min Elution: 1.6 ml MtBE	-	[2]
THMs	Pure water	Supelclean ENVI-18 (1 g/6 mL) HyperSepTM C_{18} (1 g/6 mL)	Conditioning: 4 mL ACN, 4 ml H_2O vs 50 µL MeOH, 50µL H_2O Loading: 100 mL, 0.8 mL/min vs 100 µL Washing: 100 µL Elution: 1 mL MeOH vs 100 µL MeOH	33-74 30-82	[3]
THMs	Drinking water	Supelclean ENVI-18 (2 g/12 mL)	Conditioning: 2 mL ACN, 2 ml H_2O Loading: 1 L, 15 mL/min Elution: 5 mL pentane	96.5	[4]
HAAs	Aqueous environments	LiChrolut EN (200 mg/6 mL)	Conditioning: 5 mL MeOH, 3 ml H_2O (pH < 2.5) Loading: 50 mL, 5 mL/min Washing: 1 mL H_2O (pH < 2.5) Elution: mix 0.5 mL H_2O + 3.5 mL MeOH-Acetone (v/v)	35-69	[5]
HAAs	Drinking water	Silicabond SAX (300 mg/3 mL)	Conditioning: 2x10 mL MeOH, 10 mL H_2O Loading: 50 mL, 1.5 mL/min Washing: 10 mL MeOH Elution: MeOH (10% H_2SO_4)	70-115	[6]
HAAs	Tap water	LiChrolut EN (200 mg/6 mL)	Conditioning: 5 mL MeOH, 5 ml H_2O (pH < 2.5) Loading: 500 mL, 5 mL/min Washing: 0.5 mL H_2O Elution: 2 mL MeOH	37-85	[7]
HAAs	Drinking water	LiChrolut EN (500 mg/6 mL)	Conditioning: 5 mL MeOH, 5 ACN, 5 mL H_2O (pH < 2.5) Loading: 100 mL, 5 mL/min Washing: 1 mL H_2O (pH < 2.5) Elution: 4 x 2 mL of mix. DBA/ACN (95:5, v/v)	60-102	[8]
HAAs	Drinking water	LiChrolut EN (200 mg/3 mL)	Conditioning: 2x3 mL MeOH, 3 mL H_2O (pH < 0.3) Loading: 50 mL, 1 mL/min Washing: 0.5 mL H_2O Elution: 3 mL H_2O (NaOH: 10 mM)	62-88	[9]
HAAs	Hospital wastewater	LiChrolut EN (200 mg/3 mL)	Conditioning: 2x3 mL MeOH, 3 mL H_2O (pH < 0.3) Loading: 50 mL, 2 mL/min Washing: 1mL H_2O Elution: 1 mL H_2O (NaOH: 1 mM)	64-91	[10]

HAAs	Drinking water	Silicabond SAX (300 mg/3 mL)	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 50 mL, 2 mL/min Elution: 3 mL H_2O (10% H_2SO_4)	-	[9]
HAAs	-	MEPS: Silica-C_{18} (4 mg)	Conditioning: 100 µL MtBE, 100 µL H_2O (pH = 4). Loading: 25x100 µL, 5 µL/s Washing: 100 µL H_2O Elution: 20 µL MtBE	83-117	[11]
HAcAms	Drinking water	Oasis HLB	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 500 mL, 3-5 mL/min Washing: 5 mL H_2O: MeOH (95:5, v/v) Elution: 5mL MeOH	78	[12]
Elucidation of unknown OXBPs - HBQs	Drinking water	Oasis HLB (200 mg/6 mL)	Conditioning: 6 mL MeOH (0.25% FA), 6 mL H_2O (0.25% FA) Loading: 500 mL, 2 mL/min Washing: 6 mL H_2O (0.25% FA) 6 mL H_2O: MeOH (50:0, v/v with 0.25% FA) Elution: 6 mL MeOH (0.25% FA)	-	[13]
Toxic and bioactive disinfection by-products	Wastewater effluents	Oasis HLB	Conditioning: 6 mL MeOH, 6 mL H_2O Loading: 900 mL, 10 mL/min Washing: 5 mL H_2O Elution: 8 mL MeOH	-	[14]
Toxic and bioactive disinfection by-products (e.g. AOX, AOCl, AOBr)	Swimming pool water	Oasis HLB (1 g/20 mL)	Conditioning: 10 mL MtBE, 20 mL MeOH, 10 mL H_2O (pH=1) Loading: 2 L (pH = 1) Elution: 20 mL MeOH + 10 mL MtBE under gravity	-	[15]
Toxic and bioactive disinfection by-products	Drinking water	Oasis HLB (1 g/20 ml)	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 2 L (pH = 3) Elution: 10 mL MeOH, 10 mL hexane: acetone (1:1, v/v)	60	[16]
Toxic and bioactive disinfection by-products	Source waters	HyperSep ™	Conditioning: 10 mL MeOH, 10 mL H_2O Loading: 100 mL (pH = 2) Elution: 4 mL MeOH, 4 mL acetone, 4 mL ACN	-	[17]

Abbreviations: DBA: Dibutylamine, ACN: Acetonitrile, MtBE: Methyl tert-butyl ether, HBQs: Halobenzoquinones, HANs: Haloacetonitriles, HKs: Haloketones, HNMs: Halonitromethanes, HAcAms: Haloacetamides, HALs : Haloacetaldehydes.

References

[1] M.L. Hladik, M.J. Focazio, M. Engle, Discharges of produced waters from oil and gas extraction via wastewater treatment plants are sources of disinfection by-products to receiving streams, Sci. Tot. Environ. 466 (2014) 1085-1093.

[2] R. Chinn, T. Lee, S. Krasner, M. Dale, S. Richardson, J. Pressman, T. Speth, R. Miltner, J. Simmons, Solid-phase extraction of 35 DBPs with analysis by GC/ECD and GC/MS, Proceedings of the American Water Works Association Water Quality Technology Conference, Denver, CO, 2007.

[3] L.D. Alexandrou, M.J. Spencer, P.D. Morrison, B.J. Meehan, O.A. Jones, Micro versus macro solid phase extraction for monitoring water contaminants: A preliminary study using trihalomethanes, Sci. Tot. Environ. 512 (2015) 210-214.

[4] M. Di Gioia, A. Leggio, A. Le Pera, A. Liguori, A. Napoli, C. Siciliano, SPE-GC-MS analysis of chloroform in drinking water, Chromatographia 60 (2004) 319-322.

[5] R. Loos, D. Barceló, Determination of haloacetic acids in aqueous environments by solid-phase extraction followed by ion-pair liquid chromatography-electrospray ionization mass spectrometric detection, J. Chrom. A 938 (2001) 45-55.

[6] S. Waseem, M.P. Abdullah, Validation of a solid phase extraction technique for the determination of halogenated acetic acids in drinking water, Sains Malays. 39 (2010) 227-231.

[7] D. Martınez, F. Borrull, M. Calull, Comparative study of a solid-phase extraction system coupled to capillary electrophoresis in the determination of haloacetic compounds in tap water, J. Chrom. A 827 (1998) 105-112.

[8] M. Prieto-Blanco, M. Alpendurada, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, S. Machado, C. Gonçalves, Improving methodological aspects of the analysis of five regulated haloacetic acids in water samples by solid-phase extraction, ion-pair liquid chromatography and electrospray tandem mass spectrometry, Talanta 94 (2012) 90-98.

[9] S. Waseem, I.U. Mohsin, Evolution and fate of haloacetic acids before and after chlorination within the treatment plant using SPE-GC-MS, Am. J. Anal. Chem. 2 (2011) 522-532.

[10] Y.-X. Sun, G. Ping, Determination of haloacetic acids in hospital effluent after chlorination by ion chromatography, J. Environ. Sci. 19 (2007) 885-891.

[11] A.M.C. Ferreira, M.E.F. Laespada, J.L.P. Pavón, B.M. Cordero, In situ derivatization coupled to microextraction by packed sorbent and gas chromatography for the automated determination of haloacetic acids in chlorinated water, J. Chrom. A 1318 (2013) 35-42.

[12] W. Chu, N. Gao, D. Yin, S.W. Krasner, M.R. Templeton, Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization, J. Chrom. A 1235 (2012) 178-181. [13] Y. Zhao, F. Qin, J.M. Boyd, J. Anichina, X.-F. Li, Characterization and determination of chloro-and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water, Anal. Chem. 82 (2010) 4599-4605.

[14] K. Watson, G. Shaw, F. Leusch, N. Knight, Chlorine disinfection by-products in wastewater effluent: Bioassay-based assessment of toxicological impact, Water Res. 46 (2012) 6069-6083.

[15] R.Y. Yeh, M.J. Farre, D. Stalter, J.Y. Tang, J. Molendijk, B.I. Escher, Bioanalytical and chemical evaluation of disinfection by-products in swimming pool water, Water Res. 59 (2014) 172-184.

[16] P.A. Neale, A. Antony, M.E. Bartkow, M.J. Farre, A. Heitz, I. Kristiana, J.Y. Tang, B.I. Escher, Bioanalytical assessment of the formation of disinfection byproducts in a drinking water treatment plant, Environ. Sci. Technol. 46 (2012) 10317-10325.

[17] Z. Fan, S. Gong, X. Xu, X. Zhang, Y. Zhang, X. Yu, Characterization, DBPs formation, and mutagenicity of different organic matter fractions in two source waters, Int. J. Hyg. Envir. Heal. 217 (2014) 300-306.

SM 6. Recent SPME and SBSE-based methods for OXBP analysis

Matrix	Analytes	Sorbent nature	Configuration	Time / temperature /salt	Method	Recoveries LQ	Ref
Drinking water	THMs	PDMS	HS-SPME	20 min / 35 °C / NaCl (25%, w/v)	GC- ECD	- 4.5-18 ng/L	[1]
Soft drink	THMs	CAR/PDMS	HS-SPME	15 min / 30 °C / NaCl (20%, w/v)	GC- ECD	> 90% 0.6-1.4 µg/L	[2]
Drinking water	THMs	CAR/PDMS	HS-SPME	30 min / 35 °C / NaCl (25%, w/v)	GC- ECD	- 15-30 ng/L	[3]
Drinking water	THMs	CAR/PDMS	HS-SPME	30 min / 37.5 °C / NaCl (25%, w/v)	GC- ECD	- -	[4]
Swimming pool water	THMs	PDMS	HS-SPME	25 min / 45 °C / NaCl (25%, w/v)	GC- ECD	- 14-40 µg/L	[5]
Drinking water	THMs	PDMS/DVB	HS-SPME	20 min / 40 °C / NaCl (35%, w/v)	GC- ECD	88-109% 0.2-1.6 µg/L	[6]
Drinking water	THMs	DVB/CAR/PDMS	DI-SPME	30 min / Room temperature / -	GC-MS	80-119% 0.06-2.1µg/L	[7]
Tap water and swimming pool water	HAAs	CAR/PDMS	HS-SPME	60 min / - / 5 g of Na_2SO_4	GC-MS	- -	[8]
Tap water	HAAs	PDMS	HS-SPME	10 min / 25 °C / -	GC- ECD	- 0.06-2.1µg/L	[9]
Drinking water	I-THMs	CWX/DVB	HS-SPME	30 min / room temp. / NaCl (25%, w/v)	GC- ECD	- 3.6-9 ng/L	[10]

Water	THMs, I-THMs	CAR/PDMS/DVB	HS-SPME	15 min / 70 °C / NaCl (25%, w/v)	GC-MS	84-103% 3-60 ng/L	[11]
Water	ClCN, BrCN	CAR/PDMS/DVB	HS-SPME	15 min / Room temp. / NaCl (25%, w/v)	GC-ECD	- 0.6-2.5µg/L	[12]
Drinking water	HNs	DVB/CAR/PDMS	HS-SPME	15 min / 40 °C / $NaSO_4$ (30%, w/v)	GC-MS	- 3-240 ng/L	[13]
Drinking water	HKs	DVB/CAR/PDMS	HS-SPME	15 min / 40 °C / 6 g of Na_2SO_4	GC-MS	- 35-1200 ng/L	[14]
Drinking water	HANs, HKs, chloropicrin	DVB/CAR/PDMS	HS-SPME	- / - / -	GC-MS	- 2-180 ng/L	[15]
Drinking water	I-THMS, HNMs, HANs	DVB/CAR/PDMS	HS-SPME	30 min / 40 °C / $(NH_4)SO_4$ (25%, w/v)	GC-MS	- 3-165 ng/L	[16]
Drinking water	Unknown OXBPs	CAR/PDMS	HS-SPME	30 min / - / -	GC-MS	- -	[17]
Water	Unknown OXBPs	CAR/PDMS	HS-SPME	30 min / - / -	GC-MS	- -	[18]
Water	Unknown OXBPs	CAR/PDMS	HS-SPME	- / Room temperature / NaCl (20%, w/v)	GC-MS	- -	[19]
Water	THMs	PDMS	DI-SBSE	30 min / - / -	TD-GC-HRMS	- -	[20]
Tap and river water	CPs	PDMS	DI-SBSE	- / Room temperature / -	TD-GC-MS	95% 3-6 ng/L	[21]

Abbreviations: HANs: Haloacetonitriles, HKs: Haloketones, TD: Thermal Desorption, CPs: Chlorophenols, HNMs: Halonitromethanes.

References

[1] M. Bahri, M. Driss, Development of solid-phase microextraction for the determination of trihalomethanes in drinking water from Bizerte, Tunisia, Desalination 250 (2010) 414-417.
[2] M.S. Dos Santos, E. Martendal, E. Carasek, Determination of THMs in soft drink by solid-phase microextraction and gas chromatography, Food Chem. 127 (2011) 290-295.
[3] D.-H. Cho, S.-H. Kong, S.-G. Oh, Analysis of trihalomethanes in drinking water using headspace-SPME technique with gas chromatography, Water Res. 37 (2003) 402-408.
[4] M. Aguirre-González, G. Taborda-Ocampo, C. Dussan-Lubert, C. Nerin, M. Rosero-Moreano, Optimization of the HS-SPME technique by using response surface methodology for evaluating chlorine disinfection by-products by GC in drinking water, J. Brazil. Chem. Soc. 22 (2011) 2330-2336.
[5] R. Maia, M. Correia, I.M.B. Pereira, V.M. Beleza, Optimization of HS-SPME analytical conditions using factorial design for trihalomethanes determination in swimming pool water samples, Microchem. J 112 (2014) 164-171.
[6] P.M. San Juan, J.D. Carrillo, M.T. Tena, Fibre selection based on an overall analytical feature comparison for the solid-phase microextraction of trihalomethanes from drinking water, J. Chrom. A 1139 (2007) 27-35.
[7] A. Lara-Gonzalo, J.E. Sánchez-Uría, E. Segovia-García, A. Sanz-Medel, Critical comparison of automated purge and trap and solid-phase microextraction for routine determination of volatile organic compounds in drinking waters by GC-MS, Talanta 74 (2008) 1455-1462.
[8] M. Sarrión, F. Santos, M. Galceran, In situ derivatization/solid-phase microextraction for the determination of haloacetic acids in water, Anal. Chem. 72 (2000) 4865-4873.
[9] B. Hammami, M. Driss, Development of dry derivatization and headspace solid-phase microextraction technique for the GC-ECD determination of haloacetic acids in tap water, J. Anal. Chem. 68 (2013) 671-679.
[10] B. Cancho, F. Ventura, M.T. Galceran, Solid-phase microextraction for the determination of iodinated trihalomethanes in drinking water, J. Chrom. A 841 (1999) 197-206.
[11] S. Allard, J.W. Charrois, C.A. Joll, A. Heitz, Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid phase microextraction and gas chromatography mass-spectrometry, J. Chrom. A 1238 (2012) 15-21.
[12] B. Cancho, F. Ventura, M.T. Galceran, Simultaneous determination of cyanogen chloride and cyanogen bromide in treated water at sub-μg/L levels by a new solid-phase microextraction-gas chromatographic-electron-capture detection method, J. Chrom. A 897 (2000) 307-315.
[13] I. Kristiana, C. Joll, A. Heitz, Analysis of halonitriles in drinking water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1225 (2012) 45-54.
[14] M. Serrano, M. Silva, M. Gallego, Determination of 14 haloketones in treated water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1407 (2015) 208-215.
[15] F.R. Kermani, A.-M. Tugulea, J. Hnatiw, V.H. Niri, J. Pawliszyn, Application of automated solid-phase microextraction to determine haloacetonitriles, haloketones, and chloropicrin in Canadian drinking water, Water Qual. Res. J. Can. 48 (2013) 85-98.
[16] Q. Luo, X. Chen, Z. Wei, X. Xu, D. Wang, Z. Wang, Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation, J. Chrom. A 1365 (2014) 45-53.
[17] C. Heffner, I. Silwal, J.M. Peckenham, T. Solouki, Emerging technologies for identification of disinfection byproducts: GC/FT-ICR MS characterization of solvent artifacts, Environ. Sci. Technol. 41 (2007) 5419-5425.
[18] I.K. Silwal, J.C. Rasaiah, J.E. Szulejko, T. Solouki, Proton transfer reactions of halogenated compounds: Using gas chromatography/Fourier transform ion cyclotron resonance mass spectrometry (GC/FT-ICR MS) and ab initio calculations, Int. J. Mass Spectrom. 293 (2010) 1-11.
[19] G. Alvarez-Rivera, M. Llompart, C. Garcia-Jares, M. Lores, Identification of halogenated photoproducts generated after ultraviolet-irradiation of parabens and benzoates in water containing chlorine by solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1349 (2014) 105-115.
[20] N. Jakubowska, B. Henkelmann, K.-W. Schramm, J. Namiesnik, Optimization of a novel procedure for determination of VOCs in water and human urine samples based on SBSE coupled with TD-GC-HRMS, J. Chromatogr. Sci. 47 (2009) 689-693.
[21] M. Kawaguchi, Y. Ishii, N. Sakui, N. Okanouchi, R. Ito, K. Saito, H. Nakazawa, Stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography-mass spectrometry for determination of chlorophenols in water and body fluid samples, Anal. Chim. Acta 533 (2005) 57-65.

SM 7. Recent applications of SPE for the extraction of OXBPs from water samples

Some of the most recent applications of SPE for the extraction of OXBPs from water samples are summarized in Table 3 of the manuscript. Non-polar phases are the most frequently used. They comprise silica-based reversed-phase (e.g. C18) and polymer based materials. Styrene divinylbenzene copolymers (PS-DVB) and chemically modified PS-DVB, to which polar groups are introduced, are the most commonly used polymeric supports. These polar groups provide dipole-dipole and H-bonding interaction sites, leading to the simultaneous extraction of polar and non-polar analytes in one SPE step. SPE with a functionalized PS-DVB material (Bond Elut PPL, Agilent Technologies) was applied by Lavonen et al. for the non-target analysis of OXBPs from a water surface submitted to chlorination and monochloramination [1]. A total of 499 OXBPs were identified, including 230 compounds not previously reported in literature. Stalter et al. developed and evaluated methods for extracting nonvolatile and volatile DBPs from chlorinated and chloraminated drinking water to minimize the loss of analytes [2]. For nonvolatile DBPs, solid-phase extraction (SPE) with TELOS ENV as solid phase performed superior compared to ten other sorbents. SPE yielded >70% recovery of nonpurgeable AOXs. For volatile DBPs, cryogenic vacuum distillation performed unsatisfactorily; purge and cold-trap with crushed ice achieved recoveries of 50-100% for trihalomethanes and haloacetonitriles and approximately 60-90% for purged AOX from tap water. SPE was also coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to concentrate and identify polar halobenzoquinones that had not been reported in literature before [3]. In this study, a hydrophilic/lipophilic balanced N-vinylpyrrolidone and divinylbenzene copolymer sorbent (Oasis HLB, Waters) were used. SPE, together with an evaluation of the biological activity of the extracts, is an effective methodology for assessing the presence and formation of potentially toxic OXBPs. Several SPE protocols have been reported in literature. As an example, Yeh et al. studied the bioactivity of DBPs formed in swimming pool water by subjecting SPE extracts to a battery of bioassays addressing different modes of action. In this work, the authors examined various solid phases for their extraction efficacy by evaluating AOX recovery and compatibility with bioassays. The tested phases included Oasis HLB, XAD2/8, a mixed cartridge containing coconut charcoal (Supelclean, Sigma Aldrich) and HLB (Sigma-Aldrich), Isolute ENV+ (highly cross-linked polystyrene-based polymer, John Morris), Lichrolut EN (Merck Millipore), Oasis WAX (mixed-mode weak anion exchange sorbent for strong acids, Waters) and Oasis MAX (mixed-mode anion exchange sorbent for acids, Waters). They found excellent recoveries for AOCl, with ENV+ and Lichrolut, and for AOBr, with HLB and ENV+. The Oasis HLB cartridge was chosen as the best sorbent because it produced very low background toxicity [4]. The Oasis HLB sorbent (with a battery of bioassays) was also used to assess the formation of DBPs in drinking water by Neale et al. and in wastewaters by Watson et al. and Neale et al., whereas Fan et al. used PS-DVB sorbent (HyperSep Retain, Thermo Scientific) to characterize the mutagenicity of DBPs formed in two source waters [5,6,7]. With regard

to the screening of targeted known OXBPs, Chinn et al. compared SPE using PS-DVB sorbent (Bond Elut-PPL from Agilent) with LLE based on EPA method 551.1 for extracting 35 OXBPs from waters. The authors came to the conclusion that the recovery rate for SPE was around 22% lower on average compared to the LLE method. However, they underlined that SPE still achieves over twice the concentration factor and can produce twice the response in comparison with LLE [8]. In addition, Hladik et al. successfully used Oasis HLB to determine 27 OXBPs in water, obtaining recovery rates between 65 and 103% and a residual standard deviation (RSD) lower than 14% for all compounds [9]. Several studies have shown the successful utilization of SPE for HAA extraction. Martínez et al. compared four different commercial sorbents, namely LC-SAX (a quaternary ammonium anion exchanger), LiChrolut EN, Envi-Carb (a graphitized carbon black) and Oasis HLB, in a solid phase extraction process to recover various haloacetic acids from water samples. They found that the best sorbent was LiChrolut EN (PS-DVB from Merck Millipore) which gave recovery values between 37 and 85% in the pre-concentration of 500 mL of tap water samples [10]. Prieto-Blanco et al. studied the recovery of HAAs from water samples using Oasis HLB, Isolute ENV (hyper cross-linked hydroxylated PS-DVB from Bitage) and Lichrolut EN [11]. They found that Isolute ENV offered a better recovery rate for monohalogenated acetic acids (MHAAs), whereas Lichrolut EN offered a better rate for all tested HAAs, with an average recovery of 80%. Similar results were reported by Sun and Ping on the recovery of HAAs from chlorinated hospital effluent, using C18 pretreatment to reduce the samples' turbidity and LiChrolut EN to extract the targeted compounds [12]. Chu et al., who compared five SPE cartridges for extracting 13 haloacetamides from drinking water samples, achieved the highest average recovery rates with Oasis HLB (78%), followed by Oasis MCX (68%), MAX (50%), WCX (43%) and WAX (19%) [13]. An exciting development in recent years has been the design of molecularly imprinted polymers (MIPs), which can be used in SPE applications for the selective enrichment of various compounds. To the best of our knowledge, no studies to date have reported on the use of MIPs for extracting OXBPs from water samples. Micro SPE, also known as Microextraction by Packed Sorbent (MEPS), is a very recent development in the field of solid phase extraction. Micro SPE uses the same sorbents as conventional SPE columns, but in lower amounts and with very small particle sizes (a bed weight of 1-4 mg and a particle size of 3 µL for MEPS, compared to 100-1,000 mg and 50-60 µm for conventional SPE), thus offering a greater surface area for adsorption. MEPS cartridges are handheld, and only require microliters for the conditioning and elution steps. They are also sealed from the external environment so losses of volatile compounds are minimal. However, despite their clear advantages, there are currently very few studies on the performance of MEPS for the extraction of OXBPs. Casas Ferreira et al. applied MEPS using C18 as the sorbent for extracting haloacetic acids in chlorinated water. The obtained recovery rates ranged from 83 to 117%, revealing the efficacy of MEPS for extracting HAAs from water [14]. In a very recent study, Alexandrou et al. compared micro and macro SPE methods, both using C18 as the adsorbent, for monitoring THMs in water. They found that µ-SPE yields substantially better recovery

rates for trichloromethane (50 vs 30%), bromodichloromethane (72 vs 53%) and tribromomethane (83 vs 74%), and comparable recovery rates for dibromochloromethane (63 vs 67%) [15]. The disadvantage of MEPS is that it is unable to process relatively complex matrices (e.g. water-rich suspended solids) or highly concentrated samples, which first need to be diluted and fully dissolved in the solvent, otherwise the barrel insert and needle assembly can be easily blocked.

References

[1] E.E. Lavonen, M. Gonsior, L.J. Tranvik, P. Schmitt-Kopplin, S.J. Köhler, Selective chlorination of natural organic matter: identification of previously unknown disinfection byproducts, Environ. Sci. Technol. 47 (2013) 2264-2271.

[2] P.A. Neale, A. Antony, M.E. Bartkow, M.J. Farré, A. Heitz, I. Kristiana, J.Y.M. Tang B.I. Escher, Bioanalytical assessment of the formation of disinfection byproducts in a drinking water treatment plant, Environ. Sci. Technol. 46 (2012) 10317-10325.

[3] R. Huang, W. Wang, Y. Qian, J.M. Boyd, Y. Zhao, X.-F. Li, Ultra pressure liquid chromatography-negative electrospray ionization mass spectrometry determination of twelve halobenzoquinones at ng/L levels in drinking water, Anal. Chem. 85 (2013) 4520-4529.

[4] R.Y. Yeh, M.J. Farre, D. Stalter, J.Y. Tang, J. Molendijk, B.I. Escher, Bioanalytical and chemical evaluation of disinfection by-products in swimming pool water, Water Res. 59 (2014) 172-184.

[5] D.I. Stalter, P. LI, E. O'Malley, J.Y. Tang, M. Revalor, M.J. Farré, K. Watson, U. von Gunten, B.I. Escher, Sample enrichment for bioanalytical assessment of disinfected drinking water: concentrating the polar, the volatiles, and the unknowns, Environ Sci. Technol. 50 (2016) 6495-6505.

[6] Z. Fan, S. Gong, X. Xu, X. Zhang, Y. Zhang, X. Yu, Characterization, DBPs formation, and mutagenicity of different organic matter fractions in two source waters, Int. J. Hyg. Envir. Heal. 217 (2014) 300-306.

[7] K. Watson, G. Shaw, F. Leusch, N. Knight, Chlorine disinfection by-products in wastewater effluent: Bioassaybased assessment of toxicological impact, Water Res. 46 (2012) 6069-6083.

[8] R. Chinn, T. Lee, S. Krasner, M. Dale, S. Richardson, J. Pressman, T. Speth, R. Miltner, J. Simmons, Solidphase extraction of 35 DBPs with analysis by GC/ECD and GC/MS, Proceedings of the 2007 Water Quality Conference, 2007, pp. 1-20.

[9] M.L. Hladik, M.J. Focazio, M. Engle, Discharges of produced waters from oil and gas extraction via wastewater treatment plants are sources of disinfection by-products to receiving streams, Sci. Tot. Environ. 466 (2014) 1085- 1093.

[10] D. Martınez, F. Borrull, M. Calull, Comparative study of a solid-phase extraction system coupled to capillary electrophoresis in the determination of haloacetic compounds in tap water, J. Chrom. A 827 (1998) 105-112.

[11] M. Prieto-Blanco, M. Alpendurada, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, S. Machado, C. Gonçalves, Improving methodological aspects of the analysis of five regulated haloacetic acids in water samples by solid-phase extraction, ion-pair liquid chromatography and electrospray tandem mass spectrometry, Talanta 94 (2012) 90-98.

[12] Y.-X. Sun, G. Ping, Determination of haloacetic acids in hospital effluent after chlorination by ion chromatography, J. Environ. Sci. 19 (2007) 885-891.

[13] W. Chu, N. Gao, D. Yin, S.W. Krasner, M.R. Templeton, Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization, J. Chrom. A 1235 (2012) 178-181.

[14] A.M.C. Ferreira, M.E.F. Laespada, J.L.P. Pavón, B.M. Cordero, In situ derivatization coupled to microextraction by packed sorbent and gas chromatography for the automated determination of haloacetic acids in chlorinated water, J. Chrom. A 1318 (2013) 35-42.

[15] L.D. Alexandrou, M.J. Spencer, P.D. Morrison, B.J. Meehan, O.A. Jones, Micro versus macro solid phase extraction for monitoring water contaminants: A preliminary study using trihalomethanes, Sci. Tot. Environ. 512 (2015) 210-214.

SM 8. Recent studies using SPME for the extraction of OXBPs

San Juan et al. compared three different commercial fibers: PDMS/DVB, CW/DVB and DVB/CAR/PDMS. Despite the fact that the extraction efficiency values were higher with the CAR-PDMS fiber, the PDMS-DVB coating was chosen because it was superior in terms of its detection and quantification limits, repeatability and linear range [1]. Lara-Gonzalo et al. compared PDMS, PA, CAR/PDMS and DVB/CAR/PDMS fibers, and chose DVB/CAR/PDMS which, despite having a slightly lower extraction efficiency than the CAR/PDMS fiber, provided finer chromatographic peaks [2]. The SPME technique has also been explored for the extraction of other families of OXBPs. The experiments conducted by Cancho et al. showed that CWX/DVB was the most suitable fiber for the determination of I-THMs [3]. In another study, the same authors, who compared six commercial fibers (PMDS, PA, CAR/PDMS, CWX/DVB, PDMS/DVB and DCB/CAR/PDMS) concluded that DCB/CAR/PDMS was the most suitable fiber for extracting cyanogen chloride and cyanogen bromide from water [4]. More recently, Allard et al. evaluated the extraction efficiency of five commercial fibers for the simultaneous extraction of 10 trihalomethanes, including regulated THMs and I-THMs, from water. CAR/PDMS/DVB fiber was chosen as a good compromise, to offer a good recovery rate for all THMs [5]. However, DVB/CAR/PDMS fiber was found to be the best choice for the extraction of halonitriles and haloketones [6, 7]. In the study by Kermani et al., five SPME fibers were tested for the simultaneous extraction of 20 OXBPs, namely six I-THMs, eight HANs, and six HNMs, from water [8]. DVB/CAR/PDMS fiber was found to be the most suitable for all target compounds. The approach based on HS-SPME in combination with GC/MS has been extensively employed for the isolation and structural elucidation of previously unknown OXBPs in different types of water [9, 10, 11]. In a study reported by Silwal et al., LLE and HSSPME techniques were compared for sample preparation prior to GC/MS analysis. Fiber coated with CAR/DVB was used. Based on the averaged mass measurement accuracy, multiple solvent artifacts were identified in the case of LLE. It was also shown that solvent-less SPME reduced potential interferences from solvent stabilizers [10]. Alvarez-Rivera et al. identified a total of five halogenated phenols as by-products of hydroxybenzoates using HS-SPME sampling with a CAR/DVB-coated fiber [11]. It is apparent from these two examples that SPME can be a powerful tool to help identify non-ionic unknown OXBPs.

References

[1] P.M. San Juan, J.D. Carrillo, M.T. Tena, Fibre selection based on an overall analytical feature comparison for the solid-phase microextraction of trihalomethanes from drinking water, J. Chrom. A 1139 (2007) 27-35.

[2] A. Lara-Gonzalo, J.E. Sánchez-Uría, E. Segovia-García, A. Sanz-Medel, Critical comparison of automated purge and trap and solid-phase microextraction for routine determination of volatile organic compounds in drinking waters by GC-MS, Talanta 74 (2008) 1455-1462.

[3] B. Cancho, F. Ventura, M.T. Galceran, Solid-phase microextraction for the determination of iodinated trihalomethanes in drinking water, J. Chrom. A 841 (1999) 197-206.

[4] B. Cancho, F. Ventura, M.T. Galceran, Simultaneous determination of cyanogen chloride and cyanogen bromide in treated water at sub-µg/L levels by a new solid-phase microextraction-gas chromatographic-electroncapture detection method, J. Chrom. A 897 (2000) 307-315.

[5] S. Allard, J.W. Charrois, C.A. Joll, A. Heitz, Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid-phase microextraction and gas chromatography mass-spectrometry, J. Chrom. A 1238 (2012) 15-21.

[6] I. Kristiana, C. Joll, A. Heitz, Analysis of halonitriles in drinking water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1225 (2012) 45-54. [7] M. Serrano, M. Silva, M. Gallego, Determination of 14 haloketones in treated water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1407 (2015) 208-215.

[8] F.R. Kermani, A.-M. Tugulea, J. Hnatiw, V.H. Niri, J. Pawliszyn, Application of automated solid-phase microextraction to determine haloacetonitriles, haloketones, and chloropicrin in Canadian drinking water, Water Qual. Res. J. Can. 48 (2013) 85-98.

[9] C. Heffner, I. Silwal, J.M. Peckenham, T. Solouki, Emerging technologies for identification of disinfection byproducts: GC/FT-ICR MS characterization of solvent artifacts, Environ. Sci. Technol. 41 (2007) 5419-5425.

[10] I.K. Silwal, J.C. Rasaiah, J.E. Szulejko, T. Solouki, Proton transfer reactions of halogenated compounds: Using gas chromatography/Fourier transform ion cyclotron resonance mass spectrometry (GC/FT-ICR MS) and ab initio calculations, Int. J. Mass Spectrom. 293 (2010) 1-11.

[11] G. Alvarez-Rivera, M. Llompart, C. Garcia-Jares, M. Lores, Identification of halogenated photoproducts generated after ultraviolet-irradiation of parabens and benzoates in water containing chlorine by solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1349 (2014) 105-115.

SM 9. Applications of static head space and dynamic head space for the extraction of OXBPs from water samples

- ***Static head space***

The SHS technique offers many advantages, such as simple sample preparation, inexpensive equipment, automated extraction and analysis, and the use of small sample volumes. As with the extraction methods described above, soluble salts have been used to increase the efficacy of HS sampling by increasing the ionic strength of the liquid phase [1]. For basic and acidic compounds, the recovery rate for HS sampling can be increased by adjusting the pH of the sample. The SHS-GC methodology has frequently been used for the determination of THMs in water. An example of this is the method proposed by Montesinos et al. for the analysis of six ITHMs found in beverages, in addition to four common THMs. Another example relates to the use of SHS in combination with GC/MS for the determination of halonitromethanes in tap and swimming pool water, providing a LQ between 0.09 and 1.8 µg/L and a RSD of about 6.0% [2]. More recently, SHS-GC/MS was applied for the simultaneous determination of 16 volatile and semi-volatile OXBPs in drinking water, including THMs, HNMs and HANs, achieving a LQ between 30 and 600 ng/L and an RSD of about 5.6%. In this work, the authors also compared the SHS-GC/MS method with the reference EPA method 551.1, based on LLE with MtBE (LLE-GC/MS). They found that LLE-GC/MS according to EPA method 551.1 was slightly less precise than the developed method, with average RSD values of 6.5 ± 0.8% (intraday) and 7.4 ± 0.8% (inter-day), and that it also provided a higher limit of detection with average values of 142 ± 136 versus 65 ± 58 ng/L [3]. A methodology based on the injection of analytes into a mass spectrometer after SHS has also been described recently. This methodology was applied by Serrano et al. for the determination of THM indices in drinking water. The authors found a high level of agreement between results obtained with the developed method and the reference method, corroborating the good performance of the SHS-MS approach [4].

- ***Dynamic head space***

Regarding the analysis of OXBPs, Nikolaou et al. compared four different analytical methods, based on LLE-GC/ECD, LLE-GC/MS, P&T-GC/MS and SHS-GC/MS, for determining THMs, HANs, HKs, chloral hydrate and chloropicrin in drinking water. They concluded that SHSGC/MS had acceptable recovery rates for THMs (< 0.2 µg/L), but that the detection limits were higher, while the P&T-GC/MS method gave good results for THMs (< 0.05 µg/L), but had high detection limits for the other volatile chlorination by-products, which usually occur in drinking water at very low concentrations [5]. More recently, Lara-Gonzalo et al. presented a comparison between P&T and HS-SPME methods to evaluate their efficiency in the simultaneous determination of eight volatile organochlorine compounds (including THMs) in drinking water samples. They found that both methods provided good recovery rates and limits of detection (LODs) at the sub-ppb level (< 0.7µg/L) [6]. However, the

SPME procedure is still faster than the P&T technique (2 minutes versus 15 minutes). As previously mentioned, P&T sensitivity can be improved by increasing the sample volume. However, to achieve very low detection limits, the necessary water volumes could be excessively high. A purge and trap continuous flow system (P&T-CF) has been developed to process high volumes of water for trace analyses. Unlike conventional P&T systems, the water sample is constantly renewed, resulting in high recovery rates. With a P&T-CF pre-concentration system coupled with GC/MS, LQ values ranging between 30 and 150 ng/L have been obtained for the on-line monitoring of trihalomethanes in drinking water [7]. For rapid desorption from strong sorbents, high desorption temperatures are often needed. This can lead to spurious peaks on the chromatogram due to decomposition products from the adsorbents and the degradation of thermally unstable compounds [6]. In a variant of the P&T technique, known as "closed-loop stripping analysis (CLSA)", the purge gas is constantly passed through an adsorbent localized in a closed circuit. After the purging period, the trapped organic compounds are extracted from the adsorbent by elution with a small amount of a suitable solvent. In its original version, the sorbent phase and the eluent were, respectively, activated carbon and carbon disulfide. The CLSA method is suitable for the analysis of a broad spectrum of organic compounds of intermediate volatility and molecular weight, which makes this approach more suitable for both targeted and untargeted screening purposes. Additionally, this extraction technique offers the possibility of using other analytical methods, such as liquid chromatography, for the analysis of organic extracts. The CLSA-GC/ECD method was evaluated by Kampioti et al. for the determination of 16 common non-polar and semi-polar halogenated DBPs (THMs, HANs, HAAs, HKs, chloropicrin and chloral hydrate) in drinking water. After purging the water samples for two hours, activated carbon (1.5 mg) used as the adsorbent was extracted with CS2. Despite its lowest extraction yields (5-94%), the developed CLSA-GC/MS method was able to quantify studied compounds at levels as low as 0.5 ng/L for THMs, 1 ng/L for HANs and 45-72 ng ng/L for HAAs [8]. These results also clearly show that the CLSA technique is more sensitive than P&T for the extraction of several families of OXBPs. This methodology was used subsequently by the same group to investigate the presence of DBPs belonging to different classes (THMs, HANs, HAAs, HKs, chloropicrin and chloral hydrate) in Greek drinking water [9]. An investigation was carried out by Joll et al. using CLSA followed by GC-MS analysis to identify by-products formed from terpenoids during the simulated chlorination of natural waters. In this work, dichloromethane was used as the eluent. Many non-halogenated by-products have been identified as well as regulated THMs [10]. Despite the great potential of the CLSA technique, its use for the extraction of OXBPs remains marginal, evidenced by the very few papers addressing this kind of methodology.

References

[1] I. Montesinos, M. Gallego, Speciation of common volatile halogenated disinfection by-products in tap water under different oxidising agents, J. Chrom. A 1310 (2013) 113-120.

[2] I. Montesinos, M. Gallego, Headspace gas chromatography-mass spectrometry for rapid determination of halonitromethanes in tap and swimming pool water, Anal. Bioanal. Chem. 402 (2012) 2315-2323.

[3] I. Montesinos, M. Gallego, How the inclusion of treated water in Beverages influences the appearance of halogenated volatile organic compounds, J. Agr. Food Chem. 62 (2014) 10240-10247.

[4] A. Serrano, M. Gallego, Rapid determination of total trihalomethanes index in drinking water, J. Chrom. A 1154 (2007) 26-33.

[5] A.D. Nikolaou, T.D. Lekkas, S.K. Golfinopoulos, M.N. Kostopoulou, Application of different analytical methods for determination of volatile chlorination by-products in drinking water, Talanta 56 (2002) 717-726.

[6] A. Lara-Gonzalo, J.E. Sánchez-Uría, E. Segovia-García, A. Sanz-Medel, Critical comparison of automated purge and trap and solid-phase microextraction for routine determination of volatile organic compounds in drinking waters by GC-MS, Talanta 74 (2008) 1455-1462.

[7] T.-C. Chen, G.-R. Her, On-line monitoring of trihalomethanes in drinking water using continuous-flow purge and cryofocusing gas chromatography-mass spectrometry, J. Chrom. A 927 (2001) 229-235.

[8] A.A. Kampioti, E.G. Stephanou, Simultaneous determination of halogenated neutral and acidic disinfection by-products in drinking water by closed-loop stripping extraction and capillary gas chromatography, J. Chrom. A 857 (1999) 217-229.

[9] E.S. Adamantia kampioti, An inclusive study of the presence of the disinfection by-products in Greek drinking water. 9th International Conference on Environmental Science and Technology, Rhodes Island, Greece, 2005.

[10] C.A. Joll, M.J. Alessandrino, A. Heitz, Disinfection by-products from halogenation of aqueous solutions of terpenoids, Water Res. 44 (2010) 232-242.

Formation and determination of organohalogen by-products in water - Part III. Characterization and quantitative approaches

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Abstract

Used as oxidizing biocides, halogen-containing oxidizing agents react with naturally occurring organic matter as well as inorganic compounds, leading to the formation of a wide range of organohalogen by-products (OXBPs). Despite the increase in the number of quantified OXBPs and the improved sensitivity of analytical methods, the mass balance (MB), defined as the percentage of known halides attributed to known, quantified OXBPs divided by the adsorbable organic halide (AOX) values, remains significantly below 100%. As a result, the toxicological and ecotoxicological effects of exposure to OXBPs cannot be assessed precisely. It is crucial to identify new and unknown OXBPs and to increase the scope and sensitivity of the analytical techniques employed. This article aims at providing the reader with a critical overview of the different analytical methods currently employed for OXBP characterization and MB improvement.

Keywords: Organohalogen; By-products; Water; Analysis; Characterization

Abbreviations

AOX	Adsorbable organic halide
APCI	Atmospheric pressure chemical ionization
APPI	Atmospheric pressure photoionization
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
CID	Collision induced dissociation
COC	Cold on-column
DBPs	Disinfection by-products
DNPH	2,4-dinitrophenylhydrazine
ECD	Electron capture detector
EI	Electron ionization
ENCI	Electron capture negative ionization
ESI	Electrospray ionization
FAIMS	Field asymmetric ion mobility spectrometry
FT-ICR/MS	Fourier transform ion cyclotron resonance mass spectrometry
GC	Gas chromatography
HAA(s)	Haloacetic acid(s)
HANs	Haloacetonitriles
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High performance liquid chromatography
HS	Headspace
IC	Ion chromatography
LC	Liquid chromatography
LPME	Liquid phase microextraction
MB	Mass Balance
MIMS	Membrane introduction mass spectrometry
SRM	Selected reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MTBSTFA	N-Methyl-N-tert-butyl-dimethylsilyl-trifluoroacetamide
NCI	Negative chemical ionization
NLS	Neutral loss scanning
OXBP(s)	Organohalogen by-product(s)
PFBHA	Pentafluorobenzylhydroxylamine
PIS	Precursor ion scanning
PTV	Programmed temperature vaporization
SPE	Solid phase extraction
SPME	Solid phase microextraction
SIM/SIR	Selected ion monitoring/recording
THMs	Trihalomethanes
UAOX	Unknown AOX
UPLC	Ultra-performance liquid chromatography

III.1 Introduction

This manuscript is the third and final part of a comprehensive review of recent research on the formation and determination of organohalogen by-products (OXBPs) in water. It provides an overview of the analytical procedures used to identify and quantify OXBPs in water samples. The first of the trio of articles (Part I) is devoted to a survey of recent research on the formation of OXBPs; it also discusses the relevance of the AOX global parameter often used to estimate their concentrations in different types of water [1]. The second article (Part II) deals with the sample preparation techniques commonly employed prior to OXBP analysis and compares their advantages and drawbacks [2]. The three main analytical methods for OXBP identification and quantification are: (i) "targeted screening", based on the determination of one or more known groups of OXBPs, for which analytical standards are commercially available and analytical methods have been developed and optimized, (ii) "suspect screening", employed when prior information indicates that a given compound may be present in the sample [3] and (iii) "non-targeted screening" approaches implemented to identify new or unexpected substances in a sample when no prior information is available (except the presence of halides in the chemical structure of the molecules). Over the past three decades, many OXBPs have been identified, mostly through gas chromatography-mass spectrometry (GC-MS). More recently, analytical methods using liquid chromatography coupled with mass spectrometry (LC-MS) have been developed to uncover highly polar OXBPs not identified in earlier GC-MS studies. Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS) has recently emerged as a particularly useful technique in the characterization of unknown high molecular weight OXBPs, ultimately leading to an improved mass balance (MB). Following a discussion in the first section of this paper concerning the chemical derivatization of OXBPs, which is often required prior to analysis, especially in the case of polar OXBPs, the main advanced analytical methodologies applied to determine different OXBPs are reviewed. Consideration is given to the type of technique used (separation or spectroscopic), including various combinations thereof, as well as to recent developments and future trends in the identification of unknown OXBPs.

III.2 Chemical derivatization of OXBPs

Chemical derivatization is a widely used technique in sample pretreatment for chromatographic analysis. The reasons for its use vary, based on whether the analysis is performed by liquid or gas chromatography and depending on the physicochemical properties of the analytes. It is clear from extensive scientific evidence that some OXBPs are composed of molecules that have a polar character, a low molecular weight and a neutral structure. In GC analysis, chemical derivatization is principally used to improve the volatility and thermal stability of the analytes. With LC and GC techniques, it is employed to increase the molecular weight of the OXBPs and/or to introduce functional groups that enable increased specificity and sensitivity for particular types of detection. Chemical derivatization also serves to accentuate differences between sample compounds in order to facilitate chromatographic separation. The choice of derivatization reagent mainly depends on the reactive functional group(s) of the targeted compounds and the purpose of the research.Table 1, at the end of this section, presents the main derivatization agents used to derivatize OXBPs prior to their GC or LC analysis.

Table 1. Main types of derivatives used for analysis of polar OXBPs

Derivatization reagent	Functional groups	Methods	References
Diazomethane	Carboxylic acids	GC/ECD	[19, 20, 21, 22]
		GC-NCI/MS	[23]
Methanol	Carboxylic acids	GC/ECD	[24, 25, 26]
Dimethylsulfate	Carboxylic acids	GC-EI/MS	[27, 28]
Pentafluorobenzylbromide	Carboxylic acids	GC-ECNCI/MS	[29]
n-Octanol	Carboxylic acids	GC-EI/MS	[30]
N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide	Carboxylic acids		[31]
5-chloro-2,2,3,3,4,4,5,5-octafluoropentylchloroformate	Alcohols, amines, carboxylic acids	GC-NCI/MS	[18]
Bis(trimethylsilyl)trifluoroacetamide (BSTFA)	Alcohols, phenols	GC-MS	[32, 33]
Pentafluorobenzylhydroxylamine (PFBHA)	Aldehydes, ketones, haloacetaldehydes	GC-MS	[34, 35]
Dinitrophenylhydrazine (DNPH)	Aldehydes, ketones, hydroxyacids	LC-MS	[14]
4-(2-(trimethylammonio)ethoxy) benzenaminiumdibromide	Aldehydes, carboxylic acids		[36]
O-(carboxymethyl hydroxylamine) (CMHA)	Aldehydes, ketones		[37]

In the area of polar OXBP analysis by gas chromatography, the majority of studies reported in the literature concern haloacetic acids (HAAs) and haloacetaldehydes. The most popular detection methods reported for HAAs are those employing post-column derivatization by methylation, using diazomethane, acidic methanol (H_2SO_4-MeOH) or boron trifluoride (BF_3) in methanol as reagents. Other alcohols such as ethanol, propanol, and n-octanol have also been tested [4]. Because of its good methylation efficiency and its rapid reactivity at low temperature (approximately 15 minutes), diazomethane was, for many years, the preferred reagent. This derivation process is the subject of three standards: Standard Method 6251B, US EPA 552 and NF EN ISO 23631 (2006). However, diazomethane has proven to be an extremely toxic alkylating agent with potential carcinogenic effects and its laboratory use is now considered hazardous. BF_3, on the other hand, forms fluoroboron compounds by reaction with atmospheric oxygen and methanol, which makes it prone to instability. Currently, acidic methanol is the most frequently used derivatizing reagent; its use is recommended by US EPA methods 552.2-5 and 552.1. Despite the tendency of tribromoacetic acid, and to a lesser extent dibromochloroacetic and bromodichloroacetic acids, to undergo partial decarboxylation during preparation by esterification of their corresponding methyl ester derivatives [5,6], the acidic methanol derivatization procedure has been the subject of numerous studies seeking to improve its efficiency. In a second type of approach, derivatization is carried out either before or during OXBP extraction. The most frequently described method involves the methylation of HAAs accomplished directly in a water sample using dimethyl sulfate ($SO_4(CH_3)_2$). The extraction can be achieved using a headspace (HS), solid phase microextraction (SPME), liquid phase microextraction (LPME) technique. Examples of sample preparation processes used prior to GC-MS analysis of OXBPs are given in Supplementary material 1.

High performance liquid chromatography (HPLC) is, by its very nature, more compatible with aqueous matrices and polar compounds than gas chromatography. However, known polar OXBPs are characterized by a low molecular weight and a lack of intrinsic chromophores or fluorophores. Consequently, direct LC analysis of these compounds continues to be hindered by poor chromatographic performance and low sensitivity. Chemical derivatization offers an alternative solution, providing enhanced chromatographic performance and detectability of low molecular weight compounds through the introduction of the specific moieties into their base structures. Phenols, alcohols, amino acids, amines, amides, carboxylic acids, aldehydes and ketones can all be converted into fluorescent derivatives [7,8]. Derivatization is frequently used in LC-MS applications to enhance ionization efficiency and selectivity. Ketones and aldehydes are neutral compounds; hence, the ionization efficiencies of these functional groups in electrospray ionization are usually low. Detection sensitivity is enhanced by introducing a permanently charged or proton affinitive moiety to the target

analytes. Proton-affinitive derivatization mostly involves the introduction of nitrogen containing bases, such as amino groups. Hydroxylamine, which reacts with ketones to form oximes, has been used to derivatize a wide range of substances [9]. The most widely used of the permanently charged moieties is quaternary ammonium. The use of this type of reagent for derivatization of the carbonyl groups in ketones and aldehydes has been reported in the literature [10,11]. Several reagents have been developed for the derivatization of carboxylic acids, with an ionization moiety and/or a reaction site, as well as a suitable structure to enhance the sensitivity of MS detection. The most commonly used derivatization agents for short-chain carboxylic acids are 2-hydrazinoquinoline, 2-hydrazinopyridine, and 2-picolylamine [12]. One of the reagents most frequently used to perform the derivatization of alcohols and phenols is dansyl chloride [11]. The use of other reagents, such as isonicotinoyl azide, 2-picolylamine, and mono-(dimethylaminoethyl) succinyl imidazole is also reported. Amines and amides are both protonated under acidic conditions and can, in consequence, be analyzed directly by ESI (electrospray ionization)/MS. However, the high polarity and low molecular weight of some of these compounds make their isolation from water very difficult. Chemical derivatization yields less polar and high molecular weight derivatives that provide better separation from interfering compounds and enable more sensitive detection in ESI. Amines can be derivatized using dansyl chloride or alkyl chloroformates like 9-fluorenylmethoxycarbonyl or 4-nitrobenzyl chloroformate [11]. In ESI negative mode, the incorporation of acidic moieties in the analyte structure (i.e., aromatic nitro groups, which are easily deprotonated) appears to increase ionization sensitivity. In most instances, 2,4-dinitrophenylhydrazine (DNPH) is used for this purpose. The derivatization of carbonyls with DNPH to form 2,4-dinitrophenylhydrazones derivatives, followed by LC-MS analysis using an ESI or APCI (atmospheric pressure chemical ionization) interface in negative mode, has become the method of choice for the determination of carbonyl compounds. The approach based on chemical derivatization followed by LC-MS analysis is often adopted for the screening of multiple classes of known compounds (such as steroids, sugars, organic pollutants, pharmaceutics, etc.). However, to date, few authors have used this approach for the quantitative analysis of OXBPs. In the work of Zwiener et al., an analytical method was developed for determining different forms of carbonyl compounds in disinfected water, which again involved DNPH derivatization combined with LC-MS in ESI negative mode [13]. Application of this method in the analysis of water samples from swimming pools enabled the identification of several carbonyl compounds, including aldehydes, ketones, hydroxybenzaldehyde, and dicarbonyl compounds. The strategy of combining chemical derivatization with LC-MS or GC-MS offers many advantages for the identification and structure elucidation of unknown compounds. In an ideal scenario, each derivatization reagent would react with one specific functional group in the molecules of interest. This means that unknown molecules can be stained by targeting their chemical functions. As far as LC-MS-based methods are concerned, most studies have focused on identifying carbonyl compounds using DNPH. One example of this general approach is the

work published by Richardson et al., in which the derivatization of a series of C_3 to C_{10} aldehydes and ketones was studied LC-MS [14,15].

In research aimed at identifying new by-products, the use of chemical derivatization coupled with GC-MS analysis is more frequent. A number of derivatization reagents can be used, depending on the functional groups available. In addition to those already mentioned, others have been specifically developed to improve the identification of unknown OXBPs. In the study by Le Lacheur et al., pentafluorobenzylhydroxylamine (PFBHA) was used as a derivatization reagent for aldehydes and keto-acids; this reagent reacts with aldehydes and ketones to form non-polar oximes that can be easily extracted and concentrated [16]. For the simultaneous recovery and pre-concentration of mono- and multi-functional compounds, it is necessary to use several derivatizing reagents, each of which reacts selectively with a particular functional group. An example of this approach is provided in the study by Weinberg. In this work, a reaction scheme was developed, through the combined use of several extraction phases and functional group-specific derivatization reagents, which enabled the simultaneous recovery and pre-concentration of mono- and multi-functional carbonyl-containing species [17]. Subsequent target-specific derivatization, using PFBHA, diazomethane and BSTFA, led to the identification of aldo- and ketoacids, as well as hydroxy carbonyl species, and also enabled to identify aldehydes, ketones, dialdehydes and ketoaldehydes.

Vincenti et al. examined a method for the aqueous-phase derivatization of carboxyl, hydroxyl and amine groups [18]. The authors compared the use of four highly-fluorinated alkyl and aryl chloroformates for direct water derivatization of carboxyl groups to the corresponding esters, hydroxyl groups to carbonates, and amines to carbamates. Of the four derivatizing reagents tested, 5-chloro-2,2,3,3,4,4,5,5-octafluoropentylchloroformate provided the best performance, with good reaction efficiency, good chromatographic and spectroscopic properties, and low detection limits (10-100 fmol) In addition, the derivatives obtained were reported to be stable with respect to hydrolysis and amenable to analysis by GC. The unknown compounds were identified by interpretation of their mass spectrum. Table 1summarizes the different derivatization reagents, classified by the type of derivatization reaction and the method applied in OXBPs analysis by LC and GC.

III.3 OXBP analysis methods

Recent advances in instrumentation have resulted in significant progress being made in the detection, the unambiguous structural identification and the determination of OXBP concentrations. Without contest, mass spectrometry has become a central technique in the field of research on organohalogen by-products. MS allows detection and accurate quantitation of known OXBPs and provides abundant information for structural elucidation of unknown ones. MS is generally preceded by online chromatographic separation in order to increase the sensitivity, and also reduce the complexity of mass

spectra. For this purpose, gas and liquid chromatography are the most commonly used separation techniques. As mentioned in the introduction section, the analytical approaches for the identification and quantitation of OXBPs may be classified into three main categories: target analysis, suspect analysis, and non-target analysis. A brief summary of these three approaches is schematically illustrated in Figure 1. The first category of methods involves determining of one or more known groups of OXBPs, for which reference standards are commercially available, and analytical methods have been optimized. This approach is carried out to quantitative determination of the occurrence, distribution, stability, and fate of known target OXBPs in water samples from source under investigation. The goal of suspect screening analysis, also referred to as "known unknowns", is to confirm the presence or absence of chemicals belonging to a list of compounds that have already been identified as potential OXBPs, but for which target analytical methods are not available. The identification of known-unknowns with an increasing confidence is possible thanks: searching in mass spectra databases, identification of the molecular ion, isotopic pattern analysis and mass spectral fragmentation interpretation. It should however be emphasized that the suspect screening approach provides structural information for identification of OXBPs but remains unable to quantify them in the absence of commercially available analytical standards. In general, OXBPs clearly identified become the target compounds in the future investigations particularly if their toxicity is proved. In contrast to suspect analysis, non-target screening, also called "unknown screening" analysis, aims at the identification of OXBPs for which no priori information is available. The information about the compounds to be identified is derived exclusively from exploration of chromatograms and mass spectra. Usually, the first step in non-target screening analysis, as well as in suspect screening analysis, consists in comparing the chromatogram of a real sample with that of a reference sample (i.e. blank, untreated sample) in order to reduce the amount of data to be analyzed, and to identify the most relevant chromatographic peaks. This time consuming-step when performed manually is currently automatically achieved thanks to ad hoc commercial algorithms. These compare mass chromatograms to find significant differences in terms of retention time, and mass spectrum. This automated research allows a reduction of time and effort, as well as an elimination of human error. As example, in figure 2 we presented two full-scan chromatograms obtained after solid phase extraction of river water samples taken upstream (A), and in the circuit (B) of an electric power plant using monochloramine to disinfect its cooling system. Both chromatograms are rich in chromatographic peaks, and similar in appearance so it appears very difficult to identify the chromatographic peaks of unknown OXBPs inside of a peak "drill" on the basis of a manual comparison. Figure 2Cillustrates the results of a typical experiment attempting to decrease the number of chromatographic peaks and background noise using a comparison algorithm. In this case, some differences between the two SPE extracts are easily identified. This section provides a non-exhaustive overview of recently published instrumental

methods at both quantification and identification purposes of OXBPs with a discussion of their respective advantages and limitations.

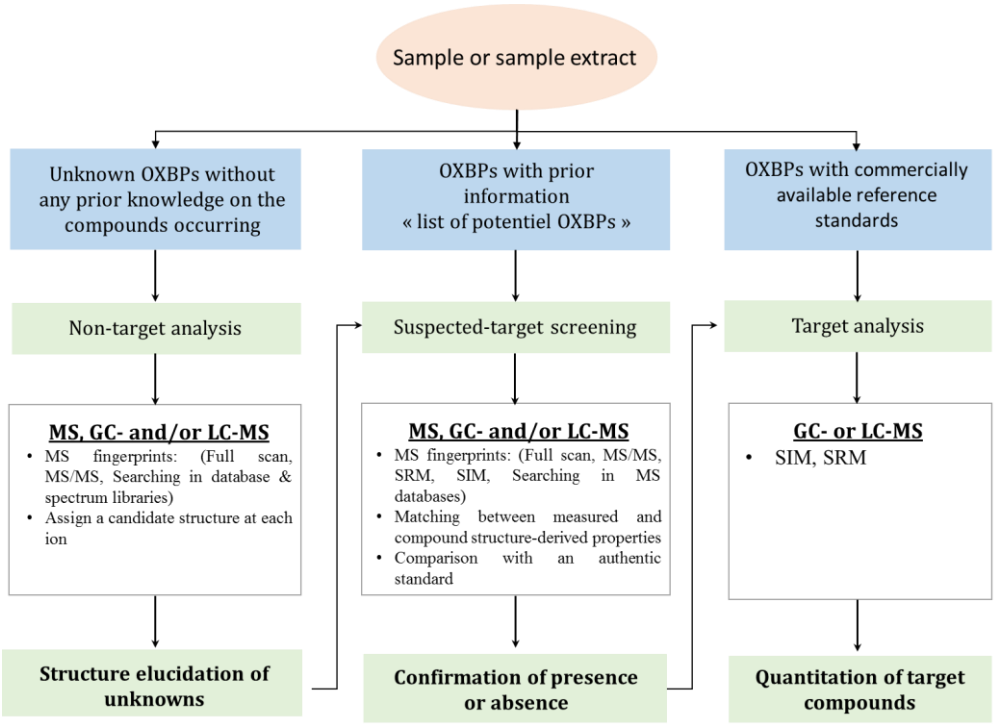

Figure 1. Schematic presentation of all three approaches used for (i) quantitative target analysis, (ii) suspect screening analysis, and (iii) non-target screening analysis of unknown OXBPs in water samples by using mass spectrometry-based methodologies

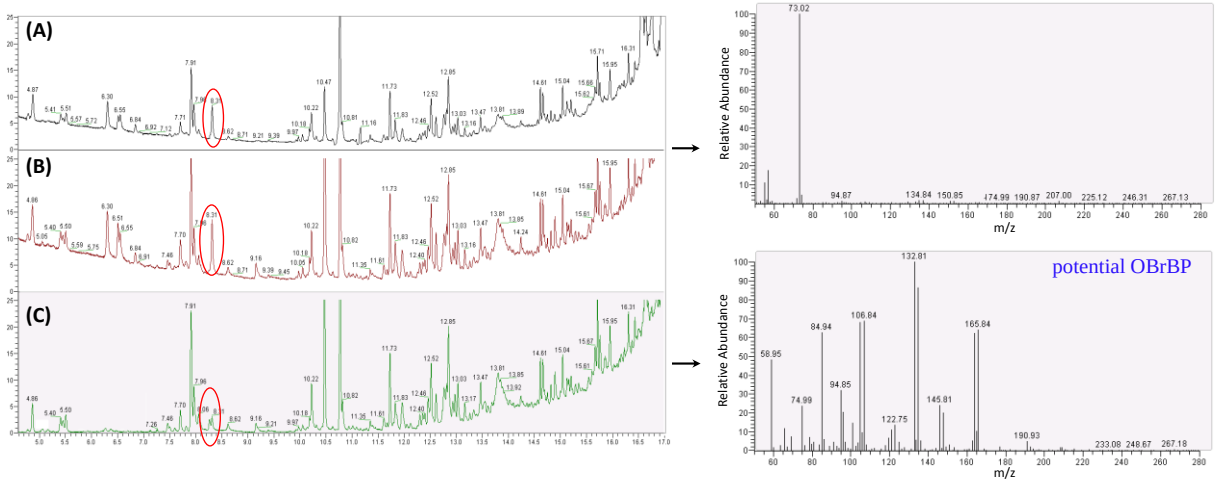

Figure 2. Comparison between total ion chromatograms obtained after solid phase extraction of the same: the first one was obtained from water not treated (A), while the second was obtained from water

treated with monochloramine. The automatic comparison using an algorithm (C) reduces the number of chromatographic peaks, facilitating the detection of relevant signals (tr = 8.31 min).

III.3.1 Gas chromatography and GC-MS coupling

OXBPs are most frequently identified and quantified using gas chromatography. The most commonly reported stationary phase for OXBP separation is composed of 5% phenyl and 95% dimethylpolysiloxane, frequently in columns of 30 m in length, with an inner diameter of 0.25 mm and a film thickness of 0.25 µm, although a wide variety of columns have been employed in different studies. Due to the relatively low concentrations of target analytes in water, injection techniques are used to introduce the samples, the most common of which are hot splitless injection and programmed temperature vaporization (PTV). Chemical derivatization is often used to expand the range of analytes accessible to GC and the injection temperature optimized to minimize thermal degradation. It has been reported that some trihalonitromethanes, trihaloacetic acids and trihaloacetonitriles degrade at temperatures above 200, 180, and 170 °C, respectively [38,39]. PTV has several advantages over other injection techniques, including decreased analyte discrimination during the injection step, better recovery of thermodegradable compounds and the adverse effects of the presence of non-volatiles in the sample on the injection process are less pronounced. PTV allows the introduction of large sample volumes: the inlet port is maintained at a low value; the solvent evaporated in the injector liner is discharged via the split exit. After the venting step, the injector is rapidly heated and analytes are transferred onto the front of the analytical column. In this configuration, sample volumes of up to several hundred µL can be introduced, drastically improving the LODs. An analytical method published by Serrano et al. was based on micro LLE combined with large-volume injection (30 µL) GC-MS for the determination of seven halo-acetaldehydes in treated water. The LODs obtained (6-20 ng/L) were significantly lower than those normally achieved by hot injection techniques [40]. An alternative technique for minimizing thermal degradation is direct on-column injection. In cold on-column (COC) injection mode, the organic extracts are directly introduced through a needle into the capillary column or retention gap, at low temperature. Comparative experiments between COC and conventional splitless injection modes for halonitromethane (HNM) determination have recently been conducted by Chen et al. They found that COC mode minimized the thermal degradation of HNMs, especially dibromochloro- and tribromo-nitromethanes in water. Both debromo- and denitro-products of HNMs were observed in the splitless injection mode at 117 °C and 170 °C. COC injection mode is best suited for the analysis of extracts from tap water samples, in which the amount of non-volatile material is relatively small, and is not recommended for samples that contain non-volatile residue [41].

Experiments have been conducted with both electron capture detectors and mass spectrometers for the detection and quantification of OXBPs. The sensitivity of the ECD depends on the nature, number and even the positions of the halogen atoms in the molecules, and generally increases with the number and atomic weight of the halogen atoms. The use of a GC/ECD system is suggested in numerous standard methods (such as EPA Methods 552.2 and 552.3, and NF EN ISO 23631) for the determination of several classes of disinfection by-products (DBPs), including THMs, HAAs and haloketones [42,43]. GC/ECD methods have been shown to provide LODs ranging from 12 to 170 ng/L for regulated HAAs and from 3 to 55 ng/L for THMs. The GC/ECD approach coupled with LLE has also been used for determining HANs, THMs and HAAs, achieving LODs in the ranges of 7-70 ng/L, 5-40 ng/L and 10-200 ng/L, respectively [44,45]. Despite the ECD selectivity, GC/ECD methods can be subject to interference by unknown OXBPs as well as by chemical artifacts [46]. The use of MS as a detector provides additional selectivity, which reduces potential interference and improves LODs. Additional benefits are that analytes do not need to be fully resolved to be identified and quantitated, as is the case when using ECD. In GC-MS, EI remains the most commonly ionization mode used for determining OXBPs; it provides mass spectra rich in fragment ions, which can be used to identify chemicals based on library database spectra (such as the NIST and Wiley databases, which together include several hundreds of spectra). While EI in full scan mode provides more specificity for the identification and structural confirmation of target OXBPs, this mode is not commonly used for their quantitative determination due to its low sensitivity. EI/MS operated in SIM (selected ion monitoring) or SRM (selected reaction monitoring) mode is the most common approach. Some standard methods use GC-MS, such as EPA Method 524.2, which employs GC-EI/MS for THM analysis, or NF EN ISO 23631, which utilizes GC-EI/MS for HAA analysis. The electron capture negative ionization (ENCI) mode is a somewhat similar detection technique to ECD. The principal advantages of these two techniques over EI are their potential for improving sensitivity by up to a 100-fold improvement in sensitivity, and the higher degree of selectivity they offer, as only a limited number of analytes, such as those containing a halogen atom, a nitro group, or an extended aromatic ring system, are prone to efficient electron capture. Conversely, several halogenated compounds do not form abundant, stable molecular or fragment ions in ENCI mode and only the halide fragment ions (m/z 35/37, m/z 79/81 and m/z 127) can be monitored. This reduces the specificity of the detection method by MS. In summary, GC appears to be a method of choice for OXBP compounds determination, especially with PTV and COC injectors, which allow large volume injection and prevent thermal degradation. Due to its high selectivity and specificity in the SIM and SIR modes, GC-MS - used in the EI and/or ENCI modes is increasingly preferred over GC-ECD. A brief summary of more recent reported GC-MS methods for OXBP analysis are presented in Table 2. A more detailed description of some of these methods are also given in Supplementary material 2.

Table 2. Recent reported GC-MS methods for OXBP analysis in water samples (references are given in Supplementary Material 2).

Analytes	Approach	Pre-treatment	Injection modes	GC Column	MS method	Acquisition mode	LODs (ng/L)
HAs	Target	LLE	PTV (30 µL)	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	6-20
HNMs	Target	HS-SDME	Split, 17 0°C (2 µL, 1:20 ratio)	TRB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	80-1200
I-THMs	Target	HS-SPME	PTV	ZB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	1-20
Halonitriles	Target	HS-SPME	Splitless, 200 °C	ZB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	0.9-80
HKs	Target	HS-SPME	Splitless, 250 °C	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	15-600
HNMs, HAAms	Target	LLE	Splitless, 140 °C	ZB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	80-6000 / 80-2000
THMs, HNMs	Target	MLLE	PTV (30 µL)	SLB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	18-60 / 9-400
THMs, HNMs, and HANs	Target	HS	Split, 170 °C (1:20 ratio)	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	10-20 / 30-140 / 30-200
I-THMs, HNMs, HANs	Target	SPME	Splitless	TRB-5 (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI(70eV)-qMS	SIM	2-70
HAAs	Target	LLE	Splitless, 210 °C	HP-5MS (30 m, 0.25 mm i.d.,	EI-tqMS	SRM	10-80

				0.25 µm film thickness)			
THMs, HANs, and HKs	Target	LLE	Splitless, 180 °C	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (45 to 80 eV)-tqMS	SRM	3-14
Halobenzoquinones	Target	SPE + derivatization (MTBSTFA)	Splitless, 270 °C	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI(70eV)-qMS	SIM	0.03-0.9
Halobenzoquinones	Non-target	SPE	PTV (20 µL)	HP-5MS (30 m, 0.25 mm i.d., 0.25µm film thickness)	EI (70eV)/PCI-IT MS	Full-scan, MS/MS	-
Halohydrins	Non-target	LLE	Splitless	Rtx-5 (50 m, 0.25 mm i.d., 0.25µm film thickness)	EI (70eV)/PCI-TOF	Full-scan	-
170 volatiles and semi-volatiles DBPs	Non-target	SPE	Splitless, 280 °C	DB5-MS (30 m, 0.25 mm i.d., 0.25 µm film thickness) and BPX 50 (2 m, 0.1 mm i.d., 0.1 µm film thickness)	EI (70eV)-qMS	Full-scan	-
Bromoimidazoles, bromoanilines, and bomomethanesulfenic acid esters	Non-target	XAD resin	Splitless, 250 °C	Rtx-5 (50 m, 0.25 mm i.d., 0.25µm film thickness)	EI (70eV)-msMS	Full-scan	-
OIBPs	Non-target	XAD resin	Splitless, 280 °C	TR-5MS (60 m, 0.25 mm i.d., 0.25 µm film thickness)	EI-q-orbitrap MS	Full-scan	-

Abbreviations: TOF: time-of-flight, qMS: single quadrupole MS, msMS: high resolution magnetic sector, IT: ion trap MS, EI: electron ionization, PCI: positive chemical ionization, SIM: single ion monitoring, SRM: selected reaction monitoring, PTV: programmed temperature vaporization injector, Single drop microextraction in the headspace mode, HS: static headspace, LLE: liquid-liquid extraction, MLLE: micro liquid-liquid extraction, SPE: solid-phase extraction, MTBSTFA: N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide, OIBPs: organoiodine by-products, HAs: Haloacetaldehydes, HNMs: halonitromethanes, HS-SDME: HKs: haloketones.

