Tesis Doctoral

INNOVACIONES EN EL CONTROL DE SUBPRODUCTOS DE DESINFECCIÓN VOLÁTILES

INNOVATIONS IN THE CONTROL OF VOLATILE DISINFECTION BY-PRODUCTS

María Isabel Montesinos González

Febrero 2015

TITULO: *Innovaciones en el control de subproductos de desinfección volátiles. Innovations in the control of volatile desinfection by-products*

AUTOR: *María Isabel Montesinos González*

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Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

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Universidad De Córdoba

Departamento de Química Analítica

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LA DIRECTORA,

Fdo. **Mercedes Gallego Fernández**
Catedrática del Departamento de Química
Analítica de la Universidad de Córdoba

Trabajo presentado para aspirar al
Grado de Doctor en Química

LA DOCTORANDA,

Fdo. **María Isabel Montesinos González**
Licenciada en Química

Dña. **Mercedes Gallego Fernández**, Catedrática del Departamento de Química Analítica de la Universidad de Córdoba, en calidad de directora de la Tesis Doctoral presentada por la Licenciada en Química, Dña. María Isabel Montesinos González,

INFORMA: Que la Tesis Doctoral "INNOVACIONES EN EL CONTROL DE SUBPRODUCTOS DE DESINFECCIÓN VOLÁTILES" ha sido desarrollada en los laboratorios del Departamento de Química Analítica de la Universidad de Córdoba y del Departamento de Química de la Universidad de Ioannina y que, a su juicio, reúne todos los requisitos exigidos a este tipo de trabajo.

Y para que conste y surta los efectos pertinentes, expide el presente informe en la ciudad de Córdoba, 15 de Diciembre de 2014.

Mercedes Gallego Fernández

Mediante la defensa de esta Memoria de Tesis Doctoral se pretende optar a la obtención de la Mención de “Doctorado Internacional” habida cuenta de que la doctoranda reúne los requisitos para tal mención:

1. Cuenta con los informes favorables de dos doctores pertenecientes a instituciones de Enseñanza Superior de países distintos a España.
2. Uno de los miembros del tribunal que ha de evaluar la Tesis pertenece a un centro de Enseñanza Superior de otro país distinto a España.
3. Parte de la defensa de la Tesis Doctoral se realizará en una lengua distinta de las lenguas oficiales en España.
4. La doctoranda ha realizado una estancia de cuatro meses en el Departamento de Química de la Universidad de Ioannina (Grecia), que ha contribuido a su formación y permitido desarrollar parte del trabajo experimental de esta Memoria.

Agradezco a la Consejería de Innovación, Ciencia y Empresa de la Junta de Andalucía la concesión de una beca de Formación de Personal Investigador correspondiente al proyecto P09–FQM–4732DGI, que ha hecho posible mi dedicación a este trabajo durante cuatro años, así como la financiación recibida por parte del Ministerio de Ciencia e Innovación para el desarrollo de las investigaciones recogidas en esta Memoria encuadradas en los Proyectos "Estrategias analíticas miniaturizadas en el control de contaminantes emergentes en la desinfección del agua" (CTQ2010–17008) y "Control de subproductos de desinfección emergentes en aguas y alimentos" (CTQ2013–42701).

Además agradecer a los organismos eidA3 y ceiA3 la subvención recibida para la realización de estancias en el extranjero, requisito imprescindible para la obtención de la mención internacional en el título de doctor.

TÍTULO DE LA TESIS: INNOVACIONES EN EL CONTROL DE SUBPRODUCTOS DE DESINFECCIÓN VOLÁTILES

DOCTORANDA: Mª Isabel Montesinos González

INFORME RAZONADO DE LA DIRECTORA DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La doctoranda **Mª Isabel Montesinos González** superó con sobresaliente los estudios de Tercer Ciclo (Máster de Química Fina Avanzada, 2009-2010). La realización de la investigación recogida en la Memoria ha permitido a la doctoranda adquirir una formación extensa en el campo de la cromatografía de gases-espectrometría de masas acopladas a técnicas de espacio de cabeza estático (SHS) y micro-extracción. Se ha seleccionado como especies diana 10 THMs, 9 HNMs y 6 HANs incluidos en la fracción volátil más importante de los subproductos de desinfección del agua. Se ha desarrollado métodos miniaturizados de extracción líquida como alternativa a la extracción líquida convencional, en consonancia con una "Química Verde", para minimizar el consumo de disolventes orgánicos y el impacto ambiental.

A lo largo de estos años se ha colaborado con las dos plantas de potabilización de aguas de Córdoba. Por primera vez, se ha evaluado la influencia de las diferentes etapas de tratamiento de la planta así como de la red de distribución en la formación/eliminación de más de 40 especies que pueden generarse en el agua tras su desinfección. Se ha realizado la primera incursión bibliográfica en la detección de trihalometanos (THMs) en bebidas (zumos, néctares, refrescos, etc.); el trabajo ha tenido una gran repercusión y ha sido elegido directamente por el Editor de la revista americana para su publicación abierta a toda la comunidad. La doctoranda ha realizado una estancia de 4 meses en una universidad griega sintetizando nuevos materiales nanoparticulares con posibilidades analíticas. El trabajo de investigación se ha materializado en la publicación de 5 artículos científicos en revistas del primer cuartil del área de Química Analítica fundamentalmente y en 2 publicaciones aceptada/enviada; y 4 comunicaciones a Congresos nacionales e internacionales. La Memoria ha sido evaluada por investigadores europeos, expertos en el tema, con una calificación de Excelente.

Por todo ello, considero que la investigación desarrollada y recogida en esta Memoria, reúne a mi juicio todos los requisitos necesarios en cuanto a originalidad, innovación y calidad, por lo que autorizo la presentación de la Tesis Doctoral de **Mª Isabel Montesinos González** para que mediante la defensa de la misma opte a la obtención de la Mención de "Doctorado Internacional".

Córdoba, 16 de Diciembre de 2014

Firma de la directora

Fdo.: Mercedes Gallego Fernández

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HIPÓTESIS, OBJETIVOS E HITOS

HYPOTHESIS, AIMS AND MILESTONES

La desinfección del agua es reconocida como uno de los mayores logros en el campo de salud pública del siglo XX. Sin embargo, los desinfectantes, además de desactivar los organismos patógenos, reaccionan con la materia orgánica presente en el agua generando los llamados subproductos de desinfección (DBPs). Los DBPs formados durante el proceso de desinfección suponen un grave problema para la salud pública ya que algunos de ellos han sido clasificados como carcinógenos. Estudios epidemiológicos han revelado que estos compuestos también pueden estar relacionados con problemas de desarrollo y de reproducción asociados al crecimiento y desarrollo del feto, así como con malformaciones congénitas. Estos estudios han estado siempre centrados en los DBPs regulados, como son los trihalometanos (THMs) y los ácidos haloacéticos (HAAs) clorados y bromados en detrimento de otros DBPs emergentes no regulados como son los DBPs nitrogenados (N-DBPs), donde están incluidos los halonitrometanos (HNMs), los haloacetonitrilos (HANs), las haloacetamidas, las nitrosaminas, etc. La agencia de protección ambiental americana (U.S. EPA) ha establecido un nivel máximo contaminante de 80 o 60 µg/L para el total de 4 THMs o 5 HAAs en agua potable. Sin embargo, esta agencia no ha clasificado el potencial toxicológico de los N-DBPs, aunque distintos ensayos *in vitro* indican que estos compuestos son potencialmente más cito- y genotóxicos que los THMs. Es conveniente por lo tanto conocer las condiciones en las que estas especies se forman y para ello lo inmediato es desarrollar métodos analíticos rápidos, sensibles y robustos para la determinación de las mismas y así ampliar el conocimiento sobre estas nuevas especies en el agua potable.

En este contexto, es de señalar que no existen métodos específicos desarrollados hasta la fecha para la determinación de los 9 HNMs, ya que las escasas incursiones realizadas hasta el inicio de esta Tesis Doctoral han empleado métodos generales de VOCs clorados y que la única especie de HNM estudiada ha sido tricloronitrometano. El primer problema planteado fue la obtención de estándares de HNMs ya que la mayoría no se pueden obtener a través de las casas comerciales usuales. La síntesis de los mismos nos la hicieron en un laboratorio especializado canadiense (Orchid Cellmark; New Westminster) que posteriormente ha pasado a Cansyn (Toronto). La obtención de HNMs además de otros DBPs empleados en esta

Memoria se ha conseguido a través de estos laboratorios previa solicitud (y a precios elevados), con periodos para la síntesis de varios meses.

En base a estas premisas, el contenido de esta Memoria se orientó al diseño, desarrollo y aplicación de nuevas metodologías rápidas/miniaturizadas para la determinación de N-DBPs en aguas tratadas. Las primeras especies seleccionadas fueron los 9 HNMs clorados y bromados que pueden formarse en el agua tratada. Como se trata de especies volátiles la técnica empleada a lo largo de la Memoria fue la cromatografía de gases con espectrometría de masas (GC–MS).

Con el fin de alcanzar los objetivos establecidos, se plantean los siguientes hitos:

1. **Selección de las condiciones cromatográficas de estos compuestos.**

   Los HNMs son especies térmicamente inestables y por lo tanto el establecimiento de las temperaturas en el cromatógrafo de gases, interfase y fuente de ionización para evitar/minimizar la descomposición de los HNMs, es el primer aspecto a considerar.

2. **Evaluación de la microextracción en fase líquida con una gota para la extracción de HNMs, siguiendo la tendencia de la "Química verde".**

   Esta técnica emplea disolventes con presiones de vapor muy bajas y puntos de ebullición muy elevados (1-octanol, dodecano, diexil éter, etc.) con objeto de minimizar la evaporación del disolvente durante la extracción. Sin embargo, estos disolventes no son compatibles con las condiciones cromatográficas de temperaturas convencionales porque ensucian la columna y la fuente del espectrómetro. Por ello este hito se plantea evaluando disolventes con presiones de vapor bajas y puntos de ebullición intermedios como decano, o-xileno, 1-hexanol, etc.

3. **Desarrollo de un nuevo método rápido y robusto para la determinación de HNMs por espacio de cabeza estática (HS) acoplado a GC–MS.**

   De todas las técnicas separativas que se pueden acoplar a un cromatógrafo de gases probablemente es la HS la más simple y robusta. Aunque son especies volátiles estudios realizados por nuestro grupo han demostrado la importancia del empleo de modificadores orgánicos en la técnica HS. Se estudiará en este apartado la adición de alícuotas de disolventes orgánicos y de sales para favorecer la volatilidad de los HNMs.

4. **Evaluación de varios agentes de decloración para la conservación de los 9 HNMs en muestras de agua potable.**

   Los desinfectantes comunes usados en el proceso de desinfección interfieren en la determinación de los DBPs a menos que sean enmascarados por un agente de decloración. Un estudio riguroso de varios agentes de decloración comunes será llevado a cabo para la selección del más adecuado.

Durante el desarrollo de la Tesis Doctoral se comprueba que los métodos desarrollados para HNMs se pueden extender a otros DBPs que aunque ampliamente estudiados por estar regulados, como los THMs, pueden aportar información a la hora de hacer las aplicaciones a aguas u otras muestras. Dentro de los THMs se incluirán por su novedad los yodados no regulados que son más tóxicos que sus homólogos clorados y bromados. Además y como continuación del estudio de N-DBPs se incluyen otros DBPs nitrogenados como son los haloacetonitrilos (HANs) dado el especial interés de estas especies nitrogenadas en la última década y su similar naturaleza con los HNMs. Con la inclusión de estas nuevas familias de DBPs surgen nuevos hitos:

5. **Diseño y evaluación analítica de nuevos métodos de (micro) extracción para varias familias de DBPs volátiles: THMs, HNMs y HANs.**

   Las técnicas de microextracción descritas hasta la fecha tienen importantes ventajas bien conocidas con algunas limitaciones asociadas a la naturaleza de los extractantes (constantes de distribución bajas y con poca o nula compatibilidad con la cromatografía de gases por ser poco volátiles). Se tratará de implementar la miniaturización de la técnica LLE convencional empleando microlitros de extractante y con la ventaja de combinarla con la inyección de prácticamente todo el extracto en un inyector de volúmenes elevados con programación de temperatura.

6. **Evaluación de la influencia del tipo de desinfectante, y de los principales parámetros cualitativos del agua en la concentración y especiación de THMs, HNMs y HANs.**

   La concentración y especiación de los DBPs está afectada por muchos parámetros cualitativos del agua (pH, nitrito, nitrato, bromuro, materia órganica, etc.) así como con las condiciones operacionales de la desinfección como son el tipo de desinfectante (cloro, dióxido de cloro, cloraminas, ozono, etc.) y dosis.

7. **Aplicación de todas las metodologías implementadas (microextracción y HS) para la determinación de DBPs volátiles en aguas potables tanto de grifo como de piscina.**

   Las aguas de consumo se dividen en dos grupos: aguas no tratadas como las minerales y tratadas como las de grifo. Dentro de las aguas tratadas también se incluyen las de piscina. Ambos tipos de aguas tratadas (grifo y piscina) son objeto de estudio en esta Memoria debido a que al estar desinfectadas contienen DBPs.

8. Evaluación de la evolución de DBPs dentro de una planta potabilizadora y en la red de distribución.

En este estudio se ha requerido la colaboración de una empresa de aguas potables que ha aportado las muestras durante un año (483 muestras) y ha determinado numerosos parámetros de calidad de las aguas tomadas durante ese periodo, incluyendo parámetros adicionales a los rutinarios de la empresa.

9. Aplicación de la técnica HS para la determinación de 10 THMs en bebidas.

Finalmente y aunque fuera del contexto de esta Memoria que se centra en el análisis de aguas potables, se ha desarrollado un método para la determinación de algunos analitos dianas de la Memoria en bebidas. Estos compuestos pueden aparecer como consecuencia de la inclusión de agua potable como ingrediente o por contacto con la misma durante la elaboración de dichas bebidas. Además se incluyen otras especies halogenadas volátiles por su limitación en aguas potables además de los THMs.

Water disinfection is recognized as one of the greatest achievements in the area of public health of the XX century. Despite that, disinfectants disable pathogenic organisms, they also react with organic matter of water causing the formation of disinfection by-products (DBPs). DBPs formed during the disinfection process are a severe public health problem since some of them have been classified as carcinogens. It was revealed by epidemiological studios that these compounds may also be related to developmental and reproduction problems associated with the growth and development of the fetus, as well as congenital malformations. These studies have always been focused on regulated DBPs such as chlorinated and brominated trihalomethanes (THMs) and haloacetic acids (HAAs) at the expense of unregulated emerging DBPs, such as nitrogen DBPs (N-DBPs) including: halonitromethanes (HNMs), haloacetonitriles (HANs), haloacetamides, nitrosamines, etc. U.S. Environmental Protection Agency (U.S. EPA) has established in drinking water a permitted total concentration of 80 and 60 μg/L for the 4 THMs and 5 HAAs, respectively. However, this agency has not classified the toxicological potential of N-DBPs, despite that different *in vitro* assays indicate that these compounds are potentially more cyto- and genotoxic than THMs. A necessity arises to extend the knowledge for these new species and to know the conditions related to their formation in drinking water; in order to achieve this, the development of fast, sensitive and robust analytical methods for the determination of these compounds is critical.

In this context, it is noteworthy that it has not been developed any specific method to date for the determination of the 9 HNMs since the few contributions, until the beginning of this Doctoral Thesis, have used general methods for chlorinated VOCs, being trichloronitromethane the only studied specie of HNM. Obtaining HNMs standards was the first proposed problem since most of them cannot be supplied by usual commercial companies. Their synthesis were made by a specialized Canadian laboratory (Orchid Cellmark; New Westminster) which has subsequently changed to Cansyn (Toronto). Thus, obtaining HNMs and other DBPs employed in this Report has been achieved through these laboratories upon request (but a high prices), with synthesis times of several months.

Based on these premises, the content of this Report has been focused on the design, development and application of new fast/miniaturized methodologies for the determination of N-DBPs in treated water. The first species selected were 9 chlorinated and brominated HNMs that may be formed in treated water. These compounds are volatile species, so gas chromatography with mass spectrometry (GC–MS) has been the technique employed throughout the Report.

In order to achieve the established objectives, the following milestones are proposed:

1. **Selection of the chromatographic conditions for these compounds.**

   HNMs are thermally unstable species, thus it is firstly mandatory to set the temperatures in the gas chromatograph, as well as the interface and ion source temperatures in order to avoid/ minimize their decomposition.

2. **Evaluation of single drop microextraction to extract HNMs, following the trend of "Green Chemistry".**

   This technique employs low vapour pressure and high boiling point solvents (1-octanol, dodecane, diexil ether, etc.) to minimize their evaporation during the extraction. However, these solvents are not suitable for GC–MS since they dirty the chromatographic column and the source of the spectrometer because of the conventional temperatures reached in chromatography. Therefore, this milestone is outlined for the evaluation of solvents with low vapour pressures and intermediate boiling points, such as decane, o-xylene, 1-hexanol, etc.

3. **Development of a new, fast and robust method for the determination of HNMs by static headspace (HS) coupled to GC–MS.**

   Static headspace is the most simple and robust technique among all the separation techniques that can be coupled to a gas chromatograph. Some studies done by our group have demonstrated the significance of using organic modifiers in the HS technique despite the fact that the whole array species studied are volatile. In this section, the addition of aliquots of organic solvents and salts will be studied to favour the HNMs volatilities.

4. **Evaluation of various dechlorinating agents for the conservation of the 9 HNMs in drinking water.**

   Disinfectant species commonly used in the disinfection process interfere with the determination of DBPs unless they are masked by a dechlorinating agent. A rigorous study of various common dechlorinating agents will be carried out in order to select the most suitable one.

During the development of the Doctoral Thesis it is verified that the methods developed for HNMs can be spread to other DBPs that have been widely studied since they are regulated, such as THMs; therefore, this may provide information for the development of applications in water or other samples. Unregulated iodinated THMs will be included as they are more toxic than their chlorinated and brominated homologous. In addition to the study of N-DBPs, other nitrogenous DBPs, such as haloacetonitriles (HANs), are included due to the significant interest on nitrogenous compounds during the last decade and because of their similar nature with HNMs. New milestones arise with the inclusion of these new families:

5. **Design and analytical evaluation of new methods for (micro) extraction of various volatile DBPs families: THMs, HNMs and HANs.**

    Microextraction techniques described to date have significant advantages but with some limitations associated with the nature of the extractant (low distribution constants and compatibility with gas chromatography due to its low volatility). It will consist of implementing the miniaturization of the LLE technique which uses microlitres of extractant and allows the injection of the total extract into a large volume injector with programme temperature vaporizer which will be a further advantage.

6. **Evaluation of the influence of the type of disinfectant, and the main quality water parameters in the concentration and speciation of THMs, HNMs and HANs.**

    The concentration and speciation of DBPs is influenced by some water quality parameters (pH, nitrite, nitrate, bromide, organic matter, etc.) and disinfection operational conditions such as the type of disinfectant (chlorine, chlorine dioxide, chloramines, ozone, etc.) and dose.

7. **Application of whole methodologies implemented (microextraction and HS) for the determination of volatile DBPs in treated water, both tap and pool.**

    Drinking water is divided in two groups: untreated, such as mineral, and treated, such as tap water; being pool water included as treated. Both types (tap and pool) are included in this report since having been disinfected they contain DBPs.

8. Evaluation of the evolution of DBPs throughout a drinking water treatment plant and the distribution network.

This study has required the collaboration of a drinking water company which has provided all the samples during a year (483 samples); this company has determined many quality parameters of the water that were taken during that period, including additional parameters apart from its daily routine.

9. Application of HS technique for the determination of 10 THMs in beverages.

Finally, although besides the context of this Report, which is focused on the analysis of drinking water, a new method for the determination of some of the target analytes of the Report in beverages has been developed. The occurrence of these compounds in beverages can be explained as a result of the inclusion of drinking water as an ingredient or of the contact with it during the elaboration of those beverages. Moreover, other halogenated volatile species have been included in addition to THMs due to their limitation in drinking water.

CAPÍTULO 1

INTRODUCCIÓN

1. Desinfección del agua

El agua juega un papel primordial en el desarrollo de los seres vivos sobre la tierra, pudiéndose decir que es la base de la vida, ya que la mayor parte de estos organismos, y por lo tanto también de los seres humanos, está formado por agua. Además de su función biológica, el agua es utilizada por los hombres en multitud de usos: doméstico, comercial, industrial, agrícola y público, entre otros, siendo por tanto primordial controlar su calidad. Durante siglos el hombre ha sufrido enfermedades como el cólera, la fiebre amarilla o la fiebre tifoidea entre muchas otras, cuyo origen era mal interpretado. Sin embargo, no fue sino hasta principios del siglo XIX cuando los científicos obtuvieron un mayor entendimiento de las fuentes y efectos de los contaminantes del agua. Durante la parte final del siglo XIX y principios del siglo XX, como consecuencia de los avances científicos, los diseños de la mayoría de sistemas de tratamiento de agua para el consumo humano fueron aplicados dada la necesidad de eliminar los microorganismos que estaban causando dichas enfermedades. Pero fue sobre todo el uso del cloro como desinfectante, la clave para reducir los brotes de enfermedades en esas épocas [1].

La desinfección del agua significa la extracción, inactivación o eliminación de los microorganismos patógenos existentes. La efectividad de un proceso de desinfección se mide por el porcentaje de organismos muertos o inactivos dentro de un tiempo, y condiciones de pH y temperatura prefijados. Desde entonces, varios tipos de desinfectantes se han usado, siendo los más comunes el cloro, hipoclorito de sodio, hipoclorito de calcio, cloraminas, dióxido de cloro y ozono. Otros desinfectantes como el bromo, yodo, plata o rayos ultravioleta no son empleados de forma generalizada. Cada uno de los desinfectantes usados tiene sus ventajas e inconvenientes en función de su coste, eficacia, estabilidad o facilidad de aplicación. En general, el cloro es el desinfectante más empleado debido a su efectividad y bajo coste. En España, por ley (RD 140/2003) debe mantenerse un nivel máximo de cloro libre residual de 1 mg/L a lo largo del sistema de distribución. En cualquier caso el tratamiento aplicado debe garantizar una serie de parámetros microbiológicos y físico-químicos que hagan el agua potable apta para su consumo. Además, la constante evolución de la sociedad ha hecho

necesario una reevaluación continua de las técnicas de desinfección para garantizar un tratamiento del agua lo más completo y eficaz posible.

2. Estación de tratamiento de agua potable

Hay unos procesos generales durante el tratamiento de las aguas dependiendo de las condiciones del agua a tratar. Estos pasos son: la captación del agua bruta, tamizado y/o eliminación de gruesos, preoxidación, coagulación + floculación/decantación, filtración/adsorción y desinfección. También es posible, dependiendo del caso, la incorporación de procesos complementarios que pueden requerirse en determinadas circunstancias, como pueden ser la aireación, el ajuste de pH o el ablandamiento del agua [2].

- **Captación del agua:** No existe una forma general aplicable a todo tipo de aguas, pero este proceso debe hacerse de forma que se consiga el agua con la mayor facilidad posible, con el mínimo gasto económico y con el menor número de medios. Lo más importante a considerar es la calidad de dicha agua, ya que cuanto mayor sea su pureza, menores tratamientos habrá que realizar sobre ella.

- **Tamizado y/o eliminación de gruesos:** Es una operación física para retirar materias que por sus propiedades o tamaño, podrían dificultar el posterior tratamiento. Se efectúa en dos etapas claramente diferenciadas; en una primera etapa de desbaste se eliminan los sólidos de mayor tamaño. Esto se consigue mediante rejas y tamices de diferentes tamaños. La siguiente fase es la de desarenado que tiene como objetivo extraer del agua las partículas sólidas decantables directamente (> 200 micras) principalmente de carácter mineral. El desarenado puede realizarse conjuntamente con el desengrase que elimina las grasas, aceites y en general los flotantes antes de que pasen a la siguiente etapa.

- **Preoxidación:** Este tratamiento se utiliza para eliminar sustancias químicas (iones ferrosos y manganosos, amoniaco, nitritos o materias orgánicas oxidables) y agentes patógenos (bacterias, algas, etc.). La preoxidación es utilizada por las plantas potabilizadoras,

principalmente en las épocas del año en las que el proceso potabilizador estándar no es capaz de conseguir la calidad requerida para el agua. Por otra parte, en muchas ocasiones el proceso de preoxidación es sustituido por la utilización de permanganato potásico, que presenta una acción más eficaz frente al hierro y al manganeso.

- **Coagulación + floculación/decantación:** La coagulación + floculación tienen como objetivo sustraer las sustancias coloidales del agua, cuya estabilidad hace que no se puedan eliminar por una simple decantación. Estas sustancias coloidales cargadas, principalmente con carga negativa, se caracterizan por su gran estabilidad en disolución. Mediante la coagulación se consigue la desestabilización de dichas partículas coloidales, a partir de la neutralización de sus cargas eléctricas, produciéndose la agrupación de estas partículas mediante el contacto de unas con otras. El proceso de floculación que sigue al anterior, consiste en una agitación suave y lenta donde las partículas entran más en contacto, se unen unas a otras formando partículas mayores que son más fáciles de separar. Existen varios reactivos químicos usados industrialmente: sulfato de alúmina, cloruro férrico, policloruro de aluminio, policlorosulfato de aluminio y sales clorosulfatadas de Al e Fe. En la fase de decantación se produce la separación de dichos flóculos del agua tratada utilizando la fuerza de la gravedad, que hace que las partículas más pesadas que el agua se separen sedimentándose.

- **Filtración/adsorción:** Esta etapa se utiliza para los posibles flóculos que hayan escapado del proceso de decantación. El fundamento de los filtros se basa en el paso de una mezcla sólido-líquido a través de un medio más o menos poroso que retendrá los sólidos permitiendo el paso del líquido. Dependiendo del tamaño concreto de los sólidos con relación a los poros, la filtración podrá radicarse bien en la superficie del medio filtrante, bien en profundidad o en ambas zonas. El material filtrante más usado es la arena silícea, de diferente calidad granulométrica en función del uso

concreto. Otros materiales pueden ser carbones con distinto grado de mineralización (que también actúan como adsorbentes), tierras calizas, etc. En el caso de la adsorción se usan materiales capaces de fijar en su superficie moléculas extraídas del agua. Es un fenómeno de transferencia de masa que depende de la propia capacidad adsorbente de la sustancia concreta y de la concentración de impureza a adsorber. El adsorbente más usado en el tratamiento de potabilización de agua es el carbón activo.

- **Desinfección:** Este último proceso consiste en la utilización de un desinfectante que permita destruir los últimos microorganismos. El más usado es el cloro, aunque ya se están incluyendo desinfectantes como el dióxido de cloro, cloraminas, ozono y radiación ultravioleta entre otros. El cloro es aplicado en forma gaseosa o líquida (hipoclorito), las cloraminas se forman combinando cloro con amoniaco, el dióxido de cloro es producido por la reacción de clorito de sodio con cloro o ácido clorhídrico, el ozono es producido por descarga eléctrica a través del aire o el oxígeno y la radiación ultravioleta usando dicha radiación a ciertas longitudes de onda.

3. Subproductos de desinfección (DBPs). Formación y parámetros influyentes

Los subproductos de desinfección (disinfection by-products, DBPs) son compuestos formados por la reacción del desinfectante con la materia orgánica natural, y los iones bromuro y/o yoduro presentes del agua [3]. La primera vez que los científicos se percataron de la presencia de estos compuestos fue en la década de los 70. Rook y Bellar observaron que el cloro reaccionaba con la materia orgánica presente en el agua para formar trihalometanos (THMs). Rook en 1974 descubrió en el agua de consumo de Rotterdam que el cloro libre reaccionaba con la materia orgánica del agua formando una amplia gama de sustancias, a las cuales identificó como los primeros DBPs: cloroformo, bromodiclorometano, dibromoclorometano y bromoformo [4]. Por otra parte, en ese mismo año, Bellar descubrió la presencia de haluros orgánicos en el agua de consumo. Al analizar el agua del rio Ohio, usada para la producción de agua potable, descubrieron que el

cloroformo se hallaba a concentraciones muy bajas en el rio, pero que después de la desinfección en la planta de tratamiento aumentaban dichas concentraciones [5]. Como resultado de estos descubrimientos, nuevas investigaciones se fueron dando y actualmente en la bibliografía se pueden encontrar más de 600 DBPs [3]. Los estudios se han centrado en los DBPs regulados como son los THMs y ácidos haloacéticos (HAAs) en detrimento de otros DBPs emergentes no regulados, como son los DBPs nitrogenados (N-DBPs), dentro de los cuales están los halonitrometanos (HNMs), halocetonitrilos (HANs), haloacetamidas, etc.

Como se ha descrito antes, la formación de DBPs implica la reacción de un desinfectante con precursores orgánicos e inorgánicos. La materia orgánica natural (NOM) es una mezcla de vegetación en descomposición o sustancias húmicas, mientras que la fracción inorgánica proviene de fuentes tales como: intrusión de agua salada, agua congénita y salmueras de yacimientos petrolíferos. Además, esta reacción se ve afectada por parámetros como el pH, la temperatura y el tiempo. La formación de DBPs emergentes sigue la misma vía pero con algunas diferencias. Además de la NOM, incluyen materia orgánica de algas (AOM), material orgánico antropogénico y materia orgánica procedente de los vertidos de las plantas de tratamiento de aguas residuales (EfOM) [6]. Dentro de los precursores orgánicos es importante conocer la composición de la NOM, puesto que determina su eliminación durante el tratamiento del agua, así como su participación en la formación de DBPs. Las fracciones más hidrófobas y ácidas de la NOM son los precursores de los DBPs. Se ha encontrado que la formación de DBPs es proporcional al contenido de carbono aromático de la NOM. Por otra parte AOM y EfOM tienen contenido de nitrógeno orgánico que ha sido relacionado con los precursores de los N-DBPs. Otras fuentes como son los materiales orgánicos antropogénicos tales como los productos farmacéuticos, productos de cuidado personal, herbicidas, pesticidas, y surfactantes, que han entrado en las aguas siendo origen de las actividades humanas, han sido implicados en la formación de DBPs, ya que muchos de ellos tienen anillos aromáticos que pueden reaccionar con oxidantes [7]. En el caso de los precursores inorgánicos los iones bromuro y yoduro han sido identificados como fuente de halógenos en la formación de DBPs bromados y yodados. Los iones bromuro y yoduro presentes en muchas aguas de

origen, se pueden oxidar rápidamente a ácido hipobromoso (HOBr) y ácido hipoyodoso (IOH) durante la desinfección. HOBr y IOH pueden reaccionar con la NOM para formar DBPs bromados y yodados, de una manera que es análoga a las reacciones con el ácido hipocloroso (HOCl). La concentración y la distribución de las especies de DBPs se ven influidas por la relación de concentración de estos iones [3].

La formación de los diferentes grupos de DBPs se asocia con grupos funcionales específicos dentro de la materia orgánica, lo que ha provocado el estudio de los mecanismos de formación de los DBPs con los diferentes desinfectantes empleados. En el caso de los mecanismos de formación de los THMs hay varios tipos de precursores. Entre las estructuras fenólicas identificadas, el resorcinol se ha considerado como uno de los principales precursores de THMs [8,9]. El mecanismo de formación del cloroformo a partir de la halogenación de compuestos dihidroxi aromáticos ha sido previamente investigado [10]. En la **Figura 1** se muestra el mecanismo propuesto por Boyce y Hornig [10], que incluye una serie de reacciones comenzando por una sustitución electrofílica, seguida de la apertura oxidativa del anillo aromático, adición y descarboxilación. La reacción tiene lugar en el carbono activado no sustituido entre los dos grupos funcionales hidroxilos. Finalmente, la fragmentación de la molécula (en A) forma el trihalometano, mientras que la ruptura (en B) permite la formación de un ácido haloacético.

Figura 1. Mecanismo de cloración del resorcinol. La ruptura de la molécula en A forma $CHCl_3$ y en B origina $CHCl_3COOH$.

Sin embargo, otras estructuras como son los compuesto fenólicos, carbohidratos [11], β-dicetonas [12] y algunos ácidos carboxílicos [13] que se pueden transformar en cetoácidos tales como el ácido cítrico [14], también pueden ser responsables de la formación de THMs. En la **Figura 2**

se muestra el esquema propuesto por Navalon y col. [11] de la cloración de carbohidratos.

Figura 2. Esquema de la cloración de un carbohidrato en la formación de THMs.

Mientras que en la **Figura 3** se muestra una posible ruta de formación de THMs a partir del ácido 5,7-dioxooctanoico [13].

Figura 3. Mecanismo de cloración del ácido 5,7-dioxooctanoico.

Otros precursores son los aminoácidos, presentes en el agua por la presencia de algas. Hong y col. [15] han estudiado la cloración de veinte aminoácidos en la formación de THMs; siendo el triptófano y la tirosina los aminoácidos que originan niveles más altos de estos compuestos.

En el caso de los N-DBPs los precursores provienen de fuentes distintas, en el caso de los HNMs uno de estos precursores es el nitrito que puede oxidarse y producir compuestos orgánicos nitrados como son el nitrometano [16] y el nitrofenol [17], los cuales bajo una cloración producen tricloronitrometano (TCNM). Otros compuestos como son las aminas primarias se ha demostrado que también son precursores de estos compuestos. Joo y Mitch [18], proponen un mecanismo de formación de TCNM a partir de monometilamina (ver **Figura 4**).

Figura 4. Esquema de la formación de TCNM a partir de la cloración de monometilamina.

En este esquema se observa como la amina se diclora con posterior oxidación para formar un nitroalcano. Los halonitroalcanos se forman rápidamente a través de la adición de cloruros al anión nitronato formado por la desprotonación del nitroalcano. Sin embargo, se obtuvieron rendimientos bajos lo que indica que no es la principal vía de formación de HNMs. Otros precursores son la fracción hidrófila de la NOM como pone de manifiesto Hu y col. [19], destacando que esta fracción muestra mayores rendimientos en la formación de HNMs que las otras fracciones que componen la NOM. Además proponen una vía de formación del TCNM a partir de la cloración del ácido aspártico (**Figura 5**). Otros precursores del TCNM durante la cloraminación son los aminoácidos, dipéptidos, purinas y pirimidinas.

Figura 5. Vía hipotética de la formación de TCNM a partir de la cloración de ácido aspártico.

En el caso de HANs también se han encontrado a los aminoácidos como precursores. El dicloroacetonitrilo (DCAN) se puede formar a partir de la cloración de aminoácidos como el ácido aspártico, la tirosina y el triptófano [20]. Otros investigadores [21] han descubierto que el DCAN se forma durante la cloraminación de aminoácidos como el ácido glutámico, citosina, cisteína y el triptófano. Ellos lo achacan a que tanto el ácido glutámico, la cisteína y el triptófano contienen estructuras del tipo R'–CH_2–$CH(NH_2)$–COOH, donde el grupo funcional carboxílico (COOH) en un α-aminoácido puede sufrir fácilmente una descarboxilación. El grupo funcional R', COOH–CH_2– en el ácido glutámico, HS– en la cisteína y el grupo indol en el triptófano puede descomponerse durante la cloraminación. En el caso de la citosina, la escisión del anillo posiblemente podría generar una estructura similar a [–CH_2–$CH(NH_2)$–]. Ellos concluyen que compuestos con estructuras del tipo R_1–CH_2–$CH(NH_2)$–R_2 o que sus intermediarios clorados contengan la estructura [–CH_2–$CH(NH_2)$–] como grupo funcional tienden a generar DCAN, si R_1 y R_2 (de la estructura anterior) pueden ser retirados fácilmente. Prueba de ello es el esquema que proponen de las vías de reacción del ácido glutámico durante su cloraminación (**Figura 6**).

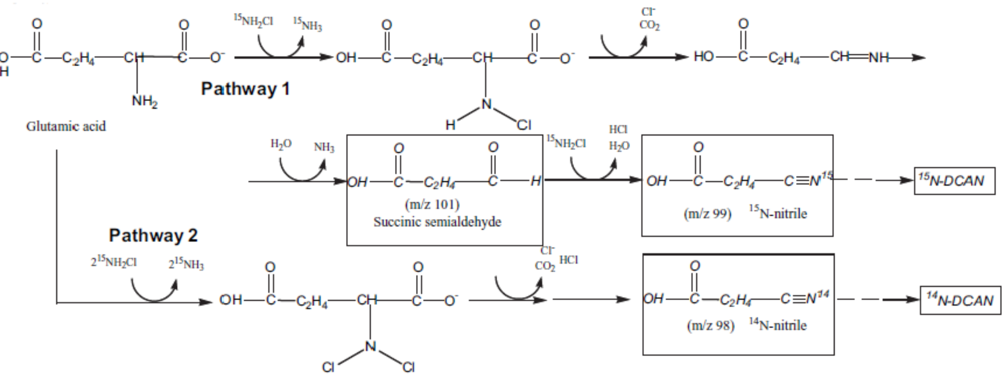

Figura 6. Vías de reacción durante la cloraminación del ácido glutámico en la formación del DCAN.

Por otra parte, Dotson y col. [6] demostraron que la formación de HANs es mayor durante la cloración de la fracción hidrófila de la NOM que de las otras fracciones. Otros investigadores [22] evaluaron la formación de DBPs durante la cloración y la cloraminación de materia orgánica procedente de algas, la cual contiene una alta cantidad de nitrógeno orgánico. En este caso los rendimientos de la formación de los N-DBPs (donde se incluyen el TCNM y el DCAN) son distintos según el proceso de desinfección, siendo la cloración la que mayor cantidad de N-DBPs produce. En la **Figura 7** se pueden ver las vías propuestas en la formación de los N-DBPs (en este caso del TCNM y del DCAN) a partir de la materia orgánica procedente de las algas en función del proceso de desinfección

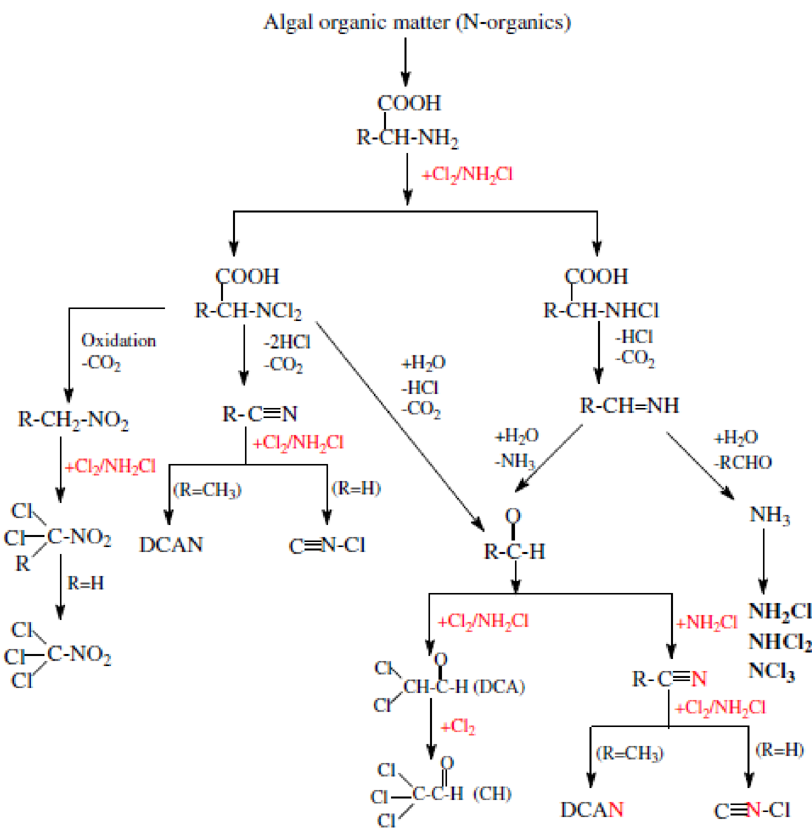

Figura 7. Diferentes vías propuestas para la formación de N-DBPs durante la cloración o cloraminación de la materia orgánica de las algas.

Como conclusión a todo lo indicado anteriormente no se conocen las vías exactas de formación de los DBPs, aunque se sabe que hay diferentes factores que afectan a la formación de estos compuestos, como son el tratamiento de desinfección así como su dosis y tiempo de contacto, la cantidad de materia orgánica, la temperatura del agua y el pH, así como el contenido de ciertas sustancias inorgánicas (bromuros, yoduros, nitritos, etc.). Un parámetro importante es la cantidad de materia orgánica, que está directamente relacionada con la formación de DBPs [23]. En el caso del pH hay discrepancias en función del grupo de DBPs, las concentraciones de THMs y HNMs incrementan [24–26] mientras que las concentraciones de HANs disminuyen [27] a medida que aumenta el pH del agua. La temperatura es otro parámetro importante, la concentración de estas tres familias de DBPs en discusión aumenta con el incremento de la temperatura [28]. Otros parámetros muy influyentes en la concentración y especiación de los distintos DBPs son el tipo de desinfectante, dosis y tiempo de reacción. La formación de los DBPs esta favorecida a mayor dosis de desinfectante y mayor tiempo de reacción [26], en el caso del tipo de desinfectante, cada desinfectante produce una gama de DBPs diferentes, dependiendo de las características del agua, de las condiciones del proceso de desinfección e incluso de la combinación de los desinfectantes usados. La cloración produce la mayor cantidad de DBPs halogenados, siendo los THMs y HAAs los más abundantes, la cloraminación produce concentraciones más bajas de DBPs (mayoritariamente THMs y HAAs), pero contribuye como fuente de nitrógeno en la formación de los N-DBPs. Por otra parte, el dióxido de cloro, es el que menor concentración de DBPs produce, y en el caso del ozono este promueve la oxidación de muchos compuestos que pueden formar DBPs [7]. Finalmente, la concentraciones de iones bromuro, yoduro y nitritos entre otros, afecta de manera significativa a la proporción y especiación de cada grupo de DBPs [25,29,30].