III.3.2 Liquid chromatography and LC-MS coupling

LC-MS is an ideal tool for the analysis of polar and high molecular weight organohalogen compounds. In comparison to GC, the resolution of HPLC is often not sufficient to separate a wide range of compounds chromatographically, resulting in poor OXBP separation. One approach to minimize this limitation involves the use of LC columns packed with sub-2 µm stationary phase particles (ultra-performance liquid chromatography: UPLC). Chromatographic separation is frequently accomplished using reversed-phase columns. However, highly polar compounds (i.e., ionic compounds such as HAAs) are not effectively retained by this type of stationary phase. Hydrophilic interaction liquid chromatography (HILIC) and ion chromatography provide alternative approaches, enabling effective separation of small polar compounds on polar stationary phases. Electrospray ionization is particularly well suited for the analysis of high polarity substances such as HAAs. This ionization mode is also widely used for less polar OXBP such as halophenols and halobenzoquinones. There are logically very few studies reporting the use of atmospheric pressure chemical ionization for OXBP analysis since this ionization mode is usually more effective for low polarity compounds. Nevertheless, a sensitive method using LC-APCI-MS has been reported for the determination of haloacetamides (see Supplementary material 3). LC/MS can be employed in non-targeted screening techniques for the identification of unknown OXBPs. This is achieved through ion fragmentation by collision-induced dissociation (CID) tandem mass spectrometry and by combining several scan modes, including precursor ion, product ion, and neutral loss scanning. Product ion data is obtained by mass selecting the ions of interest, fragmenting these ions in a collision cell, and mass separating the product ions for their subsequent detection. Neutral loss scanning (NLS) enables the detection of all ionized compounds that lose a specific moiety of mass. Precursor ion scanning (PIS) can be used for detecting all ionized compounds that produce a given fragment ion upon CID. In this MS/MS acquisition mode, the mass spectrometer scans across a mass range in the first quadrupole, fragments all ions in the collision cell, and then selects one specific product ion in the second quadrupole. Following this process, the MS software identifies the precursor ions, which were originally selected targeted ions. Application of this high resolution precursor ion scanning method was recently demonstrated by Zhai et al. for the selective determination of brominated DBPs formed during the chloramination of drinking water. As many as 29 aliphatic, aromatic or nitrogenous polar Br-DBPs were detected; several compounds identified in this work had not been previously reported in literature [47]. NLS and PIS scan modes are very important because they enable the identification of the chromatographic peaks and characteristic ions of the molecules of interest. Each precursor ion is then subjected to CID to obtain product ion mass spectra. These ions enable the de novo reconstruction of the primary

structure of the parent ions from the spectrum of fragment ions. As OXBPs may generate halide ions under CID, the monitoring of m/z 35 and 37 (Cl^-), m/z 79 and 81 (Br^-), and m/z 127 (I^-) in PIS mode can selectively detect the OXBPs present in a sample. In addition, mass spectrometry can determine the nature of the halogen bond in a given analyte by comparing the peak intensities of halogen ions produced under different collisional energies (cleavage of the C-X bond occurs at about 5 eV for halogen atoms attached to an aliphatic chain and 15 eV for halogens attached to an unsaturated or aromatic ring) [48]. Despite its importance, one problem with the PIS approach is its lack of specificity. This problem is commonly encountered when performing PIS using a low resolution triple quadrupole mass spectrometer. In fact, even with the most effective stationary phase, LC alone cannot separate all of the isobaric compounds and unit mass resolution is too low for separation of nominally isobaric ions. Consequently, LC-MS/MS experiments using unit resolution PIS may include several isobaric parent ions that bias and hence complicate the compositional interpretation of target compounds. The selectivity of precursor ion experiments can nonetheless be significantly increased by using high resolution and high accuracy time mass spectrometers. In summary, UPLC and HILIC techniques should be preferred over classical HPLC for OXBPs analysis for their ability to achieve better chromatographic separations. Already known as the best detection mode for LC-MS quantitation, tandem mass spectrometry may also be helpful for the determination of unknown OXBPs owing to particular acquisition modes such as NLS and PIS. The main limitation of low resolution mass spectrometers such as triple quadrupole instruments is their inability to separate isobaric ions; that is why they are gradually replaced by high and ultrahigh resolution detectors (see section II.3). Table 3 summarizes several recent LC-MS methods for the analysis of OXBPs in water samples. A more detailed description of some of these methods are also given in Supplementary material 3.

Table 3. Recent reported LC-MS methods for OXBP analysis in water samples (references are given in Supplementary Material 3).

Analytes	Approach	Extraction	Injection	LC Column	MS analyzer	Acquisition mode	LODs (ng/L)
HAcAms	Target	SPE	10 µL	Hypersil GOLD C18 (100 × 2.1 mm i.d., 5 µm particule size)	APCI-tq MS	SRM	7.6-19.7
HBQs	Target	SPE	20 µL	Luna C18 (100 × 2.0 mm i.d., 3 µm particule size)	ESI-tq MS	SRM	0.5-37.9
HBQs	Non-target and target	SPE	10 µL	BEH C18 (100 x 2.1 i.d., 1.7µm particule size)	ESI-TOF MS ESI-tq MS	SRM	0.2-6.6
HAAs	Target	DI	30 µL	BEH HILIC column	ESI-tq MS	SRM	80-2700
HAAs	Target	DI	15 µL	BEH C8 (100 × 2.1 mm i.d., 1.7 µm particule size)	ESI-tq MS	SRM	160-1440
I-HAAs	Target	DI	1000 µL	Gemini C18 (150 x 4.6 mm i.d., 3 µm particule size)	ESI-tq MS	SRM	0.5-2.3
OBrBPs	Non-target	LLE	Infusion, UPLC (5 µL)	HSS T3 column (100 × 2.1 mm i.d., 1.8 µm particule size)	ESI-qTOF MS	PIS (m/z 79 and 81), SRM, and MS/MS	-
HBQs	Non-target	SPE	20 µL	Luna C18 (100 × 2.0 mm i.d., 3 µm particule size)	ESI-qTOF MS ESI-tq MS	MS/MS, and SRM	-
OBrBPs	Non-target	LLE	-	HSS T3 column (100 × 2.1 mm i.d., 1.8 µm particule size) and, UFLC-XR LC 75 x 3.0mm i.d., 2.2 µm)	ESI-tq MS and, ESI-IT-TOF-MS	PIS (m/z 79 and 81), and MS/MS	-
Iodo-trihydroxybenzenesulfonic	Non-target	LLE (MtBE)	7.5 µL	-	ESI-tq MS	PIS (m/z 126.9), SRM, and MS/MS	-

acids							
Halopyrroles	Non-target	LLE	7.5 µL	HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS	PIS (m/z 79 and 81), SRM, and MS/MS	-
OXBPs	Non-target	LLE	5 µL	HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS	PIS (m/z 126.9), SRM, and MS/MS	-
3 iodinated acetic acids, 6 carbonaceous phenolic I-DBPs, and 2 nitrogenous phenolic I-DBPs	Non-target	-	5 µL	HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS	PIS (m/z 126.9), SRM, and MS/MS	-
OXBPs derived from sulfonamide antibiotics	Non-target	DI	20 µL	Atlantis T3 (3 mm x 150 mm, 3 µm particule size)	ESI-q-orbitrap MS	Full-scan and MS/MS	-
Polar OBrBPs	Non-target	LLE	Infusion	-	ESI-tq MS	PIS (m/z 79 and 81)	-
Trihalo-hydroxy-cyclopentene-diones	Non-target			HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS and ESI-IT-orbitrap MS	Full-scan, PIS, SRM, and MS/MS	-
Polar OIBPs	Non-target	SPE	Infusion	-	ESI-FT-ICR MS (9.4 T)	Full-scan (m/z 150-800)	-
Polar OBrBPs	Non-target	SPE	Infusion	-	ESI qFT-ICR MS (9.4T)	Full-scan, MS/MS	-
Polar OClBPs	Non-target	SPE	Infusion	-	ESI FT-ICR MS (12 T)	Full-scan	-

Abbreviations: tqMS: triple quadrupole MS, IT: ion trap, FT-ICR: Fourier transform ion cyclotron resonance, ESI: electrospray ionization, APCI: atmospheric pressure chemical ionization, PIS: precursor ion scan, SRM: selected reaction monitoring, UPLC: ultra-performance liquid chromatography, DI: direct injection, SPE: solid-phase extraction, LOD: limit of detection, OClBPs: organochlorine by-products, OBrBPs: organobromine by-products, OIBPs: organoiodine by-products, HBQs: halobenzoquinones, MtBE: ethyl tert-butyl ether.

III.3.3 High and ultrahigh resolution mass spectrometry

The accurate mass obtained by a high resolution mass spectrometer can be used to determine elemental composition, which provides valuable additional information for elucidating the structure of unknown compounds. Time of flight (TOF) and orbitrap mass spectrometers both have the ability to acquire accurate masses; they allowed the discovery of new DBPs [49, 50]. Nowadays, the commonly used mass analyzer for structure elucidation of unknown compounds is the TOF, often associated to a second mass analyzer such as a quadrupole (QTof), another TOF (TOF-TOF) or an ion trap, to acquire tandem mass spectrometry data. Up to date, the studies published with Orbitrap MS in the area of identifying OXBPs remain very limited. Ultrahigh resolution mass spectrometry with a Fourier transform ion cyclotron resonance mass spectrometer (FTICRMS) has also been used to explore the chemical nature of unknown OXBPs. The FTICRMS is currently the most powerful mass spectrometer in terms of resolution and accuracy. However, it is not widely used as yet, even in research laboratories, because of its high cost. FTICRMS enables the determination of both the empirical formula of a molecule and its characteristic fragments, which can then be confirmed by matching the MS fragmentation patterns with authentic chemical standards [51]. In a study by Zhang et al., an FTICRMS operated in negative mode was used to characterize and monitor the reactivity of organic matter in drinking water without chromatography coupling, in order to obtain a better understanding of the OXBP formation mechanisms; 659 compounds with one chlorine atom and 348 with two chlorine atoms were detected without proposed chemical structures [52]. Of all the OXBPs identified, only seven had been reported in previous studies. Many of the 710 OXBPs with high molecular weight were later identified in the study by Lavonen et al., in raw and drinking water treated with different chlorine-based oxidants and extracted with SPE. The authors asserted, however, that many of these OXBPs were found prior to the disinfection step [53]. In another study by Zhang et al., an FTICRMS was used to characterize low molecular weight compounds in DOM samples from a drinking water treatment plant. In total, 357 OXBPs with one chlorine atom and 199 with two chlorine atoms were assigned, without structural identification, with a high level of confidence by accurate mass analysis combined with Kendrick mass defect plots. This later was used to classify homologous compounds differing by only a given number of base units (in this case CH_2) and to arrange the chlorinated OXBPs into class species, the members of which share the same mass defect [54]. The resulting diagram is useful in reducing the number of signals obtained in a mass spectrum during analysis of complex mixtures, in classifying compounds with the same mass defect in the same line and also in facilitating the assignment of a chemical formula. To obtain more information on the elementary composition of a complex mixture, a completely different approach was used by Roach et al. to characterize petroleum samples. In this study, the authors used Kendrick mass defect diagrams in order to further reduce the number of signals and regroup molecules into several bases [55]. The CH_2, H_2, O, S, etc. bases are frequently used; however, the addition of a halogen base, to regroup poly

halogenated compounds on the basis of a halogen mass defect, has never been mentioned in literature. Moreover, the assignment of a chemical formula to the measured masses remains complicated, even with high resolution m/z measurements. In the study by Herzsprung and Hertkorn, a protocol was proposed to improve chemical formula assignment for each molecular mass obtained in high resolution, by determining the difference between the double-bond equivalents and the number of oxygen atoms [56]. This protocol has been compared to several other approaches, such as Kendrick mass defect combined with Van Krevlen diagrams, and another automated method that uses search algorithms based on functional group relationships, as reported by Koch et al. [57]. Recently, Zhang et al. applied the same methodology used in their earlier 2012 work to identify brominated by-products in chlorinated artificial drinking water. In this study, they identified 441 compounds with one bromine atom and 37 compounds with two bromine atoms. CID experiments were also performed and revealed neutral CO_2 losses, indicating that the unknown brominated compounds were polar and rich in carboxylic functions [58]. More recently, Wang and al. adopted a similar methodology to characterize unknown I-DBPs in chloraminated/chlorinated water spiked with iodide and humic substances. In total, 178 formulas for single iodine-containing products, 13 formulas for double iodine-containing products, and 15 formulas for single-chlorine/single-iodine-containing products were detected in the chloraminated water sample, while only nine formulas for single-iodine-containing products and six formulas for single-chlorine/single-iodine-containing products were found in the chlorinated water sample. Most I-DBPs had corresponding chlorine-containing analogs with identical CHO compositions. Based on a modified aromaticity index, the authors concluded that more than 68% of the I-DBPs had aromatic structures or polycyclic aromatic structures [59]. As mentioned above, the fist interest of using a high resolution mass spectrometer is to distinguish between isobaric ions and thus between isomeric species which are frequently encountered among some OXBPs. High resolution also permits to increase the selectivity of quantitation methods through reduction of matrix compounds interferences. For this purpose, orbitrap and Q-TOF analyzers will be preferred over FTICRMS for their very high scanning rate. Conversely, FTICRMS will be rather chosen for the construction of Kendrick or Van Krevlen diagrams - allowing a global vision of the OXBPs present in a very complex matrix - because its ultrahigh resolution (up to 10 000 000 vs about 500 000 for an upscale orbitrap) leads to much more accurate diagrams. Without any doubt, the FT-ICR and orbitrap MS will dominate the field of structural identification of OXBPs in the near future, particularly if we know that high molecular weight material contributes to significant portion of the AOX (up to 80%)

III.3.4 Membrane-introduction-mass spectrometry

Membrane introduction mass spectrometry (MIMS) is a direct, continuous, on-line measurement technique, which is particularly suitable for the analysis of volatile molecules. It is based on the use of a semi-permeable membrane, which acts as an interface between the aqueous samples and the mass

spectrometer [60, 61]. Owing to their porosity and hydrophobicity, semi-permeable membranes only allow certain substances to pass through, these being almost exclusively non-polar and moderately-polar compounds dissolved in water. This barrier effect applies to both water and interfering matrix substances. The membrane allows the simultaneous extraction and enrichment of analytes introduced with an inert carrier gas - usually helium - into the mass spectrometer. Different membrane materials have been tested and polydimethylsiloxane has been found to be the most effective. The extracted molecules are ionized and detected in the mass spectrometer. MIMS systems are often created by laboratory scientists themselves, who adapt and modify their existing GC-MS instruments.

Numerous applications for water samples have been reported in relation to the analysis of THMs, inorganic chloramines, halocyanogens and haloacetonitriles (HANs) [62], with low LODs being obtained for several compounds. For example, Yang and Shang developed an analytical method for analyzing cyanogen chloride and cyanogen bromide in environmental samples using the MIMS technique and reported an LOD of 1.7 µg/L for both of the analyzed compounds [63]. In the study by Chang and Her, MIMS was coupled with fast gas chromatography for on-line monitoring of THMs in water samples, achieving LODs in the range of 2 to 8 ng/L [64]. Although this technique is simple and easy to implement, it is important to note not only that its application is limited to hydrophobic, volatile and low molecular weight OXBPs but that this can be further restricted by matrix interference [65].

III.3.5 Field asymmetric ion mobility spectrometry-mass spectrometry

Field asymmetric ion mobility spectrometry (FAIMS) is a separation technique for gas-phase at atmospheric pressure. A large number of non-halogenated ions from the polar compounds contained in natural organic matter are quite labile; they can rapidly dissociate into small fragments before their detection and consequently increase the chemical background in mass spectrometry. This poses a major problem for the detection and further identification of OXBPs in water samples, especially those present at trace level. When used as an interface between an ESI source and a mass spectrometer, a FAIMS analyzer was shown to suppress the chemical interference of natural organic matter, enhance the signal-to-noise ratio and lower the limit of detection. In the study by Ells et al., ESI/FAIMS/MS was used for the identification of six HAAs in tap water. The detection limits in this study were in the 5 to 40 ng/mL range without pre-concentration, derivatization or chromatographic separation prior to analysis [66]. In a similar approach, Gabryelski et al. used the same coupling method to monitor HAAs in drinking water samples. The detection limits obtained were much lower than those achieved with conventional GC/ECD and GC-MS methods [67]. ESI/FAIMS/MS can also be applied to research on unknown OXBPs: in the study conducted by Sultan et al.,

ESI/FAIMS/MS and MS/MS were used in an attempt to elucidate the structural identification of unknown polar organohalogen by-products in drinking water. This study resulted in the identification of chlorinated diacids, never revealed in previous studies [68]. In summary, the main interest of FAIMS is that it affords the possibility to avoid GC or LC separation and thus reduces the analysis time so that labile OXBPs do not have time to degrade. It further allows decreasing detection thresholds of OXBPs by reducing the chemical interference of natural organic matter.

III.4 Conclusion

It is clear that a deeper knowledge of the nature of unidentified OXBPs is now required. AOX measurement provides a window into the study of these compounds, as the most direct estimate of unknown organohalogen by-products is obtained by subtracting the major KOXBPs from the measured AOX. Several approaches have been devised over the last three decades for identifying in real and in laboratory water samples the majority of the hitherto undetected OXBPs (80% in the case of monochloramination treatment). Most of the analytical methods used in these approaches are based on GC-MS and LC-MS coupling, due to the sensitivity of these techniques and the ability they provide to screen for many if not most products from water samples. The preparation of water samples represents a key step, as the loss of a large percentage of unknown OXBPs before analysis can clearly be attributed to sample pre-treatment and preparation procedures. In addition, large quantities of natural organic matter and interferents can affect the sensitivity of analytical methods and, consequently, the identification of OXBPs at trace level.

Based on the reports in scientific publications it can be concluded that the majority of OXBPs that have as yet escaped detection consist of compounds of high molecular weight. High-resolution mass spectrometry has been used in recent years to improve the detection and identification of high molecular weight compounds in complex mixtures and environmental samples. The use of ESI or APCI-FTICRMS can help minimize or avoid the interference associated with compounds of the same nominal mass. However, it is important to note that other classes of OXBPs, which are likely to be present in the water sample, are not ionized by ESI or APCI, whether in positive or negative ion mode. This is one of the reasons why some compounds cannot be found in mass spectra. In addition, the lack of expertise in the interpretation and analysis of fragmentation spectra for high molecular weight structures frequently deters any attempt at structural elucidation and thereby reduces the likelihood of MB improvement. Atmospheric pressure photoionization (APPI) tends to ionize less polar and smaller molecules than ESI and APCI and provides complementary mass spectra. Although time-consuming and costly, using all three ionization modes for each sample appears the best solution for detecting the maximum number of OXBPs in a water sample.

References

[1] A. Kinani, S. Kinani, B. Richard, M. Lorthioy, S. Bouchonnet, Formation and determination of organohalogen by-products in water. Part 1. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method, TrAC Trends Analytic. Chem. 85 (2016) 273-80

[2] A. Kinani, S. Kinani, S. Bouchonnet, Formation and determination of organohalogen by-products in water. Part II. Sample preparation techniques for analytical approaches, TrAC Trends Analytic. Chem. 85 (2016) 281-94

[3] M. Bourgin, E. Bichon, J.-P. Antignac, F. Monteau, G. Leroy, L. Barritaud, M. Chachignon, V. Ingrand, P. Roche, B. Le Bizec, Chlorination of bisphenol A: Non-targeted screening for the identification of transformation products and assessment of estrogenicity in generated water, Chemosphere 93 (2013) 2814-2822

[4] M. Sarrion, F. Santos, M. Galceran, Solid-phase microextraction coupled with gas chromatography-ion trap mass spectrometry for the analysis of haloacetic acids in water, J. Chrom. A 859 (1999) 159-171

[5] M. Cardador, A. Serrano, M. Gallego, Simultaneous liquid-liquid microextraction/methylation for the determination of haloacetic acids in drinking waters by headspace gas chromatography, J. Chrom. A 1209 (2008) 61-69

[6] A.M. Pawlecki-Vonderheide, D.J. Munch, J.W. Munch, Research associated with the development of EPA method 552.2, J. Chromatogr. Sci. 35 (1997) 293-301.

[7] P.S. Simone, P.L. Ranaivo, G. Geme, M.A. Brown, G.L. Emmert, On-line monitoring of nine haloacetic acid species at the μgL−1 level using post-column reaction-ion chromatography with nicotinamide fluorescence, Anal. Chim. Acta 654 (2009) 133-140

[8] D. Hou, J. Fan, L. Han, X. Ruan, F. Feng, W. Liu, F. Zheng, Determination of small halogenated carboxylic acid residues in drug substances by high performance liquid chromatography-diode array detection following derivatization with nitro-substituted phenylhydrazines, J. Chrom. A 1438 (2016) 46-56

[9] B.-L. Qi, P. Liu, Q.-Y. Wang, W.-J. Cai, B.-F. Yuan, Y.-Q. Feng, Derivatization for liquid chromatography-mass spectrometry, TrAC Trends in Anal. Chem. 59 (2014) 121-132

[10] M. Eggink, M. Wijtmans, A. Kretschmer, J. Kool, H. Lingeman, I.J. de Esch, W.M. Niessen, H. Irth, Targeted LC-MS derivatization for aldehydes and carboxylic acids with a new derivatization agent 4-APEBA, Anal. Bioanal. Chem. 397 (2010) 665-675

[11] O. Al-Dirbashi, T. Fukushima, Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry for biomedical analysis, Drug. Discov. Ther. 1 (2007) 108-118

[12] Y. Lu, D. Yao, C. Chen, 2-Hydrazinoquinoline as a derivatization agent for LC-MS-based metabolomic investigation of diabetic ketoacidosis, Metabolites 3 (2013) 993-1010.

[13] C. Zwiener, T. Glauner, F. Frimmel, Method optimization for the determination of carbonyl compounds in disinfected water by DNPH derivatization and LC-ESI-MS-MS, Anal. Bioanal. Chem. 372 (2002) 615-621

[14] S.D. Richardson, T.V. Caughran, T. Poiger, Y. Guo, F. Crumley, Identification of polar drinking water disinfection by-products with LC/MS, Proceedings of the Georgia Water Resources Conference, University of Georgia, 1999.
[15] S.D. Richardson, T.V. Caughran, T. Poiger, Y. Guo, F.G. Crumley, Application of DNPH derivatization with LC/MS to the identification of polar carbonyl disinfection byproducts in drinking water, Ozone: Sci. Eng. 22 (2000) 653-675
[16] R.M. Le Lacheur, L.B. Sonnenberg, P.C. Singer, R.F. Christman, M.J. Charles, Identification of carbonyl compounds in environmental samples, Environ. Sci. Technol. 27 (1993) 2745-2753
[17] H.S. Weinberg, Modern approaches to the analysis of disinfection by-products in drinking water, Philos. T. Roy. Soc. A 367 (2009) 4097-4118
[18] M. Vincenti, F. Fasano, M. Valsania, P. Guarda, S. Richardson, Application of the novel 5-chloro-2,2,3,3,4,4,5,5-octafluoro-1-pentyl chloroformate derivatizing agent for the direct determination of highly polar water disinfection byproducts, Anal. Bioanal. Chem. 397 (2010) 43-54
[19] A. Kanan, T. Karanfil, Formation of disinfection by-products in indoor swimming pool water: The contribution from filling water natural organic matter and swimmer body fluids, Water Res. 45 (2011) 926-932
[20] Standard method 6251B, Disinfection by-products: haloacetic acids and trichlorophenol, micro liquid-liquid extraction GC method, in: A.D. Eaton, L.S. Clesceri (Eds.), Standard Methods for the Examination of Water and Wastewater (19th ed.) Washington, DC: American Public Health Association, 1995.
[21] M.M. Domino, B.V. Pepich, D.J. Munch, P.S. Fair, Optimizing the determination of haloacetic acids in drinking waters, J. Chrom. A 1035 (2004) 9-16
[22] J. Mathew, R. McMillin, J. Gandhi, S. Mohsin, S. Czyborra, Trace level haloacetic acids in drinking water by direct injection ion chromatography and single quadrupole mass spectrometry, J. Chromatogr. Sci. 47 (2009) 505-509
[23] S.E. Duirk, C. Lindell, C.C. Cornelison, J. Kormos, T.A. Ternes, M. Attene-Ramos, J. Osiol, E.D. Wagner, M.J. Plewa, S.D. Richardson, Formation of toxic iodinated disinfection by-products from compounds used in medical imaging, Environ. Sci. Technol. 45 (2011) 6845-6854
[24] A.D. Nikolaou, S.K. Golfinopoulos, M.N. Kostopoulou, T.D. Lekkas, Determination of haloacetic acids in water by acidic methanol esterification-GC-ECD method, Water Res. 36 (2002) 1089-1094
[25] P. Varanusupakul, N. Vora-Adisak, B. Pulpoka, In situ derivatization and hollow fiber membrane microextraction for gas chromatographic determination of haloacetic acids in water, Anal. Chim. Acta 598 (2007) 82-86
[26] Y.H. Wang, P.K. Wong, Determination of dichloroacetic acid and trichloroacetic acid in drinking water by acidic methanol esterification and headspace gas chromatography, Water Res. 39 (2005) 1844-1848
[27] M.J. Cardador, A. Serrano, M. Gallego, Simultaneous liquid-liquid microextraction/methylation for the determination of haloacetic acids in drinking waters by headspace gas chromatography, J. Chrom. A 1209 (2008) 61-69
[28] M.J. Cardador, M. Gallego, Optimisation and comparison of several microextraction/methylation methods for determining haloacetic acids in water using gas chromatography, Anal. Bioanal. Chem. 396 (2010) 1331-1343

[29] M. Jia, W.W. Wu, R.A. Yost, P.A. Chadik, P.W. Stacpoole, G.N. Henderson, Simultaneous determination of trace levels of nine haloacetic acids in biological samples as their pentafluorobenzyl derivatives by gas chromatography/tandem mass spectrometry in electron capture negative ion chemical ionization mode, Anal. Chem. 75 (2003) 4065-4080

[30] M. Saraji, A.A.H. Bidgoli, Single-drop microextraction with in-microvial derivatization for the determination of haloacetic acids in water sample by gas chromatography-mass spectrometry, J. Chrom. A 1216 (2009) 1059-1066

[31] A.M. Casas Ferreira, M.E. Fernández Laespada, J.L. Pérez Pavón, B. Moreno Cordero, In situ derivatization coupled to microextraction by packed sorbent and gas chromatography for the automated determination of haloacetic acids in chlorinated water, J. Chrom. A 1318 (2013) 35-42

[32] M. Terasaki, Y. Takemura, M. Makino, Paraben-chlorinated derivatives in river waters, Environ. Chem. Lett. 10 (2012) 401-406

[33] J. Tillner, C. Hollard, C. Bach, C. Rosin, J.-F. Munoz, X. Dauchy, Simultaneous determination of polycyclic aromatic hydrocarbons and their chlorination by-products in drinking water and the coatings of water pipes by automated solid-phase microextraction followed by gas chromatography-mass spectrometry, J. Chrom. A 1315 (2013) 36-46

[34] J.W.A. Charrois, Analysis of emerging disinfection by-products in drinking water, in: R.A Maeyers (Ed), Encyclopedia of analytical chemistry, applications, theory and instrumentation, John Wiley & Sons, Ltd, Chichester, 2011.

[35] C.H. Jeong, C. Postigo, S.D. Richardson, J.E. Simmons, S.Y. Kimura, B.J. Mariñas, D. Barcelo, P. Liang, E.D. Wagner, E.D., M.J. Plewa, Occurrence and comparative toxicity of haloacetaldehyde disinfection byproducts in drinking water. Environ. Sci. Technol. 49 (2015) 13749-13759

[36] M. Eggink, M. Wijtmans, A. Kretschmer, J. Kool, H. Lingeman, I.P. Esch, W.A. Niessen, H. Irth, Targeted LC-MS derivatization for aldehydes and carboxylic acids with a new derivatization agent 4-APEBA, Anal. Bioanal. Chem. 397 (2010) 665-675

[37] C. Zwiener, S.D. Richardson, Analysis of disinfection by-products in drinking water by LC-MS and related MS techniques, TrAC Trends in Anal. Chem. 24 (2005) 613-621

[38] Y.-C. Ma, C.-Y. Chiang, Evaluation of the effects of various gas chromatographic parameters on haloacetic acids disinfection by-products analysis, J. Chrom. A 1076 (2005) 216-219

[39] Q. Luo, X. Chen, Z. Wei, X. Xu, D. Wang, Z. Wang, Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation, J. Chrom. A 1365 (2014) 45-53

[40] M. Serrano, M. Silva, M. Gallego, Micro liquid-liquid extraction combined with large-volume injection gas chromatography-mass spectrometry for the determination of haloacetaldehydes in treated water, J. Chrom. A 1218 (2011) 8295-8302

[41] H. Chen, J. Yin, M. Zhu, C. Cao, T. Gong, Q. Xian, Cold on-column injection coupled with gas chromatography/mass spectrometry for determining halonitromethanes in drinking water, Anal. Meth. 8 (2016) 362-370

[42] J.W. Hodgeson, J. Collins, R.E. Barth, D. Becker, D.J Munch, J.W. Munch, A.M. Pawlecki, Environmental Protection Agency Method 552.2 Determination of haloacetic acids and dalapon in drinking water by liquid-liquid extraction, derivatization and gas chromatography with electron capture detection, Cincinnati, OH, 2003, available at: http://www.caslab.com/EPA-Methods/PDF/552_2.pdf, accessed May 2016.

[43] NF EN ISO 23631. Water quality - Determination of dalapon, trichloroacetic acid and selected haloacetic acids - Method using gas chromatography (GCD-ECD and/or GC-MS detection) after liquid-liquid extraction and derivatization, 2006.

[44] A.D. Nikolaou, T.D. Lekkas, S.K. Golfinopoulos, M.N. Kostopoulou, Application of different analytical methods for determination of volatile chlorination by-products in drinking water, Talanta 56 (2002) 717-726

[45] R.R. Kozani, Y. Assadi, F. Shemirani, M.M. Hosseini, M. Jamali, Determination of trihalomethanes in drinking water by dispersive liquid-liquid microextraction then gas chromatography with electron-capture detection, Chromatographia 66 (2007) 81-86

[46] M. Domino, B. Pepich, D. Munch, P. Fair, Y. Xie, Method 552.3 Determination of haloacetic acids and dalapon in drinking water by liquid-liquid microextraction, derivatization, and gas chromatography with electron capture detection, United States Environmental Protection Agency, Cincinnati, OH, 2003, available at: http://www.caslab.com/EPA-Methods/PDF/552_2.pdf, accessed May 2016.

[47] H. Zhai, X. Zhang, X. Zhu, J. Liu, M. Ji, Formation of brominated disinfection byproducts during chloramination of drinking water: new polar species and overall kinetics, Environ. Sci. Technol. 48 (2014) 2579-2588

[48] Z. Deng, X. Yang, C. Shang, X. Zhang, An electrospray ionization-tandem mass spectrometry method for differentiating chlorine substitution in disinfection by-product formation, Environ. Sci. Technol. 48 (2014) 4877-4884

[49] S. Richardson, C. Postigo, Discovery of new emerging dbps by high-resolution mass spectrometry, in: Perez, Eichhorn and Barcelo (Eds) Applications of time-of-flight and orbitrap mass spectrometry in environmental, food, doping, and forensic analysis. Comprehensive Analytical Chemistry. Vol. 71, 2016, pp 335-356

[50] C. Postigo, C.I. Cojocariu, S.D. Richardson, P.J. Silcock, D. Barcelo, Characterization of iodinated disinfection by-products in chlorinated and chloraminated waters using Orbitrap based gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 408 (2016) 3401-3411

[51] C. Heffner, I. Silwal, J.M. Peckenham, T. Solouki, Emerging technologies for identification of disinfection byproducts: GC/FT-ICR MS characterization of solvent artifacts, Environ. Sci. Technol. 41 (2007) 5419-5425

[52] H. Zhang, Y. Zhang, Q. Shi, J. Hu, M. Chu, J. Yu, M. Yang, Study on transformation of natural organic matter in source water during chlorination and its chlorinated products using ultrahigh resolution mass spectrometry, Environ. Sci. Technol. 46 (2012) 4396-4402

[53] E.E. Lavonen, M. Gonsior, L.J. Tranvik, P. Schmitt-Kopplin, S.J. Köhler, Selective chlorination of natural organic matter: Identification of previously unknown disinfection byproducts, Environ. Sci. Technol. 47 (2013) 2264-2271

[54] H. Zhang, Y. Zhang, Q. Shi, S. Ren, J. Yu, F. Ji, W. Luo, M. Yang, Characterization of low molecular weight dissolved natural organic matter along the treatment trait of a waterworks using Fourier transform ion cyclotron resonance mass spectrometry, Water Res. 46 (2012) 5197-5204

[55] P.J. Roach, J. Laskin, A. Laskin, Higher-order mass defect analysis for mass spectra of complex organic mixtures, Anal. Chem. 83 (2011) 4924-4929

[56] P. Herzsprung, N. Hertkorn, W. von Tümpling, M. Harir, K. Friese, P. Schmitt-Kopplin, Understanding molecular formula assignment of Fourier transform ion cyclotron resonance mass spectrometry data of natural organic matter from a chemical point of view, Anal. Bioanal. Chem. 406 (2014) 7977-7987

[57] B.P. Koch, T. Dittmar, M. Witt, G. Kattner, Fundamentals of molecular formula assignment to ultrahigh resolution mass data of natural organic matter, Anal. Chem. 79 (2007) 1758-1763

[58] H. Zhang, Y. Zhang, Q. Shi, H. Zheng, M. Yang, Characterization of unknown brominated disinfection byproducts during chlorination using ultrahigh resolution mass spectrometry, Environ. Sci. Technol. 48 (2014) 3112-3119

[59] X. Wang, J. Wang, Y. Zhang, Q. Shi, H. Zhang, M. Yang, Characterization of unknown iodinated disinfection byproducts during chlorination/chloramination using ultrahigh resolution mass spectrometry, Sci. Total Environ. 554-555 (2016) 83-88

[60] R.C. Johnson, R.G. Cooks, T.M. Allen, M.E. Cisper, P.H. Hemberger, Membrane introduction Mass Spectrometry: Trends and applications, Mass Spectrom. Rev. 19 (2000) 1-37

[61] T.M. Allen, M.E. Cisper, P.H. Hemberger, C.W. Wilkerson Jr, Simultaneous detection of volatile, semivolatile organic compounds, and organometallic compounds in both air and water matrices by using membrane introduction mass spectrometry, Int. J. Mass Spectrom. 212 (2001) 197-204

[62] J. Li, E.R. Blatchley, Volatile disinfection byproduct formation resulting from chlorination of organic-nitrogen precursors in swimming pools, Environ. Sci. Technol. 41 (2007) 6732-6739

[63] X. Yang, C. Shang, Quantification of aqueous cyanogen chloride and cyanogen bromide in environmental samples by MIMS, Water Res. 39 (2005) 1709-1718

[64] C.C. Chang, G.R. Her, On-line monitoring trihalomethanes in chlorinated water by membrane introduction-fast gas chromatography mass-spectrometry, J. Chrom. A 893 (2000) 169-175

[65] K. Fischer, E. Fries, W. Körner, C. Schmalz, C. Zwiener, New developments in the trace analysis of organic water pollutants, Appl. Microbiol. Biotechnol. 94 (2012) 11-28

[66] B. Ells, D.A. Barnett, K. Froese, R.W. Purves, S. Hrudey, R. Guevremont, Detection of chlorinated and brominated byproducts of drinking water disinfection using electrospray ionization-high-field asymmetric waveform ion mobility spectrometry-mass spectrometry, Anal. Chem. 71 (1999) 4747-4752

[67] W. Gabryelski, F. Wu, K.L. Froese, Comparison of high-field asymmetric waveform ion mobility spectrometry with GC methods in analysis of haloacetic acids in drinking water, Anal. Chem. 75 (2003) 2478-2486

[68] J. Sultan, W. Gabryelski, Structural identification of highly polar nontarget contaminants in drinking water by ESI-FAIMS-Q-TOF-MS, Anal. Chem. 78 (2006) 2905-2917

Supplementary Materials (SM)

SM 1. Examples of sample preparation processes including derivatization used prior to GC-MS analysis of OXBPs

In the work of Cardador et al., liquid phase microextraction and methylation were performed simultaneously using tetrabutylammonium hydrogen sulfate (TBA-HSO4) as the ion-pairing agent, and 150 µL of n-pentane as extractant. After being shaken for 3 minutes at room temperature, samples were heated at 60 °C for 20 minutes in order to evaporate the n-pentane containing HAA methyl esters. It was demonstrated that n-pentane increases the derivatization yields of methyl haloacetates and minimizes decomposition of the HAAs in water and the corresponding formation of THMs. The analysis was subsequently performed using HS-GC-MS, yielding very low detection limits ranging from 0.02 to 0.40 µg/L [1]. In the study by Sarrion et al., HAAs were derivatized (in the presence of TBA-HSO4) and extracted in one step and the resulting methyl esters were analyzed by HS-SPME-GC-MS, attaining LODs similar to those obtained by Cardador et al. [2]. Other examples of this approach are provided by Saraji and Bidgoli and Al-shatri et al., who used n-octanol as both extracting solvant and derivatization reagent. Saraji and Bidgoli reported LODs between 0.1 and 1.2 µg/L using single-drop microextraction (1.8 µL) in combination with a GC-MS system, while Al-shatri et al. found LODs ranging from 0.05 to 0.57 µg/L by injecting 0.2 µL of extract into a GC-MS system after dispersive liquid-liquid microextraction using 1 mL of n-octanol [3,4]. A similar method is reported by Ferreira et al., who employed in situ aqueous derivatization of HAAs with 2,2,2-trifluroethylamine, followed by automatic extraction of their in situ amide derivatives by means of the microextraction by packed sorbent (MEPS) technique [5]. In the methodology proposed by Benanou et al., HAAs were extracted using AG1-X8 resin and methylated in-column during their elution with acidic methanol. However, a second LLE with cyclohexane was needed to extract the HAA methyl esters from the methanol. In this work, the LODs obtained with the GC/ECD analysis were between 0.1 µg/L and 0.2 µg/L [6]. In some studies, HAA derivatization has been carried out after evaporation of the sample to dryness and the esters formed then extracted from organic solvents for their subsequent analysis by GC [7].

EPA Method 556.1 and Standard Method 6252 are commonly used for analysis of haloacetaldehydes. Both methods use O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) for aqueous phase derivatization of aldehydes to their pentafluorobenzyl oximes. After a reaction time of two hours at 35 °C followed by liquid-liquid extraction with hexane, the oxime derivatives are analyzed by gas chromatography. Recently, Chen et al. investigated silylation derivatization for the rapid screening of haloacetamides in water. Their procedure was based on salt-assisted liquid-liquid extraction combined with silylation with N-methyl-N-(tert-butyldimethylsilyl)- trifluoroacetamide (MTBSTFA) performed directly in the GC liner; this is referred to as “injection-port silylation”. The optimal conditions involved 4 mL of ethyl acetate as extractant and a 10 mL water sample (pH 7) containing 3 g of

Na2SO4. After vortex extraction for only 1 minute followed by centrifugation, 10 μL of the extract (mixed with 1 μL of MTBSTFA) was analyzed by injection-port silylation GC-MS. The LOQs were determined to be 0.03 to 0.3 μg/L [8]. One disadvantage of silylation is that the derivatives are sensitive to moisture. Sample extracts must be thoroughly dried before derivatization and care has to be taken when handling the derivatized samples to prevent moisture ingress, as this would result in derivative degradation.

Sample preparation for the strong mutagen (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX)) and its halogen analogs: 3-chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone, 3-chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone and 3-bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone) in water generally involves the acidification of the samples to pH 2 followed by an enrichment procedure consisting of either LLE (with methylene chloride) or solid phase extraction (SPE). The hydroxyl group in MX and its analogs can be derivatized by alkylation, acylation or silylation [9,10,11,12,13], however derivatization by methylation, typically performed with methanol containing 2% H_2SO_4, is the most frequently used method. This procedure has certain advantages, including its simplicity and a low reaction temperature (60 °C). Its main drawback is that the fragment $[M\text{-}CH3O]^+$ used for quantitative analysis in GC-MS has a low abundance in comparison to the peaks at m/z 147 (ions present in septum and column bleed) and 149 (characteristic phthalate ions) which, despite their high intensity, are not characteristics suitable for use in MX identification by low resolution mass spectrometry. Other alkylating reagents, such as ethanol, propyl alcohol and butyl alcohols have therefore been tested. Derivatization with sec-butanol has been presented as a method that demonstrates significantly lower GC-MS detection limits. Acylation has also been explored for the derivatization of halogenated hydroxy-furanones. This method addresses several of the difficulties encountered in methylation derivatization, such as the lack of selectivity and/or sensitivity of the formed ions, the long derivatization reaction time and the labor intensive procedures. A method involving the derivatization of MX with N-methyl-bis-trifluoroacetamide, following sample enrichment by SPE and eluent evaporation prior to GC-MS analysis, was found to provide a LOD of 7.7 ng/L [15]. Here, the derivatization reaction time was relatively short (2 hours, 90 °C) in comparison to methylation with alcoholic acidic solutions, which can last up to 12 hours [14]. Despite the low abundance of the molecular ion of the trifluoroacetylated MX at m/z 314, the mass spectrum contains abundant fragment ions at m/z 201 and 199, which allow the unambiguous identification of MX. Several methods involving derivatization by silylation have also been reported. A procedure based on LLE with methylene chloride and on-line derivatization (in the injector liner) with the N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation reagent provided an LOD of 3 ng/L in GC-MS using selective ion monitoring. More recently, Planas et al. proposed a similar procedure in terms of sample extraction and derivatization, but used a GC-MS/MS method to perform the analysis, obtaining LODs ranging from 0.3 to 0.9 ng/L [16]. In the method developed by Rezemini et al., MX

was extracted from water by SPME. The SPME fibers were then inserted into a vial containing BSTFA, where they remained for 5 minutes under magnetic agitation. After adsorption, each fiber was introduced into the GC injection port to achieve thermal desorption and in situ derivatization. Although this method was simple, fully automated and did not require organic solvents, the LOD was high (30 ng/L) [17].

References

[1] M. Cardador, A. Serrano, M. Gallego, Simultaneous liquid-liquid microextraction/methylation for the determination of haloacetic acids in drinking waters by headspace gas chromatography, J. Chrom. A 1209 (2008) 61-69

[2] M. Sarrión, F. Santos, M. Galceran, In situ derivatization/solid-phase microextraction for the determination of haloacetic acids in water, Anal. Chem. 72 (2000) 4865-4873

[3] M. Saraji, A.A.H. Bidgoli, Single-drop microextraction with in-microvial derivatization for the determination of haloacetic acids in water sample by gas chromatography-mass spectrometry, J. Chrom. A 1216 (2009) 1059-1066

[4] M.A. Al-shatri, A.A. Nuhu, C. Basheer, Determination of haloacetic acids in bottled and tap water sources by dispersive liquid-liquid microextraction and GC-MS analysis, Sci. World J. (2014) ID 695049.

[5] A.M.C. Ferreira, M.E.F. Laespada, J.L.P. Pavón, B.M. Cordero, In situ derivatization coupled to microextraction by packed sorbent and gas chromatography for the automated determination of haloacetic acids in chlorinated water, J. Chrom. A 1318 (2013) 35-42

[6] D. Benanou, F. Acobas, P. Sztajnbok, Analysis of haloacetic acids in water by a novel technique: simultaneous extraction-derivatization, Water Res. 32 (1998) 2798-2806

[7] M. Sarrion, F. Santos, M. Galceran, Solid-phase microextraction coupled with gas chromatography-ion trap mass spectrometry for the analysis of haloacetic acids in water, J. Chrom. A 859 (1999) 159-171

[8] T.-L. Chen, S.-H. Tzing, W.-H. Ding, Rapid screening of haloacetamides in water using salt-assisted liquid-liquid extraction coupled injection-port silylation gas chromatography-mass spectrometry, J. Chrom. A 1422 (2015) 340-344

[9] C. Kubwabo, B. Stewart, S.A. Gauthier, B.R. Gauthier, Improved derivatization technique for gas chromatography-mass spectrometry determination of 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone in drinking water, Anal. Chim. Acta 649 (2009) 222-229

[10] J. Nawrocki, P. Andrzejewski, L. Kronberg, H. Jeleń, New derivatization method for determination of 3-chloro-4 (dichloromethyl)-5-hydroxy-2 (5H)-furanone in water, J. Chrom. A 790 (1997) 242-247

[11] J. Nawrocki, P. Andrzejewski, H. Jeleń, E. Wasowicz, Derivatization of the mutagen MX (3-chloro-4 (dichloromethyl)-5-hydroxy-2 (5H)-furanone) with butyl alcohols prior to GC-MS analysis, Water Res. 35 (2001) 1891-1896

[12] P. Rantakokko, M. Yritys, T. Vartiainen, Matrix effects in the gas chromatographic-mass spectrometric determination of brominated analogues of 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone, J. Chrom. A 1028 (2004) 179-188

[13] C. Planas, F. Ventura, J. Caixach, J. Martín, M.R. Boleda, M. Paraira, Analysis of 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone (MX) and its brominated analogues in chlorine-treated water by gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC-QqQ-MS/MS), Talanta 144 (2015) 145-156

[14] A.L. Rezemini, J.M. Vaz, L.R. Carvalho, Solid-phase microextraction for determination of 3-chloro-4-(dichloromethyl)-5-hydroxy-2 [5H]-furanone in water, J. Braz. Chem. Soc. 19 (2008) 922-928.

SM 2. Methods using GC-MS for OXBP analysis and references related to Table 2

The number of papers reporting the use of GC-EI/MS for the analysis of unregulated and emerging OXBPs has increased considerably in the last few years. Serrano et al. reported a sensitive method for the determination of seven haloacetaldehydes in water, using LLE and GC-EI/MS in SIM mode. LODs ranging between 6 and 20 ng/L were obtained [1]. In another study, Montesinos et al. developed a method for investigating the formation and occurrence of nine halonitromethanes (HNMs) in treated water. They used HS-SDME as the extraction method followed by GC-EI/MS in SIM mode. This approach provided LODs ranging from 0.08 to 1.2 µg/L [2]. A method based on HS-SPME and GC-EI/MS in SIM mode was developed by Allard et al. for the simultaneous analysis of 10 iodinated trihalomethanes (I-THMs) in water, with LODs obtained in the range 1 to 20 ng/L [3]. In the study reported by Kristiana et al., HS-SPME and GC-EI/MS (in SIM mode) were used to analyze eight halonitriles in drinking water, achieving LODs ranging from 0.9 to 80 ng/L [4]. Based on a similar methodology, Serrano et al. developed an approach for determining 14 haloketones in water; reported LODs were in the range of 15 to 600 ng/L [5]. Liew et al. reported a multi-residue method for the determination of six HNMs and four haloacetamides in drinking water, based on LLE followed by GC-EI/MS in SIM mode. Their method provided LODs from 80 to 6000 ng/L and from 80 to 2000 ng/L, for HNMs and haloacetamides respectively [6]. Montesinos and Gallego developed a method for the simultaneous determination of THMs and nine HNMs in water, using micro LLE followed by GC-MS analysis in EI and SIM modes. They reported LODs between 18 and 60 ng/L for THMs and between 9 and 400 ng/L for HNMs [7]. The same authors used a methodology based on HS-GC-EI/MS (in SIM mode) for the simultaneous determination of THMs, six HNMs and six HANs, achieving LODs in the ranges of 10 to 20 ng/L for the THMs, 30 to 140 ng/L for HNMs, and 30 to 200 ng/L for HANs [8]. Recently, Luo et al. reported the development of a multi-residue method to determine 20 OXBPs belonging to I-THMs, HNMs, and HANs in drinking water, using SPME-GC-EI/MS in SIM mode. This method achieved LODs in the ranges of 2 to 70 ng/L, 6 to 50 ng/L and 1 to 50 ng/L for the I-THMs, HNMs, and HANs, respectively [9]. GC-EI/MS in SRM mode, following LLE extraction and acidic methanol derivatization, was used by Li et al. for the determination of 10 HAAs in water. LODs were between 10 and 80 ng/L for all 10 HAAs [10]. The same team used a similar approach for determining volatile OXBPs belonging to the THM, HAN and HK families of halogenated byproducts, as well as chloropicrin. The method they developed offered rapid chromatographic analysis (10 minutes), good chromatographic separation and LODs between 3 and 14 ng/L [11].

References

[1] M. Serrano, M. Silva, M. Gallego, Micro liquid-liquid extraction combined with large-volume injection gas chromatography-mass spectrometry for the determination of haloacetaldehydes in treated water, J. Chrom. A 1218 (2011) 8295-8302

[2] I. Montesinos, M. Cardador, M. Gallego, Determination of halonitromethanes in treated water, J. Chrom. A 1218 (2011) 2497-2504

[3] S. Allard, J.W. Charrois, C.A. Joll, A. Heitz, Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid-phase microextraction and gas chromatography mass-spectrometry, J. Chrom. A 1238 (2012) 15-21

[4] I. Kristiana, C. Joll, A. Heitz, Analysis of halonitriles in drinking water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1225 (2012) 45-54.

[5] M. Serrano, M. Silva, M. Gallego, Determination of 14 haloketones in treated water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1407 (2015) 208-215

[6] D. Liew, K.L. Linge, C.A. Joll, A. Heitz, J.W.A. Charrois, Determination of halonitromethanes and haloacetamides: An evaluation of sample preservation and analyte stability in drinking water, J. Chrom. A 1241 (2012) 117-122

[7] I. Montesinos, M. Gallego. Solvent-minimized extraction for determining halonitromethanes and trihalomethanes in water, J. Chrom. A 1248(2012) 1-8

[8] I. Montesinos, M. Gallego, Speciation of common volatile halogenated disinfection by-products in tap water under different oxidising agents, J. Chrom. A 1310 (2013) 113-120

[9] Q. Luo, X. Chen, Z. Wei, X. Xu, D. Wang, Z. Wang, Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation, J. Chrom. A 1365 (2014) 45-53

[10] W. Li, Y. Liu, J. Duan, D. Mulcahy, Determination of ten haloacetic acids in water using gas chromatography-triple quadrupole mass spectrometry, Anal. Meth. 5 (2013) 2258-2266

[11] Y. Liu, J. Duan, W. Li, Q. Lai, C.P. Saint, D. Mulcahy. Determination of volatile disinfection byproducts in water by gas chromatography-triple quadrupole mass spectrometry. Anal. Lett. 48 (2015) 188-203.

SM 2. Recent reported GC-MS methods for OXBP analysis in water samples

Analytes	Approach	Pre-treatment	Injection modes	GC Column	MS method	Acquisition mode	LODs (ng/L)	Ref.
Has	Target	LLE	PTV (30 µL)	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	6-20	[1]
HNMs	Target	HS-SDME	Split, 17 0°C (2 µL, 1:20 ratio)	TRB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	80-1200	[2]
I-THMs	Target	HS-SPME	PTV	ZB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	1-20	[3]
Halonitriles	Target	HS-SPME	Splitless, 200 °C	ZB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	0.9-80	[4]
HKs	Target	HS-SPME	Splitless, 250 °C	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	15-600	[5]
HNMs, HAAms	Target	LLE	Splitless, 140 °C	ZB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	80-6000 / 80-2000	[6]
THMs, HNMs	Target	MLLE	PTV (30 µL)	SLB-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	18-60 / 9-400	[7]
THMs, HNMs, and HANs	Target	HS	Split, 170 °C (1:20 ratio)	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (70eV)-qMS	SIM	10-20 / 30-140 / 30-200	[8]
I-THMs, HNMs, HANs	Target	SPME	Splitless	TRB-5 (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI(70eV)-qMS	SIM	2-70	[9]
HAAs	Target	LLE	Splitless, 210 °C	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI-tqMS	SRM	10-80	[10]
THMs, HANs, and HKs	Target	LLE	Splitless, 180 °C	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI (45 to 80 eV)-tqMS	SRM	3-14	[11]

Halobenzoquinones	Target	SPE + derivatization (MTBSTFA)	Splitless, 270 °C	HP-5MS (30 m, 0.25 mm i.d., 0.25 µm film thickness)	EI(70eV)-qMS	SIM	0.03-0.9	[12]
Halobenzoquinones	Non-target	SPE	PTV (20 µL)	HP-5MS (30 m, 0.25 mm i.d., 0.25µm film thickness)	EI (70eV)/PCI-IT MS	Full-scan, MS/MS	-	[13]
Halohydrins	Non-target	LLE	Splitless	Rtx-5 (50 m, 0.25 mm i.d., 0.25µm film thickness)	EI (70eV)/PCI-TOF	Full-scan	-	[14]
170 volatile and semi-volatile DBPs	Non-target	SPE	Splitless, 280 °C	DB5-MS (30 m, 0.25 mm i.d., 0.25 µm film thickness) and BPX 50 (2 m, 0.1 mm i.d., 0.1 µm film thickness)	EI (70eV)-qMS	Full-scan	-	[15]
Bromoimidazoles, bromoanilines, and bomomethanesulfenic acid esters	Non-target	XAD resin	Splitless, 250 °C	Rtx-5 (50 m, 0.25 mm i.d., 0.25µm film thickness)	EI (70eV)-msMS	Full-scan	-	[16]
OIBPs	Non-target	XAD resin	Splitless, 280 °C	TR-5MS (60 m, 0.25 mm i.d., 0.25 µm film thickness)	EI-q-orbitrap MS	Full-scan	-	[17]

Abbreviations: TOF: time-of-flight, qMS: single quadrupole MS, msMS: high resolution magnetic sector, IT: ion trap MS, EI: electron ionization, PCI: positive chemical ionization, SIM: single ion monitoring, SRM: selected reaction monitoring, PTV: programmed temperature vaporization injector, Single drop microextraction in the headspace mode, HS: static headspace, LLE: liquid-liquid extraction, MLLE: micro liquid-liquid extraction, SPE: solid-phase extraction, MTBSTFA: N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide, OIBPs: organoiodine by-products, HAs: Haloacetaldehydes, HNMs: halonitromethanes, HS-SDME: HKs: haloketones

References

[1] M. Serrano, M. Silva, M. Gallego, Micro liquid-liquid extraction combined with large-volume injection gas chromatography-mass spectrometry for the determination of haloacetaldehydes in treated water, J. Chrom. A 1218 (2011) 8295-8302

[2] I. Montesinos, M. Cardador, M. Gallego, Determination of halonitromethanes in treated water, J. Chrom. A 1218 (2011) 2497-2504

[3] S. Allard, J.W. Charrois, C.A. Joll, A. Heitz, Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid-phase microextraction and gas chromatography mass-spectrometry, J. Chrom. A 1238 (2012) 15-21

[4] I. Kristiana, C. Joll, A. Heitz, Analysis of halonitriles in drinking water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1225 (2012) 45-54.

[5] M. Serrano, M. Silva, M. Gallego, Determination of 14 haloketones in treated water using solid-phase microextraction and gas chromatography-mass spectrometry, J. Chrom. A 1407 (2015) 208-215

[6] D. Liew, K.L. Linge, C.A. Joll, A. Heitz, J.W.A. Charrois, Determination of halonitromethanes and haloacetamides: An evaluation of sample preservation and analyte stability in drinking water, J. Chrom. A 1241 (2012) 117-122

[7] I. Montesinos, M. Gallego, Solvent-minimized extraction for determining halonitromethanes and trihalomethanes in water, J. Chrom. A 1248(2012) 1-8

[8] I. Montesinos, M. Gallego, Speciation of common volatile halogenated disinfection by-products in tap water under different oxidizing agents, J. Chrom. A 1310 (2013) 113-120

[9] Q. Luo, X. Chen, Z. Wei, X. Xu, D. Wang, Z. Wang, Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation, J. Chrom. A 1365 (2014) 45-53

[10] W. Li, Y. Liu, J. Duan, D. Mulcahy, Determination of ten haloacetic acids in water using gas chromatography-triple quadrupole mass spectrometry, Anal. Meth. 5 (2013) 2258-2266

[11] Y. Liu, J. Duan, W. Li, Q. Lai , C.P. Saint, D. Mulcahy, Determination of volatile disinfection byproducts in water by gas chromatography-triple quadrupole mass spectrometry, Anal. Lett. 48 (2015) 188-203

[12] R. Rodil, J.B. Quintana, G. Basaglia, M.C. Pietrogrande, R. Cela, Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry, J. Chrom. A 1217 (2010) 6428-6435

[13] R. Rodil, J.B. Quintana, R. Cela, Oxidation of synthetic phenolic antioxidants during water chlorination, J. Hazard. Mater. 199 (2012) 73-81

[14] K.J. Jobst, V.Y. Taguchi, R.D. Bowen, M.A. Trikoupis, J.K. Terlouw, Identification of halohydrins as potential disinfection by-products in treated drinking water, Int. J. of Spectrosc. 2011 (2011) 1-7

[15] C. Li, D. Wang, N. Li, Q. Luo, X. Xu, Z. Wang, Identifying unknown by-products in drinking water using comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry and in silico toxicity assessment, Chemosphere 163 (2016) 535-543

[16] E.J. Daiber, D.M. DeMarini, S.A. Ravuri, H.K. Liberatore, A.A. Cuthbertson, A. Thompson-Klemish, J.D. Byer, J.E. Schmid, Me.Z. Afifi, E.R. Blatchley, S.D. Richardson, Progressive increase in disinfection byproducts and mutagenicity from source to tap to swimming pool and spa water: impact of human inputs, Environ. Sci. Technol. 50 (2016) 6652-6662

[17] C. Postigo, C.I. Cojocariu, S.D. Richardson, P.J. Silcock, D. Barcelo, Characterization of iodinated disinfection by-products in chlorinated and chloraminated waters using orbitrap based gas chromatography-mass spectrometry, Anal. Bioanal. Chem. 408 (2016) 3401-3411

SM 3. Methods using LC-MS for OXBP analysis and references related to Table 3

LC-MS and LC-MS/MS have been recently applied in selected reaction monitoring (SRM) mode to the quantitative determination of several classes of OXPBs in waters. In the study reported by Chu et al., a method for determining 13 haloacetamides in drinking water was presented, based on solid phase extraction (SPE) followed by LC(C18)-APCI/MS/MS operating in negative-ion MRM mode. LODs ranging from 7.6 to 19.7 ng/L were obtained [1]. Zhao et al. used LC(C18)-ESI/MS/MS operating in negative-ion SRM mode to analyze eight halobenzoquinones (HBQs) in chlorinated drinking water and to investigate their formation from phenolic compounds under different disinfection and treatment processes. The HBQs were extracted from water by SPE. The proposed method achieved LODs in the range of 0.5 to 37.9 ng/L [2,3]. More recently, Huang et al. described a method for determining 12 HBQs in drinking water using SPE-UPLC(C18)-ESI/MS/MS operating in negative mode, yielding LODs in the sub-ng/L range [4]. Chen et al. reported a UPLC(HILIC)-ESI/MS/MS method, operated in negative mode, for the quantification of 10 HAAs (including monoiodoacetic acid) in drinking water. LODs in the range of 0.08 to 2.7 μg/L were achieved without pre-concentration [5]. Comparable sensitivity was obtained by Meng et al. with a C18 column [6]. LC(C18)-ESI/MS/MS operated in negative ion and SRM modes was employed by Li et al. to directly analyze four iodoacetic acids, namely monoiodoacetic acid, chloro-iodoacetic acid, bromo-iodoacetic acid and diiodoacetic acid, in drinking water without previous preparation. LODs below 2 ng/L were obtained for all target analytes [7]. Today, the technology of LC-MS with precursor ion scanning is well-developed and this has become the preferred method for the structural elucidation of polar OXBPs. In recent years, this methodology has been successfully applied for the identification of more than 50 new polar OXBPs [8,9,10,11]. In the study by Ding et al., UPLC-ESI/MS/MS was used to study the nature of brominated disinfection by-products formed during the chlorination of saline sewage effluents. Measurements were taken by a triple-quadrupole mass analyzer. Fifty-four compounds were detected using precursor ion scan mode for bromide ions (m/z 79 and 81). In the product ion scan mode, six detected compounds were identified, for the first time, as wastewater DBPs. These included: bromomaleic acid, 5-bromosalicylic acid, 3,5-dibromo-4-hydroxybenzaldehyde, 3,5-dibromo-4-hydroxybenzoic acid, 2,6-dibromo-4-nitrophenol, and 2,4,6-tribromophenol [8]. Another study, using a similar methodology to characterize unknown DBPs in chlorinated saline wastewater, was reported by Yang and Zhang. In this research, a series of halopyrroles, including tetrabromopyrrole, tribromochloropyrrole, tribromoiodopyrrole, and tribromopyrrole, were newly identified as wastewater DBPs in a chlorinated saline wastewater effluent [11]. Pan and Zhang applied UPLC-ESI/MS/MS to the detection and identification of polar, halogenated DBPs in simulated drinking water samples. Polar chloro-DBPs and bromo-DBPs were selectively identified by setting the PIS to m/z 35/37 and m/z 79/81. With this approach, the authors successfully identified 11 new aromatic halogenated DBPs classified into four groups: dihalo-4-hydroxybenzaldehydes, dihalo-4-hydroxybenzoic acids, dihalo-salicylic acids, and

trihalo-phenols [12]. More recently, Gong and Zhang identified new iodinated DBPs in chlorinated saline wastewater effluents. Molecular structure elucidation and identification were performed using UPLC-ESI/MS/MS operated in precursor ion scan mode at m/z 127 (iodide ions) and product ion scan mode. Several polar iodinated DBPs were detected, among which a new group was identified: iodo-trihydroxybenzenesulfonic acids [10]. Using the same approach, Pan et al. recently identified 11 new polar iodinated DBPs in drinking water [13].

References

[1] W. Chu, N. Gao, D. Yin, S.W. Krasner, M.R. Templeton, Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization, J. Chrom. A 1235 (2012) 178-181

[2] Y. Zhao, F. Qin, J.M. Boyd, J. Anichina, X.-F. Li, Characterization and determination of chloro-and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water, Anal. Chem. 82 (2010) 4599-4605

[3] Y. Zhao, J. Anichina, X. Lu, R.J. Bull, S.W. Krasner, S.E. Hrudey, X.-F. Li, Occurrence and formation of chloro-and bromo-benzoquinones during drinking water disinfection, Water Res. 46 (2012) 4351-4360

[4] R. Huang, W. Wang, Y. Qian, J.M. Boyd, Y. Zhao, X.-F. Li, Ultra pressure liquid chromatography-negative electrospray ionization mass spectrometry determination of twelve halobenzoquinones at ng/L levels in drinking water, Anal. Chem. 85 (2013) 4520-4529

[5] C.-Y. Chen, S.-N. Chang, G.-S. Wang, Determination of ten haloacetic acids in drinking water using high-performance and ultra-performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. Sci. 47 (2009) 67-74

[6] L. Meng, S. Wu, F. Ma, A. Jia, J. Hu, Trace determination of nine haloacetic acids in drinking water by liquid chromatography-electrospray tandem mass spectrometry, J. Chrom. A 1217 (2010) 4873-4876

[7] Y. Li, J.S. Whitaker, C.L. McCarty, Analysis of iodinated haloacetic acids in drinking water by reversed-phase liquid chromatography/electrospray ionization/tandem mass spectrometry with large volume direct aqueous injection, J. Chrom. A 1245 (2012) 75-82

[8] G. Ding, X. Zhang, M. Yang, Y. Pan, Formation of new brominated disinfection byproducts during chlorination of saline sewage effluents, Water Res. 47 (2013) 2710-2718

[9] H. Zhai, X. Zhang, X. Zhu, J. Liu, M. Ji, Formation of brominated disinfection byproducts during chloramination of drinking water: new polar species and overall kinetics, Environ. Sci. Technol. 48 (2014) 2579-2588

[10] T. Gong, X. Zhang, Detection, identification and formation of new iodinated disinfection byproducts in chlorinated saline wastewater effluents, Water Res. 68 (2015) 77-86

[11] M. Yang, X. Zhang, Halopyrroles: a new group of highly toxic disinfection byproducts formed in chlorinated saline wastewater, Environ. Sci. Technol. 48 (2014) 11846-11852

[12] Y. Pan, X. Zhang, Four groups of new aromatic halogenated disinfection byproducts: effect of bromide concentration on their formation and speciation in chlorinated drinking water, Environ. Sci. Technol. 47 (2013) 1265-1273

[13] Y. Pan, W. Li, H. An, H. Cui, Y. Wang, Formation and occurrence of new polar iodinated disinfection byproducts in drinking water, Chemosphere 144 (2016) 2312-2320.

SM 3. Recent reported LC-MS methods for OXBP analysis in water samples

Analytes	Approach	Extraction	Injection	LC Column	MS analyzer	Acquisition mode	LODs (ng/L)	Ref.
HacAms	Target	SPE	10 µL	Hypersil GOLD C18 (100 × 2.1 mm i.d., 5 µm particule size)	APCI-tq MS	SRM	7.6-19.7	[1]
HBQs	Target	SPE	20 µL	Luna C18 (100 × 2.0 mm i.d., 3 µm particule size)	ESI-tq MS	SRM	0.5-37.9	[2,3]
HBQs	Non-target and target	SPE	10 µL	BEH C18 (100 x 2.1 i.d., 1.7µm particule size)	ESI-TOF MS ESI-tq MS	SRM	0.2-6.6	[4]
HAAs	Target	DI	30 µL	BEH HILIC column	ESI-tq MS	SRM	80-2700	[5]
HAAs	Target	DI	15 µL	BEH C8 (100 × 2.1 mm i.d., 1.7 µm particule size)	ESI-tq MS	SRM	160-1440	[6]
I-HAAs	Target	DI	1000 µL	Gemini C18 (150 x 4.6 mm i.d., 3 µm particule size)	ESI-tq MS	SRM	0.5-2.3	[7]
ObrBPs	Non-target	LLE	Infusion, UPLC (5 µL)	HSS T3 column (100 × 2.1 mm i.d., 1.8 µm particule size)	ESI-qTOF MS	PIS (m/z 79 and 81), SRM, and MS/MS	-	[8]
HBQs	Non-target	SPE	20 µL	Luna C18 (100 × 2.0 mm i.d., 3 µm particule size)	ESI-qTOF MS ESI-tq MS	MS/MS, and SRM	-	[9]
ObrBPs	Non-target	LLE	-	HSS T3 column (100 × 2.1 mm i.d., 1.8 µm particule size) and, UFLC-XR LC 75 x 3.0mm i.d., 2.2 µm)	ESI-tq MS and ESI-IT-TOF-MS	PIS (m/z 79 and 81), and MS/MS	-	[10]
Iodo-trihydroxybenzenesulfonic	Non-target	LLE (MtBE)	7.5 µL	-	ESI-tq MS	PIS (m/z 126.9), SRM, and MS/MS	-	[11]

acids								
Halopyrroles	Non-target	LLE	7.5 µL	HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS	PIS (m/z 79 and 81), SRM, and MS/MS	-	[12]
OXBPs	Non-target	LLE	5 µL	HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS	PIS (m/z 126.9), SRM, and MS/MS	-	[13]
3 iodinated acetic acids, 6 carbonaceous phenolic I-DBPs, and 2 nitrogenous phenolic I-DBPs	Non-target	-	5 µL	HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS	PIS (m/z 126.9), SRM, and MS/MS	-	[14]
OXBPs derived from sulfonamide antibiotics	Non-target	DI	20 µL	Atlantis T3 (3 mm x 150 mm, 3 µm particule size)	ESI-q-orbitrap MS	Full-scan and MS/MS	-	[15]
Polar ObrBPs	Non-target	LLE	Infusion	-	ESI-tq MS	PIS (m/z 79 and 81)	-	[16]
Trihalo-hydroxy-cyclopentene-diones	Non-target			HSS T3 (1.2 mm x 100 mm, 1.8 µm particule size)	ESI-tq MS and ESI-IT-orbitrap MS	Full-scan, PIS, SRM, and MS/MS	-	[17]
Polar OIBPs	Non-target	SPE	Infusion	-	ESI-FT-ICR MS (9.4 T)	Full-scan (m/z 150-800)	-	[18]
Polar ObrBPs	Non-target	SPE	Infusion	-	ESI qFT-ICR MS (9.4T)	Full-scan, MS/MS	-	[19]
Polar OclBPs	Non-target	SPE	Infusion	-	ESI FT-ICR MS (12 T)	Full-scan	-	[20]

Abbreviations: tqMS: triple quadrupole MS, IT: ion trap, FT-ICR: Fourier transform ion cyclotron resonance, ESI: electrospray ionization, APCI: atmospheric pressure chemical ionization, PIS: precursor ion scan, SRM: selected reaction monitoring, UPLC: ultra-performance liquid chromatography, DI: direct injection, SPE: solid-phase extraction, LOD: limit of detection, OClBPs: organochlorine by-products, OBrBPs: organobromine by-products, OIBPs: organoiodine by-products, HBQs: halobenzoquinones, MtBE: ethyl tert-butyl ether

References

[1] W. Chu, N. Gao, D. Yin, S.W. Krasner, M.R. Templeton, Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization, J. Chrom. A 1235 (2012) 178-181

[2] R. Huang, W. Wang, Y. Qian, J.M. Boyd, Y. Zhao, X.-F. Li, Ultra pressure liquid chromatography-negative electrospray ionization mass spectrometry determination of twelve halobenzoquinones at ng/L levels in drinking water, Anal. Chem. 85 (2013) 4520-4529

[3] Y. Zhao, J. Anichina, X. Lu, R.J. Bull, S.W. Krasner, S.E. Hrudey, X.-F. Li, Occurrence and formation of chloro-and bromo-benzoquinones during drinking water disinfection, Water Res. 46 (2012) 4351-4360

[4] R. Huang, W. Wang, Y. Qian, J.M. Boyd, Y. Zhao, X.-F. Li, Ultra pressure liquid chromatography-negative electrospray ionization mass spectrometry determination of twelve halobenzoquinones at ng/L levels in drinking water, Anal. Chem. 85 (2013) 4520-4529

[5] C.-Y. Chen, S.-N. Chang, G.-S. Wang, Determination of ten haloacetic acids in drinking water using high-performance and ultra-performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. Sci. 47 (2009) 67-74

[6] L. Meng, S. Wu, F. Ma, A. Jia, J. Hu, Trace determination of nine haloacetic acids in drinking water by liquid chromatography-electrospray tandem mass spectrometry, J. Chrom. A 1217 (2010) 4873-4876

[7] Y. Li, J.S. Whitaker, C.L. McCarty, Analysis of iodinated haloacetic acids in drinking water by reversed-phase liquid chromatography/electrospray ionization/tandem mass spectrometry with large volume direct aqueous injection, J. Chrom. A 1245 (2012) 75-82

[8] G. Ding, X. Zhang, M. Yang, Y. Pan, Formation of new brominated disinfection byproducts during chlorination of saline sewage effluents, Water Res. 47 (2013) 2710-2718

[9] Y. Zhao, F. Qin, J.M. Boyd, J. Anichina, X.-F. Li, Characterization and determination of chloro-and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water, Anal. Chem. 82 (2010) 4599-4605

[10] H. Zhai, X. Zhang, X. Zhu, J. Liu, M. Ji, Formation of brominated disinfection byproducts during chloramination of drinking water: new polar species and overall kinetics, Environ. Sci. Technol. 48 (2014) 2579-2588

[11] T. Gong, X. Zhang, Detection, identification and formation of new iodinated disinfection byproducts in chlorinated saline wastewater effluents, Water Res. 68 (2015) 77-86

[12] M. Yang, X. Zhang, Halopyrroles: a new group of highly toxic disinfection byproducts formed in chlorinated saline wastewater, Environ. Sci. Technol. 48 (2014) 11846-11852

[13] Y. Pan, X. Zhang, Four groups of new aromatic halogenated disinfection byproducts: effect of bromide concentration on their formation and speciation in chlorinated drinking water, Environ. Sci. Technol. 47 (2013) 1265-1273

[14] Y. Pan, W. Li, H. An, H. Cui, Y. Wang, Formation and occurrence of new polar iodinated disinfection byproducts in drinking water, Chemosphere, 144 (2016) 2312-2320

[15] M. Wang, D.E. Helbling, A non-target approach to identify disinfection byproducts of structurally similar sulfonamide antibiotics, Water Res. 102 (2016) 241-251

[16] X. Zhang, J.W. Talley, B. Boggess, G. Ding, D. Birdsell, Fast selective detection of polar brominated disinfection byproducts in drinking water using precursor ion scans. Environ. Sci. Technol. 42 (2008) 6598-6603

[17] Y. Pan, W. Li, A. Li, Q. Zhou, P. Shi, Y. Wang, A new group of disinfection byproducts in drinking water: Trihalo-hydroxy-cyclopentene-diones. Environ. Sci. Technol. 50 (2016) 7344-7352

[18] X. Wang, J. Wang, Y. Zhang, Q. Shi, H. Zhang, Y. Zhang, M. Yang, Characterization of unknown iodinated disinfection byproducts during chlorination/chloramination using ultrahigh resolution mass spectrometry. Sci. Total Environ. 554-555 (2016) 83-88

[19] H. Zhang, Y. Zhang, Q. Shi, H. Zheng, M. Yang, Characterization of unknown brominated disinfection byproducts during chlorination using ultrahigh resolution mass spectrometry. Environ. Sci. Technol. 48 (2014) 3112-3119

[20] E. E. Lavonen, M. Gonsior, L.J. Tranvik, P. Schmitt-Kopplin, S.J. Köhler, Selective chlorination of natural organic matter: identification of previously unknown disinfection byproducts. Environ. Sci. Technol. 47 (2013) 2264-2271.