4. Toxicidad y normativa de DBPs volátiles

Los DBPs generados durante el proceso de desinfección suponen un grave problema para la salud pública ya que algunos de ellos han sido clasificados como carcinógenos (siendo relacionados con el cáncer de vejiga, riñón, estómago, páncreas, recto o colon) y estudios epidemiológicos han revelado que estos compuestos también pueden estar relacionados con problemas de desarrollo y de reproducción asociados al crecimiento y desarrollo del feto, así como con malformaciones congénitas [1,31–33]. El objetivo principal de la evaluación toxicológica de los DBPs ha estado siempre dirigido hacia los THMs y HAAs ya que son los DBPs predominantes en el agua potable. En este sentido, los THMs han sido considerados por la EPA como potenciales agentes cancerígenos y mutágenos, estando clasificados el cloroformo, el bromodiclorometano y el bromoformo como probables carcinógenos humanos (grupo B2, con suficiente evidencia en estudios con animales), y el dibromoclorometano como posible carcinógeno humano (grupo C, con limitada evidencia de estudios con animales) [34]. Además, la Agencia Internacional para la Investigación del Cáncer incluye al cloroformo y al bromodiclorometano dentro del grupo de sustancias consideradas como posibles carcinógenos para el ser humano (grupo 2B), aunque clasifica al dibromoclorometano y al bromoformo dentro del grupo 3 (sustancias que no pueden ser consideradas como carcinógenas por falta de evidencia experimental) [35]. Sin embargo ninguna de estas agencias ha clasificado el potencial toxicológico de los N-DBPs, aunque distintos ensayos toxicológicos *in vitro* indican que estos compuestos son potencialmente más cito- y genotóxicos que los THMs [36,37]. Aunque en lo que sí coinciden todos los estudios, es en que los DBPs yodados son mas tóxicos que sus homólogos bromados, y estos a su vez más tóxicos que sus homólogos clorados [1,3,38].

Debido a que se conoce los efectos adversos que los DBPs provocan en la salud, las autoridades competentes han establecido límites máximos para estos compuestos en reglamentaciones referidas a la calidad del agua potable. Canadá en 1978 fue el primero en establecer un valor de referencia para que los THMs totales (se entiende como concentración total de THMs a la suma de cloroformo, bromodiclorometano, dibromoclorometano y bromoformo) no excedieran de un máximo de 350 µg/L. Ya en 1979, la

agencia de Protección Ambiental de los Estados Unidos (U.S. EPA) reguló la presencia de estos compuestos en las aguas de consumo limitando su concentración máxima permitida a 100 µg/L para el total de THMs [39] y en 1984, la Organización mundial de la salud (WHO) propuso un valor máximo para el cloroformo de 30 µg/L.

Pero las nuevas investigaciones sobre la formación, la aparición y toxicidad de los THMs, así como los datos obtenidos de las concentraciones de THMs en los sistemas de distribución del agua potable, han llevado a la revisión y modificación de las normativas establecidas previamente. Así, la WHO en 1993 cambió el valor máximo para el cloroformo a 200 µg/L y en 2005 lo volvió a incrementar a 300 µg/L siendo este último el valor actual, asignado a la exposición del agua potable del 50% al 75%. Además valores máximos recomendables para las otras especies de THMs fueron publicadas por la WHO siendo 60, 100 y 100 µg/L para el bromodiclorometano, dibromoclorometano y el bromoformo, respectivamente. Posteriormente, en 1996, la U.S. EPA rebajó el límite hasta los 80 µg/L vigentes hoy en día [40]. En España, a partir del 1 de enero de 2009, el nivel máximo permitido de THMs totales es de 100 µg/L [41], mientras que los valores límite establecidos por las normativas de aguas en otros países son de 100 µg/L en Japón y 250 µg/L en Australia. Sin embargo no hay regulación similar para los N-DBPs hasta la fecha, únicamente la WHO recomienda unos valores máximos de 20 y 70 µg/L para el dicloroacetonitrilo y el dibromoacetonitrilo [42].

5. Métodos analíticos para la determinación de DBPs volátiles

Los métodos analíticos para la determinación de trihalometanos (THMs), halonitrometanos (HNMs) y haloacetonitrilos (HANs) implica tres etapas claramente diferenciadas: muestreo, extracción y separación/determinación de los analitos. En la bibliografía utilizada en este apartado, puede observarse que los métodos propuestos se han dirigido a la determinación de THMs en su gran mayoría, dado su necesario control en aguas potables. Incluso casi todas las modalidades de microextracción que se han ido desarrollando en las dos últimas décadas, en la modalidad de espacio de cabeza, han utilizado THMs como especies modelo. Las referencias a N-DBPs volátiles son escasísimas y recientes.

5.1. Muestreo

Teniendo en cuenta la elevada volatilidad de estos compuestos, deben tomarse una serie de precauciones para evitar pérdidas por evaporación así como posibles contaminaciones cruzadas. El método de la U.S. EPA 551.1 [43] indica que las muestras deben contener 100 mg/L de cloruro amónico para eliminar el cloro residual libre. Además los recipientes para recoger dichas muestras deben ser llenados hasta el borde y sin que haya burbujas para evitar posibles pérdidas. Por último, las muestras deben ser refrigeradas a 4 °C hasta el momento de su análisis.

5.2. Extracción

El objetivo de esta etapa es extraer los analitos de la matriz, para ello se debe poner en contacto con un extractante (sólido, líquido o gas) en unas determinadas condiciones de tal forma que se debiliten las interacciones analito-matriz.

5.2.1. Extracción líquido-líquido

La extracción líquido-líquido (LLE) se basa en la transferencia de un analito desde la muestra acuosa a otro disolvente líquido inmiscible con ella. La LLE ha sido la técnica más ampliamente utilizada para llevar a cabo la extracción de compuestos volátiles como son THMs, HNMs y HANs del agua. Los métodos desarrollados para la determinación de dichos

compuestos se han basado en el método oficial 551.1 propuesto por la U.S. EPA con pequeñas modificaciones [19,25,27,44–46].

Sin embargo, la LLE convencional conlleva un elevado coste y un consumo elevado de disolventes orgánicos que en su mayoría son tóxicos. Para solventar dichas desventajas, aparecen las técnicas de microextracción en fase líquida (LPME) que tienen como objetivo principal el desarrollo de métodos rápidos y efectivos, que reduzcan considerablemente el coste de la etapa de preparación de la muestra, no sólo desde el punto de vista económico sino también medioambiental. Dentro de ellas se pueden distinguir varias modalidades como se describen a continuación:

- **Microextracción en una gota de disolvente (SDME):**

Esta técnica se basa en la distribución de los analitos entre la disolución acuosa (muestra) y una microgota (comúnmente unos pocos microlitros) de un disolvente orgánico inmiscible con el agua que está suspendida en la punta de una microjeringa. Hay dos modalidades, una consiste en exponer la gota al espacio de cabeza situado sobre la muestra y la otra sumergiendo la gota en la muestra. En ambos casos los analitos migran desde la muestra hasta la gota de extractante en función de su afinidad por él. Es importante seleccionar un disolvente que tenga una alta afinidad por los analitos y baja solubilidad en agua. Las desventajas de esta técnica se deben a la inestabilidad de la gota, sobre todo por la presencia de altas concentraciones de materia orgánica y/o de partículas sólidas, o incluso si se aplican elevadas velocidades de agitación a la muestra o largos tiempos de extracción. Por tanto, la aplicabilidad de esta técnica se limita a extractos limpios mayoritariamente.

La SDME se ha utilizado para la determinación de THMs en agua. Tor y Aydin [47] han desarrollado un método en la modalidad directa seleccionando *n*-hexano como extractante, mientras Zhao y col. [48] optaron por la modalidad de espacio de cabeza empleando 1-octanol como extractante.

- **Microextracción líquido-líquido dispersiva (DLLME)**

Esta técnica permite la extracción y concentración simultánea de los analitos, su fundamento se basa en el uso de un sistema ternario de disolventes, constituido por la fase acuosa (muestra) y una mezcla de dos disolventes orgánicos, uno miscible con el agua (que funciona como agente dispersante) y otro inmiscible con el agua y miscible con el agente dispersante y de mayor densidad (que opera como extractante).

La mezcla dispersante-extractante se pone en contacto con la fase acuosa en el interior de un vial cónico, se puede observar la formación de una emulsión lo que incrementa el contacto entre las fases, favoreciéndose el paso de los analitos de la muestra al extractante. Finalmente se centrifuga y se separan las dos fases, en el fondo del vial esta el extracto (debido a la mayor densidad del extractante) y por otro lado la muestra acuosa.

Kozani y col. [49] han usado la DLLME para la determinación de THMs en muestras de agua usando disulfuro de carbono como extractante y acetona como agente dispersante obteniendo bajos límites de detección (LODs) entre 5 y 40 ng/L.

- **Microextracción en fase líquida con una gota sólida (SDLPME)**

En 2007, Khalili-Zanjani y col. [50] desarrollaron un método de LPME que se basa en usar una microgota de un disolvente que flota sobre la superficie de la muestra acuosa mientras esta se agita de forma magnética. Después de finalizar la extracción, el vial de muestra se coloca en un baño de agua fría durante unos minutos. El extracto solidificado se transfiere a un pequeño vial cónico con una espátula, el cual se funde en el vial inmediatamente a temperatura ambiente. Es importante saber que el disolvente usado como extractante debe tener un punto de ebullición alto y de fusión cercano a la temperatura ambiente (en un intervalo de 10–30 ºC), además de una presión de vapor baja. La SDLPME también ha sido usada para la determinación de THMs en muestras de agua, usando 1-undecanol como extractante [51].

- **Microextracción en fase líquida en fibra hueca (HF–LPME)**

Para solventar el problema de la inestabilidad de la gota en la técnica SDME, se introdujo la HF–LPME. Esta nueva técnica está basada en el uso de fibras huecas como soporte de fases orgánicas inmiscibles con una fase acuosa que contiene a los analitos. Estas fibras huecas son membranas porosas e hidrofóbicas, normalmente de polipropileno. En este caso el extractante se sitúa en los poros y/o en el interior de esta fibra hueca mediante una jeringa. Vora-adisak y Varanusupakul [52] utilizaron esta técnica usando 1-octanol como disolvente orgánico para impregnar los poros y llenar el interior de una fibra hueca de polipropileno con el fin de determinar THMs en muestras de agua.

5.2.2. Extracción líquido-sólido

La extracción en fase sólida (SPE) es una alternativa a la LLE ya que emplea una menor cantidad de disolvente. El fundamento de la SPE se basa en la diferente afinidad que presenta el analito (o matriz) por una fase sólida, algunos compuestos quedan retenidos en ella mientras otros pasan inalterados. Posteriormente, si los analitos de interés han quedado retenidos, éstos pueden eluirse con una pequeña cantidad de disolvente. Sin embargo, a pesar de que la SPE es una de las técnicas de extracción más empleadas en el análisis de compuestos orgánicos en aguas, son escasísimas las aplicaciones para la determinación de compuestos orgánicos volátiles debido fundamentalmente al riesgo de pérdidas por evaporación a causa de la alta volatilidad de estos compuestos.

Pero al igual que en la LLE, una serie de modalidades de SPE basadas en la microextracción de los analitos han aparecido en los últimos años en la bibliografía con el objeto de eliminar los inconvenientes asociados a la SPE. Dentro de las cuales podemos destacar:

- **Microextracción en fase sólida (SPME)**

Esta modalidad fue introducida por Pawliszyn [53] a principios de la década de los noventa y comercializada posteriormente por Supelco. Se trata de una técnica que elimina el uso de disolventes orgánicos, que además de ser caros son muchas veces nocivos y contaminantes. La SPME se basa en la extracción de los analitos utilizando una fibra de sílice fundida recubierta con una fase de un material sorbente de naturaleza

polimérica, seguida de una desorción de los analitos mediante temperatura o un disolvente orgánico. La SPME no persigue la extracción cuantitativa de los analitos sobre la fibra, sino que se establece un equilibrio entre su concentración en las distintas fases implicadas en el proceso de extracción.

La etapa de extracción puede llevarse a cabo en dos modalidades, la directa donde la fibra se expone directamente a la muestra líquida y los analitos pasan a la fibra y la de espacio de cabeza. La modalidad directa es más utilizada en el caso de analitos poco volátiles y matrices sencillas con bajos niveles de interferencias. La modalidad en espacio de cabeza (HS–SPME) expone la fibra al espacio de cabeza que hay sobre la muestra. Los analitos pasan de la muestra al espacio de cabeza y posteriormente a la fibra. Con esta modalidad se evita la retención sobre la fibra de compuestos indeseados de alto peso molecular que pueden estar presentes en la matriz. Por otro lado, posibilita la realización de tratamiento agresivos sobre la muestra (utilización de pH ácidos extremos y/o agentes oxidantes…) sin peligro de dañar la fibra. Esta modalidad es útil para la extracción de compuestos volátiles, semivolátiles o incluso poco volátiles, siempre y cuando su solubilidad en la muestra sea baja con lo que puede forzarse su paso al espacio de cabeza aumentando la temperatura o añadiendo una sal.

Esta técnica presenta una serie de ventajas frente a las técnicas de preconcentración mencionadas anteriormente ya que es muy simple, presenta un bajo coste, puede ser automatizada, requiere pequeños volúmenes de muestra y generalmente no precisa del uso de disolventes para llevar a cabo la preconcentración. Sus limitaciones están relacionadas principalmente con la baja reproducibilidad de las fibras.

La modalidad HS–SPME es la más utilizada en los últimos años para la determinación de compuestos orgánicos volátiles. Allard y col. [54] han determinado 10 THMs usando una fibra de tres fases (carboxen/polidimetilsiloxano/divinilbenceno), mientras Kristiana y col. [55] usando la misma fibra han determinado 8 HANs en muestras de agua. En el caso de extraer un conjunto más variado de estos compuestos (4 THMs, 4 HANs y TCNM), la fibra que mejor rendimientos proporcionó fue una de dos fases (carboxen/polidimetilsiloxano) [56]. Aunque en un trabajo reciente, Luo y col. [57] han propuesto de nuevo la fibra de tres fases (carboxen/polidimetilsiloxano/divinilbenceno) para la determinación de 20

compuestos que incluye 6 THMs yodados, 6 HNMs y 8 HANs en muestras de agua.

5.2.3. Extracción en fase de vapor

Las técnicas de extracción en fase gaseosa, tales como purga y trampa o espacio de cabeza estático, tienen en común el reparto de los analitos entre la matriz de la muestra y el seno de una fase gaseosa, permaneciendo en la matriz de la muestra los compuestos no volátiles de alto peso molecular. Las técnicas de espacio de cabeza pueden ser aplicadas al análisis de compuestos orgánicos volátiles o semivolátiles, además es una técnica de las más utilizadas ya que la fase extractante (aire, helio o nitrógeno) es compatible con la cromatografía de gases.

- **Espacio de cabeza dinámico**

La extracción en espacio de cabeza dinámico, también llamado purga y trampa, emplea varias etapas y permite una extracción exhaustiva y cuantitativa de los compuestos orgánicos volátiles. Al contrario que en la extracción con la modalidad de espacio de cabeza estático, los analitos volátiles no alcanzan el equilibrio entre la matriz y la fase gaseosa ya que están siendo extraidos continuamente de la muestra. La extracción consta de dos etapas, en la primera etapa el gas portador pasa a través de la muestra para purgar los compuestos orgánicos volátiles de la misma. Estos compuestos son recogidos cuantitativamente usando una trampa de material sorbente o una trampa fría. En la segunda etapa se produce la desorción térmica de los analitos retenidos en la trampa con posterior introducción en el sistema en el que serán separados y cuantificados. Las principales desventajas de esta modalidad con respecto a la modalidad estática son: la compleja instrumentación requerida, posibles interferencias por el vapor de agua, la contaminación cruzada y la generación de espuma en la superficie de la muestra.

Esta técnica ha sido comúnmente usada para la determinación de THMs tanto en muestras de agua [58–60] como en bebidas (incluyendo zumos y cervezas) [61]. En el caso de los N-DBPs, Nikolaou y col. [45] han usado esta técnica para determinar un conjunto de compuestos orgánicos volátiles, que incluyen 4 THMs, 6 HANs y TCNM, pero no consigue

recuperar 2 HANs y el TCNM. Los autores lo atribuyen a que probablemente la trampa no es capaz de retener eficazmente estos compuestos o que sufran reacciones de descomposición.

- **Espacio de cabeza estático (headspace, HS)**

En esta modalidad la muestra se introduce en un vial dejando un volumen libre sobre ella. El vial se cierra herméticamente y se introduce en un horno, de modo que los compuestos volátiles se separan de la matriz y al cabo de un tiempo se establece el equilibrio entre ambas fases. Se basa por tanto en el reparto de los analitos entre la muestra (líquida o sólida) y una fase gaseosa. Posteriormente, una alícuota de la fase gaseosa, en equilibrio termodinámico con la fase condensada, se introduce en el cromatógrafo de gases para su análisis. Los parámetros que afectan al reparto de analitos entre la fase acuosa y la gaseosa (temperatura y fuerza iónica), junto con los volúmenes de muestra y espacio de cabeza (relación de fases) tienen que ser controlados. La temperatura afecta a los compuestos altamente solubles, mientras que el volumen afecta a los menos solubles [62].

Inicialmente la introducción de la muestra se hacía de forma manual tomando una cantidad conocida de la fase gaseosa con una jeringa, pero estos sistemas son muy pocos reproducibles. Actualmente se ha automatizado mediante el uso de módulos automáticos con un sistema dosificador electroneumático. Estos sistemas poseen un brazo mecánico que introducen los viales en el horno donde se calienta la muestra a la vez que se agita de forma ligera para favorecer la transferencia de los analitos desde la muestra a la fase gaseosa. Posteriormente, una válvula de inyección de 6 vías, la cual se encuentra conectada a un bucle, recoge la fracción gaseosa que posteriormente es arrastrada por una corriente de helio hasta el cromatógrafo de gases. Este proceso ó el funcionamiento de estos automuestradores consta de 3 etapas fundamentales: equilibración, presurización y transferencia de la muestra [63].

La elección de la temperatura y tiempo de equilibración durante el desarrollo del método garantiza un procedimiento robusto. La temperatura es un parámetro muy importante, ya que a mayor temperatura menor tiempo de equilibración es necesario, porque la solubilidad de los analitos

disminuye conforme la temperatura aumenta, consiguiéndose un equilibrio entre las dos fases más rápido. Sin embargo, hay que tener en cuenta las especies termolábiles para evitar su degradación y los disolventes orgánicos presentes, ya que su evaporación puede aumentar el riesgo de explosión del vial por saturación o incluso competir con los analitos por el espacio de cabeza disminuyendo su sensibilidad. Una alternativa al aumento de la temperatura es la adición de sales para incrementar la fuerza iónica y favorecer la volatilización de los analitos más solubles. Otro factor a tener en cuenta es la relación entre las dos fases, de manera que a mayor volumen de muestra mayor aumento de la señal analítica, aunque hay que tener en cuenta el nivel máximo de muestra para que la aguja del automuestrador no entre en contacto con la muestra del vial.

Después de que se alcance el equilibrio, el gas del espacio de cabeza está listo para ser transferido al inyector del cromatógrafo de gases. El mecanismo más común para efectuar esta transferencia implica la presurización del vial con un gas inerte a través de una aguja hueca calentada, seguida de la liberación de la presión cuando el espacio de cabeza sale por la misma aguja hasta el bucle de muestra.

La última etapa es la transferencia de la muestra, en la que una corriente de helio arrastra la muestra gaseosa desde el bucle hasta el portal de inyección del cromatógrafo de gases a través de la válvula de 6 vías. La cantidad de analito que entra en el cromatógrafo de gases es proporcional al volumen del bucle que suele ser de 1–3 mL.

Esta técnica no necesita etapa de preconcentración lo que permite acortar el tiempo de análisis y simplificar el procedimiento analítico. Además no emplea disolventes orgánicos, es simple y automatizable, la suma de todas estas ventajas ha hecho que sea una de las técnicas más usadas para la determinación de compuestos orgánicos volátiles como son los THMs [9,45,58,64]. Otra modalidad propuesta más recientemente ha sido el acoplamiento del espacio de cabeza a un inyector de vaporización con temperatura programable, aumentando así más la sensibilidad [65,66]. El espacio de cabeza directamente acoplado al espectrómetro de masas ha sido usado para screening [67] o para la determinación directa de THMs [68] en muestras de agua. En el caso de los N-DBPs, Nikolaou y col. [45] han desarrollado un método de espacio de cabeza manual para la

determinación de compuestos clorados volátiles que incluye 4 THMs, 6 HANs y TCNM, pero no obtienen muy buenos límites de detección comparados con otros métodos desarrollados en el mismo trabajo. La modalidad de HS no extrae a dos especies (CAN y BAN) y ofrece LODs entre 0.5 y 20 μg/L para el resto de N-DBPs frente a la LLE con la que se obtienen LODs entre 0.007 y 0.070 μg/L extrayendo todos los N-DBPs estudiados.

5.3. Técnicas cromatográficas

La cromatografía de gases (GC) es la técnica más eficiente para el análisis de compuestos volátiles y semivolátiles, ya que ésta se adecua perfectamente a las propiedades físico-químicas de estos compuestos como son la volatilidad, estabilidad térmica y baja polaridad. La GC es una técnica de separación que se basa en la diferente volatilidad de los solutos y en la distinta capacidad de interacción de los mismos con la fase estacionaria. Su rango de aplicación se limita a sustancias térmicamente estables, ya que las muestras deben ser introducidas en la columna en fase gaseosa, lo que, en el caso de muestras líquidas requiere de un proceso previo de volatilización a altas temperatura [69]. Por lo general, está restringida a la separación de compuestos con un peso molecular menor de 1000 a una temperatura máxima de trabajo de aproximadamente 400 ºC, dentro de estos límites como se ha indicado antes la única limitación existente es la estabilidad térmica de los analitos. En general, se utilizan columnas capilares de longitudes entre 30 y 60 metros, conteniendo fases estacionarias apolares o moderadamente polares y capaces de soportar temperaturas elevadas. Para realizar la separación se inyecta una pequeña cantidad de la muestra a separar en una corriente de gas inerte a elevada temperatura, esta corriente de gas atraviesa la columna cromatográfica en donde se separaran los componentes de la mezcla.

Para la determinación de compuestos orgánicos volátiles en muestras de agua se han utilizado diferentes columnas cromatográficas. Son columnas capilares de sílice fundida recubiertas de una fase líquida. En general la fase estacionaria es de dimetilpolisiloxano (no polar) que se puede combinar con diferentes grupos fenilo o cianopropilfenilo, para alcanzar distintos grados de polaridad.

En algunas aplicaciones se ha utilizado la GC rápida. Con esta técnica es posible reducir el tiempo de análisis en una medida considerable, lo que implica un aumento de la productividad en el laboratorio. La escala de tiempo ha cambiado de 1 a 2 órdenes de magnitud y separaciones que requerían 20 min o más por cromatografía de gases convencional se pueden llevar a cabo en 20 s. Otra ventaja de esta modalidad es que permite un mayor número de repeticiones de cada muestra, las cuales se realizan en el mismo tiempo que se requeriría con la cromatografía de gases convencional. Esto proporciona un mayor número de resultados analíticos y por lo tanto una mayor frecuencia de muestreo [70]. Dentro de este contexto se pueden distinguir varias modalidades en función de que se caractericen por el empleo de columnas relativamente cortas (2–20 m), un diámetro interno de la columna más pequeño (0.03–0.25 mm), un mayor espesor de la fase estacionaria (0.5–1.4 µm) o por un programa de temperatura rápido (50–70 °C/min) [71].

La GC rápida se ha utilizado para la determinación de THMs, como es el caso presentado por Brown y Emmert [72] que usan una columna corta (VB-5: 15 m x 0.53 mm x 1.0 µm) para alcanzar una separación rápida. Con esta columna y una rampa de temperatura rápida, el tiempo de ciclo es de 7 min. Otros investigadores usando una columna convencional HP-5MS (30 m x 0.25 mm x 0.25 µm), manteniendo 10 °C durante 1.5 min con posterior elevación de la temperatura a 120 °C (40 °C/min) y manteniéndola constante 1.5 min, consiguen un tiempo de ciclo de 5.5 min [73]. Otro claro ejemplo de la utilización de esta estrategia es la usada por Chang y Her [74], quienes usando una columna capilar muy corta de DB-5MS (5 m x 0.25 mm x 0.25 µm) y manteniendo la temperatura a 50 °C durante el análisis, consiguen reducir el tiempo de ciclo a 3 min.

5.4. Técnicas de detección

En la determinación de compuestos orgánicos volátiles por cromatografía de gases se emplean detectores tales como: detector de ionización de llama, detector fotométrico de llama, detector de captura de electrones [45,47–49,52,56,58] y detector de espectrometría de masas [44–46,51,54,55,57–60,64–68], aunque son los dos últimos sin duda los más empleados en la actualidad. El detector de captura de electrones,

responde de forma muy selectiva frente a compuestos que presenten grupos con elevada afinidad electrónica, en particular halógenos y grupos nitro, ofreciendo frente a este tipo de compuestos una respuesta de 10^6–10^7 veces superior a la que muestra frente a los hidrocarburos. Por lo que este detector está indicado para la determinación de THMs, HNMs y HANs. No obstante, la espectrometría de masas es la que ofrece mayores ventajas, ya que proporciona información cualitativa y cuantitativa de manera más selectiva. Por ello, el acoplamiento cromatografía de gases-espectrometría de masas es en la actualidad la técnica más empleada en el análisis de contaminantes orgánicos volátiles en muestras de interés medioambiental [75]. Dentro de los distintos tipos de analizadores de masas (cuadrupolo, trampa iónica, sector magnético o tiempo de vuelo) el cuadrupolo es el más empleado para el análisis de contaminantes ambientales, debido fundamentalmente a que proporciona una elevada sensibilidad, una adecuada información cualitativa y unos resultados cuantitativos adecuados con un relativo bajo mantenimiento. Además, la popularidad de estos analizadores proviene de su relativamente bajo coste, simplicidad de manejo y la facilidad con la que se puede controlar electrónicamente su resolución [76].

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CAPÍTULO 2

HERRAMIENTAS ANALÍTICAS

El trabajo experimental desarrollado en esta Memoria se ha llevado a cabo gracias al uso de una serie de herramientas analíticas, entre las que se incluyen estándares y reactivos, material de laboratorio, aparatos e instrumentación de diferente naturaleza. En esta sección de la Memoria se enumeran dichas herramientas y se describen aquellas que se han considerando más relevantes.

1. Estándares y reactivos

1.1. Analitos (patrones)

Los analitos utilizados para llevar a cabo la investigación fueron de pureza analítica o superior. Los compuestos objeto de estudio en la presente Memoria se enumeran agrupados por familias, en la siguiente tabla, así como su estructura, el disolvente usado para la preparación de los estándares y la casa comercial que lo suministra.

La mitad de estos estándares no se pudieron obtener a través de las casas comerciales usuales, por lo que la síntesis de los mismos se realizaron originalmente en los laboratorios Orchid Cellmark (New Westminster) y posteriormente por Cansyn (Toronto, Canada) previa solicitud, aunque a precios elevados (~100 €/100 mg).

Compuesto	Estructura	Disolvente[a]	Casa comercial
Trihalometanos			
Triclorometano	Cl, Cl — C — H, Cl	Metanol	Sigma–Aldrich (Madrid, España)
Bromodiclorometano	Cl, Br — C — H, Cl	Metanol	Sigma–Aldrich (Madrid, España)

Dibromoclorometano	Cl Br — C — H Br	Metanol	Sigma–Aldrich (Madrid, España)
Tribromometano	Br Br — C — H Br	Metanol	Sigma–Aldrich (Madrid, España)
Dicloroiodometano	Cl I — C — H Cl	Metanol	Cansyn (Toronto, Canada)
Bromocloroiodometano	Cl Br — C — H I	Metanol	Cansyn (Toronto, Canada)
Dibromoiodometano	Br Br — C — H I	Metanol	Cansyn (Toronto, Canada)
Clorodiiodometano	Cl I — C — H I	Metanol	Cansyn (Toronto, Canada)
Bromodiiodometano	Br I — C — H I	Metanol	Cansyn (Toronto, Canada)
Triiodometano	I I — C — H I	Metanol	Sigma–Aldrich (Madrid, España)
Halonitrometanos			
Cloronitrometano	Cl H — C — NO_2 H	Acetato de etilo	Cansyn (Toronto, Canada)

Dicloronitrometano		Acetato de etilo	Cansyn (Toronto, Canada)
Tricloronitrometano		Acetato de etilo	Sigma–Aldrich (Madrid, España)
Bromonitrometano		Acetato de etilo	Sigma–Aldrich (Madrid, España)
Bromocloronitrometano		Acetato de etilo	Cansyn (Toronto, Canada)
Dibromonitrometano		Acetato de etilo	Cansyn (Toronto, Canada)
Bromodicloronitrometano		Acetato de etilo	Cansyn (Toronto, Canada)
Dibromocloronitrometano		Acetato de etilo	Cansyn (Toronto, Canada)
Tribromonitrometano		Acetato de etilo	Cansyn (Toronto, Canada)
Haloacetonitrilos			
Cloroacetonitrilo		Metanol	Sigma–Aldrich (Madrid, España)

Dicloroacetonitrilo	Cl Cl—C—CN H	Metanol	Sigma–Aldrich (Madrid, España)
Tricloroacetonitrilo	Cl Cl—C—CN Cl	Metanol	Sigma–Aldrich (Madrid, España)
Bromoacetonitrilo	Br H—C—CN H	Metanol	Sigma–Aldrich (Madrid, España)
Bromocloroacetonitrilo	Br Cl—C—CN H	Metanol	Dr.Ehrenstorfer (Augsburg, Alemania)
Dibromoacetonitrilo	Br Br—C—CN H	Metanol	Alfa Aesar (Karlsruhe, Alemania)

[a]Disolvente utilizado para la preparación del estándar.

Además, a lo largo de la Memoria se han utilizado fluorobenceno y 1,2-dibromopropano, ambos suministrados por Sigma–Aldrich (Madrid, España) como estándares internos para corregir el posible error cometido en la manipulación de la muestra y/o en la inyección manual en el cromatógrafo de gases-espectrómetro de masas. Las razones por las cuales se han seleccionado estos compuestos han sido su similitud con los analitos estudiados y su ausencia en la matriz de la muestra.

Para su correcta conservación, todos los estándares se mantuvieron en un lugar oscuro y seco, y a la temperatura que recomienda el fabricante. Disoluciones estándar de 1 g/L tanto de los analitos como de los estándares internos fueron preparadas y almacenadas en frascos de vidrio ámbar. Asimismo, distintas disoluciones de trabajo fueran preparadas diariamente en agua mineral (libre de DBPs) mediante dilución de las

anteriores. Para su correcta conservación, todas las disoluciones estándares se almacenaron en frascos de vidrio ámbar a –20 ºC.

1.2. Disolventes orgánicos

A lo largo de esta Memoria se han utilizado varios disolventes orgánicos de pureza cromatográfica para la preparación de estándares y como extractantes. Los disolventes empleados durante la realización de la Memoria fueron los siguientes:

- Acetato de etilo.
- Metanol.
- Metyl *tert*-butil éter.
- 1-Octanol.
- 1-Hexanol.
- o-Xileno.
- Decano.
- *n*-Hexano.
- *n*-Pentano.
- Ciclohexano.

1.3. Otros reactivos

- Ácidos y bases. En algunas ocasiones ha sido necesario el ajuste del pH de las muestras para favorecer los procesos de extracción y las reacciones, así como la conservación de la muestra. Para ello se han utilizado fundamentalmente ácido sulfúrico e hidróxido sódico.

- Sales. Se emplearon para favorecer el proceso de extracción, entre ellas se encuentra cloruro sódico, cloruro potásico y sulfato sódico. Esta última ha sido la sal empleada en la mayoría de los casos. Además ha sido usada como desecante para los extractos orgánicos antes de su inyección en el cromatógrafo de gases-espectrómetro de masas. Otras sales como sulfito sódico, tiosulfato sódico, sulfato amónico y ácido ascórbico, se han usado como agente declorante para eliminar el cloro residual y así evitar la formación de DBPs durante el transporte, conservación y manipulación de las muestras de agua tratada.

2. Material de laboratorio

- Microjeringas de 5 y 100 μL, esta última con punta roma.

- Micropipetas de hasta 100, 200, 1000, 10000 μL.

- Material de vidrio de diferente volumen como matraces aforados, vasos de precipitados, botes de vidrio ámbar, así como vidrio de reloj y varillas de vidrio.

- Barras agitadoras magnéticas.

- Viales de vidrio de diferente capacidad entre 2 y 20 mL (según la aplicación desarrollada) con fondo plano para la preparación de las muestras. El cierre de dichos viales se realizó mediante septa de silicona/PTFE (Supelco, Madrid) sellados o a rosca dependiendo del tipo de vial empleado.

- Botes de polietileno de 1 L para la toma de muestras de agua.

- Guantes de látex y máscara de protección.

- Contenedores de plástico de diferentes tamaños para la correcta gestión de residuos de laboratorio.

3. Aparatos

Durante el desarrollo del trabajo experimental realizado en esta Tesis Doctoral, se emplearon los siguientes aparatos e instrumentos comunes en el laboratorio analítico:

- Balanza analítica de precisión (Oahus, modelo Explorer).
- pH-metro (Crison, modelo micropH 2000).
- Vortex (Kelheim, Alemania).
- Placas calefactoras con agitadores magnéticos.

4. Instrumentación

La cromatografía de gases-espectrometría de masas ha sido la técnica empleada para la separación/determinación de trihalometanos, halonitrometanos y haloacetonitrilos a lo largo de toda la Memoria. Se han empleado tres instrumentos distintos.

- La inyección de los extractos en el método de microextracción en una gota de disolvente (Capítulo 3) y U.S. EPA 551.1 (Capítulo 3) se ha llevado a cabo empleando un cromatógrafo de gases modelo Fisons GC 8000 top acoplado a un espectrómetro de masas MD 800 Voyager de Thermo (Madrid). La inyección se realizó en modo *split*, empleando una relación de flujo de 1:20 en todos los casos.

- La inyección de elevados volúmenes en el método de microextracción líquido-líquido (Capítulo 3) y U.S. EPA 551.1 (Capítulo 3) se ha realizado en un cromatógrafo de gases HP 7890A equipado con un inyector de elevados volúmenes y de temperatura programable con cabeza sin septa G2619A, con un *liner multi-notch* desactivado (Part No. 5183-2041), en el modo de venteo del disolvente, y acoplado a un espectrómetro de masas HP 5975C (Agilent Technologies, Palo Alto, CA, USA) con detector de triple eje.

- Los experimentos llevados a cabo por espacio de cabeza estático (Capítulos 4 y 5) y U.S. EPA 551.1 (Capítulo 4) se han realizado empleando un automuestreador de espacio de cabeza HP 7694 (Capítulo 4) o G1888 (Capítulo 5) acoplado a un cromatógrafo de gases HP 6890N y un espectrómetro de masas HP 5973N de la firma Agilent Technologies (Palo Alto, CA, USA). La inyección de la muestra se ha realizado siempre a través de una línea de transferencia inerte de silicosteel que conecta el automuestreador de espacio de cabeza con el inyector del cromatógrafo de gases.

El gas portador empleado fue Helio 6.0 suministrado por Air Liquide (Sevilla) y el caudal que se utilizó fue 1.0 mL/min. La separación de los analitos se realizó empleando columnas cromatográficas, no polares de 5%-

fenil–95%-metilpolisiloxano, convencionales de distintas casas (TRB-5 de Teknokroma, SLB-5MS de Supelco, HP-5MS o HP-5MS UI de Agilent) todas de 30 m de longitud, 0.25 mm de d.i. y un espesor de fase estacionaria de 0.25 μm.

CAPÍTULO 3

Diseño de métodos de microextracción en fase líquida. Aplicación a aguas tratadas

La técnica de preconcentración tradicionalmente más utilizada es la LLE convencional pero como es bien conocido, tiene varias limitaciones relacionadas en su mayoría con el consumo excesivo de disolventes orgánicos. Siguiendo los principios de una "Química verde" se han diseñado las técnicas de LPME con vistas a simplificar los procedimientos y reducir el volumen de disolventes, no sólo desde el punto de vista económico sino también medioambiental. Dentro de este Capítulo de la Tesis se profundiza en la aplicación de la microextracción en una gota de disolvente (SDME) para la determinación de halonitrometanos (HNMs), así como el uso de la microextracción líquido-líquido (MLLE) para la determinación conjunta de trihalometanos (THMs) y HNMs en muestras de agua tratada.

El primer problema surgió en la obtención de los estándares dado la inexistencia de casas comerciales que los suministrara. El primer laboratorio que nos lo sintetizó fue Orchid Cellmark. El siguiente problema se debió al desconocimiento de estos nuevos analitos frente a las temperaturas convencionales de trabajo en el inyector, línea de transferencia y fuente de ionización del espectrómetro de masas. El estudio reveló que temperaturas entre 200 y 250 ºC en la fuente de ionización no produce descomposición de ningún HNM, sin embargo temperaturas superiores a 170 ºC en el inyector no deben usarse para evitar la descomposición de las especies trihalogenadas. Una vez establecidas las condiciones GC–MS se estudió las posibilidades que la SDME en modalidad espacio de cabeza, presentaba para la extracción de HNMs, lo cual se corresponde con la primera parte de este Capítulo. En primer lugar se estudiaron los parámetros relacionados con el tipo de extractante y volumen de la gota obteniéndose los mejores resultados con 2.5 µL de 1-hexanol. De las variables químicas las más influyentes fueron el pH de la muestra (3.0–3.5) y la adición de sal (3 g de Na_2SO_4 para 10 mL de muestra). Además se estableció la eficacia del proceso de microextracción debido a lo baja que son en las técnicas LPME. La eficiencia de la extracción del método propuesto en relación a la LLE convencional fue en términos generales del 10–20% (exceptuando 3 HNMs) la cual es bastante elevada para este tipo de técnicas. En este contexto, cabe destacar los elevados factores de preconcentración, que se consiguen, así como los bajos LODs (media, 0.5 µg/L) y alta precisión (RSD, 8%) proporcionadas. Además,

se validó con el método EPA 551.1, propuesto para compuestos orgánicos volátiles halogenados que incluye al tricloronitrometano entre ellos.

En la segunda parte de este Capítulo se aborda por primera vez una nueva modalidad de microextracción líquida que se basa en una miniaturización de la LLE convencional. Para ello se reduce el volumen de extractante a niveles de microlitros pero a diferencia de las otras modalidades LPME, la MLLE que aquí se propone tiene las connotaciones de la convencional. Así, la MLLE es exhaustiva, se alcanza el equilibrio entre ambas fases y se pueden emplear disolventes orgánicos convencionales (*n*-hexano, acetato de etilo, MTBE, etc.) con buenas prestaciones cromatográficas a diferencia de los generalmente empleados en otras modalidades de LPME (1-octanol, 1-hexanol, decano, o-xileno, etc.). Esta modalidad de microextracción se ha combinado con un inyector de temperatura programable y elevados volúmenes (PTV–LVI) en el modo *solvent vent*. En condiciones generales, el PTV se programa de modo que, en el momento en que la muestra es inyectada, el *liner* está a una temperatura inferior al punto de ebullición de los analitos y la válvula de desecho está abierta. Como consecuencia, el disolvente se elimina a través de dicha válvula mientras que los analitos, de mayor punto de ebullición, permanecen condensados en el *liner*. Una vez eliminado el disolvente, la válvula de desecho se cierra y los analitos son transferidos a la columna mediante un rápido calentamiento del *liner*. Esta modalidad presenta la ventaja de que todo el extracto puede inyectarse sin generar apenas residuos. De este modo se consigue mejorar significativamente la sensibilidad del método analítico respecto a los inyectores convencionales en los que se introduce volúmenes de muestras de 1–2 µL.