CHAPITRE II – MATERIELS ET METHODES

L’objectif de cette deuxième partie est de présenter et justifier l’ensemble des aspects expérimentaux développés et/ou utilisés au cours de ce travail de thèse.

Cette partie se découpe en deux sous-parties :

- La première sous-partie présente les sites d’étude choisis et explicite la stratégie de prélèvement et de conditionnement mise en œuvre.
- La seconde sous-partie présente les protocoles et appareils de mesure utilisés pour l’analyse des paramètres physico-chimiques classiques liés à la qualité des eaux (COD, absorbance UV_{254}, ions halogénures, etc.).

I. Sites d'étude et plan d'échantillonnage

I.1 Choix des sites CNPE d'étude

Les sous-produits organohalogénés (SPOX) diffèrent par leur nature et leur concentration en fonction des caractéristiques physico-chimiques de l'eau de rivière et des modalités de traitement employées. Ainsi, pour prendre en compte les particularités relatives à la qualité de l'eau, trois sites CNPE ont été échantillonnés : Cattenom, Saint-Laurent et Chooz. Les raisons ayant motivé la sélection de ces trois CNPE relèvent à la fois de leur localisation géographique (CNPE alimentés par trois cours d'eau différents), et de leurs teneurs en AOX relativement élevées (environ 50 µg éq. Cl/L) comparativement aux autres sites d'EDF traitant à la monochloramine.

I.2 Positionnement des points de prélèvement

Pour chaque site d'étude, les échantillons d'eau ont été prélevés en deux endroits : (i) dans la rivière, en amont de la CNPE et, (ii) dans le circuit de refroidissement (CRF) au niveau du « bassin froid ». Les prélèvements ont été effectués à des emplacements équivalents en amont des sites d'étude.

I.3 Période et fréquence d'échantillonnage

Afin de mieux prendre en compte l'influence de la variabilité temporelle des échantillons d'eaux sur la formation des SPOX, les prélèvements se sont déroulés de fin juillet à début d'octobre 2016, avec des intervalles intra-prélèvements compris entre 6 et 16 jours. Six campagnes de prélèvements ont eu lieu pour les CNPE de Cattenom et de Chooz, et quatre pour la CNPE de Saint-Laurent, soit 16 campagnes au total. Pour chaque site, les dates de prélèvements ont été planifiées de façon à respecter les périodes de début de traitement et les dates préétablies d'arrêt des tranches. Notons qu'un prélèvement hors période de traitement a été effectué pour les CNPE de Saint-Laurent et Chooz afin d'obtenir un point « 0 ». Pour des raisons pratiques et logistiques, les sites n'ont pas été échantillonnés en parallèle mais à tour de rôle. Les prélèvements ont été effectués les lundis et mercredis des semaines ne comportant pas de jours fériés. En dépit de quelques difficultés ponctuelles dues à des arrêts intempestifs du traitement à la monochloramine sur les tranches présélectionnées, les prélèvements ont été réalisés conformément au planning préétabli.

I.4 Prélèvement, conditionnement et expédition des échantillons

Les prélèvements d'eaux ont été confiés à l'entreprise prestataire CARSO qui a mobilisé son personnel en charge des prélèvements pour les programmes de suivis réglementaires des sites d'étude ; les recommandations de la norme ISO 5667-3 ont été suivies [1]. Dans le but d'éviter tout risque de « contamination croisée », des flacons-types ont été attribués à chaque site et chaque point de

prélèvement. L'échantillonnage a consisté en la réalisation de prélèvements manuels, ponctuels, au moyen de sceaux. Les échantillons ont été prélevés dans des flacons de verre de 2 L pré-conditionnés avec une solution d'acide ascorbique à 1,6 g/L (2 mL par 2 L d'eau). Les flacons ont été remplis à ras-bord, sans débordement (perte de réactifs) et sans aucune bulle d'air afin d'éviter la perte de SPOX volatils. Des échantillons d'eau ont également été prélevés dans des contenants en plastique de 5 L sans conditionnement préalable. Pour les deux points de prélèvement (amont et bassin froid), un volume total de 40 L d'eau a été échantillonné. Le tableau II-1 récapitule le nombre et le volume d'échantillons d'eau prélevés par site et par campagne.

Tableau II-1. Nature, volume et nombre d'échantillons d'eau prélevés par site et par campagne

Points de prélèvement	Volume et nombre d'échantillons prélevés	
	Conditionnement à l'acide ascorbique	Sans conditionnement
Eau de rivière, amont du CNPE	15 x 2 L (30 litres)	2 x 5 L (10 litres)
Circuit, bassin froid	15 x 2 L (30 litres)	2 x 5 L (10 litres)

Conjointement aux prélèvements destinés à EDF R&D, des prélèvements ont été réalisés puis envoyés au laboratoire d'analyse de la société CARSO pour l'analyse des paramètres relatifs à la qualité physico-chimique de l'eau (voir chapitre III-RESULTATS ET DISCUSSION).

Immédiatement après prélèvement, les flacons sont placés dans des glacières contenant des blocs réfrigérants pour maintenir leur température entre 4 et 8 °C pendant le transport jusqu'au laboratoire d'analyse. L'acheminement des prélèvements aux laboratoires d'EDF R&D a été assuré par une société spécialisée dans le transport urgent : CETUP (Compagne Européenne de Transports Urgents Personnalisés). Les échantillons ont été livrés le jour-même du prélèvement. Dès réception, ceux-ci ont été soit traités, soit stockés au réfrigérateur à 4 °C ± 2 °C. Dans tous les cas, les échantillons ont été traités dans les vingt-quatre heures suivant leur réception. Pour chaque point de prélèvement, un aliquot d'environ 2 L a été congelé en cas de besoin d'investigations complémentaires.

II. Analyses physico-chimiques des paramètres classiques

Les échantillons d'eau prélevés ont fait l'objet d'analyses réalisées à la fois sur le terrain et au laboratoire. Trois catégories de paramètres ont été suivies :

(i) les paramètres relatifs au fonctionnement des CNPE,

(ii) les paramètres relatifs à la qualité physico-chimique de l'eau,

(iii) la nature des SPOX produits et leurs paramètres globaux.

Chacun des paramètres a été mesuré sur l'ensemble des points d'échantillonnage ou sur une partie de ceux-ci, selon que cela ait été jugé pertinent ou non en termes d'intérêt scientifique et de ressources disponibles. Ce paragraphe traite des deux premières catégories de paramètres.

II.1 Paramètres liés au fonctionnement des CNPE

La première catégorie de paramètres a été déterminée sur le terrain par les préleveurs de la société CARSO (Venissieux, Lyon) parallèlement aux opérations de prélèvements. Elle comprend les paramètres suivants : pH, température, chlore résiduel libre (CRL) et chlore résiduel total (CRT).

Le dosage du CRL et CRT a été effectué à l'aide de la méthode colorimétrique à la N,N-Diéhylphénylène-1,4-diamine (DPD) par dissolution de pastilles pour l'analyse du CRL (DPD1) et du CRT (DPD3), selon le principe de la méthode normalisée NF EN ISO 7393-2 [2]. La limite de quantification de cette méthode est de 50 µg éq. Cl_2/L. La coloration produite a été mesurée grâce à un spectrophotomètre HACH (Pocket Colorimeter II) avec des cuves de 1 cm. La température de l'eau a été mesurée à tous les points à l'aide d'un thermomètre à alcool standard.

Le pH a été déterminé par électrochimie avec un pH-mètre ProfiLine Multi 3320, WTW, selon la norme NF EN ISO 10523 [3].

II.2 Paramètres relatifs à la qualité physico-chimique de l'eau

Les mesures relatives à la qualité physico-chimique de l'eau ont concerné principalement deux paramètres : la matière organique (naturelle et anthropique) et les ions halogénures.

Les indicateurs de la présence de matière organique qui ont été suivis sont le carbone organique dissous (COD) et l'absorbance dans l'ultraviolet à la longueur d'onde 254 nm, noté UV_{254}. Ces deux paramètres sont complémentaires : le COD renseigne sur la quantité de matière organique disponible dans le milieu et l'UV_{254} indique sa réactivité et donc sa tendance à former certains SPOX. Globalement, pour une eau donnée, plus la concentration en COD et l'absorbance UV_{254} sont élevées, plus la réaction entre la matière organique et la monochloramine est importante. L'absorbance spécifique $SUVA_{254}$ qui correspond à la valeur de l'absorbance UV_{254} divisée par la teneur en COD permet de déterminer l'aromaticité de la matière organique contenue dans un échantillon d'eau. Cette grandeur peut donc être utilisée en théorie comme un moyen d'estimer le potentiel de formation des SPOX d'une eau donnée. La concentration en ions halogénures joue un rôle important dans la spéciation des SPOX. Plus une eau est riche en ions bromure, plus les sous-produits bromés seront en quantité importante relativement aux chlorés.

Ces analyses ont été confiées au laboratoire d'analyse de la société CARSO. L'absorbance UV a été mesurée à une longueur d'onde de 254 nm à l'aide d'un spectrophotomètre UV-visible DR/4000 U (HACH) en cuve de 5 cm, après filtration membranaire à 0,45 µm.

Le COD est dosé dans la phase dissoute issue de la filtration sur filtre de cellulose (HVLP, Millipore) de diamètre de pores 0,45 µm, suivant la méthode normalisée NF EN 1484 [4]. Le filtrat contenant le COD est acidifié à pH = 2 avec de l'acide orthophosphorique à 5% afin de transformer les ions carbonates et bicarbonates en dioxyde de carbone (CO_2). Ce dernier sera éliminé par barbotage à l'azote. Cette étape permet l'élimination du carbone inorganique dissous. Ensuite, le COD est dosé par détection infrarouge du CO_2 libéré suite à une réaction d'oxydation chimique du COD présent dans l'échantillon avec le persulfate de sodium à 100 °C. La quantité de CO_2 détectée est proportionnelle à la quantité de COD. L'appareil utilisé est un COT-mètre de marque Shimadzu (TOC 5000A), calibré à l'aide d'une solution standard de phtalate de potassium diluée à différentes concentrations. Les résultats obtenus sont exprimés en mg éq. C/L. La limite de quantification de cette méthode est de 0,3 mg éq. C/L.

Les ions chlorure et bromure ont été mesurés par chromatographie ionique selon la norme NF EN ISO 10304-1, tandis que les iodures ont été analysés par spectroscopie d'émission optique sur plasma induit par laser (ICP/AES) sur une chaine Optima 7300 (Perkin Elmer, Villebon-sur-Yvette), suivant la norme NF EN ISO 11885 [5,6]. La limite de quantification est de 100 µg/L pour les ions chlorure, 50 µg/L pour les ions bromure et 1 µg/L pour les ions iodure.

II.3 Analyse des sous-produits organohalogénés par des méthodes physico-chimiques

II.3.1 Extraction des sous-produits organohalogénés sur phase solide (SPE)

Compte tenu de la complexité de la matrice « eau de rivière » et des faibles teneurs en SPOX attendues, une étape d'extraction s'imposait afin d'isoler les composés d'intérêt des interférents et de les concentrer à des niveaux compatibles avec les sensibilités des outils analytiques utilisés. A ce jour, cette étape d'enrichissement est couramment réalisée par extraction en phase liquide-liquide au méthyl-tert-butyl éther (MTBE). Dans le cadre de ce travail de thèse, notre choix s'est porté sur l'extraction sur phase solide, puisqu'elle offre le double avantage de concentrer avec des facteurs d'enrichissement très élevés et d'être compatible avec de nombreuses méthodes séparatives. La littérature scientifique ne recense pour le moment que peu d'applications de cette technique à l'extraction des SPOX.

Dans les paragraphes suivants, nous présentons le principe d'extraction sur phase solide, succinctement puisque celui-ci est déjà largement décrit dans la littérature scientifique [7], ainsi que l'instrumentation utilisée et les protocoles mis en place.

II.3.1.1 Principe de l'extraction sur phase solide (SPE)

Le principe de l'extraction sur phase solide repose sur la distribution des composés à extraire entre une phase liquide : l'échantillon, et une phase solide : l'adsorbant. Cette distribution est gouvernée par des mécanismes de rétention basés sur les multiples interactions entre les molécules à extraire, les sites actifs de la phase adsorbante et la phase liquide (eau et solvants d'élution). Le déroulement d'une préparation d'échantillon par SPE comprend typiquement quatre étapes. La première est le conditionnement de l'adsorbant contenu dans la cartouche de l'extraction. Cette étape permet d'activer les groupements fonctionnels de l'adsorbant à l'aide d'un solvant adéquat. Lors de la deuxième étape, l'échantillon est chargé sur le support d'extraction ; les analytes ainsi qu'une partie des interférents sont retenus sur la phase stationnaire. Cette étape est suivie par un lavage visant à éluer les composés indésirables. La dernière étape permet d'éluer les analytes d'intérêt par un solvant ou un mélange de solvants organiques présentant une plus forte affinité pour les analytes que la phase stationnaire. La SPE tire son véritable intérêt de sa simplicité, sa consommation réduite en solvants organiques, son automatisation et sa diversité en termes de supports d'extraction disponibles ou en cours de développement. Un état de l'art des méthodes d'extraction des SPOX par SPE est donné au chapitre I.

II.3.1.2 Instrumentation

Les extractions par SPE ont été effectuées à l'aide d'un extracteur automatique Autotrace 280 (Thermo Fisher Scientific, Courtaboeuf, France). Cet automate d'extraction, présenté figure II-1, permet d'automatiser les étapes de percolation d'échantillon, de lavage, de séchage et d'élution des cartouches d'extraction. Il est adapté à des échantillons de grand volume et à des cartouches d'extraction de 6 mL.

Figure II-1. Automate SPE Autotrace 280 (Thermo Fisher Scientific, Courtaboeuf, France)

II.3.1.3 Protocoles expérimentaux

Deux protocoles d'extraction ont été développés. Le premier est destiné à l'extraction de 11 acides haloacétiques (AHA), le second à l'extraction de 26 composés appartenant à six familles différentes de SPOX (4 THMs, 4 I-HMs), 1 HAL, 6 HKs, 4 HNMs, et 7 HANs). Le choix de la phase stationnaire est une étape primordiale pour l'extraction sur phase solide. Ce choix est évidemment dicté par la nature des composés à analyser et par le volume d'échantillon à extraire. Plusieurs cartouches SPE de phases stationnaires différentes ont été testées. Une description détaillée des adsorbants testés, des essais effectués et des protocoles finalement retenus est donnée dans la partie II, chapitre III (RESULTATS ET DISCUSSION) du présent manuscrit.

II.3.2 Fractionnement par ultrafiltration (UF) des SPOX

Les substances chimiques composant les AOX se trouvent sous forme d'un mélange hétérogène de composés. L'identification structurale de ces composés, présents généralement à l'état de traces dans

des matrices complexes, est une opération très difficile à accomplir. Par ailleurs, dans ce contexte, une question demeure : Comment cibler spécifiquement des molécules potentiellement toxiques ? Une approche consiste à simplifier ces mélanges par fractionnement, selon les propriétés physico-chimiques (hydrophobicité, propriétés acido-basiques, taille, et). Le fractionnement des AOX en fractions plus homogènes est très intéressant à de nombreux égards ; il permet de :

- simplifier l'identification des SPOX par le recours à la technique analytique la mieux adaptée à la fraction isolée,
- déterminer le taux de recouvrement entre les AOX mesurés dans chacune des fractions collectées et les AOX mesurés dans l'échantillon mère, ce qui permet de déterminer des indicateurs globaux quant aux catégories de substances où le manque d'information structurale est le plus important,
- comparer la toxicité globale de l'échantillon à celle de chacune des fractions, pour diriger l'effort d'élucidation structurale sur les fractions les plus toxiques.

Les méthodes de fractionnement les plus fréquemment employées reposent sur l'utilisation de techniques d'adsorption sur des colonnes de résines - ioniques et/ou non ioniques -, sur des techniques de séparation par taille ou par extraction liquide-liquide. Le fractionnement selon la taille des molécules peut être réalisé soit par ultrafiltration ou par chromatographie à perméation de gel (également appelée chromatographie d'exclusion stérique (SEC)).

Dans le cadre de ce travail de thèse, nous avons opté pour le fractionnement par ultrafiltration (UF). Comparativement aux autres techniques de fractionnement précitées, l'UF présente l'avantage de ne nécessiter aucune étape de prétraitement de l'échantillon et, de ce fait, on peut raisonnablement supposer qu'elle ne modifie pas de manière significative la nature chimique des constituants des AOX. L'UF permet également de concentrer et de purifier l'échantillon.

II.3.2.1 Principe de fractionnement par ultrafiltration (UF)

Cette technique utilise des membranes microporeuses dont le diamètre des pores est compris entre 1 et 100 nm. La séparation dépend principalement de la taille mais aussi de la structure tridimensionnelle des analytes. Les molécules dont la taille est supérieure à la taille des pores de la membrane sont retenues par cette dernière alors que les petites molécules et l'eau la traversent. La force motrice est la différence de pression de part et d'autre de la membrane. L'efficacité d'une membrane d'UF est généralement caractérisée par son seuil de coupure, qui correspond à une rétention pratiquement totale (90% le plus souvent) [8]. L'UF permet de séparer les constituants d'une solution en deux fractions : la première, appelée « retentât », où vont se concentrer les molécules de tailles supérieures au seuil de

coupure de la membrane et la seconde, appelée « perméat » ou « filtrat », qui contient les molécules de tailles inférieures à ce seuil.

II.3.2.2 Protocole et dispositifs expérimentaux

L'équipement d'UF utilisé est un système de filtration frontale « Amicon », commercialisé par la Société Merck Millipore (St-Quentin-en-Yvelines, France). Ce dernier est représenté par la figure II-2. Il est composé de cellules à agitation (modèle 8400) de 400 mL, alimentées en ligne par des réservoirs à échantillons de 800 mL.

Figure II-2. Dispositif d'ultrafiltration Millipore (St-Quentin-en-Yvelines, France)

Des membranes en acétate de cellulose régénérée présentant quatre seuils de coupure différents ont été testées : 10.000 Da, 5000 Da, 3000 Da et 1000 Da (Merck Millipore, St-Quentin-en-Yvelines, France).

Avant le fractionnement, les échantillons d'eau sont filtrés sous vide réduit à l'aide de filtres Whatman GF/F en fibres de verre, de porosité moyenne 0,45 µm, préalablement rincés à l'eau ultra pure. Cette étape indispensable permet de séparer la phase particulaire de la phase dissoute et d'éviter ainsi le colmatage des membranes. Un procédé de fractionnement par UF comporte trois étapes : le conditionnement de la membrane, le fractionnement et la régénération de la membrane.

II.3.2.2.1 Conditionnement des membranes

Pour éviter toute contamination des échantillons, un nettoyage rigoureux des membranes est nécessaire avant le fractionnement pour éliminer les produits de conditionnement utilisés par les fabricants comme le bisulfite de sodium (antibactérien) et la glycérine (antigel), par exemple. Leur nettoyage s'effectue en deux temps. Les membranes sont d'abord plongées dans un cristallisoir contenant une solution d'hydroxyde de sodium à 0,1 M pendant 30 min, puis abondamment rincées à

l'eau ultra pure. Elles sont ensuite placées dans les cellules d'ultrafiltrations où elles sont à nouveau lavées en percolant de l'eau ultra pure pendant une demi-journée.

II.3.2.2.2 Fractionnement des échantillons d'eau

500 mL d'échantillon sont introduits dans le réservoir à échantillon, puis soumis à un flux d'azote (N 5.0) à une pression comprise entre 3 et 4 bars. Cette valeur a été choisie comme un compromis entre les deux considérations antagonistes suivantes : la pression doit être suffisamment élevée pour permettre le fractionnement d'un volume important d'échantillon sur des temps courts, mais elle ne doit pas être trop élevée de manière à éviter le passage forcé d'AOX dont la taille est plus grande que le seuil de coupure de la membrane utilisée. Les dix premiers millilitres d'échantillon passés au travers de la membrane sont écartés pour éviter toute dilution de l'ultrafiltrat. Une agitation à 200 trs/min est appliquée afin de réduire l'épaisseur de la couche de polarisation. Il s'agit de la formation d'une couche de plus haute concentration en composés de hauts poids moléculaires à proximité de la membrane provoquant un gradient de concentration en sens inverse du flux. De nombreux travaux ont montré que l'augmentation progressive de la concentration en carbone organique dans le retentât entraînait le passage de molécules de masse théoriquement supérieure au seuil de coupure de la membrane. L'ultrafiltration est arrêtée avant le passage de la totalité de l'échantillon (environ 50 mL restent dans la cellule pour un volume initial de 500 mL). Le retentât obtenu est récupéré par lavage de la membrane avec un volume de 100 mL d'eau ultra pure sous ultrasons. Les fractions récupérées sont caractérisées par une mesure d'AOX.

II.3.2.2.3 Régénération des membranes

Après le fractionnement, les membranes sont régénérées par un lavage abondant à l'eau ultra pure, puis avec une solution d'hydroxyde de sodium à 0,1 M pendant 30 min. Il est avéré que le stockage des membranes dans l'eau, ne permet pas de conserver leurs propriétés physico-chimiques. Pour cette raison, nous avons opté pour un stockage dans une solution eau/éthanol (90:10, v/v) à 4 °C. Ces conditions permettent d'éviter un développement bactérien susceptible de réduire les performances des membranes.

II.3.3 Analyse des composés organohalogénés *via* le paramètre AOX

II.3.3.1 Principe de la mesure

La mesure d'AOX est réalisée selon la méthode standard régie par la norme NF EN ISO 9562 « Qualité de l'eau - dosage des composés organohalogénés adsorbables sur charbon actif (AOX) » (AFNOR, 2005) [9]. Celle-ci comporte trois étapes successives: (i) le traitement de l'échantillon, (ii)

l'adsorption des AOX sur charbon actif et, (iii) l'analyse des AOX adsorbés par titrage argentimétrique (microcoulométrie).

II.3.2.2 Protocole et dispositifs expérimentaux

La procédure analytique pour la détermination des AOX est la suivante : un volume de 100 mL d'échantillon d'eau est introduit dans une fiole conique rodée de 250 mL. Le pH est ajusté à une valeur inférieure à 2 par ajout de 100 µL d'acide nitrique concentré (65%). Un volume de 5 mL d'une solution tampon à pH 2 d'acide nitrique et de nitrate de sodium 0,2 M est ajouté. L'ajout de nitrate de sodium empêche l'adsorption excessive des ions halogénures inorganiques (chlorures, bromures, iodures) sur le charbon actif. Ensuite, 50 mg de charbon actif en poudre (CAP; de granulométrie comprise entre 10 et 50 µm, d'indice d'iode supérieur à 1050) sont ajoutés à l'échantillon qui, après fermeture de la fiole conique, est agité pendant 1 heure sur une table à agitation orbitale (figure II-3). C'est la phase d'adsorption sur charbon actif.

Figure II-3. Table d'agitation orbitale utilisée durant l'étape d'adsorption des SPOX sur charbon actif

A l'issue de la phase d'adsorption, le charbon actif chargé en AOX est filtré sur un fritté en quartz à l'aide d'une unité de filtration sous pression positive d'azote « Xprep-3 » (*TE Instruments*, Delft, Pays-Bas) (figure II-4). Ce système permet la filtration simultanée de trois échantillons. La vitesse d'écoulement de l'échantillon est ajustée entre 10 et 20 mL/min. Le gâteau de filtration est ensuite lavé trois fois par un volume total de 25 mL d'une solution de nitrate de sodium 0,01 M (lavage fractionné par 5, 10 puis 10 mL) pour éliminer le résiduel en halogénures inorganiques, puis séché sous flux d'azote.

Le fritté est ensuite introduit dans l'analyseur d'AOX. L'analyseur utilisé est un « *Xplorer* » (*TE Instruments*, Delft, Pays-Bas), commercialisé en France par la Société *Opheleia Instruments* (Soreze, France). L'appareil comprend un passeur automatique de frittés « *Newton-20* » (*TE Instruments*, Delft, Pays-Bas) permettant d'introduire jusqu'à 21 frittés d'échantillons et de standards. Dans ce modèle de passeur, une légère pression d'azote est appliquée dans l'enceinte de confinement des frittés afin de

protéger les échantillons d'une contamination par l'atmosphère du laboratoire. L'appareil possède un système intégré de destruction de l'acide acétique, ce qui permet de l'installer sur une simple paillasse hors sorbonne. La combustion du CAP est réalisée à 1000 °C sous courant d'oxygène (figure II-4). Les gaz formés (halogénures d'hydrogène) sont absorbés par une solution aqueuse d'acide acétique à 75% et les ions halogénures correspondant sont mesurés par microcoulométrie. Les autres paramètres de fonctionnement sont les suivants : température du four de pyrolyse à 1000 °C, température de la ligne de transfert four-cellule électrochimique à 200 °C, gaz de combustion: oxygène (99,99% minimum) à 300 mL/min, température de la cellule de titrage à 21 °C)

Figure II-4. Photographies de l'analyseur d'AOX modèle Xplorer (à gauche) et de l'unité de filtration module Xprep-3 (à droite), (Opheleia Instruments, Sorèze, France)

La mesure d'AOX comprend les SPOX volatils, dissous et colloïdaux ainsi que les composés liés à la matière particulaire. La teneur en AOX est exprimée en µg/L équivalent chlorures (aucune discrimination n'est faite entre composés chlorés, bromés et iodés). La limite de quantification de cette méthode est de 10 µg éq. Cl/L.

II.3.4 Analyse des composés organohalogénés par C-IC

II.3.4.1 Principe de la mesure

Une méthode permettant la spéciation des AOX en fractions chlorées (AOCl), bromées (AOBr) et iodées (AOI) a été spécifiquement élaborée dans le cadre de ce travail de thèse. Les phases d'adsorption des composés organohalogénés sur CAP (Charbon Actif en Poudre) et de combustion en atmosphère oxygénée sont équivalentes à la procédure décrite dans la norme EN ISO 9562 [9]. En revanche, les gaz produits dans le four (CO_2, H_2O, HX, X_2…) sont entrainés par l'oxygène dans un module d'absorption où ils sont piégés dans une solution d'absorption de volume connu. Un volume constant de la solution d'adsorption est par la suite automatiquement injecté dans une chaîne de chromatographie ionique. A partir des teneurs en ions halogénures mesurées, les valeurs d'AOCl, AOBr et AOI peuvent être calculées.

II.3.4.2 Instrumentation

Le système analytique C-IC (*Combustion Ion Chromatography*) utilisé dans le cadre de cette étude est un ensemble entièrement automatisé commercialisé par la société Metrohm (Villebon-sur-Yvette, France). Il se compose d'un four de combustion (multi X 2500, Analytik Jena), d'un système d'absorption des gaz de combustion (920 Absorber, Metrohm) et d'un chromatographe ionique (881 Compact IC, Metrohm) à détection conductimétrique (figure II-5).

Figure II-5. Photographie du couplage C-IC (Metrohm, Villebon-sur-Yvette, France)

La combustion est automatiquement autorégulée en durée et en intensité. A cet effet, le four de combustion est équipé d'un capteur qui mesure l'intensité de la lumière produite par la combustion et ajuste en conséquence le déplacement de la nacelle à échantillons à l'intérieur du four de combustion. Ainsi, la durée de combustion est optimisée pour assurer une combustion complète (pas de charbonnement : apparition de dépôts solides dans une combustion).

Le chromatographe ionique est constitué d'une pompe isocratique à double piston en série, d'un système de dégazage, d'une colonne de pré-concentration pour anions de type Metrosep A PCC 2/4.0 (65 µm ; 1 mm x 4 mm ; Methrom), d'une pré-colonne Metrosep A Supp 4/5 Guard/4.0 column (5 µm ; 5 mm x 4 mm ; Metrohm), d'une colonne analytique Metrosep A Supp 4 (9 µm ; 250 mm x 4 mm ; Metrohm) et d'un détecteur conductimétrique.

Le détecteur est précédé d'un système de suppression chimique destiné à améliorer la sensibilité. Ce dernier est composé d'un suppresseur d'ions (MSM, pour Metrohm Suppressor Module) et de CO_2 (MCS, pour Metrohm CO_2 Suppressor). Le MSM consiste en trois micro-cartouches remplies d'une résine échangeuse de cations ($NaX + RSO_3H \rightarrow XH + RSO_3Na$). Pendant qu'une cartouche est utilisée pour la suppression, la deuxième cartouche est régénérée en parallèle avec un acide dilué (acide sulfurique) alors que la troisième est rincée avec de l'eau. Une cartouche de suppresseur fraîchement régénérée est donc toujours disponible pour chaque analyse. Le MCS est, quant à lui, composé d'une membrane perméable aux gaz, entourée d'une chambre où règne une dépression importante.

II.3.4.3 Paramètres optimisés et performances analytiques

La mise en œuvre de la méthode de spéciation des AOX en fractions « chlorés », « bromés » et « iodés » a fait l'objet d'un travail de développement et d'optimisation. Les paramètres analytiques retenus ainsi que les performances obtenues sont détaillés dans la partie III du chapitre III (RESULTATS ET DISCUSSION) du présent manuscrit.

II.3.5 Analyse « ciblée » des SPOX volatils et semi-volatils par GC-MS

II.3.5.1 Choix des SPOX à suivre

Les SPOX ciblés dans le cadre de cette étude ont été sélectionnés suite à un travail de veille bibliographique et de synthèse des données issues d'études d'EDF R&D antérieures. La liste finale des SPOX retenue a été établie sur la base des trois critères de sélection suivants :

(i) intégrer l'ensemble des SPOX réglementés dans l'eau destinée à la consommation humaine,

(ii) intégrer les SPOX déjà étudiés par EDF afin d'asseoir les connaissances relatives à leur occurrence dans les eaux de refroidissement des CNPE,

(iii) élargir le spectre des SPOX historiquement suivis en intégrant des molécules jusqu'à présent jamais recherchées par EDF R&D et pour lesquelles des standards analytiques sont disponibles.

Au total 37 substances ont été sélectionnées : 8 halométhanes (chloroforme, bromodichlorométhane, dibromochlorométhane, bromoforme, iodoforme, chloroiodométhane, dichlorométhane, bromoiodométhane), 6 haloacétamides (chloroacétamide, dichloroacétamide, trichloroacétamide, bromoacétamide, tribromoacétamide, iodoacétamide), 11 acides haloaacétiques (acides monochloroacétique, dichloroacétique, trichloroacétique, monobromoacétique, dibromoacétique, tribromoacétique, bromodichloroacétique, e chlorodibromoacétique, iodoacétique, diiodoacétique), 6 haloacétonitriles (chloroacétonitrile, dichloroacétonitrile, trichloroacétonitrile, bromoacétonitrile, dibromoacétonitrile, iodoacétonitrile), 3 halonitrométhanes (bromonitrométhane, bromochloronitrométhane, bromodichloronitrométhane), 5 haloacétones (1,1-dichloropropanone, 1,3-dichloropropanone, 1,1,3-trichloropropanone, 1,1,1-trichloropropanone, 1,1,3,3-tetrachloropropanone) et un haloacétaldéhyde (tribromoacétaldéhyde).

II.3.5.2 Approche analytique et instrumentation

Deux méthodes d'analyse ont été développées puis validées. Celles-ci reposent sur une approche analytique couplant une extraction sur phase solide (SPE) suivie d'une analyse par chromatographie

en phase gazeuse couplée à la spectrométrie de masse en tandem. La première méthode vise le dosage de 11 acides haloacétiques ; la deuxième est une méthode multi-résidus permettant de suivre 26 SPOX.

Le système analytique utilisé est un « TSQ Quantum Ultra », commercialisé par la société Thermo Fisher Scientific (figure II-6).

Figure II-6. Photographie du couplage GC-MS utilisé (Thermo Fisher Scientific, Courtaboeuf, France).

II.3.5.3 Paramètres optimisés et performances analytiques associées

Les deux méthodes SPE GC-MS développées sont décrites plus en détail au chapitre III (RESULTATS ET DISCUSSION) du présent manuscrit. Elles sont présentées sous forme d'articles scientifiques prêts à être soumis pour publication aux revues scientifiques à comité de lecture suivantes : « *Analyst* », « *Journal of Chromatography A* », « *Talanta* » ou « *Rapid Communications in Mass Spectrometry* ».

II.3.6 *Screening* « non ciblé » des SPOX volatils et semi-volatils par GC-MS

L'analyse dite « non ciblée » vise à identifier des SPOX inconnus et/ou non ciblés par les deux méthodes d'analyse mises en œuvre dans le cadre de ce travail. Cette approche, basée sur l'utilisation de la spectrométrie de masse, repose sur la comparaison de signaux acquis sur les extraits SPE des échantillons traités et non traités, ainsi que sur l'exploitation des spectres de masses obtenus. Les analyses ont été réalisées sur l'appareillage décrit au paragraphe précédent, avec les mêmes conditions d'injection et de séparation chromatographique développées dans le cadre des deux méthodes ciblées. L'acquisition des spectres de masse est en revanche réalisée en mode « balayage complet »

(communément appelé *Full scan*), en ionisation électronique (EI), sur une gamme de rappports m/z compris entre 45 et 650 Th.

Afin de mieux repérer les pics chromatographiques des SPOX, une deuxième analyse cette fois-ci en ionisation chimique négative (NCI/MS, *Negative Chemical Ionization/Mass Spectrometry*) par attachement (ou capture) électronique a été effectuée. Son principe consiste à attacher des électrons de faible énergie cinétique sur les analytes d'intérêt. En générale, plus l'affinité électronique d'une molécule est forte, plus son aptitude à la capture des électrons est efficace. La NCI par attachement électronique, mode d'ionisation similaire à la détection par ECD (pour *Electron Capture Detection*), est jusqu'à 100 fois plus sensible que l'EI. Elle augmente également la sélectivité dans une matrice complexe, riche en matière organique, étant donné qu'un nombre limité d'analytes tels que ceux contenant un atome d'halogène, sont sujets à une capture électronique efficace [10,11]. L'analyse par GC-NCI/MS des échantillons d'eau a été effectuée dans les mêmes conditions d'injection et d'analyse chromatographique qu'en EI. Les températures de la ligne de transfert et de la source ont été respectivement maintenues à 280 et 150 °C. L'acquisition a été effectuée en mode *Full scan* de 30 à 600 m/z à une vitesse 200 ms/scan. Le méthane a été utilisé comme gaz de thermalisation des électrons à un débit de 2 mL/min.

II.3.7 *Screening* non ciblé des SPOX polaires par une approche couplant marquage chimique et analyse par LC-MS

Les études récentes ont montré que les SPOX polaires peuvent représenter une part non négligeable des sous-produits organohalogénés totaux produits [7, 11-12]. De fait de leur polarité, l'analyse de cette catégorie de composés nécessite de disposer de méthodes spécifiques permettant leur séparation et leur détection. Les SPOX polaires sont des composés peu volatils et, de ce fait, plus communément analysés par chromatographie en phase liquide couplée à la spectrométrie de masse (LC-MS). L'analyse des SPOX polaires par LC-MS peut néanmoins être entravée par une mauvaise séparation chromatographique et une faible efficacité d'ionisation. En phase inverse, les colonnes les plus couramment utilisées ne sont pas capables de retenir et de séparer efficacement certains composés hydrophiles, notamment ceux de faibles poids moléculaires. De plus, l'identification de tels composés, présents à l'état de traces dans des mélanges très complexes, sur la base de leurs spectres LC-MS en mode d'acquisition « *Full Scan »* n'est pas toujours possible.

II.3.7.1 Approche analytique

Les SPOX polaires visés dans le cadre de notre étude sont ceux comportent au moins un des groupements fonctionnels suivants : acide carboxylique, aldéhyde, cétone, alccol, phénol, amide,

amine. Une parade possible permettant leur identification consiste à les dériver chimiquement afin de faciliter leur identification *via* une recherche ciblée de la partie marquée des molécules. Cette dérivation chimique améliore également la stabilité de certains SPOX thermolabiles, notamment carbonylés, ainsi que la sensibilité des mesures, ce qui rend l'identification structurale plus aisée [13-14]. Dans

II.3.7.2 Instrumentation

La séparation chromatographique est réalisée sur un chromatographe en phase liquide à haute performance (Acquity, Waters™, France) équipé d'un injecteur automatique d'échantillons. Elle est menée en phase inverse avec une colonne Varian, Pursuit XRS ULTRA (longueur : 100 mm, diamètre : 2 mm, taille des particules : 2,8 µm). Le spectromètre de masse utilisé en LC-MS est un "Q-TOF Premier" de la société Waters™ (figure II-8). C'est un spectromètre de masse en tandem utilisant un analyseur quadripolaire (Q : Quadrupole) couplé à un analyseur à temps de vol (TOF : *Time-Of-Flight*), qui permet d'obtenir des mesures de masses exactes. L'ionisation des composés est réalisée par électronébullisation (*ElectroSpray Ionization*, ESI).

Figure II-8. Photographie du système Q-TOF utilisé

II.3.7.4 Paramètres optimisés et performances analytiques

La méthodologie globale d'identification des SPOX polaires par une approche couplant marquage chimique et analyse par LC-MS a fait l'objet d'une optimisation discutée dans la partie IV, chapitre III (RESULTATS ET DISCUSSION) du présent manuscrit.

II.3.8 *Screening* non ciblé des SPOX de hauts poids moléculaires par FT-ICR/MS

La spectrométrie de masse à transformée de Fourier (FT-ICR/MS pour *Fourier Transform Ion Cyclotron Resonance Mass Spectrometer*) est un instrument très performant, offrant une très haute précision en masse mesurée et une très haute résolution. Pour de plus amples informations sur ce type de spectromètre de masse, se référer aux articles de Comisarow *et al*., 1974, Marshall *et al.,* 1996 et Belov *et al.,* 2001 [26-28]. Jusqu'ici principalement utilisé dans les approches de types « -omiques », le FT-ICR/MS a été mis à contribution dans plusieurs études récentes pour tenter d'apporter des informations structurales sur les SPOX de hauts poids moléculaires. Il est à préciser que la résolution en masse du spectromètre FT-ICR permet de s'affranchir des interférences susceptibles d'être induites par les composés isobariques (de même masse nominale). La précision en masse offre la possibilité d'accéder à la masse exacte et donc à la formule brute de la molécule et de ses fragments caractéristiques.

II.3.8.1 Protocole expérimental et instrumentation

Dans cette étude, l'analyse des extraits SPE a été effectuée sur un FT-ICR/MS SolariX XR 9.4 T (Bruker Daltonik GmbH, Bremen, Allemagne) en ESI en modes positif et négatif (figure II-9). Les extraits ont d'abord été dilués 10 fois puis injectés à un débit de 3 µL/min. Les paramètres d'ionisation par ESI ont été ajustés comme suit : tension d'émission à 4,0 kV, tension de la colonne capillaire d'introduction à 4,5 kV, tension à l'extrémité de la colonne capillaire à -320 V. Différentes gammes de rapports m/z ont été analysées séparément pour gagner en sensibilité : 150-500, 500-1000, 1000-1500 m/z. La taille des données a été fixée à 8 M de mots, et le nombre de scans minimal à 250 scans pour chaque spectre.

Figure II-9. Photographie du spectromètre de masse FT-ICR SolariX XR 9.4 T (Bruker Daltonik GmbH, Bremen, Allemagne)

Le spectromètre de masse FT-ICR a été étalonné en mode externe par une solution diluée d'acide phosphorique pour toutes les gammes de rapports m/z. La calibration interne a été effectuée à l'aide d'espèces moléculaires organiques homologues issues de la matière organique (sous forme de $C_xH_yO_z(CH_2)_n$) présente dans chaque échantillon, grâce à un diagramme de Kendrick. Nous reviendrons sur ce diagramme dans la partie 6 du chapitre III (RESULTATS ET DISCUSSION).

II.3.8.2 Méthode d'analyse des données

La recherche de formules brutes de SPOX a été effectuée à partir de la contribution atomique suivante : $^{12}C_{0-100}$, $^{13}C_{0-2}$, $^{1}H_{0-200}$, $^{14}N_{0-3}$, $^{16}O_{0-30}$, $^{32}S_{0-2}$, $^{35}Cl_{0-6}$, $^{37}Cl_{0-4}$, $^{79}Br_{0-4}$, $^{81}Br_{0-4}$ et $^{127}I_{0-2}$, en respectant les critères de validation suivants :

- l'erreur sur la masse doit être inférieure à 1 ppm,
- la somme des atomes d'hydrogène et d'halogène doit représenter au moins 1/3 du nombre d'atomes de carbone, et ne pas excéder 2C + N + 2 (C et N représentant respectivement le nombre d'atomes de carbone et d'azote dans la formule brute proposée),
- les masses et formules attribuées doivent respecter la règle d'azote,
- le nombre d'atomes d'azote et d'oxygène ne doit pas dépasser le nombre d'atomes de carbone.

Ces critères garantissent que les formules brutes attribuées sont à un niveau de confiance élevé et peuvent exister chimiquement. L'analyse des données a été réalisée à l'aide du logiciel Bruker Compass Data Analysis (Bruker Daltonik GmbH, Bremen, Allemagne).

II.4 Méthodes bio-analytiques – outils d'aide à l'identification des SPOX

II.4.1 Démarche générale

Dans le cadre de ce travail de thèse, nous avons souhaité explorer une approche « combinée » basée sur le couplage entre les outils bio-analytiques et les méthodes physico-chimiques d'analyse pour l'identification structurale des SPOX. Dans le cas de l'analyse d'un très grand nombre d'échantillons pour lesquels la nature chimique des composés constitutifs d'une part importante d'AOX est inconnue, l'élaboration de profils d'activités (éco)toxicologiques (criblage biologique) offre la possibilité d'identifier les échantillons les plus actifs (préoccupants). Ainsi, l'effort d'identification physico-chimique pourra être orienté en priorité vers ces échantillons.

Il convient de préciser que l'objectif premier des essais biologiques réalisés dans le cadre de ce travail de thèse était d'apporter des indications sur l'existence probable de substances actives dans les échantillons étudiés afin d'orienter les travaux d'identification vers les échantillons les plus actifs. Il ne s'agit donc pas d'une caractérisation fine de la toxicité des échantillons.

II.4.2 Outils bio-analytiques disponibles

Il ne s'agit pas de ce paragraphe n'est pas de dresser une synthèse exhaustive des outils biologiques permettant d'établir les profils d'activités (éco)toxicologiques, mais de présenter les principes et modes opératoires associés aux tests bio-analytiques employés dans le cadre de cette étude.

De par leur rapidité d'exécution, leur facilité de mise en œuvre, leur bonne sensibilité et le fait de travailler avec de petits volumes d'échantillons, les tests *in vitro* sont particulièrement adaptés aux études de criblage haut débit. De plus, les outils *in vitro* sont souvent basés sur des mécanismes de réponse relativement simples et préétablis, leur conférant une spécificité vis-à-vis d'une ou plusieurs familles de substances. La toxicité, la génotoxicité, les effets mutagènes ou les perturbations endocriniennes sont autant d'activités biologiques référencées dans les études visant la caractérisation des SPOX [29-32]. Les tests de génotoxicité et de mutagénicité sont de loin les plus fréquemment rencontrés dans la littérature scientifique traitant de la problématique des SPD. Plusieurs modèles biologiques ont été rapportés ; le test d'Ames sur des souches de *Salmonella Typhimurium* demeure l'essai le plus utilisé. Des essais comparatifs ont montré de manière concluante que chaque système d'essais *in vitro* engendre pour le même type d'activité biologique des résultats différents. A titre d'exemple, Le Curieux et al. (1996) ont comparé la sensibilité de trois essais *in vitro* (test d'Ames-fluctuation, test micronoyau triton et SOS chromotest) pour la caractérisation du potentiel génotoxique de plusieurs SPOX (THMs, HANs, et HPs) [33]. Les résultats obtenus ont révélé une différence de sensibilité entre les trois modèles biologiques. Ce constat montre que le choix du bio-essai *in vitro* est une étape déterminante qui conditionne les résultats et *in fine* la nature des substances incriminées à identifier. Il souligne également la nécessité de réaliser une batterie de tests impliquant divers types cellulaires et différents systèmes de métabolisation afin de cibler des composés d'une plus large diversité de comportement.

II.4.3 Outils bio-analytiques employés

Dans un souci d'optimisation des moyens, deux activités biologiques ont été privilégiées : la cytotoxicité (lignée cellulaire de truite, RTG-2) et la génotoxicité (SOS Chromotest). Le test SOS Chromotest a été choisi en raison de sa spécificité envers un large panel de SPOX ainsi que pour la simplicité et la rapidité de sa mise œuvre comparativement au test d'Ames [34-35]. Les essais exploratoires réalisés ont consisté à analyser les échantillons d'eaux brutes sans pré-concentration pour les essais de cytotoxicité et des extraits organiques SPE reconstitués dans le DMSO (Diméthylsulfoxyde) pour l'activité génotoxique. La concentration d'échantillon fait toujours débat aujourd'hui à cause de plusieurs questions récurrentes, parmi lesquelles : la proportion des composés extraits et leur composition reflètent-elles bien l'échantillon original et sa toxicité ? Le protocole d'extraction adopté est-il adéquat pour la nature des composés génotoxiques présents ?

Malgré les incertitudes scientifiques qui demeurent, la nécessité de concentrer les échantillons fait consensus aujourd'hui, principalement à cause de la sensibilité des tests biologiques actuels et du fait que les composés génotoxiques sont généralement présents à des concentrations très faibles.

Avec l'objectif d'évaluer la contribution des SPOX les plus fréquemment détectés dans les eaux monochloraminées à l'activité globale mesurée dans échantillons analysés, les SPOX ont également été individuellement analysés pour évaluer leur activité intrinsèque.

Tous les essais *in vitro* ont été réalisés par le laboratoire TOXEM au Havre.

II.4.3.1 Test in vitro de cytotoxicité sur la lignée cellulaire de truite (RTG-2)

D'une manière générale, un test de cytotoxicité consiste à comptabiliser le pourcentage de cellules vivantes après exposition d'une culture cellulaire à une gamme de concentrations d'un toxique. Les cellules utilisées pour les essais de cytotoxicité sont des fibroblastes de gonades de truites arc-en-ciel (RTG-2, pour *Rainbow Trout Gonad cell line-2*). Le protocole expérimental comporte trois phases distinctes : (i) ensemencement des cellules, (ii) exposition des cellules aux échantillons à tester et (iii) mesure de l'activité biologique.

II.4.3.1.1 Ensemencement des cellules

L'objectif de cette étape est de produire une quantité suffisante de cellules pour l'expérimentation. Après décongélation, les cellules sont amplifiées dans des flasques de 75 cm^2, dans un milieu de culture composé de L15 Leibovitz, supplémenté avec 10% de Sérum de Veau Fœtal (SVF), 2 mM de L-glutamine, 1% d'acides aminées non essentiels, 100 unités par mL de pénicilline et 0,1 mg/mL de streptomycine. Les flasques sont maintenues à 21 °C dans une atmosphère humide (incubateur à CO_2) contenant 5% de CO_2 et 95% d'air. La trypsination est réalisée lorsque les cellules atteignent un pourcentage compris entre 80 et 90% de confluence. Les cellules sont comptées, puis ensemencées dans des plaques de culture blanches de 96 puits (CellStar®, Greiner Bio-one, D. Dutcher, Brumath, France) à une densité de 5000 cellules par puits dans 100 µL de milieu de culture complet, puis incubées à nouveau pendant 24 heures à 21 °C.

II.4.3.1.2 Exposition des cellules aux échantillons à analyser

Après avoir proliféré pendant 24 heures en plaques à 96 puits, les cellules sont exposées en séries à des dilutions de chaque type d'échantillon à analyser : les échantillons d'eau issus du bassin froid et de l'amont des CNPE étudiées ainsi que des solutions standards de plusieurs SPOX. Les échantillons sont répartis à raison de 200 µL par puit. Sept niveaux de concentrations par échantillon, chacun testé en triplica (trois puits), sont expérimentés de manière à établir des courbes dose-réponse. Le milieu de

culture est utilisé comme témoin négatif (100% de viabilité cellulaire), la doxorubicine (N° CAS : 25316-40-9) comme témoin positif (cytotoxicité). Afin de vérifier le fonctionnement correct des lignées, une gamme de dilution de doxorubicine est testée. Les cellules sont ensuite à nouveau incubées à 21 °C pendant 4 jours.

II.4.3.1.3 Lecture des plaques

Au terme des 4 jours d'exposition, les plaques sont vidées et un milieu de culture contenant le réactif Cell Tracker Green CMFDA (CTG CMFDA, 5-chloromethylfluorescein diacétate, Thermo Fisher Scientific, St. Louis, États-Unis) à une concentration de 1,5 µM est ajouté à chaque puit. Les cellules sont ensuite incubées pendant 45 minutes à 21 °C. Après incubation, la fluorescence est mesurée à la longueur d'onde de 528 nm (excitation à 485 nm) à l'aide d'un lecteur de plaque 96 puits (SpectraMax M5e, Moleculardevices, Royaume Unis). Comme schématisé sur la figure II-10, le CTG est une molécule qui devient fluorescente lorsque les groupements acétate sont clivés par des enzymes, plus particulièrement les estérases, présentes à l'intérieur des cellules viables (vivantes). Seules les cellules ayant survécu lors de l'exposition aux échantillons analysés présentent la capacité de transformer le CTG, tandis que les cellules mortes sont inactives. Plus l'intensité de fluorescence est importante plus l'activité enzymatique et le nombre de cellules métaboliquement viables sont élevés.

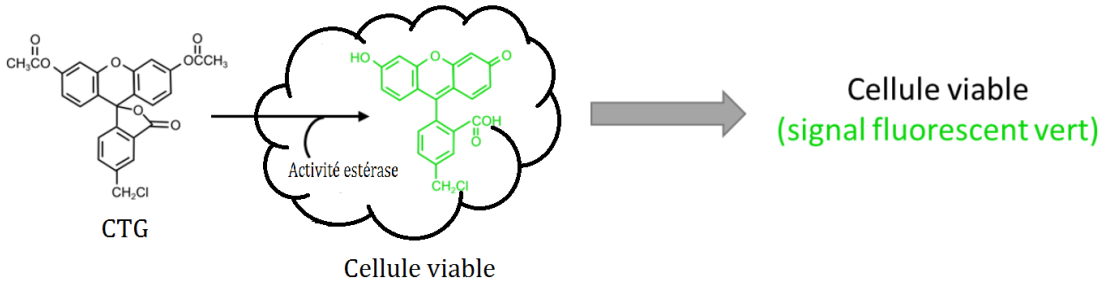

Figure II-10. Mécanisme d'action du réactif CTG utilisé pour distinguer les cellules viables des cellules mortes

II.4.3.1.4 Traitement des données et expression des résultats

Les résultats obtenus en unité de fluorescence ont ensuite été exprimés en pourcentage de fluorescence par rapport au témoin (contrôle positif). A chaque niveau de concentration, le pourcentage de survie (ou de viabilité cellulaire) est obtenu par la formule ci-après.

$$\%\ de\ viabilité = 100\ x\ \frac{Fluorescence\ dans\ le\ puits\ traité}{Fluorescence\ dans\ le\ puits\ contrôle\ négatif}$$

100% correspondant à la viabilité des cellules non mises en contact avec la doxorubicine (contrôle négatif). Les données ont été analysées à l'aide du logiciel SigmaPlot®. Ce logiciel permet d'établir des courbes dose-réponse et de déterminer la concentration entraînant un pourcentage de mortalité donné (LD 50% par exemple, LD pour *Lethal Dose*). En comparant la valeur de la LD50 d'un SPOX de référence à celles des échantillons analysés, les résultats peuvent être exprimés en quantité équivalente de ce composé.

II.4.3.2 Mesure du potentiel génotoxique au moyen du test SOS Chromotest

Le SOS Chromotest est un test de génotoxicité mettant en œuvre une souche bactérienne d'*Escherichia coli* (PQ37) modifiée génétiquement. Ce test, décrit par Quillardet *et al*. en 1982, consiste à déterminer la capacité d'une substance chimique à induire l'expression de l'un des composants du système de réparation d'urgence « SOS », le gène sfiA [36]. A ce dernier est couplé un vecteur d'expression dans la souche modifiée, le gène LacZ. La souche bactérienne modifiée est également caractérisée par une suppression de la région du LacZ normal, ce qui rend l'expression du gène LacZ strictement dépendante de celle du gène sfiA. Ainsi, plus il y a de dommages induits dans le génome de la souche modifiée suite à l'action d'un génotoxique, plus la souche exprime fortement les gènes sfiA et LacZ. La mesure de l'activation de réparation s'opère indirectement grâce au gène LacZ codant pour l'enzyme β-galactosidase dont l'activité est dosée par colorimétrie en présence d'un réactif chromogénique, l'ortho-nitrophényl β-D-galactopyranoside (ONPG). Les échantillons très toxiques peuvent inhiber la synthèse protéinique (cytotoxicité) et occasionner une sous-estimation de l'induction de la β-galactosidase. Afin de contourner ce problème, la mesure d'une protéine ne répondant pas à l'activation du système SOS, la phosphatase alcaline, est réalisée en parallèle.

Le protocole expérimental comporte trois phases : (i) ensemencement des cellules, (ii) exposition des cellules aux échantillons à tester et (iii) mesure de l'activité biologique.

II.4.3.2.1 Ensemencement des cellules

Une culture (préculture) de nuit de la souche PQ37 dans le milieu LB (*Lysogeny Broth*) additionné d'ampicilline (30 µg/mL) est ensemencée à 37 °C sous agitation continue à 250 rpm.

Le SOS Chromotest s'effectue généralement en suivant deux modes opératoires parallèles. Le premier permet de détecter les génotoxiques directs (ou actifs) qui sont des substances chimiques capables d'altérer directement la structure de l'ADN. Le deuxième permet de mettre en évidence les progénotoxiques qui sont des composés nécessitant une activation métabolique (semblable, par exemple, à celle retrouvée chez les mammifères) avant d'exprimer leur génotoxicité. L'activation métabolique est simulée par l'ajout aux bactéries d'un extrait hépatique de rongeur préalablement

traité par un inducteur enzymatique (Arochlor) et additionné de co-facteurs (mélange d'activation appelé Mix S9).

Pour l'évaluation du potentiel génotoxique, la préculture est diluée dans le milieu LB de façon à obtenir une densité comprise entre 6 et 8.10^6 CFU/mL. En ce qui concerne l'étude du potentiel progénotoxique, la préculture est diluée pour obtenir la même densité en utilisant un mélange d'activation S9 à 30%, préparé dans le tampon suivant : KCl 33 mM, $MgCl_2$ 8 mM, D-glucose-6-phosphate 5 mM, NADP 4 mM, NaH_2PO_4 10 mM. Dans des plaques 96 puits (Greiner, France) sont ajoutés dans chaque puit 190 µL de la culture et 10 µL de l'échantillon à tester. Les solutions mères et de travail (standards et échantillons) sont préparées dans le DMSO. Six concentrations par extrait SPE ont été testées (500, 1000, 2500, 5000, 10000 et 50000 ppm). Le DMSO est utilisé comme témoin négatif. Afin de s'assurer du bon fonctionnement du test, deux contrôles positifs sont réalisés avec le mutagène 4-Nityro Quinoline N-Oxide (4NQO) pour le test sans activation métabolique et le pro-mutagène Benzo[a]pyrene (B(a)P) pour les expériences avec activation métabolique. Les plaques sont ensuite exposées 3 heures sous agitation continue (250 rpm) à 37 °C.

II.4.3.2.2 Lecture des plaques

Au terme des trois heures d'exposition, les plaques sont centrifugées pendant 20 min (1200 x g à 37 °C) et le surnageant éliminé. Le culot est remis en suspension dans 200 µL de tampon préparé comme suit : 4,85 g de tris(hydroxy-methyl) aminométhane sont ajoutés à 200 mL d'eau ultrapure puis le pH de la solution est ajusté à 7,8 avec de l'acide chlorhydrique. Ensuite, 100 µL de chaque suspension sont transférés dans les puits vides d'une nouvelle plaque. La dernière phase du test consiste à mesurer l'activité enzymatique résultante. Cette détermination est réalisée en dosant la quantité de β-galactosidase et d'alcaline phosphatase à l'aide de deux chromogènes : l'ONPG, substrat jaune pour la β-galactosidase et le PNPP (p-NitroPhényle Phosphate disodium), substrat jaune pour l'alcaline phosphatase. Pour ce faire, 100 µL de la solution de chromogène combiné (ONPG à 4 mg/mL ou PNPP à 1 mg/mL) préparée dans le tampon SOS Chromogen (400 mL de méthanol, 5 mL de toluène, 100 mL de N, N-dimethyl formamide dans 1 L d'eau ultrapure, pH aux alentours de 7,8), sont ajoutés à tous les puits des microplaques (potentiel pro- et génotoxique) puis laissés à incuber 1 à 2 heures (selon la coloration) à 37 °C (100 rpm). La coloration est mesurée par un spectrophotomètre à microplaques (Biotek Synergy HT) à la longueur d'onde 405 nm (DO_{405}) pour l'activité génotoxique, et à 405 nm (DO_{405}), pour déterminer la viabilité des bactéries.

II.3.3.2.3 Traitement des données et expression des résultats

Les valeurs d'absorbance à 405 nm (DO_{405}) sont corrigées à la hausse en tenant compte de la viabilité bactérienne en utilisant un facteur de correction (FC) calculé de la façon suivante :

$$FC = \frac{Aa}{Ab}$$

Les acronymes Aa et Ab représentent respectivement les valeurs de l'absorbance de l'activité de la β-galactosidase et de l'activité de la phosphatase alcaline dans les puits en absence d'échantillon (témoin) et dans les puits en présence d'une concentration donnée d'échantillon testé.

Après avoir effectué la correction précédente, les résultats des tests SOS sont exprimés en SOS induction Factor (SIF) déterminés selon la formule suivante :

$$SIF = \frac{FC\ échantillon\ corrigé}{FC\ témoin\ négatif}$$

Les facteurs d'induction normalisent les réponses et permettent ainsi la comparaison entre les composés génotoxiques et progénotoxiques. Par définition, le témoin a un FI de 1,0. Plusieurs chercheurs préconisent d'utiliser un FI de 1,5 comme seuil de significativité génotoxique. La réponse (pro)génotoxique peut s'exprimer sous forme de relation de dose-réponse, notamment pour les génotoxiques forts.

Références

[1] NF EN ISO 5667-3. Qualité de l'eau - Échantillonnage - Partie 3 : conservation et manipulation des échantillons d'eau, Sampling. Part, Mai 2013
[2] NF EN ISO 7393-2. Qualité de l'eau - Dosage du chlore libre et du chlore total - Partie 2 : méthode colorimétrique à la N, N-diéthylphénylène-1,4 diamine destinée aux contrôles de routine, Mars 2000
[3] NF EN ISO 10523. Qualité de l'eau - Détermination du pH, Mai 2012
[4] NF EN 1484. Analyse de l'eau - Lignes directrices pour le dosage du carbone organique total (TOC) et carbone organique dissous (COD), Juillet 1997
[5] NF EN ISO 10304-1. Qualité de l'eau - Dosage des anions dissous par chromatographie des ions en phase liquide - Partie 1 : dosage du bromure, chlorure, fluorure, nitrate, nitrite, phosphate et sulfate, Juillet 2009
[6] ISO 11885:2007. Qualité de l'eau - Dosage d'éléments choisis par spectroscopie d'émission optique avec plasma induit par haute fréquence (ICP-OES), Août 2007
[7] A. Kinani, S. Kinani, S. Bouchonnet, Formation and determination of organohalogen by-products in water – Part II. Sample preparation techniques for analytical approaches, TrAC Trends in Analytical Chemistry, 85, Part C (2016) 281-294
[8] G.R. Aiken, Evaluation of ultrafiltration for determining molecular weight of fulvic acid, Environmental Science & Technology, 18 (1984) 978-981
[9] NF EN ISO 9562. Qualité de l'eau - Dosage des halogènes des composés organiques adsorbables (AOX), Mars 2005
[10] H.R. Buser, Selective detection of brominated aromatic compounds using gas chromatography/negative chemical ionization mass spectrometry, Analytical Chemistry, 58 (1986) 2913-2919.
[11] A. Kinani, S. Kinani, S. Bouchonnet, Formation and determination of organohalogen by-products in water. Part III. Characterization and quantitative approaches, TrAC Trends in Analytical Chemistry, 85, Part C (2016) 295-305.
[12] A. Kinani, S. Kinani, B. Richard, M. Lorthioy, S. Bouchonnet, Formation and determination of organohalogen by-products in water. Part I. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method, TrAC Trends in Analytical Chemistry, 85, Part C (2016) 273-280.
[13] S.U. Bajad, W. Lu, E.H. Kimball, J. Yuan, C. Peterson, J.D. Rabinowitz, Separation and quantitation of water soluble cellular metabolites by hydrophilic interaction chromatography-tandem mass spectrometry, Journal of chromatography A, 1125 (2006) 76-88.
[14] F. Xu, L. Zou, Y. Liu, Z. Zhang, C.N. Ong, Enhancement of the capabilities of liquid chromatography–mass spectrometry with derivatization: general principles and applications, Mass spectrometry reviews, 30 (2011) 1143-1172.
[15] T. Santa, Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry, Biomedical Chromatography, 25 (2011) 1-10.
[16] T. Higashi, T. Ichikawa, S. Inagaki, J.Z. Min, T. Fukushima, T. Toyo'oka, Simple and practical derivatization procedure for enhanced detection of carboxylic acids in liquid chromatography–electrospray ionization-tandem mass spectrometry, Journal of pharmaceutical and biomedical analysis, 52 (2010) 809-818.
[17] A.J. Cartwright, P. Jones, J.C. Wolff, E.H. Evans, Derivatisation of carboxylic acid groups in pharmaceuticals for enhanced detection using liquid chromatography with electrospray ionisation tandem mass spectrometry, Rapid communications in mass spectrometry, 19 (2005) 1058-1062.
[18] S.J. Barry, R.M. Carr, S.J. Lane, W.J. Leavens, S. Monte, I. Waterhouse, Derivatisation for liquid chromatography/electrospray mass spectrometry: synthesis of pyridinium compounds and their amine and carboxylic acid derivatives, Rapid communications in mass spectrometry, 17 (2003) 603-620.
[19] T. Hayama, Y. Sakaguchi, H. Yoshida, M. Itoyama, K. Todoroki, M. Yamaguchi, H. Nohta, Fluorous derivatization combined with liquid chromatography/tandem mass spectrometry: a method for the selective and sensitive determination of sialic acids in biological samples, Rapid Communications in Mass Spectrometry, 24 (2010) 2868-2874.
[20] M.S. Rashed, L.Y. Al-Ahaidib, O.Y. Al-Dirbashi, M. Al Amoudi, M.M. Al-Sayed, Z. Rahbeeni, Z. Al-Hassnan, A. Al-Dbaas, M. Al-Owain, M.N. Luanaigh, Tandem mass spectrometric assay of succinylacetone in urine for the diagnosis of hepatorenal tyrosinemia, Analytical biochemistry, 339 (2005) 310-317.
[21] A. Sakuragawa, T. Yoneno, K. Inoue, T. Okutani, Trace analysis of carbonyl compounds by liquid chromatography–mass spectrometry after collection as 2, 4-dinitrophenylhydrazine derivatives, Journal of Chromatography A, 844 (1999) 403-408.
[22] H.L. Lord, J. Rosenfeld, V. Volovich, D. Kumbhare, B. Parkinson, Determination of malondialdehyde in human plasma by fully automated solid phase analytical derivatization, Journal of Chromatography B, 877 (2009) 1292-1298.

[23] S.J. Barry, R.M. Carr, S.J. Lane, W.J. Leavens, C.O. Manning, S. Monté, I. Waterhouse, Use of S-pentafluorophenyl tris (2, 4, 6- trimethoxyphenyl) phosphonium acetate bromide and (4- hydrazino- 4-oxobutyl)[tris (2, 4, 6- trimethoxyphenyl) phosphonium bromide for the derivatization of alcohols, aldehydes and ketones for detection by liquid chromatography/electrospray mass spectrometry, Rapid communications in mass spectrometry, 17 (2003) 484-497.
[24] C.C. Lai, C.H. Tsai, F.J. Tsai, C.C. Lee, W.D. Lin, Rapid monitoring assay of congenital adrenal hyperplasia with microbore high- performance liquid chromatography/electrospray ionization tandem mass spectrometry from dried blood spots, Rapid Communications in Mass Spectrometry, 15 (2001) 2145-2151.
[25] H. Hong, Y. Wang, Derivatization with Girard reagent T combined with LC-MS/MS for the sensitive detection of 5-formyl-2'-deoxyuridine in cellular DNA, Analytical chemistry, 79 (2007) 322-326.
[26] M.B. Comisarow, A.G. Marshall, Fourier transform ion cyclotron resonance spectroscopy, Chemical physics letters, 25 (1974) 282-283.
[27] A.G. Marshall, S. Guan, Advantages of high magnetic field for Fourier transform ion cyclotron resonance mass spectrometry, Rapid Communications in Mass Spectrometry, 10 (1996) 1819-1823.
[28] M.E. Belov, E.N. Nikolaev, G.A. Anderson, K.J. Auberry, R. Harkewicz, R.D. Smith, Electrospray ionization-Fourier transform ion cyclotron mass spectrometry using ion preselection and external accumulation for ultrahigh sensitivity, Journal of the American Society for Mass Spectrometry, 12 (2001) 38-48.
[29] M.G. Narotsky, G.R. Klinefelter, J.M. Goldman, A.B. DeAngelo, D.S. Best, A. McDonald, L.F. Strader, A.S. Murr, J.D. Suarez, M.H. George, Reproductive toxicity of a mixture of regulated drinking-water disinfection by-products in a multigenerational rat bioassay, Environmental Health Perspectives (Online), 123 (2015) 564.
[30] S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research, Mutation Research/Reviews in Mutation Research, 636 (2007) 178-242.
[31] D. Stalter, E. O'Malley, U. von Gunten, B.I. Escher, Fingerprinting the reactive toxicity pathways of 50 drinking water disinfection by-products, Water research, 91 (2016) 19-30.
[32] T. Manasfi, M. De Méo, B. Coulomb, C. Di Giorgio, J.-L. Boudenne, Identification of disinfection by-products in freshwater and seawater swimming pools and evaluation of genotoxicity, Environment international, 88 (2016) 94-102.
[33] F. Le Curieux, S. Giller, D. Marzini, A. Brice, F. Erb, Utilisation de trois tests de génotoxicité pour l'étude de l'activité génotoxique de composés organohalogénés, d'acides fulviques chlorés et d'échantillons d'eau (non concentrés) en cours de traitement de potabilisation, Revue des sciences de l'eau/Journal of Water Science, 9 (1996) 75-95.
[34] P. Quillardet, M. Hofnung, Le SOS chromotest: des cellules bactériennes pour détecter et caractériser produits et radiations génotoxiques, Radioprotection, 29 (1994) 539-556.
[35] M. Ruiz, D. Marzin, Genotoxicity of six pesticides by Salmonella mutagenicity test and SOS chromotest, Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 390 (1997) 245-255.
[36] P. Quillardet, O. Huisman, R. D'Ari, M. Hofnung, The SOS chromotest: direct assay of the expression of gene sfiA as a measure of genotoxicity of chemicals, Biochimie, 64 (1982) 797-801.

CHAPITRE III –RESULTATS ET DISCUSSION

Ce chapitre est consacré à la description des résultats expérimentaux et à leur interprétation ; il inclut deux parties.

La première partie expose les développements méthodologiques effectués pendant ces travaux de thèse. Trois des méthodes analytiques développées sont présentées sous forme d'articles scientifiques prêts à être soumis pour publication dans les revues scientifiques à comité de lecture « Journal of Chromatography A », « Talanta » et « Rapid Communications in Mass Spectrometry ». Les deux premières publications décrivent les développements de techniques d'extraction par SPE et d'analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse en tandem pour la quantification de 11 acides haloacétiques (AHA) et 26 autres SPOX. La troisième publication présente le développement et l'optimisation d'une méthode de spéciation des AOX en fractions « chlorés », « bromés » et « iodés ». Des rappels de notions, de méthodes ou de problématiques sont présentés afin que les articles puissent être lus séparément. Une partie des résultats de l'application des méthodes développées à l'analyse d'échantillons d'eau des sites d'étude est également exposée.

La seconde partie est dédiée à la caractérisation des échantillons d'eau issus des sites d'étude (CNPE de Saint-Laurent, Chooz et Cattenom). Elle présente et discute les résultats obtenus et s'attache à les comparer à des résultats antérieurs.

Partie 1

I- Développement et validation d'une méthode d'analyse ciblant 11 acides haloacétiques par SPE et GC-MS

Les acides haloacétiques (AHA) potentiellement formés lors du traitement des eaux brutes aux oxydants chlorés sont au nombre de 9, ce sont des acides chloroacétiques, bromoacétiques et mixtes (chlorés et bromés). Il s'agit de la famille de sous-produits de désinfection la plus importante. Les AHA les plus communément mesurés dans les eaux de consommation et de piscines sont les acides trichloroacétique, dichloroacétique, et, à de plus faibles fréquences et concentrations, les acides monochloroacétique, monobromoacétique et dibromoacétique. Nos travaux antérieurs (résultats EDF R&D non publiés) ont mis en évidence la présence des acides monochloroacétique, dichloroacétique, trichloroacétique et bromochloroacétique dans les eaux monochloraminées. Les autres acides haloacétiques n'ont pas été mesurés à des concentrations supérieures aux limites de quantification des méthodes employées. Dans certaines de ces études, seule une partie des 9 AHA a été suivie, faute de méthode d'analyse sensible et "large spectre". Par ailleurs, les études les plus récentes tendent à montrer que la monochloramine produit des sous-produits organoiodés, comme les acides iodoacétiques. Compte tenu de ces élements, nous avons développé et validé une méthode d'analyse ciblant 11 AHA. Celle-ci repose sur une extraction sur phase solide (SPE) suivie d'une dérivatisation au méthanol acidifié et d'une analyse par GC-EI-MS/MS. Plusieurs cartouches SPE ont été testées. Oasis-HLB, Bakerbond SDB, LiChrolut EN et Bakerbond Carbon fournissent des résultats comparables pour les HAAs mais la phase Bakerbond SDB a finalement été retenue car elle a montré par ailleurs de meilleurs résultats pour un grand nombre d'autres sous-produits organohalogénés. La fonction réponse, les limites de quantification de la méthode et l'exactitude de la méthode ont été évaluées selon la norme NF T 90-210. Selon les HAAs étudiés, des seuils de quantification compris entre 0,01 et 0,50 $\mu g.L^{-1}$ ont été atteints en utilisant un volume initial d'échantillon de 1 L. La méthode développée offre des améliorations notables par rapport aux méthodes utilisées dans nos études antérieures. Elle permet en particulier de percoler des volumes d'échantillon très supérieurs (jusqu'à 1 L). Elle permet également de réaliser l'analyse des acides haloacétiques réglementés et non réglementés en une seule injection, avec une sensibilité meilleure, en particulier pour l'acide monochloroacétique réputé très difficile à analyser. Les performances de cette méthode en termes de spécificité et de sélectivité ont été démontrées sur des échantillons d'eau prélevés en rivières (en amont des CNPE).

La méthode développée a par la suite été appliquée sur des échantillons d'eau prélevés en bassins froids. Afin d'éviter toute mésinterprétation, les résultats n'ont pas été présentés et les rivières échantillonnées ont été anonymisées dans les articles suivants. La Moselle, la Meuse et la Loire ont été appelées respectivement "River 1", "River 2" et "River 3".

Comme précisé précédemment, les résultats de l'analyse des échantillons d'eau prélevés au niveau des sites d'étude sont présentés dans la deuxième partie de ce chapitre.

Combination of solid-phase extraction with acidic methanol derivation and GC-MS/MS analysis for the determination of haloacetic acids in aqueous samples

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Abstract

This article presents a sensitive, selective and specific method for the determination of several classes of haloacetic acids in water samples. This method involves solid phase extraction with acidic methanol derivation and GC-MS/MS coupling analysis. Several SPE cartridges were tested. Oasis-HLB, Bakerbond SDB, LiChrolut EN and Bakerbond Carbon provide comparable results but the Bakerbond SDB has finally been retained because it has been shown to provide the best results for a large class of organohalogenated disinfection by-products. Response function, method quantification limits, and accuracy of the method have been assessed according to the French Standard NF T 90-210. The calibration curves for all studied HAAs had consistent slopes with $R^2 > 0.99$. Depending on the HAA considered, quantification thresholds between 0.01 and 0.50 $\mu g.L^{-1}$ were reached using a sample volume of 1 L. The developed method offers improvements over previous reported works including higher sample volume (1 L), analysis of regulated and unregulated HAAs in a single injection, and better sensitivity in particular for monochloroactic acid. Applied to analyzing fifteen water samples from three different rivers in France, the developed method has detected the presence of five HAAs including: monochloroacetic acid (in 100% of the samples, < 0.5 – 1.85 $\mu g.L^{-1}$), dichloroacetic acid (87%, < 0.05 – 0.22 $\mu g.L^{-1}$), trichloroacetic acid (93%, < 0.05-0.52 $\mu g.L^{-1}$), dibromoacetic acid (53%, < 0.01-0.40 $\mu g.L^{-1}$), tribromoacetic acid (20%, < 0.05 - 0.14 $\mu g.L^{-1}$) and bromodichloroacetic acid (6%, <0.05 $\mu g.L^{-1}$).

Keywords: Haloacetic acids, river water, solid phase extraction, GC-MS coupling, tandem mass spectrometry

1. INTRODUCTION

Haloacetic acids (HAAs) are among the main organohalogenated by-products (OXBPs) identified with regular frequency in water [1]. They are mainly formed from disinfection treatments using halogen-based inorganic oxidants, such as chlorine (HOCl), chlorine dioxide (ClO_2) and monochloramine (NH_2Cl) [2]. With trihalomethanes, HAAs are still considered to be the dominant OXBPs groups on a weight basis in drinking water [3]. Their formation mechanism is now well understood and their concentration levels in drinking water are regulated in various countries [4]. HAAs are also widespread environmental contaminants [5]. However, their presence in the aquatic environments is not only related to human activities but also to natural sources [6,7]. Indeed, some HAAs have been found applications in various fields. For example, trichloroacetic acid (TCAA) is used as selective herbicide, etching or pickling agent in the surface treatment of metals, and auxiliary in textile finishing, while monochloracetic acid (MCAA) is mainly used as chemical intermediate for the synthesis of a wide variety of useful chemicals (e.g. drugs, dyes, and pesticides) [8-10]. Thus, HAAs may enter aquatic ecosystems through many routes, including discharge of waters treated by halogen-based biocides, degradation of halogenated organic compounds, and runoff from polluted sols, atmospheric deposit, as well as natural production [10-15].

HAAs have attracted since a long time considerable attention of both scientists and regulators due to their potential adverse effects on human and aquatic organisms. Indeed, the laboratory in vitro and in vivo studies showed that HAAs are cytotoxic, genotoxic, mutagenic, and teratogenic [16-18]. The recent toxicology studies found that the toxicity of HAAs follow the order: I > Br > Cl, which highly correlated to the SN2 reactivity, αC-X bond length and αC-X dissociation energy [19,20]. Also, several haloacetic acids have been shown to produce developmental and/or reproductive toxicity [21]. Because of their potential effects on human health, the world Health Organization (WHO) has suggested guideline values for MCAA, dichloroacetic acid (DCAA), and TCAA at 20, 50 and 200 µg.L-1, respectively [2]. In the USA, HAAs are regulated by the Environment Protection Agency (USA-EPA), which has established a Maximum Contaminant Level (MCL) of 60 $\mu g.L^{-1}$ for the sum of MCAA, MBAA (monobromoacetic acid), DCAA, DBAA (dibromoacetic acid) and TCAA concentrations [2]. A guideline value for these same five HAA species also exists in Canada, at concentration of 80 $\mu g.L^{-1}$. However, to date, no regulations have been promulgated in drinking water for HAAs in the European Union (EU). In order to protect freshwater aquatic organisms, INERIS (French National Institute for Industrial Environment and Risks) has proposed for the MCAA an Environmental Guide Value of 2.5 $\mu g.L^{-1}$ as Maximum Acceptable Concentration, and a predicted no effect concentration for the TCAA of 0.017 $\mu g.L^{-1}$[22]. More recently, brominated and chlorinated HAAs (9 HAAs) have been chosen by the ECHA (European Chemicals Agency) as relevant disinfection by-products for the environmental risk assessment under European biocide law (Regulation (EU) 528/2012) [23]. To evaluate the effects of HAAs on the aquatic ecosystem, there is a

need for a sensitive and specific method of analysis to quantify these compounds at trace concentrations in river water. To date, few have studied the HAAs concentrations ("background values") in river waters as well as their effects on aquatic ecosystem.

The current standard methods for HAAs analysis are based on liquid-liquid extraction (LLE) followed by gas chromatography detection using either electron capture or mass spectrometric detectors [552.2 EPA Method; ISO 23631:2006]. Due to their low volatility and polar nature, HAAs are converted to their methyl ester derivatives prior to GC analysis. Nowadays, GC/MS is gradually replacing GC/ECD in analysis laboratories and is increasingly acknowledged as the technique of choice for HAAs analysis in water samples. Up to date, a few studies using mass spectrometry operated in Multiple Reaction Monitoring (MRM) mode in comparison with Selected Ion Monitoring (SIM) mode have been described in the literature, while unambiguous HAAs analysis in the presence of a wide range of OXBPs (potential interferers) justified the use of this acquisition mode [24,25]. It is well known that mass spectrometry in MRM mode confers high specificity and reduces the risk of potential interferences related to the complexity of the matrix, providing unmatched sensitivity and selectivity in trace analysis. The additional benefits are that HAAs do not have to be fully resolved to be identified and quantitated, as it is required when using conventional ECD, or SIM mode (in the case of isobaric product ions). Moreover, LLE is increasingly criticized because it is time consuming, labor intensive, and it involves - to achieving a high concentration factors - large volumes of organic solvents (i.e. methanol and methyl-tert-butyl-ether), which are undesirable for health and disposal reasons. Solid phase extraction (SPE) is known to provide a better selectivity than LLE, thus allowing to extent the use of the method to aqueous samples rich in dissolved organic matter. Furthermore, SPE allows important pre-concentration through its ability to percolate large amounts of sample; it also limits consumption of organic solvents. However, as far as the authors are aware, no study reports its use to extracting HAAs from river waters.

The aim of the present work was to develop an alternative, sensitive and fast analytical method to simultaneously measure 11 HAAs including 5 USA-EPA regulated HAAs (MCAA, DCAA, TCAA, MBAA, and dDBAA), 4 unregulated HAAs (tribromoacetic acid (TBAA), bromochloroacetic acid (BCAA), dichlorobromoacetic acid (DCBAA) and dibromochloroacetic acid (DBCAA)), as well as 2 iodinated emerging HAAs (monoiodoacetic acid (MIAA), and dioodoacetic acid (DIAA)) in river water samples. For this purpose, the analytical approach used combines solid phase extraction, chemical derivatization and GC-MS/MS analysis in the MRM mode.

2. EXPERIMENTAL SECTION

2.1. Reagents and chemicals

A mixed standard containing monobromoacetic acid (MBAA), bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), monochloroacetic acid (MCAA), dibromochloroacetic acid (DBCAA), dibromoacetic acid (DBAA), dichloroacetic acid (DCAA), tribromoacetic acid (TBAA) and trichloroacetic acid (TCAA) (EPA 552.2 Haloacetic Acids Mix, 2000 $\mu g.mL^{-1}$ each component in MTBE, > 99%) was obtained from Sigma Aldrich (Saint-Quentin Fallavier, France). All other target analytes were obtained as neat compounds. Iodoacetic acid (IAA, 98%), 1,2-dibromopropane (internal standard, 97%) and sulphuric acid were purchased from Sigma Aldrich (Saint-Quentin Fallavier, France), as well as L-ascorbic acid (99%) employed to neutralize residual oxidants. Diiodoacetic acid (DIAA, 90%) was supplied from Santa Cruz Biotechnology (Heidelberg, Germany). 2,3-dibromopropionic acid (surrogate, 1000 $\mu g.mL^{-1}$ in MTBE) was obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Methanol (MeOH, HPLC grade, 99.8%) and methyl-tert-butyl-ether (MTBE, GC grade, 99.8%) were purchased from Merck (Darmstadt, Germany).

2.2. Sample preparation process

All samples were collected in 2 L amber bottles. 2 mL of L-ascorbic acid at 1.6 $g.L^{-1}$ have been introduced into each bottle prior to water collection in order to quench residual oxidant immediately after sampling, thus preventing any continued formation of HAAs during the holding time between sample collection and analysis [26]. A volume of 20 µL of 2,3-dibromopropionic acid surrogate standard (50 $\mu g.mL^{-1}$ in MTBE) was added to each sample on its arrival at the laboratory. Each one was gently mixed, adjusted to pH < 2 with concentrated sulphuric acid (1% v:v) and transferred into a 1 L glass bottle. The « AutoTrace 280 » (Thermo Fisher Scientific, Courtaboeuf, France) automated solid-phase extraction (SPE) system was used to extract the analytes from water. Six types of widely used adsorbing materials including Strata® SDB-L (500 mg, 6mL) from Phenomenex (Le Pecq, France), Bakerbond Carbon (500 mg, 6 mL), Bakerbond SDB-1 (200 mg, 6 mL) and Bakerbond C18 (500 mg, 6 mL) from Interchim (Montluçon, France), Oasis-HLB (500 mg, 6 mL) from Waters (Guyancourt, France), and LiChrolut® EN (200 mg, 6 mL) from Merck (Fontenay-sous-Bois, France) were evaluated in terms of selectivity and recovery yields. All cartridges were compared according to the same extraction procedure. They were conditioned with 5 mL of MeOH and 10 mL of acidified water (H_2SO_4 10% v:v). Sample volumes of 1 L were loaded on the cartridges, and the aqueous solution was eluted as waste at a flow rate of 5 $mL.min^{-1}$. After percolation, the cartridges were rinsed with 5 mL of acidified water (H_2SO_4 10% v:v) and the sorbents were dried for 10 minutes. The retained HAAs were eluted with 5 mL of acidified methanol (H_2SO_4 10% v:v) followed by 3 mL of MTBE. The resulting elutes were collected in 20 mL conical amber glass vessels, sealed hermetically and placed in a water bath regulated at 50 °C for 2 h to allow derivatization of HAAs to their methyl esters. After that, samples vials were cooled to 4 °C for 10 min and 7.5 ml of an aqueous sodium sulfate solution at a concentration of 150 $g.L^{-1}$ were added. The vials were shaken for 2 min and

allowed to stand for 10 min. The aqueous layer was discarded before quenching of the acid-catalyzed esterification of HAAs. The organic phase was neutralized by adding 1 mL of an aqueous saturated sodium bicarbonate solution (89 $g.L^{-1}$). The sample vials were shaken again for 2 min in a vortex mixer and allowed to stand for 5 min. 500 µL of the MTBE extract were transferred into an amber vial along with 10 µL of 1,2-dibromopropane (500 $\mu g.mL^{-1}$ in MTBE) as internal standard. Finally, 1.5 µL of the MTBE extract was analyzed by GC-MS. The surrogate standard was used to monitor errors during sample preparation (both extraction and chemical derivatization), whereas the internal standard was employed to monitor the GC-MS performance. For each targeted HAA, the relative recovery was calculated as the percentage ratio between its GC-MS area response obtained with the tested SPE phase to that obtained with the SPE phase leading to the highest GC-MS area response.

2.3. Instrumentation and GC-MS/MS analytical conditions

GC-MS analysis of SPE extracts were performed on a Thermo Fisher Scientific « Trace GC Ultra » gas chromatograph equipped with a « Triplus » autosampler and coupled with a « TSQ Quantum XLS » triple quadrupole mass spectrometer (Thermo Fisher Scientific, Courtaboeuf, France). The chromatographic separation was carried out on a 30 m « TG-5MS » (5% phenyl, 95% methylpolysiloxane) capillary column (internal diameter: 0.25 mm, film thickness: 0.25 µm) from Thermo Fisher Scientific. High purity helium (99.9995%) was used as the carrier gas at a constant flow of 1.4 $mL.min^{-1}$. All experiments were performed by automatically injecting 1.5 µL using a 10 µL syringe into a programmed temperature vaporization injector (PTV) in splitless mode. The PTV conditions were: injection temperature, 180 °C; cleaning temperature, 270 °C; splitless time, 2.00 min; split flow, 30 $mL.min^{-1}$; and cleaning time, 4.00 min. In order to trap the analytes at column head, the oven temperature was maintained at 35 °C for 5.00 min, then raised at 10 $°C.min^{-1}$ to 110 °C followed by an increase to 200 °C at 20 $°C.min^{-1}$, for a total duration of 17 min. The solvent delay was set at 4.50 min. Transfer line temperature, and ion source temperatures were kept at 280 and 250 °C, respectively. In a first approach, acquisition was carried out in the EI full scan mode from 50 to 450 m/z at 100 $ms.scan^{-1}$. The mass spectrometer was operated in the electron ionization mode at 70 eV. The filament emission current was set at 25 µamps in the full scan mode and at 50 µamps for MS/MS experiments. The electron multiplier voltage was set at 1040 V by automatic tuning (3.10^5 gain). MS/MS experiments were performed with argon as collision gas at a nominal pressure of 1 mTorr. The collision induced dissociation (CID) parameters were subject to optimization and are given below.

3. RESULTS AND DISCUSSION

3.1. GC-MS/MS characteristics

Parameters of the mass spectrometer and gas chromatograph were optimized by injecting a mixture solution of all HAAs in MTBE each one at 20 µg.mL^{-1}. Table 1 gives the main characteristics of the GC-MS/MS method: retention times, transitions retained and corresponding collision energies. The upper part of Figure 1 displays the chromatogram obtained after SPE of a mixture of all HAAs each one at 5 µg.L^{-1} and the surrogate and internal standards at 4 µg.L^{-1}, respectively. Under optimized GC conditions, most of the targeted HAAs are well separated with the exception of BCAA and MIAA as well as DBCAA and 2,3-dibromopropionic acid (surrogate standard, SA).

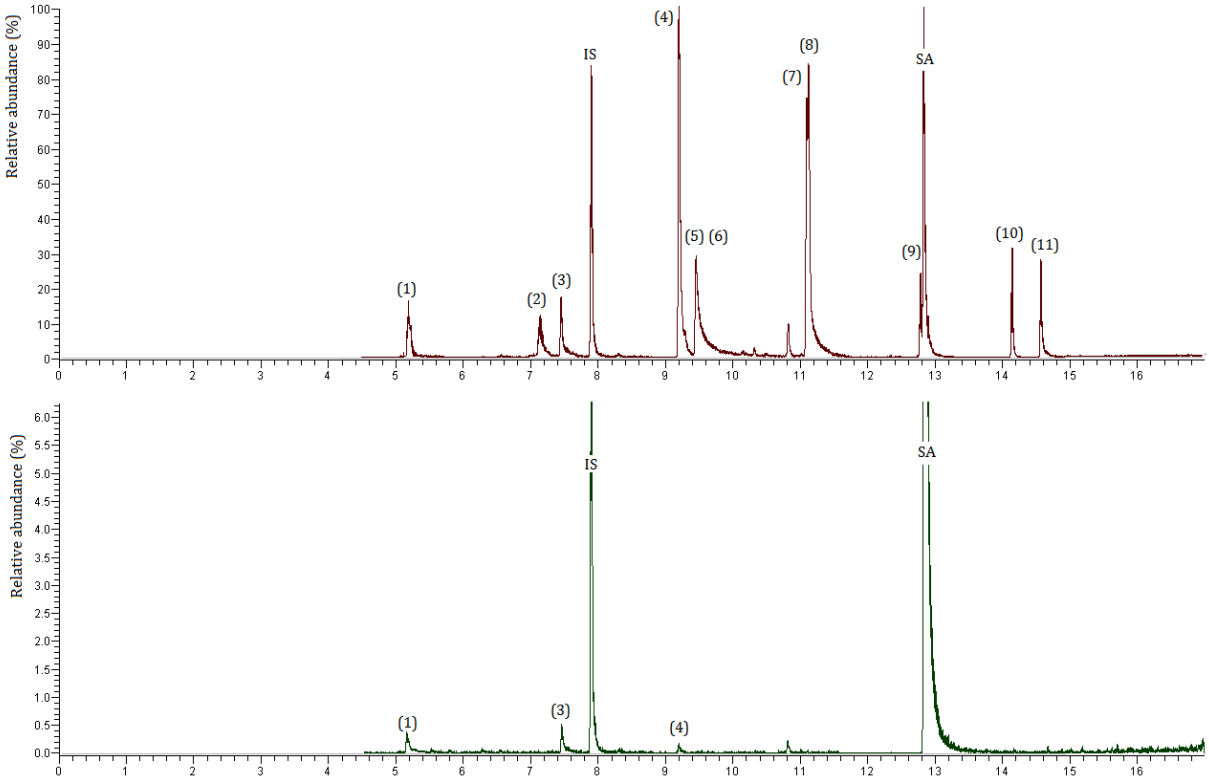

Figure 1. Chromatograms of the reference solution of HAA at 5 µg.mL^{-1} (above) and of a real sample taken on August 22nd 2016 (below). 1: MCAA, 2: MBAA, 3: DCAA, IS: 1,2-dibromopropane, 4: TCAA, 5: BCAA, 6: MIAA, 7: DBAA, 8: BDCAA, 9: DBCAA, SA: 2,3-dibromopropionic acid, 10: TBAA, 11: DIAA.

Table 1. Main characteristics of the GC-MS/MS method

Compound	Retention time (min)	Transitions for quantitation (in bold) and confirmation (m/z)	Collision energy (V)	LOQ (µg.L-1)	Calibration range	Response function	R2
Monochloroacetic acid	5.13	**77.0 → 49.0** 59.0 → 43.0	10 5	0.50	0.50-5.00	Linear	0.998
Monobromoacetic acid	7.19	**120.9 → 92.9** 72.0 → 42.0	10 7	0.50	0.50-5.00	Linear	0.998
Dichloroacetic acid	7.45	**82.9 → 48.0** 76.0 → 48.0	30 10	0.05	0.05-5.00	Quadratic	0.994
1,2-dibromopropane	7.90	**121.0 → 41.0** 123.0 → 41.0	10 10	concentration = 4 µg.L^{-1}			
Trichloroacetic acid	9.20	**116.9 → 81.9** 141.0 → 113.0	30 5	0.05	0.05-5.00	Quadratic	0.992
Monoiodoacetic acid	9.44	**169.0 → 141.0** 73.0 → 45.0	10 10	0.25	0.25-5.00	Linear	0.993
Bromochloroacetic acid	9.44	**129.0 → 48.0** 76.0 → 48.0	50 10	0.05	0.05-5.00	Quadratic	0.994
Bromodichloroacetic acid	11.11	**162.9 → 81.9** 140.9 → 112.9	40 7	0.05	0.05-5.00	Quadratic	0.994
Dibromoacetic acid	11.08	**172.9 → 91.9** 119.9 → 91.9	47 12	0.01	0.01-5.00	Quadratic	0.995
Dibromochloroacetic acid	12.79	**206.8 → 127.9** 186.9 → 158.9	40 7	0.01	0.01-5.00	Quadratic	0.993
2,3-dibromopropionic acid	12.84	**165.0 → 133.0** 186.9 → 106.0	10 10	concentration = 4 µg.L^{-1}			
Tribromoacetic acid	14.06	**250.8 → 171.8** 230.9 → 202.9	45 10	0.05	0.05-5.00	Quadratic	0.992
Diiodoacetic acid	14.46	**325.9 → 171.0** 266.8 → 139.9	15 30	0.01	0.01-5.00	Quadratic	0.999

3.2. Solid phase extraction

Results of the tests conducted on SPE cartridges are summarized in Table 2, on the basis of recovery yields and relative standard deviation (RSD) for n = 3. Performances are very poor for the Bakerbond C18 phase and not satisfying for the Strata SDB-L one, which leads for MCAA to a recovery yield of only 4% compared to that obtained with a Bakerbond Carbon phase. Compared with other stationary phases, this later tends to favor the recovery of low molecular weight HAAs over that of high molecular weight HAAs. In terms of mean recovery yields on all the selected HAAs, Oasis-HLB, Bakerbond SDB, LiChrolut EN and Bakerbond Carbon provide comparable results with relative recovery yields ranging from 31 to 100% depending to the HAAs and cartridge considered. This work being part of a larger study dedicated to the determination of the AOX (adsorbable organohalogenated compounds) parameter [2], the Bakerbond SDB phase has finally been retained because it provides the best results for several classes of organohalogenated disinfection by-products [27]. In the present work, this sorbent allowed achieving the best recovery yields for almost all the studied HAAs but MCAA and MBAA while providing satisfying results for these latter. Our results are in good agreement with some published studies. Martínez et al. compared four different commercial sorbents, namely LC-SAX (a quaternary ammonium anion exchanger), LiChrolut EN, Envi-Carb (a graphitized carbon black) and Oasis HLB, in a solid phase extraction process to recover various haloacetic acids from water samples. They found that the best sorbent was LiChrolut EN (PS-DVB from Merck Millipore) which provided recovery values between 37 and 85% in the pre-concentration of 500 mL of tap water samples [28]. Prieto-Blanco et al. studied the recovery of HAAs from water samples using Oasis HLB, Isolute ENV (hyper cross-linked hydroxylated PS-DVB from Bitage) and Lichrolut EN [29]. They found that Isolute ENV offered a better recovery rate for monohalogenated acetic acids (MHAAs), whereas Lichrolut EN offered a better rate for all tested HAAs, with an average recovery of 80%. Similar results were reported by Sun and Ping on the recovery of HAAs from chlorinated hospital effluent, using C18 pretreatment to reduce the samples' turbidity and LiChrolut EN to extract the targeted compounds [30].

Table 2. Recoveries of 11 HAAs from 1 L of river water using six SPE commercial sorbents

HAA	OASIS-HLB (500 mg)		Bakerbond SDB (200 mg)		Strata® SDB-L (500 mg)		LiChrolut® EN (200 mg)		Bakerbond Carbon (500 mg)		Bakerbond C18 (500 mg)	
	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD
MCAA	45	8	31	7	4	1	32	4	100	15	0	0
MBAA	94	16	73	15	12	2	71	7	100	17	1	0
DCAA	100	15	97	11	44	10	98	3	89	21	3	1
TCAA	100	11	95	12	70	18	98	5	69	19	12	2
MIAA	92	19	96	12	35	8	100	5	80	17	2	0
BCAA	100	21	96	14	68	18	97	9	79	23	5	2
BDCAA	100	11	100	7	85	29	98	6	61	20	14	4
DBAA	100	17	100	15	86	23	99	8	76	24	9	2
DBCAA	100	16	100	11	90	32	92	6	56	22	19	6
TBAA	93	13	100	8	91	40	87	8	47	24	27	10
DIAA	100	20	96	15	92	31	88	3	52	20	27	6

[a] % average recovery of SPE cartridges

[b] RSD: relative standard deviation (n=3)

3.3. Method performances

The developed method was validated under the optimized conditions. Response functions, quantification limits, and correctness (error and precision) have been assessed according to the French method validation standard NF T90-210, already described for the validation of an analytical method dedicated to monochloramine determination [31]. Table 1 provides the performances achieved for each HAA, in terms of limit of quantitation and correlation coefficient on the calibration domain. Various regression models including linear, quadratic and cubic models were adjusted and compared in order to determine the most suitable regression model of each HAA. The most suitable mathematical models for describing the relationship between studied HAAs and its analytical response were the linear model for the MCAA, MBAA, and MIAA, and the quadratic model for the others targeted HAAs. A good regression was obtained with correlation coefficients equal or higher than 0.990 for all the studied HAAs. As can been seen on Table 1, the LOQs values range from 0.01 to 0.50 $\mu g.L^{-1}$ depending of the considered HAA; they are widely lower than those reported by ISO 23631 standard method (from 0.5 to 10 $\mu g.L^{-1}$) and comparable to those reported by Li et al. with a method combining LEE with GC-MS/MS analysis (from 0.03 to 0.24 $\mu g.L^{-1}$) [25]. Compared with LLE, SPE tends to be relatively more efficient for MCAA and, to a lesser extent, to brominated HAA, a little less efficient for other species. The trueness and precision (accuracy) of the method were determined using river water samples spiked with HAAs standard solutions at three levels of concentration: low (LOQ), middle (5 x LOQ), and high (10 x LOQ). The river water samples were also analyzed before spiking to determine the possible presence of target analytes. The correctness of the developed method was proven since the obtained accuracy profiles respect the chosen acceptance limits, set at ± 60% for LOQ, ± 20% between 5 x LOQ and 10 x LOQ.

3.4. Application to real river water samples

The developed method was applied to quantitative determination of HAAs in untreated river water samples. These samples were collected between July and September 2016 in three rivers water in France: Meuse, Moselle, and Loire. The characteristics of water samples were presented in Table 1 in the Supporting information. The very good selectivity of developed method is illustrated on Figure 1; the bottom part of it displaying the chromatogram of a real river water (taken from the Moselle River, on August 22nd 2016), in which were detected MCAA, DCAA and TCAA in trace amounts. Values of 1.34 $\mu g.L^{-1}$, < 0.05 $\mu g.L^{-1}$ and 0.12 $\mu g.L^{-1}$ were determined for the concentrations in MCAA, DCAA and TCAA, respectively. The results of the analysis of fifteen river water samples are summarized in Table 3.

Table 3. HAAs occurrence ($\mu g.L^{-1}$) in the three studied rivers.

HAAs	LOQs ($\mu g.L^{-1}$)	Moselle (n = 6)						Meuse (n = 5)					Loire (n = 4)			
		S_1	S_2	S_3	S_4	S_5	S_6	S_1	S_2	S_3	S_4	S_5	S_1	S_2	S_3	S_4
MCAA	0.50	1.39	1.42	1.34	0.90	0.51	1.85	1.48	< LOQ	1.11	1.59	1.02	nd	1.17	< LOQ	0.95
DCAA	0.05	nd	< LOQ	< LOQ	< LOQ	nd	0.12	0.09	< LOQ	< LOQ	< LOQ	< LOQ	0.06	0.22	< LOQ	< LOQ
TCAA	0.05	0.52	0.37	0.12	0.11	0.22	0.29	< LOQ	0.06	0.06	0.14	0.12	nd	< LOQ	< LOQ	< LOQ
MBAA	0.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DBAA	0.01	0.05	nd	nd	0.40	0.05	0.05	< LOQ	nd	nd	nd	< LOQ	nd	nd	0.02	0.01
TBAA	0.05	< LOQ	nd	nd	0.14	0.14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BCAA	0.05	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
BDCAA	0.05	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DBCAA	0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MIAA	0.25	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DIAA	0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Abbreviations: nd: not detected (concentration < LOD). n: number of water samples analyzed for each river water, Si: Sampling dates (see table SI-1, in supporting information)

As can be seen form Table 3, the HAAs were detected in all the water samples analyzed. Five among the eleven target HAAs were detected at least once above their LOQ. MCAA, DCAA, TCAA, and DBAA were measured in all studied rivers, while TBAA and BDCAA was observed only in water samples from the Moselle river. Their magnitude of the concentration was in the following order: MCAA (< 0.5-1.85 $\mu g.L^{-1}$) > TCAA (< 0.05-0.52 $\mu g.L^{-1}$) > DCAA (0.05-0.22 $\mu g.L^{-1}$) > DBAA (< 0.01-0.40 $\mu g.L^{-1}$) > TBAA (< 0.05 - 0.14 $\mu g.L^{-1}$) > BDCAA (<0.05 $\mu g.L^{-1}$). MCAA was the most abundant among the six detected HAA species. In the water samples where it was detected at concentrations above LOQ, its mass concentrations were found to be accounted for 56 to 91% of the total HAAs concentration. Unfortunately, there is a very little published data reporting HAAs concentrations in surface water (lake and river waters). The available data concern mostly TRCAA. The concentrations of TCAA in the three studied rivers are in good agreement with those reported in lakes and rivers in Europe, which have been found in most water bodies in a range from <0.03 to 1.88 $\mu g.L^{-1}$ [10,32-35]. For example, Frank et al., in their survey encompassing four German rivers (nine samples), found the TCAA at concentrations ranging between 0.12 and 0.6 $\mu g.L^{-1}$ [32]. However, in the work of Loos & Barcelo, TCAA concentrations up to 308 $\mu g.L^{-1}$ were measured in river water in Portugal [31]. These authors also measured MCAA, DCAA, BCAA, BDCAA, and TBAA at 36, 1-3, 29, 7-48, and 26-42 $\mu g.L^{-1}$, respectively. To our knowledge, this is the first time that HAAs rather than MCAA, DCAA, DBAA, and BCAA have been measured in river waters.

4. CONCLUSION

This article presents a sensitive, selective and specific method for the determination of several classes of HAAs in water samples. In terms of LOQs, the developed method provides performances widely better than those reported by ISO 23631 standard method and comparable to those of the method reported by Li et al. [25]. Methods selectivity cannot be compared only based on figures displaying chromatograms but selectivity is assumed to be systematically better in SPE than in LLE [26]. SPE allows percolation of large sample volumes and is compatible with the principles of sustainable chemistry for it uses small amounts of organic solvents in comparison with LLE processes. When the developed method was applied to the determination of the HAAs in water samples from three different rivers in French, the results indicate a quasi-systematic presence of five of the eleven target HAAs (MCAA, DCAA, TCAA, DBAA, and TBAA). The developed method is currently employed by our research group for simultaneous extraction and determination of HAAs in rivers waters.

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References

[1] S.D. Richardson, C. Postigo, Drinking Water Disinfection By-Products. Chapter 4, The Handbook of Environmental Chemistry: Emerging Organic Contaminants and Human Health. Springer, New York, NY, 20:93-137, 2012.
[2] A. Kinani, S. Kinani, B. Richard, M. Lorthioy, S. Bouchonnet, Formation and determination of organohalogen by-products in water - Part I. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method, TrAC Trends Analytic. Chem. 85, 2016, 273-80
[3] P. Singer, H. Weinberg, S. Krasner, H. Arora, I. Najm, Relative dominance of HAAs and THMs in treated drinking water, AWWA Research Foundation and American Water Works Association, ISBN 1-58321-117-9, 2002.
[4] M. Deborde, U. Von Gunten, Reactions of chlorine with inorganic and organic compounds during water treatment - kinetics and mechanisms: a critical review, Water Res. 42 (2008) 13-51.
[5] M.L. Hanson, K.R. Solomon. Haloacetic acids in the aquatic environment. Part I: macrophyte toxicity, Environmental Pollution 130 (2004) 371-383.
[6] B.F. Scott, D. MacTavish, C. Spencer, W.M.J. Strachan, D.C.G. Muir, Haloacetic acids in Canadian lake waters and precipitation. Environ. Sci. Technol. 34 (2000), 4266-4272.
[7] B.F. Scott, C. Spencer, C.H. Marvin, D. MacTavish, D.C.G. Muir, Distribution of haloacetic acids in the water column of the Laurentian Great Lakes and Lake Malawi. Environ. Sci. Technol. 36 (2002), 1893-1898.
[8] A. McCulloch, Trichloroacetic acid in the environment, Chemosphere 47 (2002) 667–686
[9] M.L. Hanson, P.K. Sibley, D.A. Ellis, S.A. Mabury, D.C.G. Muir, K.R. Solomon They have attracted since a long time considerable attention of both scientists and regulators due to their adverse biological effects on human and aquatic organisms. Aquatic Toxicology 61 (2002) 251-273.
[10] European Union Risk Assessment Report – Monochloracetic acid (MCAA). EUR 21403 EN (2005). http://europa.eu.int.
[11] G. Haiber, G. Jacob, V. Niedan, G. Nkusi, H.F. Schöler, The occurrence of trichloroacetic acid (TCAA) – indications of a natural production?, Chemosphere 33 (1996) 839–849.
[12] M.R. Heal, C.A. Dickey, K.V. Heal, R.T. Stidson, M. Matucha, J.N. Cape. The production and degradation of trichloroacetic acid in soil: Results from in situ soil column experiments, Chemosphere 79 (2010) 401–407.
[13] E.J. Hoekstra, E.W.B. de Leer, U.A.T. Brinkman, Findings supporting the natural formation of trichloroaceticacid in soil, Chemosphere 38 (1999) 2875–2883.
[14] E.J. Hoekstra, E.W.B. de Leer, U.A.T. Brinkman, Mass balance of trichloroacetic acid in the soil top layer, Chemosphere 38 (1999) 551–563.
[15] M. Matucha, M. Gryndler, P. Schröder, S.T. Forczek, H. Uhlířová, K. Fuksová, J. Rohlenová, Chloroacetic acids-Degradation intermediates of organic matter in forest soil. Soil Biology & Biochemistry 39 (2007) 382–385.
[16] J.A. Pals, J.K. Ang, E.D. Wagner, M.J. Plewa. Biological mechanism for the toxicity of haloacetic acid drinking water disinfection byproducts. Environ Sci Technol. 13 (2011) 5791-5797.
[17] S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutat. Res. 636(2007) 178-242.
[18] Y. Kargalioglu, B.J. McMillan, R.A. Minear, M.J. Plewa, Analysis of the cytotoxicity and mutagenicity of drinking water disinfection by-products in Salmonella typhimurium. Teratogen. Carcinogen. Mutagen. 22 (2002) 113-128.
[19] M.J. Plewa, J.E. Simmons, S.D. Richardson, E.D., Wagner, Mammalian Cell Cytotoxicity and Genotoxicity of the Haloacetic Acids, A Major Class of Drinking Water Disinfection By-Products. Environmental and Molecular Mutagenesis 51(2010), 871-878.
[20] M.J. Plewa, E.D. Wagner, S.D. Richardson, A.D. Thruston, Y.T. Woo, A.B. McKague, Chemical and biological characterization of newly discovered iodoacid drinking water disinfection byproducts, Environ. Sci. Technol. 38(2004) 4713-4722.
[21] E.S. Hunter III, E. Rogers, M. Blanton, Ann Richard, N. Chernoff. Bromochloro-haloacetic acids: Effects on mouse embryos in vitro and QSAR considerations. Reproductive Toxicology 21 (2006) 260-266.
[22] Valeurs Guides Environnementales – INIRIS (http://www.ineris.fr/substances/fr/page/9).
[23] Guidance on the Biocidal Products RegulationVolume V, Guidance on Disinfection By-Products. (https://echa.europa.eu)
[24] L.U.R. Chiavelli, L.C. Figueiredo, R.T.R. Almeida, T. Claus, S.A. Maruyama, W.F. Costa, Validation and application of a GC-MS method for the determination of haloacetic acids in drinking water, J. Serb. Chem. Soc. 80(11) (2016) 1273-1282.

[25] W. Li, Y. Liu, J. Duan, D. Mulcahy, Determination of ten haloacetic acids in water using gas chromatography-triple quadrupole mass spectrometry, Anal. Methods 5 (2013) 2258-2266.
[26] A. Kinani, S. Kinani, S. Bouchonnet, Formation and determination of organohalogen by-products in water - Part II. Sample preparation techniques for analytical approaches, TrAC Trends Analytic. Chem. 85, 2016, 281-94.
[27] A. Roumiguières, A. Kinani, S. Bouchonnet, S. Kinani, Development and validation of a multiclass method for the determination of organohalogen disinfectant by-products in water samples using solid phase extraction and gas chromatography-tandem mass spectrometry, J. Chrom. A, submitted.
[28] D. Martınez, F. Borrull, M. Calull, Comparative study of a solid-phase extraction system coupled to capillary electrophoresis in the determination of haloacetic compounds in tap water, J. Chrom. A, 827 (1998) 105-112.
[29] M. Prieto-Blanco, M. Alpendurada, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, S. Machado, C. Gonçalves, Improving methodological aspects of the analysis of five regulated haloacetic acids in water samples by solid-phase extraction, ion-pair liquid chromatography and electrospray tandem mass spectrometry, Talanta 94 (2012) 90-98.
[30] Y.-X. Sun, G. Ping, Determination of haloacetic acids in hospital effluent after chlorination by ion chromatography, J. Environ. Sci. 19 (2007) 885-891.
[31] S. Kinani, S. Layousse, B. Richard, A. Kinani, S. Bouchonnet, A. Thomas, F. Sacher, Selective and trace determination of monochloramine in river water by chemical derivatization and liquid chromatography/tandem mass spectrometry analysis, Talanta 140 (2015) 189-197.
[32] R. Loos, D. Barcelo. Determination of haloacetic acids in aqueous environments by solid-phase extraction followed by ion-pair liquid chromatography–electrospray ionization mass spectrometric detection, J. Chrom. A, 938 (2002) 45-55.
[33] H. Frank , D. Renschen, A. Klein, H. Scholl, Trace analysis of airborne haloacetates, Journal of High Resolution Chromatography, 18(1995) 83-88.
[34] H.F. Schöler, F. Keppler, I.J. Fahimi, V.W. Niedan. Fluxes of trichloroacetic acid between atmosphere, biota, soil, and groundwater. Chemosphere 52 (2003) 339-354.
[35] A. McCulloch. Trichloroacetic acid in the environment. Chemosphere 47 (2002) 667–686.

Supplementary Materials (SM)

Table SI-1. Water quality characteristics of the river waters analyzed in this study.

River water source	Sampling date	DOC (mg C.L^{-1})	A_{254} (cm^{-1})	SUVA (L/mg.m)	Halides concentrations (mg.L^{-1})		
					Cl^-	Br^-	I^-
3	27/07/2016	4.6	0.070	1.52	17.8	< 0.1	< 1
	10/08/2016	2.7	0.073	2.70	18.6	< 0.1	< 1
	24/08/2016	2.6	0.074	2.84	18.3	< 0.1	< 1
	07/09/2016	2.5	0.071	2.84	20.5	< 0.1	< 1
2	04/08/2016	4.7	0.161	3.42	12.4	< 0.1	< 1
	18/08/2016	2.7	0.062	2.30	15.6	< 0.1	< 1
	01/09/2016	2.3	0.061	2.65	17.9	< 0.1	< 1
	15/09/2016	2.4	0.065	2.71	19.0	< 0.1	< 1
	22/09/2016	2.2	0.069	3.13	19.5	< 0.1	< 1
1	01/08/2016	3.9	0.078	2.00	329	0.3	< 1
	08/08/2016	4.1	0.079	1.93	393	0.4	< 1
	22/08/2016	3.6	0.074	2.05	354	0.4	< 1
	29/08/2016	3.6	0.075	2.08	374	0.4	< 1
	05/09/2016	3.6	0.077	2.14	329	0.4	< 1
	12/09/2016	3.7	0.138	3.73	618	0.7	< 1

Nomenclature: A_{254}: Absorbance at 254 nm. DOC: Dissolved organic carbon. SUVA: Specific ultraviolet absorbance.

II- Développement et validation d'une méthode multi résidus ciblant 26 SPOX volatils par SPE et GC-MS

Nous détaillons dans l'article ci-après le développement et la validation d'une nouvelle méthode d'analyse multi résidus par extraction sur phase solide (SPE) et analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse en tandem (GC-EI-MS/MS) pour la quantification de 26 SPOX dans les eaux. L'intérêt de développer une telle méthode émane d'une volonté d'élargir le spectre de SPOX ciblés, d'optimiser les délais de réponse et de rationaliser des coûts analytiques. Les SPOX ciblés comprennent 4 trihalométhanes (THM), 4 iodohalométhanes (I-HM), 1 haloacétaldéhyde (HAL), 6 halocétones (HK), 4 halonitrométhanes (HNM) et 7 haloacétonitriles (HAN). Les critères de sélection de ces SPOX sont décrits au paragraphe II.3.5 du chapitre II. Une attention particulière a été portée à l'abaissement des limites de quantification. Les performances de la méthode développée ont été évaluées selon le référentiel normatif NF T90-210. La nouvelle méthode offre des améliorations substantielles par rapport aux méthodes précédemment utilisées (études EDF R&D antérieures). Elle fournit des limites de quantification (LOQ) comprises entre 3 et 3000 $ng.L^{-1}$. Ses performances en termes de spécificité et de sélectivité ont été démontrées en analysant des échantillons d'eau de rivière et de robinet.

Development and validation of a multiclass method for the determination of organohalogen disinfectant by-products in water samples using solid phase extraction and gas chromatography-tandem mass spectrometry

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Abstract

An analytical method employing solid phase extraction (SPE) and gas chromatography-tandem mass spectrometry (GC-MS/MS) has been developed for quantitative determination of organohalogen disinfectant by-products (OXBPs) in water samples. The singularity of this method lies in its ability to measure 26 organohalogen by-products belonging to six different chemical classes in a single run. Target analytes include 4 trihalomethanes (THMs), 4 iodohalomethanes (I-HMs)], 1 haloacetaldehyde (HAL), 6 haloketones (HKs), 4 halonitro-methanes (HNMs), and 7 haloacetonitriles (HANs). The sample preparation procedure includes pretreatment with ascorbic acid to quench residual oxidants, followed by analyte enrichment using solid-phase extraction. Five SPE sorbents were tested. The best results for the majority of target analytes were obtained using a styrene-divinylbenzene copolymer phase and methyl tert-butyl ether (MTBE) as the elution agent. GC/MS analysis was performed using electron ionization (EI) and selected reaction monitoring for analyte detection. The performance of the method was assessed per the French standard NF T90-210. The method showed LOQs ranging from 3 to 3000 ng.L^{-1}. The applicability of the method has been demonstrated by analyzing both river water and tap water samples. The OXBPs detected most often in the tap water were DBCM (in 100% of the samples, 4.3 – 4.7 µg/L), BDCM (100%, 1.3 – 1.7 µg/L), TBM (100%, 0.8 – 4.4 µg/L), TCM (100%, 0.6 – 0.7 µg/L), DBAN (75%, 0.5 – 0.9 µg/L), with DCAN and 1,1,1-TCP detected at concentrations around the LOQ levels. In the monochloramine-treated river water samples, the only OXBPs measured at concentrations above the limit of quantification were TCM and 1,1-DCP. To the best of our knowledge, this is the first SPE-GC-MS/MS method enabling the analysis of this many OXBPs. Additionally, this study provides novel information regarding the occurrence of several OXBPs in

French tap and river water samples, which will aid future risk assessment, management and control initiatives.

1. INTRODUCTION

Halogen-based inorganic oxidants such as chlorine (HOCl), chlorine dioxide (ClO_2), and monochloramine (NH_2Cl), are widely used as oxidants in water treatment processes in order to inactivate pathogenic microorganisms, control biofouling and reduce the risk of introducing invasive species into local aquatic systems. These biocides also react with naturally occurring organic matter, as well as with inorganic compounds (such as bromide and iodide) and anthropogenic contaminants, leading to the formation of a wide range of undesirable organohalogen by-products (OXBPs) [Kinani et al., 2016a].

Since the discovery of the carcinogenic effect of trihalomethanes (THMs) in the mid-1970s, extensive studies have been conducted to detect and identify other organohalogen compound by-products. Recent advances in analytical techniques have resulted in the identification of a large and constantly increasing number of OXBPs, with nearly 700 halogenated compounds identified to date in a variety of water sources. The classes of organohalogen by-products most commonly detected in water are the trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), haloketones (HKs), haloaldehydes (HALs), halonitromethanes (HNMs) and haloacetamides (HAcAms) [Richardson et al. 2012]. Some of these compounds have been proved or are suspected to cause various cancers, mutagenicity, teratogenicity, reproductive effects, toxicity and endocrine disruption in animals and humans, especially when exposure is continuous or repeated over long periods [Plewa and Wagner, 2009; Chowdhury et al. 2009].

Only THMs and HAAs are the subject of current drinking water regulations. In the U.S., for example, the EPA has established a maximum contaminant level (MCL) for total THMs (TTHM: chloroform, dichlorobromomethane, chlorodibromomethane, and bromoform) and for the cumulative concentration of five HAAs (HAA5: monochloro-, monobromo-, dichloro-, dibromo-, and trichloroacetic acid) at 80 and 60 $\mu g.L^{-1}$, respectively. Within the European Union, only THMs are covered by legislation, which sets the maximum allowable TTHM concentration at a value of 100 $\mu g.L^{-1}$. Regarding surface waters, monochloroacetic acid (MCAA) has been listed as a relevant substance in the Water Framework Directive, with a provisional environmental quality standard value of 0.58 $\mu g.L^{-1}$. OXBPs are also regulated by EU Biocides Regulation 528/2012, which makes the submission of an environmental risk assessment of by-products resulting from biocide release/discharge into European surface and groundwater a condition of the authorization of biocidal products. A list of by-products, including 4 THMs, 9 HAAs, and 4 HANs (dichloroacetonitrile, trichloroacetonitrile, chlorobromoacetonitrile, and dibromoacetonitrile), has recently been established for this purpose [ECHA, 2016]. The lowering of current maximum permissible levels as well as the regulation of novel OXBPs are currently under

study in many countries, leaving significant challenges for the water treatment industry to face in the near future [Ma et al., 2014].

Among the OXBPs identified to date, THMs and HAAs are generally well documented and particularly so in chlorinated drinking water. Information on the occurrence of other known halogenated by-products remains limited however and is insufficient to enable understanding the processes by which they are formed or to evaluate their potential health and environmental impacts and how these can be controlled. We therefore need to expand our knowledge of these classes of by-products and, in this context, analytical methods allowing identification and quantification of these substances in water matrices are an important prerequisite. Analysis of a wide range of OXBPs is not an easy task given their formation at low concentrations in the complex aquatic matrices like river water and wastewater. Although numerous analytical methods for the determination of organohalogen by-products have been reported in the literature over the past few decades, these are mainly based on the analysis of very few compounds at a time, often those belonging to a specific "family" of OXBPs. A recently published review by Kinani et al. (2016b) has highlighted the advantages and limitations of these methods. The principal analytical techniques employed in the analysis of volatile and semi-volatile OXBPs are gas chromatography with electron capture detection (GC-ECD) or coupled with single quadrupole mass spectrometry operated in electron ionization (EI) or electron capture negative ionization (ECNI) mode. With regard to relative sensitivity, ECNI provides significantly lower Limits of Detection (LODs) than EI. Conversely, ENCI leads to the detection of only the halide fragment ions (m/z 35/37, m/z 79/81 or m/z 127) for several halogenated compounds, thus greatly compromising selectivity. The determination of a large number of OXBPs requires more selective techniques, such as tandem mass spectrometry (MS/MS). MS/MS using triple quadrupole instruments offers particularly high sensitivity and selectivity in the selected reaction monitoring (SRM) mode, making it an attractive technique for the quantification of multiple classes of halogen by-products. However, despite its clear benefits, SRM detection is still an emerging technology and currently less applied for OXBPs analysis than selected ion monitoring (SIM), especially when coupled with GC.

Regarding extraction methods, applications of a variety of procedures such as purge and trap (P&T), liquid-liquid extraction (LLE), liquid-phase micro extraction (LPME), solid phase extraction (SPE) and solid phase micro-extraction (SPME) have been reported [Lara-Gonzalo et al., 2008; Aguilera-Herrador et al., 2008; Kinani et al., 2016b]. LLE with methyl tert-butyl ether (MTBE) as the extractant remains the most widely used technique for the extraction of volatile and semi-volatile OXBPs from water samples, although it has a limited sample throughput capacity (30 to 100 mL) and requires the use of a relatively large volume of MTBE for analyte extraction (6 mL of MTBE per 100 mL of sample). P&T and headspace techniques provide only relatively good sensitivity for highly volatile OXBPs, like THMs [Nikolaou et al., 2005; Allard et al., 2012]. Compared to conventional LLE, SPE has a number of advantages, including: (i) higher recoveries with concentration factors for target

analytes of up to three orders of magnitude, achieving detection limits at the nanogram per liter level, (ii) the variety of stationary phases currently available or under development, allowing to target a wide range of molecules (iii) less use of organic solvents and (iv) the possibility of automation.

Due to the diversity of chemical and physical properties of OXBPs, their determination involves using several single-class methods, each being specific to one class or to a number of chemicals. This strategy is particularly appropriate because sample analyses are conducted in the optimal system configuration for each family of compounds, ensuring the best analytical performance in terms of sensitivity and specificity is achieved for all target compounds. The downside, however, is the necessity of subjecting each sample to several analyses, which not only requires a large amount of sample but also makes this approach proportionately more time consuming and expensive than other analytical methods. At the same time, the need for more accurate characterization of the spatial and temporal variability of OXBP concentrations necessitates increased sampling frequency, with a consequent increase in the number of samples to be analyzed. There is thus a need to develop fast and cost-effective analytical methods that enable high-throughput analysis of OXBPs. A limited number of multiclass analytical methods have to date been developed for organohalogen by-products determination in water. In 2012, Liew et al. presented a method, based on LLE and subsequent analysis by GC-EI/MS in SIM mode, for the determination of six halonitromethanes (HNMs) and four haloacetamides in drinking water, achieving LODs from 0.08 to 6 µg.L^{-1} and from 0.08 to 2 µg.L^{-1} for HNMs and haloacetamides respectively. Montesinos and Gallego, (2013) developed a method for the simultaneous determination of THMs and nine HNMs in water, using micro LLE followed by GC-MS analysis in EI and SIM modes. They reported LODs between 18 and 60 ng.L^{-1} for THMs and between 9 and 400 ng.L^{-1} for HNMs. In another study, these authors implemented a methodology based on HS-GC-EI/MS (in SIM mode) for the simultaneous determination of THMs, six HNMs and six HANs, achieving LODs ranging from 10 to 20 ng.L^{-1} for the THMs, from 30 to 140 ng.L^{-1} for the HNMs and from 30 to 200 ng.L^{-1} for HANs [Montesinos and Gallego, 2013]. Recently, Luo et al. (2014) reported the development of a multi-residue method using SPME-GC-EI/MS in SIM mode to determine 20 OXBPs, belonging to the I-THM, HNM and HAN classes, in drinking water. This method achieved LODs in the ranges of 2 to 70 ng.L^{-1}, 6 to 50 ng.L^{-1} and 1 to 50 ng.L^{-1} for the I-THMs, HNMs and HANs, respectively. A method offering rapid chromatographic analysis (10 minutes) with good separation and LODs between 3 and 14 ng.L^{-1} was developed by Li et al. (2013), who employed GC-EI/MRM mode in combination with LLE for the determination of volatile OXBPs belonging to the THM, HAN and HK classes. SPE has already been coupled to GC-EI/MS to analyze THMs [Alexandrou et al., 2015] and HAAs [Sadia and Pauzi, 2009] but only one study reports the use of SPE-GC/MS for multiclass determination of OXBPs [Chinn et al., 2007]. The latter method, using 200 mg of styrene divinylbenzene resin as adsorbent material (Varian Bond; Elut-PPL, 200 mg / 3 mL, Palo Alto, CA) and MTBE as extraction eluent, did not perform as well as was expected because the

LODs obtained were too high (1 to 5 µg.L^{-1}). To the best of our knowledge, a methodology based on SPE-GC-EI-MS/MS has never been applied to the sensitive quantification of a wide range of organohalogen by-products.

The goals of the present study were to develop and validate a sensitive multiclass analytical method for the determination of 26 organohalogen by-products belonging to six different classes in water samples. The target analytes were 4 trihalomethanes (THMs), 4 iodomethanes (I-HMs), one haloacetaldehyde (HAL), 6 haloketones (HKs), 4 halonitromethanes (HNMs) and 7 haloacetonitriles (HANs). This method is based on solid-phase extraction and gas chromatography coupled to mass spectrometry operating in both SIM and SRM modes. Simultaneous analysis of 26 OXBPs with different structures and physicochemical properties requires finding the best compromise in the selection of the various analytical parameters. The experimental parameters affecting the extraction efficiency as well as the sample analysis were investigated and optimized to improve sensitivity and selectivity. The method developed was evaluated according to the French standard NF T90-210 in terms response functions, method quantification limits, and correctness. This method was then applied to the determination of 26 OXBPs in tap and river water and the results obtained were compared with respect to the nature of oxidant (monochloramine versus chlorine).

2. MATERIALS AND METHODS

2.1. Reagents and chemicals

Aqueous solutions were prepared with ultrapure water (specific resistance of 18 MΩ.cm^{-1} at 25 °C) produced by a PURELAB Chorus 1 water purification system supplied by Veolia Water Technologies (Wissous, France). A mixed standard containing bromochloroacetonitrile (BCAN), dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN), 1,1-dichloro-2-propanone (1,1-DCP), 1,1,1-trichloropropanone (1,1,1-TCP), trichloroacetonitrile (TCAN) and trichloronitromethane (TCNM) (EPA 551B halogenated volatiles mix, 2000 µg.mL^{-1} each component in acetone, purity > 99%) was obtained from Sigma Aldrich (Saint-Quentin, Fallavier, France). All other target analytes were obtained as neat compounds with purities of between 86.5% and 99%. Trichloromethane (TCM, 99%), bromodichloromethane (BDCM, 98%), dibromochloro-methane (DBCM, 98%), tribromomethane (TBM, 99%), triiodomethane (TIM, 99%), chloroiodomethane (CIM, 97%), bromoiodomethane (BIM, 97%), chloroacetonitrile (CAN, 99%), bromoacetonitrile (BAN, 97%), iodoacetonitrile (IAN, 98%), bromonitromethane (BNM, 90%), chloropropanone (CP, 95%), 1,3-dichloro-2-propanone (1,3-DCP, > 95%), 1,1,3-trichloropropanone (1,1,3-TCP, 86.5%), tribromoacetaldehyde (TBAL, 97%), and 1,2-dibromopropane (internal standard, 97%) were purchased from Sigma Aldrich (Saint-Quentin, Fallavier, France). Dichloroiodomethane (DCIM, 95%), bromochloronitromethane (BCNM, 85-90%), bromodichloronitromethane (BDCNM, 90-95%)

and 1,1,3,3-tetrachloro-propanone (1,1,3,3-TCP, 90-95%) were supplied by Santa Cruz Biotechnology (Heidelberg, Germany). Methanol (MeOH, HPLC grade, 99.8%) and methyl-tert-butyl-ether (MTBE, GC grade, 99.8%) were purchased from Merck (Darmstadt, Germany). The L-ascorbic acid (99%) used to neutralize the residual oxidants was acquired from Sigma Aldrich (Saint-Quentin, Fallavier, France). The physicochemical properties of the compounds studied are provided in Supplementary material 1.

2.2. Standard solutions

For each neat compound, 10 mL of stock standard solution was prepared in MTBE (at concentrations between 0.8 and 2.2 $mg.mL^{-1}$). Individual standard stock solutions were stored in amber glass vials at -18 °C for 3 weeks. Working standard solutions (10 $\mu g.mL^{-1}$) were prepared daily from the stock solutions by serial dilutions with MTBE and used to make mixture solutions with which to spike the blank water samples (ultrapure and river water) for SPE and GC-MS optimization.

2.3. Sample collection

The developed method was applied to the tap and river water samples. River water samples were collected between July and September 2016 at three localities in France where the river water is treated with monochloramine: from the Meuse river, the Moselle river, and the Loire river. Chlorinated tap water was collected in the Paris region (France) by drawing samples from the laboratory's water supply system. All samples were collected in 2 L amber bottles, and filled with no headspace to prevent volatilization of OXBPs. All sample bottles were washed in lab dishwashers using ultrapure water and baked at 70 °C for at least 2 hours prior to use. The river water samples were transported to the laboratory in refrigerated road vehicles immediately after their collection. Once received at the laboratory, the samples were kept in the dark at 4 °C and analyzed as soon as possible within a period of not more than 48 hours after collection.

2.4. Sample preparation

2.4.1. Pretreatment

The sample pretreatment involves a neutralization step to quench any residual oxidant before SPE extraction. L-ascorbic acid has proven to be the most suitable reducing agent in the analysis of several families of organohalogen by-products, many of which have been found to degrade when other quenching agents, such as sodium sulphite, sodium arsenite, sodium borohydride and ammonium chloride, are employed [Liew et al., 2012; Kristiana et al., 2014; Kinani et al., 2016a]. Ascorbic acid was therefore selected as the preferred reducing agent for the 26 target OXBPs. 2 mL of L-ascorbic acid at 1.6 $g.L^{-1}$ was introduced into each bottle (2 L) prior to sampling. The amount of ascorbic acid required for complete neutralization of oxidants is sample site-specific. The amount used here was

sufficient to neutralize 0.65 mg $Cl_2.L^{-1}$, which corresponds to about twice the amount needed. The excess ascorbic acid did not have a negative impact on the quality of analysis of the target chemicals by SPE-GC/MS. No additional sample preservation procedure was applied. A 100 µL volume of 1,2-dibromo-propane (internal standard) solution at a 10 $\mu g.mL^{-1}$ concentration was added into each water sample in the laboratory, in order to obtain a concentration of 1 $\mu g.L^{-1}$ prior to SPE extraction.

2.4.2. Solid phase extraction procedure

Water samples were mixed gently and transferred into a 1 L glass bottle. The "AutoTrace 280" automated SPE system (Thermo Fisher Scientific, Courtaboeuf, France), which is able to process six samples simultaneously, was used to extract the target OXBPs from water. To ensure the best possible recovery was achieved for all target compounds, five types of widely used SPE cartridges were evaluated for extraction efficiency. The nature and properties of these cartridges are given in Table 1. The same extraction procedure was followed for all recovery experiments to enable a reliable and direct comparison of the respective cartridges. All of these were conditioned successively with 5 mL of MeOH and 10 mL of ultrapure water. Sample volumes of 1 L were then loaded onto the cartridges and the aqueous solution passed directly through to waste at a flow rate of 5 $mL.min^{-1}$. After sample percolation, the cartridges were rinsed with 5 mL of ultrapure water. The sorbents were not dried during the conditioning and sample loading steps. The retained OXBPs were eluted twice with an aliquot of 0.8 mL of MTBE and the organic extracts were transferred into 300 µL GC vials for analysis. This corresponds to a concentration factor of 625. The resulting extracts were immediately analyzed by GC/MS.

Table 1: Nature and properties of tested SPE cartridges

SPE Cartridge	Dimensions	Sorbent material	Supplier
Bakerbond SDB-1	40-150 µm[a], 200 mg[b], 6 mL[c]	Styrene-divinylbenzene copolymer	Interchim
Strata® SDB-L	60-150 µm, 500 mg, 6 mL	Styrene divinylbenzene	Phenomenex
Bakerbond Carbon	(-), 500 mg, 6 mL	Activated carbon	Interchim
Oasis-HLB	60 µm, 500 mg, 6 mL	Divinylbenzene-co-N-vinylpyrrolidone copolymer	Waters
LiChrolut® EN	40-120 µm, 500 mg, 6 mL	Ethylvinylbenzene-divinylbenzene copolymer	Merck

[a] Particle size; [b] Sorbent bed weight; [c] SPE tube volume; (-): Data not reported

2.5. Instrumentation and analytical conditions

GC-MS analysis of the SPE extracts was performed on a Thermo Fisher Scientific "Trace GC Ultra" gas chromatograph equipped with a "Triplus" autosampler and coupled to a "TSQ Quantum XLS" triple quadrupole mass spectrometer (Thermo Fisher Scientific, Courtaboeuf, France). The chromatographic separation was carried out on a 30 m "TG-5MS (5% phenyl, 95% methylpolysiloxane) capillary column (internal diameter: 0.25 mm, film thickness: 0.25 µm) from Thermo Fisher Scientific. High purity helium (99.9995%) was used as the carrier gas at a constant flow of 1 $mL.min^{-1}$. All experiments were performed by automatic injection with a 10 µL syringe of 1.2 µL into a programmed temperature vaporization injector (PTV) operated in splitless mode. The PTV conditions were: injection temperature: 60 °C; vaporization and transfer ramps: 870 and 300 $°C.min^{-1}$; vaporization and transfer temperatures: 200 and 220 °C; cleaning temperature, 280 °C; splitless time, 1.35 min; split flow, 50 $mL.min^{-1}$; and evaporation, transfer and cleaning times: 1.15, 0.2 and 1.00 min, respectively. To ensure the analytes would be trapped at the column head, the oven temperature was maintained at 35 °C for 3.6 min and then ramped at 20 $°C.min^{-1}$ up to 210 °C, followed by an increase to 280 °C at a rate of 60 $°C.min^{-1}$, where it was held for 1.5 min. The transfer line and ion source temperatures were kept at 280 °C and 250 °C, respectively and the solvent delay was set to 2.8 min.

As a first step, data was acquired in EI full scan mode, scanning from 50 to 450 m/z at 100 ms/scan. The mass spectrometer was operated in the positive electron ionization mode at 70 eV, with the filament emission current set at 25 µA. The electron multiplier voltage was set to 1040 V by automatic tuning (3.10^5 gain) and the mass spectrometer was automatically tuned using ions produced by the electron ionization of perfluorotributylamine. For the MS/MS experiments, argon at a nominal pressure of 1 mTorr was used as the collision gas. The filament current was set to twice the value used in the full scan experiments. The collision induced dissociation (CID) parameters were subject to optimization and are discussed in section 3.2. Instrument control, data acquisition and processing were performed using the Xcalibur software (Thermo Fisher Scientific, Courtaboeuf, France). The GC-MS analysis time was 15 minutes.

3. RESULTS AND DISCUSSION

3.1. Optimization of injector temperature and chromatographic separation

The first problem to solve when using gas chromatography for the analysis of organohalogen compounds is the potential thermal decomposition of the analytes of interest. It has been reported that HANs and HNMs are thermally unstable and decompose under commonly used injection port temperatures (170 - 250 °C) [Chen et al., 2002; Montesinos et al., 2011]. Given the boiling points of certain analytes, such as iodoform (218 °C), temperatures above 170 °C (corrected by the pressure) should be applied to ensure effective transfer of all target compounds into the GC column. PTV

injection has been used to minimize degradation of thermally unstable OXBPs as well as to introduce large sample volumes. Several factors can influence the efficiency of PTV injection, including liner design (material, internal volume, shape, presence or absence of packing material), vaporization and transfer ramps, vaporization and transfer temperatures, splitless time and split flow [Engewald et al., 1999].

Two GC inlet liners were tested: a PTV metal liner (1 mm ID x 2.75 mm OD x 120 mm L) and a PTV glass liner packed with glass wool (1 mm ID x 2.75 mm OD x 120 mm L). Although both are able to accept injection volumes of up to 20 µL, an intermediate volume of 10 µL was selected in order to achieve a relatively high concentration factor. Vaporization and transfer ramps were set at 14.5 °C.min^{-1} on the basis of the suppliers' recommendations. Given the low boiling point of MTBE (BP 55.2 °C), the initial injection and solvent evaporation temperatures were tested in the 35 - 60 °C range. The 35 °C lower bound corresponds to the lowest temperature that can be attained in a reasonable period of time without the need for cryogenic cooling of the inlet, while 60 °C is the maximum temperature that can be applied owing to the boiling temperature of chloroform, which is 61 °C. These tests revealed that low temperatures (< 60 °C) combined with long solvent vent times (up to 15 min) result in peak broadening and splitting. Regardless of the solvent vent time applied (1 to 10 min), when both the initial inlet and solvent evaporation temperature were set at 60 °C, a loss of most of the volatile analytes was observed. This is due to the proximity of the MTBE and target analyte boiling points. According to previous studies, separation of analytes from the solvent without significant loss requires a PTV initial temperature of 250 °C below the boiling point of the most volatile analytes [Štajnbaher and Zupančič-Kralj, 2008]. Subsequent experiments were therefore carried out with the PTV injector operated in splitless injection mode without solvent venting, using an injection volume of 1.2 µL with the optimized parameters presented in section 2.5. These operating conditions have been optimized to ensure gradual transfer of all analytes into the analytical column without decomposition. All the compounds of interest were detected and no degradation peak was observed when standard solutions containing 50 µg.mL^{-1} of each of the target compounds were analyzed separately by GC/MS in full-scan mode (m/z 45-500). The gradual and simultaneous evaporation of analytes and solvent probably prevents degradation of thermally labile compounds. This result may also be due to shorter residence times of the sample vapors in the injector compared with standard split/splitless injection. Although examination of the chromatograms of some analytes reveals some impurities, their area ratios relative to the target analytes remain below 15%: 1,1,3-TCP (1,1,1,3-TCP and 1,1,3,3-TCP), BCNM (DBCNM and DBNM), BDCNM (DBCNM), and BNM (DBNM). With the exception of 1,1,3,3-TCP, which is a degradation product of 1,1,3-TCP and whose intensity is too low to be considered during the calibration, the impurities are not produced through thermal decomposition of the target compounds.

The GC oven temperature program was optimized to obtain the best possible chromatographic separation for all target OXBPs. A standard mixture containing each target analyte at a concentration of 50 µg.mL^{-1} in MTBE was used for optimization tests. The MS was operated in full-scan mode over the 45 - 500 Th range. In addition to the 26 compounds targeted in this study, the initial list included six haloacetamides (monochloroacetamide, dichloroacetamide, trichloroacetamide, monobromoacetamide, tribromoacetamide and iodo-acetamide). These compounds were subsequently excluded from the list because they produce chromatographic peaks with significant tailing. Co-eluted compounds were injected individually to ensure unambiguous identification of the corresponding characteristic fragment ions.

To provide the necessary conditions for solvent condensation, the initial oven temperature was set at 35 °C. This temperature was chosen because chloroform and MTBE peaks cannot be separated above 35 °C, while lower temperatures would require the installation of a cryogenic cooling system, as mentioned beforehand. Four oven temperature ramp rates were tested in order to achieve the best chromatographic separation within the shortest possible runtime: 35, 20, 15 and 10 °C.min^{-1}. At 35 °C.min^{-1}, many components were co-eluted. Reducing the oven ramp rate to 20 °C.min^{-1} improved chromatographic separation for most of the co-eluted analytes. Below 20 °C.min^{-1}, however, CP, 1,1-DCP, BCAN and 1,1,1-TCP were still coeluting with CAN, DCAN, DCIM, and BDCNM, respectively. Ramp rates below 20 °C.min^{-1} led to an increase in analysis time with no further gain in terms of chromatographic separation. Moreover, a significant reduction in the signal-to-noise ratios, manifested by a broadening of the chromatographic peaks, was observed when decreasing the temperature ramp. Based on these results, 20 °C.min^{-1} was selected as the optimum value for the temperature ramp rate. A GC-EI/MS chromatogram of a standard mixture of the 26 target OXBPs at 20 µg.mL^{-1} is presented in Figure 1.