Con esta técnica se abordó la determinación conjunta de THMs y HNMs dado que son también DBPs volátiles y sobre todo dada la importancia en su control debido a que están regulados por distintos organismos y países en aguas de consumo. La selección del extractante es la clave en esta modalidad MLLE. Se ensayaron *n*-hexano, MTBE y acetato de etilo, siendo este último el seleccionado por las notables ventajas que reporta. El volumen de extractante fue de 200 µL para 9 mL de agua ya que volúmenes inferiores dificultaban la recogida del extracto. Las variables instrumentales relacionadas con el PTV–LVI también se estudiaron además

de las químicas como la influencia del pH de la muestra, tipo y cantidad de sal así como tipo de agitación y tiempo. En lo referente a la eficiencia de la extracción del proceso de MLLE en relación a la LLE convencional fue del ~85%, la cual es muy favorable teniendo en cuenta la relación muestra acuosa/extractante (~40) tan grande empleada en el proceso miniaturizado. En este Capítulo se aborda también el estudio de la influencia de diversos agentes declorantes para la conservación de aguas tratadas dadas las controversias descritas en la bibliografía, siendo solo necesaria la acidificación de la muestra en el momento de la recogida para mantener la estabilidad de estos compuestos durante 2 días a 4 ºC. Finalmente cabe resaltar la elevada sensibilidad del método debido a la combinación de la MLLE con el PTV–LVI–GC–MS obteniéndose LODs medios de 30 ng/L y RSD del 6%.

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of halonitromethanes in treated water

I. Montesinos, M.J. Cardador, M. Gallego*

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071, Córdoba, Spain

Journal of Chromatography A 1218 (2011) 2497

Abstract

As halonitromethanes (HNMs) have begun to play an increasingly important role as disinfection by-products, the development of a highly sensitive method for their analysis has become a priority. The mass spectrometric behavior of the 9 HNMs revealed that trihalonitromethanes are more unstable than di- or monohalonitromethanes under common chromatographic conditions. The absence of a comprehensive method for HNMs has given rise to the development of the first method for the whole array of these species, involving the selection of a solventless technique. Single drop microextraction in the headspace mode (HS–SDME) was selected as it is inexpensive and easy to operate. Comparative measurements through EPA liquid-liquid extraction (LLE) method for halogenated volatile compounds, show this approach to be superior to the manual LLE procedure (the average limits of detection (LODs) for the 9 HNMs were 0.5 and 1 μg/L for the HS–SDME and EPA methods, respectively), adequate precision (8.2 and 7.0% for HS–SDME and EPA methods, respectively) and does not consume excessive solvent since the total extract (~2 μL) was injected completely into the GC–MS instrument. The method was used to measure HNMs in treated water and the results were compared to the EPA method in parallel.

1. Introduction

Water is a scarce commodity and a fundamental resource for the human being since it plays a decisive role in health. Since the end of the XIX century, water disinfection has been used to reduce the incidence of illnesses. Chemical disinfectants (chlorine, chloramines, ozone, chlorine dioxide, etc.) are effective in killing harmful microorganisms in drinking water, but they also oxidize organic matter that forms disinfection by-products (DBPs) [1–4]. Although more than 600 DBPs have been reported in the literature, only 11 are currently regulated [1,5]. Among the unregulated DBPs, the 9 halonitromethanes (HNMs) receive special attention because of their potentially high toxicity and their occurrence in final waters at some treatment facilities [6]. Chloropicrin (trichloronitromethane, TCNM) has been the most commonly measured example in this class followed by bromonitromethane (BNM) and bromopicrin (tribromonitromethane, TBNM), which are a potential concern for toxicity [7,8]. Average concentrations in treated water containing bromide [9] have been reported between 0.1 and 10 μg/L for some HNMs and between 0.9 and 1.5 μg/L for TCNM in wastewater treatment plant effluents [10]. Despite the increasing amount of the literature on HNMs, there has been little systematic research reported on a whole array of HNM species due to the lack of commercial chemical standards in all the species, which only became available in the early 2000s. For this reason it has only recently become possible to establish the formation and speciation characteristics of HNMs as well as the factors controlling their formation in drinking waters [10–12]. In these studies, liquid-liquid extraction has been employed as a preliminary step, using the EPA methods proposed to determine halogenated volatile organic compounds (VOCs) in water [13], in order to determine some HNMs by gas chromatography–electron capture detection (GC–ECD) [9–12] or by GC mass spectrometry (MS) [14]. Other alternatives for VOCs (including TCNM) such as solid phase microextraction (SPME) with GC–MS and Purge & Trap-GC–MS [15] have also been used. Chloropicrin or TCNM (the first HNM identified as a DBP) in a mixture of other chlorine VOCs, prepared with distilled water, has been determined for headspace (HS)–SPME–GC–ECD between 0.1 and 2.5 μg/L [16] and by HS and manual injection into a GC–MS [17] or GC–ECD [18] with limits of detection (LODs) of 0.5 or 2.5 μg/L,

respectively. However, these methods heat the samples in the injector at 175–250 ºC, which favor up to 50% decomposition of TCNM [14]. In summary, neither have proper methodologies been developed for the 9 HNMs nor has any study of the chromatographic temperatures of the GC–MS been found to minimize/eliminate the decomposition of the 9 compounds. Only BNM and TCNM have been found in the NIST (No. 69) or Wiley spectral library database, although mass spectral ions of the 9 HNMs have been reported in the bibliography [6].

As outlined above, to date the EPA methods (for halogenated VOCs) using GC to determine some HNMs in water require liquid-liquid extraction with methyl tert-butyl ether (MTBE) which implies great solvent consumption and cost. To overcome such problems, recent research activities have been oriented toward the development of miniaturized sample preparation techniques like SPME [19] and liquid phase microextraction (LPME) [20–23]. In DBPs, LPME has been used for the determination of trihalomethanes (THMs) and more recently for haloacetic acids [24] but never for HNMs. In order to find precedents applying LPME methods to HNMs, we have to refer to the 4 THMs which are also volatile DBPs. Two similar methods based on single drop microextraction (SDME)–GC–ECD are proposed for the 4 THMs with similar LODs (0.2–0.4 μg/L) using 1-octanol [25] or *n*-hexane [26] as extractant. Direct hollow fiber (HF)-LPME–GC–ECD uses 25 μL of 1-octanol at 35 ºC, providing LODs of 0.01–0.2 μg/L [27]. The most recent method to determine the 4 THMs by LPME–GC–MS [28] is based on the solidification of a floating organic microdrop (7 μL of 1-undecanol) with enrichment factors up to 480-fold (LODs, 0.03–0.08 μg/L), but it requires drastic extraction conditions (15 min at 60 ºC) which are related to low recoveries.

Taking into consideration the foregoing, the aims of this work have been: (i) to propose the first method for the whole array of HNM species in water because 7 HNMs, although still very expensive, are now commercially available (in addition to the 2 HNMs, TCNM and BNM, that have always been on the market); (ii) to develop a solventless technique in which only one drop of an organic solvent is employed, as occurs in LPME; (iii) to avoid/minimize the decomposition of HNMs during heating in the injection port of GC and/or hot transfer line/ion source of the MS, which can complicate their identification in treated water; and (iv) to obtain enough

sensitivity to determine the 9 HNMs at ng/L levels in treated water samples. The proposed HS–LPME–GC–MS method consists of a simple and fast extraction stage using a microdrop of organic solvent at the tip of a commercial microsyringe to extract the 9 HNMs from the water sample under soft conditions. The method is nearly solvent-free since the total extract was injected into the GC–MS instrument. For the first time a rigorous study has been tackled on the impact of 9 HNM decomposition in the injection port of the gas chromatograph as well as of the ion source of the mass spectrometer on the mass spectra for all 9 HNMs, since only four of them had been studied previously.

2. Experimental

2.1. Chemicals

Chloronitromethane (CNM, 90–95%), dichloronitromethane (DCNM, 95%), bromochloronitromethane (BCNM, 85–90%), bromodichloronitromethane (BDCNM, 90–95%), dibromonitromethane (DBNM, 90%), dibromochloronitromethane (DBCNM, 90–95%) and tribromonitromethane (TBNM, 90–95%) standards were supplied by Orchid Cellmark (New Westminster, Canada), while trichloronitromethane (TCNM, 99%), and bromonitromethane (BNM, 90%) were purchased from Sigma–Aldrich (Madrid, Spain) and the internal standard, fluorobenzene, from Fluka (Madrid, Spain). The solvents, 1-octanol, o-xylene, decane and 1-hexanol were purchased from Sigma–Aldrich. Ethyl acetate, methyl tert-butyl ether (MTBE) and sulfuric acid were supplied from Merck (Darmstadt, Germany). Potassium chloride, sodium chloride, anhydrous sodium sulfate, anhydrous magnesium sulphate and ammonium sulfate (dechlorinating agent) were purchased from Panreac (Barcelona, Spain). Stock standard solutions containing 1 g/L of individual halonitromethane and cumulative solutions (0.1 g/L) were prepared in ethyl acetate and stored frozen in amber glass vials at –20 °C. More dilute cumulative solutions were prepared daily in mineral water (free of DBPs) at the microgram per liter level.

2.2. Apparatus

Sample analysis was performed with a Fisons 8000 GC instrument interfaced to a Voyager mass spectrometer and controlled by a computer running MASSLAB software (Thermo, Madrid, Spain). The gas chromatographic separation was achieved on a 30 m × 0.25 mm i.d., 0.25 μm film TRB-5 capillary column coated with a stationary phase of 5%-phenyl–95%-methylpolysiloxane and supplied by Teknokroma (Barcelona, Spain). All injections were made in the split mode (1:20 split ratio) by setting the injector temperature at 170 °C. The GC oven temperature program was: 40 °C (3 min) and then raised at 40 °C/min to 140 °C (2 min) and 180 °C (3 min). The helium carrier gas (6.0 grade purity, Air Liquid, Seville, Spain) was set at 1 mL/min. The mass spectrometer was used in the following conditions: ion source temperature, 200 °C; transfer line temperature, 200 °C; electron impact ionization mode, 70 eV; scan range from *m/z* 30–255; time for solvent delay, 2 min. Optimization experiments were conducted in total ion chromatography (TIC) mode at 3.5 scans/s. The ions selected for identification and quantification of HNMs (SIM mode) are listed in **Table 1**; *m/z* values for fluorobenzene (IS) were: 50, 70, 96 (base peak).

2.3. Sample collection and preservation

Water samples were collected in amber glass bottles of 125 mL with poly(tetrafluoroethylene) screw caps. The bottles, containing 1.7 g of ammonium sulfate as the quenching reagent of residual chlorine [29], were completely filled to avoid evaporation of volatile compounds. To validate the sampling protocol for the analysis of HNMs, the storage time of the sample at 4 °C was studied using mineral water fortified with 5 μg/L of HNMs (except to TBNM, 10 μg/L). The studies were conducted over 10 days; the results indicated that the concentrations of CNM, DCNM, TCNM, BNM and BCNM remained constant for 7 days, whereas DCBNM, DBNM, DBCNM and TBNM only for 1 day. Thus, samples were stored at 4 °C and analyzed within 1 day of collection. For analysis, 10 mL of water sample (prepared as described below) was placed in 15 mL glass vials.

2.4. HS–SDME–GC–MS procedure

A 5 µL GC microsyringe model 87925 from Hamilton (Teknokroma, Barcelona) was used to perform the SDME experiments. Ten milliliter water samples or mineral water containing between 0.2 and 300 µg/L of each halonitromethane and 20 µg/L of fluorobenzene (IS) were placed in a 15 mL glass vial containing 3 g (2.1 mol/L) of Na_2SO_4 and the pH was adjusted at ~3.2 by adding 30 µL of 0.1 mol/L H_2SO_4. A stirring bar (1.3 cm long) was added to the vial, which was closed immediately with a screw cap equipped with a silicon septum. Afterward the vial was stirred in a vortex mixer for 2 min in order to dissolve the salt and then placed in a water bath. A 2.5 µL volume of 1-hexanol was withdrawn into the microsyringe, the needle tip was inserted through the silicone septum and the 2.5 µL drop of extractant exposed to the headspace of the sample stirred at 600 rpm for 20 min at 30 ºC. After extraction, the drop was retracted back into the microsyringe and the total extract (~2 µL) injected into the GC instrument.

2.5. LLE procedure (EPA Method 551.1)

Liquid-liquid extraction for the determination of HNMs in water was performed in triplicate following the EPA method 551.1 [13] proposed for the determination of halogenated VOCs. Samples were collected in 62 mL amber bottles with a poly(tetrafluoroethylene) screw cap containing 0.8 g of ammonium sulfate and without headspace to avoid evaporation of VOCs. A 12 mL aliquot was withdrawn from the sample bottle and discarded and the pH was adjusted at 4.5–5.5 with diluted H_2SO_4. Fifty µicrolitre of a 10 mg/L standard solution of fluorobenzene (IS), 3 mL of extracting solvent (MTBE), 20 g of Na_2SO_4 and 1 g of copper sulfate were added to the remaining sample (50 mL) and the vial was stirred for 4 min; once the HNMs were extracted, the vial was left to stand for 2 min in order to separate both phases. Then, 1 mL of the upper MTBE layer was transferred to a 2 mL glass vial and 0.1 g of sodium sulfate was added to dry the extract. Finally 2 µL of the extract was injected into the GC–MS instrument.

3. Results and discussion

Gas chromatography/mass spectrometry (GC–MS) has been the primary analytical tool used to identify DBPs in drinking water. A few trihalomethyl compounds partially decompose in the injection port of GC (forming mainly haloforms) or the GC–MS transfer line (the resulting mass spectra are a mixture of the native compound and decomposition products) [14]. Among LPME techniques, SDME is the most popular because it is inexpensive, does not require any equipment and is easy to operate; also the headspace mode provides the best resolution for VOCs [30]. Factors that influence extraction efficiency should be established, such as the organic solvent, sample pH, salting-out effect, and physical parameters.

3.1. Gas chromatography/mass spectrometry optimization conditions

Instrumental and analytical conditions can have a significant effect on determining halonitromethanes. The HNMs are thermally unstable and can decompose under temperatures commonly used in the injection port, hot transfer line and in the ion source during GC–MS analysis. A study of the behavior of some HNMs (mainly bromopicrin) in GC–MS analysis is carried out by Chen et al. [14]. To date there is no information either on the influence of temperatures on the GC–MS in the determination of mono and dihalonitromethanes or on the mass spectrometer ion source temperature for the 9 HNMs. That is why this paper embarks on a rigorous study of the influence of the temperature in the GC injection port and the mass spectrometer ion source. For this purpose, 1 µL of a standard solution containing 50 µg/mL of each HNM in ethyl acetate was injected into the GC at different injection port temperatures between 150 and 250 °C. This parameter affects trihalonitromethanes in a different way than it does mono and dihalonitromethanes. In fact, trihalonitromethanes decompose above 170 °C, their peak areas at 250 °C were 45% relative to values obtained at 150 °C, which is in agreement with the above study [14]. The major decomposition products are haloforms, which are probably formed by hydrogen abstraction from solvents due to the trihalomethyl radical. On the other hand, neither mono- nor dihalonitromethanes decomposed in the interval of temperatures assayed. This can be observed clearly in **Figure 1**

where the peak areas of trihalonitromethanes decreased at a GC injection port temperature of 250 °C relative to 170 °C, whereas for mono- and dihalonitromethanes the analytical signals remain constant in both instances. This fact can hinder the identification and quantification of other DBPs also present in treated water, such as THMs. For example, the formation of chloroform and bromoform as the main decomposition products of TCNM and TBNM, respectively (with GC injection port temperatures above 170 °C) could contribute to overestimations of chloroform and bromoform concentrations in treated water samples, possibly allowing the presence of TCNM and TBNM to go undetected in the original drinking water.

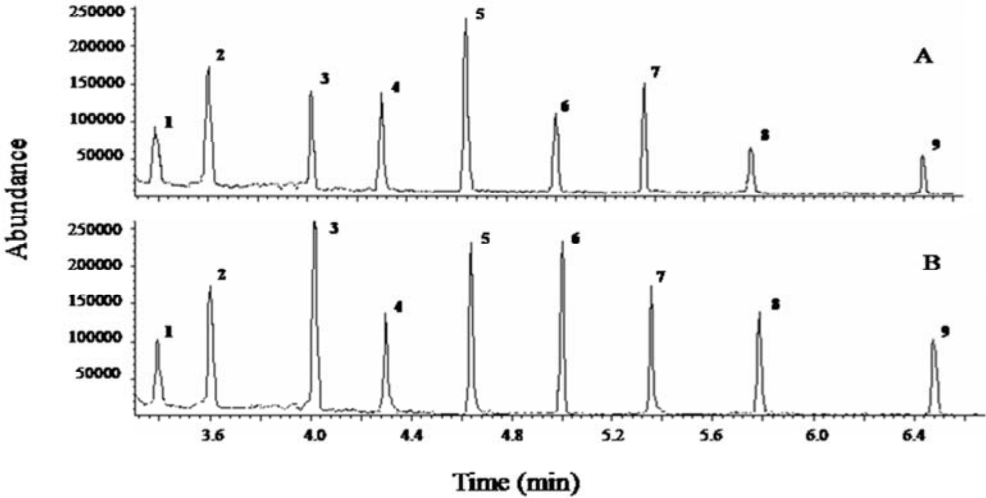

Figure 1. GC–MS total ion chromatograms for the 9 HNM standards using different injection temperatures (A) 250 °C and (B) 170 °C. Peak identification: CNM (1); DCNM (2); TCNM (3); BNM (4); BCNM (5); BDCNM (6); DBNM (7); DBCNM (8); TBNM (9).

In the present study the transfer line of the GC–MS was heated to a temperature (200 °C) similar to the highest temperature in the GC program (180 °C). Thus, only the effect of the mass spectrometer ion source temperature was checked for the 9 HNMs in the range 200–250 °C. None of the 9 species showed a decrease in the peak area, nor were halomethanes detected. So, it can be concluded that there was no evidence of decomposition in the 9 compounds up to 250 °C (since their mass spectra

confirmed their identities), although they probably could decompose at higher temperatures. In conclusion, the selected temperatures were 170 ºC for the injection port and 200 ºC for both the transfer line and the ion source of the mass spectrometer, to avoid/minimize HNM decomposition.

Finally, after obtaining the mass spectra in the best chromatographic conditions for the 9 HNMs, the most significant ions for unequivocal identification were selected. For this purpose, the criteria employed were sensitivity (selecting the most abundant peak, base peak) and selectivity (selecting the characteristic ions of each compound). **Table 1** shows the three ions selected for the identification of HNMs (quantification one in boldface print), their relative abundance and their corresponding fragments.

Table 1. Mass spectral ions selected for identification and quantification (boldfaced) of halonitromethanes

Compound	Mol wt	*m/z* (relative abundance) → [fragment ion]
CNM	95	49 (100) → $[CH_2Cl]^+$, 51 (42) → $[CH_2Cl]^+$, 46 (12) → $[NO_2]^+$
BNM	139	93 (100) → $[CH_2Br]^+$, 95 (95) → $[CH_2Br]^+$, 46 (10) → $[NO_2]^+$
DCNM	129	83 (100) → $[CHCl_2]^+$, 85 (71) → $[CHCl_2]^+$, 46 (8) → $[NO_2]^+$
DBNM	217	173 (100) → $[CHBr_2]^+$, 171 (67) → $[CHBr_2]^+$, 46 (10) → $[NO_2]^+$
BCNM	173	129 (100) → $[CHClBr]^+$, 127 (87) → $[CHClBr]^+$, 46 (9) → $[NO_2]^+$
TCNM	163	117 (100) → $[CCl_3]^+$, 119 (96) → $[CCl_3]$+, 46 (1) → $[NO_2]^+$
TBNM	295	251 (100) → $[CBr_3]^+$, 253 (98) → $[CBr_3]^+$, 46 (6) → $[NO_2]^+$
BDCNM	207	163 (100) → $[CCl_2Br]^+$, 161 (70) → $[CCl_2Br]^+$, 46 (20) → $[NO_2]^+$
DBCNM	251	207 (100) → $[CClBr_2]^+$, 209 (77) → $[CClBr_2]$+, 46 (11) → $[NO_2]^+$

None of the halonitromethanes show molecular ions in their mass spectra and their base peaks correspond to molecular weight less 46 Da, which is a consequence of losing a nitro group $[M–NO_2]^+$ [6]. In the ion source, when an electron impacts on a neutral molecule, the molecule is ionized and gives off an extra electron. When a molecule loses an electron, it acquires a positive charge and an unpaired electron and therefore the ion becomes a cation-radical. When the atom is highly electronegative, it will tend to gain the electron and will remain as a radical. The nitro group and the halogens are electronegative both capturing the electron to form

radicals. The mechanism of fragmentation of these compounds (TCNM as model) is as follows:

$$[Cl_3CNO_2] + e^- \rightarrow [Cl_3CNO_2]^{+\bullet} + 2e^-$$

$$[Cl_3CNO_2]^{+\bullet} \rightarrow [Cl_3C]^+ + NO_2^\bullet$$

$$[Cl_3C]^+ \rightarrow [Cl_2C]^{+\bullet} + Cl^\bullet$$

$$[Cl_2C]^{+\bullet} \rightarrow [ClC]^+ + Cl^\bullet$$

Taking into account that the remaining fragments contained different combinations of halogen atoms (chlorine and/or bromine) which presented specific mass spectra due to their isotopic abundance ratios [31], the fragments selected for the unequivocal identification of each compound were chosen based on the different isotopic signals provided for each analyte. Chlorine and bromine atoms have two stable isotopes of 35 and 37 amu, and 79 and 81 amu, respectively. Thus, molecules that contain chlorine and/or bromine atoms provide M+2 peaks related with their isotopes. By way of example, for the identification of trichloronitromethane (whose electron ionization mass spectrum appears in **Figure 2**), the *m/z* ratios 117 (100% abundance) and 119 (96% abundance) were selected because the three chlorine atoms in the fragment could be identified due to the isotopic relative abundance of both ions. The nitro group (*m/z* 46) was selected as the third fragment ion for the identification of halonitromethanes in spite of its low abundance since this *m/z* ratio was specific for nitro derivatives suggesting the presence of a NO_2 group, so it could be used for the unequivocal identification of HNMs versus THMs and other halogenated hydrocarbons.

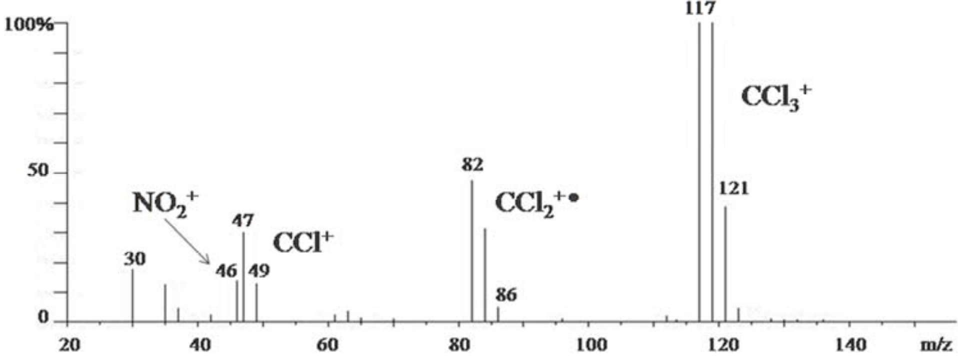

Figure 2. Electron ionization mass spectrum of trichloronitromethane (TCNM).

3.2. Selection of extraction solvent and droplet volume

It is essential to select a proper organic solvent for the establishment of a HS–SDME method, which is related to the chemical nature of the target compounds. As there is no study to date on this variable for HNMs, a variety of water-immiscible organic solvents were considered as the possible extractant. The uncertainty associated with the LPME technique, mainly the partial evaporation of the drop, was corrected by the use of an internal standard. Preliminary experiments with 2 µL of drop were examined at 30 °C using 10 mL of spiked mineral water samples at a concentration of 100 µg/L of the 9 HNMs and 20 µg/L of fluorobenzene (IS) containing 3 g of NaCl (in vials of 15 mL) under the following conditions at an extraction time 15 min and stirring rate of 600 rpm. All the extraction experiments were performed by measuring the relative peak area of each halonitromethane to the internal standard using the average of three replicate measurements (after that the different peak areas of fluorobenzene between solvents were normalized). After extraction, the drop was retracted and 1 µL of the extract injected into the GC–MS instrument. As can be seen in **Figure 3**, 1-hexanol provided the best extraction efficiency for 5 HNMs (CNM, BNM, BCNM, DBCNM and TBNM) whereas 1-octanol (the most commonly used in LPME techniques) only provided slight advantages for 3 HNMs (DCNM, BDCNM and DBNM); it did not extract to TBNM and scarcely did so to CNM and BNM. In addition 1-octanol required higher temperatures (BP ~200 °C) in the injection port, chromatographic column and mass spectrometer ion

source than 1-hexanol (BP ~160 ºC), which, as mentioned above, is related to the decomposition of the trihalonitromethanes (see **Figure 1**) to halomethanes among other compounds. Decane and o-xylene provided the poorest results and therefore were discarded. In conclusion, 1-hexanol showed the best extraction efficiency and adapted itself to the temperatures established for GC–MS analysis. The relative peak areas increased with increasing solvent volume although when the drop exceeded 2.5 µL, its manipulation was more elaborate and less reliable. Furthermore, large injection volumes resulted in a more extensive band broadening in capillary GC. Considering these factors, 2.5 µL of 1-hexanol (~2 µL of extract) was selected as the extractant since it provided the best extraction efficiency, good reproducibility (RSD ~10%) and has a boiling point of 157 ºC (vapour pressure, 0.947 Torr at 25 ºC), which prevented its retention (or is insignificant) either into the column or in the mass spectrometer ion source at optimal conditions.

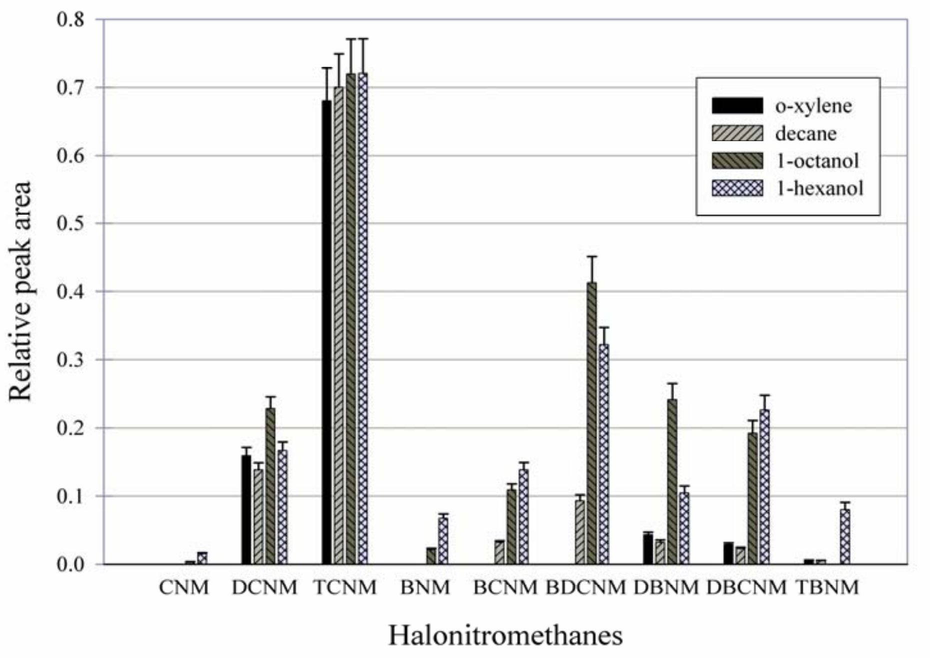

Figure 3. Effect of selection of solvent on HS–SDME technique for 100 µg/L of each HNMs using 2 µL of extractant. Error bars are the standard deviation for three measurements.

3.3. Effect of chemical variables

The only documentation about the influence of chemical parameters on HNMs is related with bromopicrin (TBNM) which is destroyed by common dechlorination agents (e.g. ascorbic acid) and requires a 3.5–4.0 pH to minimize base-catalyzed hydrolysis in water [14]. Therefore, the first chemical variable studied was the sample pH for the 9 HNMs because no information was available for these compounds. A few drops of diluted sulfuric acid solutions were used to adjust the pH of the aqueous sample in the acid region. As displayed in **Figure 4**, chloropicrin (TCNM) was not affected by the sample pH in any interval assayed and only minimally by DCNM, BCNM and BNM, whereas BDCNM, DBCNM, CNM and DBNM were influenced by the sample pH, especially TBNM. Since the pH of the sample was related to extraction efficiency, the best results for the simultaneous extraction of the 9 HNMs were obtained at pH 3.0–3.5. To minimize sample manipulation, the aqueous sample was adjusted at pH ~3.2 by adding 30 μL of 0.1 mol/L H_2SO_4 per 10 mL of sample.

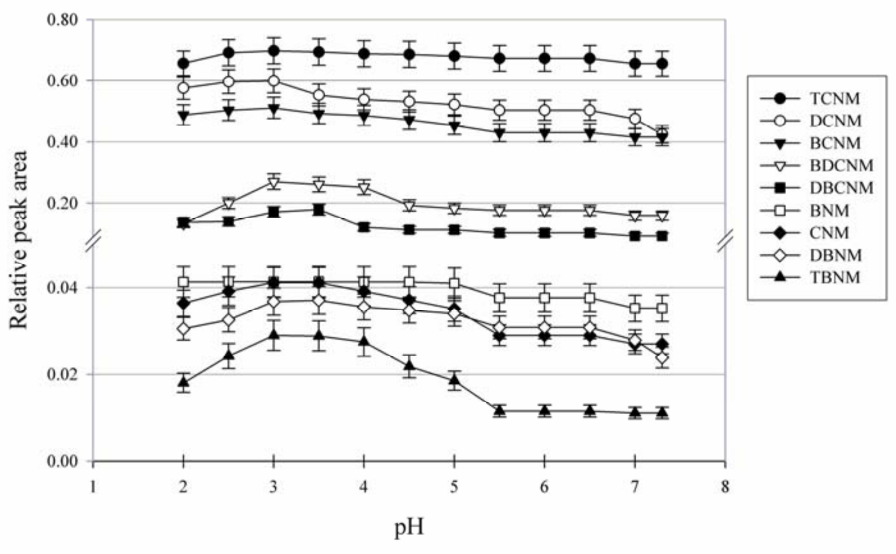

Figure 4. Influence of pH on the volatilization/extraction of the 9 HNMs from aqueous samples with 1-hexanol. Error bars are the standard deviation for three measurements.

A series of extraction experiments were carried out with a 1-hexanol drop (2.5 µL) by adding different salts (NaCl, KCl, Na_2SO_4 and $MgSO_4$) from 0 to 5 g to 10 mL of spiked mineral water samples (100 µg/L) at 30 ºC for all HNMs. Better conditions for the extraction of all HNMs were achieved by adding salts. In the first approach, anhydrous $MgSO_4$ was discarded since during the initial extraction, a good bit of heat is generated by exothermic hydration after the addition of the salt (3 g, 2.5 mol/L) to the samples. In one sense, this heat generation aids the extraction speed or efficiency of HNMs while, on the other hand, too much heat may lead to a loss of the droplet [32]. **Figure 5** shows that KCl as well as NaCl provided the poorest peak areas even at high concentrations (~7 mol/L) whereas Na_2SO_4 was the better choice. The data confirmed that sodium sulfate increased the extraction efficiency for the 9 HNMs to double the amount for some compounds (CNM, DCNM, BNM, BCNM, BDCNM and DBCNM). Thus, the best conditions for the 9 HNMs extraction were performed with 3 g of Na_2SO_4 per 10 mL of sample.

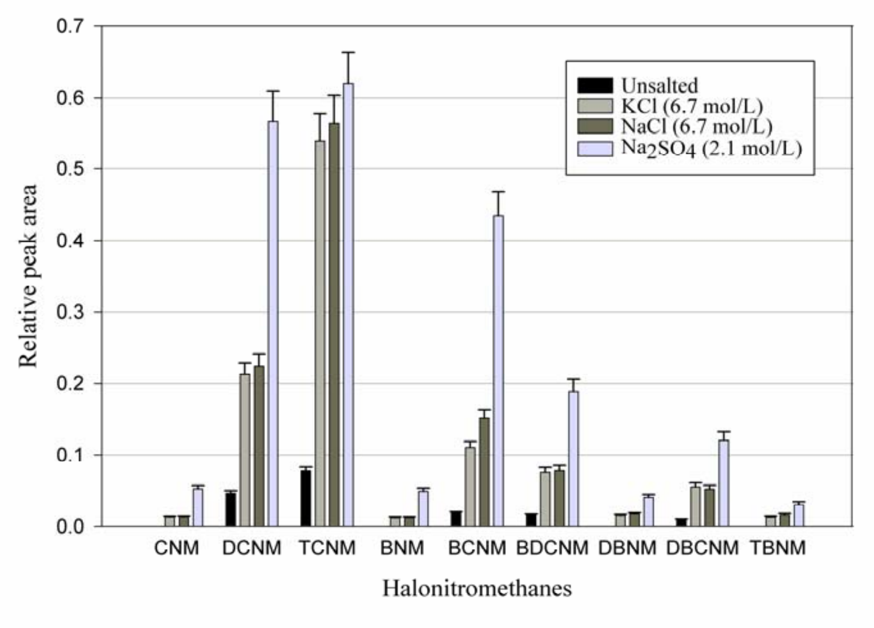

Figure 5. Feasibility for using different salts at variable concentrations on the extraction of the 9 HNMs. Error bars are the standard deviation for three measurements.

The influence of the water sample and headspace volume was examined from 5 to 10 mL in 15 mL sample vials (headspace volume from 10 to 5 mL). In all experiments the amount of each HNM was 0.5 μg whereas the volume of the sample changed. The experimental results showed that the extraction efficiency increased as the sample volume grew since the volume of gaseous phase (headspace) was minimized, which was in agreement with other HS–LPME methods [33]. A sample volume of 10 mL (in 15 mL vials) was adopted considering that when 3 g of salt was added and agitated using stirrer, the volume increased to ~11.5 mL; this ensured that the drop of extractant would not come into contact with the aqueous sample during the extraction step.

3.4. Optimization of physical parameters

The variation in extraction efficiency as a function of extraction time was studied with a 1-hexanol drop in the interval 5–40 min. The HS–SDME experiments of over 40 min extraction time could not be used due to the evaporation of the solvent in the air, which seriously influenced the accuracy of the results. The relative peak areas increased as the extraction time (~20%) rose to 15 min, above which it remained constant. To ensure maximum extraction, an extraction time of 20 min was selected for further experiments. For volatile analytes, the extraction temperature had a double impact on HS–SDME. At a higher temperature, diffusion coefficients in both water and headspace were higher and the extraction time could be shorter, but the partition coefficients for the analyte between the organic solvent and the gaseous phase were lower [30]. The effect of temperature was studied by exposing the 1-hexanol drop for 20 min in the headspace of 10 mL HNMs working solutions, in triplicate, between 25 and 40 ºC. For temperatures over 40 ºC, there was a faster solvent evaporation of the drop. As expected, the amounts of HNMs extracted increased at 30–35 ºC, above which the relative signals decreased by ~10%. The last optimized study was the stirring rate. Agitation of the sample solution enhanced the mass transfer in the aqueous phase, induced convection in the headspace, and consequently reduced the time for reaching a thermodynamic equilibrium. At stirring rates above 800 rpm resulted in the instability of the vials causing the dislodgement of the organic drop from the needle. At stirring

rate lower than 500 rpm the extraction efficiency decreased. Therefore, a stirring rate of 600 rpm was adopted in the method proposed.

Finally, fluorobenzene was selected in terms of volatility as internal standard to correct the uncertainty associated with the LPME technique and the injection of the extract into the GC instrument.

3.5. Efficiency of the HS–SDME process

The HS–SDME theory indicates that an organic compound initially present in the aqueous phase is volatilized and then a dynamic equilibrium is established between the concentration of the compound in the headspace and that of the analyte in the organic solvent drop. The yield of the volatilization/extraction process was jointly evaluated using an aqueous solution containing 15 μg/L of each HNM (except TBNM, 30 μg/L) in 2.1 mol/L Na_2SO_4 at pH ~3.2. In this experiment, five consecutive extractions of the same sample were carried out with a fresh drop of 2.5 μL of 1-hexanol, and the percentage of analytes extracted was calculated. The relative extraction yield was calculated using a normalization method in which the sum of the analytical signals obtained in the five sequential extractions was assigned a value of 100%. From these results, about 35% and 25% of the HNMs were extracted in the first and second extraction, above which the relative extraction yield decreased slowly. The results of this study showed that the highest fraction of the 9 HNMs was obtained in the first extraction. On the basis of the above considerations, although there was a lot of carry-over, only one extraction step was recommended in order to increase the sensitivity of the method because two sequential extractions provided a higher quantity of residues at the expense of a lower signal.

The following study focused on the average yield of the HS–SDME method by comparing the traditional LLE processes in the 9 HNMs. First, 1 mL of mineral water containing 60 μg (except TBNM, 120 μg) of each HNM and 30 μg of fluorobenzene in 2.1 mol/L Na_2SO_4 at pH ~3.2 was extracted with 1 mL of 1-hexanol in quintuplicate; the extraction efficiency of the manual LLE was calculated through calibration curves constructed with standards prepared directly in 1-hexanol. The average efficiency of the manual extraction after 5 min of agitation was 75% (for TCNM, BDCNM, DBCNM and TBNM), 65% (for DCNM and BCNM) and 40% (for CNM, BNM

and DBNM); the other fractions of analytes were extracted in subsequent extractions in the remaining aqueous phase. The results were compared to those obtained with 10 mL of an aqueous solution containing 150 ng of individual halonitromethanes (except TBNM, 300 ng) prepared in the above conditions in quintuplicate, using the HS–SDME method and 2.5 μL of 1-hexanol. In these conditions the theoretical concentration in both extracts (from LLE and HS–SDME methods) was similar (60 μg/mL for 8 HNMs and 120 μg/mL for TBNM). The extraction efficiency of the HS–SDME method related to the LLE one was ~20% (for TCNM, BDCNM, DBCNM and TBNM), ~10% (for DCNM and BCNM) and ~3% for (CNM, BNM and DBNM). The results obtained showed that both in the manual and in the microextraction techniques, trihalonitromethanes were the most favorably extracted compounds due to their higher solubility in 1-hexanol, taking into account their lower polarity. Therefore the pre-concentration factor of the method proposed ranged between ~120 and ~800 for monohalonitromethanes and trihalonitromethanes, respectively.

3.6. Quantitative calibration and reproducibility

Several analytical curves for standards in mineral water over the concentration range 0.2–300 μg/L of HNMs were obtained by plotting the analyte to the internal standard peak area against the analyte concentration. The 12-point calibration curve for each halonitromethane throughout the experimental concentration range showed good linearity with the correlation coefficients (r) of ≥0.991. The limits of detection were defined as the concentration of the analyte that provided a chromatographic peak area equal to three times the regression standard deviation ($S_{y/x}$) divided by the slope of the calibration graph [34], ranging from 0.06 μg/L for TCNM to 1.2 μg/L for TBNM. The reproducibility of the method proposed (analyzing 11 mineral water samples spiked with 5 μg/L of each HNM; 10 μg/L for TBNM) was good, with average RSD values of 8.2 ± 1.7% (within-day) and 9.3 ± 1.8% (between-day). As can be seen in **Table 2**, the HS–SDME method was very sensitive and allowed the determination of DCNM, TCNM, BCNM and BDCNM at ng/L levels; the brominated compounds and CNM were those that presented the least sensitivity. The high degree of sensitivity achieved for TCNM was noteworthy (chloropicrin) since it is the compound usually detected in drinking water.

Table 2. Analytical figures of merit for the determination of HNMs by HS-SDME and EPA 551.1 methods

Compound	HS-SDME					EPA 551.1				
	LOD (µg/L)	Linear range (µg/L)	Recovery (%)[a]	RSD (%)		LOD (µg/L)	Linear range (µg/L)	Recovery (%)	RSD (%)	
				Within-day	Between-day				Within-day	Between-day
CNM	0.9	3.0–300	90,92	8.5	9.4	0.3	1.0–300	90,96	6.6	7.8
DCNM	0.07	0.3–300	94,95	6.5	7.6	0.09	0.3–300	95,98	5.7	6.8
TCNM	0.06	0.2–300	95,98	6.2	7.1	0.1	0.4–300	94,99	5.9	6.8
BNM	0.9	3.0–300	91,96	8.6	9.5	0.3	1.0–300	89,97	6.8	8.0
BCNM	0.08	0.3–300	94,96	6.8	7.8	0.09	0.3–300	92,96	5.4	6.5
BDCNM	0.2	0.7–300	95,97	8.0	9.3	0.5	1.7–300	90,97	7.4	8.2
DBNM	0.9	3.0–300	91,93	8.6	9.8	0.2	0.7–300	94,95	6.6	7.7
DBCNM	0.3	1.0–300	94,97	8.8	9.8	1.0	3.3–300	93,98	8.4	9.2
TBNM	1.2	4.0–300	90,93	12.0	13.2	6.0	20–300	90,94	10.1	11.2

[a]The first and second data corresponds to the average percent recoveries for the low and high amount levels, respectively.