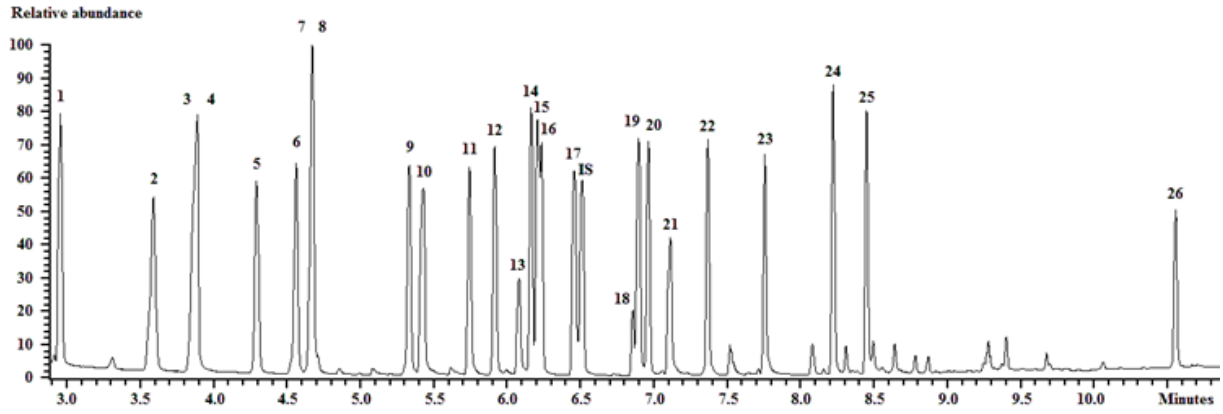

Figure 1. Chromatogram obtained by GC-EI/MS analysis with EI at 70 eV of a standard mixture containing 20 µg.mL^{-1} of each target analyte in MTBE. Compounds are: (1) TCM, (2) TCAN, (3) CP, (4) CAN, (5) BDCM, (6) CIM, (7) 1,1-DCP, (8) DCAN, (9) TCNM, (10) BAN, (11) DBCM, (12)

BIM, (13) BNM, (14) BCAN, (15) DCIM, (16) 1,1,1-TCP, (17) BCNM, (18) BDCNM, (19) 1,3-DCP, (20) TBM, (21) IAN, (22) DBAN, (23) 1,1,3-TCP, (24) TBAL, (25) 1,1,3,3-TCP, and (26) TIM

3.2. Optimization of MS/MS conditions

A standard of each of the targeted OXBPs at 10 $\mu g.mL^{-1}$ in MTBE was injected and scanned over the mass range from 45 to 500 Th, in order to select precursor ions for the MS/MS experiments. Two SRM transitions per compound were chosen to ensure adequate selectivity. Collision energy (CE) values from 0 to 50 V were tested. For most analytes, the best signal-to-noise ratio was observed at a CE value with which the precursor ion was not fully fragmented. In the case of BNM, the most abundant fragment ions were [$^{79}BrCH_2^+$] and [$^{81}BrCH_2^+$], corresponding to nominal m/z ratios of 93 and 95, respectively. These ions could not be fragmented without loss of sensitivity. Proper operation of the SIM mode was impracticable under the chromatographic conditions used because the retention times of BNM and BIM (5.92 min and 6.17 min, respectively) are too close to allow effective removal of argon from the collision cell between the elution of the two compounds. A pseudo single ion monitoring (SIM) corresponding to a collision energy of 0 V was therefore applied. The EI-MS/MS product-ion mass spectra of 1,3-DCP (6.89 min) and 1,1,3-TCP (7.77 min) also displayed similar fragmentation patterns; however, they are well separated chromatographically. Table 2 summarizes the optimized SRM transitions and collision energies used for confirmation and quantification of the 26 target OXBPs.

Table 2. Optimized parameters for analysis of the 26 target OXBPs in the SRM mode

Analytes	Retention time (min)	Time window (min)	Transition 1[a], CE 1[b]	Transition 2[a], CE 2[b]
TCM	2.95	0.4	83.0 → 47.0 (27 V)	117.9 → 83.0 (16 V)
TCAN	3.59	4.02	108.0 → 73.0 (26 V)	81.9 → 47.0 (27 V)
CP	3.89	1.30	92.0 → 43.0 (3 V)	94.0 → 43.0 (3 V)
CAN	3.90	1.30	75.0 → 48.0 (6 V)	77.0 → 50.0 (6 V)
BDCM	4.30	0.36	83.0 → 48.0 (30 V)	128.9 → 48.0 (33 V)
CIM	4.58	0.62	175.9 → 49.0 (11 V)	177.9 → 49.0 (10 V)
1,1-DCP	4.66	1.21	91.0 → 63.0 (5 V)	83.0 → 48.0 (27 V)
DCAN	4.68	1.21	74.0 → 47.0 (18 V)	81.9 → 47.0 (26 V)
TCNM	5.34	0.59	116.9 → 81.9 (25 V)	81.9 → 47.0 (20 V)
BAN	5.44	1.71	119.0 → 40.0 (10 V)	121.0 → 40.0 (10 V)
DBCM	5.75	0.77	128.9 → 48.0 (35 V)	207.8 → 128.9 (12 V)
BIM	5.92	0.77	219.8 → 92.9 (21 V)	221.8 → 94.9 (9 V)
BNM	6.09	0.77	94.9 → 94.9 (0 V)	92.9 → 92.9 (0 V)
BCAN	6.17	1.12	154.9 → 74.0 (8 V)	74.0 → 47.0 (15 V)
DCIM	6.21	1.12	209.9 → 83.0 (11 V)	83.0 → 48.0 (34 V)
1,1,1-TCP	6.24	0.77	97.0 → 61.0 (8 V)	125.0 → 43.0 (15 V)
BCNM	6.46	0.35	126.9 → 48.0 (31 V)	128.9 → 48.0 (30 V)
IS	6.52	0.35	121.0 → 41.1 (8 V)	123.0 → 41.1 (8 V)
BDCNM	6.86	0.99	162.9 → 81.9 (24 V)	160.9 → 81.9 (25 V)
1,3-DCP	6.89	1.29	77.0 → 49.0 (8 V)	79.0 → 51.0 (8 V)
TBM	6.97	0.99	251.8 → 172.9 (9 V)	172.9 → 91.9 (42 V)
IAN	7.12	2.27	166.9 → 127.0 (13 V)	166.9 → 40.0 (15 V)
DBAN	7.37	0.99	198.9 → 120.0 (8 V)	118.0 → 90.9 (15 V)
1,1,3-TCP	7.77	0.98	77.0 → 49.0 (9 V)	79.0 → 51.0 (7 V)
TBAL	8.22	0.98	172.9 → 93.0 (45 V)	174.9 → 93.0 (45 V)
1,1,3,3-TCP	8.45	0.6	111.0 → 83.0 (8 V)	195.9 → 83.0 (15 V)
TIM	10.56	1.8	393.7 → 266.8 (9 V)	266.8 → 139.9 (44 V)

[a] Transition 1 is used for quantitative analysis and transition 2 for confirmation; [b] CE: Collision energy

3.3. Optimization of the solid-phase extraction procedure

The extraction efficiency of SPE is influenced by many factors, such as the pH and volume of the sample, the eluent type and flow rate, as well as the physicochemical properties of the stationary phase. In order to achieve the highest possible sensitivity, a water sample volume of 1 L was selected

for the SPE recovery experiments. Several previous studies have reported that the pH of the water sample affected OXBP stability. In general, increasing the water pH increases the rates of decomposition of many OXBPs, such as haloacetic acids and HANs, and the consequent formation of THMs [Kinani et al., 2016b]. Conversely, however, a lowering of the pH to values below 4.5 leads to the degradation of other compounds, such as haloacetamides. There was no examination of the pH effect in this study. As all of the studied compounds are present in neutral form in the pH range of the river and tap water samples used in this study (pH values of 7-9), an intermediate pH value of 8 was selected to ensure the stability of all the compounds.

LLE using MTBE as the solvent remains, at present, the most commonly used extraction method for the analysis of volatile OXBPs. MTBE was consequently selected as the elution solvent for the experiments. An elution volume of two 0.8-mL aliquots was used to maximize the extraction recovery of the target analytes while also minimizing the elution of interfering components trapped on the sorbent during the percolation step. The volume of recovered elution solvent varied from one SPE cartridge to another, depending on the amount and structure of the adsorbent phase. The SPE recovery tests showed that a partial loss of most volatile compounds occurred during solvent drying, whether with a nitrogen blowing concentrator or using a rotary evaporator. For this reason, the extract concentration step was omitted from the SPE procedure.

Five of the most frequently used commercial SPE cartridges were compared in order to select the best stationary phase. The cartridges tested were the Bakerbond SDB-1, Strata® SDB-L, Bakerbond Carbon, OASIS-HLB, and LiChrolut® EN. The extraction procedure described in Section 2.4.2 was followed for each test and cartridge type. For the recovery estimates, water samples were spiked with a mixture of the 26 OXBPs at two levels of concentration (ranging from 0.01-5 $\mu g.L^{-1}$ and from 0.5-10 $\mu g.L^{-1}$). Concentrations at each level were individually chosen based on the sensitivity of the procedure for each compound. All experiments were performed in triplicate and the results were expressed as a relative percentage of the most intense signal for each cartridge to the highest signal observed for all cartridges. For each analyte, the value of 100% corresponds to the highest intensity observed in any of the tests performed on the various cartridges. The results are schematically illustrated in Figure 2.

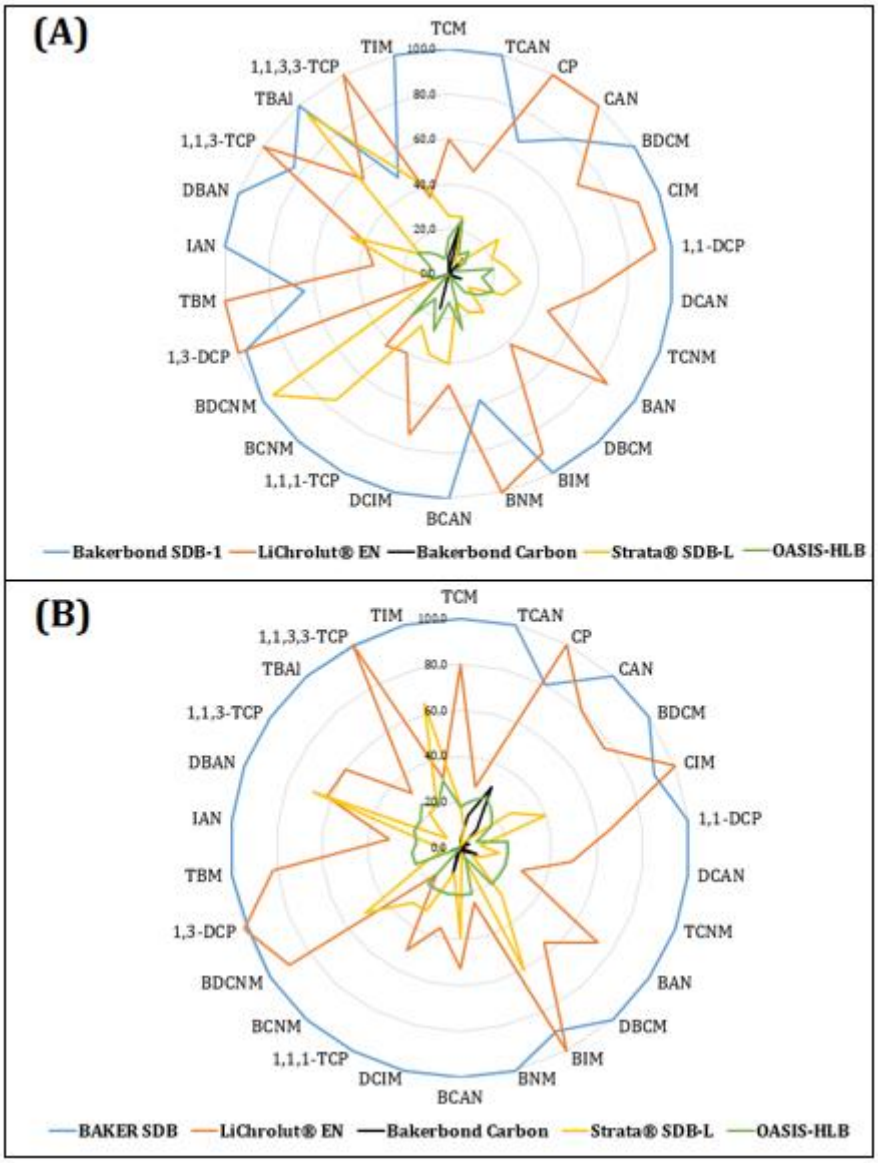

Figure 2. Comparison of the OXBP recoveries (%) obtained with the five selected sorbents (A: at 0.01-5 µg.L^{-1}, B: at 0.5-10 µg.L^{-1})

As shown in the figure 2, the best extraction results were obtained with Bakerbond SDB-1 (which has been retained for further development), followed by LiChrolut® EN and Strata® SDB-L, while OASIS-HLB and Bakerbond Carbon yielded much lower recoveries. The low yields obtained with the Bakerbond Carbon cartridges are a noteworthy and unexpected result as organohalogen compounds are known for their strong affinity with black carbon (coal).

3.4 Method evaluation

The performance of the whole method (SPE followed by GC-EI-MS/MS analysis) was validated for river water analysis according to the procedure described in the French standard NF T90-210. The

validation parameters were: response function (linearity), limit of quantification and accuracy (trueness and precision). The acceptance criteria were first defined and tests were then conducted to verify the validity of the method by checking that the results were within the pre-determined acceptable limits of performance for each validation parameter.

To determine the response function, five calibration curves, each plotted with seven concentration levels, were constructed over five days. The calibration curves were obtained by plotting the ratio of the peak area of a given analyte to that of the corresponding internal standard against the concentrations of the spiked analyte. Several mathematical functions, with weighting ($1/x$, $1/x^2$) and without, were applied to the calibration curve and compared for goodness of fit to obtain the best response functions. The response functions were considered satisfactory if the back-calculated concentrations all fell within the acceptance range of ± 30% of the nominal value (initial spike concentration) for the lowest concentration (LLOQ) and the difference was less than 30% at all other concentrations within the calibration range.

For determination of LOQs, river water samples were spiked with the OXBP mixture at low concentrations and then analyzed by SPE GC-EI-MS/MS. From the chromatograms obtained, the expected LOQ was estimated for each OXBP as the concentration with a signal-to-noise ratio of 10:1. Next, five water samples spiked with a standard mixture containing all analytes at the estimated LOQ concentrations were prepared over five different days and analyzed by SPE GC-EI-MS/MS. In the absence of regulatory requirements as to the accuracy at the limit of quantification for the target OXBPs, a maximum permissible deviation (MPD) of 60% was used, as recommended by standard NF T90-210.

Ideally, accuracy (trueness and precision) should be verified using certified reference samples and a MPD based on regulatory requirements, official standards or even on specifications provided by a client or the laboratory itself. However, as no certified reference materials were available for the target OXBPs and matrices of interest, fortified water samples were used to evaluate the method trueness. This was determined from the assay recovery results for each compound at three different concentration levels: low (at LOQ values), medium, and high. Recovery tests were run on five separate days with each sample measured twice a day (n=10 replicates per concentration level). The criteria for acceptance of the validation data were fixed as follows: MPD from the mean of ± 60% at LOQ values, ± 30% at mid-level, and ± 20% at high-level concentrations. The results obtained for the various validation parameters are summarized in Table 3. The developed method provides LOQ values ranging from 3 to 3000 $ng.L^{-1}$. The calibration curves are either linear or quadratic, with correlation coefficients (r^2) between 0.950 and 0.999. The correctness of this developed method has been demonstrated by recovery results within the established acceptance limits.

Table 3. Results for the validation parameters of the developed method for the determination of 26 OXBPs in water samples

Analytes	Concentration range ($\mu g.L^{-1}$)	r^2	LODs ($\mu g.L^{-1}$)	LOQs	Correctness (%)	
					ML	HL
TCM	0.003 – 1.5	0.998	0.001	0.003	21	14
BDCM	0.01 – 1.5	0.997	0.003	0.010	14	14
DBCM	0.01 – 1.5	0.999	0.003	0.010	11	7
TBM	0.1 – 7.5	0.988	0.033	0.100	28	26
TIM	0.5 – 7.5	0.998	0.167	0.500	93	61
DCIM	0.05 – 1.5	0.999	0.016	0.050	14	15
CIM	0.01 – 1.5	0.981	0.003	0.010	6	7
BIM	0.01 – 1.5	0.998	0.003	0.010	16	9
CP	3 – 10	0.950	1.00	3.000	5	8
1.1-DCP	0.25 – 1.5	0.998	0.080	0.025	11	9
1.3-DCP	2 – 7.5	0.985	0.667	2.000	3	11
1.1.1-TCP	0.10 – 1.5	0.990	0.033	0.100	19	8
1.1.3-TCP	1 – 7	0.967	0.334	1.000	12	7
1.1.3.3-TCP	0.75 – 7.5	0.991	0.250	0.750	8	11
TBAL	2 – 8.5	0.963	0.667	2.000	27	19
CAN	0.5 – 10	0.990	0.167	0.500	22	25
DCAN	0.05 – 1.5	0.995	0.016	0.050	8	8
TCAN	0.01 – 1.5	0.998	0.003	0.010	15	6
BCAN	0.05 – 1.5	0.995	0.016	0.050	11	13
BAN	2 – 10	0.997	0.667	2.000	9	13
DBAN	0.05 – 1.5	0.994	0.016	0.050	16	15
IAN	0.5 – 10	0.974	0.167	0.500	6	12
BNM	2 – 10	0.987	0.667	2.000	16	28
BCNM	0.25 – 7.5	0.996	0.085	0.250	12	9
TCNM	0.003 – 1.5	0.995	0.001	0.003	3	10
BDCNM	2 – 10	0.955	0.667	2.000	14	27

Abbreviations: ML: mid-level concentration; HL: high level of concentration.

3.5. Application of the method to the analysis of real water samples

After validation, the developed method was applied to study the occurrence of the 26 target OXBPs in tap water as well as in river water. Eighteen river water samples and four tap water samples were analyzed using the methods described in the previous sections. The analytical results are summarized in Table 4. Seven of the targeted compounds, namely DCAN, BCAN, DBAN, TCM, BDCM, TBM and DBCM were systematically detected in tap water (n = 4) in the respective ranges: 114-137, 137-581, 464-944, 583-744, 1265-1651, 802-4556 and 4327-4691 $ng.L^{-1}$, with DBCM being the most

abundant OXBP. The measured concentrations of the THMs are in agreement with those typically found in treated (chlorinated) drinking water in France and Europe, which are in the range of 0.5 to 80 µg.L^{-1} for TCM, < 1 to 50 µg.L^{-1} for BDCM, 0.1 to 50 µg.L^{-1} for DBCM and < 0.1 to 50 µg.L^{-1} for TBM. In untreated river water samples, the THMs were detected occasionally, and only TCM was found in all water samples at concentrations ranging from < 10 to 36 ng.L^{-1}. The measured THMs could originate from discharges resulting from human activities; particularly TCM, which is ubiquitously present in surface and groundwater [Rook, 1974; Bellat et al., 1974]. They could also be of biogenic origin: several studies have demonstrated that TCM can be naturally formed through biogeochemical processes. In treated river water samples, only CP, 1,1-DCP, DCAN and TCM were detected occasionally at concentrations below the LOQ for CP and DCAN, from <3 to 9 ng.L^{-1} for TCM, and from < 250 to 1260 ng.L^{-1} for 1,1-DCP. In comparison to tap water, the concentrations of measured OXBPs were lower. These results confirm once again that monochloramine produces less undesirable by-products than chlorine.

Table 4. Results obtained from the monitoring of 26 target OXBPs in chlorinated tap water, as well as both untreated and monochloraminated river water samples

Analytes	LOQ ($ng.L^{-1}$)	Concentrations ($ng.L^{-1}$)						
		Tap water (n=4)	RW 1 (n=3)		RW 2 (n=3)		RW 3 (n=3)	
			untreated	treated	untreated	treated	untreated	treated
TCM	3	583-744	8-13	nm-9	36	4	5	nd
BDCM	10	1265-1651	nm	nd	nm	nd	nd	nd
DBCM	10	4327-4691	nd	nd	11	nd	nd	nd
TBM	100	802-4456	nm	nd	nm	nd	nm	nd
TIM	500	nd	nd	nd	nd	nd	nd	nd
DCIM	50	nd	nd	nd	nd	nd	nd	nd
CIM	10	nd	nd	nd	nd	nd	nd	nd
BIM	10	nd	nd	nd	nd	nd	nd	nd
CP	3000	nd	nd	nd	nd	nm	nd	nm
1,1-DCP	250	nd	nd	nm	nd	1260	nd	263
1,1,1-TCK	100	nm-104	nd	nd	nd	nd	nd	nd
1,1,3-TCP	1000	nd	nd	nd	nd	nd	nd	nd
1,1,3,3-TCP	750	nd	nd	nd	nd	nd	nd	nd
TBAL	2000	nd	nd	nd	nd	nd	nd	nd
CAN	500	nd	nd	nd	nd	nd	nd	nd
DCAN	50	114-137	nd	nd	nd	nm	nd	nm
TCAN	10	nd	nd	nd	nd	nd	nd	nd
BAN	2000	nd	nd	nd	nd	nd	nd	nd
DBAN	50	464-944	nd	nd	nd	nd	nd	nd
BCAN	50	137-581	nd	nd	nd	nd	nd	nd
IAN	500	Nd	nd	nd	nd	nd	nd	nd
TCNM	10	Nd	nd	nd	nd	nd	nd	nd
BNM	2000	Nd	nd	nd	nd	nd	nd	nd
BCNM	250	Nd	nd	nd	nd	nd	nd	nd
BDCNM	2000	Nd	nd	nd	nd	nd	nd	nd

Abbreviations: RW = River Water, nm = detected but not measured (<LOQs), nd = not detected (<LODs)

4. CONCLUSION

A robust, sensitive method employing SPE and GC-MS/MS operated in SRM mode has been developed for quantitative determination of organohalogen disinfectant by-products in water samples. While previously published GC/MS methods have been specific to one class or to a limited number of chemicals, the singularity of the present method is its ability to measure 26 OXBPs belonging to six different chemical classes in a single run. The applicability of the method was confirmed by analyzing several water tap water and river water samples, with the results showing method LOQs ranging from 3 to 3000 $ng.L^{-1}$. Given these performances, this method constitutes a useful tool that allows the study of several categories of organohalogen by-products for which data on the occurrence, formation and fate, as well as information regarding their potential health and environmental impacts are still limited.

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References

Allard, S. Charrois, J.W.A. Joll, C.A. Heitz, A. (2012). Simultaneous analysis of 10 trihalomethanes at nanogram per liter levels in water using solid-phase microextraction and gas chromatography mass-spectrometry. *Journal of Chromatography A*, 1238, 15-21.

Aguilera-Herrador, E. Lucena, R. Cárdenas, S. Valcárcel, M. (2008). Determination of trihalomethanes in waters by ionic liquid-based single drop microextraction/gas chromatographic/mass spectrometry. *Journal of Chromatography A*, 1209, 76-82.

Alexandrou, L.D. Spencer, M.J.S. Morrisson, P.D. Meehan, B.J. Jones, O.A.H. (2015). Micro versus macro solid phase extraction for monitoring water contaminants: A preliminary study using trihalomethanes. *Science of the Total Environment*, 512-513, 210-214.

Bellar, T.A. Lichtenberg, J.J. Kroner, R.C. (1974). The occurrence of organohalides in chlorinated drinking waters. *Journal American Water Works Association*, 66, 703-706.

Chen, P.H. Richardson, S.D. Krasner, S.W. Majetich, G. Glish, G.L. (2002). Hydrogen abstraction and decomposition of bromopicrin and other trihalogenated disinfection byproducts by GC/MS. *Environmental Science Technology* 36: 3362-3371.

Chinn, R. Lee, T. Krasner, S. Dale, M. Richardson, S. Pressman, J. Speth, T. Miltner, R. Simmons, J. E. (2007). Solid-Phase Extraction of 35 DBPs with Analysis by GC/ECD and GC/MS. American Water Works Association, Denver, CO, 1-20.

Chowdhury, S. Champagne, P. McLellan, P.J. (2009). Models for predicting disinfection byproduct (DBP) formation in drinking waters, a chronological review. *Science Total Environment*, 407, 4189-4206.

ECHA 2016. Guidance on the Biocidal Products Regulation. Volume V, Guidance on Disinfection By-Products.

Engewald, W. Teske, J. Efer, J. (1999). Programmed temperature vaporisers-based large volume injection in capillary gas chromatography. *Journal of Chromatography A*, 842, 143-161.

Kinani, A. Kinani, S. Bouchonnet, S. (2016b). Formation and determination of organohalogen by-products in water – Part II. Sample preparation techniques for analytical approaches. *Trends in Analytical Chemistry*, 85, 281-294.

Kinani, A. Kinani, S. Richard, B. Lorthioy, M. Bouchonnet, S. (2016a). Formation and determination of organohalogen by-products in water – Part I. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method. *Trends in Analytical Chemistry*, 85, 273-280.

Kristiana, I. Lethorn, A. Joll, C. Heitz, A. (2014). To add or not: The use of quenching agents for the analysis of disinfection by-products in water samples. *Water Research*, 59, 90-98.

Lara-Gonzalo, A. Sánchez-Ura, J.E. Segovia-García, E. Sanz-Medel, A. (2008). Critical comparison of automated purge and trap and soli-phase microextraction for routine determination of volatile organic compounds in drinking waters by GC-MS. *Talanta*, 74, 1455-1462.

Liew, D. Linge, K.L. Joll, C.A. Heitz, A. Charrois, J.W.A. (2012). Determination of halonitromethanes and haloacetamides: An evaluation of sample preservation and analyte stability in drinking water, *Journal of Chromatography A*, 1241, 117-122.

Liu, Y. Duan, J. Li, W. Lai, Q. Saint, C.P. Mulcahy, D. (2015). Determination of volatile disinfection byproducts in water by gas chromatography-triple quadrupole mass spectrometry. *Analytical Letters*, 48, 188-203.

Luo, Q. Chen, X. Wei, Z. Xu, X. Wang, D. Wang, Z. (2014). Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation, *Journal of Chromatography A*, 1365, 45-53.

Ma, H. Li, Y. Zhang, H. Shah, S. M. Chen, J. (2014). Salt-assisted dispersive liquid–liquid microextraction coupled with programmed temperature vaporization gas chromatography–mass spectrometry for the determination of haloacetonitriles in drinking water. *Journal of Chromatography A*, 1358, 14-19.

Montesinos, I. Cardador, M. J. Gallego, M. (2011). Determination of halonitromethanes in treated water. *Journal of Chromatography A*, 1218, 2497-2504.

Montesinos, I. Gallego, M. (2013). Speciation of common volatile halogenated disinfection by-products in tap water under different oxidising agents, *Journal of Chromatography A*, 1310, 113-120.

Nikolaou, A. D. Golfinopoulos, S. K. Rizzo, L. Lofrano, G. Lekkas, T. D. Belgiorono, V. (2005). Optimization of analytical methods for the determination of DBPs: Application to drinking waters from Greece and Italy. *Desalination*, 176, 25-36.

Plewa, M.J. Wagner, E.D. (2009) Mammalian Cell Cytoxicity and Genotoxicity of Disinfection By-Products. Water Research Foundation, D., CO, USA.

Richardson, S.D. Postigo C. (2012). Drinking water disinfection by-products, Chapter 4, in: The Handbook of Environmental Chemistry: Emerging Organic Contaminants and Human Health. *Springer*, 20, 93-137.

Rook, J.J. (1974). Formation of haloforms during chlorination of natural waters. *Journal of Water Treatment*, 23, 234-243.

Sadia, W. Pauzi, A. (2009). SPE-GC-MS for the Determination of Halogenated Acetic Acids in Drinking Water. *Chromatographia*, 69, 1447-1451.

Štajnbaher, S. Zupančič-Kralj, L. (2008). Optimisation of programmable temperature vaporizer-based large volume injection for determination of pesticide residues in fruits and vegetables using gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1190, 316-326.

III- Fiabilisation des mesures d'AOX et développement d'une méthode de spéciation de ces derniers en fractions chlorées, bromées et iodées

Le paramètre AOX permet d'estimer la teneur en composés organohalogénés totaux présents dans un échantillon d'eau. Il s'agit d'un paramètre réglementaire qui fait l'objet d'un suivi régulier sur les rejets et qui est pris en compte pour le calcul des redevances aux agences de l'eau pour pollution d'origine non domestique. Le paramètre AOX revêt également un intérêt « scientifique » puisqu'il permet de calculer des bilans de matière et ainsi d'estimer le pourcentage d'AOX non imputé à des sous-produits organohalogénés connus. La mesure d'AOX fait l'objet d'une norme internationale NF EN ISO 9562. Toutefois, les essais de comparaison interlaboratoires (accrédités par le COFRAC) réalisés par EDF R&D ont mis en évidence une dispersion importante des résultats obtenus avec une tendance à la surestimation, ce qui témoigne d'une difficulté partagée par les laboratoires d'analyse à mesurer ce paramètre. Or, comme le montre les équations 1 et 2 ci-dessous, toute surestimation des teneurs en AOX conduira automatiquement à surestimer la teneur en SPOX inconnus. Ce constat nous a amené à réexaminer la méthode de mesure du paramètre AOX afin de déterminer les causes possibles des résultats incohérents obtenus par certains laboratoires. Les résultats expérimentaux obtenus sont présentés dans la première partie de l'article ci-après. Ces derniers mettent en évidence un effet matrice très important dû aux ions chlorure. Contrairement à la prescription de la norme NF EN ISO 9562 qui précise que les chlorures n'interfèrent avec la mesure d'AOX qu'à partir d'une teneur de 1 g éq. Cl/L, les résultats obtenus dans le cadre de la présente étude confirment sans équivoque l'hypothèse d'un « effet positif » des chlorures à des concentrations plus faibles (200 mg éq. Cl/L). En revanche les ions iodure et bromure n'interfèrent pas sur la mesure d'AOX aux concentrations habituellement retrouvées dans les eaux de rivières. Des modifications ont été apportées au protocole de lavage normalisé. Celles-ci se sont montrées efficaces pour éliminer les chlorures jusqu'à 1 g éq. Cl/L.

$$\textbf{Eq. 1} \qquad \%\,\mathbf{KAOX} = \Sigma\, \frac{\mathbf{n \times 35.45 \times Ci/Mi}}{\mathbf{AOX}} \times \mathbf{100}$$

$$\textbf{Eq. 2} \qquad \%\,\mathbf{UAOX} = \mathbf{100} - \%\,\mathbf{KAOX}$$

$$\textbf{Eq. 3} \qquad \mathbf{AOX_{C\text{-}IC}} = \mathbf{AOCl + AOBr + AOI}$$

La seconde partie de cet article décrit le développement et l'optimisation d'une méthode d'analyse permettant d'établir le taux d'incorporation des différents halogénures (Cl, Br, I) dans les AOX. Cette spéciation chimique des AOX en fractions chlorées (AOCl), bromées (AOBr) et iodées (AOI) est importante pour plusieurs raisons. D'une part, le devenir et la toxicité des SPOX dépendent des formes chimiques sous lesquelles ils existent. Comme nous l'avons évoqué au premier chapitre, plusieurs

études récentes ont établi un lien direct entre la toxicité et la nature de l'halogénure incorporé dans le SPOX étudié. En général, cette toxicité suit l'ordre : I >> Br > Cl. D'autre part, la spéciation des AOX permet de déterminer des bilans de matière en fonction de chaque type d'halogénure. Cette information est particulièrement intéressante dans les études qui visent l'identification des SPOX inconnus puisque cette spéciation offre la possibilité d'apprécier dans quelle(s) fraction(s) le manque de connaissances est le plus important. Pour l'ensemble de ces considérations, nous avons donc mis au point une méthode de spéciation des AOX. Cette méthode, notée C-IC (pour *Combustion Ion Chromatography*), repose sur une adsorption des sous-produits organohalogénés sur charbon actif, suivie d'une combustion à 1000 °C. Les gaz d'halogénure d'hydrogène résultants de la combustion sont par la suite absorbés dans une solution absorbante puis analysés en ligne par chromatographe ionique. Après optimisation des paramètres de combustion, d'absorption et d'analyse par chromatographie ionique, la méthode a été validée selon le référentiel normatif NF T 90-210. Elle a ensuite été appliquée avec succès à l'analyse d'échantillons d'eau de rivières issus des sites d'étude. La comparaison des résultats obtenus par cette méthode avec ceux de la méthode d'AOX conventionnelle montre une bonne concordance.

Par ailleurs, il est connu que les deux approches de détermination des AOX, coulométrique et C-IC, induisent des pertes. Celles-ci résultent de plusieurs facteurs, comme évoqué au chapitre I. Afin d'établir des bilans de matière plus précis (ratio SPOX connus corrigé du rendement analytique/AOX), ces pertes ont été évaluées pour chacun des 43 SPOX étudiés. Les rendements analytiques obtenus varient de 4,4 à environ 100%, en fonction des SPOX étudiés et de l'approche analytique employée (coulométrique *versus* C-IC). Les rendements analytiques obtenus par l'approche C-IC se sont montrés similaires ou supérieurs à ceux obtenus par la méthode AOX conventionnelle.

Determination of adsorbable organic halogen in water samples by combustion–microcoulometry versus combustion–ion chromatography titration

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Abstract

The effectiveness of the nitrate wash protocol according to ISO 9562 standard method to eliminate interferences caused by halide ions was examined. A false-positive AOX values were observed when chlorides concentrations exceed 100 ppm. The improvements realized efficiently remove chlorides bias even when their concentrations reach 1000 ppm. Secondly, an analytical method for chemical speciation of AOX into adsorbable organic chlorine (AOCl), bromine (AOBr) and iodine (AOI) was developed. This method combines adsorption of organic halogen compounds (OXBPs) onto activated carbon, combustion of the activated carbon, absorption of the resulting gases in an adsoption solution, and analysis of trapped halides by on-line ion chromatography (abbreviated here as C-IC). The main influence parameters were investigated. The following optimal conditions were established: aqueous solution containing 2.4 mM sodium bicarbonate/2.0 mM sodium carbonate, and 2% acetone (v/v) as mobile phase, 2 mL of aqueous sodium thiosulfate (500 ppm) as absorbing solution, 0.2 $ml.min^{-1}$ as water inlet flow rate, and 5 min as post-combustion time. This method was validated according to NF T90-210 standard method. Calibration curves fitted through a quadratic equations show coefficients of determination (r^2) greater than 0.9998, and RSD less than 5%. The LOQs were at 0.9, 4.3, and 5.7 µg eq $Cl.L^{-1}$ for AOCl, AOBr, and AOI, respectively. The accuracy and precision were well within predefined acceptable limits. The applicability of the validated method was demonstrated by the analysis of twenty-four water samples from three Rivers in France. The measurements reveals AOX amounts above 10 µg $Cl.L^{-1}$ in all untreated samples, suggesting the presence of organohalogen compounds in the sampled rivers.

1. INTRODUCTION

Halogen-based biocides, such as chlorine (HOCl), chlorine dioxide (ClO_2), and monochloramine (NH_2Cl), are widely used in water treatment processes in order to inactivate pathogenic microorganisms, control biofouling and prevent risk of introducing invasive species into local aquatic ecosystems [1]. These biocides react also with naturally occurring organic matter, inorganic compounds (e.g. bromide and iodide), and anthropogenic contaminants, leading to the formation of a vast and heterogeneous range of unwanted organohalogen byproducts (OXBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs) [1].

Methodologies for monitoring OXBPs are mainly of two different types. The first one uses targeted analysis to quantify known OXBPs as individual species (compound-by-compound), while the second, referred to as "integrative" or "global" analysis, measures the sum of OXBPs regardless of their chemical identity. The specific analysis of all known OXBPs would not only be very time consuming and prohibitively expensive but simply impossible due to limits in the available analytical methodologies for many of them. Indeed, only known byproducts for which analytical methods with sufficient accuracy and sensitivity are available can be measured. Consequently, targeted analysis inevitably misses unidentified OXBPs and a major part of known compounds. This raises the importance to develop integrative analytical methodologies capable of measuring total amounts of OXBPs to achieve better monitoring and management of these chemicals in treated waters. All OXBPs can be quantitatively evaluated thanks to the Adsorbable Organic Halogen parameter (AOX, where X = Cl, Br, and I). This analytical parameter provides a measure of sum halogens incorporated into organic chemicals, without information about the structure or nature of the organic compounds to which halogens are bounded. The AOX analytical procedure involves three main stages, namely: (i) enrichment and concomitant isolation of OXBPs from acidified water samples by adsorption on activated carbon, (ii) washing activated carbon with nitrate solution to remove interfering inorganic halides, and (iii) mineralization of adsorbed organohalogen compounds followed by on-line analysis of released halogenides (HX) by argentometric titration (usually microcoulometry). The detection method is based on the high affinity of chloride, bromide and iodide ions towards silver (Ag^+) ions. AgF however is more soluble in water (1.8 g/L at 25 °C), explaining why organofluorides are not accounted in the AOX measurements [1]. The silver-based microcoulometric titration is incapable to differentiate between halides, making the AOX method inappropriate to halogen speciation. By convention, AOX values are expressed as micrograms equivalent chloride per liter (µg $Cl.L^{-1}$).

The halogen-specific AOX determination, namely adsorbable organic chlorine (AOCl), bromine (AOBr) and iodine (AOI), is very important since it has been recently demonstrated that organiodine and organobromine byproducts tend to be more toxic than their chlorinated analogues [2,3,4]. Thus, differential determination of AOCl, AOBr, and AOI provides information on the toxicity and chemical nature of the byproducts formed, as well as it enables to understand the effect of halides concentrations

on the formation and distribution of OXBPs [5,6]. Halogen-specific AOX can be analyzed according to two main different ways. Neutron active ion analysis (NAA) is an EPA approved method that involves irradiation of activated carbon by a neutron flux, followed by detection of the radioactive halogens (^{38}Cl, ^{80}Br, and ^{128}I) by gamma-ray spectrometry. Although highly sensitive and specific, the NAA method remains inadequate for routine analysis due to its high cost and skill level required [7,8]. The second approach involves hydropyrolysis of AOX, collection of HX formed in an adsorption solution followed by analysis of the collected halides. Ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP/MS) are the most widely used techniques for quantitating halide ions in trapping solutions [9]. Other alternative detection methods have also been reported. Pontius et *al.* developed a method that uses inductively coupled plasma atomic emission spectroscopy (ICP-AES) for determination of halides [10]. More recently, Pan and Zhang described a detection method based on liquid chromatography (LC) coupled with mass spectrometry (MS) for iodide detection by setting selected ion recording on m/z 127 [11]. LC/MS, ICP/MS, and AES have in common the drawback of being very expensive and requiring extra skill. LC/MS suffers also from the formation of adducts (isobaric interferences) in the mobile phase and in the ionization source. Ion chromatography has been presented in several studies as a technique of choice for halides determination because of its ease of use, robustness, and affordable price. A further advantage is that IC also allows AOX determination by summing AOCl, AOBr, and AOI, as well as analysis of adsorbable organic fluorine (AOF). IC can be coupled either online or offline with a combustion furnace. Recently, several analytical instruments companies have developed online couplings, commercialized under the name of "combustion ion chromatography", C-IC. To date, there have been relatively few studies examining the potential of the C-IC technique for the analysis of sub-groups of AOX in water samples, almost all of these based on offline couplings [1].

AOX, and halogen-specific AOX are also metrics particularly useful for determining to what extent the known and measured byproducts are representative of the full amount of formed OXBPs. From the amounts of OXBPs (Ci) obtained by targeted analytical methods (e.g., GC/MS), their respective molar mass (Mi), as well as molar mass of chloride (M_{Cl} = 35.45 g.mol^{-1}), the percentage part of known and unknown AOX (respectively KAOX and UAOX), can be calculated using the equations 1 and 2 presented below. In eq. 1, n represents the number of halogen atoms in each OXBP. Known *vs* unknown AOCl, AOBr, and AOI can be determined in an analogous manner.

Eq. 1 $$\% \, KAOX = \Sigma \frac{n \times 35.45 \times Ci/Mi}{AOX} \times 100$$

Eq. 2 $$\% \, UAOX = 100 - \% \, KAOX$$

Eq. 3 $$AOX_{C\text{-}IC} = AOCl + AOBr + AOI$$

Today, over than 500 OXBPs have been identified. Several studies have investigated the occurrence of several of them in treated waters from various sources. According to Kinani et *al.*, 40 to 70% of the AOX formed by chlorine are attributed to KOXBPs, while the value is less than 20% on average for monochloramine, suggesting that several unknown and unidentified OXBPs still need to be identified [1]. Recent researches have shown that a significant fraction of uncounted AOX could be attributed to unidentified polar and high-molecular weight OXBPs unamenable to be detected by gas chromatography, which is usually used for structural identification of unknown compounds [12-16]. An elevated UAOX-to-AOX ratio could be also related to an overestimation of AOX actual values. It has been reported that incomplete removal of inorganic halides during the wash step by a nitrate solution causes positive bias [17,18,19]. The data on the amount of halides beyond which a significant bias occurs remain heterogeneous and contradictory while it is crucial to measure the AOX and its sub-groups with great accuracy and repeatability for the best estimation of the UAOX values. In addition, to our knowledge, only chloride effects have been examined. On the other hand, adsorption-pyrolysis approaches measure only the amount of OXBPs retained on activated carbon and trapped in the adsorption solution. For example, polar, low molecular weight, and relatively hydrophilic OXBPs are known to be weakly adsorbed onto activated carbon, and therefore poorly recovered [7,20]. For a rigorous and accurate assessment of the KAOX percent, the amount of OXBPs lost during the various stages of the AOX analysis procedure must be taken into account in the calculations of the mass balance (Eq. 1), as it is the case for targeted analyzes. However, so far, very few studies have been devoted to recoveries study of known OXBPs, and only a limited number of chemicals have been examined. Thus, there is a manifest need for further studies to evaluating the recovery efficiency of known OXBPs by the AOX method.

The objectives of this study were to: (i) find a suitable washing protocol to remove inorganic halides, in order to obtain reliable and precise AOX results; (ii) determine the recoveries of 43 OXBPs belonging to nine different chemical classes including 8 halomethanes, 11 haloacetic acids (HAAs), 6 haloacetamides (HAcAms), 6 haloacetonitrils (HANs), 4 Haloketones (HKs), 4 halophenols (HPs), 2 halopropanes. 1 Haloacetaldehyde, and 1 halonitromethane; (iii) develop and optimize conditions for halogen-specific AOX determination by on-line combustion ion chromatography (C-IC) and finally; (iv) analyze river water samples having been disinfected by monochloramine using the C-IC developed method, in order to assess its suitability for the simultaneous determination of AOX and its sub-groups.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals

Aqueous solutions were prepared in ultrapure water (18 MΩ cm at 25 °C) produced by a PURELAB Chorus 1 water purification system (Veolia Water Technologies. Wissous. France). All reagents used were of analytical grade or higher purity. Sodium nitrate ($NaNO_3$. ≥ 99.5%), nitric acid (HNO_3. ≥ 65%), acetic acid (CH_3COOH. ≥ 96%), and sulfuric acid (H_2SO_4. > 98%), were purchased from VWR International (Fontenay-sous-Bois. France). Working solutions for the AOX method were prepared in accordance with the ISO 9562: 2004 standard method. Two kinds of high purity powdered activated carbon were employed: the first one marketed by TE Instruments (grain size 10-50µm) for use with its AOX analyzer and the second one supplied by Metrohm (grain size 30-63µm) for C-IC experiments. Sodium thiosulfate ($Na_2S_2O_3$ > 98%) was purchased from VWR International (Fontenay-sous-Bois. France), sodium sulfide nonahydrate (Na_2S, $9H_2O$ >99.99%) and *hydrogen peroxide* solution (H_2O_2, 30% w/w) were purchased from Sigma Aldrich Saint-Quentin Fallavier. France) and sodium sulfite (Na_2SO_3. > 98%) was purchased from Merck (Fontenay-sous-Bois. France).

Commercially analytical standards of fluoride, chloride, bromide, and iodide each one at 1000 ppm were provided by Metrohm (Villebon-sur-Yvette, France). A multi-element solution at 1 ppm in each halide was prepared to calibrate the ion chromatograph. Primary nitrite, nitrate, sulfate, sulfite and, thiosulfate standards (each one at 1000 ppm), assumed to be potential interfering ions, were prepared by dissolving the appropriate amounts of their corresponding salts in water. All the stock solutions were stored in plastic bottles, and kept refrigerated at 4 °C until used. Solvents and chemicals used in the preparation of IC eluents included sodium bicarbonate with purity more than 99.7%, sodium carbonate with purity more than 99.9% (Sigma-Aldrich. Saint-Quentin-Fallavier, France), and HPLC-grade acetone (Merck, Fontenay-sous-Bois, France). For recovery experiments, 43 OXBPs belonging to nine different chemical classes were studied. These include 8 halomethanes, 11 haloacetic acids (HAAs), 6 haloacetamides (HAcAms), 6 haloacetonitrils (HANs), 4 Haloketones (HKs), 4 halophenols (HPs), 2 halopropanes, 1 Haloacetaldehyde, and 1 halonitromethane. They have been selected for their frequent occurrence in treated water and commercial availability of their analytical standards. Their commercial sources, purities, molecular weights, boiling points and solubilities are given in Table 1, Supporting Information 1. The selected OXBPs exhibit different volatilities, hydrophobicities, and solubilities in water. Individual standard stock solutions of each chemical at 1 ppm were prepared in methanol and immediately stored in amber glass vials at 4 °C until use. The working aqueous solutions were prepared daily by diluting appropriate volumes of the stock solutions in water.

2.2. Sample collection

The developed methods were applied to river water samples, which were collected between July and October 2016 at three locations in France (Meuse River, Moselle River, and Loire River), before and after treatment with monochloramine. The water samples characteristics are given in Table 1,

Supporting Information 2. All the samples were collected in 2 L amber bottles containing 2 mL of L-ascorbic acid at 1.6 $g.L^{-1}$ to quench the monochloramine residual, and filled without headspace to prevent volatilization of OXBPs. The amount of ascorbic acid used in this study is sufficient to neutralize 0.65 mg $Cl_2.L^{-1}$, which corresponds to about twice the monochloramine residual amount in sampled waters. Once collected, the water samples were transported to the laboratory in refrigerated road vehicles. Upon receipt at the laboratory, samples were kept in the dark at 4 °C and analyzed as soon as possible, always within 48 h after collection.

2.3. Determination of AOX using the adsorption–pyrolysis–microcoulometric method

AOX content was analyzed using an AOX analyzer (Xplorer) equipped with an autosampler (Newton) from Trace Elemental Instruments (Opheleia Instruments, France). For enrichment onto granular activated carbon, the batch shaker procedure according to the ISO 9562 standard method was used. Briefly, after collection the water samples were immediately quenched of remaining residual oxidant by 1 N sodium sulfite (10 mL per 1 L of sample). 100 mL of each sample were introduced into 250 mL Erlenmeyer flasks and acidified to pH < 2 by addition of 0.2 mL of concentrated nitric acid 65% (w/w), followed by 5 mL of acidic nitrate solution and 50 mg activated carbon powder. The flasks were hermetically sealed and automatically shaken on an orbital shaker (KS 500, Labo Moderne, France) at 150 rpm and room temperature for one hour. Samples were then filtered through a quartz frit to recover the carbon particles using an automatic filtration unit equipped with three independent filtration channels (Xprep-3, Ophelia Instruments, France). Samples were percolated through filters at a flow rate of 5 $mL.min^{-1}$ using nitrogen at a pressure of 30 psi. To remove trapped inorganic halides, the “filtration cakes” were washed thrice with a 0.01 N nitrate solution (15.5 mg NO_3^- per sample) for a total volume of 25 mL. The filters were then placed on the AOX analyzer autosampler for introduction into the combustion furnace. A weak nitrogen stream was applied within the enclosure of autosampler, in order to prevent samples contamination by laboratory air. The temperature furnace in which circulates oxygen at a flow rate of 300 $mL.min^{-1}$ was kept at 1000 °C. The resulting combustion gases were directed to a tube filled with concentrated sulphuric acid to trap water and interfering compounds. The dried and clean gasses were lead into a temperature controlled titration cell (30 °C) containing acetic acid (70%, w/w), where halide ions were quantified by microcoulometry. The AOX analyzer performs an analysis in about 10 minutes per sample.

Prior to AOX determination in water samples, a preliminary quality control was carried out. The proper performance of the equipment was tested by three procedures: (i) checkup of microcoulometric titration cell recovery, (ii) verification of the procedure recovery using organic standard solution at a concentration close to the expected concentration in real samples, and (iii) method blank analysis. The recovery of the microcoulometric cell was determined daily by injecting 50 µL of a standard solution

of 2 mM NaCl directly in the titration cell. A relative standard deviation (RSD %) of less than 2.5% was required. Before each measurement series, the complete procedure (enrichment by adsorption onto activated carbon and microcoulometric detection) was controlled by analyzing a standard solution of 4-chlorophenol at 50 µg $Cl.L^{-1}$. A RSD of less than 10% was required to accept and validate the analytical results.

2.4. Determination of halogen-specific AOX parameters using the adsorption–pyrolysis–ion chromatography method

Determination of halogen-specific AOX parameters was performed using an on-line C-IC system from Metrohm (Villebon-sur-Yvette, France). It comprises a combustion furnace, a gas absorption unit (920 Absorber Module), and an ion chromatograph (881 Compact IC pro). The IC system was equipped with a dual isocratic pump, an automated membrane eluent degassing, a sample injector equipped with a 250 µL sample loop, and a suppressed conductivity detector. Instrumental control, data acquisition and data processing were performed using the MagIC Net 2.2 for Windows chromatography software (Metrohm). A schematic diagram of the instrument is given in Figure 1, Supporting Information 1.

The enrichment procedure was similar to that described in Section 2.2. However, the employed C-IC system requiring filters with dimensions different from those compatibles with the Opheleia AOX analyzer, filtration was achieved using the automatic filtration unit AFU 3 from Analytik Jena. After filtration, the filters (containing the activated carbon onto which the organic halogens were adsorbed) were automatically introduced into the combustion tube thanks to a boat control unit. The combustion was conducted in a pure oxygen stream at a constant flow rate of 300 $mL.min^{-1}$. The filters were introduced into a hot zone at 400 °C and gradually moved inside the combustion furnace to where the temperature reached 1050 °C. A sensor measured the intensity of the flame produced and controlled the speed of introduction of the sample into the furnace, thereby avoiding soot formation and optimizing the duration of the combustion. A post-combustion time was applied in order to burn remaining organic compounds. Argon delivered at 100 $mL.min^{-1}$ was used as carrier gas. During the combustion, water was introduced in the furnace using a dosing control unit. At the end of combustion, the inner walls of the combustion tube as well as the gas transfer lines were rinsed with 1 and 2 mL, respectively, of ultrapure water, in order to ensure that all produced halogens were collected in the absorbing solution. The combustion gases and rinsing waters were trapped in 2 mL of an absorption solution containing a strong reducing agent. The concentration of halide ions was determined by automatically injecting 200 µL of the absorbing solution into the IC column. After injection, the absorption tube was washed with the absorption solution to prepare the next analysis.

The chromatographic column was a Metrosep A Supp 4 analytical column (4 x 250 mm, 9 µm particle size) having a stationary phase of polyvinyl alcohol with quaternary ammonium groups, that was safeguarded with a Metrosep A Supp 4/5 Guard/4.0 column (4 x 5 mm, 5 µm particle size) supplied by

Metrohm. The column was maintained at 30 °C in the chromatograph oven. Detection was performed using a suppressed conductivity detector. Conductivity suppression was performed using a system combining a chemical suppressor and a carbon dioxide suppressor, respectively MSM II and MCS, from Metrohm. The IC instrument was equipped with a dosing control unit, allowing to perform calibration curves from only one standard solution by accurate injection of decreasing volumes.

3. RESULTS AND DISCUSSION

3.1 AOX analysis using the adsorption–pyrolysis–microcoulometric titration approach

3.1.1 Investigation of the interferences caused by inorganic halides on AOX measurements

It is well known that artefacts can easily be produced with the AOX method. During the enrichment phase, activated carbon may adsorb halides, some of which being detectable by microcoulometry, that can thus impact the measure of OXBPs in a water sample. Washing the activated carbon with a nitrate solution constitutes a key step for removing adsorbed halides. Although the ISO 9562 standard method specifies that washing of the filtration cake with 25 mL of a 0.01 M nitrate solution (corresponding to 15.5 mg NO_3^- per 100 mL of sample) is effective for removing chlorides up to 1000 ppm, several studies have indicated that chlorides may cause positive interference even at lower concentrations [17,21-23]. Dilution of the water samples may minimize this interference but also leads to dilution of the AOX contents, which is especially problematic when the AOX concentration is close to the limit of quantitation (10 µg $Cl.L^{-1}$). Experiments were conducted aiming at providing additional information on the effectiveness of the ISO 9562 washing procedure and evaluating the eventual positive bias caused by inorganic halides on AOX measurements. They were based on the analysis of ultrapure water (OXBPs free) spiked with halides at concentrations ranging from 1 to 1000 $mg.L^{-1}$ for chloride, 25 to 200 $mg.L^{-1}$ for bromide, and 10 to 100 $\mu g.L^{-1}$ for iodide. These values are representatives of halides concentrations in natural freshwaters, where they are expected to vary between 1-1000 $mg.L^{-1}$, 0.01-1000 $\mu g.L^{-1}$, and 0.5-100 $\mu g.L^{-1}$, for chloride, bromide, and iodide, respectively [24-25]. Analyses were performed following the protocol described in Section 2.2. The experiments were realized in triplicate. Data were corrected by subtracting procedure blank values and expressed as a mean ± standard deviation. Figure 1 (A. B and C) summarizes the obtained results.

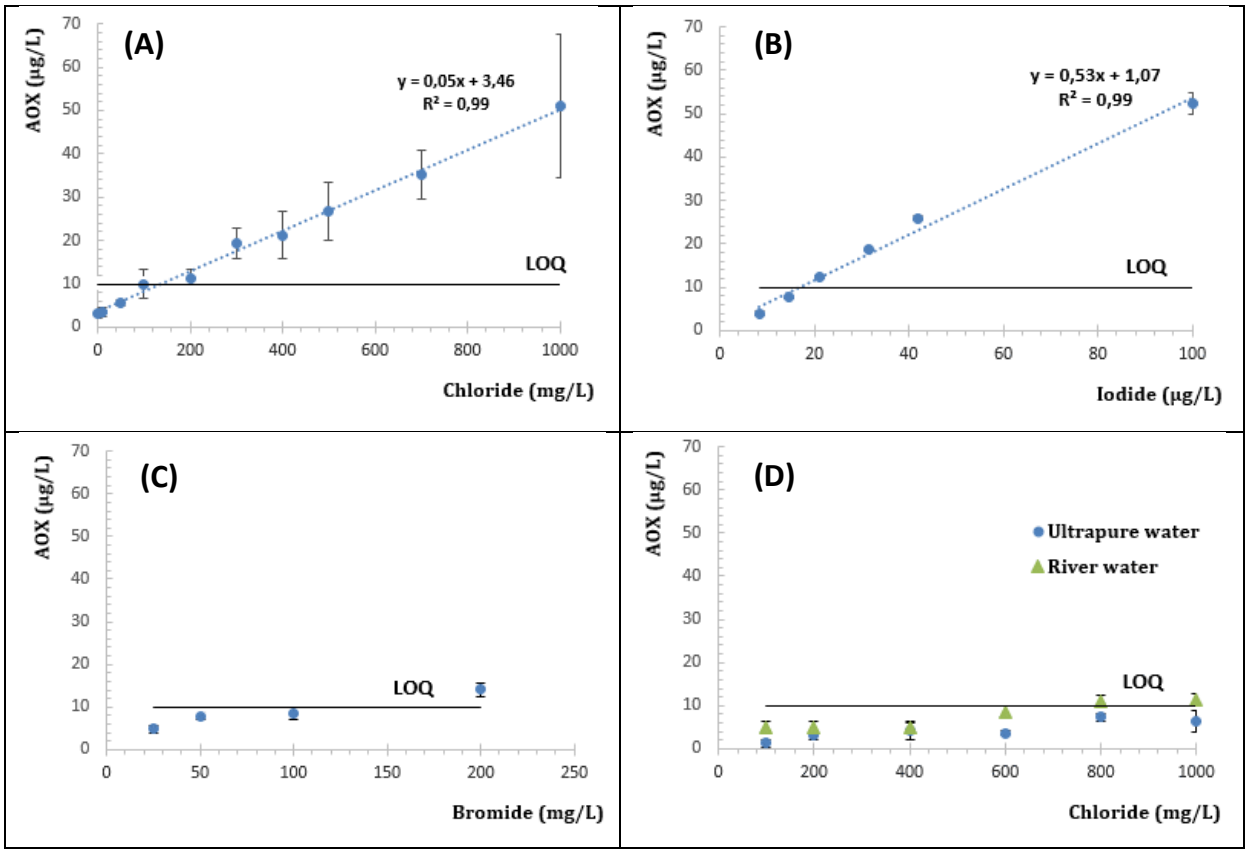


Figure 1. Interference of halogen ions concentrations on the AOX responses (A: ultrapure water spiked with chloride ions. B: ultrapure water spiked with iodide ions. C: ultrapure water spiked with bromide ions, and D: river and ultrapure waters spiked with chloride ions)

The results in Figure 1-A show that AOX responses increase when increasing chloride concentrations. An excellent correlation (r^2 = 0.99) was calculated for the linear regression between inorganic chloride concentrations against AOX response, indicating a positive interference caused by inorganic chlorides. This interference produces a measurable false-positive AOX value even at a chloride concentration of 100 ppm. The chloride interference evidenced in this study can lead to erroneous conclusions when analyzing real samples, since chloride concentrations higher than 100 mg $Cl.L^{-1}$ may be found in natural fresh waters, as previously specified. For example, chloride concentrations in Moselle River water (France) may reach more than 400 $mg.L^{-1}$ [26-27]. According to Figure 1-A, it can be reasonably expected that AOX measurement according to the ISO 9562 wash protocol overestimates the AOX amounts in this water source from 13 to 27 µg $Cl.L^{-1}$ approximately. As can be seen from data on Figure 1-C, bromide ions up to 100 $mg.L^{-1}$ do not interfere with AOX determination. Concerning iodide ions, the magnitude of interference was more important than that induced by chloride ones, as illustrated on Figure 1-D. The slope of the regression line is 0.53 with a correlation coefficient of 0.99, indicating that approximately 50% of the inorganic iodine amount is measured as AOX in the analyzed samples. However, inorganic iodide (iodide and iodate ions) concentrations in natural waters are typically very low. The reported mean value for river waters is about 5 $µg.L^{-1}$ [28-29]. At this

concentration, it is unlikely that inorganic iodide cause measurable false positive values. However, increased iodide concentrations can be found in source waters from areas impacted by salt water and wastewater intrusion or with special geological formation [30].

3.1.2 Elimination of chloride interference on the determination of OXBPs by the AOX method

Removal of inorganic chloride interference is crucial for more accurate determination of AOX amounts, and thus of UAOX. Overestimation of AOX values leads to exaggerate the part of unknown OXBPs. This is of particular concern in the case of monochloramine because it produces lower concentrations of OXBPs compared to other halogen-based inorganic oxidants. To eliminate chloride interference, numerous factors were identified as possible influence parameters, including concentration and/or volume of the nitrate washing solution, the washing operating mode, and memory effects of sample filtration reservoirs. Several studies have shown that increasing nitrate concentration and/or volume improves inorganic halides removal efficiency but unfortunately causes the loss of numerous OXBPs [31]. In order to obtain effective chloride removal without sacrificing recovery of OXBPs, it has been decided to not modify either volume or concentration of the nitrate solution. The mechanism of incomplete removal of inorganic chlorides during the washing phase might be related to a preferential passage of the nitrate solution through "channels" formed as consequence of excessive drying of the filtration cake after sample loading. To verify this hypothesis, the following procedure was tested. After loading of the water samples, the nitrogen flow was stopped before complete drying of activated carbon. For minimization of memory effects, the filters containing filtration cakes were removed and connected to cleaned sample containers. A 2 mL spray of ultrapure water was applied to re-suspend the activated carbon before it was washed three times with a 0.01 N nitrate solution for a total volume of 25 mL, without complete drying between each fraction. This designed protocol was applied to the analysis of both river and ultrapure water samples spiked with chloride at concentrations ranging from 100 to 1000 $mg.L^{-1}$. Figure 1-D summarizes the obtained results. As can been seen, all obtained values are below the quantitation limit of the AOX method (l0 µg $Cl.L^{-1}$), demonstrating the effectiveness of our modified washing procedure to eliminate interference due to chloride ions up to 1000 $mg.L^{-1}$. Hence, this washing protocol was applied for all further experiments.

3.1.3 Recovery of OXBP compounds

For a rigorous assessment of the KAOX percent, the loss of OXBPs that might occur during the AOX procedure should be taken into account when calculating the mass balance (Eq. 1), as it is the case for targeted analyzes. For this purpose, the recoveries of 43 OXBP compounds belonging to nine different chemical classes were evaluated. Experiments consisted in analyzing water samples prepared from OXBPs stock solutions in ultrapure water. Three concentration levels were tested: 25, 50, and 75 µg $Cl.L^{-1}$). Synthetic solutions were considered as real samples and treated according to the procedure

described in Section 2.2. Experiments were repeated on three different days for each solution. The recovery was calculated as percentage by comparing the obtained concentration with the theoretical one. To evaluate the efficiency of the adsorption phase on activated carbon, experiments were carried out by directly analyzing 50 mg of activated carbon on which a volume corresponding to a concentration of 50 µg $Cl.L^{-1}$ of each tested OXBP had been dropped. Table 1 provides a summary of results.

Table 1. Recoveries of selected OXBPs obtained by conventional AOX method (n = 3)

Group	OXBPs	Recovery % ± RSD - AOX procedure (n = 3)		Recovery % ± RSD - C-IC procedure (n = 3)	
		Complete procedure	DA	Complete procedure	DA
Halomethanes 6 THMs + 2 HMs	Chloroform	53.1 ± 2.8	50.5 ± 0.8	36.6 ± 1.4	86.6 ± 2.6
	Bromodichloromethane	65.8 ± 4.3	65.4 ± 2.3	63.5 ± 1.0	86.9 ± 0.7
	Dibromochloromethane	71.4 ± 4.0	86.7 ± 1.3	74.8 ± 3.2	90.0 ± 0.3
	Bromoform	78.0 ± 1.7	78.5 ± 0.3	74.3 ± 0.8	89.5 ± 1.5
	Iodoform	88.6 ± 2.1	96.9 ± 1.0	91.5 ± 4.2	96.4 ± 0.4
	Dichloroiodomethane	84.2 ± 4.2	79.3 ± 1.7	85.5 ± 4.2	93.4 ± 0.4
	Chloroiodomethane	69.9 ± 2.1	75.6 ± 1.6	96.5 ± 1.3	97.5 ± 1.4
	Bromoiodomethane	81.0 ± 2.3	77.9 ± 1.4	98.5 ± 3.3	97.5 ± 1.4
Halopropanes	1,2-Dibromopropane	83.1 ± 1.6	77.2 ± 2.2	93.1 ± 1.6	97.2 ± 0.2
	1,2,3-Trichloropropane	92.3 ± 3.9	93.1 ± 1.6	97.3 ± 3.9	98.1 ± 1.6
Haloacetamides	Chloroacetamide	4.4 ± 1.2	97.5 ± 1.0	90.0 ± 4.7	90.0 ± 3.3
	Dichloroacetamide	34.7 ± 1.4	97.8 ± 2.3	96.1 ± 2.3	98.1 ± 3.8
	Trichloroacetamide	79.2 ± 2.0	86.2 ± 1.2	91.8 ± 4.6	91.8 ± 3.4
	Bromoacetamide	6.8 ± 2.6	95.8 ± 0.6	96.5 ± 1.3	96.5 ± 1.0
	Tribromoacetamide	92.2 ± 0.3	93.7 ± 0.6	93.7 ± 3.3	96.7 ± 2.7
	Iodoacetamide	32.4 ± 3.0	92.9 ± 1.0	94.9 ± 0.6	95.3 ± 0.7
Haloacetic acids	Monochloroacetic acid	28.3 ± 2.3	79.0 ± 0.1	44.6 ± 0.7	96.9 ± 0.9
	Dichloroacetic acid	60.4 ± 3.3	92.0 ± 0.7	70.0 ± 2.7	96.1 ± 2.0
	Trichloroacetic acid	75.0 ± 3.8	92.9 ± 1.4	83.0 ± 1.7	92.3 ± 0.9
	Monobromoacetic acid	48.6 ± 2.6	77.1 ± 2.1	53.3 ± 0.7	97.9 ± 2.7
	Dibromoacetic acid	83.8 ± 4.1	92.7 ± 1.8	79.7 ± 2.0	92.3 ± 0.9
	Tribromoacetic acid	60.5 ± 1.7	81.3 ± 1.0	85.6 ± 3.0	96.8 ± 1.2
	Bromochloroacetic acid	70.0 ± 4.1	90.7 ± 2.5	90.9 ± 5.4	98.1 ± 4.7
	Bromodichloroacetic acid	75.5 ± 0.2	90.0 ± 0.7	75.3 ± 2.3	93.4 ± 2.7
	Dibromochloroacetic acid	65.4 ± 5.8	89.6 ± 0.7	72.7 ± 1.4	96.4 ± 0.9
	Iodoacetic acid	56.8 ± 4.1	93.8 ± 0.5	56.6 ± 2.6	100.0 ± 1.1
	Diiodoacetic acid	105.0 ± 2.5	105.0 ± 0.4	104.5 ± 1.9	106.3 ± 1.8
Haloacetonitriles	Chloroacetonitrile	7.4 ± 2.6	82.5 ± 1.0	92.9 ± 4.2	92.2 ± 2.8
	Dichloroacetonitrile	66.6 ± 3.1	93.1 ± 0.2	97.4 ± 2.2	96.9 ± 2.7
	Trichloroacetonitrile	83.7 ± 3.0	94.8 ± 0.3	91.6 ± 6.0	94.6 ± 4.6
	Bromoacetonitrile	19.4 ± 2.0	82.5 ± 0.8	92.6 ± 5.4	92.7 ± 4.8
	Dibromoacetonitrile	91.5 ± 1.0	101.2 ± 0.9	97.2 ± 2.5	97.2 ± 2.4
	Iodoacetonitrile	54.4 ± 3.0	94.5 ± 0.5	99.3 ± 0.5	99.6 ± 0.5
Halonitromethanes	Bromonitromethane	55.9 ± 0.7	110 ± 0.8	93.4 ± 2.1	93.4 ± 2.1
Haloacetaldehydes	Tribromoacetaldehyde	90.8 ± 2.2	99.6 ± 0.1	95.4 ± 5.8	104.3 ± 1.7
Haloketones	Chloropropanone	34.1 ± 1.7	97.0 ± 0.1	56.7 ± 2.3	99.2 ± 1.3
	1,1-Dichloropropanone	82.2 ± 1.3	104.0 ± 0.5	93.4 ± 0.8	100.9 ± 0.8
	1,3-Dichloropropanone	72.2 ± 1.8	101.7 ± 1.7	82.9 ± 1.2	101.1 ± 0.4
	1,1,3-Trichloropropanone	105.6 ± 4.2	118.5 ± 0.3	104.9 ± 1.4	120.7 ± 1.2
	1,1,1-Trichloropropanone			78.4 ± 2.2	98.6 ± 1.6
Halophenols	4-Chlorophenol	93.9 ± 2.1	97.9 ± 0.1	99.1 ± 2.5	99.9 ± 0.3
	4-Bromophenol	91.5 ± 1.0	98.0 ± 0.3	98.0 ± 1.9	100.0 ± 0.6
	4-Iodophenol	90.7 ± 3.2	95.9 ± 0.6	98.4 ± 2.5	101.4 ± 0.6
	2,4,6-Tribromophenol	93.4 ± 1.0	95.5 ± 1.8	100.5 ± 1.7	100.9 ± 2.7

Abbreviations: DI: Direct analysis of activated carbon on which a volume corresponding to a concentration of 50 µg eq $Cl.L^{-1}$ of each tested OXBP has been dropped.

The AOX method provided recoveries below 100% for all studied compounds except 1.1.3-Trichloropropanone and Diiodoacetic acid. Mean recoveries were within a range of 53.1-88.6% for halomethanes (HMs and THMs), 28.3-105.0% for HAAs, 34.1-105.6% for HKs, 7.4-83.7% for HANs, 4.4-92.2% for HAcAms, 83.1-92.3% for halopropanes, 90.7-93.9% for HPs, 55.9% for bromonitrometane, and 90.8% for tribromoacetaldehyde. The RSD values were below 6% for all tested compounds, indicating a good reproducibility. Similar values were found by Reckhow et *al.* who employed the conventional AOX method using columns packed with activated charcoal to extract THMs and HAAs [32]. Sullivan et *al.* concluded that low molecular weight AOX are poorly recovered by the shaker method, while high molecular weight AOX are essentially quantitatively recovered [33]. The obtained recoveries vary greatly between OXBPs. The lowest recoveries were detected for the more polar compounds such as monobromoacetamide, monochloroacetonitrile, monobromoacetonitrile, monochloroacetic acid and dichloroacetamide. The poor recovery of polar compounds might be due to their limited adsorption on activated carbon and their high solubility in water. Conversely, the more bulky hydrophobic compounds, such as halophenols and halopropanes, show recovery values close to 100%. In general, recoveries values tend to increase with increasing the number and molar weight of halogen atoms. This was the case for HAAs for example, where the recovery increases in the order MCAA (28.3%) < MBAA (48.6%) < MIAA (56.8%) < DCAA (60.4%) < TCAA (75.0%) < DBAA (83.8%) < DIAA (105.5%). It should be noted that for a given chemical, hydrophobicity increases and volatility decreases with the number of halogen atoms and their molar weight. A correlation analysis showed strong positive correlation ($0.912 < r^2 < 0.996$) between recovery yields and hydrophobicity of studied OXBPs (Figure 2, Supporting Information 2). The low recovery yields of THMs could be expected given their volatility. Indeed, during the combustion phase, a competition occurs between volatilization and decomposition by pyrolysis. The volatilization phenomenon leads to lower AOX values since only the part of adsorbed analytes that is converted into halides during pyrolysis is taken into account in the AOX measurements. The degree of losses varies considerably depending on the chemical nature of the OXBPs examined, as evidenced by the results provided by direct injection into the AOX analyzer. Figure 3 (Supporting Information 3) shows a good linear relationship between recovery yields and volatility of halomethanes with a correlation coefficient greater than 0.66. The low recovery yield of TBAA (60.5%) in comparison to that of DBAA (83.6%) is probably related to its degradation during pyrolysis. Mia and Chiang reported that injection of underivatized HAAs into the gas chromatograph injector causes their thermal decomposition into halomethanes [34]. These transformation by-products may be thereafter volatilized, as explained below. The formation of species untitratable with the microcoulometric detector during OXBPs combustion such as HOX, X_2Also, it might be possible that OXBPs undergo degradation during sample preparation and/or adsorption on activated carbon, leading to lower recoveries. For example, Li et *al.* found that a portion of AOCl can be reduced to chloride by activated carbon [36]. On the other hand, the ISO 9562 standard method recommends adding sodium sulfite to

remove residual oxidant and nitric acid (to a pH < 2), in order to preserve AOX as well as to convert ionic OXBPs to neutral forms (e.g. haloacetates to HAAs). The effects of quenching agents on the preservation of OXBPs have been widely investigated and discussed. For example, Chu et *al.* found that a relatively large amount of sodium sulfite, sodium thiosulfate or ascorbic acid degraded HAcAm compounds to some degree, especially the brominated and iodinated ones [37]. In another study, Liew et al. showed that the tri-HNMs degraded in the presence of sodium sulfite [38].

Our results highlight the importance of integrating the recoveries obtained by the AOX batch method for an accurate assessment of the KAOX percent. Based on the recovery values summarized in Table 1, a correction factor may be calculated for each studied OXBPs, with the aim to correct its contribution to the AOX value (Section 1. Eq. 1).

3.2. Optimization of C-IC operating conditions

The third objective of this work was to develop a robust, efficient and friendly C-IC method for chemical speciation of AOX. The method has been optimized by studying the effect of various combustion, adsorption and chromatographic parameters. The details and highlights of method development are discussed in the following sections.

3.2.1 Ion chromatography conditions - optimization and interference study

The method was optimized by changing the analytical conditions in order to obtain the most suitable chromatographic separation. The analytical column used in this work is commonly employed for routine anion separation [39]. Mobile phase types and possible interference from several ions were studied. Given the low stability of the employed stationary phase at high pH values (pH > 12), a carbonate/bicarbonate mixture was selected as eluent. Three different mobile phase compositions were used and their effect on the separation of halides ions was studied: phase A was composed of ultrapure water containing 1 mM Na_2CO_3/2 mM $NaHCO_3$, phase B was composed of ultrapure water containing 2.4 mM Na_2CO_3/2.0 mM $NaHCO_3$ and phase C was composed of water containing 2.4 mM Na_2CO_3/2.0 mM $NaHCO_3$ with 2% of acetone. The anions derived from the quenching reagent (SO_3^{2-}), the nitrate wash solution (NO_3^-, NO_2^-), as well as the absorption solution ($S_2O_3^{2-}$, SO_4^{2-}) may affect chromatographic resolution. Their possible interferences with halide ions were investigated. Each anion was injected individually in IC to determine its retention time prior to injection in a mixture solution. Results were evaluated on the basis of peak shape and chromatographic resolution. Figure 2 shows an example of ion chromatograms obtained from a standard mixture of halide ions and five potentially interfering anions.

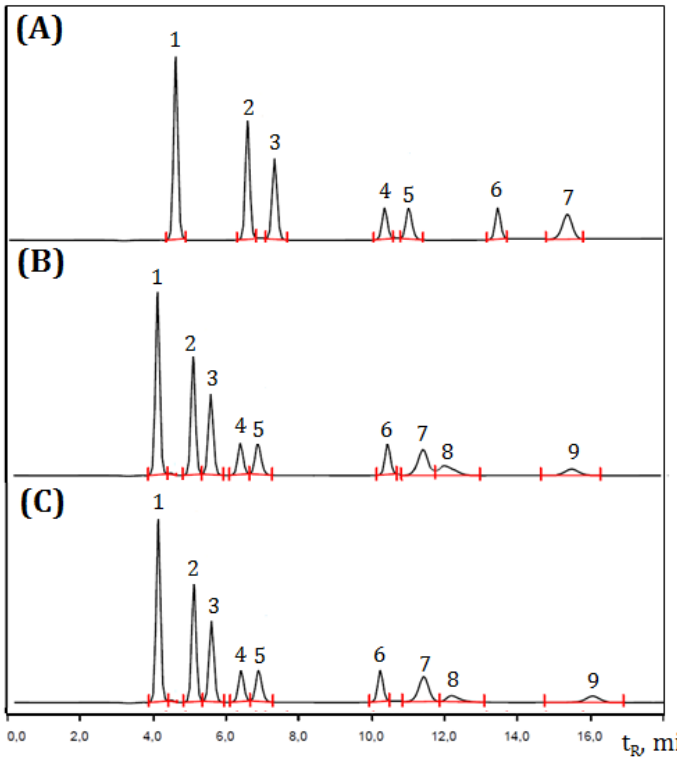


Figure 2. Comparison of ion chromatograms obtained with the different mobile phase tested: (A) mobile phase A; (B) mobile phase B; and (C) mobile phase C. The chromatographic peaks represents the following anions: fluoride (1), chloride (2), nitrite (3), bromide (4), nitrate (5), sulfite (6), sulfate (7), iodide (8), and thiosulfate

As expected, the retention times decreases for all anions when increasing carbonate concentration, and the use of acetone as organic modifier produces sharper peaks, and reduces retention times of hydrophobic ions. The best separation was achieved using the mobile phase C. With this mobile phase composition, inorganic halides were resolved from each other and also from other common inorganic anionic species expected to interfere with their determination. It has been thus selected for halides separation.

3.2.2 Optimization of absorption conditions

Ultrapure water was selected as absorbing solvent due to its high compatibility with ion chromatography. Pyrohydrolysis of organohalogen compounds may form, in addition of halide ions (X^-), several other inorganic halogen-containing species such as molecular halogens (X_2), and hypohalogen acids (HOX) [40-41]. Molecular halogens may be generated according to the Deacon-like reaction (Reaction 3). Based on the thermodynamic predictions, the molar Br_2-to-HBr ratio is much higher than that of Cl_2-to-HCl. At 900 °C, it has been estimated that this ratio equals 30% for bromine, whereas chlorine is largely in the HCl form. According to Miyake et *al.*, the I_2-to-HI ratio may be greater than that of bromine because iodine has lower electronegativity [42]. To convert

possible X_2 and HOX into X^- forms in the combustion gases, a chemical reducing agent should be added in the absorption solvent. Many reducing agents have been reported in the literature [43]. The reducing agent should meet several stringent requirements, such as efficient conversion of inorganic halogen species into halide ions and compatibility with ion chromatography; it must not interfere with halides determination. Four reducing chemicals including hydrogen peroxide, sodium sulfite, sodium thiosulfate, and sodium sulfide were evaluated. The volume of the absorbing solution was kept constant at 2 mL. According to the results obtained (Table 3, Supporting information 3), sodium thiosulfate was chosen due to its better suitability for IC determinations as well as its efficiency to recover all halogens. The required amount was evaluated testing concentrations ranging from 0 to 600 ppm. Figure 3 presents the effect of the concentration of sodium thiosulfate on the absorption efficiency.

$$4HX + O_2 \rightleftharpoons 2X_2 + 2H_2O \qquad \text{Reaction 1}$$

$$2Na_2S_2O_3 + HOCl \longrightarrow Na_2S_4O_6 + NaCl + NaOH \qquad \text{Reaction 2}$$

$$Na_2S_2O_3 + 4Cl_2 + 5H_2O \longrightarrow 2NaHSO_4 + 8HCl \qquad \text{Reaction 3}$$

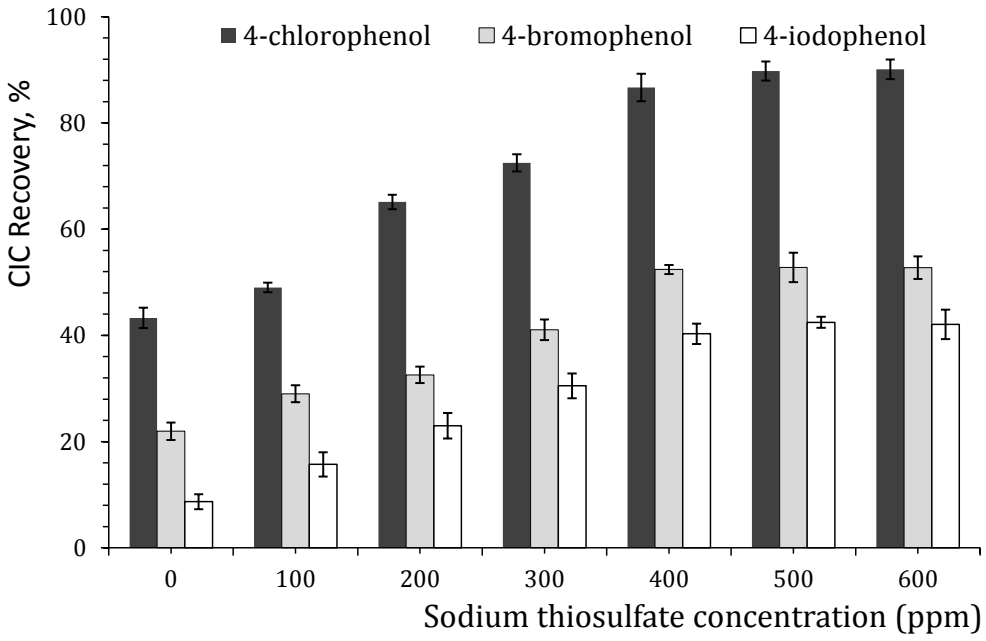


Figure 3. Effect of sodium thiosulfate concentration on the absorption efficiency. Pyrohydrolysis conditions were 0.1 mL^{-1} min as flow rate of water inlet, and 5 min. as a post-combustion time. Determination by C-IC (error bars are the standard deviation; n = 3)

As can be seen in Figure 3, recoveries lower than 50% were obtained for all halogens in the absence of sodium thiosulfate. They decreases in the following order: Cl (43%) > Br (22%) > I^- (9%), which seems in accordance with thermodynamic predictions. Halides' recoveries increase gradually as the concentration of sodium thiosulfate was increased then remain constant at above 400 ppm. These

results clearly denote the formation of inorganic halogen-containing species other than halides ions. The comparison of yields obtained in the presence of sodium thiosulfate at a concentration above 400 $mg.L^{-1}$ and those obtained without sodium thiosulfate suggests that released halogens are predominantly X_2 and/or HOX. The reason for the high ratio of X_2 and/or HOX-to-HX may be partly explained by a lack of water molecules during the combustion step. Indeed, according to Reaction 1, water is a product of the Deacon reaction; the equilibrium of this reaction moves to the right when the system contains low amounts of water. Therefore, an aqueous solution of sodium thiosulfate at 500 ppm was considered as the suitable absorbing solution in this work.

3.2.3 Optimization of combustion conditions

Different parameters of the combustion program have to be adjusted to obtain a complete combustion and guarantee that all halogens are detected. They include flow rate of combustion gas, combustion temperature, water inlet flow rate and combustion duration. According to the literature and instructions provided by the C-IC supplier, oxygen flow rate and furnace temperature were set at 300 $mL.min^{-1}$ and 1050 °C, respectively [43-44]. Water inlet flow rate and post-combustion duration were optimized.

Water inlet. Ultrapure water is continuously introduced into the furnace during the combustion stage. The water vapor plays mainly three roles: first, as carrier gas, it takes out the gases formed in the combustion tube and reduces chemisorption effects; second, it participates in chemical reactions of pyrohydrolysis as shown by Reaction (4), and third, it limits corrosion of the combustion tube (in quartz) by shifting the equilibrium of Reaction (5) towards the left [45,46].

$$C_nH_{2n+1}X + H_2O \longrightarrow C_nH_{2n+1}OH + HX \qquad \text{Reaction 4}$$

$$SiO_2 + 4\,HF \rightleftharpoons SiF_4 + 2H_2O \qquad \text{Reaction 5}$$

The total amount of introduced water depends on the combustion time and of the selected flow rate. Accurate water addition using a dosing control unit eliminates the need to add phosphate to serve as an internal standard to correct volume variations between experiments in combustion IC methods [37]. The water inlet flow rate is a parameter to be optimized because increasing it also increases the volume of the absorption solution, causing dilution of the trapped halides. In order to evaluated this parameter, 50 mg of activated carbon filed with 50 µL of a mixture of 4-chloro-, 4-bromo-, and 4-iodo-phenol, each one at 50 µg $Cl.L^{-1}$ were analyzed by C-IC. Two flow rates of water inlet were tested: 0.1, and 0.2 $mL.min^{-1}$ (maximum flow rate allowed). The following absorption and combustion conditions were applied: 2 mL of aqueous sodium thiosulfate at 500 ppm as absorbing solution and 5 min as post-combustion time. Experiments were conducted in triplicate. Results are summarized in Figure 4.

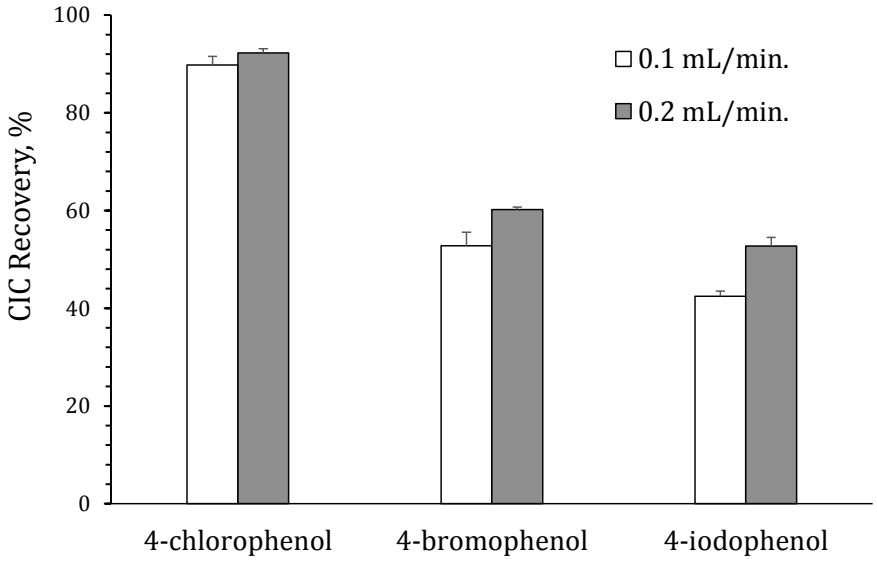


Figure 4. Effect of water inlet flow rate on the recovery of halide ions. Pyrohydrolysis and absorption conditions using: 5 min of post-combustion time, and 2 mL of aqueous sodium thiosulfate solution at 500 ppm. Determination by C-IC (error bars are the standard deviation; n = 3)

Unlike chloride ions, which were recovered at more than 90%, bromide and iodide ions were recovered at less than 60%, even at a water flow rate of 0.2 mL.min^{-1}. As can be seen on Figure 4, increase of the water flow rate from 0.1 to 0.2 mL exhibits considerable influence on the recovery yields of bromide and iodide ions (10 to 20% increase), whereas it has only a weak effect on chloride ions (3% increase). It can be concluded that improvement of the combustion process prevails over the dilution caused by the increased flow rate in the case of iodide and bromide ions, while both phenomena are balanced concerning chloride ions (final volume of the absorption solution 6 mL vs 7mL). Therefore, 0.2 ml.min^{-1} was selected as the optimum water inlet flow rate for further experiments.

Post-combustion time. In order to ensure an optimal burning of the activated carbon, a post-combustion time must be applied in addition to the combustion time automatically determined. In order to evaluate this parameter, 50 mg of activated carbon filed with 50 µL of a mixture of 4-chloro-. 4-bromo- and 4-iodo-phenol, each one at 50 µg Cl.L^{-1} were analyzed by C-IC. Three post-combustion times were investigated: 5, 7, and 10 min. Experiments were conducted in triplicate using 2 mL of an absorbing aqueous solution of sodium thiosulfate at 500 ppm with a water inlet flow rate of 0.2 mL.min^{-1}.Results are summarized in Figure 5.

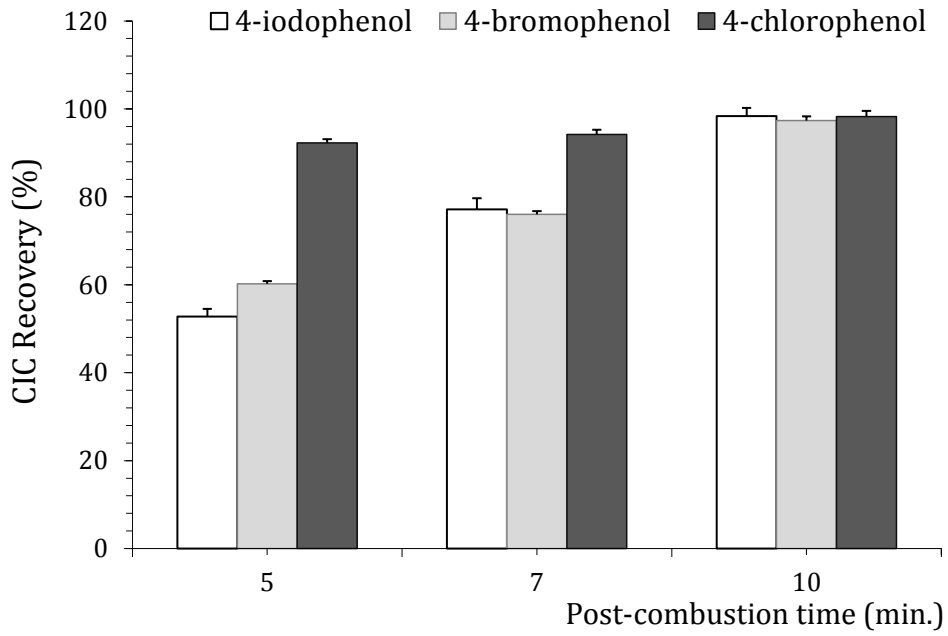


Figure 5. Influence of post-combustion time on the combustion efficiency. Pyrohydrolysis and absorption conditions were respectively 0.2 mL.min^{-1} as flow rate of water inlet, and 2 mL of aqueous sodium thiosulfate solution at 500 ppm. Determination by C-IC (error bars are the standard deviation; n = 3).

As shown in Figure 5, no significant effect of post-combustion time on recovery yields of chlorides was observed, whereas bromide and iodide ions recoveries increase significantly with the post-combustion time. The best recoveries (> 97%) were achieved with a 10 min post-combustion time for the three halogens. 10 min was thus adopted as the optimum post-combustion time and was selected for further experiments. It is important to point out that recovery improvement might not be directly related to an improvement of the burning process due to an increase of activated carbon residence time in the combustion furnace. In fact, increasing the post-combustion time automatically increases both the amounts of water introduced in the pyrohydrolysis unit and the time of combustion gases into the absorbing solution. These two parameters may act concomitantly. In addition, no memory effects were revealed, showing that the total combustion time was sufficient to achieve complete transfer of combustion gases into the absorption solution.

3.3. C-IC performances

The developed method was validated according to the French NF T90-210 standard, already described in our study dedicated to monochloramine determination [47]. This normative document provides statistical tools for the initial evaluation of quantitative methods in the field of water physico-chemical analysis. The validation parameters were the response function, limit of quantification and correctness. Acceptance criteria were first defined and tests were conducted to check the validity of the method using these pre-determined criteria.

3.3.1 Response functions

Ion chromatography was calibrated by injecting a decreasing volume of halides in aqueous mixture, each one at 120 µg $Cl.L^{-1}$. For the determination of the response function, five calibration curves, each plotted with six concentration levels (120, 60, 24, 12, 6, and 3 µg $Cl.L^{-1}$), were produced over five different days. The calibration curves were obtained by plotting the ratio of the peak area of each halide against the concentration. In order to obtain the best calibration curves, several mathematical functions, with and without weighting (1/x, $1/x^2$) were applied and compared. The best relationship between concentration and response was obtained with the quadratic model. All halides showed determination coefficients (r^2) higher than 0.9998, and RSD less than 5%. Table 2 lists calibration and validation data for the analytical method.

Table 2. Calibration and validation data for the analytical proposed method

Halogen	Concentration range (µg $Cl.L^{-1}$)	R^2	LOQs (µg $Cl.L^{-1}$)	Accuracy (%)		
				25 µg $Cl.L^{-1}$	50 µg $Cl.L^{-1}$	75 µg $Cl.L^{-1}$
Cl (AOCl)	3-120	0.9998	0.9	< 7.6	< 5.0	< 2.9
Br (AOBr)	3-120	0.9998	4.3	< 8.8	< 5.2	< 3.4
I (AOI)	3-120	0.9998	5.7	< 4.3	< 3.7	< 0.3

Note: % Accuracy = % (trueness & precision), calibration was examined by injecting halides in aqueous mixture in the IC system, correctness was evaluated by analyzing water samples spiked with 4-chlorophenol, 4-bromophenol and, 4-iodophenol by C-IC after extraction on activated carbon

3.3.2 Limits of quantification

First, a presupposed limit of quantification (LOQ) has been estimated for each halide as the concentration corresponding to a signal-to-noise ratio equal to 10. Next, standard mixtures containing all the halides at the presupposed LOQs were prepared over five different days, and analyzed by ion chromatography. In the absence of any regulatory requirements, a maximum acceptable deviation of 60% was fixed, as recommended by standard NF T90-210. The experimental LOQs for chloride, bromide and iodide were 0.9, 4.3, and 5.7 µg $Cl.L^{-1}$, respectively while those reported in the literature were in the range of 3.0-9.0, 2.7-7.1, and 1.6-8.2 µg $Cl.L^{-1}$ for AOCl, AOBr, and AOI, respectively [7,11,19,48-49].

3.3.3 Method accuracy

Ideally, correctness (precision and trueness) should be verified using samples with a certified reference value and a maximum acceptable deviation based on regulatory requirements, official standards or even specifications provided by a client or laboratory itself. Since no certified reference materials were

available for AOCl, AOBr and AOI in water samples, the C-IC procedure accuracy was evaluated by analyzing water samples spiked with 4-chloro-, 4-bromo- and, 4-iodo-phenol, as model compounds of AOCl, AOBr, and AOI, respectively. These solutions were considered as real samples and therefore treated according to the procedure described in Section 2.2. The accuracy was assayed over five separate days at three different concentration levels for each OXBP model: 25, 50, and 75 µg $Cl.L^{-1}$. On each day of analysis, each water sample was measured twice. The maximum acceptable deviation was fixed at ± 10 %. The correctness of the method has been proven since the results were within the chosen acceptance limit (see Table 2).

3.3.4 Recovery of OXBP compounds using the C-IC detection approach

The developed method was applied to recovery studies of OXBPs according to the methodology described in Section 3.1.3. The determined recoveries are shown in Figure 2. Mean recoveries were within a range of 36.6-98.5% for halomethanes (HMs and THMs), 44.6-104.5% for HAAs, 56.7-104.9 for HKs, 91.6-99.3% for HANs, 90.0-96.9 for HAcAms, 93.1-97.3 for halopropanes, 99.1-100.5 for HPs, 93.4% for bromonitromethane, and 95.4 for tribromoacetaldehyde. The RSD values were below 6% for all the compounds, indicating a good reproducibility of measurements. Compared with existing methods, better results were obtained in this work [7,42,50]. The same hypotheses made in paragraph 3.1.3 may be considered to explain the differences between doping and measured concentrations. In a general way, the recovery yields from combustion-microcoulometric titration and C-IC were comparable. However, there were some exceptions. The developed C-IC method provides substantially improved yields for CIM (96.5% vs. 69%), CAcAm (90.0% vs. 4.4%), DCAN (96.1% vs. 34.7%), BAcAm (96.5% vs. 6.8%), IAcAm (94.9% vs. 32.4%), MCAA (44.6% vs. 28.3%), TBAA (85.6% vs. 60.5%), CAN (92.9% vs. 7.4%), DCAN (97.4% vs. 66.6%), BAN (92.6 vs. 19.4%), IAN (99.3% vs. 54.4%), BNM (93.4% vs. 55.9%), and chloroketone (56.7% vs. 34.1%). Assuming that the main difference between both approaches concerns the combustion gases trapping step, the improvement in recoveries seems to indicate formation of inorganic halogen-containing species (e.g., HOX, X_2, and $^{-}OX_3$) during the combustion phase, which are undetectable by microcoulometric titration. In the case of the C-IC method, these species are converted to halides in the absorption solution before being measured by ion chromatography.

3.4. Application to real river water samples

In order to test the applicability of the C-IC method to real samples, monochloramine-treated and untreated water samples from three different sites were analyzed and the results were compared to those obtained with the AOX standard method. These samples were collected between July and October 2016 in three rivers in France: Meuse, Moselle, and Loire. The characteristics of water samples are presented in Table 1 (Supporting information 2). Table 3 shows the results of AOCl,

AOBr, AOI, and AOX determination. The AOX values derived from the C-IC (AOX_{C-IC}) approach were obtained by summing the amount of measured AOCl, AOBr, and AOI.

In order to test the applicability of the C-IC method to real samples, halogen-based biocide treated and untreated water samples from three various rivers in France were analyzed and the results were compared to those obtained with the AOX standard method. Table 3 shows the results of AOCl, AOBr, AOI, and AOX determination. The AOX values derived from the C-IC (AOX_{C-IC}) approach were obtained by summing the amount of measured AOCl, AOBr, and AOI.

The measurements conducted in the untreated water samples revealed AOX concentrations above the LOQ value of 10 µg $Cl.L^{-1}$ in all samples. The AOX content of the river water samples decreases in the following order: River 1 (mean: 17.4 ± 1.5 µg $Cl.L^{-1}$, n = 6) ≃ River 2 (mean: 17.9 ± 0.6 µg $Cl.L^{-1}$, n = 6) < River 3 (mean: 13.2 ± 1.5 µg $Cl.L^{-1}$, n = 4), traducing the occurrence of significant levels of organohalogen compounds in the studied river waters. These "unknown compounds" could originate from discharges resulting from anthropogenic activities, as well as biogenic emissions. Despite the significant difference in the physic-chemical properties of the sampled rivers, similar ranges of AOX were obtained.

The AOX concentrations in treated water samples were found to be higher due to the formation of organohalogen byproducts resulting from the reaction of the halogen-based biocide with organic materials. Overall, the AOX values obtained by microcoulometric titration and those determined by ion chromatography (sum of AOCl, AOBr, and AOI) were in the same range, varying from 26.3 to 58.5 µg $Cl.L^{-1}$, and from 29.3 to 58.7 µg $Cl.L^{-1}$, respectively. The relationship between these two approaches was examined and the results are shown in the Figure 4 (Supporting Information 3). A regression slope of 0.98 and a correlation coefficient greater than 0.97 indicate a good agreement between the two methods, offering additional support to the accuracy of the developed method. The slope value, close to 1 suggests that the measured OXBPs in real water samples behave in the same way with both methods. This is consistent with literature data according which a significant portion of AOX can be attributed to high molecular weight-OXBPs [51-52].

As can be seen in Table 3, AOCl accounts for a major portion of AOX values in all the analyzed water samples, followed by AOBr, whereas AOI concentrations are below LOQ. On weight concentration basis, AOCl accounted for 81.2 to 89.7% of the AOX in the treated water samples taken from Loire River, for 90.9 to 97.7% in the treated water samples taken from Meuse River, and for 83.9 to 95.1% in the water samples taken from Moselle River. The AOCl-to-AOX ratios in this study are comparable with the values reported by others researchers [49-50]. The low proportion of AOBr compared to AOCl can be explained by both low bromide ions concentrations in the studied river water samples and by a low reactivity of monochloramine. In principle, monochloramine is a weak oxidant that oxidizes iodide to hypoiodous acid (HOI) which can then react with natural organic matter to form

iodinated by-products [1]. The absence of AOI can be related to a low level of iodide ions in treated river water samples.

Table 3. Results of AOCl, AOBr, AOI, and AOX determination

River	Sampling date	Sampling location	Halogen-specific AOX. µg eq $Cl.L^{-1}$ (n = 3)			AOX. µg eq $Cl.L^{-1}$ (n = 3)	
			AOCl	AOBr	AOI	IC detection	Microcoulometric detection
1	04/08/2016	untreated	15.0 ± 0.7	4.5 ± 0.3	< LOD	19.5 ± 2.1	18.4 ± 0.7
		treated	51.6 ± 0.2	6.1 ± 2.1	< LOD	57.7 ± 1.1	54.0 ± 1.1
	18/08/2016	untreated	14.2 ± 0.5	6.5 ± 0.1	< LOD	20.7 ± 0.9	18.3 ± 0.6
		treated	51.2 ± 1.1	5.0 ± 0.8	< LOD	56.2 ± 4.2	56.3 ± 0.7
	01/09/2016	untreated	15.0 ± 1.7	< LOD	< LOD	15.0 ± 1.7	18.3 ± 0.6
		treated	51.4 ± 5.5	< LOQ	< LOD	51.4 ± 5.5	56.9 ± 0.5
	15/09/2016	untreated	15.1 ± 1.1	4.3 ± 0.9	< LOD	19.4 ± 5.4	17.2 ± 0.6
		treated	52.6 ± 1.8	5.2 ± 0.3	< LOD	57.8 ± 5.3	55.6 ± 2.5
	22/09/2016	untreated	13.5 ± 0.3	4.3 ± 0.3	< LOD	17.8 ± 1.6	17.6 ± 1.3
		treated	50.8 ± 1.4	< LOQ	< LOD	50.8 ± 1.4	52.0 ± 3.2
	06/10/2016	untreated	15.1 ± 1.1	4.3 ± 0.9	< LOD	19.4 ± 5.4	17.1 ± 0.5
		untreated	22.6 ± 1.8	5.2 ± 0.3	< LOD	27.8 ± 2.9	21.9 ± 2.9
2	01/08/2016	untreated	10.5 ± 1.1	6.3 ± 0.3	< LOD	16.8 ± 2.6	16.0 ± 0.7
		treated	49.1 ± 0.5	9.4 ± 1.9	< LOD	58.5 ± 1.0	56.1 ± 1.2
	08/08/2016	untreated	14.6 ± 1.4	6.0 ± 1.1	< LOD	20.6 ± 4.7	17.0 ± 0.1
		treated	47.3 ± 1.2	7.1 ± 0.5	< LOD	54.4 ± 5.2	56.2 ± 1.0
	22/08/2016	untreated	13.1 ± 0.8	6.8 ± 0.1	< LOD	19.9 ± 1.5	17.5 ± 1.9
		treated	48.7 ± 2.5	4.3 ± 0.3	< LOD	53.0 ± 6.4	58.7 ± 1.2
	29/08/2016	untreated	11.5 ± 1.6	4.3 ± 0.5	< LOD	15.8 ± 4.0	16.7 ± 0.7
		treated	49.7 ± 1.1	4.6 ± 0.6	< LOD	54.3 ± 4.6	55.2 ± 0.7
	05/09/2016	untreated	8.5 ± 1.7	4.3 ± 0.3	< LOD	12.8 ± 3.4	19.6 ± 0.6
		treated	47.3 ± 2.1	6.9 ± 0.7	< LOD	54.2 ± 3.6	52.0 ± 1.9
	12/09/2016	untreated	13.1 ± 0.3	< LOQ	< LOD	13.1 ± 0.3	17.9 ± 1.0
		treated	50.2 ± 3.2	< LOQ	< LOD	50.2 ± 3.2	55.3 ± 0.5
3	27/07/2016	untreated	8.9 ± 1.6	< LOQ	< LOD	8.9 ± 1.6	11.9 ± 0.7
		treated	31.4 ± 1.0	< LOQ	< LOD	31.4 ± 1.0	33.0 ± 0.8
	10/08/2016	untreated	11.9 ± 0.3	4.3 ± 0.6	< LOD	16.2 ± 2.7	12.5 ± 0.2
		treated	31.2 ± 0.2	7.2 ± 0.4	< LOD	38.4 ± 2.4	33.8 ± 0.9
	24/08/2016	untreated	9.0 ± 0.9	< LOQ	< LOD	9.0 ± 0.9	15.4 ± 0.8
		treated	26.3 ± 0.5	< LOQ	< LOD	26.3 ± 0.5	29.3 ± 0.8
	07/09/2016	untreated	13.1 ± 0.3	< LOQ	< LOD	13.1 ± 0.3	13.2 ± 0.9
		untreated	19.7 ± 4.0	< LOQ	< LOD	19.7 ± 4.0	15.2 ± 0.5

4. CONCLUSION

AOX measurements pose a great challenge, mainly because of the interferences due to halide ions. A precise and accurate method for AOX determination is needed since very important decisions are taken on the basis of such measurements. One of the key steps of the analytical procedure of AOX concerns washing of the activated carbon with an acidic sodium nitrate solution. The tests conducted in this work showed that the washing protocol of the ISO 9562 standard method might produce false positive AOX when chloride concentrations exceed 100 ppm. For example, a concentration of 400 ppm of chloride ions may overestimate the AOX amounts by 13 to 27 µg $Cl.L^{-1}$ approximately. The improvements conducted on the washing procedure have permitted to efficiently remove chlorides bias even when they reach 1000 ppm, without increasing either the volume of the nitrate solution nor its concentration. In the second part of this work, an analytical method for chemical speciation of AOX in river water samples into adsorbable organic chlorine (AOCl), bromine (AOBr) and iodine (AOI) has been developed and validated. Under the optimized conditions, the results of the method validation were satisfactory. The best relationship between concentration and response was obtained by the quadratic model. For all halides, the method showed determination coefficients (r^2) higher than 0.9998, and RSD less than 5%. The experimental LOQs for chloride, bromide and iodide were 0.9, 4.3, and 5.7 µg $Cl.L^{-1}$, respectively. Recovery experiments using ultrapure water spiked with OXBP standards, as AOX model compounds, showed that the C-IC method provides recovery values lower than 100% but better than those obtained with the conventional AOX method. These recoveries should be taken into account for an accurate assessment of the known part of AOX. However, the microcoulometric titration and C-IC methods provide similar results on real river water samples.

References

[1] A. Kinani, S. Kinani, B.Richard, M. Lorthioy, S. Bouchonnet. Formation and determination of organohalogen by-products in water - Part I. Discussing the parameters influencing the formation of organohalogen by-products and the relevance of estimating their concentration using the AOX (adsorbable organic halide) method. Trends in Analytical Chemistry, 58 (2016) 273-280.

[2] M.J. Plewa, J.E. Simmons, S.D. Richardson, E.D. Wagner. Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. Environmental and Molecular Mutagenesis 51 (2010) 871-878.

[3] L. Zhang, L. Xu, Q. Zeng, S.-H. Zhang, H. Xie, A.-L. Liu, W.-Q. Lu. Comparison of DNA damage in human-derived hepatoma line (HepG2) exposed to the fifteen drinking water disinfection byproducts using the single cell gel electrophoresis assay. Mutation Research 741 (2012) 89-94.

[4] Y. Yang, Y. Komaki, S.Y. Kimura, H.-Y. Hu, E.D. Wagner, B.J. Marin, M.J. Plewa. Toxic Impact of Bromide and Iodide on Drinking Water Disinfected with Chlorine or Chloramines. Environmental Science & Technology 48 (2014) 12362-12369.

[5] P.A. Neale, A. Antony, M.E. Bartkow, M.J. Farré, A. Heitz, I. Kristiana, J.Y.M. Tang, B.I. Escher. Bioanalytical assessment of the formation of disinfection byproducts in a drinking water treatment plant. Environ. Sci. Technol. 46 (2012) 10317-10325.

[6] J. Tan, S. Allard, Y. Gruchlik, S. McDonald, C. A. Joll, A. Heit. Impact of bromide on halogen incorporation into organic moieties in chlorinated drinking water treatment and distribution systems. Science of the Total Environment 541 (2016) 1572-1580.

[7] J. Oleksy-Frenzel, S. Wischnack, M. Jekel. Application of ion-chromatography for the determination of the organic-group parameters AOCl, AOBr and AOI in water. Fresenius J Anal Chem. 366 (2000) 89-94.

[8] Y. Miyake, M. Kato, K. Urano. A method for measuring semi- and non-volatile organic halogens by combustion ion chromatography. Journal of Chromatography A 1139 (2007) 63-69.

[9] J.S.F. Pereira, C.M. Moreira, C.N. Albers, O.S. Jacobsen, E.M.M. Flores. Determination of total organic halogen (TOX) in humic acids after microwave-induced combustion. Chemosphere 83 (2011) 281-286.

[10] C.J. Pontius, G. Kraeger, R. Lasky. Determination of total halogen content in halogen-free fluxes by inductively coupled plasma and some limitations of ion chromatography. Proceedings of SMTA International (2015) 349-353.

[11] Y. Pan, X. Zhang. Total organic iodine measurement: A new approach with UPLC/ESI-MS for off-line iodide separation/detection. Water research 47 (2013) 163-172.

[12] X. Zhang, R.A. Minear, S.E. Barrett. Characterization of high molecular weight disinfection byproducts from chlorination of humic substances with/without coagulation using UF – SEC – ESI-MS/MS. Environ. Sci. Technol., 39 (2005) 963-972.

[13] G. Ding, X Zhang. A picture of polar iodinated disinfection byproducts in drinking water by (UPLC/)ESI-tqMS. Environ. Sci. Technol. 43 (2009) 9287-9293.

[14] Y. Pan, W. Li, H. An, H. Cui, Y. Wang. Formation and occurrence of new polar iodinated disinfection byproducts in drinking water. Chemosphere 144 (2016) 2312-2320.

[15] X. Wang, J. Wang, Y. Zhang, Q. Shi, H. Zhang,Y. Zhang, M. Yang. Characterization of unknown iodinated disinfection byproducts during chlorination/chloramination using ultrahigh resolution mass spectrometry. Science of the Total Environment 554-555 (2016) 83-88.

[16] D. Stalter, L.I. Peters, E. O'Malley, J.Y.-M. Tang, M. Revalor, M.J. Farré, K. Watson, U. von Gunten, B.I. Escher. Sample Enrichment for Bioanalytical Assessment of Disinfected Drinking Water: Concentrating the Polar, the Volatiles, and the Unknowns. Environ. Sci. Technol. 50 (2016) 6495-6505.

[17] H. Kußmaul, H. Simon, M. Sietz. Impact of halide ions on the determination of adsorbable organically bound halogens (AOX). Analytical 1 bioanalytical Chemistry 330 (1988) 532–532.

[18] C. Grøn, H.P. Dybdahl. Determination of total organic halogens (TOX); Bias from a non-halogenated organic compound. Environment International, 22 (1996) 325-329.

[19] G. Hua, D.A. Reckhow. Determination of TOCl, TOBr and TOI in drinking water by pyrolysis and off-line ion chromatography. Anal Bioanal Chem. 384 (2006) 495-504.

[20] J. Sullivan, M. Douek. Method and sample-related problems in the determination of AOX in effluents. Tappi journal 79 (1996)145-154.

[21] H. Moehseri, C.C. Lee, C.T. Kagel, H. Tuchfeld. Interferences of the total organic halogen method by inorganic chloride. 419 (1989) 419-429.

[22] J.G. Douglas, H.D. Meznarich, J.R. Olsen, G.A. Ross, M. Stauffer. Investigation of the Total Organic Halogen Analytical Method at the Waste Sampling and Characterization Facility. (Technical Report, DOI: 10.2172/940020).

[23] J.M. Symons. Technical note: Interference of Br-, BrO3-, and ClO3- with DOX determination. Journal of American Water Works Association (1995).

[24] K.A. Schwehr, P.H. Santschi. Sensitive determination of iodine species, including organo-iodine, for freshwater and seawater samples using high performance liquid chromatography and spectrophotometric detection. Analytica Chimica Acta 482 (2003) 59-71.

[25] M. Flury and A. Papritz. Bromide in the Natural Environment: Occurrence and Toxicity. Journal of Environmental Quality 22 (1993) 747-758.

[26] Gilbert A. Modèle thermo-halin de la retenue de Mirgenbach à Cettenom. Hydroécol. Appl. (1990) 37-53.

[27] W. Kloppmann, A. Bourhane, S. Schomburgk. Salinisation des masses d'eaux en France métropolitaine et dans l'Outre-mer. BRGM-ONEMA (2010).

[28] D.C. Whitehead. The Distribution and Transformations of Iodine in the Environment. Environment International 10 (1984) 321-339.

[29] R. Fuge, C.C. Johnson. The geochemistry of iodine, a review. Environmental Geochemistry and Health 8 (1986) 31-54.

[30] S.D. Richardson, F. Fasano, J.J. Ellington, F.G. Crumley, K.M. Buettner, J.J. Evans, B.C. Blount, L.K. Silva, T.J. Waite, G.W. Luther, A.B. Mckague, R.J. Miltner, E.D. Wagner, M.J. Plewa. Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. Environ. Sci. Technol. 42 (2008) 8330-8338.

[31] C.M.M. Bougeard, E.H. Goslan, B. Jefferson, S.A. Parsons. Comparison of the disinfection by-product formation potential of treated waters exposed to chlorine and monochloramine. Water Research 44 (2010) 729-740.

[32] D.A. Reckhow, G. Hua, J.K. Reckhow, P.G. Hatcher, S.A.L. Caccamise, R. Sachdeva. Characterization of Total Organic Halogen Produced During Disinfection Processes. American Water Works Association (2007).

[33] J. Sullivan, M. Douek. Method and Sample-Related Problems in the Determination of AOX in Effluents, 1994 Environmental Conference Proceedings.

[34] Y.-C. Ma, C.-Y. Chiang. Evaluation of the effects of various gas chromatographic parameters on haloacetic acids disinfection by-products analysis. Journal of Chromatography A 1076 (2005) 216-219.

[35] J.A. Sweetman, E.A. Boettner. Detection of brominated and chlorinated organics by a gas chromatographic microcoulometric detector Effect of pyrolysis tube conditions. Journal of Chromatography 112 (1981) 115-120.

[36] Y. Li, X. Zhang, C. Shang, S.W. Krasner. Evaluation and improvement of total organic bromine analysis with respect to reductive property of activated carbon. Water Research, 45 (2011) 1229–1237.

[37] W.H. Chu, N.Y. Gao, D.Q. Yin, S.W. Krasner, M.R. Templeton. Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadruple mass spectrometry with atmospheric pressure chemical ionization. Journal of Chromatography A 1235 (2012) 178-181.

[38] D. Liew, K.L. Linge, C.A. Joll, A. Heitz A, J.W. Charrois. Determination of halonitromethanes and haloacetamides: an evaluation of sample preservation and analyte stability in drinking water. Journal of Chromatography A 1241 (2012) 117-122.

[39] J. Weiss, D. Jensen. Modern stationary phases for ion chromatography. Analytical and bioanalytical chemistry. 375 (2003) 81-98.

[40] K. Liu, W.-P. Pan, J.T. Riley. A study of chlorine behavior in a simulated fluidized bed combustion system. Fuel 79 (2000) 1115–1124.

[41] M. Ni, H. Xiao, Y. Chi, J. Yan, A. Buekens, Y. Jin, S. Lu. Combustion and inorganic bromine emission of waste printed circuit boards in a high temperature furnace. Waste Management 32 (2012) 568–574.

42] Y. Miyake, M. Kato, K. Urano. A method for measuring semi- and non-volatile organic halogens by combustion ion chromatography. Journal of Chromatography A 1139 (2007) 63-69.

[43] T. Taflik, F.A. Duarte, É.L.M. Flores, F.G. Antes, J.N.G. Paniz, É.M.M. Flores, V.L. Dressler. Determination of Bromine, Fluorine and Iodine in Mineral Supplements using Pyrohydrolysis for Sample Preparation. J. Braz. Chem. Soc., 23 (2012) 488-495.

[44] Y. Noguchi, L. Zhang, T. Maruta, T. Yamane, N. Kiba. Simultaneous determination of fluorine, chlorine and bromine in cement with ion chromatography after pyrolysis. Analytica Chimica Acta 640 (2009) 106-109.

[45] A. Wagner, B. Raue, H.-J. Brauch, E. Worch, F.T. Lange. Determination of adsorbable organic fluorine from aqueous environmental samples by adsorption to polystyrene-divinylbenzene based activated carbon and combustion ion chromatography. Journal of Chromatography A, 1295 (2013) 82-89.

[46] B. Peng, D. Wu. Study on bromine release from bituminous coal during combustion. Fuel 157 (2015) 82-86.

[47] S. Kinani. S. Layousse. B. Richard. A. Kinani. S. Bouchonnet. A. Thomas. F. Sacher. Selective and trace determination of monochloramine in river water by chemical derivatization and liquid chromatography/tandem mass spectrometry analysis. Talanta 140 (2015) 189-197.

[48] I. Kristiana, H. Gallard, C. Joll, J.-P. Croué, The formation of halogen-specific TOX from chlorination and chloramination of natural organic matter isolates. Water research, 43 (2009) 4177-4186.

[49] I. Kristiana, S. McDonald, J. Tan, C. Joll, A. Heitz, Analysis of halogen-specific TOX revisited: Method improvement and application. Talanta 139 (2015) 104-110.

[50] G. Brandt, A. Kettrup. Determination of organic group parameters (AOCl, AOBr, AOs) in water by means of ion-chromatographic detection. Fresenius' Zeitschrift für analytische Chemie 327(1987) 213-219.

[51] X. Zhang. R. A. Minear. Formation, adsorption and separation of high molecular weight disinfection byproducts resulting from chlorination of aquatic humic substances. Water Research 40 (2006) 221-230.

[52] X. Zhang, R.A. Minear, S.E. Barrett. Characterization of High Molecular Weight Disinfection Byproducts from Chlorination of Humic Substances with/without Coagulation Pretreatment Using UF−SEC−ESI-MS/MS. Environmental Science & Technology 39 (2005) 963–972.

Supplementary Materials (SM)

SM 1. *Name, sources, purities and physico-chemical properties of studied compounds*

Group	Compound	Purity (%. w/w)	Source	M.W[a] (g/Mol)	B. P[b] (°C)	S[c] (g/L)	Log Kow
Trihalomethanes THMs	Chloroform	99	Sigma-Aldrich (Saint-Quentin Fallavier. France)	119.4	61	8.1	1.97
	Bromodichloromethane	98		163.8	90	3.6	2.00
	Dibromochloromethane	98		208.3	120	1.3	2.16
	Bromoform	99		252.7	149	3.1	2.40
	Iodoform	99		393.7	218	0.1	3.03
Halomethanes	Chloroiodomethane	97	Santa Cruz Biotechnology (Heidelberg. Germany)	176.4	108	1.5	
	Dichloroiodomethane	95		210.8	128	0.7	
	Bromoiodomethane	97	Sigma-Aldrich	220.8	139	0.7	
Haloacetamides HAcAms	Chloroacetamide	98	Sigma-Aldrich	93.5	112	345	-0.58
	Dichloroacetamide	97	Merck (Fontenay-sous-Bois. France)	128.0	234.3	64.5	-0.09
	Trichloroacetamide	99	Sigma-Aldrich	162.4	238	13	0.83
	Bromoacetamide	99		138.0	271.6	237	-0.49
	Tribromoacetamide	96	Santa Cruz Biotechnology	295.8	263.0	1.5	1.10
	Iodoacetamide	99	Sigma-Aldrich	185	297.1	75.6	0.04
Haloacetic acids HAAs	Monochloroacetic acid	99	Sigma-Aldrich	94.50	189	858	0.22
	Dichloroacetic acid	99		128.94	194	1000	0.92
	Trichloroacetic acid	99		163.39	196	44	1.33
	Monobromoacetic acid	99		138.95	208	93.8	0.41
	Dibromoacetic acid	97		217.84	129	22.1	0.70
	Tribromoacetic acid	99		296.74	245	1.1	
	Bromodichloroacetic acid	99		207.80	215	4.8	0.61
	Dibromochloroacetic acid	99		252.25	233	2.3	1.22
	Iodoacetic acid	98		185.9	208	24.3	
	Diiodoacetic acid	90	Santa Cruz Biotechnology	311.8	320	1.3	
Haloacetonitriles HANs	Chloroacetonitrile	99	Sigma-Aldrich	75.50	126.5	29.8	0.11
	Dichloroacetonitrile	98		109.94	112.5	33.5	0.29
	Trichloroacetonitrile	98		144.39	85.7	0.71	1.21
	Bromoacetonitrile	97		119.95	154.5	37	0.20
	Dibromoacetonitrile	90		198.84	163.1	9.6	0.47
	Iodoacetonitrile	98		166.95	183	10.4	
Halonitromethanes HNMs	Bromonitromethane	90	Sigma-Aldrich	139.94	147.5	18.7	0.31
	Bromochloronitromethane	87	Santa Cruz Biotechnology	174.38	132.7	9.2	
	Bromodichloronitromethane	92		208.83	115.5	1.0	
Haloketones HKs	1.1-Dichloropropanone	98.2	Sigma-Aldrich	126.96	117	0.003	
	1.3-Dichloropropanone	> 95		126.97	173	55.2	
	1.1.3-Trichloropropanone	86		161.41	89	n.r.	
	1.1.1-Trichloropropanone			161.41	149	7.4	
	1.1.3.3-Tetrachloropropanone	92	Santa Cruz Biotechnology	195.86	191	3.1	
Halophenols HP	4-chlorophenol	99	Sigma-Aldrich	128.55	220	24	
	4-bromophenol	99		173.01	236	14	
	4-iodophenol	99		220.01	287	9.8	
	2.4.6-tribromophenol	99		330.80	244	0.06	
Haloacetaldehyde	Tribromoacetaldehyde	97		280.74	174	n.r	

Abbreviations:-[a]M.W: Molecular Weight, [b]B.P: Boiling Point, [c]WS: Water Solubility at 25°C, [d]Log Kow: Log octanol-water partition coefficient, [e]NR: Data not reported

SM 2. *Water quality characteristics of the river waters included in this study*

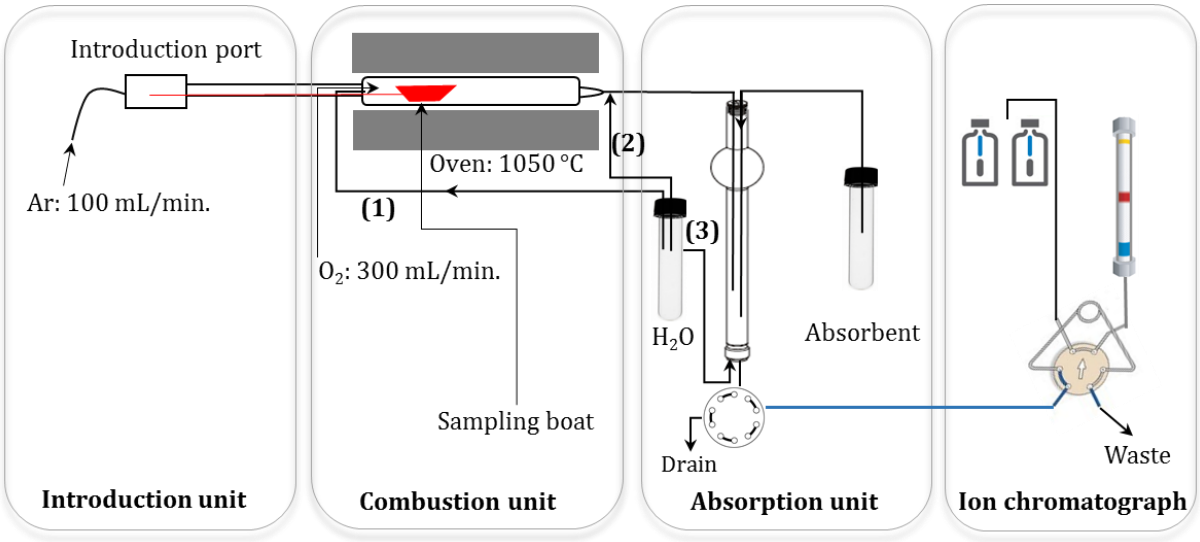
River	Sampling date	Sample	DOC (< 0.45 µg ; mg $C.L^{-1}$)	A_{254} nm (cuve 4 cm)	Cl^- ($mg.L^{-1}$)	Br^- ($mg.L^{-1}$)	I^- ($mg.L^{-1}$)
1	04/08/2016	Untreated	4.7	0.646	12.4	< 0.1	< 1
		Treated	6.6	0.807	-	-	-
	18/08/2016	Untreated	2.7	0.247	15.6	< 0.1	< 1
		Treated	3.1	0.373	-	-	-
	01/09/2016	Untreated	2.3	0.243	17.9	< 0.1	< 1
		Treated	3.2	0.398	-	-	-
	15/09/2016	Untreated	2.4	0.262	19	< 0.1	< 1
		Treated	3.6	0.372	-	-	-
	22/09/2016	Untreated	2.2	0.278	19.5	< 0.1	< 1
		Treated	3.2	0.464	-	-	-
	06/10/2016	Untreated	2.2	0.219	20.4	< 0.1	< 1
		Treated	2.6	0.320	-	-	-
2	01/08/2016	Untreated	3.9	0.313	329	0.3	< 1
		Treated	5.2	0.667	-	-	-
	08/08/2016	Untreated	4.1	0.315	393	0.4	< 1
		Treated	5.0	0.615	-	-	-
	22/08/2016	Untreated	3.6	0.297	354	0.4	< 1
		Treated	5.2	0.636	-	-	-
	29/08/2016	Untreated	3.6	0.300	374	0.4	< 1
		Treated	5.1	0.584	-	-	-
	05/09/2016	Untreated	3.6	0.307	329	0.4	< 1
		Treated	5.3	0.596	-	-	-
	12/09/2016	Untreated	3.7	0.552	618	0.7	< 1
		Treated	5.2	0.306	-	-	-
3	27/07/2016	Untreated	4.6	0.279	17.8	< 0.1	< 1
		Treated	3.5	0.383	-	-	-
	10/08/2016	Untreated	2.7	0.292	18.6	< 0.1	< 1
		Treated	3.3	0.365	-	-	-
	24/08/2016	Untreated	2.6	0.296	18.3	< 0.1	< 1
		Treated	3.3	0.386	-	-	-
	07/09/2016	Untreated	2.5	0.286	20.5	< 0.1	< 1
		Treated	3.3	0.401	-	-	-

Nomenclature: A_{254} nm: Absorbance at 254 nm, DOC: Dissolved organic carbon

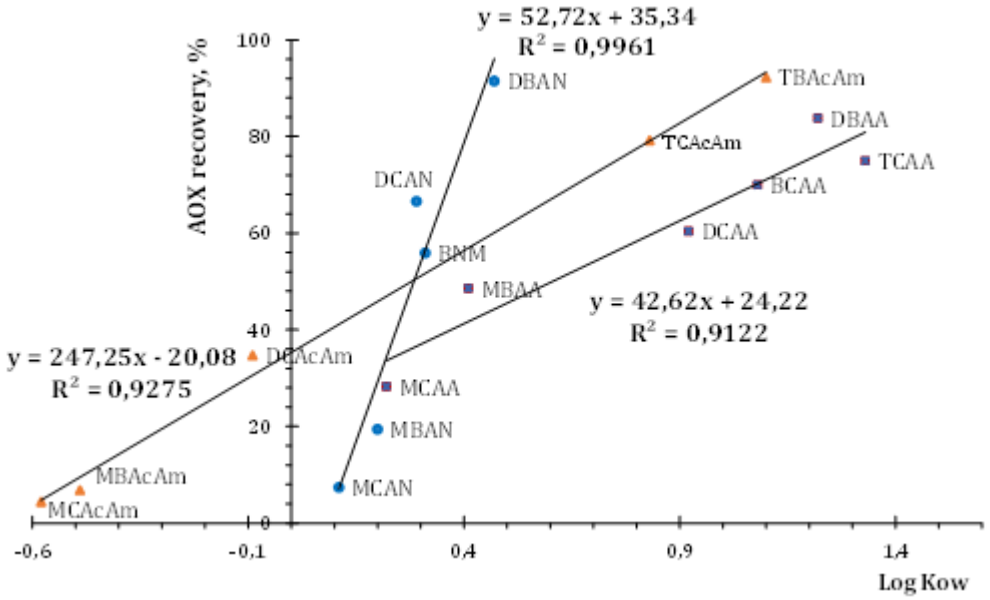
SM 3. *Advantages and drawbacks of the four absorbent solutions tested*

Absorption solution	Advantages	Drawbacks
Hydrogen peroxide	Recovery yield higher than 85% for chloride and bromide ions	Recovery yield less than 10% for iodide ion
Sodium sulfite	Recovery yield higher than 90% for chloride and bromide ions	- Decomposition of sulfite ion to sulfate ion - Recovery yield lower than 60% for iodide ion
Sodium sulfide	- Recovery yield higher than 95% for chloride and bromide ions - Recovery yield higher than 90% for iodide ion	Presence of a disruptive peak for chloride ion
Sodium thiosulfate	- Recovery yield higher than 95% for chloride and bromide ions - Recovery yield higher than 95% for iodide ion	

SM 4. *Schematic illustration of the C-IC system*

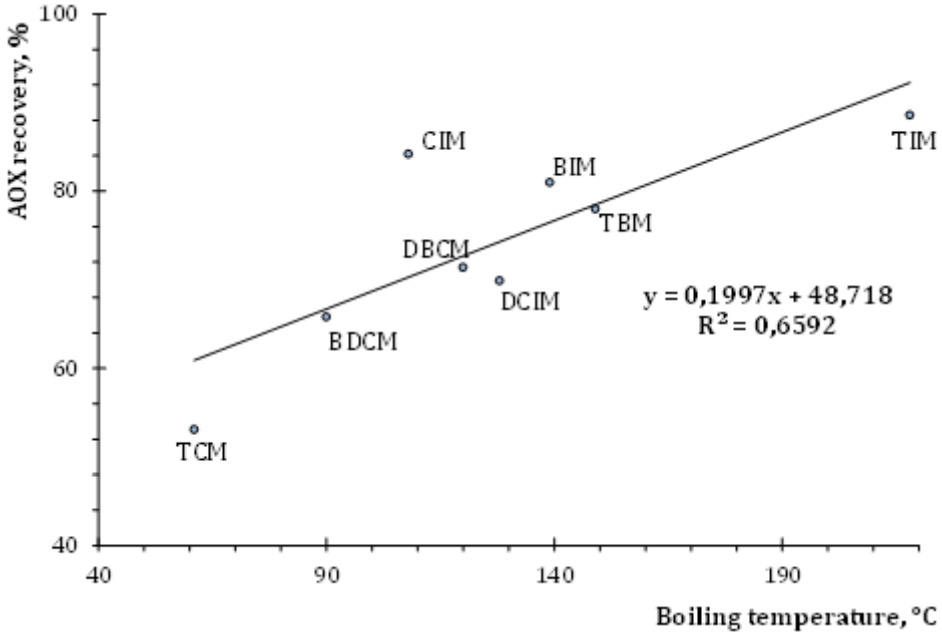


SM 5. *Relationship between recovery yields obtained using the AOX standard method and the hydrophobicity of some OXBPs.*



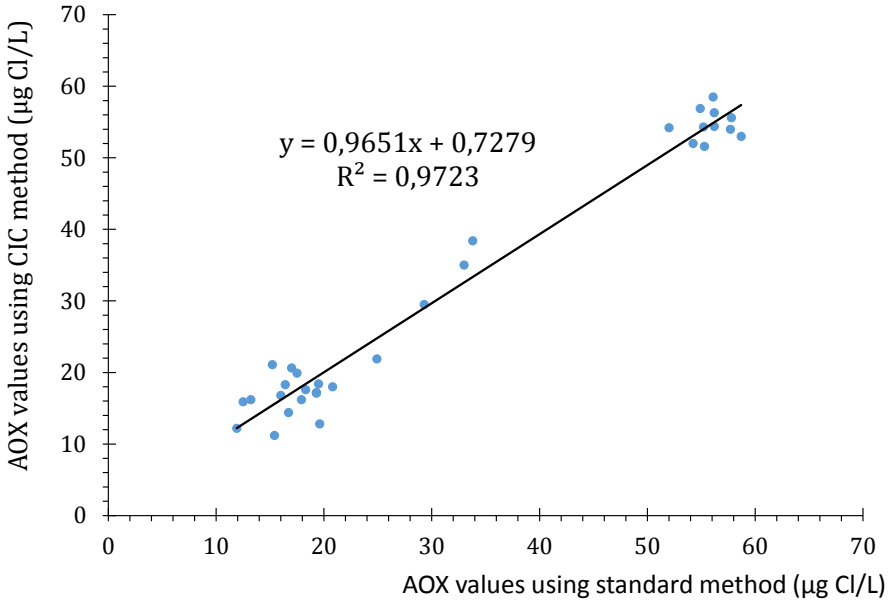
Abbreviations: MCAA: Monochloroacetic acid, MBAA: Monobromoacetic acid, DCAA: Dichloroacetic acid, BCAA: Bromochloroacetic acid, DBAA: Dibromoacetic acid, TCAA: Trichloroacetic acid, MCAcAm: Monochloroacetamide, MBAcAm: Monobromoacetamide, DCAcAm: Dichloroacetamide, TCAcAm: Trichloroacetamide, TBAcAm: Tribromoacetamide, MCAN: Monochloroacetonitrile, MBAN: Monobromoacetonitrile, BNM: Bromonitromethane, DCAN: Dichloroacetonitrile, and DBAN: Dibromoacetonitrile.

SM 6. *Relationship between the recovery yields obtained using the AOX standard method and the volatility of halomethanes.*



Abbreviations: TCM: Chloroform, BDCM: Bromodichloromethane, DBCM: Dibromochloromethane, DCIM: Dichloroiodomethane, CIM, Chloroiodomethane, BIM: Bromoiodomethane, TBM: Bromoform, and TIM: Iodoform

SM 7. *Relationship between measured AOX (standard method) and calculated AOX (C-IC method)*



IV- Développement d'une méthode d'identification des SPOX polaires par une approche couplant marquage chimique et analyse par LC-MS (screening non ciblé)

Une nouvelle approche de profilage chimique non ciblé a été développée dans le but d'identifier les SPOX polaires inconnus. Celle-ci repose sur une méthodologie couplant une dérivation précolonne des SPOX polaires suivie d'une analyse des produits de dérivation par LC-MS.

1. Optimisation des conditions analytiques

1.1 Détermination des conditions chromatographiques optimales

La phase mobile joue un rôle essentiel en LC-MS. En plus de son importance pour la séparation des composés, elle influe également sur les intensités des signaux observés. La phase mobile retenue est constituée d'un mélange binaire A-B (A : eau ultra pure avec 0,1% d'acide formique, B : méthanol (MeOH) avec 0,1% d'acide formique), délivré à un débit constant de 0,2 mL/min. Le gradient de solvants d'élution est présenté dans le tableau III-1. 10 µL d'échantillon sont injectés dans le système chromatographique.

Tableau III-1. Gradient de solvants d'élution utilisé pour les analyses par LC-MS

t (min)	A (%)	B (%)
0	60	40
10	20	80
30	60	40

1.2 Optimisation des conditions de détection par spectrométrie de masse

Les principaux paramètres de détection par spectrométrie de masse résultent d'un compromis après optimisation pour chacune des molécules modèles étudiées. La tension entre l'aiguille de nébulisation et la contre électrode est fixée à 3,0 kV. Les conditions optimisées sont les suivantes : les débits des gaz de nébulisation et de désolvatation (azote) sont respectivement de 70 et 700 L/h. Les températures de la source et de l'azote de désolvatation sont respectivement de 100 °C et 400 °C. La tension de cône est fixée à 20 V. De l'argon ultra pur (99,999%) est introduit dans la cellule de collision à un débit de 0,28 mL/min, à une pression de 3.10^{-3} mbar. Une énergie de collision de 2 eV est appliquée pour conserver les ions moléculaires protonés lors de leur traversée de la cellule. La calibration du Q-TOF est effectuée quotidiennement avec de l'acide orthophosphorique à 0,01% dans un mélange ACN:H_2O

(1:1, v/v). Les clusters de cet acide permettent de calibrer l'analyseur sur une gamme de 97 à 1762 Th. L'acquisition est effectuée simultanément en modes *full scan* et MS/MS.

2. Protocole de dérivation chimique

Les extraits SPE (*versus* standards des SPOX modèles) sont concentrés sous vide à 30 °C au moyen d'un évaporateur rotatif jusqu'à obtention d'un volume de 1 mL environ. Ils sont ensuite séchés à température ambiante sous flux d'azote. Le résidu est repris par 1 mL d'acétonitrile. 10 µL de cette solution sont transférés dans un tube contenant 200 µL de la solution de dérivatisation fraîchement préparée. Le mélange réactionnel est ensuite incubé pendant 1 heure à 60 °C. Le mélange réactionnel est refroidi rapidement dans un bain de glace, séché sous flux d'azote puis repris avec 100 µL de phase mobile à t = 0 (voir ci-dessus).

3. Optimisation des conditions opératoires de la réaction de dérivatisation

Les conditions opératoires de la réaction de dérivatisation ont été étudiées avec pour objectif d'optimiser le rendement et réduire le temps nécessaire à la préparation des échantillons.

3.1 Choix de l'agent de marquage chimique

Le choix d'un agent de dérivatisation adéquat est capital. Ce choix repose principalement sur la réactivité des groupes fonctionnels des SPOX ciblés, des performances chromatographiques à l'issue de la dérivation et de la capacité à s'ioniser de l'agent de dérivatisation [15]. L'estérification et l'amidation sont deux réactions de dérivatisation courantes pour les acides carboxyliques. Par exemple, la 2-picolylamine (PA) et la 2-hydrazinopyridine (HP) ont été utilisées pour améliorer l'efficacité d'ionisation d'acides carboxyliques faiblement ionisés dans des échantillons biologiques en formant respectivement des dérivés amide et acylhydrazide [16]. Le 2-chloro-1-méthylpyridinium, la triéthylamine et l'heptadécafluoroundécylamine constituent d'autres réactifs de dérivatisation pour les acides carboxyliques [17-19]. Pour les aldéhydes et les cétones, les réactions avec l'hydroxylamine et les hydrazines, comme la 2,4 dinitrophénylhydrazine (DNPH) et la dansyl hydrazine, ont été largement utilisées pour produire des dérivés plus stables que les composés parents [20-22]. Le bromure de (4-hydrazino-4-oxobutyl)tris (2,4,6-triméthoxyphényl) phosphonium, le réactif de Girard P et le réactif de Girard T constituent d'autres agents de dérivatisation des aldéhydes et cétones [23-25].

Nous avons testé et comparé trois agents de dérivatisation chimique parmi les plus fréquemment utilisés pour l'analyse par chromatographie liquide. Il s'agit de la 2-hydrazinoquinoline (HQ), la picolylamine (PA) et le phénylhydrazine (PH, analogue de la DNPH). Pour ce faire, neuf SPOX

modèles appartenant à différentes familles chimiques ont été sélectionnés : 4-chlorophénol, 4-aminobenzophénone, acide chloropropionique, acide dichloroacétique, acide 2,3-dibromobutyrique, 4-chlorobenzaldéhyde, 1,1-dichloroacétone, 1,1,1-trichloroacétone et monoiodoacétamide. La capacité des trois agents de dérivatisation chimique à marquer les SPOX modèles a été étudiée en procédant à l'analyse d'une solution contenant les composés d'intérêt, incubés pendant 1 heure à 60 °C. Un mélange composé du triphénylphosphine (TPP) et du 2,2'-Dithiodipyridine (DTPD) a été ajouté à l'ensemble des solutions de dérivation afin de pouvoir dériver les fonctions acide carboxyliques, comme illustré figure III-1. La dérivatisation a été réalisée conformément au protocole général décrit ci-dessus. Le tableau III-2 ci-après reprend de façon synthétique les résultats obtenus.

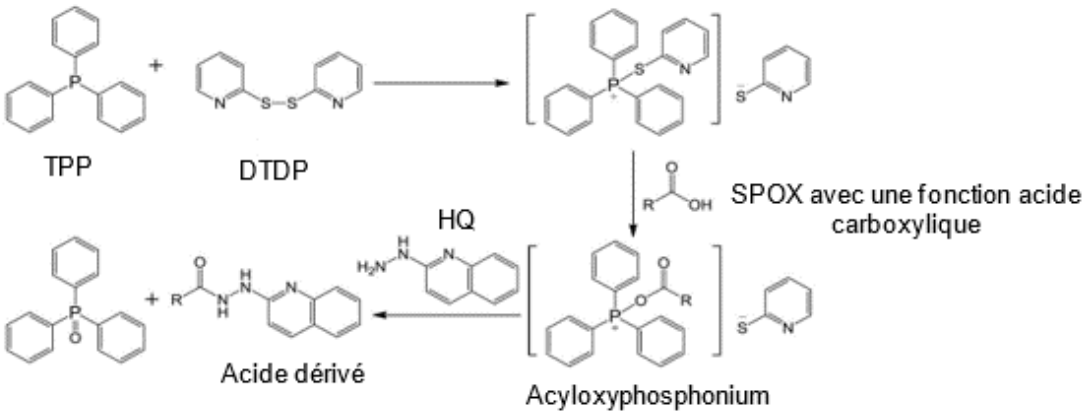


Figure III-1. Dérivatisation d'un acide carboxylique par le HQ, la TPP et le DTDP

Tableau III-2. Résultats (appréciation qualitative) des tests de dérivation menés sur des échantillons d'eau dopés avec différents composés organohalogénés

SPOX testés	Composition de la solution de dérivatisation		
	HQ, TPP, DTDP	PA, TPP, DTDP	PH, TPP, DTDP
4-chlorophénol	+	+	+
4-aminobenzophénone	+	+	+
Acide chloropropionique	++	+	+
4-chlorobenzaldéhyde	++	+	+
1,1-dichloroacétone	++	+	+
1,1,1-trichloroacétone	++	+	+
Acide dichloroacétique	++	+	+
Monoiodoacétamide	++	+	+
Acide 2,3-dibromobutyrique	++	+	+

Abréviations : nd : non détecté, (+) pic présent en faible intensité, (++) pic présent en forte intensité, HQ : 2-hydrazinoquinoline, PA : picolylamine, PH : phénylhydrazine, TPP : triphénylphosphine, DTDP : 2,2'-Dithiodipyridine.

Comme le montre le tableau III-2, les meilleurs résultats ont été obtenus avec la 2-hydrazinoquinoline qui est donc retenue pour la suite des expérimentations.

3.2 Optimisation des conditions de la réaction de dérivatisation par la HQ

Nous avons étudié l'influence de la température, du temps d'incubation et du solvant sur le rendement de la dérivatisation.

3.2.1 Sovant de dérivation

Sachant que l'ajout de solvants polaires et/ou basiques peut modifier le rendement de la réaction de dérivatisation, deux solvants ont été testés : l'acétonitrile et l'isopropanol. Les meilleures performances ont été observées avec l'acétonitrile. De plus, l'acétonitrile permet d'accroître la solubilité des composés les plus apolaires et éviter leur adsorption sur le flaconnage en verre. Ainsi ce solvant a été retenu pour la suite de nos expériences.

3.2.2 Durée de la réaction de dérivatisation

Quatre temps de dérivation ont été testés : 15, 30, 60 et 75 min. La température a été fixée à 60 °C. Comme le montre la figure III-2, le rendement de la réaction de dérivation atteint un plateau vers 60 min. Ce temps de réaction a été retenu pour la suite des expériences.

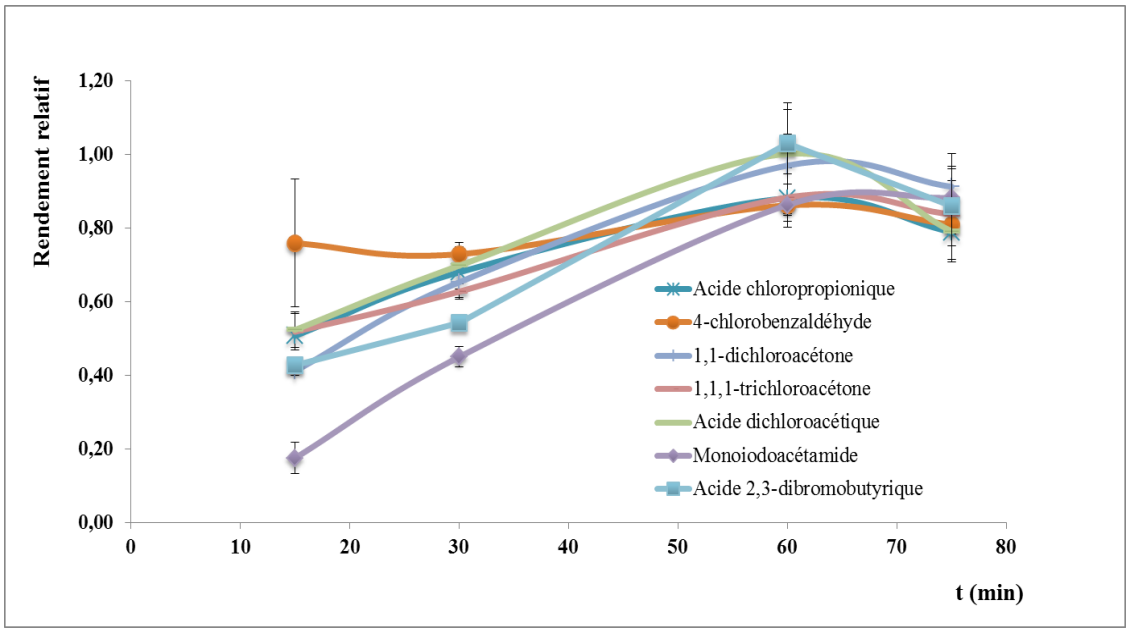


Figure III-2. Effet du temps sur le rendement de la réaction de dérivation par HQ. Les valeurs expérimentales sont exprimées en rendement relatif moyen ± écart-type (n =3).