3.7. Validation of HS–SDME with EPA method 551.1

A comparison was carried out between the proposed method and that of EPA 551.1 in order to validate the alternative proposal; in this case the best pre-concentration factor for the manual EPA alternative was used [ratio aqueous volume (50 mL)/organic volume (3 mL) = 17]. All quantitative parameters were determined as previously mentioned; for the reproducibility study, 11 mineral water samples spiked with 10 µg/L of each HNM (except TBNM, 30 µg/L) were analyzed and the results are listed in **Table 2**. The EPA method 551.1 employed in this study using GC–MS was not as sensitive as the LOD value reported (0.014 µg/L) only for TCNM using GC–ECD [13] due to the higher sensitivity achieved with ECD in halogenated compounds. In the framework of comparison, the EPA method 551.1 was slightly more precise than that of HS–SDME with average RSD values of 7.0 ± 1.5% (within-day) and 8.0 ± 1.5% (between-day), but the sensitivity as the slope of the calibration graphs was lower than that achieved by the HS–SDME method proposed (except for CNM, BNM and DBNM). As can be seen in **Table 2**, the HS–SDME method provided lower LODs (average LODs, 0.5 µg/L) than those obtained by the EPA 551.1 (average LODs, 1 µg/L) for five HNMs (DCNM, TCNM, BDCNM, DBCNM and TBNM); it is necessary to highlight that TCNM was the one generally found in drinking water. With respect to reproducibility, the EPA method 551.1 provided lower RSD values than HS–SDME; although this difference was negligible when the error introduced by the miniaturization of the LLE technique was taken into account.

In the same vein, the recoveries of both methods were also calculated using a tap water that was fortified by two different concentrations of each HNM in quintuplicate. HS–SDME method recoveries were calculated by spiking 2 and 10 µg/L for DCNM, TCNM, BCNM, BDCNM and DBCNM or 5 and 20 µg/L for the other compounds (CNM, BNM, DBNM and TBNM). In the EPA method, tap water was fortified with 2 and 10 µg/L (omitting BDCNM, DBCNM and TBNM which were spiked at 20 and 40 µg/L levels). All waters contained TCNM at detectable levels and, in this case, its concentration in the spiked samples was quantified and compared to those calculated as the sum of the native concentration in unspiked samples and spiked concentration. As can be listed in **Table 2** in the HS–SDME method, all compounds were determined with average

recoveries between 93 and 95% for the low and the high amount levels, respectively, whereas the recoveries of the EPA method ranged from 92 (at low levels) to 97% (at high levels). The good agreement between the two methods demonstrated the reliability of the proposed microextraction method.

3.8. Analysis of water samples

Recent studies examining the potential of HNM formation in drinking waters under different oxidation conditions showed that ozonation–chlorination produced the highest HNM yields, followed in the order of chlorination by ozonation–chloramination and chloramination [11]. In order to verify the effectiveness of the proposed HS–SDME method in the application of interest, 20 treated water samples (tap and swimming pool) were analyzed, including samples subjected to oxidative treatment with ozone in addition to chlorination. In the waters analyzed, only chloropicrin (TCNM) was found; the others were either not found or were beneath detection limits. **Table 3** lists the TCNM concentrations found in water treated by chlorination (samples 1–12, and all swimming pool waters) or ozonation plus chlorination (samples 13–15). The results obtained were compared to those provided by the EPA method 551.1, also listed in **Table 3**. The two methods provided similar results, although TCNM remained undetected in some water samples using the EPA method 551.1, which corroborated the good performance of the proposed HS–SDME method. In practice, the concurrent oxidation process with ozone increased the TCNM concentration, which was in agreement with previous observations by several groups [5,9,11]. There were no significant differences between tap and swimming pool waters treated only by chlorination, although the concentration of residual chlorine and organic matter was higher in swimming pools than in tap waters; therefore, the ozonation step substantially increased the formation of TCNM. The concentration of TCNM found in tap waters ranged from <0.2 to 4.3 μg/L which was in agreement with Bougeard et al. [35] who reported TCNM concentrations from non-detected to 3.4 μg/L in chlorine drinking waters and those found in waste water treatment plant effluents (0.9–1.5 μg/L) [10].

Table 3. Analysis of water samples treated by chlorination (except to 13−15 tap waters, treated also by ozonation) by the proposed (HS−SDME) and the reference (EPA 551.1) methods (n = 5)

	Concentration of TCNM found ± standard deviation (μg/L)	
	HS–SDME	EPA 551.1
Tap 1	0.3 ± 0.1	0.4 ± 0.1
Tap 2	<0.2	n.d.[a]
Tap 3	2.4 ± 0.2	3.0 ± 0.2
Tap 4	0.2 ± 0.1	<0.4
Tap 5	2.6 ± 0.2	3.3 ± 0.2
Tap 6	0.2 ± 0.1	<0.4
Tap 7	0.3 ± 0.1	<0.4
Tap 8	0.9 ± 0.1	0.8 ± 0.1
Tap 9	<0.2	n.d.
Tap 10	1.1 ± 0.1	0.8 ± 0.1
Tap 11	2.5 ± 0.2	3.0 ± 0.2
Tap 12	0.3 ± 0.1	<0.4
Tap 13	4.0 ± 0.3	3.6 ± 0.2
Tap 14	4.3 ± 0.3	4.4 ± 0.3
Tap 15	3.9 ± 0.3	3.7 ± 0.13
Swimming pool 1	2.3 ± 0.2	1.6 ± 0.2
Swimming pool 2	0.4 ± 0.1	0.5 ± 0.1
Swimming pool 3	1.7 ± 0.2	1.3 ± 0.1
Swimming pool 4	1.2 ± 0.1	1.5 ± 0.2
Swimming pool 5	0.8 ± 0.1	0.6 ± 0.1

[a]n.d. = not detected.

4. Conclusion

A comparison of the proposed method with other methods reported in the literature for the determination of chloropicrin (TCNM) in water samples by different techniques is given in **Table 4**. The proposed method was the most sensitive compared with LLE–GC–MS alternatives, omitting the LLE–GC–ECD methods due to the higher sensitivity achieved with ECD in halogenated compounds. All LLE methods require large volume of extractant; in that way, the proposed HS–SDME method offers advantages since all extract (~2 μL) was injected into the instrument without residues. Current research on halonitromethanes (HNMs) in water (viz. toxicity study,

factors controlling their formation in water) uses methods optimized for the determination of halogenated VOCs, normally EPA method 551.1. However, these methods have some pitfalls when applied to unknown analytes like HNMs. In the absence of a comprehensive method, it has been necessary to develop an alternative to take into account such important variables for the analyte as the sample pH, the type of extractant and salt, and GC–MS conditions, among others. For the first time a method has been developed to determine the whole array of 9 HNMs, taking into consideration some very important current concerns like miniaturization and environmental aspects. Moreover, the method does not substantially increase sample processing time compared to reported EPA methods, but does provide higher sensitivity and similar reproducibility to the EPA method 551.1. Therefore, the proposed HS–SDME method may be of practical utility in both sample screening and analysis.

Table 4. Comparison of the HS-SDME-GC-MS method with other related methods for determination of chloropicrin (TCNM)

Method	LOD (μg/L)	Sample volumen (mL)	Extractant volumen (mL)	Linear range (μg/L)	RSD (%)	Reference
LLE-GC-ECD	0.014	50	3	0.1–15[a]	7.7	13 (EPA Method 551.1)
LLE-GC-MS	0.1	35	2	0.25–100[a]	3.5–18.1	17 (EPA Method 551.1 modified)
LLE-GC-ECD	0.04	35	2	0.25–100[a]	2.7–8.4	17 (EPA Method 551.1 modified)
HS-GC-MS	0.5	8	–	0.25–100[a]	–	17
HS-GC-ECD	0.4[b]–2.5	5	–	–	10	18
LLE-GC-MS	0.1	50	3	0.4–300	5.9	EPA Method 551.1, this work
HS-SDME-GC-MS	0.06	10	2.5×10^{-3}	0.2–300	6.2	This work

[a]Calibration range for a mixture of halogenated VOCs including chloropicrin.
[b]Data using splitless sample injection.

Acknowledgments

This work has been supported by the CTQ2010–17008 Grant from the Spanish Ministry of Education and the P09–FQM–4732 from the Junta of Andalucian. FEDER also provided additional funding. Isabel Montesinos would like to thank P09–FQM–4732DGI for awarding a predoctoral Grant. We also thank Dr. A. Serrano for helpful discussions.

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Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Solvent-minimized extraction for determining halonitromethanes and trihalomethanes in water

I. Montesinos, M. Gallego*

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071, Córdoba, Spain

Journal of Chromatography A 1248 (2012) 1

Abstract

Halonitromethanes (HNMs) are a class of nitrogenous disinfection by-products (N-DBPs) that have so far received little attention and focused largely on trichloronitromethane. By contrast, trihalomethanes (THMs) are the most commonly regulated DBPs and have been the subject of much study. This paper reports the first miniaturized system for the simultaneous determination of the nine known HNMs and four THMs in tap and swimming pool water. Micro liquid-liquid extraction (MLLE) is an adaptation of EPA Method 551.1 using ethyl acetate instead of methyl tert-butyl ether as extractant and large injected sample volumes (30 µL) in combination with programmed temperature vaporizer–gas chromatography–mass spectrometry for improved sensitivity and selectivity. Because extraction is done with a few microlitres of organic solvent (200 µL) and practically all extract is injected into the instrument, MLLE can be regarded as a virtually solvent-free sample preparation technique. The proposed method provided an extraction efficiency of ~85%, average limits of detection (tribromonitromethane excluded) of 30 ng/L and relative standard deviations of ~6.0%. The influence of various dechlorinating agents on the stability of the thirteen target analytes in treated water was evaluated; the only salt allowing both types of compounds to be efficiently preserved was $(NH_4)_2SO_4$, but only for 1 day at 4 °C. Therefore, acidifying the sample at pH ~3.4 —the optimum value for MLLE— at the time of collection is

recommended in order to ensure that both HNMs and THMs retain their integrity for 2 days during storage at 4 ºC.

1. Introduction

Disinfection in water treatment processes is used to prevent the spread of disease through drinking water. Reactions between disinfectants and natural organic matter in water cause the formation of organic disinfection by-products (DBPs) [1–3]. The nitrite plays an important role in the formation of the nitro group of halonitromethanes (HNMs), which have received special attention because of their potential high toxicity [3,4]. Very few among the more than six hundred DBPs identified to date have been the subject of exposure or toxicological studies [3]. Epidemiological studies have revealed that long-term exposure to trihalomethanes (THMs), non-nitrogenous form of DBP, is correlated with an increased risk of cancer [3]. Comparisons of data from in vitro mammalian cell tests have shown that nitrogenous DBPs (N-DBPs) such as HNMs, haloacetonitriles and haloacetamides are all far more cytotoxic and genotoxic than are THMs and haloacetic acids [5–8]. Also, brominated nitromethanes (especially dibromonitromethane) and mixed bromochloro–nitromethanes are more cytotoxic and genotoxic than are chlorinated nitromethanes [9]. Based on increasing evidence of adverse health effects associated to these compounds, the US Environmental Protection Agency (EPA) has established a maximum contaminant level of 80 μg/L for total THMs in drinking water [10]; no similar regulation for HNMs has to date been issued. Disinfectant type is a key factor in N-DBP formation since, depending on the particular compound and reaction conditions, the nitrogen can derive from the organic precursors. Pre-ozonation prior to chlorination and chloramination significantly boosts HNM formation [11,12]. Average concentrations of HNMs from undetectable to 3.4 μg/L in treated water containing chlorine [13], and trichloronitromethane (TCNM) levels from 0.9 to 1.5 μg/L in wastewater processing effluents [12], have been reported. From the available literature, total concentrations of THMs in drinking water are very variable depending on the particular country and drinking treatment. Thus, range from 2.6 to 66 μg/L [13], 26–93 μg/L [14] and 38–78 μg/L [3] have been published; however, actually these concentrations can be much higher [15]. Disinfection of swimming pools (particularly indoor pools) can raise THM levels to 100–145 μg/L [16] and TCNM levels to 0.4–1.9 μg/L [17,18].

The determination of these compounds in water typically relies on EPA Method 551.1, which is based on liquid-liquid extraction (LLE). This method is commonly used to determine halogenated volatile organic compounds (VOCs) including THMs and TCNM in drinking water [19]. Alternative methods for determining THMs in drinking water include purge-and-trap-gas chromatography-mass spectrometry (GC–MS) [20], headspace–GC–MS (HS–GC–MS) [14], liquid-phase microextraction coupled with GC–electron capture detection [21] and solid-phase microextraction–GC–MS [22]. Two recently reported methods for the specific determination of the nine HNMs based on single drop microextraction in the headspace mode [23] or static HS–GC–MS [18] provide low detection limits (0.06–1.2 and 0.03−0.60 μg/L, respectively).

Programmed-temperature vaporization (PTV) with large volume injection (LVI) has played a prominent role in GC analysis. In the solvent vent mode, PTV not only boosts sensitivity, but also affords discrimination of low volatile compounds and reduces degradation of thermally unstable analytes. This technique was used to determine THMs in aqueous matrices with an HS autosampler connected to a PTV–GC–MS system; detection limits ranged from 0.4 to 2.6 ng/L [24].

Liquid–liquid extraction (LLE) is the preconcentration technique most widely used for the determination of HNMs and THMs in water (EPA Method 551.1 with minor modifications) [8,12,25,26]. However, there is a recent trend in sample preparation to miniaturize conventional LLE by substantially reducing the organic/aqueous ratio in accordance with the principles of “Green Chemistry” [27]. The aim of this work was to develop the first miniaturized liquid-liquid extraction (MLLE) method adapted to EPA Method 551.1 for the determination of the whole range of HNMs and THMs in water. In addition, a rigorous study of various dechlorinating agents commonly used to preserve finished drinking water was performed in order to resolve the controversies over their action. To this end, the effect of these dechlorinating agents on the stability of both types of compounds was assessed. Since extraction is done with only a few microlitres of organic solvent and practically all extract is injected into the PTV–LVI–GC–MS system, MLLE can be regarded as a solvent-minimized extraction technique.

2. Materials and methods

2.1. Chemicals and standards

Chloronitromethane (CNM, 90–95%), dichloronitromethane (DCNM, 95%), bromochloronitromethane (BCNM, 85–90%), bromodichloronitromethane (BDCNM, 90–95%), dibromonitromethane (DBNM, 90%), dibromochloronitromethane (DBCNM, 90–95%) and tribromonitromethane (TBNM, 90–95%) standards were purchased from Orchid Cellmark (New Westminster, Canada). Nitromethane (NM, 99%), trichloronitromethane (TCNM, 99%), bromonitromethane (BNM, 90%), trichloromethane (TCM, 99%), bromodichloromethane (BDCM, 98%), dibromochloromethane (DBCM, 98%) and tribromomethane (TBM, 95%) were supplied from Sigma–Aldrich (Madrid, Spain) and the internal standard, fluorobenzene, from Fluka (Madrid, Spain). *n*-Hexane, methyl tert-butyl ether (MTBE), ethyl acetate and sulphuric acid were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulphate, sodium sulphite, L-ascorbic acid, sodium thiosulphate pentahydrate and ammonium sulphate were supplied from Panreac (Barcelona, Spain). Stock standard solutions containing 1 g/L of each halonitromethane or trihalomethane and cumulative solutions (0.1 g/L) were prepared in ethyl acetate or methanol, respectively, and stored in amber glass vials at –20 ºC. More dilute cumulative solutions were prepared daily in mineral water at the microgram per liter level. In spite of the treatment of the tap water (using Milli-Q system), ultrapure water continues to present trihalomethanes (THMs) from the tap water. These compounds did not appear in the blanks performed with the commercial mineral water since it is untreated. Therefore, mineral water was proposed for DBPs determination in aqueous matrices when the analysis carried out by our group [14]. Other solvents and salts were of analytical grade or better.

2.2. Sampling and preservation

Amber glass bottles (125 mL) with poly(tetrafluoroethylene) (PTFE) screw caps were used for the collection of the treated water. Water samples were adjusted at pH ~3.4 by adding 250 µL of 0.1 M H_2SO_4, and completely filled to avoid evaporation of the volatile analytes. In this acidic condition no

dechlorinating agent for residual chlorine was necessary, as it is described in Section 3.6. In acidic medium the concentration of the 13 target analytes remained constant in the water for 2 days at 4 °C. When the time between sample collection and analysis exceeded 2 days, samples could be stored at –20 °C up to 14 days.

2.3. Micro liquid-liquid extraction procedure

A volume of 9 mL of treated water (pH ~3.4) or standard solution prepared in mineral water containing between 0.03 and 100 μg/L concentration of each HNM and THM, and 4 μg/L fluorobenzene at pH 3.0–4.0 (adjusted to pH ~3.4 by adding 20 μL of 0.1 M H_2SO_4) was added to a 10 mL glass vial and supplied with 200 μL of ethyl acetate and 3 g of Na_2SO_4, after which the vial was immediately sealed and vortexed for 1 min, followed by decantation for 2 min. Then, a volume of ~50 μL of the upper organic layer was transferred to a 0.1 mL conical glass insert that was placed inside a 2 mL amber glass GC vial containing ~10 mg of Na_2SO_4 to dry the extract. Finally, a volume equivalent to that of dried extract, 30 μL, was withdrawn by means of a 100 μL GC microsyringe furnished with a fixed needle and injected into the PTV–LVI–GC–MS instrument for analysis.

2.4. Liquid-liquid extraction (EPA Method 551.1) procedure

Liquid-liquid extraction for the determination of HNMs and THMs in water was performed in quintuplicate following EPA Method 551.1 [19] proposed for the determination of halogenated VOCs. Samples were collected in 62 mL amber bottles with a PTFE screw cap containing 0.8 g of ammonium sulphate and without headspace to avoid evaporation of VOCs with minor modifications (viz. internal standard and PTV–LVI–GC–MS detection). A 12 mL aliquot was withdrawn from the sample bottle and discarded and the pH was adjusted at 4.5–5.5 with diluted H_2SO_4. Fifty microlitres of a 10 mg/L standard solution of fluorobenzene (IS), 3 mL of extracting solvent (MTBE), 20 g of Na_2SO_4 and 1 g of copper sulphate were added to the remaining sample (50 mL) and the vial was stirred for 4 min; once the HNMs and THMs were extracted, the vial was left to stand for 2 min in order to separate both phases. Then, 1 mL of the upper MTBE layer was transferred to a 2 mL glass vial and 0.1 g of Na_2SO_4 was added to dry

the extract. Finally, 30 µL of the extract was injected into the PTV–LVI–GC–MS instrument.

2.5. PTV–LVI–GC–MS instrumentation

Experiments for the determination of the 13 analytes were carried out with a PTV–GC–MS consisted of an HP 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP 5975C mass selective detector, with Triple-Axis Detector. Separation of the different compounds was achieved with an SLB-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness) coated with a stationary phase of 5%-phenyl–95%-methylpolysiloxane supplied by Supelco. Helium was used as the carrier gas with a column flow rate of 1 mL/min (6.0 grade purity, Air Liquid, Seville, Spain). The column oven temperature program was set to an initial temperature of 40 °C for 4 min and then raised at 0.5 °C/min to 50 °C, then increased to 100 °C at 50 °C/min, and finally, increased at 80 °C/min up to 180 °C and held for 2 min. Under these conditions the total chromatographic run time was 28 min. An Agilent programmable temperature vaporization inlet (G2619A Septumless Head), with multi-notch deactivated PTV liner (Part No.5183-2041), was applied as the sample injector. The injection was carried out in programmed temperature vaporization in solvent vent mode. In the injection step, 30 µL of the sample was introduced into the liner at 45 °C. During the solvent evaporation step the temperature was kept constant for 0.01 min, and the vent flow at 60 mL/min. In the transfer step the split valve was closed and the temperature increased to 170 °C at a rate of 200 °C/min (this injector port temperature was chosen because trihalonitromethanes degrade with temperatures above 170 °C) [26]. Finally, the split valve was opened and the injector kept at 170 °C with a purge flow of 60 mL/min at 1 min, until the end of the run for cleaning purposes. The mass spectrometer was operated in electron ionization mode using a voltage of 70 eV. The transfer line and ion source temperatures were 200 and 250 °C, respectively. The solvent delay time was set to 9 min, during which the filament was turned off to protect it from the arrival of the solvent. The quadrupole MS was set in full scan mode for identification and ion selection (29–300 amu) at 3.5 scans/s and in the selected ion monitoring mode (SIM) for quantification. The ions selected for

identification and quantification of HNMs and THMs (SIM mode) are listed in **Table 1**; *m/z* values for fluorobenzene (IS) were: 50, 70, 96 (base peak).

Table 1. Analytical figures of merit of the MLLE and EPA 551.1 (with PTV-LVI-GC-MS technique) methods

Compound	m/z[a]	MLLE				EPA 551.1			
		LOD (ng/L)	Linear range (μg/L)	RSD (%)		LOD (ng/L)	Linear range (μg/L)	RSD (%)	
				Within-day	Between-day			Within-day	Between-day
TCM	47, **83**, 85	60	0.20–100	6.4	7.4	140	0.45–200	6.4	7.3
BDCM	**83**, 85, 129	20	0.07–100	5.9	6.9	50	0.15–100	5.1	6.0
DBCM	79, 127, **129**	18	0.06–100	5.6	6.3	50	0.15–100	5.3	6.4
TBM	171, **173**, 175	20	0.07–100	5.8	6.7	60	0.20–100	5.5	6.6
CNM	46, **49**, 51	15	0.05–100	5.6	6.5	100	0.30–100	5.8	7.0
DCNM	46, **83**, 85	9	0.03–100	5.2	6.1	30	0.10–100	5.1	6.1
TCNM	46, **117**, 119	15	0.05–100	5.6	6.6	60	0.20–100	5.6	6.7
BNM	46, **93**, 95	15	0.05–100	5.8	6.5	100	0.33–100	5.7	6.8
BCNM	46, 127, **129**	12	0.04–100	5.3	6.2	40	0.13–100	5.3	6.2
BDCNM	46, 161, **163**	70	0.20–100	6.3	7.0	210	0.70–100	6.6	7.6
DBNM	46, 171, **173**	20	0.07–100	6.0	7.1	120	0.40–100	5.6	6.4
DBCNM	46, **207**, 209	100	0.30–100	6.5	7.5	500	1.70–100	7.4	8.6
TBNM	46, **251**, 253	400	1.30–100	7.6	8.6	3000	9.00–100	7.8	8.7

[a]Base peaks used for quantification are boldfaced.

3. Results and discussion

3.1. Selection of the extractant

The choice of organic solvent (extractant) is governed by the chemical nature of the target compounds; thus, the solvent should have a low solubility in water and excellent GC properties. Also, its boiling point influences performance in the solvent vent injection mode. Based on the foregoing, we initially used *n*-hexane, MTBE and ethyl acetate (boiling point ~69, ~55 and ~77 ºC, respectively) as extractants on the grounds of their good selectivity for the thirteen analytes and their also good chromatographic performance. In addition, fluorobenzene was used as internal standard (IS) to correct errors associated with the partial dissolution of each organic solvent in the aqueous phase and make the

extraction efficiency in the different solvents comparable. The sample pH, the amount of salt and the aqueous/organic volume ratio were initially selected according to EPA Method 551.1. To this end, preliminary tests involving 9 mL of mineral water (adjusted to pH 4.5–5.5 with dilute H_2SO_4) spiked with a 20 μg/L concentration of each of the nine HNMs and four THMs, and supplied with 4 μg/L IS in 10 mL vials were conducted. Extraction was accomplished by using a volume of 0.5 mL of each solvent and 3.5 g of Na_2SO_4. The vial was recapped and shaken by hand for 4 min, after which it was allowed to stand for another 2 min in order to facilitate separation of the two phases. Then, about 100 μL of upper organic layer was transferred into a 2 mL conical glass insert containing ~10 mg of Na_2SO_4 and 20 μL of the extract was injected into the PTV–LVI–GC–MS instrument for analysis. A vent flow rate of 20 mL/min was used for 0.01 min. All extraction tests involved measuring the relative peak area of each analyte to IS, using the average of three replicate measurements. As can be seen from **Figure 1**, the less polar HNMs (BDCNM, DBNM, DBCNM and TBNM) were optimally extracted in *n*-hexane, whereas their more polar counterparts were more efficiently extracted in ethyl acetate. Also, the most polar HNMs (CNM and BNM) were not extracted by *n*-hexane. Overall, THMs were better extracted by ethyl acetate, but trichloromethane was extracted slightly more efficiently in MTBE. The average extraction percentage with *n*-hexane, MTBE and ethyl acetate was 28 ± 2%, 33 ± 3% and 50 ± 4%, respectively. Ethyl acetate was selected as the best choice since it provided good selectivity for all target analytes, was scarcely volatile and exhibited no significant dissolution during extraction, so it was easier to handle and provided more reproducible results than MTBE. Because the volume of organic solvent used has a strong effect on enrichment factors, we examined its influence over the range 100–500 μL. As expected, the relative peak area increased with decreasing solvent volume; below 200 μL, however, the supernatant (organic phase) was difficult to collect visually, which detracted from reproducibility in aspirated volumes. A 200 μL volume of ethyl acetate was therefore adopted to extract the nine HNMs and four THMs from 9 mL of aqueous solution. Following EPA Method 551.1 with MTBE as extractant [19], copper sulphate was used to facilitate visual distinction of the two phases. Using this colored salt in our MLLE method made it difficult to distinguish the interface water/ethyl acetate, so we

chose to omit it. According to the high miscibility of ethyl acetate in water (~20%), the extract was dried with sodium sulphate to effectively remove the water before analyzing by GC–MS.

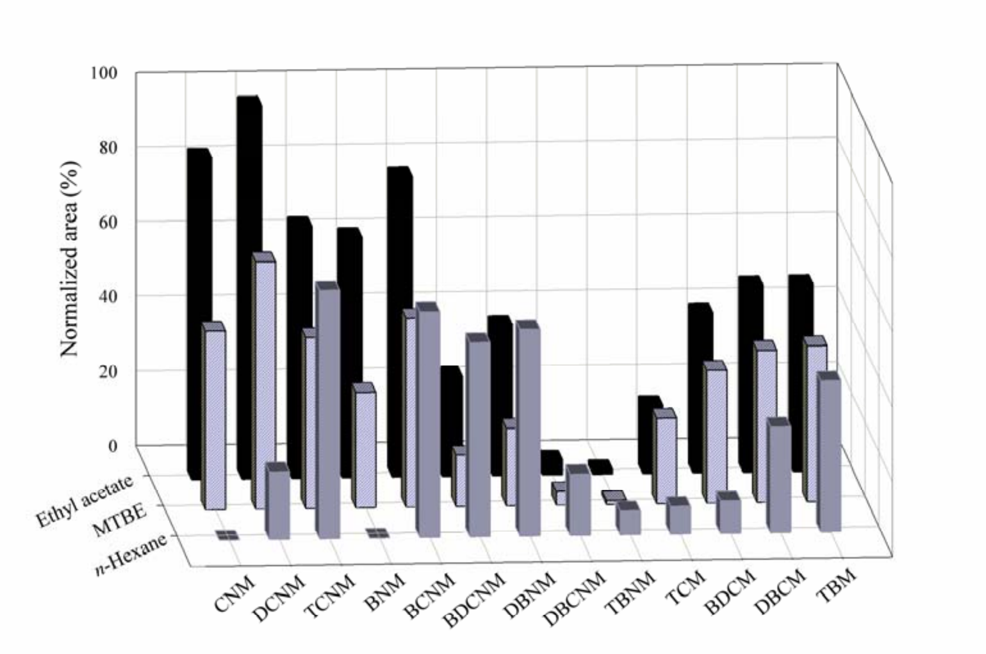

Figure 1. Influence of the extractant on performance in MLLE method. The relative peak area of each analyte was normalized to DCNM. Sample, 9 mL of mineral water spiked with a 20 µg/L concentration of each of the thirteen target analytes; extractant volume, 500 µL.

3.2. Effect of instrumental variables

The experimental variables of the PTV–LVI system were found to strongly influence analytical performance in the proposed MLLE method. PTV injections were done in four steps, namely: injection, solvent evaporation, analyte transfer and cleaning. The injection volume was influential on all compounds since a large volume can greatly increase the response of the target analytes. We used variable volumes of a standard solution containing 10 µg/mL of each of the nine HNMs and four THMs in addition to a 2 µg/mL concentration of IS in ethyl acetate for injection into the instrument. By way of example, **Figure 2** shows the effect of the

injected volume on the signals for nine representative target analytes. As can be seen, normalized areas increased with increasing injected volume except for DCNM, TCNM, CNM, BDCM and TCM (excluded in **Figure 2**), whose peak areas remained virtually above 30 μL. This result can be ascribed to the increased volatility of these compounds, which were swept out of the liner together with the solvent as the injection volume was raised. The optimum injected volume was found to be 30 μL.

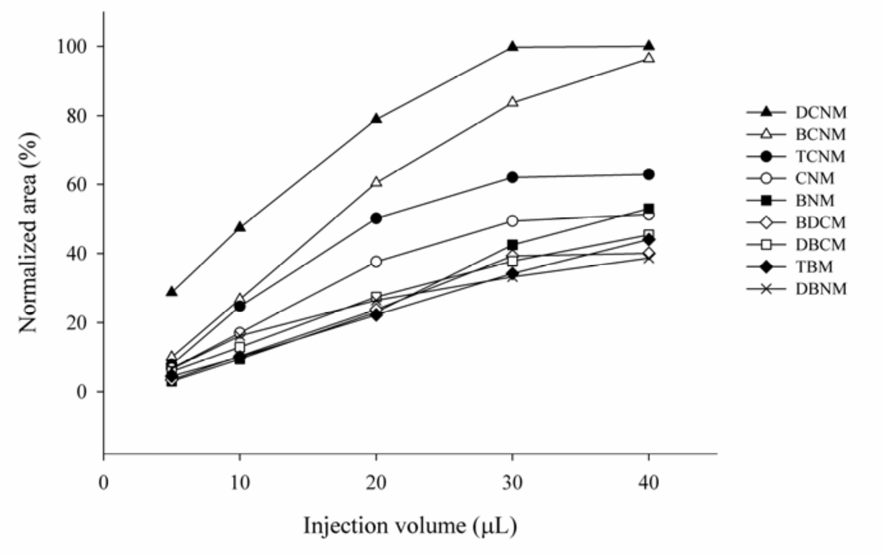

Figure 2. Influence of the sample injection volume on the PTV–LVI–GC–MS determination of nine representative analytes. Sample, 10 μg/mL of each standard dissolved in ethyl acetate.

The optimum initial inlet temperature allowing the solvent to be evaporated without analyte losses was examined over the range 45–70 °C. An initial temperature of 45 °C was selected to cool the liner because it resulted in a fairly short analysis time (10 min versus more than 20 min at lower temperatures) and increased analyte peak areas by 45–50% in relation to 70 °C. The influence of the inlet temperature programme was examined by assessing the effect of the ramp rate on sensitivity. Of the five ramp rates used (100, 200, 300, 400 and 500 °C/min), 200 °C/min provided the best results for all analytes. Too low a vent flow can cause a small amount of residual solvent to remain in liner and too high a flow can

result in analytes being swept out of it. The influence of the vent flow rate was examined over the range 20–80 mL/min and 60 mL/min for 0.01 min selected as optimal. The influence of the column pressure was examined from 7 to 12 psi and 10 psi found to be optimal. Finally, the injector was kept at 170 °C with a purge flow of 60 mL/min until the end of the run for cleaning purposes.

3.3. Influence of MLLE variables

The only reported evidence of the influence of the sample pH on the whole range of HNMs corresponds to a single drop microextraction method implemented in the headspace mode (HS–SDME) and previously developed by our group [23]. The optimum extractant and sample pH were 1-hexanol and 3.0–3.5, respectively. Recent methods for determining THMs based on LLE or liquid phase microextraction (LPME) have not been assessed for the influence of the sample pH since they usually adopt the pH recommended in EPA Method 551.1. No report on the influence of the sample pH or other chemical variables on the LLE of both groups of VOCs exists as yet. In fact, the two LLE methods for HNMs and THMs [15,19] use a pH of 4.5–5.5 and 0.4 g of Na_2SO_4 per milliliter of aqueous phase as salting-out agent. We examined the effect of the sample pH in the acid region (20 µg/L of each analyte and 4 µg/L IS) from 2.0 (adjusted with dilute sulphuric acid) to 7.4 (the pH of the aqueous solution before extraction). The influence of the sample pH was negligible for five HNMs (DCNM, BCNM, TCNM, BNM and DBNM) and all four THMs throughout the studied range. By contrast, the relative peak areas for the other four HNMs (CNM, BDCNM, DBCNM and TBNM) were markedly affected, their signals increasing with increasing sample pH up to 2.7–3.0 and then decreasing above pH 4.0. The optimum sample pH was that minimizing sample processing and found to be ~3.4, which was adjusted by adding 20 µL of 0.1 M H_2SO_4 per 9 mL of sample.

Addition of a salt in LLE facilitates transfer of the analytes from the aqueous phase [28]. We studied various types of salts with a similar (Na_2SO_4 and K_2SO_4) or different stoichiometry ($MgSO_4$ and NaCl) as salting-out agents. The type of salt was found to have a significant influence on separation of the two phases. Thus, the presence of NaCl or K_2SO_4 at a concentration of 7.9 and 2.7 M, respectively, in the aqueous phase (i.e. a

similar ionic strength) precluded visualization of the interface between layers as did the absence of salt. The addition of $MgSO_4$ at concentration of 2.0 M heated the vial owing to its exothermic hydration and reduced the extraction efficiency, especially for brominated HNMs and TCNM, which were partially degraded to their corresponding halomethanes (e.g. TCNM to trichloromethane). A Na_2SO_4 at concentration of 2.7 M provided the best extraction efficiency without degradation of HNMs. The effect of the ionic strength of the aqueous phase was assessed by using Na_2SO_4 concentrations from 1.5 to 3.0 M (saturated solution). Increasing the concentration of salt increased the relative peak areas for the thirteen target analytes up to 2.3 M (3 g of salt), above which they leveled off. An amount of 3 g of salt per 9 mL of sample was therefore chosen for quantitative analyses.

Agitating samples were found to facilitate and expedite MLLE. We examined the effect of manual, magnetic, ultrasonic and vortex agitation during extraction of the analytes for 1 min. The average relative peak areas obtained with magnetic, ultrasonic and manual agitation were ~20%, ~25% and ~85% of those provided by vortex agitation, which was assigned 100%, probably because magnetic and ultrasonic agitation somehow hindered solubilization of the salting-out agent. The influence of the vortex extraction time was studied over the range 1–4 min; based on the results, equilibrium was reached after 1 min for all analytes, so this time was selected for further testing.

3.4. Efficiency of the MLLE process

The MLLE method uses a small volume of ethyl acetate relative to the aqueous phase (the aqueous/organic volume ratio is 45), so exhaustive extraction with it is probably impossible. The MLLE extraction yield was assessed by applying the procedure described in Section 2.4 to the thirteen target analytes at a concentration of 10 µg/L each in quintuplicate. Three consecutive extractions of the same sample with fresh 200 µL aliquots of ethyl acetate were done. The average relative extraction yield was calculated in quintuplicate, using a normalization method in which the combined analytical signal obtained in the three sequential extractions was assigned a value of 100%. Based on the results, 76 ± 4% and 24 ± 1% of the thirteen

analytes was extracted in the first and second run, respectively; by contrast, the third run provided negligible extraction. The largest fraction of target analytes was therefore obtained in the first run. Thus, despite some carry-over, only one extraction run was subsequently performed in order to increase the sensitivity of the MLLE method.

In another test series, we compared the average yields obtained with the proposed MLLE method and those of conventional LLE. To this end, 1 mL of a mineral water solution containing a 0.5 μg/mL concentration of each HNM and THM, and 0.5 μg/mL IS in a 2.3 M Na_2SO_4 solution at pH ~3.4 was extracted with 1 mL of ethyl acetate in triplicate. Three consecutive extraction of the same sample with fresh 1 mL aliquots of extractant were done. The average efficiency of manual extraction for 5 min was 91 ± 5%; the remaining fraction of analytes, 8.7 ± 0.5%, was extracted from the aqueous phase in the second run. The extraction efficiency of the MLLE method was ~85% that of the LLE method, which is quite acceptable if one considers the high volume ratio used. The preconcentration factor of the proposed method was ~40; this makes our method more sensitive than the conventional EPA Method 551.1, which uses an aqueous/organic volume ratio of 17.

3.5. Analytical performance

The above-described optimum conditions for the proposed MLLE/PTV–LVI–GC–MS method were used to construct calibration curves for aqueous standards prepared in mineral water containing concentrations from 0.03–1.30 to 50–100 μg/L of the thirteen target analytes by plotting the analyte-to-internal standard peak area ratio against the amount of analyte (12 points per curve). The results are listed in **Table 1**. All target analytes exhibited good linearity, with correlation coefficients greater than 0.995. Limits of detection (LODs) were calculated as the analyte concentrations providing chromatographic peaks equal to three times the regression standard deviations ($S_{y/x}$) divided by the slope of the calibration graph [29] and found to range from 9 to 400 ng/L. Worth special note is the high sensitivity for TCNM and the four THMs (LODs of 15–60 ng/L), which are typically encountered in drinking water. The precision of the proposed method was determined by analysing 11 mineral water samples spiked with

a 5 µg/L concentration of each HNM and THM, and found to be quite good, with average relative standard deviation (RSD) values of 6.0 ± 0.6% (within-day) and 6.9 ± 0.7% (between-day). Compared to previously reported LPME methods, the LODs for HNMs provided by the proposed MLLE method (9–400 ng/L) are significantly lower than those obtained by HS–SDME–GC–MS (60–1200 ng/L) [23]. Those for THMs (30 ng/L on average) are lower than the LODs for alternatives methods such as LPME–GC–electron capture detection (ECD) [21] or HS–LPME–GC–ECD [30] (310 and 225 ng/L, respectively, on average), and similar to those of EPA Method 551.1 as implemented by LLE–GC–MS (20 ng/L) [31]. These values are better than those provided by other methods for individual HNMs or THM groups, which makes the proposed MLLE method the most sensitive for determining the whole range of HNMs and THMs reported to date.

The proposed MLLE method and EPA Method 551.1 were compared by using the procedure described in Section 2 to validate the former. All quantitative parameters were determined as stated above. Thus, reproducibility was assessed by analysing 11 mineral water samples spiked with a 10 µg/L concentration of each HNM and THM except TBNM, which was used at 30 µg/L; the results are listed in **Table 1**. The precision, with average RSD values of 5.9 ± 0.9% (within-day) and 7.0 ± 0.9% (between-day), was similar to that of the proposed method. However, there was a considerable difference in sensitivity (as slope of the calibration graph) between the two. In addition, the average LOD (TBNM excluded) was 31 ± 29 ng/L for the MLLE method and 122 ± 130 ng/L for EPA Method 551.1. The EPA Method 551.1 used here is less sensitive with GC–MS than with GC–ECD [19] owing to the increased sensitivity of ECD for halogenated compounds. By way of example, the reported LOD for BDCM with GC–ECD is 5 ng/L, which is 10 times lower than that achieved here with GC–MS (50 ng/L).

Recoveries were calculated by spiking a tap water sample with two different concentrations (5 and 20 µg/L) of each target analyte—by exception, TBNM was spiked at 15 and 30 µg/L in the EPA alternative—, all in quintuplicate (n = 5). The tap water contained TCM, BDCM, DBCM and TCNM at detectable levels so the spiked concentrations were compared with those calculated as the combination of the native concentration in unspiked

samples and the spiked concentrations. As can be listed in **Table 1**, all analytes were recovered by 95% when spiked at the lower concentration and by 98% at the higher concentration with the proposed method versus by 93 and 98%, respectively, with the EPA method. Therefore, both methods provided similar recoveries from a real water sample and no matrix effect was observed in the joint determination of HNMs and THMs in treated water samples.

3.6. Stability of HNMs and THMs in treated water

Commonly used disinfectant species such as hypochlorous acid, hypochlorite ion, chlorine dioxide, ozone and chloramines interfere with the determination of DBPs unless they are masked with a dechlorinating agent. THMs are usually preserved by adding NH_4Cl [32], ascorbic acid [30,33] or Na_2SO_3 [34] to treated water. However, there is some controversy over the most suitable dechlorinating agent for determining HNMs—studies have so mainly focused on TCNM. Thus, the original EPA Method 551.1 recommends NH_4Cl as dechlorinating agent for halogenated VOCs (TCNM included) [19]; however, a recent study suggested that using NH_4Cl to convert free chlorine to monochloramine causes the formation of small amounts of haloacetic acids (regulated DBPs) during chloramination and is therefore inadvisable [35]. More recent studies on halogenated VOCs recommend using $(NH_4)_2SO_4$ instead [36]. Others authors have proposed $Na_2S_2O_3$ [37] or ascorbic acid [38] as dechlorinating agent for halogenated VOCs (TCNM included). More recently, EPA [39] recommends two types of salts, ascorbic acid at pH 3.5 for 6 HNMs (CNM, DCNM, TCNM, BNM, BCNM and DBNM) and NH_4Cl for 3 HNMs (BDCNM, DBCNM and TBNM) which complicates the method. This section describes the efficiency of various salts used as dechlorinating agents including Na_2SO_3 [34], $(NH_4)_2SO_4$ [36], $Na_2S_2O_3$ [37] and ascorbic acid [38] in preserving treated water for the determination of the nine HNMs and four THMs.