3.2.3 Température de réaction

Après avoir optimisé le temps de réaction, nous avons testé quatre valeurs de température : 20, 40, 60 et 80 °C. Les résultats obtenus sont illustrés par la figure III-3. La variation des réponses observées souligne l'influence significative de la température sur le rendement de la réaction de dérivatisation. Sur les quatre températures de dérivatisation testées, un rendement de dérivatisation optimal a été observé à 60 °C pour les sept SPOX modèles testés (figure III-3). Cette température de réaction a été choisie pour la suite des expériences.

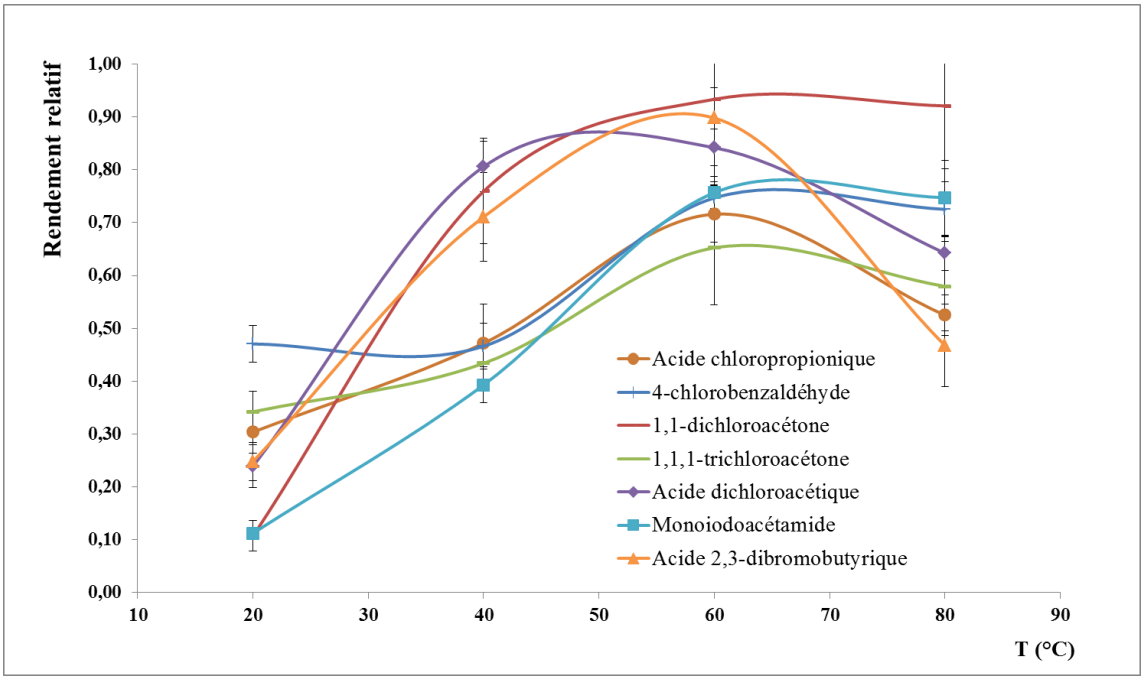


Figure III-3. Effet de la température sur la réaction de dérivation par HQ. Les valeurs expérimentales sont exprimées en rendement relatif moyen ± écart-type (n =3).

3.2.4 Concentration en agent de dérivation chimique

Un excès de réactif de marquage est une condition à priori favorable pour augmenter la cinétique et le rendement de la réaction. Etant donné que la concentration en SPOX dans les échantillons réels est inconnue, trois solutions de dérivation à base de HQ dans l'acétonitrile ont été préparées à des concentrations de 2, 20 et 200 mg/L.

Références

[1] T. Santa, Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry, Biomedical Chromatography, 25 (2011) 1-10.

[2] T. Higashi, T. Ichikawa, S. Inagaki, J.Z. Min, T. Fukushima, T. Toyo'oka, Simple and practical derivatization procedure for enhanced detection of carboxylic acids in liquid chromatography–electrospray ionization-tandem mass spectrometry, Journal of pharmaceutical and biomedical analysis, 52 (2010) 809-818.

[3] A.J. Cartwright, P. Jones, J.C. Wolff, E.H. Evans, Derivatisation of carboxylic acid groups in pharmaceuticals for enhanced detection using liquid chromatography with electrospray ionisation tandem mass spectrometry, Rapid communications in mass spectrometry, 19 (2005) 1058-1062.

[4] S.J. Barry, R.M. Carr, S.J. Lane, W.J. Leavens, S. Monte, I. Waterhouse, Derivatisation for liquid chromatography/electrospray mass spectrometry: synthesis of pyridinium compounds and their amine and carboxylic acid derivatives, Rapid communications in mass spectrometry, 17 (2003) 603-620.

[5] T. Hayama, Y. Sakaguchi, H. Yoshida, M. Itoyama, K. Todoroki, M. Yamaguchi, H. Nohta, Fluorous derivatization combined with liquid chromatography/tandem mass spectrometry: a method for the selective and sensitive determination of sialic acids in biological samples, Rapid Communications in Mass Spectrometry, 24 (2010) 2868-2874.

[6] M.S. Rashed, L.Y. Al-Ahaidib, O.Y. Al-Dirbashi, M. Al Amoudi, M.M. Al-Sayed, Z. Rahbeeni, Z. Al-Hassnan, A. Al-Dbaas, M. Al-Owain, M.N. Luanaigh, Tandem mass spectrometric assay of succinylacetone in urine for the diagnosis of hepatorenal tyrosinemia, Analytical biochemistry, 339 (2005) 310-317.

[7] A. Sakuragawa, T. Yoneno, K. Inoue, T. Okutani, Trace analysis of carbonyl compounds by liquid chromatography–mass spectrometry after collection as 2, 4-dinitrophenylhydrazine derivatives, Journal of Chromatography A, 844 (1999) 403-408.

[8] H.L. Lord, J. Rosenfeld, V. Volovich, D. Kumbhare, B. Parkinson, Determination of malondialdehyde in human plasma by fully automated solid phase analytical derivatization, Journal of Chromatography B, 877 (2009) 1292-1298.

[9] S.J. Barry, R.M. Carr, S.J. Lane, W.J. Leavens, C.O. Manning, S. Monté, I. Waterhouse, Use of S-pentafluorophenyl tris (2, 4, 6-trimethoxyphenyl) phosphonium acetate bromide and (4-hydrazino-4-oxobutyl)[tris (2, 4, 6-trimethoxyphenyl) phosphonium bromide for the derivatization of alcohols, aldehydes and ketones for detection by liquid chromatography/electrospray mass spectrometry, Rapid communications in mass spectrometry, 17 (2003) 484-497.

[10] C.C. Lai, C.H. Tsai, F.J. Tsai, C.C. Lee, W.D. Lin, Rapid monitoring assay of congenital adrenal hyperplasia with microbore high-performance liquid chromatography/electrospray ionization tandem mass spectrometry from dried blood spots, Rapid Communications in Mass Spectrometry, 15 (2001) 2145-2151.

[11] H. Hong, Y. Wang, Derivatization with Girard reagent T combined with LC-MS/MS for the sensitive detection of 5-formyl-2'-deoxyuridine in cellular DNA, Analytical chemistry, 79 (2007) 322-326.

4. Extraction et enrichissement des SPOX par extraction sur phase solide

L'extraction et l'enrichissement des SPOX est une étape clé de notre processus d'analyse global pour :

- l'analyse des composés ciblés (approche ciblée),
- la spéciation des SPOX par poids moléculaire,
- l'identification des composés inconnus (approche non ciblée) ou,
- la mise en œuvre de l'approche EDA.

Cette étape est cependant difficile à optimiser, notamment lorsque de nombreux composés sont recherchés simultanément. De par sa capacité à extraire des composés en un temps relativement court, l'absence de filtration après extraction, la faible consommation de solvant, la capacité à être automatisée et les facteurs de concentration élevés, l'extraction sur phase solide (SPE) s'impose comme un choix technique idéal pour l'extraction de SPOX dans les eaux de rivières.

Concernant l'approche ciblée, deux protocoles d'extraction par SPE avaient été développées : le premier pour extraire 11 AHA et le deuxième pour extraire 26 SPOX volatiles appartenant à différentes familles chimiques. Bien que les deux protocoles susmentionnés se soient montrés satisfaisants pour extraire l'ensemble des 33 SPOX ciblés, il est très difficile de présumer de leurs performances à extraire de manière non discriminante l'ensemble des SPOX, parmi lesquels d'éventuels composés inconnus et/ou émergents. Il était donc nécessaire d'étudier la capacité de la cartouche « Bakerbond SDB-1 » à retenir un large spectre de SPOX aux propriétés physico-chimiques variées. Cette évaluation était d'autant plus importante que la réussite de l'approche non ciblée et de l'approche EDA (Effects-directed analysis) en dépendait. Pour ce faire, plusieurs échantillons d'eau de rivières traités et non traités par la monochloramine ont été extraits par SPE, puis dilués par de l'eau MilliQ pour détermination du paramètre AOX. Les rendements d'extraction sont présentés dans le tableau III-3. Les teneurs en AOX mesurées ont été corrigées à partir des valeurs de blancs d'analyse mesurées.

Tableau III-3. Teneurs en AOX mesurées par microcoulométrie et rendements d'extraction SPE

Cours d'eau	Point de prélèvement	Date	AOX (µg éq. Cl/L)	SPE-AOX (µg éq. Cl/L)	Rendement %
Seine	Chatou	26/11/2015	22,6 ± 0,9	12,9 ± 1,2	57
		17/02/2016	15,9 ± 1,8	10,6 ± 0,6	67
		16/03/2016	20,4 ± 0,3	11,5 ± 1,1	56
		15/07/2016	16,8 ± 1,6	10,8 ± 0,4	64
Loire	Amont CNPE	27/07/2016	11,9 ± 0,7	< LOQ	-
	BF		33,0 ± 0,8	23,1 ± 0,6	70
	Amont CNPE	10/08/2016	12,5 ± 0,2	11,3 ± 0,3	90
	BF		33,8 ± 0,9	19,6 ± 2,2	58
	Amont CNPE	24/08/2016	15,4 ± 0,8	11,1 ± 0,1	72
	BF		29,3 ± 0,8	19,1 ± 1,4	65
	Amont CNPE	07/09/2016	13,2 ± 0,9	10,1 ± 0,1	76
	BF		15,2 ± 0,5	11,8 ± 0,5	77
Meuse	Amont CNPE	04/08/2016	18,4 ± 0,7	10,5 ± 0,6	57
	BF		54,0 ± 1,1	27,4 ± 1,0	51
	Amont CNPE	18/08/2016	18,0 ± 0,4	< LOQ	-
	BF		56,3 ± 0, 7	29,8 ± 2,4	53
	Amont CNPE	01/09/2016	18,3 ± 0,6	10,2 ± 1,1	56
	BF		56,9 ± 0,5	25,1± 1,4	44
	Amont CNPE	15/09/2016	17,2 ± 0,6	10,3 ± 0,9	59
	BF		55,6 ± 2,5	31,0 ± 0,8	56
	Amont CNPE	22/09/2016	17,6 ± 1,3	10,1 ± 0,8	57
	BF		52,0 ± 3,2	26,6 ± 1,9	51
	Amont CNPE	06/10/2016	17,1 ± 0,5	10,8 ± 1,2	63
	BF		21,9 ± 2,9	10,1 ± 0,2	46
Moselle	Amont CNPE	01/08/2016	16,0 ± 0,7	10,3 ± 0,3	64
	BF		56,1 ± 1,2	26,4 ± 2.5	47
	Amont CNPE	08/08/2016	17,0 ± 0,1	12,3 ± 1,2	72
	BF		56,2 ± 1,0	30,6 ± 2,3	54
	Amont CNPE	22/08/2016	17,5 ± 1,9	10,2 ± 0,4	58
	BF		58,7 ± 1,2	31,6 ± 0,8	54
	Amont CNPE	29/08/2016	16,7 ± 0,7	10,5 ± 0,3	63
	BF		55,2 ± 0,7	27,2 ± 0,9	49
	Amont CNPE	05/09/2016	19,6 ± 0,6	10,6 ± 0,4	54
	BF		52,0 ± 3,2	27,7 ± 0,6	53
	Amont CNPE	12/09/2016	17,9 ± 1,0	10,2 ± 0,2	57
	BF		55,3 ± 0,5	26,5 ± 2,3	48

Des rendements d'extraction satisfaisants avoisinant les 60% (en moyenne) ont été obtenus. Ces résultats viennent confirmer la capacité de la cartouche « Bakerbond SDB-1 » à extraire un large éventail de molécules aux propriétés physicochimiques différentes, ce qui constitue un des points essentiels au développement de l'approche « *screening* non ciblé » et l'approche EDA.

Partie 2

I. Synthèse des résultats des campagnes de mesures et de caractérisation des SPOX constitutifs des AOX sur les échantillons d'eau en provenance des CNPE de Saint-Laurent, Chooz et Cattenom

Rappelons que l'objectif de cette deuxième partie est de présenter et analyser l'ensemble des résultats obtenus sur des échantillons d'eaux prélevés en amont et aux bassins froids des sites d'étude au regard des objectifs fixés dans la cadre de ce travail de thèse. Il s'agit de replacer ces résultats dans le contexte plus général décrit dans la littérature, et de mettre en évidence les apports des travaux de thèse en termes d'amélioration des connaissances et des outils analytiques par rapport aux résultats antérieurs d'EDF R&D.

I.1 Analyse des composés organohalogénés adsorbables sur charbon actif (AOX)

La monochloramination des eaux, comme tout traitement à base d'oxydants halogénés, entraîne la formation de sous-produits organohalogénés, de structures chimiques très peu connues, qui peuvent être quantifiés de façon intégrative par la mesure du paramètre chimique AOX.

Les eaux de rivières renferment des AOX d'origine anthropique et/ou naturelle, en concentrations parfois non négligeables. Cette contribution doit être prise en considération dans le calcul des teneurs d'AOX formés par monochloramination. Cette contribution est d'autant plus importante que le mode de fonctionnement des CRF (recirculation de l'eau de refroidissement) conduit à un phénomène de concentration des eaux du circuit. Ainsi, dans le bassin froid, en supposant que les AOX entrant *via* l'eau d'appoint soient conservés (absence de réactions chimiques dans le circuit, ni de perte par déposition des AOX adsorbés sur les matières en suspension), les concentrations en AOX endogènes devraient se trouver augmentées proportionnellement au taux de concentration. Le taux de concentration dépend principalement de deux paramètres : le débit d'appoint et le débit d'évaporation. Par conséquent le taux de concentration variera d'un CNPE à l'autre. Il pourra également varier dans la journée selon les conditions météorologiques. Il est estimé par le facteur de concentration (Fc) selon deux approches différentes : physique et chimique. Le «Fc physique » est estimé en rapportant le débit d'appoint sur le débit de la purge. Le «Fc chimique » quant à lui, est le plus souvent calculé en rapportant la conductivité de l'eau du bassin froid à la conductivité de l'eau d'appoint. Toutefois, s'appuyer sur la conductivité pour estimer le «Fc chimique » pendant les périodes de traitement à la monochloramine pourrait conduire à une surestimation de ce paramètre. Plusieurs autres éléments chimiques « conservateurs », comme les ions calcium ou sodium, peuvent être utilisés. Etant donné que la formation des SPOX est intimement liée à la présence de matière organique dans les eaux d'appoint, nous avons fait le choix de nous référer au carbone organique dissous (COD) pour estimer le Fc « chimique ». Il est calculé à partir de la formule suivante : Fc

= COD (eau d'appoint) / COD (eau du bassin froid). Dans la suite de cette l'étude, une distinction sera faite entre :

- **les AOX endogènes, notés AOX_{AM}** ; ils sont obtenus par analyse des échantillons prélevés en amont des CNPE étudiés,
- **les AOX totaux, notés AOX_{BF},** qui comprennent les AOX endogènes et les AOX formés par monochloramination ; ils sont obtenus par analyse des échantillons prélevés en bassins froids des CNPE étudiés et,
- **les AOX formés par monochloramination**, obtenus grâce à la formule de calcul suivante : $\mathbf{AOX_{formés} = AOX_{BF} - Fc.AOX_{AM}}$

Les résultats de l'analyse des paramètres physico-chimiques (COD, halogénures, UV254 nm et AOX) réalisée sur l'ensemble des échantillons d'eau prélevés sont consignés dans le tableau III-4. Nous rappelons que les teneurs en AOX ont été exprimées en microgrammes équivalent chlorure contenu dans un litre d'eau analysé (µg éq. Cl/L) et que chaque échantillon d'eau a fait l'objet de trois analyses indépendantes (n= 3). Les concentrations brutes d'AOX ont été corrigées par soustraction des valeurs obtenues pour les blancs d'analyses.

Tableau III-4. Paramètres physico-chimiques mesurés dans les échantillons d'eau prélevés des trois CNPE étudiés.

Site d'étude	Date de prélèvement	Point de prélèvement	AOX (µg éq. Cl/L)	COD (mg C/L)	A_{254} nm (cuve 4 cm)	Cl^- (mg/L)	Br^- (mg/L)	I^- (mg/L)
St-Laurent	27/07/2016	Amont	11,9 ± 0,7	3,5	0,279	17,8	< 0,1	< 1
		BF T2	33,0 ± 0,8	4,6	0,383	-	-	-
	10/08/2016	Amont	12,5 ± 0,2	2,7	0,292	18,6	< 0,1	< 1
		BF T2	33,8 ± 0,9	3,3	0,365	-	-	-
	24/08/2016	Amont	15,4 ± 0,8	2,6	0,296	18,3	< 0,1	< 1
		BF T2	29,3 ± 0,8	3,3	0,386	-	-	-
	07/09/2016	Amont	13,2 ± 0,9	2,5	0,286	20,5	< 0,1	< 1
		BF T1	15,2 ± 0,5	3,3	0,401	-	-	-
Chooz	04/08/2016	Amont	18,4 ± 0,7	4,7	0,646	12,4	< 0,1	< 1
		BF T1	54,0 ± 1,1	6,6	0,807	-	-	-
	18/08/2016	Amont	18,0 ± 0,4	2,7	0,247	15,6	< 0,1	< 1
		BF T1	56,3 ± 0, 7	3,1	0,373	-	-	-
	01/09/2016	Amont	18,3 ± 0,6	2,3	0,243	17,9	< 0,1	< 1
		BF T1	56,9 ± 0,5	3,2	0,398	-	-	-
	15/09/2016	Amont	17,2 ± 0,6	2,4	0,262	19	< 0,1	< 1
		BF T1	55,6 ± 2,5	3,6	0,372	-	-	-
	22/09/2016	Amont	17,6 ± 1,3	2,2	0,278	19,5	< 0,1	< 1
		BF T2	52,0 ± 3,2	3,2	0,464	-	-	-
	06/10/2016	Amont	17,1 ± 0,5	2,2	0,219	20,4	< 0,1	< 1
		BF T1	21,9 ± 2,9	2,6	0,320	-	-	-
Cattenom	01/08/2016	Amont	16,0 ± 0,7	3,9	0,313	329	0,3	< 1
		BF	56,1 ± 1,2	5,2	0,667	-	-	-
	08/08/2016	Amont	17,0 ± 0,1	4,1	0,315	393	0,4	< 1
		BF	56,2 ± 1,0	5,0	0,615	-	-	-
	22/08/2016	Amont	17,5 ± 1,9	3,6	0,297	354	0,4	< 1
		BF	58,7 ± 1,2	5,2	0,636	-	-	-
	29/08/2016	Amont	16,7 ± 0,7	3,6	0,300	374	0,4	< 1
		BF	55,2 ± 0,7	5,1	0,584	-	-	-
	05/09/2016	Amont	19,6 ± 0,6	3,6	0,307	329	0,4	< 1
		BF	52,0 ± 3,2	5,3	0,596	-	-	-
	12/09/2016	Amont	17,9 ± 1,0	3,7	0,306	618	0,7	< 1
		BF	55,3 ± 0,5	5,2	0,552	-	-	-

Abréviations : A_{254} : Absorbance UV à la longueur d'onde 254 nm, BF : Bassin froid, COD : Carbone organique dissous, T : Tranche.

I. 1.1 Détermination des facteurs de concentration

Après traitement des résultats bruts, les facteurs de concentration minimaux, maximaux et moyens calculés pour chaque CNPE à partir des valeurs de COD sont reportés dans le tableau III-5. Les Fc de conception sont donnés à titre de comparaison.

Tableau III-5. Facteurs de concentration des CNPE étudiés

Site CNPE	n	$Fc_{minimal}$	$Fc_{maximal}$	Fc_{moyen}	CV%	$Fc_{conception}$
Saint-Laurent	4	1,27	1,32	**1,28**	3,5	**1,50**
Chooz	6	1,18	1,50	**1,35**	10,8	**1,65**
Cattenom	6	1,22	1,47	**1,38**	6,7	**1,60**

Abréviations *:* n : nombre de campagnes de mesure, Fc : Facteur de concentration, CV : Coefficient de variation

Les coefficients de variation permettent d'évaluer la précision des résultats. Ils montrent que les valeurs de Fc calculés sont toutes de bonne qualité métrologique puisque des valeurs inférieures à 10,8% ont été obtenues. Globalement les Fc chimiques calculés sont du même ordre de grandeur que les facteurs de concentration de conception. Ceci tend à indiquer que le COD serait bien conservé lors de son transit dans les circuits de refroidissement des sites d'étude. Les écarts observés entre les deux types de Fc pourraient être imputés aux incertitudes sur les mesures et/ou à une faible perte de COD dans les circuits.

1.2 Proportions d'AOX endogènes dans les eaux de rivières étudiées

Comme le montre le tableau III-4, l'analyse des échantillons d'eaux prélevés en amont des CNPE étudiés révèle la présence d'AOX à des teneurs comprises entre 11,9 et 19,6 µg éq. Cl/L (LOQ = 10 µg éq. Cl/L). Les teneurs moyennes mesurées varient de la manière suivante : Meuse (17,9 ± 0,6 µg éq. Cl/L ; n = 6) ≈ Moselle (17,4 ± 1,2 µg éq. Cl/L ; n = 6) > Loire (13,2 ± 1,5 µg éq. Cl/L ; n = 4). Dans l'ensemble, les quantités d'AOX apportées par chaque eau de rivière restent stables dans le temps, comme en témoignent les faibles valeurs d'écarts types obtenus.

Ces résultats suggèrent une présence de composés organohalogénés d'origine anthropique et/ou naturelle dans les eaux de rivières alimentant les CNPE étudiés. Leur contribution à la concentration d'AOX mesurée dans les eaux du bassin froid, après concentration dans le circuit de refroidissement, est très importante, comme nous allons le mettre en évidence dans le paragraphe suivant.

1.3 Proportions d'AOX formés suite à la monochloramination des eaux d'appoint

Du fait des temps de séjour hydraulique dans les circuits de refroidissement (2 à 14 heures), il est impossible de prélever un échantillon d'eau d'appoint de composition chimique et minérale qui soit strictement identique à celui qui sera prélevé en bassin froid. Pour des raisons pratiques, les prélèvements en amont et en bassins froids des CNPE ont été réalisés simultanément, sans tenir compte des temps de séjour dans le circuit. Pour estimer la quantité d'AOX octroyée à la monochloramination, nous avons considéré que l'eau du bassin froid est issue de l'eau de rivière en amont du CNPE au moment du prélèvement.

Pour tenir compte de l'effet de concentration dans le circuit de refroidissement, nous avons émis l'hypothèse selon laquelle les AOX contenus dans l'eau d'appoint seront concentrés de la même façon que le COD. Ainsi les concentrations en AOX attribués à la monochloramination peuvent être déduites en utilisant la formule suivante : $AOX_{formés} = AOX_{BF} - Fc \times AOX_{AM}$. Le tableau III-6 synthétise, pour chaque site d'étude, les teneurs moyennes maximales et minimales d'AOX formés lors de la monochloramination des eaux d'appoint. Précisons que les concentrations obtenues en dehors de la période de traitement à la monochloramine ont été écartées (cas du CNPE de Saint-Laurent et de Chooz). Les valeurs moyennes ont donc été calculées uniquement sur les valeurs obtenues pendant le traitement.

Tableau III-6. Concentrations d'AOX formés (en µg éq. Cl/L) mesurées dans les eaux du bassin froid des sites étudiés

Site CNPE	Année	n	$AOX_{minimale}$	$AOX_{maximale}$	$AOX_{moyenne}$	Ecart-type
Saint-Laurent	**2016**	**3***	**9,8**	**18,5**	**15,2**	**4,8**
	2011	*6*	*25,1*	*46,4*	*34,3*	*7,3*
Chooz	**2016**	**5***	**26,4**	**35,6**	**30,3**	**3,5**
	2011	*6*	*28,3*	*43,0*	*34,3*	*7,3*
Cattenom	**2016**	**6**	**23,1**	**35,3**	**31,4**	**4,5**
	2011	*0*	*ND*	*ND*	*ND*	*ND*

Abréviations : n : nombre de prélèvements, ND : données non disponibles car l'instauration du traitement à la monochloramine sur le CNPE de Cattenom n'est que récente (2015)

Les résultats obtenus montrent que la monochloramination des eaux d'appoint produit des quantités variables et souvent non négligeables d'AOX. Les teneurs moyennes formées dans les eaux du bassin froid varient entre 9,3 et 23,2 µg éq. Cl/L confirmant la formation de sous-produits organohalogénés. Les teneurs

les plus élevées ont été observées pour les eaux du bassin froid de Chooz (Meuse). Ces teneurs montrent que la majeure partie du chlore contenu dans la monochloramine injectée dans les circuits de refroidissement est consommée par des processus d'oxydation aboutissant la formation des chlorures (résiduel de monochloramine en sortie condenseur d'environ 250 µg éq. Cl_2/L *versus* teneurs en AOX formés comprises entre 9,3 et 23,2 µg éq. Cl/L). Seule une faible partie de la monochloramine injectée aboutit à la formation de sous-produits organohalogénés *via* des réactions de substitution.

Il est également important de rappeler qu'à taux de traitement identique, la monochloramination des eaux de rivières produit des quantités d'AOX beaucoup plus faibles comparée à la chloration. En effet, des concentrations avoisinant une cinquantaine de µg éq. Cl/L sont fréquemment mesurées dans les eaux de distribution. Les concentrations mesurées dans les eaux de piscines peuvent être jusqu'à 500 fois plus élevées que celles mesurées dans les eaux de distribution. Il convient de préciser néanmoins que la concentration en chlore résiduel total dans les eaux de distribution et de piscines dépasse très largement les valeurs maximales mesurées dans les bassins froids des sites d'étude.

Nous avons également rapporté dans le tableau III-6 les résultats d'AOX obtenus en 2011 en vue de leur comparaison avec ceux obtenus dans le cadre de ce travail de thèse. Nous précisons que les campagnes de mesures de 2016 et 2011 ont été effectuées à la même période de l'année, à savoir entre juillet et octobre. La comparaison des résultats montre que les teneurs en AOX formés mesurées en 2016 se situent dans la fourchette des valeurs basses relevées en 2011. Cependant cette comparaison souffre d'un manque de représentativité temporelle dû à la nature intrinsèque du mode d'échantillonnage ponctuel. En effet, il demeure délicat d'imputer d'une manière directe la baisse constatée aux modalités du traitement en vigueur et/ou aux conditions environnementales. Notons tout de même que les valeurs plus faibles d'AOX formés calculées pourraient au moins en partie être expliquées par les améliorations apportées à la méthode de mesure d'AOX. Précisons qu'en 2011 les analyses d'AOX avaient été réalisées par un laboratoire de routine accrédité par le COFRAC.

Sur le plan purement analytique, les résultats obtenus mettent en lumière un point crucial, à savoir que la monochloramine produit des composés organohalogénés en concentrations très faibles, de quelques dizaines de µg éq. Cl/L dans le meilleur des cas, qu'il convient nécessairement de concentrer afin de rendre possible leur caractérisation physicochimique par les méthodes physicochimiques conventionnelles.

2. Spéciation des AOX en fractions chlorés (AOCl), bromés (AOBr) et iodés (AOI)

Les résultats d'analyse des fractions chlorées, bromées et iodées d'AOX, après correction par soustraction des valeurs de blancs, sont résumés dans le tableau III-7. Les valeurs analytiques sont exprimées en microgrammes équivalent chlorures contenus dans un litre d'eau analysé (µg éq. Cl/L). A noter que la dernière campagne effectuée sur le site de Saint-Laurent et celle sur le site de Chooz représentent un « point zéro » qui correspond à une période de temps où le traitement à la monochloramine était en arrêt. Rappelons que les valeurs rapportées pour chacun des points de prélèvement sont les résultats des moyennes des concentrations obtenues en analysant trois fois le même échantillon.

Tableau III-7. Teneurs en AOCl, AOBr, AOI, et AOX obtenues par C-IC

Site d'étude	Prélevemnt	Point de prélevement	AOCl	AOBr	AOI	AOX (n = 3)	
						C-IC	Micro-coulometrie
Saint-Laurent	27/07/2016	Amont	8,9 ± 1,6	< LOQ	< LOD	8,9 ± 1,6	11,9 ± 0,7
		BF T2	31,4 ± 1,0	< LOQ	< LOD	31,4 ± 1,0	33,0 ± 0,8
	10/08/2016	Amont	11,9 ± 0,3	4,3 ± 0,6	< LOD	16,2 ± 2,7	12,5 ± 0,2
		BF T2	31,2 ± 0,2	7,2 ± 0,4	< LOD	38,4 ± 2,4	33,8 ± 0,9
	24/08/2016	Amont	9,0 ± 0,9	< LOQ	< LOD	9,0 ± 0,9	15,4 ± 0,8
		BF T2	26,3 ± 0,5	< LOQ	< LOD	26,3 ± 0,5	29,3 ± 0,8
	07/09/2016	Amont	13,1 ± 0,3	< LOQ	< LOD	13,1 ± 0,3	13,2 ± 0,9
		BF T1	19,7 ± 4,0	< LOQ	< LOD	19,7 ± 4,0	15,2 ± 0,5
Chooz	04/08/2016	Amont	15,0 ± 0,7	4,5 ± 0,3	< LOD	19,5 ± 2,1	18,4 ± 0,7
		BF T1	51,6 ± 0,2	6,1 ± 2,1	< LOD	57,7 ± 1,1	54,0 ± 1,1
	18/08/2016	Amont	14,2 ± 0,5	6,5 ± 0,1	< LOD	20,7 ± 0,9	18,3 ± 0,6
		BF T1	51,2 ± 1,1	5,0 ± 0,8	< LOD	56,2 ± 4,2	56,3 ± 0,7
	01/09/2016	Amont	15,0 ± 1,7	< LOD	< LOD	15,0 ± 1,7	18,3 ± 0,6
		BF T1	51,4 ± 5,5	< LOQ	< LOD	51,4 ± 5,5	56,9 ± 0,5
	15/09/2016	Amont	15,1 ± 1,1	4,3 ± 0,9	< LOD	19,4 ± 5,4	17,2 ± 0,6
		BF T1	52,6 ± 1,8	5,2 ± 0,3	< LOD	57,8 ± 5,3	55,6 ± 2,5
	22/09/2016	Amont	13,5 ± 0,3	4,3 ± 0,3	< LOD	17,8 ± 1,6	17,6 ± 1,3
		BF T2	50,8 ± 1,4	< LOQ	< LOD	50,8 ± 1,4	52,0 ± 3,2
	06/10/2016	Amont	15,1 ± 1,1	4,3 ± 0,9	< LOD	19,4 ± 5,4	17,1 ± 0,5
		BF T1	22,6 ± 1,8	5,2 ± 0,3	< LOD	27,8 ± 2,9	21,9 ± 2,9
Cattenom	01/08/2016	Amont	10,5 ± 1,1	6,3 ± 0,3	< LOD	16,8 ± 2,6	16,0 ± 0,7
		BF T1	49,1 ± 0,5	9,4 ± 1,9	< LOD	58,5 ± 1,0	56,1 ± 1,2
	08/08/2016	Amont	14,6 ± 1,4	6,0 ± 1,1	< LOD	20,6 ± 4,7	17,0 ± 0,1
		BF T1	47,3 ± 1,2	7,1 ± 0,5	< LOD	54,4 ± 5,2	56,2 ± 1,0
	22/08/2016	Amont	13,1 ± 0,8	6,8 ± 0,1	< LOD	19,9 ± 1,5	17,5 ± 1,9
		BF T1	48,7 ± 2,5	4,3 ± 0,3	< LOD	53,0 ± 6,4	58,7 ± 1,2
	29/08/2016	Amont	11,5 ± 1,6	4,3 ± 0,5	< LOD	15,8 ± 4,0	16,7 ± 0,7
		BF T1	49,7 ± 1,1	4,6 ± 0,6	< LOD	54,3 ± 4,6	55,2 ± 0,7
	05/09/2016	Amont	8,5 ± 1,7	4,3 ± 0,3	< LOD	12,8 ± 3,4	19,6 ± 0,6
		BF T1	47,3 ± 2,1	6,9 ± 0,7	< LOD	54,2 ± 3,6	52,0 ± 1,9
	12/09/2016	Amont	13,1 ± 0,3	< LOQ	< LOD	13,1 ± 0,3	17,9 ± 1,0
		BF T1	50,2 ± 3,2	< LOQ	< LOD	50,2 ± 3,2	55,3 ± 0,5

Abréviations et légendes : LOQ (AOCl) = 0,9 µg éq. Cl/L, LOQ (AOBr) = 4,3 µg éq. Cl/L, LOQ (AOI) = 5,7 µg éq. Cl/L.

Notons à ce stade que les concentrations mesurées sont toutes de bonne qualité métrologique puisque la totalité des écarts types obtenus sont faibles.

2.1 Fractions iodées d'AOX

En examinant les différents échantillons d'eau analysés (amont et bassin froid), il est important de relever qu'aucun d'entre eux ne présente des teneurs en AOI supérieures à la limite de détection de la méthode C-IC développée, soit 2 µg éq. Cl/L. Comme évoqué au premier chapitre de ce manuscrit, la concentration en ions halogénure d'une eau joue un rôle important dans la spéciation des sous-produits de désinfection formés. Plusieurs études avaient montré que la présence des ions iodure en fortes concentrations dans des eaux traitées à la monochloramine favorise la formation des sous-produits organoiodés. Les valeurs d'AOI inférieures à la limite de détection (LD) obtenues dans le cadre de notre étude peuvent paraître ainsi surprenantes dans une première approche compte tenu la réactivité de la monochloramine vis-à-vis des ions iodure. Toutefois, plusieurs hypothèses, intervenant individuellement ou de façon concomitante, peuvent être émises pour expliquer les résultats obtenus, parmi lesquelles nous pouvons citer :

- La teneur en ions iodure de l'eau d'appoint : certaines eaux de surface peuvent contenir des iodures à des concentrations élevées. Comme nous l'avons déjà évoqué (chapitre I), la monochloramine, au vu de son potentiel redox, peut oxyder facilement ces ions iodures en iode (I_2, HOI, IO^-). L'iode possède comme la monochloramine des propriétés oxydantes lui permettant de réagir à son tour sur la matière organique et produire des composés organiques iodés. Toutefois, ces sous-produits ne deviennent perceptibles qu'à des teneurs élevées en ions iodure. Dans notre cas, toutes les concentrations en ions iodure mesurées dans les échantillons prélevés en amont des sites d'étude se sont montrées inférieures à la limite de quantification (1 mg/L) ;
- Le taux de traitement (0,25 mg éq. Cl_2/L en sortie de condenseurs) : plus celui-ci s'élève, plus le taux de conversion des ions iodure en iode est favorisé. Nous pouvons donc nous attendre raisonnablement à ce que la quantité de sous-produits organoiodés augmente avec la dose de monochloramine injectée ;
- Le temps de séjour hydraulique (temps de contact) de l'eau dans les bassins : la réaction de formation des SPOX ne se fait pas instantanément. Les concentrations en sous-produits organiques iodés dans les eaux de bassins froids dépendront de leur vitesse et de leur rendement de formation à partir des différents précurseurs ;
- La sensibilité de la méthode de mesure : même si nous émettons l'hypothèse selon laquelle des sous-produits organiques iodés se sont réellement formés, ils ne peuvent être mis en évidence que lorsque leurs teneurs dépassent à la limite de quantification de la méthode de mesure employée.

La désinfection d'une eau de rivière contenant de faibles teneurs en iodures peut produire des composés organoiodés lorsqu'elle est associée à une forte dose de monochloramine et à un temps de contact élevé.

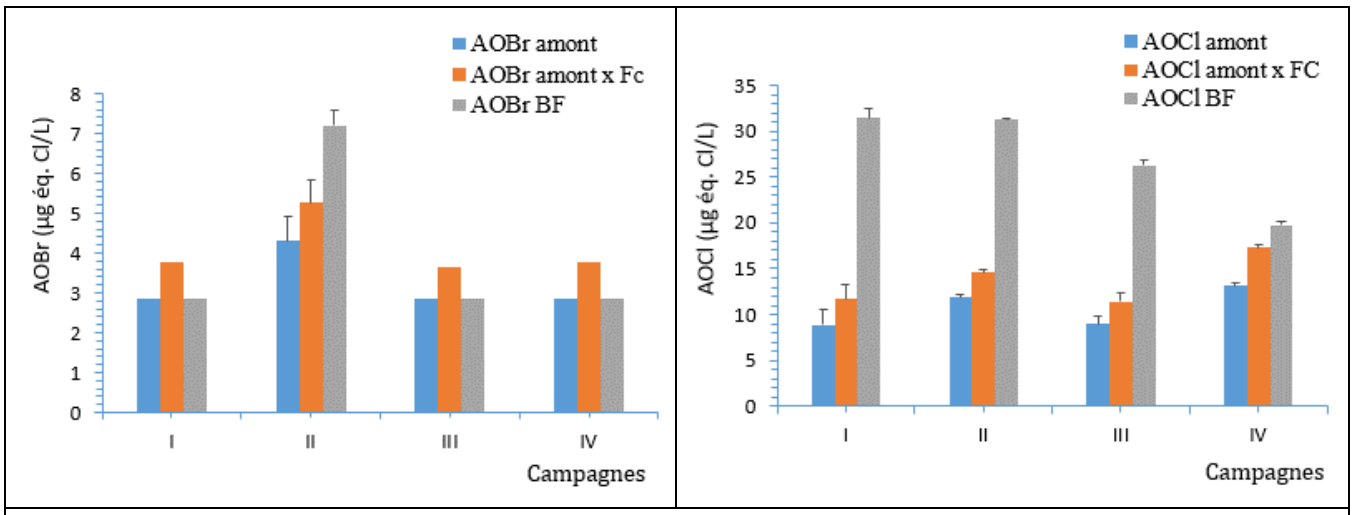
D’autres facteurs tels que la température de l’eau, sa charge organique (COD), la nature des molécules constituant son COD et son pH peuvent également influencer la formation des sous-produits organiques iodés.

2.2 Fractions bromées d’AOX

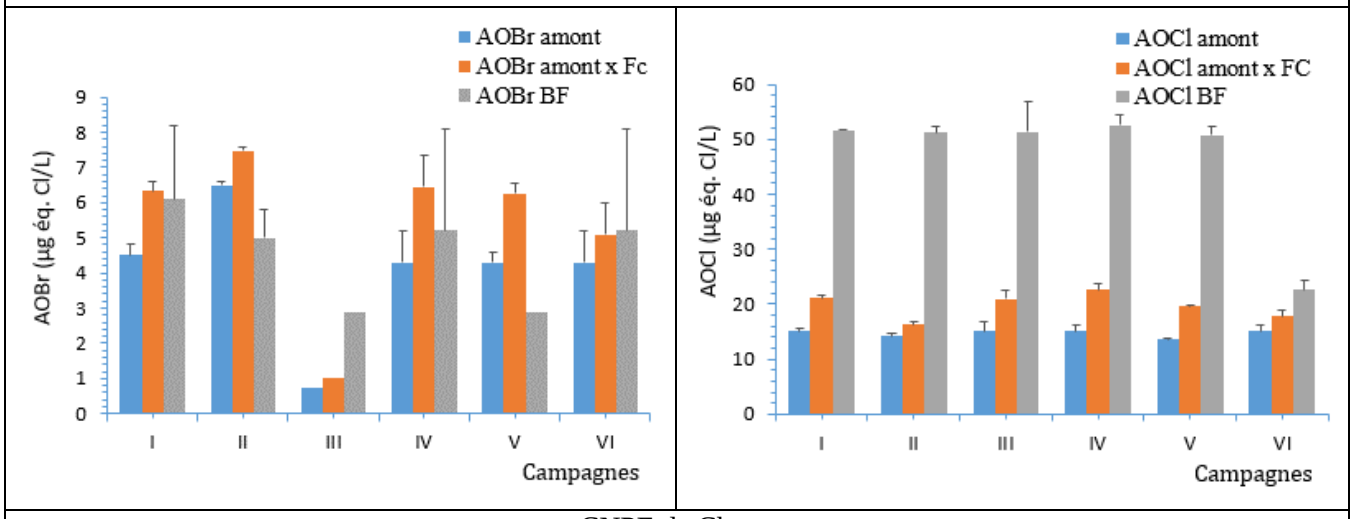
Tous les échantillons d’eau analysés contiennent des AOBr en concentrations variables et souvent supérieures à la limite de quantification (4,6 µg éq. Cl/L), ce qui indique une contribution de ces espèces aux valeurs d’AOX totaux mesurées. Les concentrations d’AOBr mesurées restent faibles et varient entre des valeurs inférieures à la limite de quantification (LQ) et 9,4 µg éq. Cl/L. Les valeurs les plus élevées ont été mesurées sur le site de Cattenom. Le phénomène de concentration des eaux dans les circuits de refroidissement n’est pas sans effet sur les teneurs en fractions chlorées et bromées d’AOX mesurées au bassin froid des sites d’étude et par conséquent sur les quantités imputées au traitement à la monochloramine. Pour évaluer le taux d’incorporation de chaque halogène dans les AOX après monochloramination, les concentrations en différentes fractions mesurées dans les eaux prélevées en amont ont été corrigées par les facteurs de concentration déterminés précédemment.

Dans le cas où les concentrations mesurées dans les échantillons prélevés aux points amont et bassin froid se révèlent inférieures à limite de détection, nous proposons de leur attribuer une valeur égale à LD/2. De la même manière, pour les échantillons dont les concentrations en AOBr sont inférieures à limite de quantification nous proposons de leur octroyer une valeur égale à (LQ + LD)/2. En appliquant ces approximations de calcul, nous minimisons raisonnablement les concentrations en AOBr endogènes et maximisons en parallèle celles d’AOBr susceptibles d’être formés par monochloramination. La figure III-4 présente les profils de concentrations en AOCl et AOBr selon le point de prélèvement et le site d’étude.

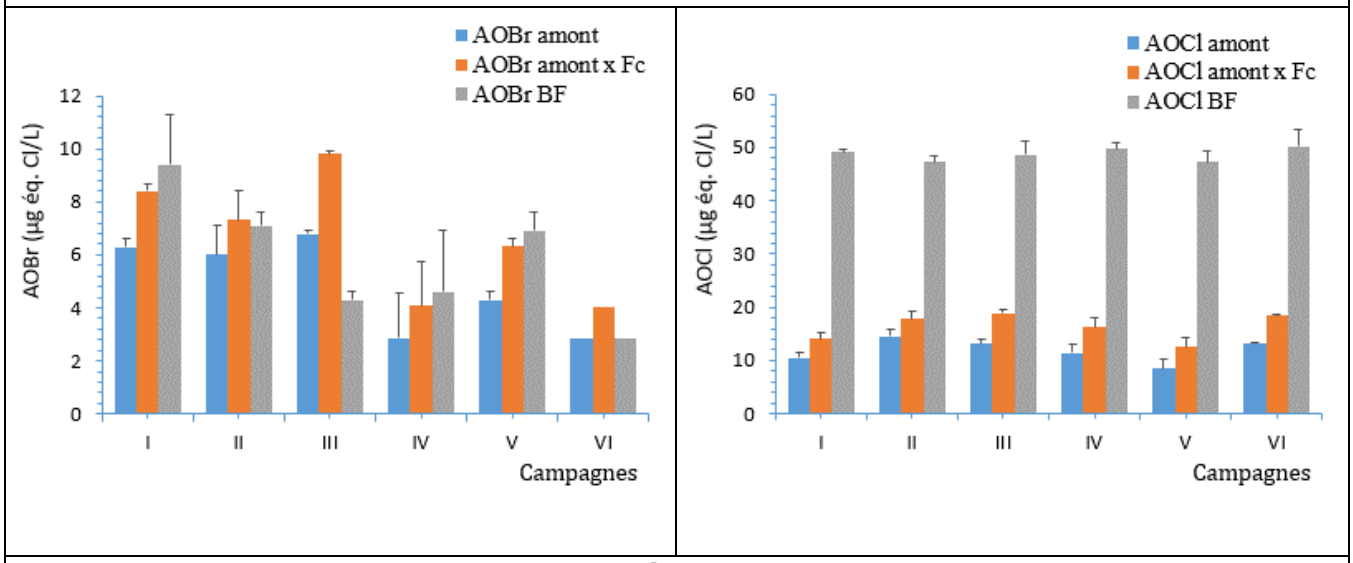
Figure III-4. Distributions des concentrations en AOCl et AOBr selon le point de prélèvement et le site d'étude



CNPE de Saint-Laurent



CNPE de Chooz



CNPE de Cattenom

L'examen des histogrammes obtenus (figure III-4) montre que les teneurs en AOBr mesurées dans les échantillons d'eau prélevés en bassins froids de Chooz et de Saint-Laurent suivent de très près celles calculées à partir des concentrations mesurées dans les eaux prélevées en amont de ces deux sites. Dans l'ensemble, les concentrations d'AOBr calculées sont supérieures ou égales à celles mesurées dans les échantillons d'eaux issus des bassins froids. Ce résultat laisse supposer que le traitement à la monochloramine tel qu'il est mis en œuvre dans les conditions de cette étude (modalités de traitement et qualité de l'eau d'appoint) ne produit pas de sous-produits organobromés en concentrations perceptibles par la méthode C-IC développée.

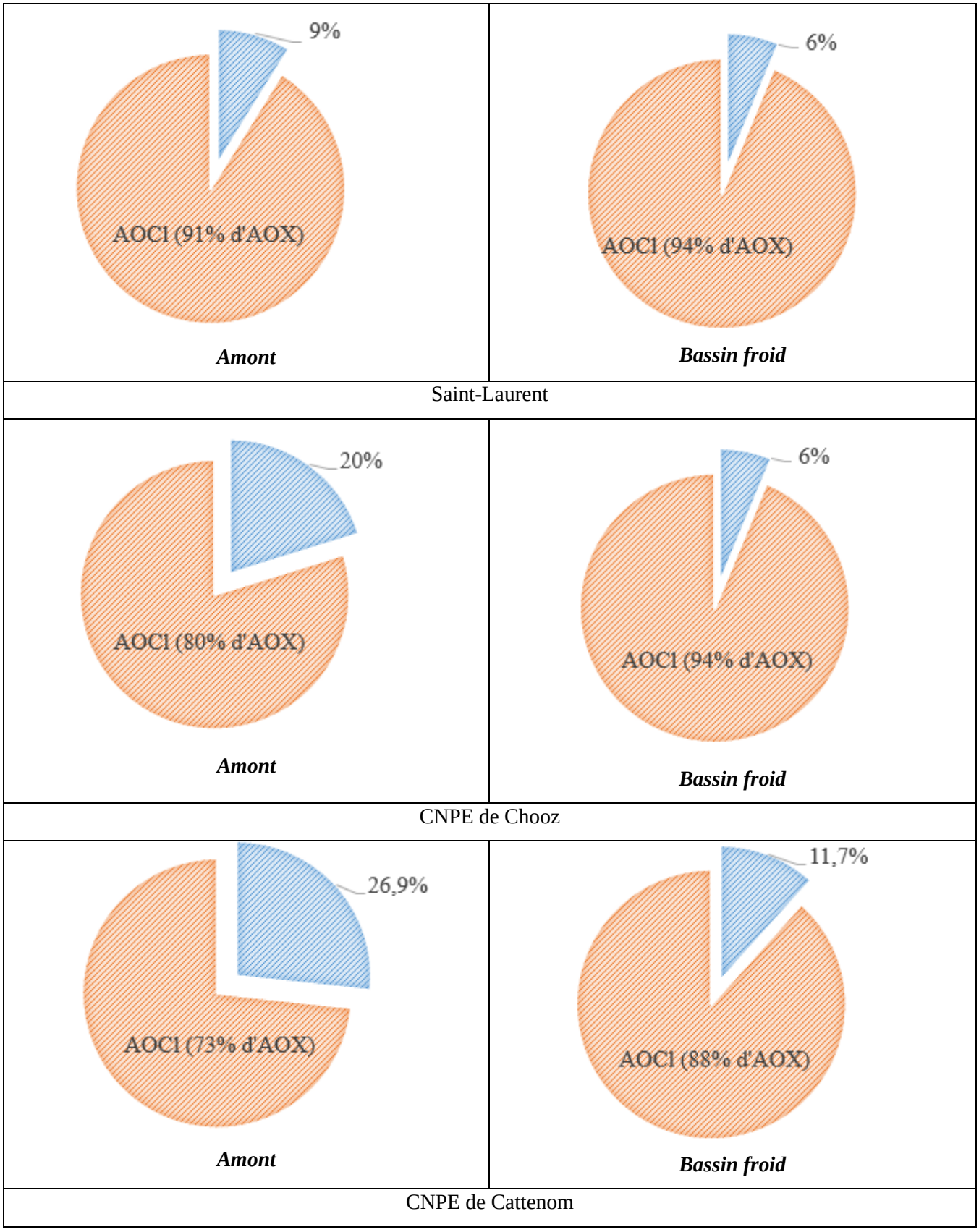
Concernant le site de Cattenom, l'allure de l'histogramme obtenu présente une tendance légèrement différente. L'examen des résultats montre que les concentrations en $AOBr_{formés}$ mesurées se révèlent légèrement supérieures à celles obtenues par calcul pour trois des six prélèvements effectués. Ces résultats sont cohérents avec les constatations réalisées à l'occasion d'une étude menée par EDF R&D en 2007 sur des échantillons d'eau en provenance du même site. Comparativement aux autres sites d'étude, la formation des sous-produits organobromés dans les eaux du bassin froid de Cattenom peut être liée à la particularité de l'eau de la Moselle riche en ions bromure (0,3-0,7 mg/L). Notons tout de même que les teneurs d'AOBr formées restent marginales en comparaison de celles d'AOCl.

2.3 Fractions chlorées d'AOX

Les teneurs moyennes en AOCl mesurées dans les échantillons d'eaux de rivières prélevés en amont des CNPE d'étude varient de la manière suivante : Meuse (14,6 ± 0,8 µg éq. Cl/L ; n = 6) > Moselle (11,9 ± 2,2 µg éq. Cl/L ; n = 6) > Loire (10,7 ± 2,1 µg éq. Cl/L ; n = 4). Ces résultats suggèrent une présence de composés organochlorés d'origine anthropique et/ou naturelle dans les eaux alimentant les CNPE étudiés. Leur contribution à la concentration d'AOX mesurée dans les eaux du bassin froid, après concentration dans le circuit de refroidissement, est très importante, comme le montrent les histogrammes de la figure III-4. Quant aux échantillons prélevés en bassins froids, les résultats obtenus montrent que la proportion de chlore incorporé dans les AOX totaux augmente après le traitement à la monochloramine, ce qui indique la formation de sous-produits organiques chlorés. Les teneurs moyennes d'AOCl formés varient de la manière suivante : Cattenom (32,5 ± 2,2 µg éq. Cl/L ; n = 6) ≈ Chooz (31,4 ± 0,6 µg éq. Cl/L ; n = 5) > Saint-Laurent (17,1 ± 2,4 µg éq. Cl/L ; n = 3).

Comme le montre la figure III-5, le chlore est l'halogène majoritaire que ce soit dans les échantillons d'eau prélevés en amont ou en bassins froids des sites d'étude. La fraction chlorée d'AOX contribue pour 88 à 94 % en moyenne aux AOX totaux formés dans le bassin froid selon le site d'étude.

Figure III-5. Répartition du COT et des AOX dans les fractions



Sur le plan métrologique, notons que les concentrations en AOX calculées en sommant les teneurs en AOCl et AOBr sont similaires à celles obtenues par la méthode conventionnelle d'AOX (tableau III-4). Ce résultat confirme le bon accord entre les deux techniques de mesure d'AOX (approche C-IC *versus* approche basée sur la microcoulométrie).

Sur le volet purement analytique, au vu des résultats obtenus, nous pouvons nous attendre à ce que les sous-produits organohalogénés formés (connus et inconnus) soient très majoritairement constitués de molécules organiques chlorées.

3. Spéciation des AOX par poids moléculaire

Les composés constitutifs d'AOX peuvent être de différentes masses molaires et donc de différentes tailles. Après filtration sur une membrane de 0,45 µm, les échantillons d'eaux de Saint-Laurent, Cattenom et Chooz ont été fractionnées par ultrafiltration sur une membrane ayant un seuil de coupure de 1 kDa, conformément au protocole expérimentale décrit dans le chapitre « matériels et méthodes ». Le choix de ce seuil de coupure vient du fait que les résultats de nos études antérieures, corroborés par des tests préliminaires, ont montré qu'environ 70 % d'AOX formés par la monochloramine possèdent une masse molaire apparente inférieure à 1 kDa. Nous rappelons que les rendements d'AOX retenus par la membrane (fraction appelée retentât) sont obtenus en divisant par cinq les concentrations obtenues par analyse d'un échantillon d'eau de 500 mL alors que les concentrations de perméat sont données par rapport à l'analyse d'une fraction de 100 mL d'eau fractionnée. Les résultats obtenus, après correction par soustraction des valeurs de blancs, sont résumés dans le tableau III-8 ci-après.

Tableau III-8. Proportion relative des AOX endogènes et des AOX formés inférieure ou supérieure à 1 kDa

Camp.	Saint-Laurent				Chooz				Cattenom			
	% AOX amont		% AOX formés		% AOX amont		% AOX formés		% AOX amont		% AOX formés	
	<1kDa	>1kDa	<1kDa	>1kDa	<1kDa	>1kDa	<1kDa	>1kDa	<1kDa	>1kDa	<1kDa	>1kDa
1	82	18	69	16	66	16	73	17	74	16	71	16
2	80	20	69	16	73	16	71	16	71	17	75	17
3	68	17	73	17	73	17	72	16	70	16	69	15
4					70	16	72	17	74	16	74	18
5					74	16	73	17	71	18	76	16
6									74	16	73	17

Notons tout d'abord que les rendements totaux obtenus en sommant les valeurs d'AOX mesurés dans le perméat et le retentât varient entre 86 à 90%. Ces valeurs inférieures à 100% peuvent être attribuées à une combinaison de trois facteurs essentiels qui sont : l'incertitude sur la mesure d'AOX, des pertes de composés d'intérêt (ex. par volatilisation ou dégradation), et la rétention irréversible de certains SPOX sur la membrane.

Les résultats obtenus indiquent que la proportion de la fraction d'AOX composée de molécules de masse molaire apparente inférieure à 1 kDa est quasi identique quels que soient l'eau et le site étudié. Elle représente en moyenne une proportion massique d'environ 70 % d'AOX totaux (AOX perméat + AOX retentât). Autrement dit, environ 30 % de composés organohalogénés formés par monochloramination des eaux de rivières possèdent une masse molaire supérieure à 1 kDa. Cette dernière fraction est selon toute vraisemblance plus importante puisque certaines molécules de tailles supérieures à celles des pores traversent la membrane. Il est estimé qu'environ 10% de molécules de masses molaires apparentes supérieures au seuil de coupure de la membrane la traversent.

Sur le plan purement analytique, les résultats obtenus mettent en évidence un point très important pour la suite de l'étude de spéciation chimique d'AOX, à savoir que le traitement à la monochloramine, tel que appliqué par les sites d'étude, produit des composés organohalogénés attribués à la fraction de taille supérieure à 1 kDa. Leur proportion en masse est supérieure à 30% d'AOX totaux formés. Or ces composés peu volatils sont donc difficilement analysables avec des techniques classiques telles que la chromatographie en phase gazeuse couplée à la spectrométrie de masse.

4. Analyse des sous-produits organohalogénés individuels (approche ciblée)

Au total 26 SPOX volatiles et 11 acides haloacétiques ont été recherchés dans les échantillons d'eau collectés en amont et au bassin froid des sites d'étude en utilisant les deux méthodes d'analyse SPE/GC-MS/MS développées à ces fins. Les tableaux III-9 à III-11 présentent, pour chaque site d'étude, les résultats obtenus. Seuls sont reportés les composés ayant été détectés.

Tableau III-9. Concentrations en µg/L des sous-produits organohalogénés mesurés dans les eaux collectées en amont et au bassin froid de Saint-Laurent.

Composé	LQ	27/07/16		10/08/16		24/08/16		07/09/16	
		AM	BF	AM	BF	AM	BF	AM	BF
Trihalométhanes									
TCM	0,003	0,008	nd	0,013	0,009	nd	nd	nd	nd
BDCM	0,010	nd	nd	< LQ	nd	nd	nd	nd	nd
TBM	0,100	< LQ	nd	nd	nd	nd	nd	nd	nd
Acides haloacétiques									
AMCA	0,500	nd	6,921	1,167	1,330	< LQ	2,284	0,954	1,070
ADCA	0,050	0,060	3,837	0,216	< LQ	< LQ	0,302	< LQ	< LQ
ATCA	0,050	nd	nd	< LQ	nd	< LQ	< LQ	< LQ	< LQ
AMBA	0,050	nd	0,960	nd	nd	nd	nd	nd	nd
ADBA	0,010	nd	0,046	nd	nd	0,025	0,025	0,012	0,034
Halopropanones									
1,1-DCPr	0,250	nd	<LQ	nd	nd	nd	nd	nd	nd

Abréviations : LQ : limite de quantification, nd : non détecté (< LD : limite de détection), TCM : chloroforme, BDCM : bromodichlorométhane, TBM : bromoforme, AMCA : acide monochloroacétique, ADCA : acide dichloroacétique, ATCA : acide trichloroacétique, AMBA : acide monobromoacétique, ADBA : acide dibromoacétique, 1,1-DCPr : 1,1 dichloropropanone.

Tableau III-10. Concentrations en µg/L des sous-produits organohalogénés mesurés dans les eaux collectées en amont et au bassin froid de Chooz

Composés	LQ	04/08/16		18/08/16		01/09/16		15/09/16		22/09/16	
		AM	BF	AM	BF	AM	BF	AM	BF	AM	BF
Trihalométhanes											
TCM	0,003	0,005	nd	0,003	nd	< LQ	nd	nd	nd	nd	nd
DBCM	0,010	nd	nd	nd	0,015	nd	nd	nd	nd	nd	nd
TBM	0,100	< LQ	nd	nd	nd	< LQ	< LQ	nd	nd	nd	nd
Halométhanes											
CIM	0,050	nd	nd	nd	nd	< LQ	< LQ	nd	nd	nd	nd
BIM	0,010	nd	nd	nd	nd	< LQ	0,019	nd	nd	nd	nd
Acides haloacétiques											
AMCA	0,500	1,477	4,387	< LQ	4,380	1,109	4,226	1,586	4,219	1,025	4,250
ADCA	0,050	0,090	2,404	< LQ	1,678	< LQ	2,137	< LQ	2,053	< LQ	2,010
ATCA	0,050	0,027	0,147	0,065	0,121	0,060	0,110	0,138	0,210	0,124	0,173
ADBA	0,010	< LQ	0,131	nd	nd	nd	0,066	nd	0,082	< LQ	0,037
ABCA	0,050	nd	0,222	nd	nd	nd	< LQ	nd	0,327	nd	0,276
Haloacétonitriles											
DCAN	0,050	nd	< LQ	nd	nd	nd	nd	nd	nd	nd	nd
Halopropanones											
CPr	3,000	nd	< LQ	nd	< LQ	nd	nd	nd	nd	nd	nd
1,1-DCPr	0,250	nd	0,263	nd	1,479	0,375	nd	nd	nd	nd	nd

Abréviations : Abréviations : LQ : limite de quantification, nd : non détecté (< LD : limite de détection), TCM : chloroforme, BDCM : bromodichlorométhane, TBM : bromoforme, CIM : chloroiodométhane, BIM : bromoiodométhane, AMCA : acide monochloroacétique, ADCA : acide dichloroacétique, ATCA : acide trichloroacétique, ADBA : acide dibromoacétique, ABCA : acide bromochloroacétique, DCAN : dichloroacétonitrile, CPr : chloropropanone, 1,1-DCPr : 1,1 dichloropropanone.

Tableau III-11. Concentrations en µg/L des sous-produits organohalogénés mesurés dans les eaux collectées en amont et au bassin froid de Cattenom

Composés	LQ	01/08/16		08/08/16		22/08/16		29/08/16		05/09/16		12/09/16	
		AM	BF	AM	BF	AM	BF	AM	BF	AM	BF	AM	BF
Trihalométhanes													
TCM	0,003	0,036	0,004	0,034	0,01	0,01	nd	0,02	nd	0,032	0,006	nd	nd
BDCM	0,010	0,036	0,004	0,034	0,01	0,01	nd	0,02	nd	0,032	< LQ	nd	nd
DBCM	0,010	0,011	nd	0,008	nd	nd	nd	0,003	nd	0,017	nd	nd	nd
TBM	0,100	< LQ	nd	< LQ	nd	0,284	nd	0,331	nd	0,158	< LQ	nd	nd
Halométhanes													
BIM	0,010	nd	nd	nd	nd	nd	Nd	nd	nd	< LQ	nd	nd	nd
Acides haloacétiques													
AMCA	0,500	1,391	6,192	1,418	7,322	1,341	4,533	0,896	4,337	0,509	5,008	1,847	3,45
ADCA	0,500	nd	2,435	< LQ	2,958	< LQ	2,385	< LQ	3,399	nd	3,40	0,123	0,53
ATCA	0,050	0,517	0,438	0,371	0,33	0,119	0,178	0,109	0,149	0,216	0,204	0,291	0,354
ADBA	0,010	0,05	0,199	nd	0,539	nd	0,036	0,4	0,04	0,046	0,123	0,048	0,08
ATBA	0,050	< LQ	< LQ	nd	nd	nd	nd	0,139	0,149	0,142	0,144	nd	0,099
ABCA	0,050	nd	0,715	nd	0,414	nd	0,326	nd	0,200	nd	0,546	nd	nd
ABDCA	0,010	0,026	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
ACDBA	0,010	nd	0,035	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Haloacétonitriles													
DCAN	0,050	nd	< LQ	nd	< LQ	nd	< LQ	< LQ	nd	nd	nd	nd	nd
Halopropanones													
CPr	3,000	nd	< LQ	nd	nd	nd	< LQ	nd	< LQ	nd	nd	nd	nd
1,1-DCPr	0,250	nd	1,26	nd	< LQ	nd	1,54	nd	1,473	nd	< LQ	nd	nd

Abréviations : LQ : limite de quantification, nd : non détecté (< LD : limite de détection), TCM : chloroforme, BDCM : bromodichlorométhane, DBCM : dibromochlorométhane, TBM : bromoforme, AMCA : acide monochloroacétique, ADCA : acide dichloroacétique, ATCA : acide trichloroacétique, ADBA : acide monobromoacétique, ATBA : acide tribromoacétique, ABCA : acide bromochloroacétique, ABDCA : acide bromodichloroacétique, ACDBA, acide chlorodibromoacétique, CPr : chloropropanone, 1,1-DCPr : 1,1 dichloropropanone.

4.1 SPOX individuels mesurés dans les échantillons d'eau prélevés en amont des sites d'étude

La distribution des SPOX détectés dans les échantillons d'eaux prélevés en amont des sites d'étude et le récapitulatif des résultats de mesures sont fournis dans le tableau III-12 ci-dessous.

Tableau III-12. Teneurs moyennes en µg/L et le degré d'occurrence des sous-produits organohalogénés détectés dans les échantillons d'eaux prélevés en amont des sites d'étude

	Loire				Meuse				Moselle			
	n	moy.	min.	max.	n	moy.	min.	max.	n	moy.	min.	max.
Trihalométhane												
TCM	2/4	0,010	0,008	0,013	3/5	0,004	< LQ	0,005	5/6	0,026	0,001	0,036
BDCM	1/4			< LQ					5/6	0,026	0,001	0,036
DBCM									4/6	0,013	< LQ	0,017
TBM	1/4			< LQ	2/5		< LQ		5/6	0,258	< LQ	0,331
Halométhanes												
BIM					1/5		< LQ		1/6			< LQ
CIM					1/5		< LQ					
Acides haloacétiques												
AMCA	3/4	1,060	< LQ	1,167	5/5	1,300	< LQ	1,586	6/6	1,234	0,509	1,847
ADCA	4/4	0,136	< LQ	0,216	5/5	0,090	< LQ	0,090	4/6	0,123	< LQ	0,123
ATCA	3/4			< LQ	5/5	0,083	0,027	0,138	6/6	0,270	0,109	0,517
ADBA	2/4	0,018	0,012	0,025	2/5			< LQ	4/6	0,136	0,048	0,400
ATBA									3/6	0,140	< LQ	0,142
ABDCA									1/6	0,026		0,026
Haloacétonitriles												
DCAN									1/6			< LQ
Halopropanones												
1,1-DCPr					1/5			0,375				

Les résultats obtenus mettent en évidence la présence de plusieurs sous-produits organohalogénés dans les échantillons d'eaux prélevés en amont des sites d'étude. Leur degré d'occurrence et leur teneur varient d'une rivière à l'autre. Les plus fréquemment détectés sont les acides monochloroacétique dichloroacétique, trichloroacétique et dibromoacétique, ainsi que le chloroforme. Ceci dénote un marquage chimique des eaux de rivières prélevées en amont des sites d'étude par des composés organohalogénés identiques à ceux susceptibles d'être produits par le traitement à la monochloramine.

Précisions toutefois que les niveaux relevés sont très faibles, en général inférieurs à la limite de quantification des méthodes analytiques communément utilisées par les laboratoires de routine. A titre d'exemple, lors des campagnes de mesures conduites par EDF R&D sur 7 sites d'étude en 2011, aucun des 14 sous-produits organohalogénés identifiés dans le cadre de ces travaux de thèse n'avait été détecté. Les analyses physico-chimiques avaient été réalisées par un laboratoire de routine accrédité par le COFRAC.

4.2 SPOX individuels mesurés dans les échantillons d'eau prélevés aux bassins froids des CNPE étudiées

Les SPOX détectés ainsi que les concentrations minimales, maximales et moyennes retrouvées sont présentés dans le tableau III-13 ci-après. Les données présentées en italique correspondent à celles de l'analyse des échantillons d'eaux prélevés en amont des sites d'étude.

Tableau III-13. Teneurs (en µg/L) en sous-produits organohalogénés détectés dans les échantillons d'eaux des bassins froids étudiés (valeurs en gras)

	Saint-Laurent				**Chooz**				**Cattenom**			
	n	moy.	min.	max.	n	moy.	min.	max.	n	moy.	min.	max.
Trihalométhane												
TCM	**1/3** *2/4*	 *0,01*	 *0,008*	**0,009** *0,013*					**3/6** *5/6*	**0,007** *0,026*	**0,004** *0,001*	**0,010** *0,036*
BDCM									**3/6** *5/6*	**0,007** *0,026*	**0,004** *0,001*	**0,010** *0,036*
DBCM					**1/5**			**0,015**				
TBM					**1/5** *2/5*			**< LQ** *< LQ*	**1/6** *5/6*	 *0,258*	 *< LQ*	**< LQ** *0,331*
Halométhanes												
BIM					**1/5** *1/5*			**0,019** *< LQ*				
CIM					**1/5** *1/5*			**< LQ** *< LQ*				
Acides haloacétiques												
AMCA	**3/3** *3/4*	**2,901** *1,060*	1,330 *< LQ*	6,921 *1,167*	**5/5** *5/5*	**4,292** *1,300*	**4,219** *< LQ*	**4,387** *1,586*	**6/6** *6/6*	**5,140** *1,234*	**3,450** *0,509*	**7,372** *1,847*
ADCA	**3/3** *4/4*	**2,069** *0,136*	**< LQ** *< LQ*	**3,837** *0,216*	**5/5** *5/5*	**2,056** *0,090*	**1,678** *< LQ*	**2,404** *0,090*	**6/6** *4/6*	**2,517** *0,123*	**0,530** *< LQ*	**3,399** *0,123*
ATCA	**1/3** *3/4*			**< LQ** *< LQ*	**5/5** *5/5*	**0,152** *0,083*	**0,110** *0,027*	**0,210** *0,138*	**6/6** *6/6*	**0,275** *0,270*	**0,149** *0,109*	**0,438** *0,517*
AMBA	**1/3**			**0,960**								
ADBA	**2/3** *2/4*	**0,035** *0,018*	**0,025** *0,012*	**0,046** *0,025*	**4/5** *2/5*	**0,079**	**0,037**	**0,131** *< LQ*	**6/6** *4/6*	**0,169** *0,136*	**0,539** *0,048*	**0,169** *0,400*
ATBA									**4/6** *3/6*	**0,131** *0,140*	**< LQ** *< LQ*	**0,149** *0,142*
ABCA					**4/5**	**0,275**	**< LQ**	**0,327**	**5/6**	**0,440**	**0,200**	**0,715**
ACDBA									**1/6** *1/6*			**0,035** *0,026*
Haloacétonitriles												
DCAN					**1/5**			**< LQ**	**3/6**			**< LQ**
Halopropanones												
CPr					**2/5**			**< LQ**	**3/6**			**< LQ**
1,1-DCPr	1/3			< LQ	**2/5** *1/5*	**0,871**	**0,263**	**1,479** *0,375*	**5/6**		**< LQ**	**1,540**

L'analyse des résultats obtenus laisse ressortir ce qui suit. Tout d'abord, notons que, sur l'ensemble des échantillons d'eaux prélevés en bassins froids, 20 sous-produits organohalogénés suivis dans le cadre de ce travail n'ont jamais été détectés à des concentrations supérieures aux limites de détection des méthodes analytiques utilisées. Il s'agit des composés organohalogénés suivants :

- Famille des halométhanes : triodométhane (LQ : 0,5 µg/L) et dichloroiodométhane (LQ : 0,01 µg/L) ;
- Acides haloacétiques : acide chlorodibromoacétique (LQ : 0,01 µg/L), acide iodoacétique (LQ : 0,01 µg/L) et acide diiodoacétique (LQ : 0,01 µg/L) ;
- Haloacétonitriles : chloroacétonitrile (LQ : 0,5 µg/L), trichloroacétonitrile (LQ : 0,01 µg/L), bromochloroacétonitrile (LQ : 0,05 µg/L), bromoacétonitrile (LQ : 2 µg/L), dibromoacétonitrile (LQ : 0,05 µg/L) et iodoacétonitrile (LQ : 0,5 µg/L) ;
- Halonitrométhanes : bromonitrométhane (LQ : 2 µg/L), bromochloronitrométhane (LQ : 0,25 µg/L), bromodichloronitrométhane (LQ : 0,003 µg/L) et trichloronitrométhane (LQ : 2 µg/L) ;
- Halopropanones : 1,3-dichloropropanone (LQ : 2 µg/L), 1,1,1-trichloropropanone (LQ : 0,1 µg/L), 1,1,3-trichloropropanone (LQ : 1 µg/L) et 1,1,3,3-tetrachloropropanone (LQ : 0,75 µg/L) ;
- Haloacétaldéhydes : tribromoacétaldéhyde (LQ : 2 µg/L)

Sur l'ensemble des 37 sous-produits de désinfection organohalogénés recherchés dans les eaux prélevées en bassins froids, 17 composés ont été détectés à des concentrations supérieures à la limite de quantification, dont certains de manière très ponctuelle. Les plus fréquemment retrouvés, tous sites confondus, sont : l'acide monochloroacétique (100% ; n =14), l'acide dichloroacétique (100% ; n = 14), l'acide trichloroacétique (86% ; n=14), l'acide dibromoacétique (86% ; n = 14), l'acide tribromoacétique (29% ; n = 4), l'acide bromochloroacétique (64% ; n = 9), le dichloroacétonitrile (29% ; n = 4), le chloropropanone (36% ; n = 5) et le 1,1-dichloropropanone (50% ; n = 7). L'acide monochloroacétique est le sous-produit organohalogénés majoritaire tous sites confondus, suivi de plus près par l'acide dichloroacétique. Ce résultat est inattendu puisque nos études antérieures avaient montré que l'acide dichloroacétique est le composé majoritaire. Cette inversion pourrait s'expliquer en partie par une difficulté dans l'analyse de l'acide monochloroacétique.

En fonction des teneurs mesurées et le taux de détection, les sites d'étude peuvent être classés dans l'ordre suivant : Cattenom > Chooz > Saint-Laurent. Les teneurs relevées sont, pour la plus part des composés suivis, du même ordre de grandeur que celles mesurées lors de nos études antérieures, mais avec une fréquence de détection plus élevée. Les améliorations substantielles apportées aux techniques de mesure en termes de sensibilité et de spécificité ont de toute évidence fortement contribué à croître le nombre de sous-produits organohalogénés détectés.

Il convient cependant d'être très prudent quant à l'interprétation des teneurs positives (> LQ) et leurs fréquences d'occurrence. En effet, comme nous l'avons évoqué précédemment, le mode de fonctionnement

des circuits de refroidissement conduit à un phénomène de concentration des eaux du bassin froid. Cette concentration pourrait conduire à la détection de certains composés organohalogénés initialement présents dans les eaux de rivières en amont des CNPE étudiés à des concentrations inférieures à la limite de quantification des méthodes utilisées. Autrement dit, les teneurs mesurées dans les échantillons d'eaux en provenance des bassins froids correspondent en réalité aux composés présents dans l'eau d'appoint, concentrés dans le circuit de refroidissement, auxquels viennent s'ajouter les quantités formées par monochloramination. Ainsi, certains composés bien qu'ils soient détectés dans les bassins froids peuvent ne pas être typiques au traitement à la monochloramine tel qu'appliqué par les sites d'étude. En revanche, les substances les plus volatiles devraient avoir leur concentration diminuée par volatilisation (échange air/eau au sein des tours de refroidissement), ce qui explique la détection de certains d'entre elles davantage dans les échantillons d'eaux prélevées en amont que dans les échantillons prélevés en bassins froids.

4.3 SPOXs attribués au traitement à la monochloramine et leur contribution au paramètre AOX

Pour tenir compte de l'effet de concentration dans le circuit de refroidissement, nous avons émis l'hypothèse selon laquelle les SPOX non volatiles contenus dans l'eau d'appoint, comme c'est le cas des acides haloacétiques, seront concentrés de la même façon que le COD. Ainsi, les teneurs imputées à la monochloramination peuvent être déduites en utilisant la formule de calcul suivante : $SPOX_{formés} = AOX_{BF} - Fc \times SPOX_{AM}$.

Toutefois, il n'est pas toujours possible de retrancher les concentrations en sous-produits provenant de l'amont quand celles-ci sont inférieures aux limites de quantification des méthodes analytiques utilisées. Pour pallier cette difficulté, nous avons posé les hypothèses de calcul suivantes. Tout d'abord, dans le cas où les concentrations mesurées dans les échantillons prélevés en amont se révèlent inférieures à limite de détection, nous proposons de leur attribuer une valeur égale à LD/2. De la même manière, pour les échantillons dont les concentrations sont inférieures à limite de quantification nous proposons de leur octroyer une valeur égale à : LQ/2 pour les échantillons prélevés en amont et, LQ pour les échantillons d'eau issus des bassins froids. Ces hypothèses de calcul permettent de minimiser raisonnablement les teneurs en SPOX « endogènes » et maximiser en parallèle celles en SPOX susceptibles d'être formés par monochloramination. Le tableau III-14 ci-dessous synthétise, pour chaque site d'étude, les résultats obtenus.

Tableau III-14. Bilan des sous-produits organohalogénés formés (en µg/L) dans les eaux du bassin froid des sites étudiés

	Saint-Laurent	**Chooz**	**Cattenom**

	n	moy.	min.	max.	n	moy.	min.	max.	n	moy.	min.	max.
Trihalométhane												
BDCM	**1/3**			**0,013**								
DBCM					**1/5**			**0,013**				
TBM	**1/3**			**0,027**	**1/5**			**0,077**				
Halométhanes												
BIM	**1/3**			**0,012**	**1/5**			**0,012**				
CIM	**1/3**			**0,038**	**1/5**			**0,038**				
Acides haloacétiques												
AMCA	**2/3**	**4,238**	**1,967**	**6,811**	**5/5**	**2,738**	**1,840**	**2,738**	**6/6**	**3,451**	**0,854**	**5,593**
ADCA	**2/3**	**1,997**	**0,236**	**3,758**	**4/5**	**1,946**	**1,649**	**2,102**	**6/6**	**2,280**	**0,357**	**3,278**
ATCA	**1/3**			**0,018**	**4/5**	**0,046**	**0,003**	**0,109**	**1/6**			**0,006**
AMBA	**1/3**			**0,927**								
ADBA	**1/3**			**0,039**	**4/5**	**0,074**	**0,030**	**0,124**	**5/6**	**0,154**	**0,012**	**0,537**
ATBA									**4/6**	**0,170**	**0,017**	**0,539**
ABCA					**4/5**	**0,200**	**0,013**	**0,314**	**5/6**	**0,429**	**0,188**	**0,704**
ACDBA									**1/6**			**0,024**
Haloacétonitriles												
DCAN					**1/5**			**0,013**	**3/6**	**0,039**	**0,038**	**0,040**
Halopropanones												
CPr					**2/5**	**2,363**	**2,300**	**2,363**	**3/6**	**2,301**	**2,278**	**2,333**
1,1-DCPr	**1/3**			**0,195**	**2/5**	**0,871**	**0,263**	**1,479**	**2/6**	**0,818**	**0,207**	**1,428**

L'examen des résultats montre que certaines teneurs calculées sont extrêmement faibles. Il est important de préciser que ces valeurs positives peuvent ne pas refléter une formation réelle de sous-produits mais tout simplement être le résultat des approximations de calcul susmentionnées.

Afin d'estimer les proportions que représentent les sous-produits organochlorés mesurés par rapport aux valeurs d'AOX formés, les concentrations ont tout d'abord été exprimées en µg éq. Cl/L, additionnées ensemble, puis divisées par les valeurs d'AOX formés. Les bilans matières obtenus sont présentés dans le tableau III-15 ci-après.

Tableau III-15. Contribution de la somme de sous-produits organohalogénés mesurés dans les AOX formés

Campagne	Saint-Laurent			Chooz			Cattenom		
	ΣSPOX	AOX	Bilan (%)	ΣSPOX	AOX	Bilan (%)	ΣSPOX	$AOX_{formés}$	Bilan (%)
1	5,00	17,4	**29**	2,96	28,2	11	4,28	34,8	12
2	0,14	18,5	**1**	4,48	35,6	**13**	4,91	35,5	14
3	0,77	9,75	8	2,20	31,4	7	3,14	33,4	9
4	-	-	-	1,93	29,8	7	3,78	31,5	12
5	-	-	-	1,14	26,4	**4**	3,63	23,1	**16**
6	-	-	-	-	-	-	0,55	30,1	**2**

Comme le montrent les résultats obtenus, le bilan massique associé aux sous-produits organohalogénés identifiés imputables au traitement à la monochloramine varie dans le temps (variabilité inter-site) et l'espace (variabilité intra-sites). La contribution moyenne des sous-produits organohalogénés mesurés dans le cadre de ce travail de thèse par rapport aux teneurs d'AOX formés est de l'ordre de 10 % (n = 14), avec des bilans de matière qui varient dans un domaine assez large, allant de 1 à 29 %. Bien que les bilans de matière établis restent faibles, la présente étude apporte néanmoins des améliorations significatives par rapport aux estimations des études antérieures. A titre d'illustration, lors d'une large campagne d'échantillonnage et de mesure réalisée en 2011 par EDF R&D sur sept sites de production traitant leurs eaux d'appoint à la monochloramine, la proportion en sous-produits organohalogénés identifiés (ACDA et AMCA) ne dépassait pas, dans le meilleur des cas, les 5,3% d'AOX formés (cas du CNPE de Saint Laurent).

Comme nous l'avons évoqué plus haut, les bilans de matière doivent être corrigés par les concentrations retrouvées par la méthode de mesure d'AOX. A titre d'exemple, la somme des sous-produits quantifiés dans les eaux de bassin froid de Cattenom le 01/08/2016 est de 5 µg éq. Cl/L avec un bilan de matière de 9%. Sachant que les sous-produits identifiés sont analysés avec un rendement de 28 à 84%, le bilan de matière recalculé dans ce cas est de 4,5%, selon l'équation donnée dans l'article 1, chapitre I :

$$\%MB\ (part\ of\ known\ AOX) = 100\ x\ \frac{\Sigma \frac{n\ x\ (OXnBPi)\ x\ M(Cl)}{M(OXnBPi)}}{AOX}$$

Avec n est le nombre d'halogènes, $OXBP_i$ sont les SPOX identifiés, M(Cl) et M(OXnBPi) sont les masses molaires du chlore et des SPOX identifiés, respectivement.

Par conséquent, les bilans de matière corrigés des rendements analytiques sont répartis comme suit :

- de 0,07 à 1,6% dans les échantillons d'eau en amont et de 0,8 à 6,1% dans les échantillons d'eau de bassin froid de Saint-Laurent ;
- de 0,2 à 1,1% dans les échantillons d'eau en amont et de 2,8 à 3,2% dans les échantillons d'eau de bassin froid de Chooz ;
- de 1 à 2% dans les échantillons d'eau en amont et de 1 à 4% dans les échantillons d'eau de bassin froid de Cattenom.

Malgré que nous avons élargi la liste de sous-produits organohalogénés recherchés et amélioré substantiellement la sensibilité des méthodes analytiques utilisées, les bilans de matière obtenus restent très faibles (< 10% en moyenne). Ce résultat indique qu'une partie importante de sous-produits organohalogénés échappe toujours aux méthodologies analytiques classiques basées sur une recherche ciblée des molécules connues. Ce constant n'est pas surprenant, il est même attendu, en tenant compte les résultats de l'étude de spéciation des AOX formés par poids moléculaire. En effet, nous avons montré que plus de 30 % d'AOX formés dans les eaux des bassins froids possèdent une masse molaire apparente supérieure 1 KDa. Cette catégorie de molécules ne se prête pas à une analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse, technique communément utilisée pour développer des méthodologies de *screening* à la fois ciblé et non ciblé.

5. *Screening* non ciblé des SPOX volatils inconnus par GC-MS

Les extraits d'échantillon d'eau collectés en amont et au bassin froid des 3 sites d'étude ont été analysés en *fullscan* en impact électronique (GC-EI-MS) et en ionisation chimique négative (GC-NCI-MS). Les données présentées dans les tableaux III-16 à III-18 montrent que toutes les eaux étudiées contiennent des composés halogénés, 55 molécules au total ont pu être extraites des 3 sites étudiés : 20 molécules pour les eaux collectées à Saint-Laurent, 12 molécules pour les eaux de Chooz et 18 molécules pour les eaux de Cattenom. Certains de ces composés ont été détectés à la fois dans les eaux brutes non traitées collectées en amont des CNPE et celles issues des eaux traitées, d'autres en revanche n'ont été mises en évidence que dans les eaux en provenance des bassins froids. A titre d'exemple, la figure III-6 présente le spectre de masse d'un composé bromé détecté dans les échantillons d'eau collectés en amont et au bassin froid de Saint-Laurent et Cattenom. Le profil du massif isotopique m/z 153/155 est caractéristique de la présence d'un seul atome de brome $^{79}Br/^{81}Br$,

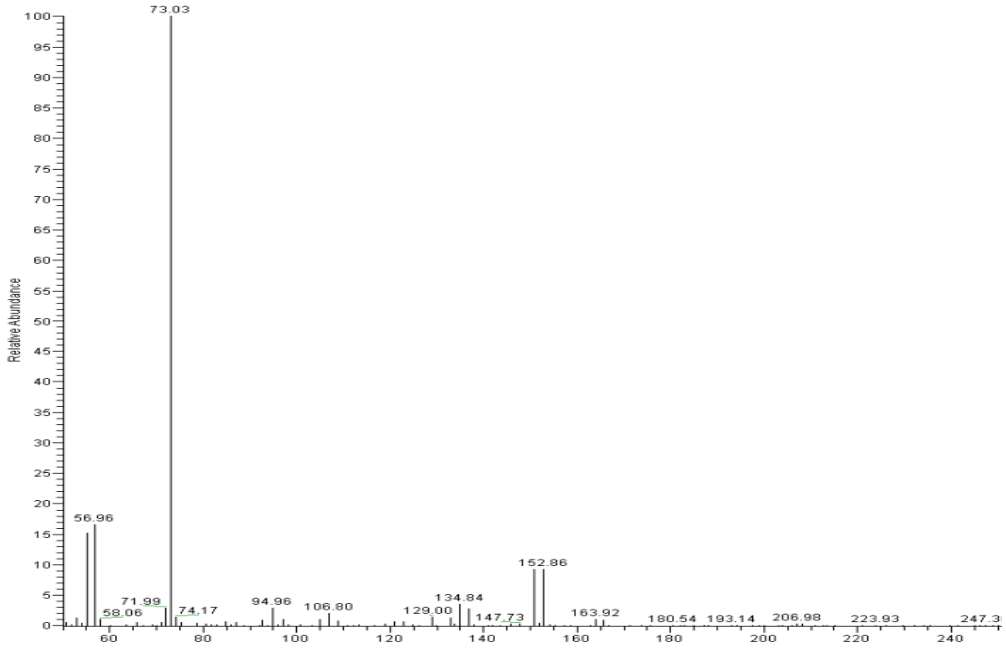


Figure III-6. Spectre de masse obtenu en ionisation électronique d'un composé bromé détecté dans les échantillons d'eau collectés en amont et au bassin froid de Saint-Laurent et Cattenom

En comparant les aires des pics chromatographiques des composés mis en évidence à celle du pic de l'étalon interne (1,2-dibromopropane), nous avons estimé que certains de ces derniers pourraient être présents à des concentrations avoisinant le 0,1 µg.L^{-1}, ce qui indique que leur contribution à la valeur d'AOX mesurée est importante. Cependant, ces informations partielles ne donnent qu'un ordre de grandeur des teneurs réellement produites. Tous les pics répertoriés dans les tableaux III-16 à III-18 ont fait l'objet d'une recherche en base de données. Le 1-bromo-2-méthyl-2-propanol (Tr = 6,48 min) a pu ainsi être identifié dans certains échantillons d'eau collectés en amont et au bassin froid de Saint-Laurent, Chooz et de Cattenom. La comparaison des aires des pics chromatographiques correspondants avec celle du pic de l'étalon interne a permis d'estimer que les concentrations en 1-bromo-2-méthyl-2-propanol ne dépassaient pas 0,1 µg/L. Ce composé a été rapporté comme étant un nouveau sous-produit de désinfection de l'eau potable chlorée et/ou monochloraminée [1]. Cependant, Y. Xie, 2016 a montré qu'il s'agit d'un artefact issu de la réaction entre le MtBE et les ions bromures ou certains sous-produits bromés [2]. Afin de trancher quant à l'existence effective de nouveaux SPOX, d'autres études doivent être effectuées pour distinguer ce qui est présent dans l'échantillon avant l'analyse et ceux susceptibles d'être formés pendant l'analyse et le traitement de l'échantillon.

Tableau III-16. Sous-produits organohalogénés détectés dans les échantillons d'eau collectés en amont et au bassin froid de Saint-Laurent

Temps de rétention (min)	Nature du composé	Prélèvement
4,84	Composé chloré	27/07/16 [(b)]
5,95	Composé bromé	27/07/16 [(b)]
6,48	1-bromo-2-méthyl-2-propanol	01/08/16 [(c)]
7,55	Composé chloré	27/07/16 [(b)]
7,73	Composé chloré	07/09/16 [(b)]
7,98	Composé chloré	27/07/16 [(b)]
8,36	Composé bromé	27/07/16 [(c)]
8,46	Composé chloré	27/07/16 [(c)] ; 10/08/16 [(c)] ; 24/08/16 [(c)] ; 07/09/16 [(c)]
8,77	Composé chloré	27/07/16 [(c)] ; 10/08/16 [(c)] ; 24/08/16 [(c)] ; 07/09/16 [(c)]
9,01	Composé chloré	24/08/16 [(c)]
9,13	Composé chloré	27/07/16 [(b)]
9,81	Composé chloré	27/07/16 [(c)] ; 10/08/16 [(c)] ; 24/08/16 [(c)] ; 07/09/16 [(c)]
9,92	Composé bromé	27/07/16 [(b)]
10,00	Composé chloré	27/07/16 [(b)] ; 10/08/16 [(b)]
10,21	Composé chloré	24/08/16 [(c)]
10,82	Composé chloré	07/09/16 [(b)] ; 24/08/16 [(b)]
11,09	Composé chloré	27/07/16 [(c)]
13,30	Composé bromé	27/07/16 [(c)] ; 10/08/16 [(c)] ; 24/08/16 [(c)] ; 07/09/16 [(c)]
13,57	Composé chloré	27/07/16 [(b)]
14,80	Composé bromé	10/08/16 [(c)] ; 24/08/16 [(c)]

[(a)] Composé détecté uniquement dans les échantillons d'eau collectés en amont, **[(b)]** au bassin froid et [(c)] en amont et au bassin froid

Tableau III-17. Sous-produits organohalogénés détectés dans les échantillons d'eau collectés en amont et au bassin froid de Chooz

Temps de rétention (min)	Nature du composé	Prélèvement
4,84	Composé chloré	04/08/16 [(b)] ; 01/09/16 [(b)] ; 15/09/16 [(b)] ; 22/09/16 [(b)]
6,48	1-bromo-2-méthyl-2-propanol	04/08/16 [(c)]
7,10	Composé chloré	04/08/16 [(c)]
7,58	Composé bromé	01/09/16 [(b)] ; 15/09/16 [(b)] ; 22/09/16 [(b)] ; 06/10/16 [(b)]
7,72	Composé chloré	01/09/16 [(b)] ; 15/09/16 [(b)] ; 22/09/16 [(b)] ; 06/10/16 [(b)]
7,98	Composé chloré	04/08/16 [(b)]; 18/08/16 [(b)] ; 01/09/16 [(b)] ; 15/09/16 [(b)] ; 22/09/16 [(b)]
9,03	Composé chloré	18/08/16 [(b)]
9,71	Composé chloré	04/08/16 [(c)] ; 18/08/16 [(c)] ; 15/09/16 [(c)] ; 22/09/16 [(c)] ; 06/10/16 [(c)]
9,92	Composé bromé	04/08/16 [(b)] ; 18/08/16 [(b)] ; 01/09/16 [(b)] ; 15/09/16 [(b)] ; 22/09/16 [(b)]
13,12	Composé bromé	01/09/16 [(b)]
14,80	Composé chloré	01/09/16 [(b)] ; 15/09/16 [(b)] ; 22/09/16 [(b)] ; 06/10/16 [(b)]
15,67	Composé bromé	04/08/16 [(c)] ; 18/08/16 [(c)] ; 01/09/16 [(c)] ; 15/09/16 [(c)] ; 22/09/16 [(c)]

[(a)] Composé détecté uniquement dans les échantillons d'eau collectés en amont, **[(b)]** au bassin froid et [(c)] en amont et au bassin froid

Tableau III-18. Sous-produits organohalogénés détectés dans les échantillons d'eau collectés en amont et au bassin froid de Cattenom

Temps de rétention (min)	Nature du composé	Prélèvement
4,79	Composé chloré	01/08/16 [(b)] ; 22/08/16 [(b)] ; 29/08/16 [(b)]
6,48	1-bromo-2-méthyl-2-propanol	01/08/16 [(c)]
7,55	Composé chloré	01/08/16 [(b)]
7,89	Composé chloré	08/08/16 [(b)]
8,36	Composé bromé	01/08/16 [(c)]
8,46	Composé bromé	01/08/16 [(b)] 22/08/16 [(b)] ; 29/08/16 [(b)] ; 05/09/16 [(b)] ;

		12/09/16 [b]
8,55	Composé chloré	01/08/16 [c]
8,78	Composé bromé	01/08/16 [c] ; 08/08/16 [c] ; 22/08/16 [c] ; 29/08/16 [c] ; 05/09/16 [c] ; 12/09/16 [c]
9,13	Composé chloré	01/08/16 [b]
9,32	Composé bromé	01/08/16 [a] ; 08/08/16 [a] ; 22/08/16 [a] ; 29/08/16 [a] ; 05/09/16 [a] ; 12/09/16 [a]
9,71	Composé chloré	01/08/16 [c] ; 29/08/16 [c] ; 05/09/16 [c]
9,92	Composé chloré	01/08/16 [c] ; 08/08/16 [c] ; 22/08/16 [c] ; 29/08/16 [c] ; 05/09/16 [c] ; 12/09/16 [c]
11,54	Composé bromé	01/08/16 [c] ; 08/08/16 [c] ; 22/08/16 [c] ; 29/08/16 [c] ; 05/09/16 [c] ; 12/09/16 [c]
11,80	Composé chloré	01/08/16 [c] ; 29/08/16 [c]
11,90	Composé chloré	29/08/16 [c] ; 12/09/16 [c]
14,42	Composé chloré	22/08/16 [c]
14,78	Composé chloré	12/09/16 [c]
15,67	Composé bromé	01/08/16 [c] ; 22/08/16 [c] ; 29/08/16 [c] ; 12/09/16 [c]

[a] Composé détecté uniquement dans les échantillons d'eau collectés en amont, **[b]** au bassin froid et [c] en amont et au bassin froid

6. *Screening* non ciblé des SPOX de hauts poids moléculaires par FT-ICR/MS

Le spectromètre de masse FT-ICR a été étalonné en mode externe avec une solution diluée d'acide phosphorique pour les gammes de rapports m/z choisies : de 150 à 800 et de 800 à 1500 Th. La calibration interne a été effectuée pour chaque échantillon d'eau à l'aide de molécules organiques homologues issues de la matière organique sous forme $CxHyOz(CH_2)n$.

La recherche de formules brutes de sous-produits organohalogénés a été effectuée à partir des critères évoqués dans la partie II.3.8 du chapitre II (MATERIELS ET METHODES).

Ces critères garantissent que les formules brutes attribuées bénéficient d'un niveau de confiance élevé et que les espèces correspondantes peuvent exister chimiquement.

La figure III-7 représente le spectre de masse en ESI+ obtenu en infusion d'un extrait SPE d'un échantillon d'eau prélevé en amont de Saint-Laurent le 10/08/2016. Cette figure illustre la complexité de l'interprétation spectrale due à la présence de multiples pics séparés de moins d'une unité de masse atomique. Ce type de résultat est souvent rencontré avec les échantillons contenant de la matière organique naturelle [3-6]. Une observation fine (zoom) montre que le spectre de masse (a) se compose d'un ensemble de signaux séparés par des écarts de 2 et 14 Da. L'écart de 2 Da des signaux est attribué à une différence de degré d'insaturation par rapport aux signaux voisins [8]. L'écart de 14 Da correspond à « CH_2 » ; il est attribué à la présence de séries homologues de composés organiques [3,9].

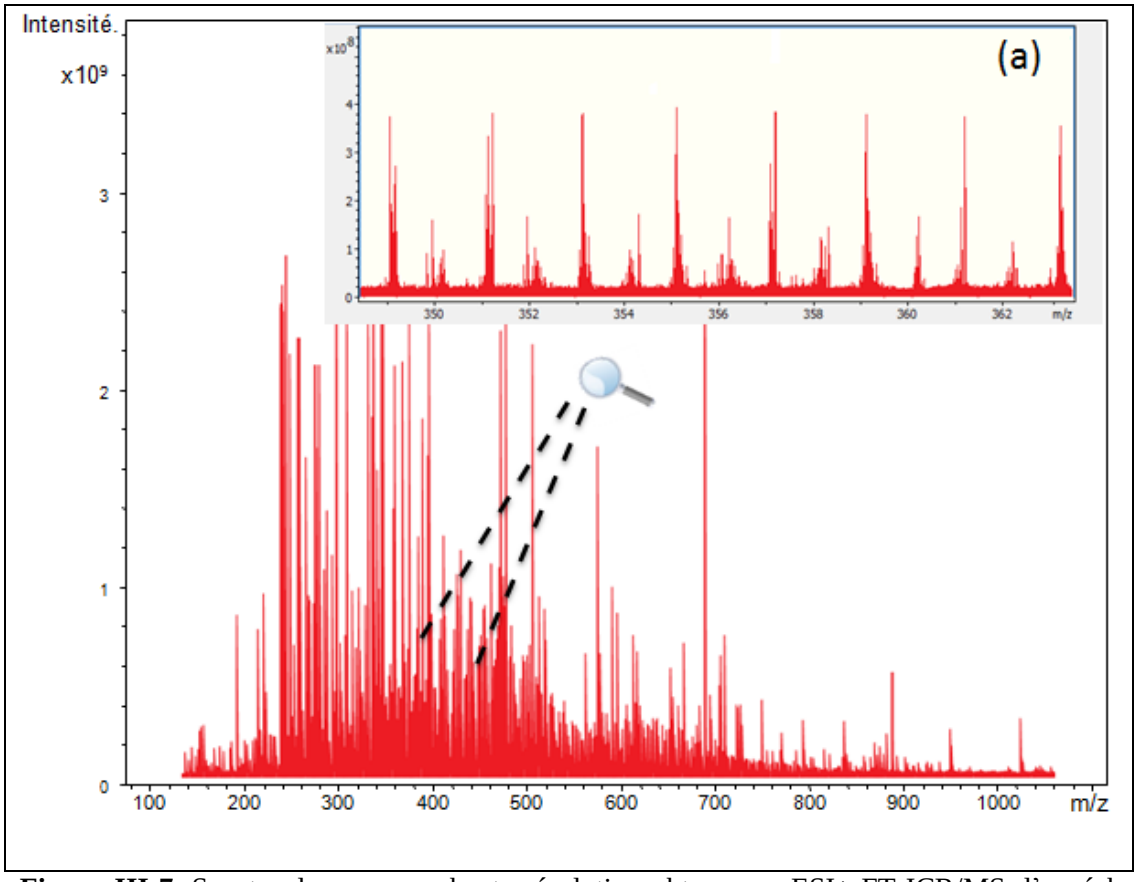


Figure III-7. Spectre de masse en haute résolution obtenu par ESI+-FT-ICR/MS d'un échantillon d'eau collecté en amont de Saint-Laurent le 10/08/2016

Le spectre présenté sur la figure III-7 contient 13095 signaux ioniques. La détermination des masses exactes des ions à moins de 1 ppm d'erreur de masse permet de déterminer la formule brute des analytes. Les données présentées dans le tableau III-19 montrent que toutes les eaux étudiées contiennent des composés halogénés à haut poids moléculaire. 17 sous-produits au total ont pu être extraits et détectés pour

l'ensemble des 3 sites étudiés. Certains sont présents uniquement dans les eaux collectées au bassin froid des CNPE.

Les spectres de masse de tous les pics listés dans le tableau III-19 ont été « vérifiés » par comparaison avec les spectres théoriques calculés par le logiciel de traitement Data Analysis. A titre d'exemple, la figure III-8 présente le spectre de masse d'un composé chloré, de formule brute $C_{31}H_{30}ClN_4O_{19}$, détecté dans les échantillons d'eau collectés en amont et bassin froid de Saint-Laurent, Chooz et Cattenom.

Il est à noter que seuls les composés chlorés ont été détectés. Les composés bromés et iodés n'ont pas été détectés du fait de leurs concentrations faibles dans les échantillons d'eau confirmées par l'analyse en C-IC.

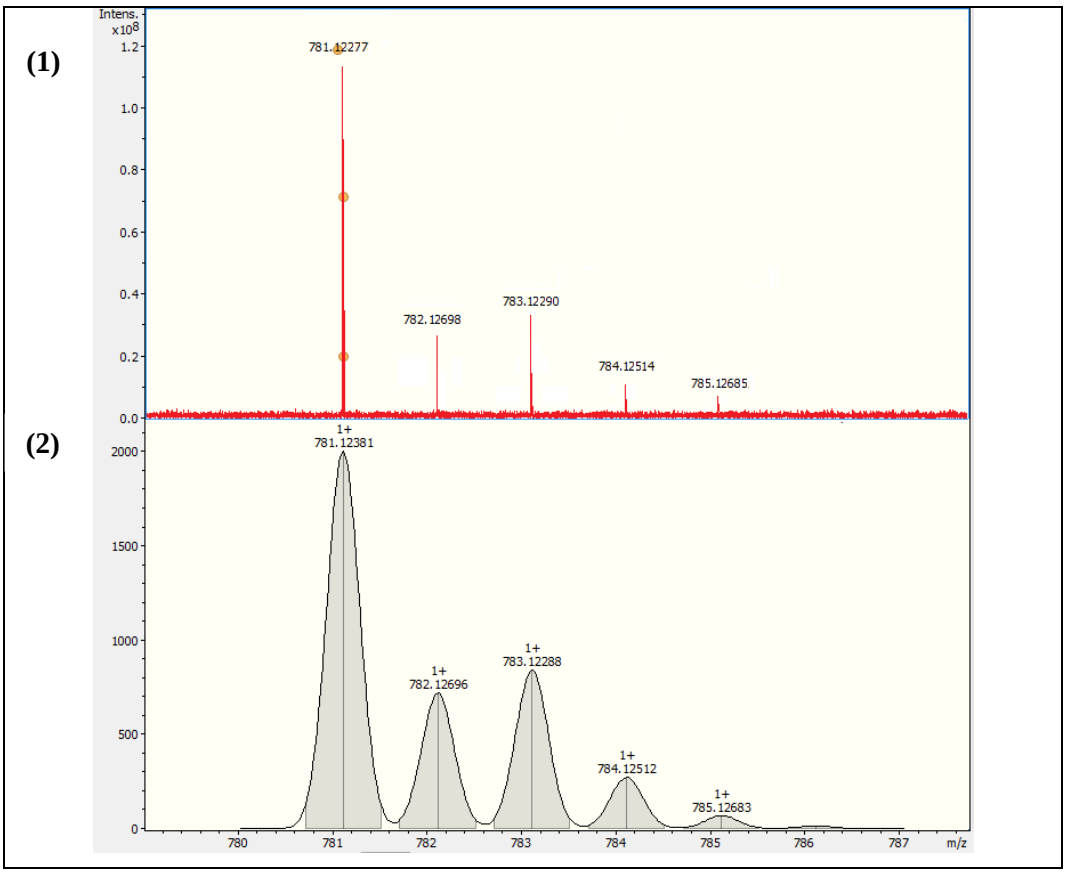


Figure III-8. Spectres de masse (1) expérimental et (2) théorique du composé chloré $C_{31}H_{30}ClN_4O_{19}$, détecté dans les échantillons d'eau collectés en amont et bassin froid de Saint-Laurent, Chooz et Cattenom

Tableau III-19. Liste des formules brutes des sous-produits organohalogénés identifiés dans les échantillons d'eau en amont et de bassin froid de Saint-Laurent, Chooz et Cattenom

Formule brute	m/z mesuré	CNPE-date
$C_7H_{17}ClN_2O$	180,102392	SLA-10/08/16 [(c)] ; SLA-24/08/16 [(c)] ; CHO-04/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_8H_{19}ClN_2O$	194,118042	SLA-27/07/16 [(b)] ; SLA-24/08/16 [(b)] ; CHO-04/08/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)] ; CAT-05/09/16 [(c)]
$C_{13}H_{22}ClN_5$	283,155825	CHO-04/08/16 [(b)] ; CHO-01/09/16 [(b)] ; CHO-15/09/16 [(b)] ; CAT-08/08/16 [(b)] ; CAT-29/08/16 [(b)] ; CAT-05/09/16 [(b)]
$C_{13}H_{28}ClN_3NaO$	300,181311	SLA-10/08/16 [(c)] ; SLA-24/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CAT-01/08/16 [(c)]
$C_{11}H_{24}ClN_4O_4$	311,148059	SLA-27/07/16 [(b)] ; SLA-10/08/16 [(b)] ; SLA-24/08/16 [(b)] ; CHO-04/08/16 [(b)] ; CHO-01/09/16 [(b)] ; CHO-15/09/16 [(b)] ; CAT-01/08/16 [(b)] ; CAT-22/08/16 [(b)] ; CAT-29/08/16 [(b)]
$C_{14}H_{22}ClN_5O$	311,150739	SLA-10/08/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_{20}H_{21}Cl_2N_6O_2$	327,109756	SLA-10/08/16 [(c)] ; SLA-24/08/16 [(c)] ; CHO-04/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_{14}H_{22}ClN_5O_2$	327,145654	SLA-10/08/16 [(c)] ; SLA-24/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-01/08/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_{17}H_{24}ClN_5$	333,171475	CHO-04/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_{18}H_{26}ClNa_2O_2$	355,141123	CHO-04/08/16 [(c)] ; CHO-18/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CHO-22/09/16 [(c)] ; CAT-01/08/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_{17}H_{24}ClN_5O_2$	365,161304	SLA-10/08/16 [(c)] ; SLA-24/08/16 [(c)] ; CHO-01/09/16 [(b)] ; CHO-15/09/16 [(b)] ; CAT-01/08/16 [(b)] ; CAT-08/08/16 [(b)] ; CAT-29/08/16 [(b)]
$C_{18}H_{28}ClN_5O$	365,197690	CHO-18/08/16 [(b)] ; CHO-01/09/16 [(b)] ; CHO-15/09/16 [(b)] ; CHO-22/09/16 [(b)] ; CAT-01/08/16 [(b)] ; CAT-08/08/16 [(b)] ; CAT-29/08/16 [(b)] ; CAT-05/09/16 [(b)]
$C_{35}H_{68}ClN_2$	551,506554	SLA-10/08/16 [(b)] ; SLA-24/08/16 [(b)] ; CHO-04/08/16 [(b)] ; CHO-01/09/16 [(b)] ; CHO-15/09/16 [(b)] ; CAT-08/08/16 [(b)] ; CAT-29/08/16 [(b)]
$C_{24}H_{36}Cl_6N_3NaO$	647,078004	SLA-10/08/16 [(b)] ; CHO-04/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_{19}H_{34}Cl_7N_{10}$	647,078211	CHO-04/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)]
$C_{21}H_{31}Cl_3N_2NaO$	647,078393	CHO-18/08/16 [(b)] ; CHO-01/09/16 [(b)] ; CHO-22/09/16 [(b)] ; CAT-

		01/08/16 [(b)] ; CAT-29/08/16 [(b)] ; CAT-05/09/16 [(b)] ; CAT-12/09/16 [(b)]
$C_{31}H_{30}ClN_4O_{19}$	781,122751	CHO-04/08/16 [(c)] ; CHO-18/08/16 [(c)] ; CHO-01/09/16 [(c)] ; CHO-15/09/16 [(c)] ; CHO-22/09/16 [(c)] ; CAT-01/08/16 [(c)] ; CAT-08/08/16 [(c)] ; CAT-29/08/16 [(c)] ; CAT-05/09/16 [(c)] ; CAT-12/09/16 [(c)]

SLA: Saint-Laurent, CHO: Chooz, CAT: Cattenom, AM: Amont, BF: Bassin froid

L'outil d'analyse de défaut de masse de Kendrick (*Kendrick Mass Defect*, KMD) est utilisé pour identifier des composés modèles, de composition élémentaire attendue, dans des spectres de masse à haute résolution de mélanges complexes [4,10]. Le concept de masse de Kendrick est de changer l'échelle de masse habituelle en une échelle de masse normalisée sur la base du groupement CH_2 (équation 1). Le KMD est alors calculé comme la différence entre la masse de Kendrick normalisée et la masse nominale observée (équation 2). Les valeurs de KMD représentent le reflet de la déviation d'une masse exacte par rapport à celles des structures homologues ne variant que par le groupement de base utilisé. En d'autres termes, les masses exactes de molécules différant d'un nombre entier de motifs CH_2 diffèrent de multiples de la masse exacte de CH_2 ; elles présentent par conséquent la même valeur de KMD. A titre d'exemple, deux composés de compositions élémentaires de $C_{20}H_{40}O_2$ et $C_{21}H_{42}O_2$ auront la même valeur de KMD. Le KMD peut être utilisé pour identifier des molécules ayant les mêmes différences de composition [4].

$$\text{Masse de Kendrick (MK)} = \text{masse observée} \times (14 / 14{,}01565) \quad (1)$$

$$\text{KMD} = (\text{masse nominale observée} - \text{MK}) \times 1000 \quad (2)$$

Des molécules peuvent différer par une autre unité de normalisation que celle de CH_2. Cette unité doit être un fragment moléculaire qui peut être ajouté ou retiré à une formule chimique sans créer de radical, telle que H_2, O, CH_2O ou X (X étant un halogène) [7,11]. Dans le cadre de ce travail, cette approche permet de vérifier si des molécules polyhalogénées sont présentes dans un échantillon d'eau.

La figure III-9 représente le diagramme de Kendrick d'un échantillon d'eau prélevé au bassin froid de Cattenom le 08/08/2016. Cette figure montre le non alignement de toutes les masses présentes (représentées par des points) selon la base du chlore, ce qui indique l'absence de sous-produits organohalochlorés contenant un écart de masse de Cl. Aucun diagramme n'a été effectué pour le brome et l'iode étant donné que le chlore est l'halogène majoritaire. Aucune molécule polychlorée n'a été détectée dans les échantillons d'eau prélevés en amont et au bassin froid des 3 CNPE.

Une nouvelle méthodologie d'analyse utilisant le concept de défaut de masse, cette fois d'ordre supérieur a été introduite par Roach *et al*, en 2011 pour l'analyse de données spectrales de masses de multiples bases

[7]. L'utilisation simultanée d'autres unités de base (par exemple O, H_2 ou halogènes) conduit à un changement systématique des défauts de masse ; elle permet par conséquent d'identifier des séries de molécules homologues d'ordre supérieur qui ont pour différence de masse des unités de base utilisées simultanément.

Le calcul de défaut de masse généralisé utilisant 3 bases différentes B_1, B_2 et B_3 est le suivant :

<u>1er ordre</u> MK_{B1} = m/z observée x (masse nominale de B_1 / masse exacte de B_1)

KMD_{B1} = (masse nominale observée - MK_{B1}) x 1000

<u>2ème ordre</u> $MK_{B1, B2} = KMD_{B1} / KMD_{B1}(B_2)$

$KMD_{B1, B2}$ = (masse nominale observée - MK CH_2) x 1000

<u>3ème ordre</u> $MK_{B1, B2, B3}$ = m/z observée x (masse nominale de CH_2 / masse exacte de CH_2)

$KMD_{B1, B2, B3}$ = (masse nominale observée - MK CH_2) x 1000

où MK est la Masse de Kendrick et KMD son défaut de masse associé (*Kendrick Mass Defect*).

Cet outil d'analyse multi-ordre permet de réaliser une investigation quant à la présence de sous-produits organohalogénés différant par trois groupements à la fois.

La figure III-10 représente le diagramme de Kendrick multi-ordre d'un échantillon d'eau prélevé au bassin froid de Cattenom le 01/08/2016. Cette figure montre le non alignement de toutes les masses présentes selon les trois bases de Cl, CH_2 et H_2 (représentées par des points), ce qui indique l'absence de sous-produits organochlorés contenant un écart de masse de CH_2 et de H_2 dans tous les échantillons d'eau prélevés en amont et au bassin froid des 3 CNPE, aucune molécule organochlorée n'a présenté ces écarts de masse, indiquant l'absence de molécules polychlorées présentant des écarts de masse de CH_2 et de H_2. Les mêmes résultats ont été obtenus en remplaçant la base de H_2 par celle de O.

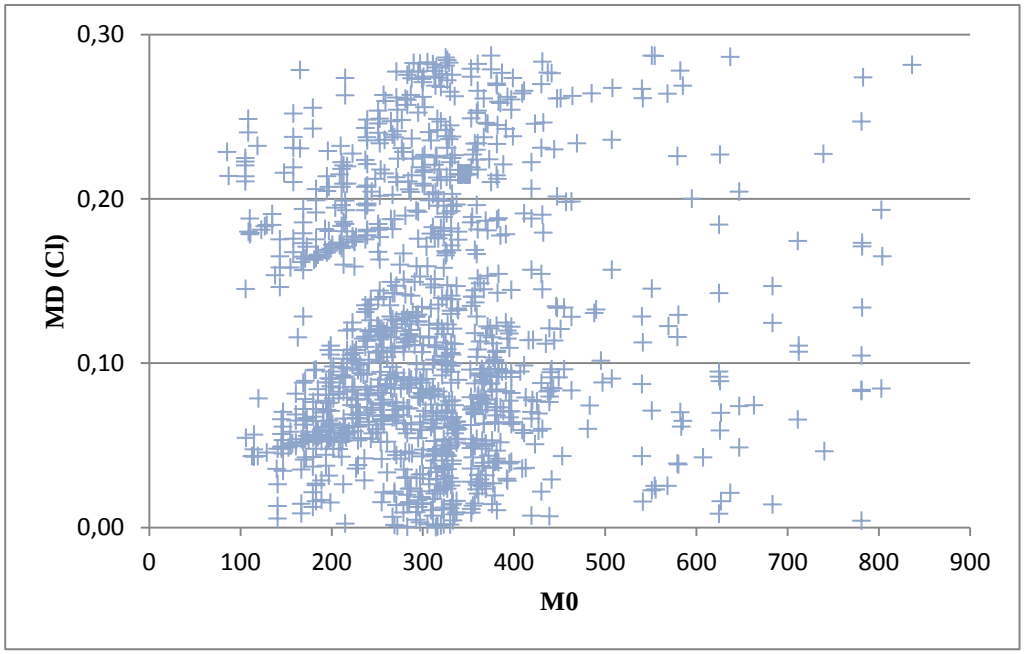


Figure III-9. Diagramme de Kendrick d'un échantillon d'eau de bassin froid de Cattenom prélevé le 08/08/2016

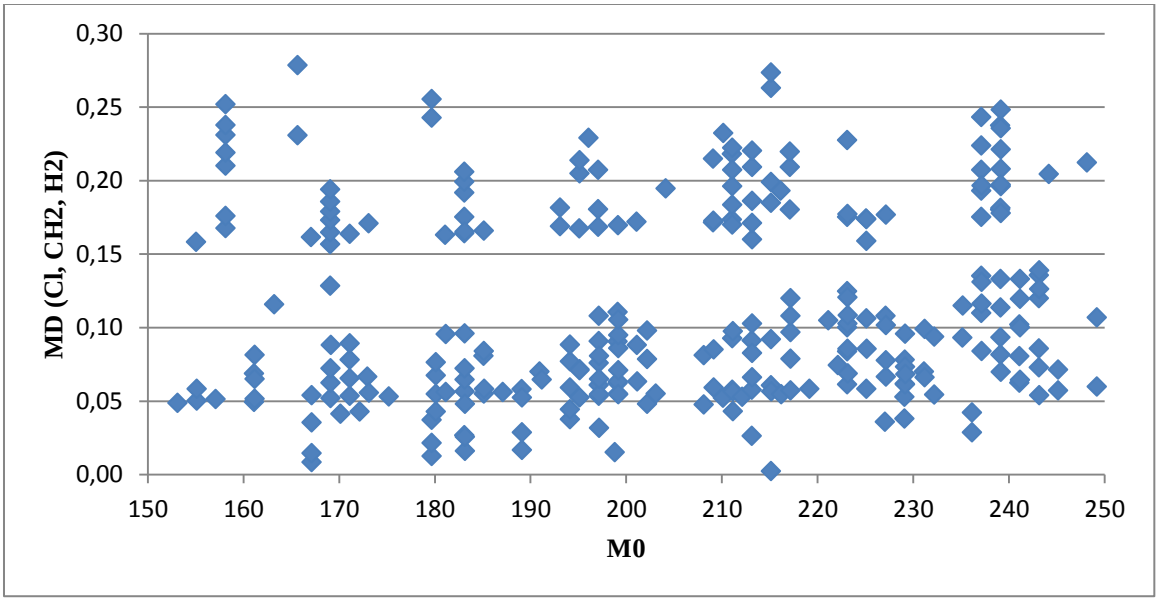


Figure III-10. Diagramme de Kendrick de 3ème ordre d'un échantillon d'eau de bassin froid de Cattenom prélevé le 08/08/2016 - m/z de 150 à 250

A l'heure du rendu de ce manuscrit, la recherche de SPOX inconnus est loin d'être aboutie. L'interprétation « manuelle » des données se poursuit mais les travaux d'identification de structures non répertoriées sont

fastidieux et s'inscrivent dans la durée. Dans ce contexte, les échantillons ont été conservés pour d'éventuelles analyses ultérieures. La complexité de la matrice pourrait nous amener à opérer un fractionnement visant à réduire les phénomènes de suppression spectrale fréquemment observés en ESI. L'acquisition récente d'une source APPI pourrait également contribuer à détecter des molécules non ou mal ionisées en ESI.

7. Bio-analyses des échantillons et extraits d'eaux des CNPE

7.1. Cytotoxicité sur cellules RTG-2 des échantillons d'eau des 3 CNPE

Les résultats des tests de cytotoxicité sur cellules RTG-2 des échantillons d'eau des 3 CNPE sont présentés dans le tableau III-20.

Tableau III-20. Cytotoxicité des échantillons d'eau collectés en amont et au bassin froid des 3 CNPE - DL_{50} ou viabilité à la dose maximale testée vis-à-vis des cellules RTG-2 en culture

Saint-Laurent				
Prélèvement	Amont		Bassin froid	
	DL_{50}	% de viabilité à 100% d'échantillon	DL_{50}	% de viabilité à 100% d'échantillon
27/07/16	-	77,85 ± 2,6	-	97,8 ± 12,3
10/08/16	-	67 ± 5,0	-	107,2 ± 6,3
24/08/16	96,7 ± 3,1	-	-	109,7 ± 2,4
07/09/16	-	108,5 ± 1,3	-	108,2 ± 7,7
Chooz				
04/08/16	-	71,5 ± 7,6	-	96,5 ± 3,6
18/08/16	99,8 ± 2,1	-	-	84,1 ± 8,4
01/09/16	-	96,8 ± 3,4	-	91,9 ± 5,3
15/09/16	-	92,8 ± 4,2	-	86,9 ± 1,4
22/09/16	-	92,8 ± 3,7	-	71,7 ± 5,9
06/10/16	100	-	-	93 ± 2,3
Cattenom				

01/08/16	-	103,23 ± 11,2	-	94,3 ± 6
08/08/16	-	67,6 ± 5,4	-	82 ± 5,3
22/08/16	-	86,9 ± 2,9	-	110,7 ± 5,7
29/08/16	-	98,9 ± 4,7	62,7 ± 1,1	
05/09/16	-	94,1 ± 3,1	-	90,8 ± 8,6
12/09/16	-	102,5 ± 5	-	94,8 ± 1,6

Les résultats présentés dans le tableau III-20 montrent que la majorité des échantillons d'eau collectés en amont et au bassin froid n'induisent pas d'effet cytotoxique significatif sur les cellules RTG-2. Le calcul d'une DL_{50} (dose létale induisant 50% de mortalité) n'est possible que pour 3 échantillons collectés en amont du site : Saint-Laurent du 24/08/16, Chooz du 18/08/16 et du 06/10/16, et pour un seul échantillon d'eau collecté en bassin froid : Cattenom du 29/08/16.

Quatre échantillons d'eau collectés en amont induisent une cytotoxicité (T-Test ; $p < 0,05$) sur les cellules RTG-2 à la concentration maximale testable (100% d'échantillon) : Saint-Laurent du 27/07/16 et du 10/08/16, Chooz du 04/08/16 et Cattenom du 08/08/16, contre un seul échantillon d'eau collecté au bassin froid : Chooz du 22/09/16. Afin de mieux comprendre l'origine de cette cytotoxicité, des tests ont été effectués sur certains sous-produits organohalogénés identifiés dans les eaux collectées en amont et au bassin froid des 3 CNPE ; les résultats correspondants sont présentés dans le tableau III-21.

Tableau III-21. Cytotoxicité des sous-produits organohalogénés modèles (DL_{50}) vis-à-vis des cellules RTG-2 en culture

Standard	**DL_{50}**
1,1-dichloropropan-2-one	3,54 µM ± 1,3
Acide chlorodibromoacétique	1,63 mM ± 1,1
Acide tribromoacétique	0,99 mM ± 1,03
Acide dichloroacétique	1,93 mM ± 1,6
Acide dibromoacétique	0,56 mM ± 1,2
Acide chloroacétique	0,51 mM ± 1,03
Acide trichloroacétique	2,12 mM ± 1,6
Acide bromochloroacétique	0,94 mM ± 1,1
Acide bromoacétique	3,6 µM ± 1,2

Le test de cytotoxicité, réalisé sur les cellules RTG-2 selon le mode opératoire précédemment décrit permet de classer les sous-produits organohalogénés modèles selon un gradient de toxicité décroissant : 1,1-dichloropropan-2-one = acide bromoacétique > acide chloroacétique = acide dibromoacétique > acide bromochloroacétique > acide tribromoacétique > acide chlorodibromoacétique > acide dichloroacétique > acide trichloroacétique.

En comparant les concentrations des sous-produits identifiés dans les eaux des 3 CNPE et leurs valeurs de DL_{50}, on constate qu'un effet cytotoxique apparent n'est observé qu'en présence de fortes concentrations des sous-produits organohalogénés identifiés. Par conséquent, la cytotoxicité de l'échantillon est probablement induite par des composés autres que ceux identifiés. A titre d'exemple, le produit le plus cytotoxique dans cette liste est le 1,1-dichloropropan-2-one, identifié dans les eaux collectées au bassin froid de Cattenom le 29/08/2016 à une concentration de 0,01 µM ; la valeur de DL_{50} de ce dernier est de 3,5 µM.

7.2 Génotoxicité des échantillons d'eau des 3 CNPE – SOS Chromotest

Les résultats des tests de génotoxicité SOS Chromotest des échantillons d'eau des 3 CNPE extraits par SPE selon les deux méthodes décrites dans les parties I et II du chapitre III (RESULTATS ET DISCUSSION) sont présentés dans les tableaux III-22 (test SOS-Chromotest sans bioactivation) et III-23 (test SOS-Chromotest avec bioactivation).

Tableau III-22. SOS *Induction Factor* sans bioactivation (-S9)

Saint-Laurent				
Prélèvement	Amont		Bassin froid	
	MTBE	MeOH	MTBE	MeOH
27/07/16	SE	SE	SE	SE
10/08/16	SE	SE	SE	SE
24/08/16	SE	SE	SE	SE
07/09/16	SE	SE	SE	SE
Chooz				
04/08/16	SE	SE	SE	SE
18/08/16	SE	SE	SE	SE
01/09/16	SE	SE	SE	SE
15/09/16	SE	SE	SE	SE
22/09/16	SE	SE	SE	SE
06/10/16	1,28 ± 0,089 à 50000 ppm	SE	SE	1,36 ± 0,09 à 50000 ppm

Cattenom				
01/08/16	SE	SE	SE	SE
08/08/16	SE	SE	SE	SE
22/08/16	SE	SE	SE	SE
29/08/16	1,34 ± 0,071 à 50000 ppm	SE	SE	SE
05/09/16	SE	SE	SE	SE
12/09/16	SE	SE	SE	SE

SE = Sans Effet

Tableau III-23. SOS *Induction Factor* avec bioactivation (+S9)

Saint-Laurent				
Prélèvement	Amont		Bassin froid	
	MTBE	MeOH	MTBE	MeOH
27/07/16	SE	SE	SE	SE
10/08/16	SE	SE	SE	SE
24/08/16	SE	SE	SE	SE
07/09/16	SE	SE	SE	SE
Chooz				
04/08/16	SE	SE	SE	SE
18/08/16	SE	SE	SE	SE
01/09/16	SE	SE	SE	SE
15/09/16	SE	SE	SE	SE
22/09/16	SE	SE	SE	SE
06/10/16	SE	SE	SE	SE
Cattenom				
01/08/16	SE	SE	SE	-
08/08/16	SE	SE	SE	1,56 ± 0,095 à 50000 ppm
22/08/16	SE	SE	SE	SE
29/08/16	SE	SE	SE	SE
05/09/16	SE	SE	SE	SE
12/09/16	SE	SE	SE	SE

SE = Sans Effet

Les résultats présentés dans les tableaux III-22 et III-23 montrent que les extraits d'échantillons d'eau collectés dans les 3 CNPE ne présentent pas d'effet potentiel génotoxique que ce soit avec ou sans activation métabolique (+S9 et -S9). Sans activation métabolique, trois extraits présentent un faible potentiel génotoxique (SIF < 1,5) mis en évidence à la plus forte concentration testée : extrait MTBE d'échantillon d'eau collecté en amont de Cattenom le 29/08/16, extrait MTBE d'échantillon d'eau collecté en amont de Chooz le 06/10/16 et un extrait MeOH collecté au bassin froid de Chooz le même jour.

Le potentiel génotoxique de ces extraits à la concentration de 50000 ppm équivaut à une concentration de NQO (génotoxique de référence sans S9) inférieure à 50 nM (concentration la plus faible testée), Avec activation métabolique, seul l'extrait MeOH d'échantillon collecté au bassin froid de Cattenom le 08/08/16 présente un potentiel génotoxique (SIF=1,56) mis en évidence à la plus forte concentration testée : ce potentiel génotoxique à 50000 ppm équivaut à celui du B(a)P (génotoxique de référence avec S9) à une concentration de 0,656 µM.

De manière générale, les extraits MeOH exercent une toxicité sur l'organisme d'essai sans activation métabolique.

Références

[1] Y, Xie, D. Reckhow, Formation of halogenated artifacts in brominated, chloraminated and chlorinated solvents, Environmental Science & Technology, 28 (1994) 1357-1360

[2] Y, Xie, Disinfection by-products in drinking water. Formation, analysis and control. 2016, pp 123-124

[3] E, B, Kujawinski, Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS): characterization of complex environmental mixtures, Environmental Forensics, 3(3-4) (2002), 207-216

[4] A, C, Stenson, W, M, Landing, A, G, Marshall, W, T, Cooper, Ionization and Fragmentation of Humic Substances in Electrospray Ionization Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry, Analytical Chemistry, 74 (17) 2002, 4397-4409

[5] M, Gonsior, M, Zwartjes, W, J, Cooper, W, Song, K, P, Ishida, L, Y, Tseng, M, K, Jeung, D, Rosso, N, Hertkorn, P, Schmitt-Kopplin, Molecular characterization of effluent organic matter identified by ultrahigh resolution mass spectrometry, Water Research, 45(9), 2011, 2943-2953

[6] P, J, Roach, Glycogen and its metabolism, Current molecular medicine, 2(2) 2002, 101-120,

[7] P, J, Roach, J, Laskin, A, Laskin, Higher-order mass defect analysis for mass spectra of complex organic mixtures, Analytical chemistry, 83(12) 2011, 4924-4929

[8] T, L, Brown, J, A, Rice, Effect of experimental parameters on the ESI FT-ICR mass spectrum of fulvic acid, Analytical Chemistry, 72(2) (2000), 384-390

[9] G, Plancque, B, Amekraz, V, Moulin, P, Toulhoat, C, Moulin, Molecular structure of fulvic acids by electrospray with quadrupole time of flight mass spectrometry, Rapid Communications in Mass Spectrometry, 15(10)(2001), 827-835

[10] C, A, Hughey, C, I, Hendrickson, R, P, Rodgers, A, G, Marshall, K, Qian, Kendrick mass defect spectrum: a compact visual analysis for ultrahigh-resolution broadband mass spectra, Analytical Chemistry, 73(19) (2001), 4676-4681
[11] L, Sleno, The use of mass defect in modern mass spectrometry, Journal of mass spectrometry, 47(2) (2012), 226-236

CONCLUSION GENERALE

Ce travail avait pour objectif d'améliorer les connaissances quant à la nature chimique des sous-produits organohalogénés constitutifs d'AOX dans les eaux de rivières traitées à la monochloramine. Sur les plans méthodologique, analytique, écotoxicologique, la synthèse des résultats obtenus permet de tirer les enseignements suivants :

Caractéristiques des AOX naturels et produits par monochloramination :

L'analyse des échantillons d'eaux prélevés en amont des CNPE étudiés a montré la présence d'AOX à des teneurs moyennes comprises entre 11,9 et 19,6 µg éq. Cl/L. Ce résultat révèle la présence de composés organohalogénés d'origine anthropique et/ou naturelle dans les eaux de rivières contribuant d'une manière importante à la concentration d'AOX mesurée dans les eaux prélevées aux bassins froids, après concentration dans le circuit de refroidissement. Concernant les échantillons d'eaux prélevés aux bassins froids des CNPE, une formation de quantités variables et non négligeables d'AOX, en moyenne entre 29,3 et 58,7 µg éq. Cl/L, a été constatée, confirmant par la suite la formation de sous-produits organohalogénés suite au traitement à la monochloramine.

Une méthode analytique utilisant le couplage C-IC pour la spéciation chimique d'AOX dans des échantillons d'eau de rivière en chlorure (AOCl), bromure (AOBr) et iodure (AOI) organiques adsorbables sur charbon actif a été développée et validée. Cette méthode a permis au premier lieu de vérifier la fiabilité de mesure d'AOX en évaluant les rendements de récupération sur des sous-produits organohalogénés modèles, et les comparer avec ceux obtenus avec la méthode « conventionnelle » par microcoulométrie. Les rendements de récupération étaient inférieurs à 100%, mais significativement supérieurs à ceux obtenus avec la méthode « classique » de mesure d'AOX. Ces rendements ont été pris en compte par la suite pour réaliser une évaluation précise du bilan de matière.

Cette méthode a été appliquée sur des échantillons d'eau de rivières brutes et traités à la monochloramine afin de spécifier et évaluer le taux d'incorporation d'halogène dans les échantillons d'eau prélevés. Cette étape a permis de démontrer la présence de composés organochlorés d'origine anthropique et/ou naturelle dans les eaux alimentant les CNPE étudiés. Leur contribution à la concentration d'AOX mesurée dans les eaux du bassin froid, après concentration dans le circuit de refroidissement, est très importante. Quant aux échantillons prélevés aux bassins froids, la proportion de chlore incorporé dans les AOX totaux a augmenté après le traitement à la monochloramine, ce qui indique la formation de sous-produits organiques chlorés. Il est à noter que la fraction chlorée d'AOX mesurée dans tous les sites étudiés contribue pour 88 à 94 % en moyenne aux AOX totaux formés dans les eaux prélevées aux bassins froids.

Une méthode de fractionnement des AOX utilisant une membrane de 1000 Da a été utilisée pour déterminer la répartition des AOX selon leur masse molaire. Dans tous les échantillons d'eau traités, les sous-produits organohalogénés inférieurs à 1000 Da représentaient 70% du total du paramètre AOX, Le rendement total en AOX après fractionnement est de l'ordre de 90%, Cette valeur est imputable à la non uniformité des pores : le seuil de coupure des membranes correspond généralement à une rétention de 90% des molécules. Ces résultats mettent en évidence le fait qu'une partie importante est constituée de composés organohalogénés peu volatiles. Ces derniers sont difficilement analysables par les techniques classiques telles que la chromatographie en phase gazeuse couplée à la spectrométrie de masse.

Spéciation des AOX naturels et générés par la monochloramine :

Deux méthodes sensibles, sélectives et spécifiques ont été développées pour la détermination d'acides haloacétiques et de 26 autres sous-produits organohalogénés connus dans des échantillons d'eau. Les résultats obtenus en appliquant ces deux méthodes sur les échantillons d'eau collectés en amont et bassin froid des 3 CNPE ont permis de déceler la présence de plusieurs sous-produits organohalogénés : l'acide monochloroacétique, l'acide dichloroacétique, l'acide trichloroacétique, l'acide dibromoacétique, l'acide tribromoacétique, le chloroforme, le bromodichlorométhane, le chlorodibromométhane et le 1,1-dichloropropanone. Malgré le nombre croissant de sous-produits organohalogénés identifiés à ce jour et la sensibilité croissante des méthodes analytiques, le bilan de matière, défini comme le pourcentage de sous-produits organohalogénés connus par rapport à la concentration en AOX, indique qu'une partie importante des sous-produits organohalogénés reste inconnue. Les bilans de matière obtenus lors de cette étude étaient inférieurs à 10%, malgré l'élargissement de la liste de sous-produits organohalogénés recherchés ainsi que l'amélioration de la sensibilité des méthodes analytiques utilisées. Ce résultat indique qu'une partie importante de sous-produits organohalogénés échappe toujours aux méthodologies analytiques classiques basées sur une recherche ciblée des molécules connues.

Les extraits d'échantillon d'eau collectés en amont et au bassin froid des 3 CNPE ont fait l'objet d'une investigation en *screening* non ciblé par GC-MS. 55 molécules au total ont pu être extraites et détectées pour l'ensemble des 3 sites étudiés. 20 molécules pour les eaux collectées à Saint-Laurent, 12 molécules pour les eaux collectées à Chooz et 18 molécules pour les eaux collectées à Cattenom. Certains de ces composés sont présents uniquement dans les eaux brutes non traitées collectées en

amont des CNPE, d'autres sous-produits sont présents dans les 3 sites étudiées. Cependant, ces sous-produits n'ont pas été caractérisés structurellement. La concentration de ces sous-produits organohalogénés est estimée à des concentrations supérieures à 0,1 $\mu g.L^{-1}$, indiquant leur contribution de manière significative à la valeur globale d'AOX. Cependant, ces informations seules ne permettent ni d'identifier ni de quantifier précisément les composés caractérisés.

En raison du manque de sensibilité du système LC-qTof/MS, les analyses en screening en LC-MS n'ont pas été effectuées. Ces derniers ont été gardés pour une analyse ultérieure.

Les extraits d'échantillons obtenus par SPE ont été analysés en haute résolution par le FT-ICR/MS. 17 sous-produits au total ont pu être extraits et détectés dans les 3 CNPE. Certains sont présents uniquement dans les eaux collectées au bassin froid. Il est à noter que seuls les composés chlorés ont été détectés. Les composés bromés et iodés n'ont pas été détectés du fait de leurs faibles concentrations dans les échantillons d'eau, confirmées par l'analyse en C-IC.

Effet cytotoxique et génotoxique des échantillons d'eau des 3 CNPE étudiées :

La majorité des échantillons d'eau collectés en amont et au bassin froid n'a pas montré d'effet cytotoxique sur les cellules RTG-2. L'attribution d'une DL_{50} n'était possible que pour 3 échantillons d'eau, collectés en amont de Saint-Laurent le 24/08/16, de Chooz le 18/08/16 et le 06/10/16, et au bassin froid de Cattenom le 29/08/16. Quatre échantillons d'eau collectés en amont induisent une cytotoxicité (T-Test ; $p < 0,05$) sur les cellules RTG-2 à la concentration maximale testable (100% d'échantillon) : les échantillons d'eau de Saint-Laurent du 27/07/16 et du 10/08/16, de Chooz du 04/08/16 et de Cattenom du 08/08/16, contre un seul échantillon d'eau collecté au bassin froid : échantillon de Chooz du 22/09/16. Afin de mieux comprendre l'origine de cette cytotoxicité, des tests ont été effectués sur les sous-produits organohalogénés modèles identifiés dans les eaux collectées en amont et au bassin froid des 3 CNPE. En comparant les concentrations des sous-produits identifiés dans les eaux des 3 CNPE et leurs valeurs de DL_{50}, on constate qu'un effet cytotoxique apparent n'est observé qu'en présence de fortes concentrations des sous-produits organohalogénés identifiés. Par conséquent, la cytotoxicité de l'échantillon n'est potentiellement induite que par des composés autres que ceux identifiés.

Pour les tests de génotoxicité, les extraits d'échantillons d'eau collectés dans les 3 CNPE montrent qu'ils ne présentent pas d'effet potentiel génotoxique, que ce soit avec ou sans activation métabolique (+S9 et -S9). Sans activation métabolique, trois extraits présentent un faible potentiel génotoxique

(SIF < 1,5) mis en évidence à la plus forte concentration testée : extrait MTBE d'échantillon d'eau collecté en amont de Cattenom le 29/08/16, extrait MTBE d'échantillon d'eau collecté en amont et un extrait MeOH collecté au bassin froid de Chooz le 06/01/16. Le potentiel génotoxique de ces extraits à la concentration de 50000 ppm équivaut à une concentration de NQO (génotoxique de référence sans S9) inférieure à 50 nM (concentration la plus faible testée). Avec activation métabolique, seul l'extrait MeOH d'échantillon collecté au bassin froid de Cattenom le 08/08/16 présente un potentiel génotoxique (SIF=1,56) mis en évidence à la plus forte concentration testée : ce potentiel génotoxique à 50000 ppm équivaut à celui du B(a)P (génotoxique de référence avec S9) à une concentration de 0,656 µM. Il est à noter que, de manière générale, les extraits MeOH exercent une toxicité sur l'organisme d'essai sans activation métabolique.

D'une manière générale, une partie importante des SPOX issus du traitement des eaux à la monochloramine demeure inconnue, conduisant à un bilan de matière insatisfaisant. Le défi majeur restant à relever à ce jour est d'identifier la partie non encore caractérisée des SPOX. Au fur et à mesure que les capacités analytiques s'accroissent, de nouveaux SPOX peuvent être identifiés. Par conséquent, la liste des SPOX visée par les méthodes analytiques ciblées développées dans le cadre de cette thèse doit être encore élargie, incluant d'autres familles de SPOX, souvent formés et identifiés à l'heure actuelle uniquement dans les eaux chlorées (halobenzoquinones, halopyrroles, halofuranones, etc.).

Les analyses de screening en GC-MS et en haute résolution par FT-ICR/MS ont permis de signaler la présence de plusieurs SPOX inconnus. Des analyses complémentaires en spectrométrie de masse en tandem sont nécessaires afin de remonter à leurs structures, et par la suite les inclure parmi les SPOX ciblés.

université PARIS-SACLAY
ÉCOLE DOCTORALE
Sciences chimiques : molécules, matériaux, instrumentation et biosystèmes

Titre : Spéciation des composés organohalogénés constitutifs d'AOX issus de la monochloramination des eaux brutes de rivières

Mots clés : AOX, composé organohalogéné, monochloramine

Résumé : Le travail de cette thèse a pour objectif de progresser sensiblement dans la spéciation chimique des composés organohalogénés constitutifs d'AOX via une démarche analytique interconnectée. Cette démarche a été appliquée sur des échantillons d'eau traités et non traités à la monochloramine. Elle a permis une amélioration du bilan de matière via la réduction d'incertitudes pendant la préparation d'échantillon, le développement des méthodes d'analyse ciblées en GC-MS permettant de doser simultanément 37 composés organohalogénés connus et la réalisation d'essais de screening non ciblé par GC-MS et en haute résolution par le FT-ICR/MS pour l'identification des composés organohalogénés inconnus.

Les résultats obtenus sur les échantillons d'eau traités et non traités à la monochloramine ont permis de révéler une contribution non négligeable des composés organohalogénés d'origine anthropique et/ou naturelle présents dans les eaux de rivières choisies à la concentration d'AOX.

Les analyses ciblées en GC-MS ont permis de révéler la présence de 16 composés organohalogénés dans les échantillons d'eau dont 9 composés identifiés d'une manière récurrente dans les 3 sites étudiés après traitement à la monochloramine.

Les investigations en screening non ciblé en GC-MS et en haute résolution par FT-ICR/MS ont permis de déceler la présence de 55 composés organohalogénés volatiles et 17 composés organohalogénés polaires inconnus dans les échantillons d'eau brutes traités et non traités à la monochloramine des 3 sites étudiés.

D'une manière générale, le bilan de matière dans le cadre de cette étude a été amélioré significativement de 1 à 29% comparativement aux études antérieures où ne dépassait pas les 5%.

Title : Speciation of organohalogen compounds constituting AOX from the monochloramination of raw river water

Keywords : AOX, organohalogen compound, monochloramine

Abstract: The aim of this thesis is to enhance significantly the chemical speciation of the organohalogen compounds constituting AOX via an interconnected analytical approach. This approach was applied to raw and monochloramined river water. It allowed an improvement of the material balance by reduction of uncertainties during the sample preparation step, the development of sensitive methods by GC-MS allowing simultaneous determination of 37 known organohalogen compounds and to perform non-targeted screening of volatile and polar unknown organohalogen compounds by GC-MS and FT-ICR/MS.
The results obtained in this study revealed a significant contribution of anthropogenic and/or natural organohalogen compounds present in the river water to the AOX concentration.

The GC-MS analyzes of monitored organohalogen compounds revealed the presence of 16 organohalogen compounds in the water samples, 9 of which were identified in a recurrent manner in the 3 sites studied after treatment with monochloramine.
Non-target screening by GC-MS and high-resolution FT-ICR/MS revealed the presence of 55 unknown organohalogen volatile compounds and 17 unknown organohalogen polar compounds in treated and non-treated raw water samples of the 3 sites studied.
In general, the material balance in this study has been significantly improved by 1 to 29% compared to previous studies where did not exceed 5%.

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