The initial test involved all nine HNMs—THMs are known to be well preserved—, which were spiked at a 40 μg/L concentration each to a tap water sample containing a 0.4 mg/L concentration of free residual chlorine. The sample contained only one HNM (TCNM), the concentration of which was determined prior to spiking the water. Aliquots of the spiked tap water containing each salt (dechlorinating agent) at a 0.1 M concentration were

placed in separate 35 mL amber glass bottles that were stored refrigerated at 4 °C for 2 h. The stability of the nine HNMs in the presence of each dechlorinating agent was assessed against freshly prepared, unpreserved tap water. After 2 h, only $(NH_4)_2SO_4$ succeeded in maintaining the response of the nine HNMs at levels similar to those obtained with freshly spiked water containing no dechlorinating agent. No chromatographic peak for any HNM was obtained in the presence of Na_2SO_3. On the other hand, peak areas remained constant for six HNMs and no signals were obtained for the other three brominated trihalogenated species (BDCNM, DBCNM and TBNM) with ascorbic acid. In the presence of $Na_2S_2O_3$, the signal for one HNM (CNM) remained constant but the other eight HNMs were degraded by the loss of halogen atoms. Complete degradation of the nine HNMs with Na_2SO_3, and of BDCNM, DBCNM and TBNM with ascorbic acid, was especially fast; thus, not even the corresponding dehalogenated products were observed in the chromatograms. Pearson et al. [40] proposed a parallel degradation pathway for TCNM in presence of zero valent iron by which DCNM, CNM, nitromethane (NM) and, finally, methylamine (MA) were formed. Accordingly, we assumed that the complete degradation of HNMs in the presence of these two dechlorinating agents can progress to NM and MA as end-products. No NM was detected with the proposed GC–MS method, however, probably because this technique is not sensitive enough for this compound. Thus, the direct injection of standards of NM in ethyl acetate provided no chromatographic peaks—not even at concentrations above 50 mg/L. Degradation of the eight HNMs (DCNM, TCNM, BNM, BCNM, BDCNM, DBNM, DBCNM and TBNM) in the presence of $Na_2S_2O_3$ was confirmed by performing the stability tests on tap water samples spiked with each HNM individually at a 40 µg/L concentration. The eight HNMs were thus degraded by ~10% (DCNM), ~65% (TCNM) and ~85–100% (brominated HNMs) relative to freshly spiked samples containing no $Na_2S_2O_3$. Degradation was a result of halogenated compounds undergoing reductive dehalogenation in the presence of reductants, the effect being more marked on brominated HNMs owing to their increased oxidation state. Gan et al. [41] showed chloroacetanilide to lose chloride in the presence of $Na_2S_2O_3$. An identical mechanism can be assigned to HNMs since their degradation also involves halogen losses. The chromatograms of **Figure 3** illustrate the degradation of BCNM (A) DBNM (B) and TCNM (C) in the

presence of $Na_2S_2O_3$ as compared to $(NH_4)_2SO_4$ (i.e. in the absence of degradation). The dehalogenation of BCNM (**Figure 3A**) involved the loss of one chlorine atom and one bromine atom, and caused the formation of two by-products (CNM and BNM) at the expense of a decrease in the signal for BCNM. This was also the case with DBNM (**Figure 3B**) since BNM appeared with $Na_2S_2O_3$ but not with $(NH_4)_2SO_4$. As can be seen from **Figure 3C**, the degradation of TCNM in the presence of $Na_2S_2O_3$ resulted in the simultaneous formation of two major by-products: DCNM and CNM. These results are consistent with others of Lee et al. [42], who found TCNM to be rapidly degraded by ferrous salts via abiotic reduction to DCNM and CNM under anoxic conditions. Our results suggest that $(NH_4)_2SO_4$ is the only dechlorinating agent efficiently preserving all nine HNMs in treated water.

It should be noted that $(NH_4)_2SO_4$ was the best dechlorinating agent for HNMs but there is not information about the stability for THMs with this salt. Therefore, the next step was to study the stability of the four THMs in the presence of 0.1 M $(NH_4)_2SO_4$. Using this salt allowed all four THMs to remain stable for at least 1 week, and the HNMs for only 1 day, at 4 ºC. It was therefore advisable to use an alternative preservative for HNMs in tap water. Halogenated VOCs (halogenated acetaldehydes) in treated water at pH 4.5 (adjusted with HCl + ascorbic acid) can be stored refrigerated at 4 ºC for least 14 days [43]. Since the proposed MLLE method required an acid medium to extract the thirteen target analytes, we assessed their stability under acidic conditions. Thus, we examined the stability of the nine HNMs and four THMs at a 10 µg/L concentration each in tap water at pH ~3.4—the optimum value for the MLLE method. Each sample was analyzed in triplicate at hourly intervals on the first day and then a higher intervals for 2 days. The results showed that the target analytes remained stable for at least 2 days in acidified treated water. Consequently, we chose to only acidify the sample at the time of collection in order to preserve both HNMs and THMs during storage at 4 ºC. Based on these results, and taking into account that the MLLE method required a sample pH of ~3.4, this step was omitted from the analysis of treated water.

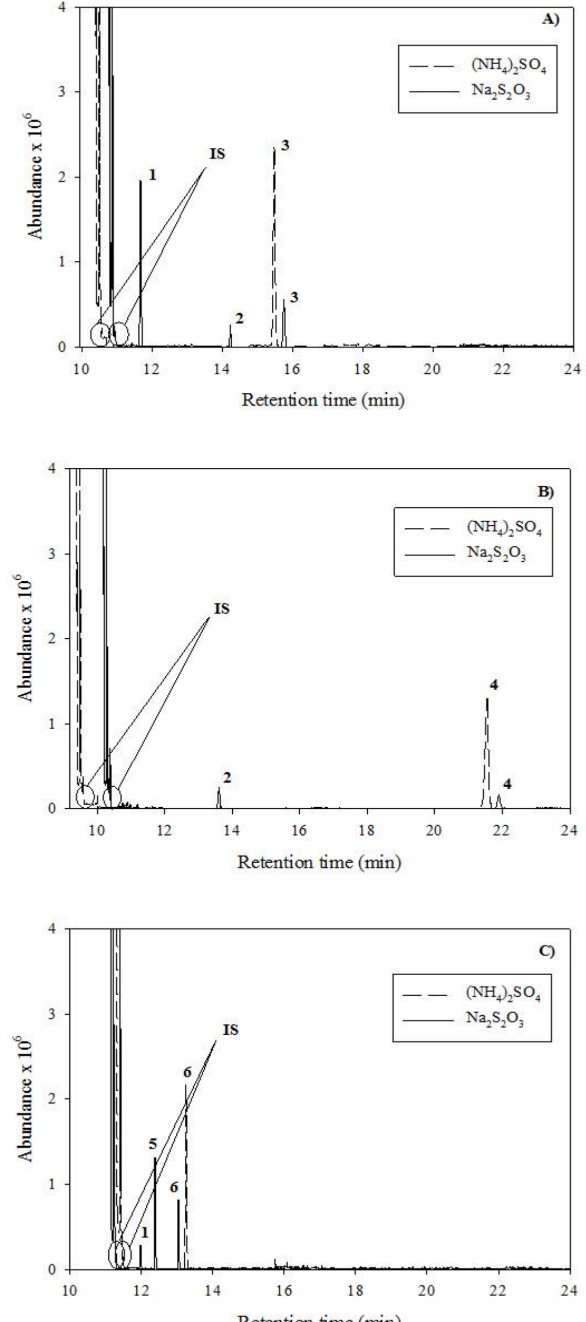

Figure 3. Stability of BCNM (A), DBNM (B) and TCNM (C) in a spiked (40 µg/L) tap water sample supplied with 0.1 M $(NH_4)_2SO_4$ (dashed line) or $Na_2S_2O_3$ (solid line) as dechlorinating agent after 2 h at 4 ºC. Overlay of both total ion chromatograms. Peak identification: CNM (1); BNM (2); BCNM (3); DBNM (4); DCNM (5); TCNM (6).

3.7. Analysis of water samples

The proposed method was used to determine HNMs and THMs in 6 tap and 3 swimming pool treated water samples. Only one HNM (TCNM) and four THMs were detected. **Table 2** lists the concentrations of the 3–5 analytes found at detectable concentrations in the chlorinated samples (swimming pool 3 excepted) or brominated samples (swimming pool 3). The results were validated against those provided by EPA Method 551.1 (see **Table 2**). The analytes not shown were either undetectable or present at levels below their LODs. Both methods provided similar results; however, EPA Method 551.1 failed to detect TCNM in some samples owing to its lower sensitivity. The concentration of TCNM in the tap samples ranged from 0.1 to 2.2 μg/L; also, the total THM concentrations were 7–50 μg/L and hence below the maximum contaminant level set by the US EPA (80 μg/L for the sum of THMs). The concentrations of TCNM found in the 3 swimming pool water samples (average 1.5 μg/L) were similar to those in the 6 tap water samples (average 1.0 μg/L), even though the concentrations of organic matter and residual chlorine were higher in the pool water samples (3 mg/L) than in the tap water samples (0.4 mg/L). This can be ascribed to a possible decomposition of HNMs in swimming pool water. For clarification purposes, two experiments in quintuplicate were conducted in parallel: (i) 2 L of swimming pool water and 2 L of tap water (both spiked with 20 μg/L of each HNM and 2 μg/L of the IS) were stored at 4 °C and analyzed within 1 day at intervals of 1 hour; and (ii) a similar process to the previous one by store both spiked samples at room temperature (~30 °C). In these experiments any dechlorinating agent was added. In relation to the stability of the 9 HNMs, the results obtained show that no significant difference was found between tap and swimming pool water. After 8 hours of storage at 4 °C some analytes (CNM, BNM, BDCNM, DBNM, DBCNM and TBNM) were partially degraded and others (DCNM, TCNM and BCNM) remained stable at least 1 day in both type of waters. The stability of the target analytes at room temperature (~30 °C) decreased drastically after 2 h of storage (DCNM, TCNM and BCNM excluded because they remained stable at least 12 h). We assumed that the possible explanation of the low concentration of HNMs (TCNM) in pool water was the result of the low reactivity of these compounds in accordance with Kanan and Karanfil [17]. This was also consistent with the low degree of HNM formation potentials in chlorinated

drinking water reported by Hu et al. [25]. However, pool water contained the highest THM concentrations owing to the presence of large amounts of disinfectants and organic matter from organic fluids from swimmers among other sources. As shown the HOBr and HOCl ratio has a marked influence on the resulting THM composition [16]. Repeated chlorination of swimming pool water under closed-loop operation tends to raise the proportions of the more chlorinated THM, the whole process leading to chloroform (TCM) enrichment relative to tap water. By contrast, brominated pool water contains abundant bromoform (TBM), which is again consistent with the preferential reaction of organic matter with HBrO over HClO.

Table 2. Analysis of treated water samples by chlorination (except to swimming pool water 3, treated by bromination) by the proposed (MLLE) and the reference (EPA 551.1) methods (n=5)

	Concentration found ± standard deviation (μg/L)									
	TCM		BDCM		DBCM		TBM		TCNM	
	MLLE	EPA 551.1	MLLE	EPA 551.1	MLLE	EPA 551.1	MLLE	EPA 551.1	MLLE	EPA 551.1
Tap 1	18 ± 1	21 ± 2	6.5 ± 0.4	6.2 ± 0.4	1.6 ± 0.1	1.3 ± 0.1	n.d.[a]	n.d.	0.1 ± 0.1	<0.2[b]
Tap 2	36 ± 3	32 ± 2	11 ± 1	13 ± 1	2.4 ± 0.2	2.8 ± 0.2	n.d.	n.d.	1.0 ± 0.1	1.2 ± 0.1
Tap 3	26 ± 2	24 ± 2	13 ± 1	16 ± 1	1.9 ± 0.1	2.1 ± 0.2	n.d.	n.d.	2.2 ± 0.1	1.9 ± 0.1
Tap 4	31 ± 2	34 ± 3	10 ± 1	9 ± 1	1.3 ± 0.1	1.5 ± 0.1	n.d.	n.d.	1.8 ± 0.1	2.1 ± 0.2
Tap 5	20 ± 1	22 ± 2	8.4 ± 0.6	8.1 ± 0.6	3.1 ± 0.2	2.7 ± 0.2	n.d.	n.d.	0.5 ± 0.1	0.9 ± 0.1
Tap 6	3.2 ± 0.2	2.9 ± 0.2	1.8 ± 0.1	2.0 ± 0.1	1.6 ± 0.1	1.3 ± 0.1	n.d.	n.d.	0.1 ± 0.1	<0.2
Swimming pool 1	56 ± 4	61 ± 4	16 ± 1	14 ± 1	n.d.	n.d.	n.d.	n.d.	2.4 ± 0.2	2.0 ± 0.1
Swimming pool 2	68 ± 5	70 ± 5	4.5 ± 0.3	4.2 ± 0.3	n.d.	n.d.	n.d.	n.d.	1.6 ± 0.1	1.3 ± 0.1
Swimming pool 3	6.6 ± 0.4	6.1 ± 0.4	3.6 ± 0.3	3.0 ± 0.3	5.7 ± 0.4	5.3 ± 0.4	11 ± 1	14 ± 1	0.5 ± 0.1	0.7 ± 0.1

[a]n.d., not detected.

[b]< limit of quantification

4. Conclusions

A new MLLE method for the joint determination of the nine HNMs and four THMs in treated water was developed. This is the first method to be adapted to EPA Method 551.1, which it outperforms in some respects, namely: (a) a reduced consumption of solvents, (b) shorter extraction times and reduced manipulation, and (c) higher preconcentration factors, with extraction efficiencies of ~85%. A rigorous study of the stability of the target analytes in the presence of various salts as dechlorinating agents revealed the best choice to be $(NH_4)_2SO_4$, which, however, was effective for the intended purpose for only 1 day. Nevertheless, the problem can be easily solved since the proposed method requires acidifying the sample, which, if done at the time of collection ensures effective preservation of the analytes for 2 days in the absence of salt. Since extraction requires only a few microlitres of organic solvent and practically all extract is injected into the PTV–LVI–GC–MS, MLLE can be regarded as a virtually solvent-free sample preparation technique. As in other LPME techniques, the difficulty encountered in visually inspecting collection of the extract and the problems posed by fiber non-uniformity in SPME can be circumvented by using a stereo microscope. The THM and TCNM concentrations found in the tap water samples fell in the low microgram-per-liter range and were thus compliant with environmental regulations; by contrast, the concentrations of THMs in swimming pool water were much higher but their maximum acceptable levels are unregulated as yet.

Acknowledgements

This work was funded in the frameworks of Projects CTQ2010–17008 (Spain's Ministry of Education) and P09–FQM–4732 (Andalusian Regional Government). FEDER provided additional funding. Isabel Montesinos is grateful for the award of a pre-doctoral grant from Project P09–FQM–4732DGI.

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CAPÍTULO 4

Desarrollo de métodos rápidos mediante HS–GC–MS. Aplicación a aguas tratadas

El análisis de compuestos orgánicos halogenados volátiles en agua se realiza principalmente mediante cromatografía de gases seguida de detección por captura de electrones o espectrometría de masas. Generalmente es necesaria una etapa de pretratamiento de la muestra, en la que los compuestos se separan de la matriz y, en algunos casos, se someten a procedimientos de preconcentración para alcanzar los niveles de sensibilidad deseados. Esta etapa, además de ser la más laboriosa, es la principal fuente de error del método analítico. Una técnica que presume de su robustez es el espacio de cabeza estático acoplado a un cromatógrafo de gases. La principal ventaja de esta configuración es que el tratamiento de la muestra se reduce al mínimo, evitando así los posibles errores asociados fundamentalmente a las pérdidas. Este Capítulo de la Memoria comprende el desarrollo de un método rápido, simple y sensible para la determinación de DBPs volátiles halogenados por HS–GC–MS, así como su aplicación a muestras de agua tratada.

Las especies diana en este Capítulo son las mismas que las contempladas en el Capítulo 3 [halonitrometanos (HNMs) y trihalometanos (THMs)] extendiéndose también a los haloacetonitrilos (HANs). En la primera parte se ha desarrollado el primer método descrito en la bibliografía para la determinación de los 9 HNMs clorados y bromados en aguas tratadas, tanto de consumo como de piscina, mediante HS–GC–MS. Los parámetros que controlan la extracción HS se optimizaron con el objetivo de obtener la mayor sensibilidad posible. En este contexto hay dos reactivos claves en la eficacia de la extracción, la adición de sales y la de un modificador químico. Al igual que se vio en los métodos contemplados en el Capítulo 3, la sal que proporcionó los mejores resultados fue Na_2SO_4. El modificador orgánico suele ser un disolvente orgánico más volátil que los analitos, que añadido a la fase acuosa favorece la eficacia de la extracción al calentar, debido a que cuando se volatiliza arrastra a los analitos. Se ensayaron varios disolventes observándose que la polaridad de los mismos y sus puntos de ebullición eran propiedades prioritarias para favorecer la volatilización de los HNMs. MTBE fue el disolvente que proporcionó los mejores resultados ya que incrementó el rendimiento de la volatilización aproximadamente 4 veces en comparación con no utilizar ninguno. Una comparación con el método SDME–GC–MS descrito en el Capítulo 3 muestra que los LODs obtenidos por HS–GC–MS son cuatro veces inferiores

a los obtenidos por microextracción líquida, con similar precisión. La validación con la alternativa EPA 551.1 pone de manifiesto que el método aquí descrito por HS–GC–MS es cinco veces más sensible que el general por LLE de la EPA para volátiles halogenados y con unas recuperaciones similares >94%. En lo referente a la determinación de HNMs en aguas de piscina cabe resaltar que solo se detectó tricloronitrometano a concentraciones entre 0.4 y 1.9 μg/L, concentraciones por otra parte similares a las del agua de consumo, a pesar de que la concentración de materia orgánica y de cloro residual es mucho mayor en piscinas.

En la segunda parte de este Capítulo se contempló la inclusión de 6 HANs porque son también DBPs nitrogenados, como los HNMs, y porque son significativamente más tóxicos que los DBPs regulados (THMs y ácidos haloacéticos). Por lo tanto en esta parte de la Memoria se incluye los 4 THMs regulados, 6 HNMs y 6 HANs. Se continuará con la línea HS–GC–MS debido a las ventajas de esta técnica en relación con las de microextracción líquida. Los parámetros químicos son similares a los del método HS–GC–MS descrito en la primera parte solo para HNMs. Así la muestra de agua se ajusta a un pH ácido, se emplea Na_2SO_4 y MTBE como modificador orgánico. La eficacia de la extracción es elevada (85–95%) con la excepción de los DBPs nitrogenados monohalogenados (cloronitrometano, bromonitrometano, cloroacetonitrilo y bromoacetonitrilo) que solo se extraen en un 60%. No obstante este dato no es relevante dado que los DBPs volátiles que se encuentran usualmente en aguas tratadas son dihalogenados y especialmente trihalogenados. El método es extraordinariamente sensible con LODs entre 10 y 200 ng/L y preciso, RSD <6%. El método aplicado a aguas de grifo permite la detección de hasta 10 de las especies estudiadas, en ocasiones a niveles de ng/L, debido a la elevada sensibilidad del mismo.

Finalmente se estudió la influencia de algunos parámetros químicos (pH, oxidabilidad, nitrito, nitrato, amonio, cloro residual y bromuro) y el proceso de desinfección (ClO_2/Cl_2, Cl_2/NH_2Cl y O_3/Cl_2) en la formación y especiación de estos compuestos a partir de muestras recogidas de diversas plantas de tratamiento de agua. Los parámetros se estudiaron en amplios intervalos: pH (7.0–8.2), oxidabilidad al permanganato (≤0.1–2.3 mg O_2/L), nitrito (≤0.01 mg/L), nitrato (≤1–50 mg/L), amonio (≤0.05–1.60 mg/L) y

cloro residual libre (0.04–0.80 mg/L). Las aguas fueron suministradas por empresas de potabilización de aguas y se seleccionaron a lo largo de un año para conseguir mayores intervalos de variabilidad. Por lo tanto este estudio se realizó con aguas reales y aptas para el consumo y en ningún caso se prepararon en laboratorios. Los resultados sugirieron que la oxidabilidad al permanganato (relacionada con la materia orgánica) y la concentración de bromuro, así como las condiciones de desinfección están directamente relacionadas con la aparición de estos compuestos, ambos en su concentración y especiación. Por tanto, el interés de este estudio permite el conocimiento de parámetros influyentes en la formación de estos DBPs lo que facilita la información adecuada para evitar su formación a mayor escala.

Anal Bioanal Chem

ORIGINAL PAPER

Headspace gas chromatography–mass spectrometry for rapid determination of halonitromethanes in tap and swimming pool water

I. Montesinos, M. Gallego*

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071, Córdoba, Spain

Analytical and Bioanalytical Chemistry 402 (2012) 2315

Abstract

Halonitromethanes (HNMs) are one of the most cytotoxic and genotoxic classes found among the unregulated disinfection by-products formed by the reaction of chemical disinfectants with natural organic matter in water. Typical methods used to determine these compounds in water (mainly trichloronitromethane) are based on the Environmental Protection Agency (EPA) method 551.1 using liquid-liquid extraction. A fast and straightforward method for the determination of the nine HNMs in water has been developed using a static headspace (HS) coupled with gas chromatography–mass spectrometry (GC–MS). Important parameters controlling headspace extraction were optimised to obtain the highest sensitivity: 250 µL of methyl tert-butyl ether (as a chemical modifier) and 6 g of anhydrous sodium sulphate were added to the water sample; an oven temperature of 80 °C and an equilibration time of 20 min were also selected. The addition of a chemical modifier favoured the volatilisation of all HNMs, increasing their signals up to approximately four times. Under optimum conditions, the method developed provides limits of detection between 0.03 and 0.60 µg/L and a relative standard deviation of ~6.0%. The developed method was validated and then compared with the reference method EPA 551.1 for the analysis of tap and swimming pool water. A good agreement in the results was observed, which corroborated the good performance of the proposed HS–GC–MS method.

1. Introduction

The disinfection of drinking waters is an essential treatment used to inactivate microbial pathogens in the water supply and ease the removal of certain physical-chemical contaminants. Drinking water disinfection by-products (DBPs) are formed when a chemical disinfectant (chlorine, chloramine, chlorine dioxide or ozone) reacts with natural organic matter and/or bromide/iodide present in drinking water supplies [1,2]. Nitrogenous organic compounds consume chlorine or chloramines, forming organic chloramines which decompose to form nitrogenous DBPs such as haloacetonitriles, cyanogens halides and halonitromethanes (HNMs) [3]. Furthermore, although more than 600 emerging DBPs have been reported in the literature, less than 100 have undergone quantitative occurrence or health effect studies [4]. Several epidemiological studies have revealed that there is an association between health effects and exposure to DBPs. In this context, even though the HNMs are present at lower concentrations than regulated trihalomethanes (THMs) and haloacetic acids (HAAs) in the USA, recent research indicate that they have higher cytotoxicity and genotoxicity than the more common DBPs [5,6]. The high degree of cytotoxicity and genotoxicity of HNMs is attributed to the great intrinsic reactivity conferred by the nitro group. On the other hand, brominated and mixed bromo-chloro-nitromethanes are more genotoxic than chlorinated nitromethanes [7]. The genotoxicity of trichloronitromethane (TCNM) and bromonitromethane in *in vivo* wing somatic mutation and recombination in the Drosophila wing-spot test [8] and in human cells treated in vitro [9] shows that both compounds are highly genotoxic. Currently, nine HNMs can be formed from chlorine, chloramine, ozone-chlorine, or ozone-chloramines disinfection; in this process, nitrite plays an important role in the formation of the nitro group of these compounds. Moreover, recent studies indicate that the HNMs may increase when ozonation is used prior to chlorination or chloramination treatment, which produces trihalogenated HNMs as the major species [10]. Most of these are also found in drinking water treated with chlorine or chloramine (without ozone), but at much lower levels, indicating that ozone may be an important component in their formation [11,12]. In treated water, the total concentration of HNMs when chlorine is applied ranged from undetectable to 3.4 µg/L [13] and between 0.9 and 1.5 µg/L for TCNM in wastewater treatment plant effluents [11].

Given the fact that high levels of free chlorine residuals continuously maintain in public swimming pools and the high level of human body excretions, swimming pools have recently been recognised as an important source of exposure to DBPs. The formation of different DBPs from these materials showed concentrations of TCNM in indoor swimming pool water between 0.7 and 1.7 μg/L [14], or 0.4–2.3 μg/L both in indoor and outdoor pools [15].

The Environmental Protection Agency (EPA) method 551.1 is commonly used for the determination of halogenated volatile organic compounds (VOCs) in drinking water [16], and adaptations of this method have been used in several studies for the measurements of HNMs by gas chromatography–electron capture detection (GC–ECD) [6,10,11,17] or by GC–mass spectrometry (MS) [18]. Other analytical methods that have been developed for the determination of VOCs (including TCNM) in drinking water were headspace solid-phase microextraction with GC–ECD [19] and Purge & Trap–GC–MS [20]. The headspace (HS) technique with GC has also been used for the determination of VOCs (including TCNM) [21] or only TCNM [22], providing limits of detection (LODs) of 0.5 or 2.5 μg/L, respectively. In both methods, 8 or 5 mL of the water sample (in 10-mL vials) was heated in a water bath at 45 or 80 °C for 40 or 30 min; then, 0.5 mL of the gas phase was withdrawn with a gas syringe and manually injected into the GC–MS [21] or GC–ECD [22], respectively. Neither of these methods study the variables involved, such as the sample pH, ionic strength, etc., which are related to the volatilisation efficiency of the compounds; in addition, low recoveries and precision (taking into account manual operation and the absence of internal standard) were obtained. However, the HS technique is a fast, simple, efficient and environment-friendly sampling method that has recently been used with GC for the analysis of some DBPs in water samples, such as THMs [23] and HAAs [24]. Recently, a specific method to determine the nine HNMs based on single-drop microextraction in the headspace mode has been proposed in order to miniaturise the extraction process, which provides low detection limits (0.06–1.2 μg/L), using 1-hexanol as the extractant [15]. An aspect not adequately evaluated to date is the application of the static HS technique for the determination of halonitromethanes, considering the advantages of the HS versus other extraction alternatives in terms of selectivity, since only

the volatile fraction from the aqueous sample is introduced into the GC instrument. The aim of this study was to develop a sensitive and straightforward method for the determination of HNMs in treated water by HS–GC–MS since practical considerations suggest the use of a single method for the determination of some classes of DBPs without compromising the results for other analytes. Furthermore, the present research is the first of its kind to include a rigorous study of the variables involved in the volatilisation of all species of halonitromethanes, with a special emphasis on the sample pH, type of salt, stability of HNMs in treated water and the use of a dechlorinating agent. The proposed method could be embraced by public laboratories which need to perform routine controls of HNMs in water.

2. Materials and methods

2.1. Standards and chemicals

TCNM (99%) and bromonitromethane (BNM, 90%) standards were supplied by Sigma-Aldrich (Madrid, Spain), while chloronitromethane (CNM, 90–95%), dichloronitromethane (DCNM, 95%), bromochloronitromethane (BCNM, 85–90%), bromodichloronitromethane (BDCNM, 90–95%), dibromonitromethane (DBNM, 90%), dibromochloronitromethane (DBCNM, 90–95%) and tribromonitromethane (TBNM, 90–95%) were purchased from Orchid Cellmark (New Westminster, Canada); the internal standard, fluorobenzene, was from Fluka (Madrid, Spain). Potassium and sodium chlorides, anhydrous sodium sulphate and ammonium sulphate (dechlorinating agent) were supplied by Panreac (Barcelona, Spain). Ethyl acetate, *n*-pentane, methyl tert-butyl ether (MTBE), *n*-hexane, cyclohexane and sulphuric acid were purchased from Merck (Darmstadt, Germany). Individual stock standard solutions of halonitromethane compounds at concentrations of 1.0 g/L and cumulative solutions (0.1 g/L) were prepared in ethyl acetate and stored frozen in amber glass vials at −20 °C. More dilute solutions were prepared daily in mineral water (free of DBPs) to obtain concentrations at the microgram per litre level.

2.2. Instrument

The experimental setup consisted of an HS autosampler HP 7694 and an HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with an HP 5973N mass selective detector. The autosampler included a robotic arm, a 44-space autosampler carousel and a HS generation unit, which combined an oven to heat the samples inside the vials and a six-port injection valve with a 3-mL loop filled with the HS fraction. The operating conditions for the HS autosampler were as follows: vial equilibration time, 20 min; oven temperature, 80 ºC; vial pressurization time, 30 s; loop fill time, 9 s; valve/loop temperature, 100 ºC. Helium (6.0 grade, Air Liquid, Seville, Spain), regulated with a digital pressure and flow controller, was used both to pressurize vials and drive the headspace formed to the injection port of the chromatograph via the transfer line at 110 ºC. Injection was done in the split mode (split ratio, 1:20) for 1 min; an HP-5MS [(5%)-phenyl(95%)-methylpolysiloxane capillary column (30-m × 0.32-mm i.d., 0.25-μm film thickness), J&W] was used. The temperature conditions were as follows: 40 ºC for 3 min and then raised at 40 ºC/min to 140 ºC, held for 2 min, and ramped at 40 ºC/min to 180 ºC, finally held for 3 min; chromatographic run time, 11.5 min. Helium carrier gas was passed at a rate of 1 mL/min; a solvent delay of 3.3 min was used. The injector, source and quadrupole temperatures were maintained at 170, 200 and 150 ºC, respectively. The MS was operated in the electron impact ionization mode using electron energy of 70 eV. Optimisation experiments were conducted in total ion chromatography mode between *m/z* 29 and 300 at 3.5 scans per second. Quantification of HNMs was performed in selected ion monitoring mode, and five different acquisition windows were defined taking into account the retention times and suitable fragments of HNMs, as shown in **Table 1** [*m/z* values for fluorobenzene (internal standard, IS)—50, 70 and 96 (base peak)]. All the scans were performed in high-resolution mode and with a dwell time of 100 ms. Total ion current chromatograms were acquired and processed using G1701DA D.01.02 Standalone data analysis software (Agilent Technologies) on a Pentium IV computer that was also used to control the whole system.

Twenty-millilitre glass flat-bottomed vials for headspace analysis with 20-mm PTFE/silicone septa caps and crimped aluminium closure (Supelco, Madrid, Spain) were also employed. Vials and septa were heated at 100 and 70 °C, respectively, overnight prior to use.

Table 1. Analytical characteristics of the determination of HNMs by the HS–GC–MS and EPA 551.1 (GC–MS) methods

Compounds	m/z[a]	HS–GC–MS				EPA 551.1			
		LOD (μg/L)	Linear Range (μg/L)	RSD (%)		LOD (μg/L)	Linear Range (μg/L)	RSD (%)	
				Within-day	Between-day			Within-day	Between-day
CNM	46, **49**, 51	0.15	0.5–300	6.2	7.0	0.20	0.7–300	6.7	7.9
DCNM	46, **83**, 85	0.04	0.2–300	5.4	6.3	0.06	0.2–300	6.1	6.9
TCNM	46, **117**, 119	0.03	0.1–300	5.2	6.0	0.08	0.3–300	5.8	6.7
BNM	46, **93**, 95	0.16	0.5–300	6.5	7.4	0.20	0.7–300	6.6	7.6
BCNM	46, 127, **129**	0.06	0.2–300	5.2	6.2	0.07	0.2–300	5.5	6.6
BDCNM	46, 161, **163**	0.07	0.2–300	5.5	6.4	0.40	1.4–300	7.6	8.7
DBNM	46, 171, **173**	0.08	0.3–300	5.8	6.7	0.20	0.7–300	6.6	7.5
DBCNM	46, **207**, 209	0.11	0.4–300	6.3	7.3	0.70	2.3–300	8.0	9.1
TBNM	46, **251**, 253	0.60	2.0–300	8.4	9.5	4.5	15–300	9.5	10.5

[a]Characteristic ions used for identification of HNMs, in boldface the quantification m/z values.

2.3. Sampling and preservation

Tap and swimming pool water samples were collected in 125 mL amber glass bottles with PTFE screw caps and without headspace in order to avoid evaporation of volatile compounds. In order to reduce any free chlorine, 1.7 g of ammonium sulphate was added to each bottle prior to sampling (0.1 mol/L). The stability of the nine HNMs in water has been previously established, resulting in the compounds only remaining stable when the water samples were stored at 4 °C and analysed within 1 day of collection [15]. When the time between sample collection and analysis exceeded 1 day, samples could be stored at –20 °C up to 7 days.

2.4. HS–GC–MS procedure

Twelve millilitres of tap or swimming pool water samples or halonitromethane standards prepared in mineral water (free of DBPs) containing between 0.1 and 300 µg/L of each HNM and 20 µg/L of fluorobenzene in 0.1 mol/L ammonium sulphate (dechlorinating agent) was added to a 20 mL glass vial with 6 g of anhydrous sodium sulphate (saturated solution). Then, 250 µL of MTBE was added and the pH was adjusted to ~3.5 by adding 20 µL of 0.1 mol/L H_2SO_4. The vial was immediately sealed and vortexed for 1 min for homogenisation purposes. Finally, the vial was placed in the 44-space autosampler carousel where the robotic arm took each one and introduced it in the HS oven to release HNMs from the liquid to the gas phase in the aforementioned conditions. In the next step, the injection valve was switched and the helium stream carried the sample loop content towards the GC–MS instrument. HNM separation through the chromatographic column was performed using the temperature programme mentioned above. Finally, each analyte was identified and quantified in the mass spectrometer using three characteristic *m/z* ratios, as can be seen in **Table 1**.

2.5. Liquid-liquid extraction (EPA method 551.1) procedure

Liquid-liquid extraction for the determination of HNMs in water was performed in quintuplicate following EPA method 551.1 [16]. Samples were collected in 62 mL amber bottles with a poly(tetrafluoroethylene) screw cap containing 0.8 g of ammonium sulphate and without headspace to avoid evaporation of VOCs. A 12 mL aliquot was withdrawn from the sample bottle and discarded; the pH was adjusted at 4.5–5.5 with diluted H_2SO_4. Fifty microlitres of a 10 mg/L standard solution of fluorobenzene (IS), 3 mL of the extracting solvent (MTBE), 20 g of Na_2SO_4 and 1 g of copper sulphate were added to the remaining sample (50 mL); the vial was stirred for 4 min. Once the HNMs were extracted, the vial was left to stand for 2 min in order to separate both phases. Approximately 1 mL of the upper MTBE layer was transferred to a 2 mL glass vial, and 0.1 g of anhydrous Na_2SO_4 was added to dry the extract. Finally, 2 µL of the extract was injected into the GC–MS instrument.

3. Results and discussion

3.1. Optimization of instrumental headspace variables

Oven temperature has a pronounced influence on the efficiency of the extraction because it has a direct impact on the equilibrium concentration of the HNMs in the headspace of the sample vial. This variable was studied using 10 mL of mineral water at pH ~3.0 (adjusted with a few drops of diluted sulphuric acid) containing 100 µg/L of individual HNM, 20 µg/L of fluorobenzene (IS) and 3 g of Na_2SO_4 in 20 mL glass vials. All the extraction experiments were performed by measuring the relative peak area of each halonitromethane to the internal standard using the average of three replicate measurements (after that the different peak areas of fluorobenzene between the temperatures were normalised). In these experiments, the sample loop temperature was kept 20 ºC higher than the oven one and the transfer line 10 ºC higher than the sample loop temperature. The oven temperature was evaluated from 50 to 80 ºC (higher temperatures were not tested in order to minimise the evaporation of water) using a vial equilibration time of 25 min, with the loop and transfer line temperatures changed accordingly. The analytical signal increased as the

temperature did; thus, 80 ºC was selected for further experiments. The time required to reach equilibrium was also studied between 5 and 30 min using an oven temperature of 80 ºC. The optimal relative peak areas were obtained above 18 min, remaining constant from this value; 20 min was chosen as the optimal vial equilibration time. Pressurization time was verified between 10 and 50 s, causing negligible changes in the abundance signal above 30 s. The loop fill time had no effect above 9 s.

3.2. Optimization of chemical variables

3.2.1. Sample pH and ionic strength

To date, there has not been any information about sample pH values to favour the generation of headspace for HNMs. The only documentation about the influence of the sample pH on the extraction of the nine HNMs from water is related to a miniaturisation method based on single-drop microextraction in the headspace mode (HS–SDME); this study showed that the optimal value of the sample pH ranged from 3.0 to 3.5 for the extraction of all HNMs in 1-hexanol [15]. Therefore, in this work, the first chemical variable studied was the sample pH for the nine HNMs since it affects the generation of the headspace; this variable was assayed over the range 2.0–7.5. A few drops of diluted sulphuric acid solutions were used to adjust the pH of the aqueous sample in the acid region (hydrochloric acid was discarded as it contains chlorine and the analytes are halogenated compounds; HNO_3 was also discarded as it is an oxidant) [15], although there was none in the original 10 mL of spiked mineral water samples (pH ~7.5). As can be seen in **Figure 1**, the volatilisation of the nine target analytes was relatively constant in the 2.8–4.2 range, decreasing their analytical signals above this value. TCNM (chloropicrin) was the least affected by the change in pH. The decrease in analytical signals could be attributed to a base-catalyzed hydrolysis of HNMs [15]. To minimise sample manipulation, the aqueous sample was adjusted to pH ~3.5 by adding 16 μL of 0.1 mol/L H_2SO_4 per 10 mL of sample.

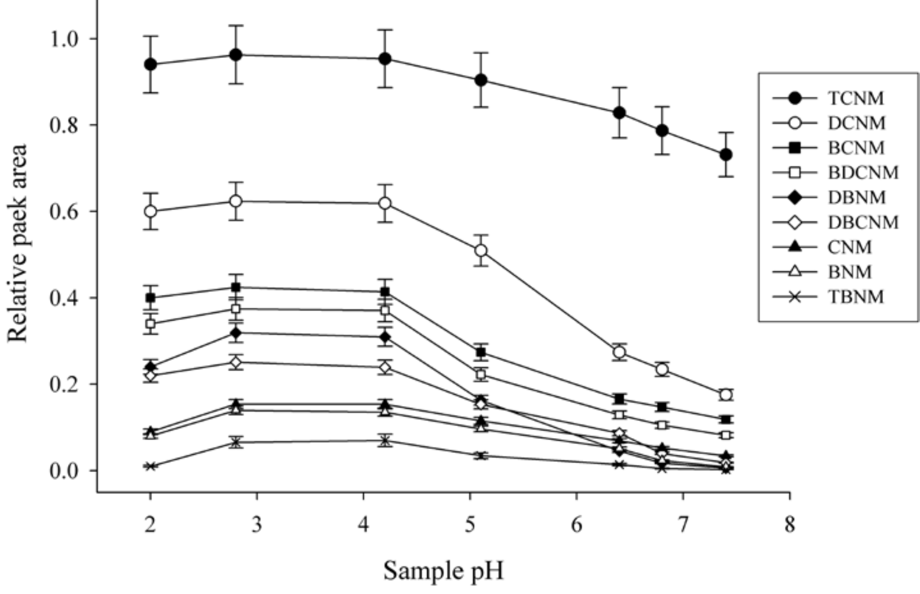

Figure 1. Influence of the sample pH on the volatilisation efficiency of the nine HNMs. Error bars are the standard deviation for three measurements.

The so-called salting-out effect is commonly used to improve the release of organic volatile compounds from an aqueous sample matrix to its headspace. The salting-out increased the ionic strength of the aqueous solution and, in this way, could decrease the solubility of target analytes and, therefore, the variation of the vapour/liquid equilibrium system. In our case, a rigorous study of this parameter was achieved as there are no studies on this effect for individual HNMs in the proposed HS methods [21,22]. Initially, three types of salt, including potassium chloride, sodium chloride and anhydrous sodium sulphate, were assayed at variable molarities up to the saturated solution. **Figure 2** shows the best results obtained for each salt. As can be seen, the addition of any type of salt provides better conditions for the volatilisation of all HNMs when compared with the unsalted experiment. The data showed that sodium sulphate increased the volatilisation efficiency of all HNMs to different degrees according to the number of halogens. Thus, the addition of 5 g Na_2SO_4 per 10 mL of water sample (saturated solution, ~3.5 mol/L of Na_2SO_4) increased the relative peak areas of approx. 6, 10 or 20 times for

trihalogenated (TCNM, BDCNM, DBCNM and TBNM), dihalogenated (DCNM, BCNM and DBNM) or monohalogenated (CNM and BNM) compounds, respectively, in relation to unsalted samples. This effect can be ascribed to the fact that the addition of salt to the aqueous phase favoured the volatilisation of moderately polar species, so monohalonitromethanes were the species most favoured by the presence of salt due to their higher polarities.

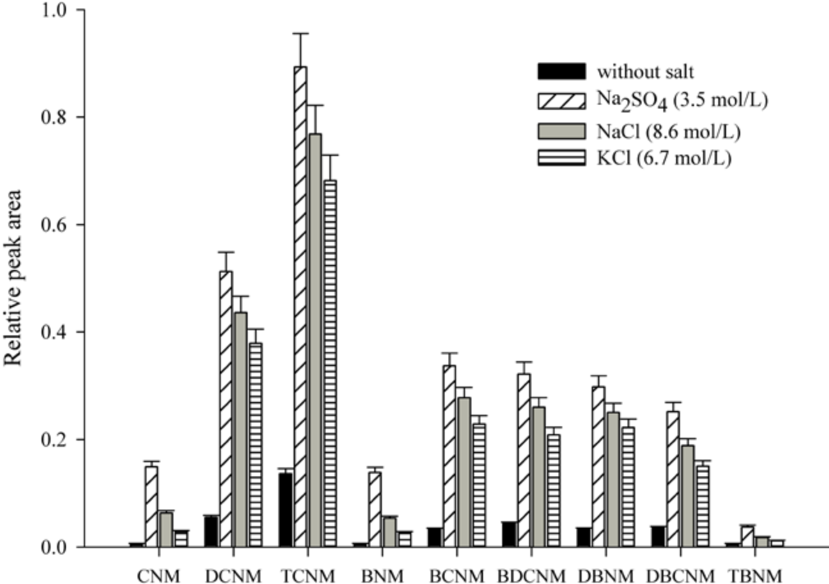

Figure 2. Effect of different type of salts and no salt addition to the aqueous phase on the volatilisation efficiency of the nine HNMs. Error bars are the standard deviation for three measurements.

3.2.2. Effect of sample volume and chemical modifier

The ratio of the volume of the aqueous and gaseous phases in the vial can affect the concentration of the HNMs in the headspace. To ensure that the autosampler needle will not come into contact with the sample during the sampling time, 12 mL of aqueous sample was taken as the highest value (when salt was added to 20 mL vials containing 12 mL water, the volume increased to ~15 mL). In order to study the effect of the sample volume on the sensitivity of the method, the volume of sample was examined from 5 to 12 mL (in 20 mL vials) containing 100 µg/L of each HNM and 20 µg/L of fluorobenzene (IS) in 3.5 mol/L Na_2SO_4 at pH ~3.5. The signal abundance increased on increasing the sample volume up to 12 mL, probably as the result of the increasing HNM concentrations in the headspace. Therefore, a sample volume of 12 mL (in 20 mL vials) was chosen as the optimal value. As the sample volume was varied in relation to the initial optimisation process (10 mL), the most prevalent variables (oven temperature, equilibration time and ionic strength) were checked again. The results obtained for 12 mL were similar to those provided for 10 mL of sample volume.

The release of volatile compounds in aqueous solution is favoured by the presence of an organic modifier, which is why modifiers may enhance the determination of DBPs by HS–GC–MS [24]. For this reason, several solvents (cyclohexane, *n*-pentane, ethyl acetate, *n*-hexane and MTBE) were individually added to the aqueous sample in order to select the most suitable one for the volatilisation of HNMs at low temperatures. The effect of each modifier was very different and divides them into two groups, namely (1) solvents that did not favour the volatilisation of HNMs, at least in a significant way, in relation to the experiment without a modifier (cyclohexane, *n*-pentane and ethyl acetate) and (2) solvents that increase the volatilisation efficiency of all HNMs (*n*-hexane and MTBE). Surprisingly, a very different behaviour was observed between solvents with similar chemical structure and polarity such as *n*-pentane and *n*-hexane. *n*-Pentane has a lower boiling point (~36 °C) than *n*-hexane (~69 °C); thus, the first one was evaporated quickly into the oven (80 °C), hindering the transport of the HNMs to the headspace. All this is visualised in **Figure 3**.

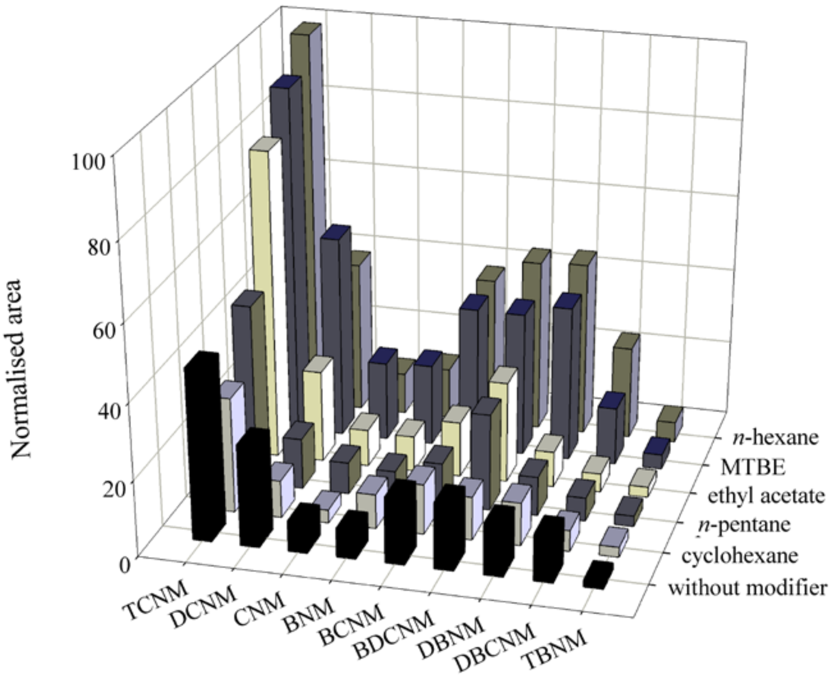

Figure 3. Influence of selection of the chemical modifier on the normalised area responses (the relative peak area of each HNM was normalised with respect to TCNM). For each experiment, 150 µL of each organic solvent was added to the water sample.

Thus, the highest peak area ratios (normalised to 100%) were obtained using 150 µL of *n*-hexane or MTBE, which provided an average increase of ~2.5 times in relation to the signal obtained without the addition of a modifier. The trihalonitromethanes were the most favoured with *n*-hexane due to their lower polarities, whereas for monohalonitromethanes, it was MTBE as a consequence of the higher polarities. Therefore, the following experiment was advocated to discriminate between the two solvents or a mixture of them. On this basis, *n*-hexane and MTBE were individually studied as modifiers from 150 to 300 µL and as a mixture at different ratios (MTBE/*n*-hexane, 75:75, 50:100, 100:50, 100:100, 100:200 and 200:100, v/v in microlitres). The best results

obtained for each solvent and the mixture of the two were illustrated in **Figure 4**. Two hundred and fifty microlitres of MTBE provided the best results in terms of normalised area for all compounds in the HS–GC–MS method since the average analytical signal increased approximately four times in relation to the signal obtained without a modifier. This can be ascribed to the fact that the volatilisation of the most volatile solvent (MTBE) swept the HNMs also.

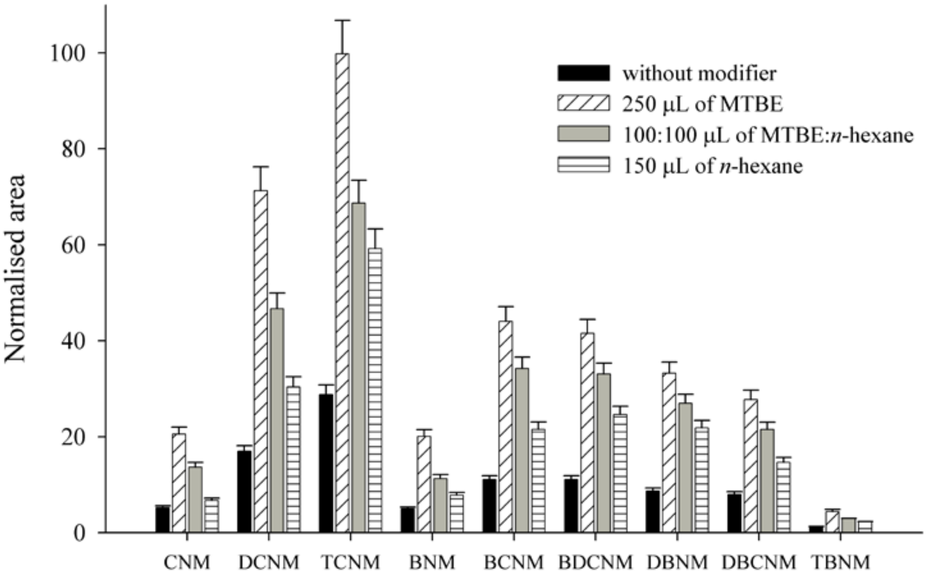

Figure 4. Effect of the volume of the MTBE and/or *n*-hexane on the normalised area responses of the nine HNMs. Error bars are the standard deviation for three measurements.

Finally, the efficiency percentage of the whole process for the nine halonitromethanes in aqueous medium was calculated in quintuplicate by a second extraction of the remaining aqueous phase by spiking 250 μL of fresh MTBE again in order to check for the absence of HNMs. The results showed that the analytical signals obtained for trihalonitromethanes were lower than 5% (no significant carryover), whereas for dihalonitromethanes and monohalonitromethanes they were 20% and 45%, respectively. The extraction efficiency of monohalonitromethanes (55%) was the lowest due to

their higher polarities and solubilities in water (~15 times more soluble than trihalonitromethanes). Therefore, although a significant carryover was obtained for monohalonitromethanes, the results obtained do not justify a second extraction. In addition, the HNMs normally present in treated water were the trihalonitromethanes which provided a good efficiency extraction (95%).

3.3. Validation of static HS–GC–MS

The performance and reliability of the proposed HS–GC–MS method was assessed by determining the linear range, analyte detectability and precision for the nine HNMs studied. Several analytical curves were constructed using 12 mL of standards in mineral water with variable amounts of analytes (0.1–300 μg/L); good correlation coefficients were obtained (higher than 0.993 in all cases). LODs, determined as the lowest concentration of the analyte that can be reliably differentiated from the background level (signal-to-noise ratio=3) [25], ranged from 0.03 to 0.60 μg/L. The precision of the proposed method, as relative standard deviation (RSD), was evaluated by analysing 11 individual standard mixtures containing 5 μg/L concentration of each halonitromethane in mineral water on the same day and three different days. The results obtained were satisfactory, with RSD average values of 6.0 ± 1.0% (within-day) and 7.0 ± 1.0% (between-day).

A comparison of the proposed method was carried out with the only method described in the bibliography for the whole array determination of halonitromethanes in water by single-drop microextraction in the headspace mode (HS–SDME–GC–MS) [15]. The LODs provided by the proposed HS–GC–MS method for DCNM, TCNM and TBNM or BDCNM and DBCNM were approximately two or three times lower than those achieved by the HS–SDME–GC–MS, whereas for CNM and BNM or DBNM, these values were approximately six or ten times lower. The RSD was slightly lower for the HS–GC–MS method than those provided by the HS–SDME–GC–MS alternative, with average values of 7.0 ± 1.5% (within-day) and 8.0 ± 1.5% (between-day) [15]. We can conclude that the limits of detection of the proposed method are approximately four times lower than the HS–SDME–GC–MS, with similar precision.

3.4. Comparison of static HS–GC–MS with EPA method 551.1

To understand the feasibility of quantitative analysis using this new method, the static headspace technique was compared with the method usually employed to determine TCNM with other halogenated VOCs in water. Thus, the proposed HS–GC–MS method was compared with that of EPA 551.1, also using GC–MS, but with this instrument, the EPA method was not as sensitive as with GC–ECD [16] because ECD provides higher sensitivity for halogenated compounds. By way of example, the LOD value reported only for TCNM using GC–ECD is 0.014 μg/L [16]. All quantitative parameters were determined, as previously mentioned, using the best pre-concentration factor for the manual EPA alternative [ratio aqueous volume (50 mL)/ organic volume (3 mL)= 17]. As listed in **Table 1**, the LODs provided by the proposed method were about five times lower than those obtained by EPA method 551.1, the low levels obtained for brominated HNMs being especially significant. The reproducibility study was evaluated by analysing 11 mineral water samples spiked with a concentration of 5 μg/L of each HNM (except for TBNM, 20 μg/L) on 1 day and on three different days. EPA method was slightly less precise than the HS–GC–MS, with average RSD values of 6.9 ± 1.3% (within-day) and 8.0 ± 1.3% (between-day), and it provided higher LODs with average values of 0.71 ± 1.43 versus 0.14 ± 0.18 μg/L for the HS–GC–MS method.

Finally, the recoveries of both methods were calculated by spiking tap water with two different concentrations of each HNM (1 and 10 μg/L; 3 and 10 μg/L for TBNM) to 12 mL of the sample for the HS–GC–MS method or with 3 and 10 μg/L (20 and 40 μg/L for TBNM) for the EPA alternative; all experiments were carried out in quintuplicate (n=5). Tap water contained TCNM at detectable levels, and in this case, its concentration in the spiked samples was quantified and compared with those calculated as the sum of the native concentration in the unspiked sample and the spiked concentration. Average recoveries of the HS–GC–MS method varied between 94% and 96%, whereas for the EPA method it ranged from 92% to 97% for the low and the high amount levels, respectively. The results of the HS–GC–MS method were in good agreement with those obtained by EPA method 551.1.

3.5. Analysis of water

The proposed HS–GC–MS method was applied to determine HNMs in treated water, including 12 tap and 6 swimming pool samples. For comparison, other untreated waters including pond, lake and river water were also analysed since TCNM (chloropicrin) can be used as fumigants for controlling soil-borne pathogens, parasitic nematodes, fungi and weeds; therefore, it can appear in these types of waters [26]. Only tap and swimming pool waters were classified as positive for TCNM: the other HNMs were either not found or were beneath detection limits. Brominated HNMs were undetected in all positive water samples because they probably contained low bromide ion concentration. The TCNM concentrations obtained were compared with those provided by EPA method 551.1, both listed in **Table 2**. Chloropicrin concentrations in tap water ranged from 0.2 to 3.0 μg/L, which were in agreement with those provided by the EPA method. TCNM was not quantified in some water samples using the EPA method because of its lower sensitivity when compared with the HS–GC–MS method. Surprisingly, the concentrations of TCNM found in swimming pool waters (between 0.4 and 1.9 μg/L) were similar to those of tap waters, although the concentration of residual chlorine and organic matter was higher in swimming pools than in tap waters. These data were in agreement with Kanan and Karanfil [14] who indicated the low reactivity of chlorine with natural organic matter to produce HNMs. Although other studies included TCNM in the determination of chlorinated VOCs in drinking water [19,21,22], chloropicrin was rarely detected since the LODs of these methods were high, preventing the methods from being applicable for determining trace concentrations of TCNM in treated water.

Table 2. Concentration of TCNM found in water samples by the proposed HS–GC–MS and EPA 551.1 (GC–MS) methods (n=5)

	Concentration of TCNM found ± standard deviation (μg/L)	
	HS–GC–MS	EPA 551.1
Tap 1	2.5 ± 0.2	2.7 ± 0.2
Tap 2	0.2 ± 0.1	<0.3
Tap 3	2.8 ± 0.2	2.4 ± 0.2
Tap 4	3.0 ± 0.2	2.7 ± 0.2
Tap 5	1.6 ± 0.1	1.9 ± 0.2
Tap 6	0.2 ± 0.1	<0.3
Tap 7	2.2 ± 0.2	2.6 ± 0.2
Tap 8	2.9 ± 0.2	3.2 ± 0.2
Tap 9	0.2 ± 0.1	<0.3
Tap 10	0.4 ± 0.1	0.4 ± 0.1
Tap 11	3.0 ± 0.2	2.8 ± 0.2
Tap 12	1.8 ± 0.1	1.5 ± 0.1
Pond 1–4	<0.03	<0.08
Lake 1	<0.03	<0.08
River 1–2	<0.03	<0.08
Swimming pool 1	1.3 ± 0.1	1.5 ± 0.1
Swimming pool 2	0.8 ± 0.1	0.7 ± 0.1
Swimming pool 3	0.4 ± 0.1	0.6 ± 0.1
Swimming pool 4	0.8 ± 0.1	0.6 ± 0.1
Swimming pool 5	1.4 ± 0.1	1.4 ± 0.1
Swimming pool 6	1.9 ± 0.1	1.6 ± 0.1

4. Conclusions

The proposed HS–GC–MS method provided lower limits of detection for determining the nine HNMs in comparison to other methods proposed for VOCs that included TCNM. All liquid-liquid extraction methods for halogenated VOCs require large volumes of extractant, except a miniaturised liquid phase microextraction (LPME) method proposed recently to determine the nine HNMs [15]. This LPME method is characterised by not consuming excessive solvent (~2.5 µL of extractant), being superior to the manual extraction procedure, the only limitation of which is the manipulation of the organic drop. The HS technique with GC has been used for the determination of VOCs (including TCNM) [21] or only TCNM [22], providing LODs of 0.5 or 2.5 µg/L, respectively. Both methods involved manual extraction and injection of the HS, which provided low recoveries and precision. In addition, in none of these methods are the variables involved in the generation of the headspace studied. The use of automatic headspace generation for introducing the sample has the advantage of not requiring prior sample treatment, reducing the experimental errors associated with this step of the analytical process. The addition of a chemical modifier (MTBE) favoured the volatilisation of HNMs, increasing the signal approximately four times in relation to the signal obtained without a modifier. The proposed HS–GC–MS method has been successfully compared with the EPA method 551.1 since a good agreement in the results was obtained after analysing a wide number of water samples. The authors believe that the proposed HS–GC–MS method could be a candidate for the daily determination of HNMs in water in official organizations.

Acknowledgment

This work has been subsidised by the CTQ2010–17008 grant from the Spanish Ministry of Education and the P09–FQM–4732 from the Junta of Andalucian. FEDER also provided additional funding. Isabel Montesinos would like to thank P09–FQM–4732DGI for their concession of a predoctoral grant.

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Journal of Chromatography A
journal homepage: www.elsevier.com/locate/chroma

Speciation of common volatile halogenated disinfection by-products in tap water under different oxidising agents

Isabel Montesinos, Mercedes Gallego*

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071, Córdoba, Spain

Journal of Chromatography A 1310 (2013) 113

Abstract

A simple and efficient method has been developed for the extraction and determination of sixteen common volatile halogenated disinfection by-products (DBPs) using the static headspace (HS) technique coupled with gas chromatography–mass spectrometry (GC–MS). The DBPs determined included trihalomethanes (THMs), halonitromethanes (HNMs) and haloacetonitriles (HANs). The extraction parameters (HS conditions, ionic strength and organic modifier) were studied in order to obtain the highest sensitivity. Under optimum conditions (water containing 250 μL of methyl tert-butyl ether and 6 g of anhydrous sodium sulphate was heated 20 min at 80 °C), the HS–GC–MS method provides limits of detection between 10 and 200 ng/L and a relative standard deviation of ~5.6%. Samples collected from genuine tap water treated with different oxidising agents (ClO_2/Cl_2, Cl_2/NH_2Cl and O_3/Cl_2) in several disinfection treatment plants were successfully analysed in order to establish their effect on the occurrence of DBPs. In parallel, the influence of the main parameters of the water (pH, conductivity, nitrite, nitrate, free residual chlorine, permanganate oxidability and bromide) was also studied. The results suggest that the permanganate oxidability (related to organic matter) and the bromide concentration as well as disinfection conditions are directly related to the occurrence of THMs, HNMs and HANs, both in their concentrations and

speciation. The method developed was compared to the reference EPA Method 551.1 for the analysis of tap water.

1. Introduction

The disinfection of drinking water and swimming pools is necessary to prevent water-borne infections. This treatment incurs the formation of undesired disinfection by-products (DBPs) by reaction of the disinfectant with organic matter [1–3]. The studies have been focused on currently regulated DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs) to the detriment of other emerging unregulated DBPs, particularly nitrogenous DBPs (N-DBPs). Recently several factors have increased interest in the study of N-DBPs since many of them imply a greater perceived health risk than regulated DBP species. Thus, comparison of data from *in vitro* geno- and cytotoxicity assays suggest that haloacetonitriles (HANs), halonitromethanes (HNMs) and haloacetamides are significantly more toxic than the regulated THMs and HAAs [4–6]. On the other hand, to reduce the formation of THMs and HAAs, water utilities are experimenting with alternatives to chlorine disinfection, although some of these emerging disinfectant combinations reduce THMs and HAAs at the expense of promoting N-DBPs [2,7]. The US Environmental Protection Agency (EPA) has set a maximum contaminant level of 80 μg/L or 60 μg/L for total THMs, or five HAAs in drinking water [8]; no similar regulation for HNMs and HANs has been issued to date. Only the World Health Organisation has published drinking-water guidelines for two of the HANs: 70 μg/L for dibromoacetonitrile (DBAN) and provisionally 20 μg/L for dichloroacetonitrile (DCAN) [9]. Ozonation prior to chlorination can change the formation of some DBPs significantly. Thus, DCAN and bromochloroacetonitrile (BCAN) were reduced in ozonated water, but haloketones and some HNMs precursors increased due to the formation of ozonated precursors [10–12]. Choi and Richardson [13] have used radiolabeled nitrite ($^{15}NO_2^-$) to show that nitrite was the source of the nitro group in two brominated HNMs produced after the ozonation-chlorination of a humic acidsolution.

The analysis of halogenated volatile organic compounds including THMs, trichloronitromethane (TCNM), DCAN, BCAN, DBAN and trichloroacetonitrile in drinking water is generally carried out by the US EPA Method 551.1 [14] based on a liquid-liquid extraction (LLE), with minor modifications [15–17]. To date, THMs have been the most widely studied

DBPs and in some cases have been taken as volatile models when a new microextraction method has been developed based on “green chemistry”. The methods include static headspace (HS) coupled to gas chromatography–mass spectrometry (GC–MS) [18], HS-programmed temperature vaporisation (PTV)–GC–MS [19], purge-and-trap (P&T)–GC–MS [20], HS-liquid phase microextraction and GC–MS [21], dispersive liquid-liquid microextraction and GC with electro capture detection (ECD) [22] and solid-phase microextraction (SPME) coupled with GC–MS [23]. HNMs have been determined in treated water by manual HS-single-drop microextraction and GC–MS [24], HS–GC–MS [25] and GC–MS after LLE (with 5 mL of methyl tert-butyl ether) [26]. Dihaloacetonitriles have been determined by LLE and GC–MS using ethyl acetate as extractant [27], and more recently six species of HANs by SPME–GC–MS at low detection limits (0.002–0.030 μg/L) [28]. Recently a new micro liquid-liquid extraction in combination with a PTV–GC–MS method has been proposed for the simultaneous determination of four THMs and nine HNMs in treated water with many advantages in terms of sensitivity [29]. Nikolaou et al. [16] have carried out a broad study for the simultaneous determination of volatile chlorination by-products in drinking water, including THMs, HNMs and HANs. In this work the authors assayed different sample preparation techniques (LLE, P&T, HS) in combination with GC–ECD and GC–MS, comparing the analytical features of each one. Other methods utilised for a similar purpose are closed loop stripping extraction and GC–ECD [30] and SPME with GC–ECD [31]. These methods only study one HNM (TCNM) [16,30,31] and in some cases [16] it is not valid for the extraction of some HANs. There will be other comments about these methods further on.

From the foregoing, it is possible to conclude that there is not a simultaneous method for the specific determination of the most relevant THMs, HNMs and HANs generally found in drinking water since those that do exist are based on the EPA Method 551.1 with minor modifications. Thereby this study aims to develop a simple analytical method for the quantification of sixteen of these volatile halogenated DBPs. The advantage of the static HS technique coupled to GC–MS for volatile compounds was taken into account for the development of a robust new method. Thus, the aims of this work were: (i) to develop the first sensitive and straightforward method for the joint speciation of 4 THMs, 6 HNMs and 6 HANs in tap water

by HS–GC–MS; (ii) to evaluate the possible influence of the main water parameters (pH, nitrite, nitrate, bromide, organic matter, etc.) in the concentration and speciation of these compounds; and (iii) to obtain information about the effect of the different disinfectants on the occurrence of these DBPs in tap water.

2. Experimental

2.1. Chemicals, materials and standard solutions

Chloronitromethane (CNM, 90–95%), dichloronitromethane (DCNM, 95%), bromochloronitromethane (BCNM, 85–90%) and dibromonitromethane (DBNM, 90%) standards were supplied by Cansyn (Toronto, Canada). Trichloronitromethane (TCNM, 99%), bromonitromethane (BNM, 90%), trichloromethane (TCM, 99%), bromodichloromethane (BDCM, 98%), dibromochloromethane (DBCM, 98%), tribromomethane (TBM, 95%), chloroacetonitrile (CAN, 99%), dichloroacetonitrile (DCAN, 98%), trichloroacetonitrile (TCAN, 98%), bromoacetonitrile (BAN, 97%) and the internal standard (IS), 1,2-dibromopropane, were purchased from Sigma–Aldrich (Madrid, Spain). Dibromoacetonitrile (DBAN, 95%) and bromochloroacetonitrile (BCAN, 0.1 g certified) were acquired from Alfa Aesar (Karlsruhe, Germany) and Dr.Ehrenstorfer (Augsburg, Germany), respectively. Ethyl acetate, methyl tert-butyl ether (MTBE), methanol and sulphuric acid were supplied from Merck (Darmstadt, Germany). Potassium and sodium chlorides, and anhydrous sodium sulphate were purchased from Panreac (Barcelona, Spain). Twenty millilitre glass flat-bottomed vials for headspace analysis with 20-mm poly(tetrafluoroethylene) (PTFE)/silicone septa caps and crimped aluminium closure (Supelco, Madrid, Spain) were also employed. Vials and septa were heated at 100 and 70 ºC, respectively, overnight prior to use. Stock standard solutions (1 g/L) of each halonitromethane (in ethyl acetate), trihalomethane (in methanol) or haloacetonitrile (in methanol) and cumulative solutions (0.1 g/L) were stored in amber glass vials at –20 ºC. More dilute cumulative solutions were prepared daily in mineral water at the microgram per litre level. In spite of the treatment of the tap water (using Milli-Q system), ultrapure water continues to present

trihalomethanes from the tap water. These compounds did not appear in the blanks performed with the commercial mineral water since it is untreated. Therefore, mineral water was proposed for DBPs determination in aqueous matrices when the analysis is carried out by our group [18].

2.2. Instrument

Sample analyses were carried out on an HP (Agilent Technologies, Palo Alto, CA) 6890 gas chromatograph-5973N mass selective detector equipped with a 7694 headspace autosampler. The autosampler consists of an oven (with capacity for 44 vials), a 3 mL loop connected to a six-port injection valve and an inert transfer line. The operating conditions for the HS autosampler were as follows: vial equilibration time, 20 min; oven temperature, 80 °C; vial pressurisation time, 30 s; loop fill time, 9 s; valve/loop temperature, 100 °C. Helium was used both to pressurise the vial and to transfer the loop content to the injection port of the gas chromatograph, which was equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness). Sample injection was done in split mode (1:20 split ratio) for 1 min. Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 1 mL/min. The injector temperature was set at 170 °C, the interface temperature maintained at 200 °C, and the source and quadrupole temperatures were maintained at 200 °C and 150 °C, respectively. The chromatographic oven temperature programme was as follows: 40 °C for 5 min and then programmed to 80 °C at 5 °C/min, held for 2 min, and ramped at 40 °C/min to 180 °C, finally held for 3 min. Mass spectra (electron impact ionisation, 70 eV) were acquired in scan mode using the m/z 29–300 at 3.5 scans per s. The ions selected for identification and quantification of THMs, HNMs and HANs (SIM mode) are listed in **Table 1**; m/z values for 1,2-dibromopropane (IS) were: 42, 121 (base peak), 123.

Table 1. Quality parameters of the HS–GC–MS and LLE EPA 551.1 methods

Compound	*m/z*[a]	HS				EPA 551.1			
		LOD (ng/L)	Linear range (µg/L)	RSD (%) Intra-day	RSD (%) Inter-day	LOD (ng/L)	Linear range (µg/L)	RSD (%) Intra-day	RSD (%) Inter-day
TCM	47, **83**, 85	10	0.03–50	5.3	6.2	180	0.60–100	6.9	7.7
BDCM	**83**, 85, 129	10	0.03–50	5.2	5.9	60	0.20–100	5.7	6.6
DBCM	79, 127, **129**	15	0.05–50	5.2	6.0	70	0.25–100	5.7	6.7
TBM	171, **173**, 175	20	0.07–50	5.5	6.4	70	0.25–100	5.9	6.8
CNM	46, **49**, 51	130	0.40–100	6.1	7.0	200	0.65–100	6.8	7.7
DCNM	46, **83**, 85	40	0.10–100	5.4	6.2	60	0.20–100	5.9	6.7
TCNM	46, **117**, 119	30	0.10–100	5.2	6.2	80	0.25–100	6.2	7.1
BNM	46, **93**, 95	140	0.45–100	6.3	7.3	200	0.65–100	6.9	7.9
BCNM	46, 127, **129**	60	0.20–100	5.4	6.2	70	0.25–100	6.5	7.3
DBNM	46, 171, **173**	80	0.25–100	5.5	6.3	200	0.65–100	7.6	8.4
CAN	40, 48, **75**	150	0.50–100	6.5	7.6	40	0.10–100	5.8	6.9
DCAN	**74**, 82, 84	30	0.10–100	5.3	6.3	30	0.10–100	5.7	6.5
TCAN	73, **108**, 110	30	0.10–100	5.5	6.4	30	0.10–100	6.1	7.0
BAN	40, **119**, 121	200	0.65–100	6.3	7.2	400	1.30–200	7.7	8.7
BCAN	**74**, 76, 155	50	0.15–100	5.6	6.5	80	0.25–100	6.4	7.2
DBAN	**118**, 120, 199	50	0.15–100	5.8	6.7	500	1.65–200	7.9	8.8

[a] Base peaks used for quantification are boldfaced.

2.3. Sample collection and analyte stability

Tap water samples were collected in amber glass bottles (500 mL with PTFE screw caps) and transported to the laboratory in coolers with icepacks, keeping them refrigerated (4 ºC) until analysis. In previous studies we demonstrated that THMs and HNMs remained stable for at least 2 days (4 ºC) when the sample was adjusted at pH ~3.5 [29]. The stability of HANs in treated water under these conditions is unknown. Therefore tap water samples were fortified with 10 µg/L of each target analyte (4 THMs, 6 HNMs and 6 HANs), acidified with diluted H_2SO_4 at pH ~3.5 and refrigerated. Each sample was analysed in quintuplicate at hourly intervals on the first day and then at higher intervals for 2 days. The results showed

that the sixteen analytes remained stable for at least 2 days in acidified treated water at 4 ºC. Consequently, we chose to only acidify the sample at the time of collection in order to preserve all compounds during the storage at 4 ºC. This is in agreement with the sample preservation of HANs by EPA Method 551.1 which recommends the acidification of the water although at higher pH values (4.8–5.5) to inhibit base-catalysed degradation of the HANs [14]. Following this, tap water was acidified at pH ~3.5 by adding 0.8 mL of 0.1 M H_2SO_4 in bottles of 500 mL, completely filled to avoid evaporation of the volatile analytes. When the time between sample collection and analysis exceeded 2 days, samples could be stored at –20 ºC up to 14 days. Samples for routine water quality parameters were collected and handled under the same conditions as samples for DBP determination. Water quality parameters (pH, conductivity, nitrite, nitrate, free residual chlorine, permanganate oxidability and bromide) were determined according to standard methods [32].

2.4. HS–GC–MS procedure

Twelve millilitres of preserved tap water samples (pH ~3.5) or aqueous standard solutions at pH ~3.5 (adjusted with 20 µL of 0.1 M H_2SO_4) containing between 0.03 and 50 µg/L (THMs) and 0.1 and 100 µg/L (N-DBPs) of each compound and 5 µg/L of 1,2-dibromopropane (IS) were added to a 20 mL glass vial with 6 g of anhydrous sodium sulphate (saturated solution). Then, 250 µL of MTBE (as modifier) was added and the vial was immediately sealed and vortexed for 1 min for sample homogenisation. Samples were analysed in quintuplicate by HS–GC–MS, using the operating conditions mentioned above. Finally, each analyte was identified and quantified in the mass spectrometer using the three characteristic *m/z* ratios listed in **Table 1**.

2.5. LLE procedure (EPA Method 551.1)

Liquid-liquid extraction for the determination of THMs, HNMs and HANs in tap water was performed in quintuplicate following EPA Method 551.1 [14]. Samples were collected in 62 mL amber bottles with a PTFE screw cap containing 0.8 g of ammonium sulphate and without headspace to avoid evaporation of VOCs. A 12 mL aliquot was withdrawn from the

sample bottle and discarded and the pH adjusted manually at 4.5–5.5 with diluted H_2SO_4. Fifty microlitres of a 10 mg/L standard solution of 1,2-dibromopropane (IS), 3 mL of extracting solvent (MTBE), 20 g of Na_2SO_4 and 1 g of pentahydrate copper sulphate were added to the remaining sample (50 mL); the vial was stirred for 4 min. Once the THMs, HNMs and HANs were extracted, the vial was left to stand for 2 min in order to separate both phases. Approximately 1 mL of the upper MTBE layer was transferred to a 2 mL glass vial and 0.1 g of anhydrous Na_2SO_4 was added to dry the extract. Finally, 2 µL of the extract was injected into the GC–MS instrument.

3. Results and discussion

3.1. Headspace variables

The most relevant variables related to generating the gaseous phase were studied using 10 mL of aqueous solution at pH ~3.5 (adjusted with diluted sulphuric acid) containing 20 µg/L of each DBPs (4 THMs, 6 HNMs and 6 HANs), 5 µg/L of 1,2-dibromopropane (IS) and 3 g of Na_2SO_4 in 20 mL glass vials. All the extraction experiments were performed by measuring the relative peak area of each analyte to the internal standard using the average of five replicate measurements (after that the different peak areas of 1,2-dibromopropane between the temperatures were normalised). In the whole study, both the sample loop and transfer line temperatures were kept at 20 and 30 °C above the oven HS unit, respectively. In order to minimise water evaporation, the oven temperature was raised to 80 ºC and the vial equilibration time from 5 to 30 min. The response ratio increased with the increase in temperature, therefore 80 °C was selected. The relative peak areas increased as the equilibration time rose to 10 min and 18 min for THMs and N-DBPs (HNMs and HANs), respectively, according to their volatility. To ensure maximum volatilisation of all sixteen compounds, an extraction time of 20 min was selected for further experiments.

3.2. Effect of chemical conditions

Preliminary analyte volatilisation tests using spiked standard samples containing different salts (potassium chloride, sodium chloride and

anhydrous sodium sulphate) were assayed at variable molarities up to the saturated solution. The best results obtained for each salt are shown in **Figure 1** for nine representative analytes (1 THM, 4 HNMs and 4 HANs). As expected, the addition of any type of salt favoured the volatilisation of all analytes when compared to the unsalted experiment. The behaviour of the 4 THMs was similar in the unsalted experiment and in the presence of the different salts, while the N-DBPs had different behaviour according to the number of halogens. Monohalogenated compounds (CNM, BNM, CAN and BAN) were practically undetected in the absence of salt. This effect can be ascribed to the different polarities of the compounds, so monohalonitromethanes and monohaloacetonitriles were the species most favoured by the presence of salt due to their higher polarities. By contrast, trihalogenated compounds (4 THMs, TCNM and TCAN) were less favoured because they are less polar. The optimal signals for the sixteen target analytes were achieved with the addition of 5 g Na_2SO_4 (3.5 mol/L) per 10 mL of water sample.

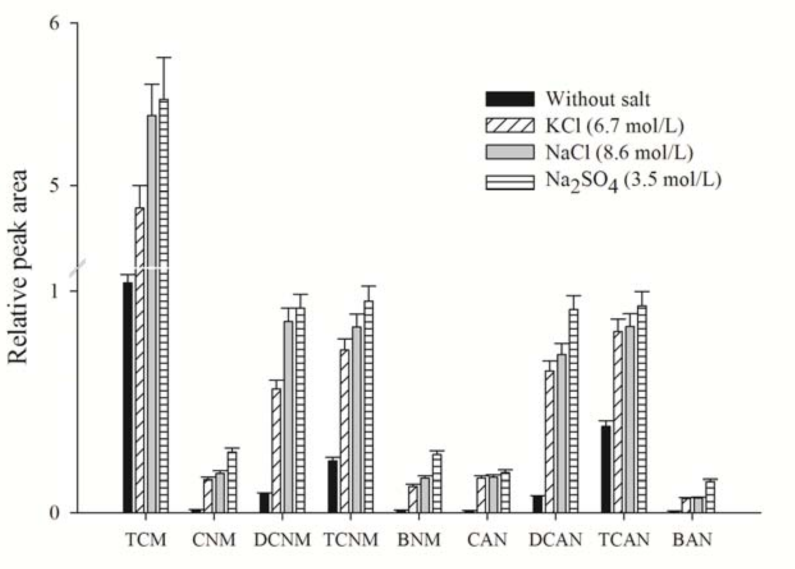

Figure 1. Influence of different type of salts (saturated solutions) on the volatilisation efficiency for nine representative volatile DBPs. Error bars are the standard deviation for five measurements.

The influence of the sample pH on the HS–GC–MS method was tested between 2.2 (adjusted with diluted sulphuric acid) and 7.4 (the genuine pH of the aqueous sample). The influence of the sample pH was negligible for THMs throughout the range studied, as it was observed in **Figure 2**. The response ratios of the 6 HNMs and 6 HANs remained relatively constant from pH 2.6 to 4.1 (HNMs) or 2.6 to 5.6 (HANs), decreasing above these values due to their base catalysed degradation [17,24]. From these results and taking into account that the preservation of the water sample for the determination of the target compounds required a sample pH of ~3.5, this pH was selected for its analysis. Thus, the adjustment of the sample pH was omitted in the analysis of tap water.

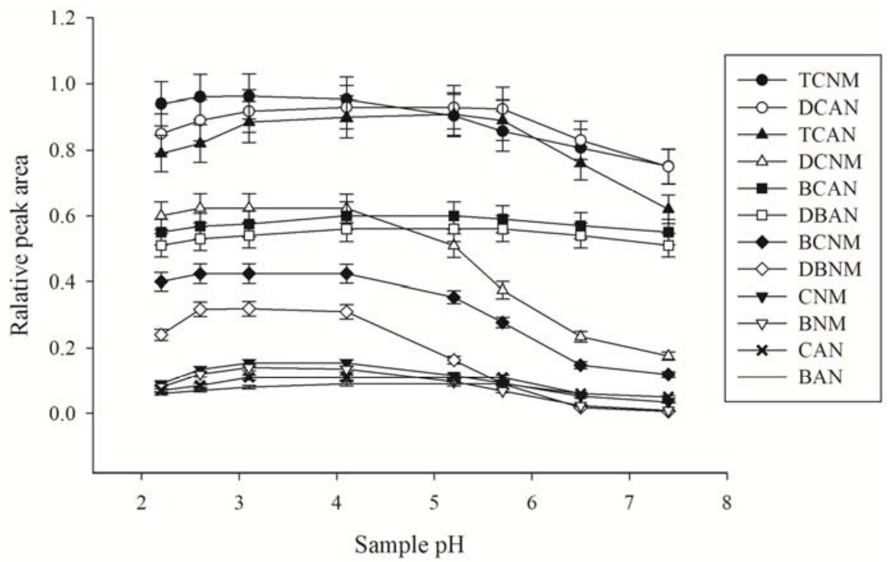

Figure 2. Effect of the sample pH on the volatilisation efficiency for THMs, HNMs and HANs. Error bars are the standard deviation for five measurements.

There is a direct relationship between both the aqueous and the headspace phases and the sensitivity of the method. The ratio between the two phases was studied taking into account that 12 mL of aqueous sample was the highest possible value (when salt was added to 20 mL vials, the volume increased to ~15 mL) to ensure that the autosampler needle would not come into contact with the sample during sampling. As could be

expected, the response ratios for all target analytes increased with the increase in sample volume to 12 mL. As the sample volume (12 mL) increased with respect to the initial optimisation process (10 mL), the most prevalent variables (HS and chemical variables) were checked again. The HS variables were unchanged but the chemical variables increased according to the sample volume for 12 mL (6 g of anhydrous sodium sulphate and 20 μL of 0.1 M H_2SO_4).

An organic modifier has proven to be a very effective additive to facilitate the release of analytes from the matrix and it is often used to accelerate volatilisation [25]. The addition of four solvents of different variable polarity (*n*-hexane, *n*-pentane, MTBE and ethyl acetate) was tested with the aim of enhancing the transfer of the 4 THMs, 6 HNMs and 6 HANs from the water sample. The behaviour of each family of DBPs was very different, so this parameter (200 μL of different solvents) was depicted individually in **Figure 3** (A, B and C). The volatilisation of THMs was the least affected with respect to the N-DBPs. Brominated THMs (**Figure 3A**) were favoured in the presence of MTBE and to a lesser extent in *n*-hexane (even TCM decreased with this modifier) as compared to volatilisation without organic solvent or with ethyl acetate. **Figure 3B** shows that MTBE was the best organic solvent for the six species of HNMs, followed by *n*-hexane mainly for brominated HNMs. For HANs (see **Figure 3C**), only MTBE again enhanced the volatilisation efficiency of the six HANs enough in relation to the experiment without a modifier or in presence of ethyl acetate. For the other solvents, not only did they not increase their signals but, in some cases, they decreased (mainly *n*-pentane for DCAN, CAN, BAN, BCAN and DBAN). In conclusion, we can say that MTBE favoured the volatilisation of the twelve N-DBPs ~50–70% in relation to the signal obtained without a modifier or with ethyl acetate; this increase was more pronounced for brominated compounds. This effect can be explained on the basis of their different volatilities: as the brominated compounds are less volatile, the advantages of the organic modifier are more prominent. So MTBE was selected as the organic modifier to increase the sensitivity of the HS–GC–MS method. The response ratio increased with the volume of MTBE up to 200–250 μL; further experiments were achieved with 250 μL.

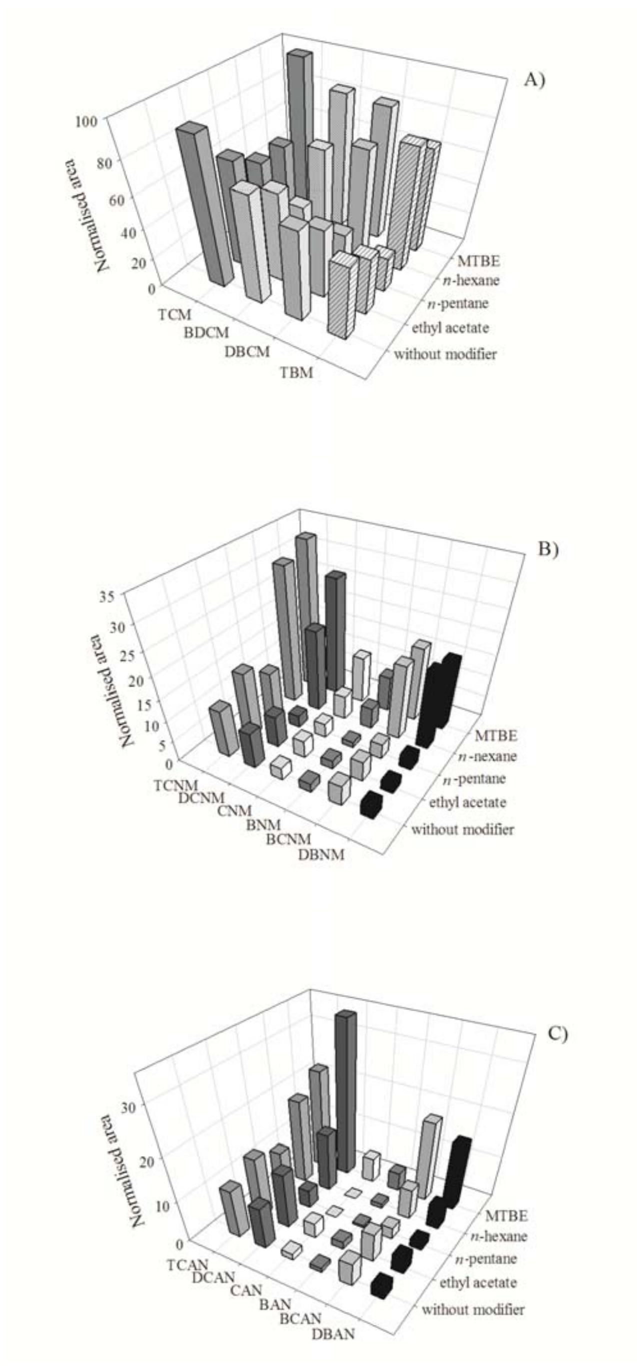

Figure 3. Influence of the organic modifier (200 µL) on the normalised area responses of THMs (A), HNMs (B) and HANs (C). The relative peak area of each analyte was normalised with respect to trichloromethane (TCM).

Finally, the yield of the whole process for the 4 THMs, 6 HNMs and 6 HANs in the aqueous matrix was evaluated using a second HS extraction of the remaining aqueous phase by spiking 250 µL of fresh MTBE again (no salt was added since the aqueous solution remained saturated). The average efficiency percentage, calculated in quintuplicate, showed that the response ratios obtained for trihalogenated compounds (4 THMs, TCNM and TCAN) were lower than 3% (no significant carryover), whereas for dihalogenated (DCNM, BCNM, DBNM, DCAN, BCAN and DBAN) and monohalogenated (CNM, BNM, CAN and BAN) compounds, they were 15% and 40%, respectively. The extraction efficiency of monohalogenated (60%) ones was lower due to their higher polarities and solubilities in water compared to other compounds. Anyway, the analytes commonly detected in treated water are trihalogenated and dihalogenated compounds, which provided an efficiency extraction of 97% and 85%, respectively.

3.3. Quantitative calibration and precision

Under the extraction conditions described, the linearity range, limits of detection (LODs), limits of quantification (LOQs), repeatability (intra-day) and reproducibility (inter-day) were measured using spiked mineral water samples at pH ~3.5 (adjusted with 20 µL of 0.1 M H_2SO_4). Results of these parameters are shown in **Table 1**. The linearity was obtained in the range 0.03–50 for THMs and 0.1–100 µg/L for N-DBPs. Correlation coefficients over 0.994 were obtained for the sixteen analytes. LODs or LOQs, defined as the minimum concentration providing chromatographic signals 3 times or 10 times higher than background noise [33], were obtained in the range 10–80 (excepting the 4 monohalogenated N-DBPs) or 30–250 ng/L (the lowest concentration of the linear range). The precision of the HS–GC–MS proposed method was checked by eleven replicate analyses of the spiked (5 µg/L) samples and presented as relative standard deviations (RSDs) in **Table 1**. The average values of RSDs were 5.6 ± 0.4% (intra-day) and 6.5 ± 0.5% (inter-day). In order to assess the chromatographic resolution and efficiency of the analytical procedure, **Figure 4** shows an HS–GC–MS chromatogram corresponding to a mineral water spiked with 5 µg/L of each compound.

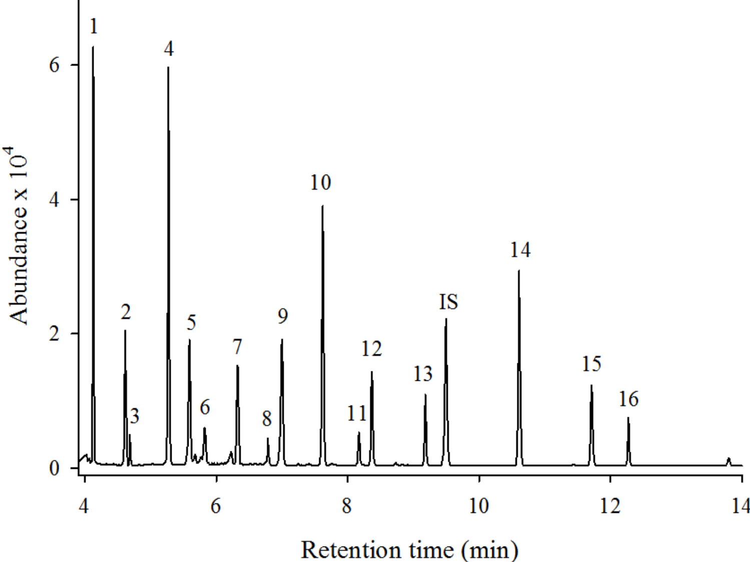

Figure 4. GC–MS chromatogram in SIM mode for mineral water sample spiked with 5 µg/L of each compound. Peak identification: TCM (1); TCAN (2); CAN (3); BDCM (4); DCAN (5); CNM (6); DCNM (7); BAN (8); TCNM (9); DBCM (10); BNM (11); BCAN (12); BCNM (13); TBM (14); DBAN (15); DBNM (16); and 1,2-dibromopropane (IS).

In the bibliography, few methods have been developed to determine the mix of the three types of DBPs here studied (THMs, HNMs and HANs), and they employed different techniques. A study comparing our developed method with these methods was performed and the results are presented in **Table 2**. The proposed HS–GC–MS method covers a greater number of compounds (16 species) than other methods (8–11 species). The LLE methods require 2 or 3 mL of extractant [14,16] and our method only 0.25 mL of MTBE as a modifier. Our method with the other manual HS alternative consumed the lowest sample volume. In relation to LLE, our method has higher sensitivity when using similar detection (GC–MS) and lower in relation to GC–ECD, especially for HANs, probably due to the ECD detector. P&T–GC–MS and manual HS with GC–MS have the advantage of solventless techniques but provide the lowest sensitivity (LODs at µg/L levels). In addition the P&T technique is unable to effectively retain TCNM, CAN and BAN, probably due to the trap or the decomposition reactions in these cases [16]. On the other hand, there are two methods with higher

sensitivity than that proposed (the closed-loop stripping extraction with GC–ECD and the SPME with GC–ECD), which can probably be ascribed to the employment of a large sample volume (900 mL) [30] and/or ascribed to ECD detection [30,31].

Table 2. Comparison of the HS–GC–MS method with other related methods for determination of THMs, HNMs and HANs

Method	Analyte	Sample volume (mL)	LODs (ng/L)	Reference
LLE and GC–ECD	4 THMs; TCNM; 4 HANs	50/3[a]	5–75; 14; 4–10	[11] (EPA Method 551.1)
LLE and GC–ECD	4 THMs; TCNM; 6 HANs	35/2[a]	5–10; 40; 7–70	[13] (EPA Method 551.1 modified)
LLE and GC–MS	4 THMs; TCNM; 4 HANs	35/2[a]	10–30; 100; 10–500	[13] (EPA Method 551.1 modified)
P&T–GC–MS	4 THMs; 4 HANs	35	10–50; 500–10x10^3	[13]
Manual HS and GC–MS	4 THMs; TCNM; 4 HANs	8	50–200; 500; 1–20 (x10^3)	[13]
Closed-loop stripping extraction and GC–ECD	4 THMs; TCNM; 4 HANs	900	0.5–0.5; 1.2; 1.1–1.1	[27]
SPME and GC–ECD	4 THMs; TCNM; 4 HANs	25	0.3–1.4; 3.2; 5.0–23.8	[28]
HS–GC–MS	4 THMs; 6 HNMs; 6 HANs	12	10–20; 30–140; 30–200	This work

[a] Extractant volume in mL.

The proposed HS–GC–MS method was compared to that of EPA 551.1 [14] in order to validate the alternative proposal. In this case, the best pre-concentration factor for the manual EPA alternative was used [aqueous/organic (50 mL/3 mL) volume ratio = 17]. All quantitative parameters were determined as stated above. Thus, repeatability and reproducibility was also assessed by analysing eleven mineral water samples spiked with a 5 µg/L concentration of each THM, HNM and HAN (excepting BAN and DBAN, 10 µg/L). EPA Method 551.1 (**Table 1**) was slightly less precise than the HS–GC–MS one because it needs more preparation sample steps, with average RSD values of 6.5 ± 0.8% (intra-day) and 7.4 ± 0.8% (inter-day), and it also provided higher LODs with average values of 142 ± 136 versus 65 ± 58 ng/L. The EPA Method 551.1 that is used here (GC–MS) is less sensitive than the EPA Method 551.1 (GC–ECD) [14] according to the different detectors used. By way of example, the reported LOD for TCNM with GC–ECD is 14 ng/L, which is much lower than what is obtained here by GC–MS (80 ng/L).

In the same vein, the recoveries of both methods were also calculated using a tap water fortified at two different concentrations (5 and 20 µg/L for THMs, or 1 and 5 µg/L for N-DBPs) of each target analyte; as an exception, BAN and DBAN were spiked at 2 and 5 µg/L in the EPA alternative. These concentrations were selected in concomitance to their concentrations in water; all experiments were carried out in quintuplicate (n = 5). The selected tap water contained TCM, BDCM, DBCM, TCNM, DCAN and BCAN and therefore the genuine concentration in the unspiked sample was initially determined. The recoveries of the proposed HS–GC–MS method ranged from 95 to 98% for the sixteen species at the two spiked levels, while those for EPA Method 551.1 were 93–96% at low and high concentration levels, respectively. These results revealed that no matrix effect was observed in the determination of the 4 THMs, 6 HNMs and 6 HANs in treated water samples under these experimental conditions.

3.4. Influence of water quality parameters and different chlorine-containing disinfectants on the levels and type of DBPs formed

The concentration and speciation of DBPs are affected by many water quality parameters and operating conditions such as the type of disinfectant and its dosage [3,34]. The influence of some water quality parameters such as pH, conductivity, nitrite, nitrate, free residual chlorine, permanganate oxidability and bromide concentration was carried out under different disinfection treatments for the occurrence of the sixteen volatile DBPs. After several months of analysing different genuine water from drinking water treatment plants (DWTPs) to obtain the widest possible range of parameters, fifteen representative tap water samples were selected. **Table 3** presents the values of the parameters selected, as well as their chlorine-containing disinfectants (chlorine, chlorine dioxide, monochloramine) and the ozone used. The ranges studied were 7.1–8.1 (pH), 119–913 μS/cm (conductivity), <1.0–46.2 mg/L (nitrate), 0.1–0.7 mg/L (free residual chlorine), 0.1–2.3 mg O_2/L (permanganate oxidability) and <0.1–0.35 mg/L (bromide); the tap water selected did not contain nitrite at significant levels (<0.01 mg/L). The results obtained in the determination of the sixteen target analytes in the fifteen waters by the proposed HS–GC–MS method were compared to those provided by EPA Method 551.1, both listed in **Table 4**. While both methods provided similar results, the EPA Method 551.1 did not allow the determination of BCAN (tap water 6–9 and 13) and DBAN (tap water 1, 2, 6 and 7) owing to its lower sensitivity.

Table 3. Values of the water quality parameters[a]

	pH	Conductivity (µS/cm)	NO_3^- (mg/L)	Free residual chlorine (mg/L)	Oxidability (mg O_2/L)	Bromide (mg/L)	Disinfectant
Tap 1	7.6	496	2.9	0.5	1.8	0.26	ClO_2/Cl_2
Tap 2	7.7	490	1.8	0.1	2.0	0.22	ClO_2/Cl_2
Tap 3	8.1	128	<1.0	0.1	1.3	<0.10	ClO_2/Cl_2
Tap 4	7.7	119	<1.0	0.7	1.3	<0.10	ClO_2/Cl_2
Tap 5	7.8	134	1.2	0.5	1.6	<0.10	ClO_2/Cl_2
Tap 6	7.9	735	8.6	0.3	0.3	0.35	ClO_2/Cl_2
Tap 7	8.1	913	18.4	0.7	0.4	0.31	ClO_2/Cl_2
Tap 8	7.1	413	46.2	0.6	0.1	<0.10	ClO_2/Cl_2
Tap 9	7.7	495	9.8	0.5	0.1	<0.10	ClO_2/Cl_2
Tap 10	7.6	261	2.3	0.6	2.3	<0.10	Cl_2/NH_2Cl
Tap 11	7.6	265	2.2	0.7	1.9	<0.10	Cl_2/NH_2Cl
Tap 12	7.4	210	6.1	0.1	1.8	<0.10	Cl_2/NH_2Cl
Tap 13	7.6	231	2.6	0.1	1.0	<0.10	O_3/Cl_2
Tap 14	7.8	334	3.0	0.1	1.5	<0.10	O_3/Cl_2
Tap 15	7.1	255	5.1	0.1	1.6	<0.10	O_3/Cl_2

[a]NO_2^-<0.01 mg/L

The majority of water quality parameters (see **Table 3**) did not significantly affect DBP formation (pH, conductivity, nitrite, nitrate and free residual chlorine values). By contrast, permanganate oxidability and bromide concentrations were directly related to the concentrations and speciation of target analytes. The parameter corresponding to oxidability (related to organic matter) is directly related to the concentration of the compounds studied as has been described by Kim et al. for DBPs in general [35]. This is seen most clearly when comparing samples 1–9, as the disinfectants (ClO_2/Cl_2) are the same and they cover a wide range of permanganate oxidability (0.1–2.0 mg O_2/L). **Figure 5** illustrates a general trend towards increasing DBP concentrations as do the oxidability concentrations, reflecting a simple linear relationship between the data obtained with a correlation coefficient of 0.895.

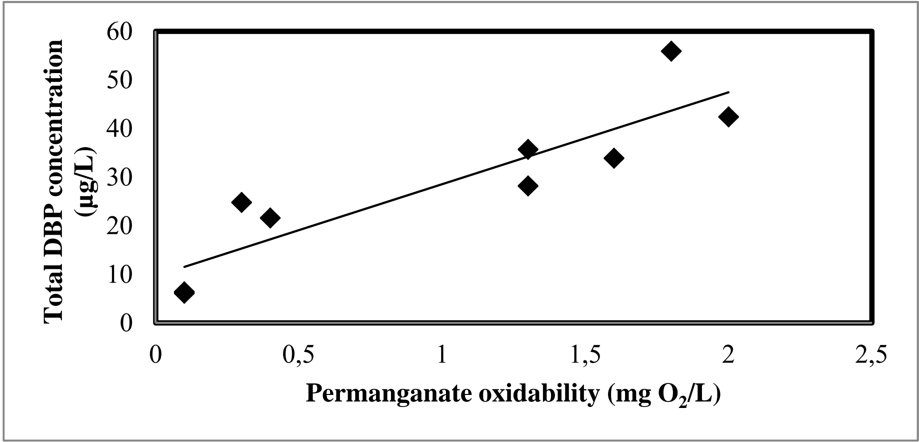

Figure 5. Sum of the DBP concentrations found in water treated with ClO_2/Cl_2 (samples 1–9 in Table 4) in relation to the permanganate oxidability of these waters.

Thus, the total concentration found of the five-eight species (**Table 4**) varied from ~6 to 42 µg/L, for an oxidability of 0.1 (samples 8 and 9) and 2.0 (sample 2) mg O_2/L, respectively. With respect to the bromide concentration, the speciation of halogenated DBPs is strongly affected by the presence of the bromide ion in natural waters. The bromide ion is oxidised by chlorine to hypobromous acid or hypobromous ion, which in turn reacts with natural organic materials, forming brominated DBPs to the detriment of chlorinated ones [1,3,12]. As can be found listed in **Table 4**, the higher concentrations of brominated DBPs were related to water containing the highest values of bromide (samples 1, 2, 6 and 7). In these samples, brominated THMs (mainly DBCM and TBM) represented 50–78% of total THMs and also shifted the distribution of di-HANs (DCAN turned into BCAN and then into DBAN) [7]. In these conditions, BCAN and DBAN represented 70–84% of total di-HANs. With respect to HNMs, DBNM was detected only in these tap waters at levels from <0.25 to 1.7 µg/L.

The type of disinfectant used in DWTPs affects both the amount of DBPs and their occurrence. The treated water samples were collected from DWTPs that employed different disinfectants as depicted in **Table 3** (ClO_2, Cl_2, NH_2Cl and O_3). To simplify the comparison, waters 6–9 with the lowest oxidability and 1, 2, 6 and 7 with the highest bromide concentrations were discarded because both parameters are a limiting factor in the occurrence

of these compounds. As can be seen in **Table 4**, there were no significant differences in the concentration of THMs using different disinfectants; thus, the average value of the total concentration of THMs found with ClO_2/Cl_2 (samples 3–5), Cl_2/NH_2Cl (samples 10–12) or O_3/Cl_2 (samples 13–15) was 30.4, 25.8 or 35.0 µg/L, respectively. According to the bibliography, chloramines can contribute to the nitrogen source in the formation of HNMs [36] and ozone can also increase their concentrations [10,12]. DBNM was undetected in the nine tap waters selected for this comparison, and TCNM was the only HNM found in tap water treated with Cl_2/NH_2Cl, whereas DCNM in addition to TCNM were found at the highest concentrations in the water treated with O_3 (average concentrations for DCNM and TCNM were 1.3 and 3.7 µg/L, respectively). For the HANs, DBAN was also undetected in these waters due to the low bromide concentration. DCAN and BCAN were found in all the samples selected for this study, the average concentrations being of (DCAN/BCAN) 1.9/0.3, 3.7/0.8 and 1.3/0.4 µg/L in water treated with ClO_2/Cl_2 (samples 3–5), Cl_2/NH_2Cl (samples 10–12) and O_3/Cl_2 (samples 13–15), respectively. This was in agreement with previous results found in the bibliography, since ClO_2 and O_3 prior to chlorination reduced HAN formation [10,34], whereas waters treated with Cl_2/NH_2Cl increased their formation [37]. This study was conducted with a small number of waters, but it is remarkable that none of them were fortified with organic matter, bromide or any type of disinfectants, since all the samples were genuine tap water.

Table 4. Results of the analyses of water by the proposed HS–GC–MS and (EPA 551.1) methods (n=5)

	Concentration found ± standard deviation (μg/L)									
	TCM	BDCM	DBCM	TBM	DCNM	TCNM	DBNM	DCAN	BCAN	DBAN
Tap 1	10 ± 1 (11)	12 ± 1 (10)	19 ± 1 (16)	5.5 ± 0.4 (5.9)	n.d.[a]	n.d.	<0.25[b]	1.5 ± 0.1 (1.2)	6.3 ± 0.4 (6.1)	1.6 ± 0.1 (<1.65)
Tap 2	9.1 ± 0.6 (9.6)	9.5 ± 0.6 (9.8)	14 ± 1 (12)	4.6 ± 0.3 (4.8)	n.d.	n.d.	<0.25	1.3 ± 0.1 (0.9)	2.6 ± 0.2 (2.1)	1.3 ± 0.1 (<1.65)
Tap 3	19 ± 1 (17)	7.2 ± 0.5 (6.9)	5.6 ± 0.4 (5.8)	1.7 ± 0.1 (1.4)	n.d.	<0.1	n.d.	1.8 ± 0.1 (2.0)	0.4 ± 0.1 (0.6)	n.d.
Tap 4	16 ± 1 (17)	5.0 ± 0.4 (4.6)	3.5 ± 0.2 (3.3)	1.3 ± 0.1 (1.6)	n.d.	<0.1	n.d.	2.1 ± 0.2 (1.9)	0.3 ± 0.1 (0.4)	n.d.
Tap 5	21 ± 1 (19)	6.8 ± 0.5 (7.1)	3.2 ± 0.2 (3.5)	0.8 ± 0.1 (1.0)	n.d.	<0.1	n.d.	1.8 ± 0.1 (2.2)	0.3 ± 0.1 (0.6)	n.d.
Tap 6	2.1 ± 0.2 (2.3)	2.6 ± 0.2 (3.0)	7.5 ± 0.5 (7.1)	8.8 ± 0.6 (8.5)	n.d.	n.d.	1.7 ± 0.1 (1.7)	0.4 ± 0.1 (0.6)	0.2 ± 0.1 (<0.25)	1.5 ± 0.1 (<1.65)
Tap 7	2.6 ± 0.2 (2.5)	2.2 ± 0.2 (2.4)	6.0 ± 0.4 (6.3)	7.9 ± 0.5 (7.7)	n.d.	n.d.	1.5 ± 0.1 (1.8)	0.4 ± 0.1 (0.4)	0.3 ± 0.1 (<0.25)	0.7 ± 0.1 (<1.65)
Tap 8	2.7 ± 0.2 (2.5)	1.6 ± 0.1 (1.3)	1.0 ± 0.1 (1.1)	n.d.	n.d.	n.d.	n.d.	0.5 ± 0.1 (0.7)	0.3 ± 0.1 (<0.25)	n.d.
Tap 9	2.8 ± 0.2 (2.6)	1.7 ± 0.1 (2.0)	1.2 ± 0.1 (0.9)	n.d.	n.d.	n.d.	n.d.	0.5 ± 0.1 (0.8)	0.2 ± 0.1 (<0.25)	n.d.
Tap 10	17 ± 1 (19)	10 ± 1 (11)	3.8 ± 0.2 (3.5)	n.d.	n.d.	1.1 ± 0.1 (1.4)	n.d.	3.5 ± 0.2 (3.0)	0.8 ± 0.1 (0.6)	n.d.
Tap 11	16 ± 1 (17)	9.8 ± 0.6 (9.5)	3.6 ± 0.3 (3.9)	n.d.	n.d.	0.9 ± 0.1 (0.6)	n.d.	3.3 ± 0.2 (3.3)	0.9 ± 0.1 (1.1)	n.d.
Tap 12	8.7 ± 0.5 (8.5)	5.9 ± 0.4 (6.3)	2.5 ± 0.2 (2.2)	n.d.	n.d.	1.8 ± 0.1 (2.0)	n.d.	4.3 ± 0.3 (4.5)	0.6 ± 0.1 (0.6)	n.d.
Tap 13	22 ± 1 (20)	12 ± 1 (10)	4.4 ± 0.3 (4.7)	n.d.	1.2 ± 0.1 (1.0)	3.8 ± 0.3 (4.0)	n.d.	1.2 ± 0.1 (0.9)	0.2 ± 0.1 (<0.25)	n.d.
Tap 14	19 ± 1 (21)	9.5 ± 0.4 (9.3)	3.2 ± 0.2 (2.9)	n.d.	1.3 ± 0.1 (1.5)	3.5 ± 0.2 (3.1)	n.d.	1.1 ± 0.1 (1.2)	0.4 ± 0.1 (0.5)	n.d.
Tap 15	20 ± 1 (17)	11 ± 1 (13)	3.8 ± 0.3 (4.1)	n.d.	1.5 ± 0.1 (1.3)	3.7 ± 0.3 (4.1)	n.d.	1.6 ± 0.1 (1.2)	0.5 ± 0.1 (0.4)	n.d.

[a] n.d., not detected.
[b] <LOQ.

4. Conclusions

This work demonstrated a fast, simple, and efficient method to extract and analyse sixteen common volatile halogenated DBPs, including THMs, HNMs and HANs, at trace levels in genuine tap water. The HS–GC–MS method is fast since no sample treatment is needed and the analysis time is less than 25 min, as in the GC–MS analysis (~ 20 min). A rigorous study of the influence of some water quality parameters and different chlorine containing disinfectants (including ozone) on the occurrence of DBPs to predict their formation and speciation showed that: (i) the permanganate oxidability (organic matter) is directly related to the occurrence of DBPs; (ii) the concentration of bromide influenced the distribution of chlorinated/brominated species. Thus, brominated DBP concentrations were more than 70% of the total DBPs formed in tap water containing a high bromide concentration; and (iii) the type of disinfectant is related to the occurrence of N-DBPs, whereas THMs were not influenced. The water treated with Cl_2/NH_2Cl increased the formation of N-DBPs (HNMs and HANs). On the other hand, ozonation contributed mainly to the occurrence of HNMs. It is noteworthy that organic matter suspended in natural water is a key precursor to these DBPs.

Acknowledgements

This work was funded in the frameworks of Projects CTQ2010–17008 (Spain's Ministry of Education) and P09–FQM–4732 (Andalusian Regional Government). FEDER provided additional funding. Isabel Montesinos is grateful for the award of a pre-doctoral grant from Project P09–FQM–4732DGI. The authors would like to thank the company EMPROACSA for their collaboration in this work both as a water supplier as well as clarification of the doubts on the issues related to water study.

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CAPÍTULO 5

Evaluación de 46 DBPs volátiles y no volátiles en una planta de potabilización.

Control de DBPs volátiles en bebidas preparadas con agua potable

Este Capítulo de la Memoria incluye los trabajos más recientes que han surgido durante la realización de esta Tesis Doctoral como consecuencia, por una parte de la colaboración de la empresa de potabilización de aguas de Córdoba y por otra, del interés de Organizaciones Internacionales como la FAO/WHO en el desarrollo de metodologías para el control de DBPs en bebidas y alimentos.

En la primera parte se recoge los estudios de control de DBPs (46 especies) en las aguas de la empresa cordobesa. Para ello se ha llevado a cabo dos estudios en paralelo: los parámetros químico-físicos (lluvia, temperatura del agua, pH, conductividad, nitrito, etc.), realizados por la propia empresa y el control de los DBPs realizados en los laboratorios del grupo. Se emplearon tres metodologías, todas por HS–GC–MS dadas las ventajas de la técnica HS frente a otras alternativas extractivas. Los DBPs volátiles halogenados (10 THMs, 6 HNMs y 6 HANs) se han evaluado por el método HS–GC–MS descrito en el Capítulo 4 para aguas. Los DBPs no volátiles [13 ácidos haloacéticos (HAAs) y 11 aldehidos alifáticos y aromáticos] se han determinado por la misma técnica pero se requiere de una etapa de derivatización habida cuenta de su escasa volatilidad. Se ha realizado un estudio espacial desde el agua bruta hasta el agua potabilizada que sale de la empresa así como en diferentes puntos de la red de distribución (24 y 48 horas). Por lo tanto se han muestreado a lo largo de distintas etapas de tratamiento en la planta potabilizadora y red de distribución, 7 puntos. La aparición de estos compuestos en las aguas tratadas varía de acuerdo a la calidad del agua de origen (precursores orgánicos e inorgánicos y parámetros cualitativos) y las operaciones llevadas a cabo en la planta de tratamiento (dosis de desinfectante y tiempo de reacción entre los precursores y el tipo de desinfectante). Hay escasa información sobre la distribución de tan elevado número de DBPs durante todas las etapas que se realizan en una planta de tratamiento de agua, ya que la mayoría de los estudios realizados hasta la fecha se han centrado en los DBPs regulados, pero hay muchos DBPs no regulados como son compuestos nitrogenados y aldehídos de los que no se tiene información global. Por lo tanto, se requieren estudios más detallados en relación al comportamiento de los diferentes DBPs bajo variaciones de las condiciones de desinfección, en orden de ser capaces de definir el esquema de

tratamiento óptimo que minimice la formación de todos los DBPs. Este estudio se ha completado con la variabilidad temporal estudiando la influencia de las cuatro estaciones del año.

Del estudio se concluyó que solo 5 aldehidos y 2 HAAs se encontraron en el agua bruta aunque a concentraciones inferiores a los µg/L. La etapa de cloración formó 3 nuevos aldehídos (incluido el benzaldehído), 5 HAAs y cloroformo; en la etapa de sedimentación siguieron aumentando las concentraciones de los analitos encontrados en la etapa anterior y se forman 3 nuevos. Los filtros de arena eliminaron sustancialmente aldehídos y HAAs (15–50%), pero incrementaron los niveles de THMs, HNMs y HANs hasta un 70%. Por último la etapa de cloraminación elevó los niveles de los compuestos ya existentes en las etapas anteriores y se forman nuevos compuestos como ácido monoiodoacético, dibromoclorometano, dicloroiodometano y bromocloroacetonitrilo. En la red de distribución los 23 DBPs encontrados (exceptuando 5 aldehidos) aumentaron su concentración a lo largo del tiempo, en mayor porcentaje en las estaciones calurosas (HAAs alrededor de un 50% y los THMs un 350%).

El agua consumida como agua de bebida y la usada en un amplio rango de aplicaciones industriales alimentarias es frecuentemente desinfectada antes de su uso. El empleo de agua tratada en la industria alimentaria puede generar contaminantes químicos en comida y bebida, como son los DBPs y VOCs. La vía más importante de exposición es la ingestión del agua tratada de consumo, que puede ocurrir por ingestión directa o como resultado de su inclusión en otros tipos de bebidas y alimentos. El objetivo planteado en la segunda parte de este Capítulo fueron las bebidas refrescantes (preparadas con agua potable) y zumos de frutas (reconstituidos con agua potable). El primer problema a resolver es desarrollar un método para este tipo de muestras dada las escasas aportaciones bibliográficas. Se incluye en este estudio 10 THMs (clorados, bromados y emergentes yodados) y 4 VOCs (que están regulados en aguas potables). Se desarrolló un método por HS–GC–MS para la determinación de las 14 especies en refrescos y zumos de frutas, aprovechando la simplicidad y robustez de la metodología previamente desarrollada para THMs en aguas. La variable más significativa en estas muestras es la

presencia de CO_2 en las bebidas carbonatadas. Cuando se abre una bebida carbonatada, la presión se reduce a la presión atmosférica, provocando la descomposición del ácido carbónico y liberando CO_2. Esta pérdida de CO_2 origina un arrastre de los compuestos más volátiles a la atmósfera. Además, el CO_2 puede competir con los analitos por el espacio de cabeza dentro del vial. La opción más simple fue la adición de NaOH hasta alcanzar un pH de 8–9 de manera que la especie prevalente es el HCO_3^-. Las ventajas del método HS–GC–MS propuesto se centran en la mínima manipulación de la muestra y elevada sensibilidad lo que permite detectar especies, en estas bebidas, desconocidas hasta la fecha como dicloroiodometano.

El estudio realizado en esta parte del Capítulo implica la evaluación de la procedencia de estos compuestos, ya que no siempre aparecen por la inclusión de agua tratada como parte de un ingrediente de la bebida sino que pueden aparecer debido a la desinfección con productos clorados de la línea de preparación y envasado de la bebida, con lo que queda retenida agua que puede ser una fuente de contaminantes. Esto se ha comprobado analizando zumos 100% exprimidos (bebidas que no poseen agua tratada como ingrediente) en los que se ha encontrado trihalometanos clorados a bajas concentraciones. En cambio, los otros tipos de bebidas contienen además de trihalometanos clorados, THMs bromados y dicloroidometano, que pueden utilizarse como marcadores de la presencia de agua tratada. La fuente principal de la presencia de THMs en zumos de frutas reconstituidos, néctares y refrescos es el agua tratada que se incluye como ingrediente; la concentración y la especiación dependen del volumen de agua añadido y del proceso de desinfección de esa agua.

Year-long evaluation of the presence of 46 disinfection by-products throughout a drinking water treatment plant

I. Montesinos, M. Serrano, M.J. Cardador, M. Silva, M. Gallego*

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071, Córdoba, Spain

Abstract

In this work, we studied a total of 46 regulated and non-regulated disinfection by-products (DBPs) including 10 trihalomethanes (THMs), 13 haloacetic acids (HAAs), 6 halonitromethanes (HNMs), 6 haloacetonitriles (HANs) and 11 aldehydes at different points in a drinking water treatment plant (DWTP) and its distribution network. Determining an increased number of compounds and using accurate, sensitive analytical methodologies for new DBPs can be useful to overcome some challenges encountered in the comprehensive assessment of the quality and safety of drinking water. This paper provides a detailed picture of the spatial and seasonal variability of DBP concentrations from raw water to water in the distribution network. Samples were collected on a weekly basis at seven different points in the four seasons of a year to acquire robust data for DBPs and supplementary quality-related water parameters. Only 5 aldehydes and 2 HAAs were found in the raw water, and all at concentrations in the low microgram-per-litre range. Chlorine dioxide caused the formation of 3 new aldehydes (benzaldehyde included), 5 HAAs and chloroform. The concentrations of DBPs present in the raw water were up to 6 times higher in the warmer seasons (spring and summer). The sedimentation process further increased their concentrations and caused the formation of three new ones. Sand filtration substantially removed aldehydes and HAAs (15–50%), but increased the levels of THMs, HNMs and HANs by up to 70%. Chloramination raised the levels of 8 aldehydes and 7 HAAs; also, it caused the formation of monoiodoacetic acid, dibromochloromethane, dichloroiodomethane and bromochloroacetonitrile

at concentrations below 0.5 μg/L. Therefore, this treatment increases the levels of existing DBPs and leads to the formation of new ones to a greater extent than does chlorine dioxide. Except for 5 aldehydes, the 23 DBPs encountered at the DWTP exit were found at increased concentrations in the warmer seasons (HAAs by about 50% and THMs by 350%). In any case, the total concentrations of all regulated DBPs (THMs and HAAs) were lower than 25 μg/L and hence below regulated values.

1. Introduction

Water disinfection has been used to improve the hygienic quality of drinking water by removing waterborne bacterial pathogens since the early twentieth century [1,2]. Chlorine is the most widely used disinfectant for this purpose by virtue of is extremely high efficiency and relatively low cost. However, studies conducted in the 1970s revealed that chlorination generated potentially harmful disinfection by-products (DBPs) [3]. Since then, several hundred DBP species have been identified and new ones continued to emerge as more accurate and precise analytical methods with determination capabilities at the trace level have become available [4]. Drinking water frequently contains the following types of DBPs in addition to trihalomethanes (THMs) and haloacetic acids (HAAs): haloacetonitriles (HANs), haloketones (HKs), trichloronitromethane (chloropicrin, CP), trichloroacetaldehyde (chloral hydrate, CH) [5–7], *N*-nitrosamines [8], aldehydes [9] and carboxylic acids [10]. Several studies have revealed potentially harmful effects on health in more than two hundred halogenated and non-halogenated DBPs [2,11,12]. This has led the United States Environmental Protection Agency to regulate acceptable levels for the most prevalent DBPs in chlorination process as the combined concentration of four THMs and five HAAs to 80 µg/L and 60 µg/L, respectively [13]. Also, the European Union has regulated the total concentration of THMs to 100 µg/L after 2008 [14], but has so far established no regulatory limit for HAAs or other DBPs.

Some authors have suggested that the formation of DBPs should be prevented at any rate because once formed, they are difficult to remove by treatments commonly used in drinking water production [15]. DBP formation and degradation studies have so far been conducted at the laboratory or pilot plant level and much more frequently in drinking water treatment plants (DWTPs). Rodriguez et al. [16] found a reduction in DBP potentials and degradation of HAAs at points with a high bioactivity such as the rapid sand filtration unit. Also, Chuang et al. [17] conducted sand column laboratory studies to explore the association between HAA biodegradation and the chlorine concentration; and Tubić et al. [18] used a pilot-scale system to assess the performance of ozone, H_2O_2/O_3 and GAC in an overall treatment to reduce DBP precursors such as natural organic

matter. Some studies on DBP formation and evolution in water distribution systems have shown HAAs and THMs to differ in spatial behaviour [16,17,19]. Others have documented the formation of halogenated DBPs including THMs, HAAs, HKs, HANs, CP and CH in water treatment plants in various cities [6,20–24]. The first study of this kind was carried out in the 1990s and involved sampling raw water and the distribution systems of three DWTPs using different disinfectants over a period of one year [20], in addition to a DWTP using chlorination and ozonation [21]. The results for the four DWTPs were similar. Thus, chlorination caused the formation of halogenated DBPs; THM levels varied all year long by effect of changes in water temperature and break-point conditions; HAAs formed and evolved differently from THMs; HANs were formed at all stages of the process and completely adsorbed in the GAC filters; HKs and CH were detected at concentrations below 1 µg/L and also adsorbed in the GAC filters; and no CP was formed during the process [21]. Golfinopoulos and Nikolau [6,22] conducted a more extensive study at four conventional DWTPs in Athens; although they detected no DBP in the raw water, they encountered all DBP categories in all chlorinated samples within the DWTP and its distribution network in all sampling periods for 10 years.

A detailed picture of the spatial and temporal variability of non-regulated DBPs (4 HANs, CP and 2 HKs) in a drinking water distribution network was recently provided by Shanks et al. [23]. In a recent survey, a total of 4 THMs and 9 HAAs including iodoform (IF) and iodoacetic acid (IAA) were detected in drinking waters from 13 DWTPs in Shangai [24]. The survey, however, focused mainly on the influence of water characteristics (8 different parameters) and disinfection treatments on the presence of IF and IAA. Neale et al. [25] used *in vitro* bioanalytical tools and quantified halogen-specific adsorbable organic halogens to examine the formation of DBPs in a DWTP. Papageorgiou et al. [9] studied the presence and fate of carbonyl compounds as ozonation by-products at a DWTP for one year. They detected up to 14 DBPs at concentrations in the region of 70 µg/L after ozonation and found them to have been removed by about 75% in the treated water.

Some natural waters are cocktails of chemical and microbial contaminants that require appropriate processing to remove various kinds

of potentially harmful substances including DBPs. Existing information about the presence of a wide range of regulated and non-regulated DBPs at different points in DWTPs and their distribution networks is scant; also, most studies in this context have targeted a single species or a few at most. This led us to undertake the present study, where we examined the presence and evolution of more than 40 DBPs including 10 THMs, 13 HAAs, 6 halonitromethanes (HNMs), 6 HANs and 11 aldehydes in a DWTP using chlorine dioxide and chloramines as disinfectants with a view to (i) assessing spatial variability in DBP concentrations from raw water to distribution network (7 sampling points) and (ii) elucidating the effect of seasonal changes on DBP concentrations and their potential relationship to water quality. The study involved sampling water at the DWTP on a monthly basis for one year.

2. Materials and methods

2.1. Reagents

Standards of the 46 DBPs (see **Table S1**) were either purchased from Sigma–Aldrich (Madrid, Spain) in 99% purity or synthesised by Cansyn (Toronto, Canada). Derivatization reagents (viz., dimethylsuphate–tetrabutylammonium hydrogen sulphate for HAAs, and *o*-2,3,4,5,6-pentafluorobenzylhydroxylamine hydrochloride for HAAs and aldehydes) were purchased from Fluka (Madrid, Spain). Stock standards solutions containing a 1 g/L concentration of each compound were prepared in methanol (aldehydes, THMs and HANs), ethyl acetate (HNMs) or MTBE (HAAs) and stored in amber glass vials at –20 °C. Working-strength standard solutions were prepared on a daily basis by dilution at the microgram-per-litre level in mineral water (untreated, DBP-free water) or commercial LC–MS Ultra-grade water (aldehyde-free water).

2.2. Water treatment at the plant

The study was carried out at a DWTP located in SE Spain that uses ClO_2/NH_2Cl as disinfectants. The DWTP processes and supplies 180 million L water/day from a reservoir (total water volume 145 hm^3) to a population of ca. 300 000.

There is no direct input of wastewater or recreational use in the reservoir —only fishing is permitted. **Figure 1** depicts the potabilization process and the location of the 7 sampling points. First, raw water pumped from the intake is pre-oxidized with ClO_2 (0.6–1.0 mg/L) as a mixture of chorine and sodium chlorite. Then, pretreated water is allowed to settle in flow solid contact clarifiers to which aluminium polychloride is added (sedimentation step). Next, clarified water is passed through rapid sand filters (filtration step). Finally, the water is further disinfected with chloramines (chloramination step), ammonia and free chlorine being added separately to the effluent and chloramines were formed in situ (2.1–2.6 mg/L). Samples in the distribution system were collected about 2.5 km (24 h) or 5.0 km (48 h) from the water treatment plant.

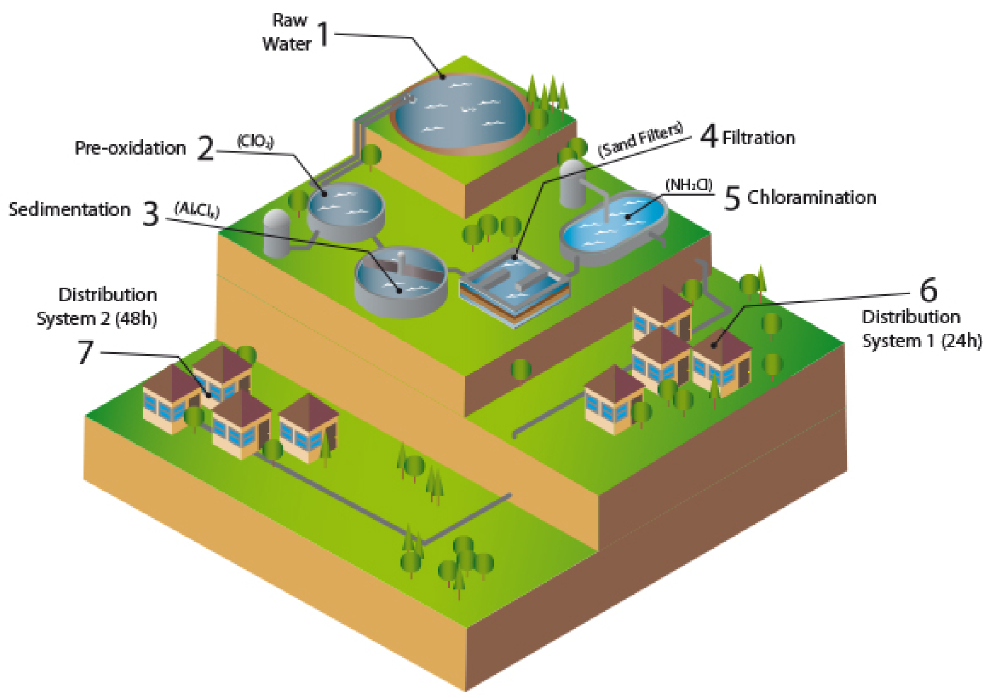

Figure 1. Schematic depiction of the drinking water treatment plant. Location of the sampling points and residence time in each.

2.3. Sampling

Water samples were collected without headspace in 1 L opaque polytetrafluoroethylene bottles and transported to the laboratory in coolers with icepacks. Samples were immediately acidified at pH 3.0-3.5 by adding 1.6 mL of 0.1 M H_2SO_4 and then refrigerated (4 ºC) until analysis. Most DBPs were stable for at least one week at 4 ºC —by exception, dibromonitromethane remained stable for only 1 day—, so the samples were stored at that temperature and analysed within 1 day of collection [26–28]. When the time between collection and analysis exceeded one day, samples were stored at −20 ºC for up to one week. In order to assess potential between-day variations, several samples were collected in triplicate at the 7 sampling points on consecutive days for a week (Monday to Sunday, n = 7 × 3 = 21) in April 2013. Samples were collected at approximately the same time (8 to 9 am) each day for a whole year. Spatial and seasonal changes were assessed in four surveys held in spring (May 2013), summer (September 2013), autumn (November 2013) and winter (February 2014), when the water temperature average was 22 ± 3 ºC, 29 ± 2 ºC, 19 ± 2 ºC and 12 ± 2 ºC, respectively. To this end, samples were collected in triplicate on a weekly basis (4 samples per month × 4 seasons, n = 16) at each of the 7 sampling points (n = 21 each week).

2.4. Analytical methods

Water samples were analysed for the 46 DBPs studied by using three static headspace gas chromatographic methods previously optimized by our group [26–28] that are briefly described in the Supplementary Material. Potential contamination during analysis was assessed by using a blank with each set of 4 samples; no DBPs were detected in the blank samples, however. **Table S1** summarizes the analytical features of the three methods. Samples for routine water quality determinations were collected and handled under the same conditions as those for DBP determinations. Water quality-related parameters (viz., pH, conductivity, ultraviolet absorbance at 254 nm, nitrite, nitrate, ammonia nitrogen, total Kjeldahl nitrogen, permanganate oxidability, chloride and bromide) were determined by using standard methods [29]. The results are listed in **Table 1.**

Table 1. Average values of water quality parameters between April 2013 and February 2014

Sampling points	Parameters	Units	Seasons			
			Spring	Summer	Autumn	Winter
Raw water (1)	Rainfall	L/m^2	27	26	70	101
	Temperature	ºC	20	28	17	11
	pH	pH units	7.4	7.2	7.4	7.6
	Conductivity	µS/cm	153	168	198	208
	UV-254[a]	cm^{-1}	0.189	0.172	0.100	0.119
	Permanganate oxidability[b]	mg O_2/L	5.7	4.6	3.9	3.5
	Nitrite	mg/L	0.015	<0.005	<0.005	<0.005
	Nitrate	mg/L	3.6	2.5	1.4	1.1
	$N-NH_3$	mg/L	0.02	<0.02	<0.02	0.03
	Org N[c]	mg/L	0.62	0.69	0.38	0.43
	Chloride	mg/L	8.1	7.6	8.3	9.5
	Bromide	mg/L	0.03	<0.01	0.02	<0.01
Pre-oxidation (2)	pH	pH units	7.4	7.1	7.4	7.6
	Conductivity	µS/cm	154	168	199	209
	UV-254	cm^{-1}	0.149	0.136	0.090	0.119
	Permanganate oxidability	mg O_2/L	5.9	4.4	3.9	3.4
	Nitrite	mg/L	0.008	<0.005	<0.005	<0.005
	Nitrate	mg/L	3.4	2.5	1.4	1.6
	$N-NH_3$	mg/L	0.03	<0.02	0.03	0.03
	Org N	mg/L	0.66	0.37	0.50	0.50
	ClO_2 dose	mg/L	1.0	0.7	1.0	0.8
Sedimentation (3)	pH	pH units	7.1	7.1	7.3	7.4
	Conductivity	µS/cm	172	185	215	221
	UV-254	cm^{-1}	0.060	0.056	0.033	0.049
	Permanganate oxidability	mg O_2/L	3.0	2.7	2.3	2.3
	Nitrite	mg/L	0.027	<0.005	<0.005	<0.005
	Nitrate	mg/L	3.4	2.8	1.5	1.4
	$N-NH_3$	mg/L	0.03	<0.02	0.02	0.03
	Org N	mg/L	0.51	0.60	0.54	0.45
	Al_xCl_3 dose	mg/L	74	59	59	52

Table 1. Continuation

Filtration (4)	pH	pH units	7.3	7.0	7.4	7.5
	Conductivity	µS/cm	175	185	215	219
	UV-254	cm^{-1}	0.049	0.050	0.029	0.044
	Permanganate oxidability	mg O_2/L	2.7	2.4	2.2	2.3
	Nitrite	mg/L	0.114	<0.005	<0.005	<0.005
	Nitrate	mg/L	3.5	2.7	1.5	1.1
	N-NH_3	mg/L	0.03	<0.02	<0.02	0.03
	Org N	mg/L	0.53	0.51	0.61	0.55
Chloramination (5)	Temperature	ºC	21	28	19	13
	pH	pH units	7.2	7.1	7.4	7.4
	Conductivity	µS/cm	173	187	216	225
	UV-254	cm^{-1}	0.048	0.051	0.033	0.048
	Permanganate oxidability	mg O_2/L	2.8	2.3	2.3	2.1
	Nitrite	mg/L	0.054	<0.005	<0.005	<0.005
	Nitrate	mg/L	3.4	2.7	1.7	1.8
	N-NH_3	mg/L	0.40	0.41	0.43	0.45
	Org N	mg/L	0.66	0.28	0.21	0.07
	NH_2Cl dose	mg/L	2.2	2.4	2.4	2.4
	Chloride	mg/L	16	14	16	13
	Bromide	mg/L	0.06	<0.01	0.04	<0.01
Distribution System (6)	Temperature	ºC	25	31	21	14
	pH	pH units	7.2	7.0	7.2	7.3
	Conductivity	µS/cm	178	193	217	240
	UV-254	cm^{-1}	0.048	0.050	0.033	0.048
	Permanganate oxidability	mg O_2/L	2.7	2.1	1.8	2.4
	Nitrite	mg/L	0.023	<0.005	<0.005	<0.005
	Nitrate	mg/L	3.4	2.6	1.5	1.9
	N-NH_3	mg/L	0.36	0.40	0.43	0.44
	Org N	mg/L	0.61	0.18	0.23	0.14
	Chloride	mg/L	16	14	14	16
	Bromide	mg/L	0.05	<0.01	<0.01	<0.01

[a] Absorbance at 254 nm.

[b] Permanganate oxidability; calculated by the oxidation of organic compounds in the water by potassium permanganate.

[c] Organic nitrogen; calculated as the difference between Total Kjeldahl nitrogen and NH_3–N.

3. Results and discussion

The DWTP under study was selected to allow comparison of (i) the different treatment steps typically conducted at a DWTP; (ii) the influence of climatic conditions (seasonal changes); and (iii) residence times in the water distribution system (spatial changes). The 46 DBPs studied were selected on the basis of their frequent or potential presence in the source (raw) or treated water.

3.1. Water parameters

Table 1 lists the average values of the quality-related parameters of the water at each sampling point during the studied period (May 2013 to February 2014). As can be seen, the quality of the raw and treated water changed between seasons. Thus, the highest content in organic carbon, expressed as permanganate oxidability, in the raw water was highest in spring (5.7 mg O_2/L), followed by summer (4.6 mg O_2/L), autumn (3.9 mg O_2/L) and then winter (3.5 mg O_2/L). However, the sedimentation step efficiently removed natural organic matter (permanganate oxidability was reduced to 2.3–3.0 mg O_2/L). As found in a previous study [30], the water contained nitrite but its concentration (< 0.005 to 0.114 mg/L) was too low for nitrogen DBPs (N-DBPs) to form. The presence of DBPs and their speciation are influenced by other factors such as the disinfectant dose used, which remained fairly constant (0.7–1.0 mg Cl_2O/L and 2.2–2.4 mg NH_2Cl/L), and the bromide concentration, which was higher in spring (0.03–0.06 mg/L) and autumn (0.02–0.04 mg/L) than in the other seasons (<0.01 mg/L). Other water parameters had little effect on the presence of DBPs.

The assessment of seasonal changes was preceded by that of between-day variability within the treatment plant by collecting samples at the different sampling points (see **Figure 1**) for seven consecutive days in spring (April 2013). As can be seen from **Table S2**, DBP concentrations varied little. Also, their variability was similar to that observed in different weeks of the same month. Therefore, changes in DBP concentrations can be ascribed to contamination of the raw water and to the DWTP treatments, and the presence of DBPs in the water was thus accurately reflected in samplings conducted one day each week. The presence and distribution

profiles of each kind of DBP in the DWTP and its distribution system are discussed below.

3.2. Presence of regulated and non-regulated DBPs in water samples collected in different seasons from the DWTP

The results discussed here are concentrations of regulated and non-regulated DBPs obtained by sampling at points 1–7 during the four seasons from May 2013 to February 2014. Spatial and seasonal changes were assessed by collecting a water sample in triplicate each week (4 samples per month per season, n = 16) at the 7 sampling points listed in **Figure 1**. Therefore, the data in **Table 2** were obtained from a total of 336 water samples. The spatial distribution and seasonal variation of the total concentrations of each family of DBPs (aldehydes, volatile DBPs and haloacetic acids) are discussed below and illustrated in **Figure 2**.

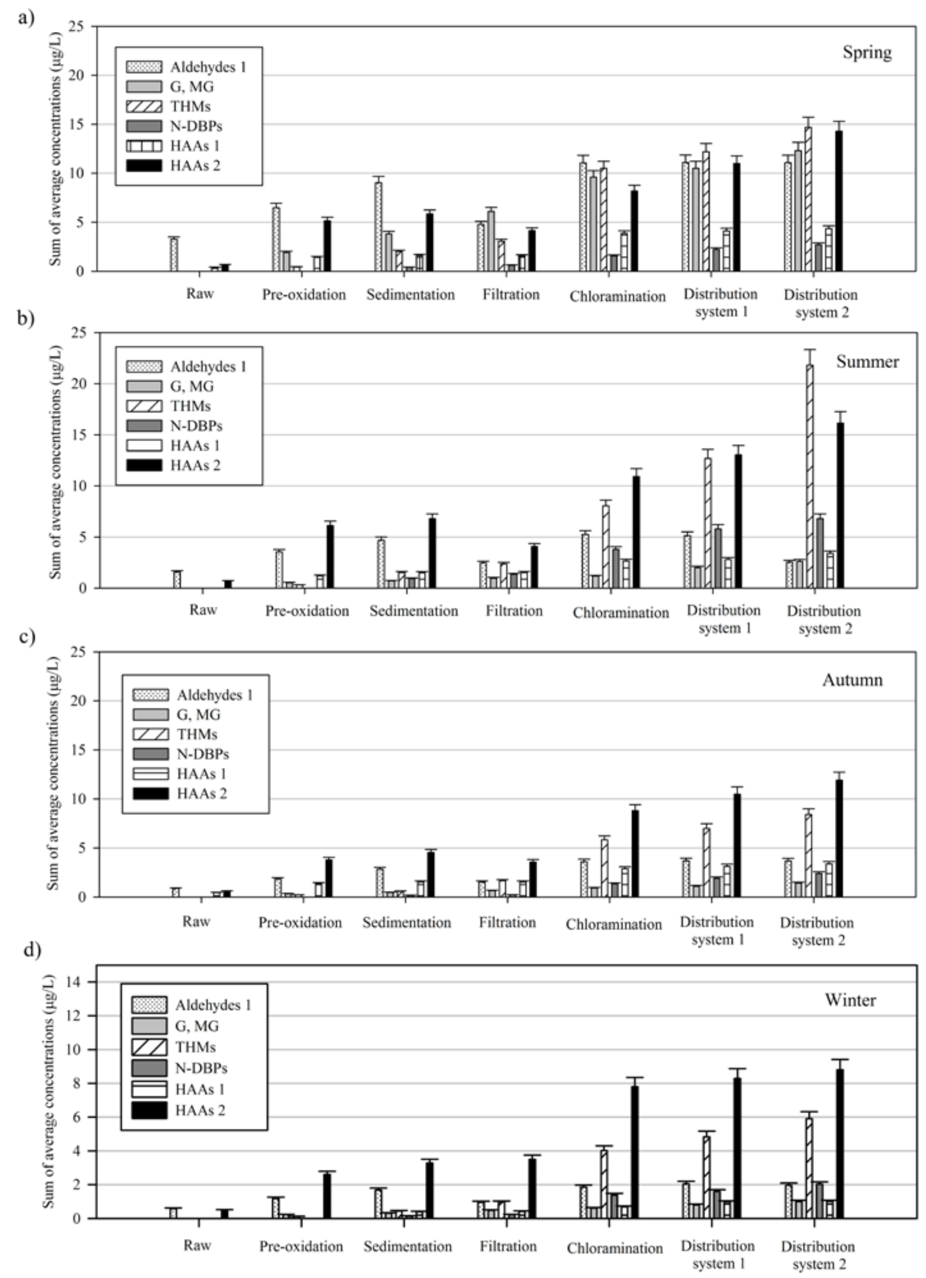

Figure 2. Combined average concentrations of DBP groups in water samples collected at the 7 sampling points in the four seasons. Error bars represent standard deviations of the mean concentrations. Aldehydes 1: C1-C5. N-DBPs: TCNM, DCAN and BCAN. HAAs 1: MCAA, MBAA, MIAA and DBAA. HAAs 2: DCAA, TCAA, BCAA and BDCAA.

3.2.1. Aldehydes

The spring season was used as reference to examine the spatial distribution of carbonyl compounds because its climatic conditions were in between those of the winter (mostly rainy) and summer (largely dry) in the study areas, and also because the levels of these compounds peaked in spring. As can be seen from **Table 2** (May 2013), the raw water (sampling point 1, SP1 in **Figure 1**) contained five of the eleven aldehydes studied, at concentrations from 0.08 (valeraldehyde, C5) to 1.8 μg/L (formaldehyde, C1). These five aliphatic aldehydes were previously found at also increased concentrations (3.6–99.6 μg/L) in two rivers in the urban area of Poznan, Poland [31]. No dicarbonyl aldehydes (G and MG) or BA were detected in the raw water, even though a recent study found G and MG at a concentration of 2.78 and 2.26 μg/L, respectively, in raw water from a Greek DWTP [9]. The presence of carbonyl compounds can probably be explained as the result of natural processes and/or anthropogenic sources. The natural sources of aldehydes are related to photodegradation of dissolved organic matter, microbial oxidation of volatile organics [31,32], direct emission from growing vegetation, biomass burning and living organisms [33]. As regards anthropogenic sources, carbonyl compounds can be the result of pollution from fuel combustion and manufacturing [31,34–36]. Pre-oxidation with chlorine dioxide (SP2) further increased the amounts of the five aliphatic aldehydes with respect their initial levels, and caused G, MG and BA to be formed (**Table 2**). This was a result of the chlorine dioxide being used in high enough doses (1.0 mg/L on average) to react with organic matter present in the raw water to form aldehydes (average permanganate oxidability 5.7 mg O_2/L; average UVA_{254} 0.189 cm^{-1}). This result is consistent with those of Dąbrowska et al. [37], who also found increased amounts of aldehydes (C1, C2, G and MG) after a chlorine dioxide treatment. It should be noted that 3-MBA, 2-EBA and 2,5-DMBA were not detected at any sampling point in the DWTP (see **Table S1**), probably because their formation requires higher concentrations of disinfectant and organic matter —as high as those used in swimming pool water, where they are usually detected [28]. A coagulation/flocculation step with Al_xCl_3 (SP3), increased the average concentrations of the eight aldehydes by about 50%, which suggests that this treatment was inefficient in their removal. These results are inconsistent with those recently reported by Papageorgiou et al.

[9] for a full-scale DWTP disinfecting water with ozone, where coagulation/flocculation reduced the levels of carbonyl compounds by 40%; these authors, however, failed to state the specific coagulant used even though the efficiency of a coagulation procedure in removing aldehydes is influenced by the type of coagulant and oxidant (ozone) dose [38,39].

The next step in the water treatment was passage through rapid sand filters (SP4). It should be noted that this is the first time the sedimentation and filtration steps at a DWTP have been examined separately; in fact, previous studies dealt with a global sedimentation/filtration process. As can be seen from **Table 2**, the filtration step was efficient in removing the five aliphatic aldehydes —reductions amounted to about 50%—; however, it also increased the average concentrations of G, MG and BA by about 40%. These results are partly consistent with those of a recent study by Papageorgiou et al. [9] where filtration removed C1, C2, C3 and C4 by 10–60% but failed to remove C5 and reduced the concentrations of G and MG. The use of rapid sand filtration to remove aldehydes is justified by the fact that sand supports biological activity and facilitates the reduction of dissolved and biodegradable carbon, ultimately facilitating the removal of aldehydes [39,40]. Changes in the concentrations of G, MG and BA (viz., the aldehydes with the highest molecular masses) on passage through sand filters were previously examined at a pilot-scale treatment plant [41] and ascribed to an increased resistance to microbiological activity relative to C1 and C2 —with lower molecular masses. Also, additional high-molecular mass dialdehydes are seemingly formed on the filter bed in the absence of biomass on the filters [41]. The second disinfection step (chloramination, SP5) also raised the concentrations of the eight aldehydes in relation to the previous step (see **Figure 2**). There exists no literature on the presence and distribution profiles of aldehydes after chloramination in DWTPs, so no comparison was possible here. In any case, our results are consistent with those of some batch experiments were similar aldehydes exhibited increased concentrations upon chloramination [42]. Based on our results, the influence of chlorine dioxide and chloramination on the presence of the eight target aldehydes was similar; in fact, the total concentration of aldehydes (SP2 and SP5) was further increased in relation to those found in the previous steps (SP1 and SP4, respectively). Finally, we examined the

variation of the water residence time along the distribution network, which conformed to a decreasing gradient of the free and total residual disinfectant concentrations, at points 24 and 48 h from the DWTP exit. As can be seen from **Table 2**, the samples, collected from SP6 and SP7, contained the five aliphatic aldehydes at similar concentrations. Also, the concentrations of G and MG increased slightly with time, whereas that of BA decreased in parallel. This is the first time the distribution profiles of aldehydes at the exit of a DWTP have been established.

Although the operating conditions in the treatment plant were adjusted to quality changes in the raw water, the average concentration of aldehydes exhibited a seasonal influence (see **Table 2**). Thus, the water contained the same five aliphatic aldehydes (C1–C5) during the four seasons; however, their combined average concentration changed considerably between seasons (from 3.27 μg/L in spring to 1.59 μg/L in summer, 0.86 μg/L in autumn and 0.58 μg/L in winter). The concentrations found each season can be correlated with UVA_{254} and the content in organic matter (as permanganate oxidability), which were higher in spring than in winter (**Table 1**); this result is consistent with that of a previous study by Papageorgiou et al. [9]. Also, seasonal changes can be correlated with the amount of rainfall, which additionally influences the concentration of aldehydes in raw water. In this study, the combined average concentration of aldehydes decreased with increasing amount of rainfall (see **Table 1**), which is consistent with the results of recent research into a potential inverse relationship between carbonyl concentrations in river water and the amount of rainfall (i.e., its dilution effect) [31]. The pre-oxidation step had a similar effect irrespective of season. Thus, it further increased the combined average concentration of the five aliphatic aldehydes in relation to the raw water, and caused G, MG to appear. Benzaldehyde was the only aromatic aldehyde detected after pre-oxidation with chlorine dioxide in spring, probably because it requires large amounts of organic matter to form. This is the first time the effect of individual DWTP processes on seasonal changes in BA concentration has been elucidated. The sedimentation step increased the average concentration of aldehydes by about 50% irrespective of season, and so did the filtration step, which additionally increased the concentrations of G, MG and BA by about 40%.

The last oxidation step (chloramination) increased the combined average concentration of aldehydes by ca. 50% in all seasons.

The concentration of aliphatic aldehydes at the DWTP exit remained fairly constant in spring, autumn and winter, but differed markedly in summer, where it decreased by about 50% at SP7 (viz., 48 h from the DWPT exit). This result can be ascribed to biodegradation of the aldehydes by effect of high microbiological activity at the high temperatures reached in the summer [9,31,41]. However, G and MG levels beyond the DWTP exit increased by 40–100% in all seasons —summer included—, probably as a result of their strong resistance to microbiological activity [41].

3.2.2. Volatile DBPs (THMs, HNMs and HANs)

Previous research showed the individual factors most strongly affecting THM formation to be the chlorine dose and residual concentration, organic matter concentration, water pH and temperature, and presence of bromide and ammonia [43,44]. As can been seen from **Table 2**, none of the 22 volatile species studied (10 THM, 6 HNMs and 6 HANs) was found in the raw water at any time. Therefore, the presence of these compounds in the treated water can be exclusively ascribed to the process of disinfection. Only chloroform (TCM) was formed in the pre-oxidation step with chlorine dioxide (SP2), probably because its formation is faster than that of other volatile species or because it requires a lower disinfectant dose or organic matter to form. The concentration of TCM was higher in the warmer months (spring and summer). The sedimentation step with aluminium polychloride (SP3) caused the formation of another THM (BDCM) and one HNM (TCNM) in the warmer seasons, and one HAN (DCAN) in all; also, it increased the concentration of TCM roughly 3.5 times in all seasons by reaction of chlorine dioxide with residual precursors present in the water. Filtration raised the levels of the four volatile species formed in the previous step by 40–70%. The increase is similar to that observed after the filtration step (20–50%) in previous studies conducted in Spain [21] but smaller than that in a more recently reported study performed in Australia (70–200%) [25]. Also consistent with previous reports [21,25,45,46], none of the compounds was removed by the sand filters. The second oxidation step with chloramines (SP5) further increased the concentrations of the compounds already present in the water 2.5–3.5 times with respect to the previous step

(see **Figure 2**) and caused three new species (DBCM, DCIM and BCAN) to appear for the first time, in all seasons. The formation of the new compounds can be ascribed to their requiring higher oxidant amounts in the disinfection step [47,48]: a 2.2 mg/L chloramine concentration versus one of 1.0 mg/L of chlorine dioxide or the presence of chloramines involve substitution reactions that could be formed new DBPs. Moreover, chloramines incorporate nitrogen into organic precursors, thereby increasing the formation of N-DBPs [48,49] in addition to iodinated DBPs [47] as previously shown in batch experiments. This is the first study examining the effect of individual DWTP treatment steps on dichloroiodomethane (DCIM) and its seasonal changes, although it was only detected after the chloramination step. Possibly, previous studies used methods that were inadequately sensitive to detect DCIM at the levels found in this work (<0.07–0.33 μg/L). DBP concentrations also change considerably with the residence time of the water in the DWTP distribution system [23,43,49]. In this work, the combined average concentration of volatile species at SP6 (i.e., 24 h from the DWTP exit) —DCIM excluded owing to its low concentration in all seasons— increased more markedly in summer (45–80%) than in the other seasons (10–30%). These results suggest that warm water temperatures favour the formation of volatile DBPs and are consistent with those of previous studies on the effect of temperature and seasonal conditions on DBP formation [23,43,49]. The concentrations of the three THMs at SP7 (i.e., 48 h from the DWTP exit) also increased more markedly in the summer (70–85%) than in the other seasons (20–40%) (see **Figure 2**). Although the N-DBP concentrations also increased in the four seasons, they were lower in the summer (10%) than in the other seasons (20–30%). This spatial variability is consistent with the increase in total volatile DBP levels with increasing water temperature and distance from the source [43]. For example, the concentrations of the four THMs in summer (**Table 2**) were approximately 1.5 and 3.0 times higher at SP6 (24 h) and SP7 (48 h), respectively, than they were in the finished water (SP5, DWTP exit). It is therefore advisable to monitor these species in the vicinity of public drinking systems in addition to DWTP exits.

The combined average concentration of the seven volatile DBPs changed considerably between months and exhibited statistically significant differences between seasons as a result. Thus, the concentration

of THMs in the finished water (SP5) peaked in spring (10 μg/L), followed by summer (8 μg/L), autumn (6 μg/L) and then winter (4 μg/L). This result is unsurprising if one considers the increased permanganate oxidability of the raw water in spring (5.7 mg O_2/L) in relation to winter (3.5 mg O_2/L). The increase in THM concentration in the warmer seasons was less marked than that found in a previous study by Wei et al. [24]: 6–10 times in summer relative to winter. Therefore, permanganate oxidability can be used as an indicator for THM precursors. The three N-DBPs evolved differently in this respect; thus, the combined average concentration was higher in the summer (4 μg/L) than in the spring (2 μg/L), the difference probably being the result of the high water temperature in the summer (29 ± 2 ºC) in relation to the other seasons. The presence of brominated DBPs is particularly important owing to their high toxicity. Bromide in the water is oxidized to hypobromous acid (HOBr) by chlorine in the form of hypochlorous acid (HOCl), which facilitates the formation of chlorinated and brominated DBPs. As can be seen from **Table 2**, brominated DBPs (DBCM and BCAN, mainly) prevailed in spring and autumn by effect of the increased concentrations of bromide (0.06 and 0.04 mg/L, respectively) relative to the other seasons (<0.01 mg/L).

3.2.3. Haloacetic acids

The spring season was used as a model to examine the spatial distribution of the thirteen HAAs studied. Only trichloroacetic acid (TCAA) and dichloroacetic acid (DCAA) were found at concentrations lower than 1 μg/L in the raw water (**Table 2**, May 2013). Monochloroacetic acid (MCAA) was also detected, albeit below quantifiable levels, in one of the four samples (SP1). Only one existing study previously detected TCAA in raw water from a DWTP; the study was conducted in Spain and TCAA found at concentrations from 0.3 to 1.6 μg/L [21]. On the other hand, DCAA and TCAA have been widely detected at variable levels from <0.10 to 2.4 μg/L in surface water, rainwater and seawater [50–54]. The presence of HAAs in natural waters can likely be explained as a result of pesticide degradation and/or atmospheric degradation of refrigerant compounds [55]. Thus, trichloroacetate (as a sodium salt, ester or amide derivative) has been used against perennial grasses, and monochloroacetate is the phytotoxic principle in the herbicides alachlor, propachlor, metazachlor and

metolachlor [56]. In addition, trichloroacetate in natural aquatic systems under anaerobic conditions is converted into dichloroacetate by anaerobic microbial degradation [55]. None of the other HAAs was detected in the raw water, which suggests that they form during water treatment.

Pre-oxidation with chlorine dioxide (SP2) caused the four brominated HAAs (MBAA, BCAA, DBAA and BDCAA) to appear for the first time as a result of the reaction of organic matter with bromide and chlorine present in the water. Chlorine dioxide increased the concentrations of DCAA and TCAA 8 and 3.5 times, respectively, from their initial levels; on the other hand, MCAA remained at similar concentrations but was detected in virtually all water samples. It should be noted that DCAA and TCAA were the prevalent HAAs, which is consistent with the results of previous studies on DWTPs conducted in Spain [21], Greece [6,22] and Canada [20,23,57,58]. The sedimentation (coagulation/flocculation) step (SP3) increased the combined average concentration of the seven HAAs by about 15% from the previous step, which suggests that this treatment was inefficient in their removal because they were present as anions at the prevailing water pH (pK_a 0.5–2.8). The next treatment step involved passage through rapid sand filters (SP4). HAAs are known to undergo biodegradation in DWTPs using highly bioactive treatments such as slow sand filtration [58]. However, removal of HAAs by effect of rapid sand filtration has rarely been observed [17,59]; by exception, Rodriguez et al. [16] found DCAA to be degraded in the warm seasons. In our study, rapid sand filtering decreased the average concentration of DCAA by about 50%, and of those BCAA and BDCAA by about 20%, through biodegradation in the filters. Thus, although HAAs were partially biodegraded, they were incompletely removed because their contact time with the rapid sand filters was too short (a few minutes) and the filters were subject to frequent backwashing —which prevented development of the biofilm needed to biodegrade the acids [17]. The second disinfection step with chloramines (SP5) further increased the combined average concentration of the seven chlorinated and brominated species from the previous step (12 versus 5.8 μg/L) and caused an iodinated species (monoiodoacetic acid, MIAA) to be formed. Previous studies have suggested that chloramination enhances the formation of iodinated DBPs [1,24]. Both chlorine dioxide and chloramines can oxidize iodide to HOI, but only chlorine dioxide can rapidly convert HOI

to iodate [24,60]. Hence, HOI has a higher likelihood to react with organic matter and form iodinated DBPs during chloramination. The concentration of this compound was relatively low (average 0.3 μg/L), probably because of the low levels of iodine present or the near-neutral pH of the water (~7.2) — in fact, the formation of MIAA requires acid conditions [60]. These results are consistent with those of a study on drinking water from thirteen DWTPs in Shangai where the peak MIAA concentration remained at sub-ppb levels most of the time and only peaked (1.7 μg/L) after chloramination [24]. Changes in the concentrations of HAAs were also substantial for their residence times in the distribution system. This can be ascribed to DBP formation reactions proceeding as free residual disinfectant was consumed while DBP precursors were still present in the water [23]. Thus, the combined average concentration of HAAs at SP6 (24 h) and SP7 (48 h) was ca. 25 and 55% higher, respectively, than that at the DWTP exit (SP5). However, our results depart from those of another study in which the concentrations of HAA (DCAA, mainly) were found to decrease through microbiological degradation in the distribution network [57]. We can therefore conclude that the two water disinfection treatments used, and the residence time in the distribution network, have a considerable impact on the evolution of HAAs—in fact, they dramatically increased the levels of these compounds. Based on the data of **Table 2**, HAAs can classified into two distinct groups in this respect. Thus, MCAA, MBAA, MIAA and DBAA were formed at low concentrations and remained at relatively constant levels in all steps except for an increase after chloramination step. On the other hand, DCAA, TCAA, BCAA and BDCAA increased in concentration in all steps except filtration. The difference is visually illustrated in **Figure 2**.

The average concentrations of HAAs also exhibited a seasonal influence (see **Table 2**). Thus, the three chlorinated HAAs found in the raw water in spring were also detected in autumn, whereas only two or one was detected in the summer and winter, respectively. TCAA was found in all seasons and raw water samples, at average concentrations of 0.4, 0.5, 0.2 and 0.5 μg/L in spring, summer, autumn and winter, respectively. The concentration of DCAA in the raw water was lower than of TCAA in the warmer months (average 0.2 μg/L), but greater by 100% in autumn and undetectable in winter. As stated above, the presence and concentrations of HAAs in natural water are highly variable; also, their changes cannot be

connected with location or date [61]. The pre-oxidation step had a similar effect in spring, summer and autumn; thus, it caused the formation of up to five new HAAs, and increased the concentrations of DCAA and TCAA, already present in raw water, 6 times. The combined average concentration of HAAs peaked at 6.5–7.3 μg/L in the warmer seasons by effect of the increased water temperatures (22−29 °C) facilitating the formation of HAAs. On the other hand, only three species (DCAA, TCAA and BCAA) were detected in the winter season, at average concentrations of 0.2–1.2 μg/L, owing to the low prevailing temperatures (12 ± 2 °C). These results are consistent with those other studies on HAAs conducted in the USA where the highest concentrations were observed in the warm seasons [57,62]. By contrast, the highest concentrations of brominated HAAs were found in spring and autumn, when the bromide levels were higher ($[Br^-]$ = 0.02 mg/L). The sedimentation step further increased the total HAA concentration of ca. 15% in any season by reaction of chlorine dioxide with residual precursors present in the water. The greatest reduction of HAA concentrations in the rapid sand filters (25–40%) occurred in the warmer seasons; in the others, concentrations remained at similar levels, probably as a result of the decreased microbiological activity in the filters at the lower temperatures. The chloramination step further increased the combined average concentration of HAAs in all seasons; also, it caused the formation of MIAA in all except winter. As in spring, an increase in HAA levels in the distribution network was observed in all seasons, which suggests that they increased with increasing residence time. As expected, and consistent with previous reports [22,30], the total HAA concentrations found at SP7 were higher in the warmer seasons (ca. 20 μg/L) than in the cooler seasons (10−15 μg/L). On the other hand, the concentrations of DCAA and TCAA in the distribution system were similar in all seasons and the latter prevailed in the summer. The different seasonal distribution of the two prevalent HAAs (DCAA and TCAA) is consistent with the results of other studies on seasonal and spatial changes in a DWTP [23,57,58]. Since DCAA and TCAA differ in their precursors [57,62], one plausible explanation for the seasonal distribution observed is that the properties of organic matter may change through the year; also, DCAA may undergo marked biodegradation under the typically warm water temperatures of summer and the prevalence of the two HAAs be altered as a result [58,63,64].

Table 2 . DBP concentrations (μg/L) from the DWTP samples collected in different seasons

Compounds	Sampling point													
	Raw (1)		Pre-oxidation (2)		Sedimentation (3)		Filtration (4)		Chloramination (5)		Distribution system 1 (6)		Distribution system 2 (7)	
	n[a]	Min–Max[b]	n	Min–Max	n	Min–Max	n	Min–Max	n	Min–Max	n	Min–Max	n	Min–Max
Spring (May 2013)														
Aldehydes														
C1	4	1.2–1.8	4	2.8–3.7	4	4.1–5.2	4	2.2–2.8	4	5.2–6.4	4	5.2–6.3	4	5.1–6.2
C2	4	0.73–1.5	4	1.7–3.2	4	2.3–3.9	4	1.1–2.6	4	2.9–4.4	4	2.5–4.1	4	2.6–3.9
C3	4	0.37–0.88	4	0.52–0.91	4	0.61–0.98	4	0.25–0.40	4	0.50–0.74	4	0.54–0.85	4	0.50–0.77
C4	4	0.10–0.25	4	0.27–0.38	4	0.34–0.53	4	0.11–0.20	4	0.27–0.44	4	0.29–0.48	4	0.26–0.45
C5	3	0.08–0.14	3	0.09–0.22	4	0.19–0.30	4	0.09–0.22	4	0.19–0.31	4	0.20–0.33	4	0.20–0.34
G	0	N.D.[c]	4	1.1–1.4	4	1.9–4.0	4	2.5–4.4	4	4.0–7.1	4	4.4–6.9	4	5.0–9.8
MG	0	N.D.	4	0.66–1.6	4	0.80–2.2	4	1.6–3.0	4	2.3–4.4	4	3.0–4.9	4	5.2–6.4
BA	0	N.D.	4	0.18–0.36	3	0.46–0.61	3	0.58–0.79	3	0.65–0.99	3	0.75–1.1	3	0.73–0.82
Volatile DBPs														
TCM	0	N.D.	4	0.32–0.60	4	1.2–1.7	4	2.1–2.6	4	7.3–8.6	4	8.5–9.4	4	10–12
BDCM	0	N.D.	0	N.D.	4	0.42–0.60	4	0.69–0.84	4	1.9–2.5	4	2.2–2.9	4	2.5–3.4
DBCM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	0.23–0.48	4	0.32–0.62	4	0.41–0.82
DCIM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	0.12–0.24	4	0.14–0.25	4	0.14–0.26
TCNM	0	N.D.	0	N.D.	4	0.15–0.37	4	0.18–0.73	4	0.62–1.3	4	0.68–1.4	4	1.0–2.4
DCAN	0	N.D.	0	N.D.	4	0.12–0.22	4	0.14–0.36	4	0.37–1.2	4	0.45–1.5	4	0.86–1.3
BCAN	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.15[d]	4	0.17–0.21	4	0.18–0.31
HAAs														
MCAA	1	<0.40	3	<0.40–0.62	3	0.43–0.72	3	<0.40–0.86	4	0.41–1.2	4	0.59–1.4	4	0.69–1.4
MBAA	0	N.D.	1	<0.40	3	<0.40–0.60	3	0.43–0.67	3	0.69–1.6	3	0.80–1.8	3	0.93–2.1
DCAA	3	<0.10–0.40	4	0.40–3.2	4	0.53–3.5	4	0.26–2.1	4	0.80–4.4	4	1.6–5.1	4	2.4–6.3
MIAA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	0.27–0.44	4	0.29–0.48	4	0.26–0.49
TCAA	3	<0.10–0.70	4	0.40–2.2	4	0.50–2.2	4	0.49–2.1	4	1.2–4.9	4	1.9–7.0	4	2.4–8.7
BCAA	0	N.D.	2	0.40–1.1	4	0.52–1.2	4	0.50–0.90	4	0.91–1.7	4	1.3–2.1	4	1.5–2.8
DBAA	0	N.D.	3	<0.20–0.90	3	<0.20–1.1	2	<0.20–0.80	3	0.65–1.9	4	0.76–2.1	4	0.87–2.4
BDCAA	0	N.D.	3	0.60–1.5	3	0.70–1.8	3	<0.30–1.4	4	1.2–2.7	4	1.6–3.3	3	2.0–4.4

Table 2. Continuation

Summer (September 2013)														
Aldehydes														
C1	4	0.55–1.3	4	0.93–2.8	4	1.5–3.4	4	0.73–2.0	4	2.3–3.4	4	2.4–3.5	4	1.0–2.1
C2	4	0.42–0.85	4	0.98–1.7	4	1.0–1.9	4	0.55–1.3	4	0.94–1.9	4	0.86–1.9	4	0.35–0.71
C3	4	0.10–0.15	4	0.16–0.28	4	0.21–0.54	4	0.13–0.25	4	0.35–0.43	4	0.36–0.41	4	0.06–0.36
C4	4	0.05–0.11	4	0.11–0.21	4	0.17–0.30	4	0.06–0.12	4	0.28–0.35	4	0.26–0.35	4	0.16–0.21
C5	3	<0.03–0.08	3	0.06–0.13	4	0.16–0.21	4	0.06–0.10	4	0.09–0.21	4	0.08–0.18	4	0.04–0.07
G	0	N.D.	4	0.34–0.49	4	0.39–0.55	4	0.46–0.71	4	0.69–0.94	4	0.81–1.7	4	1.0–2.3
MG	0	N.D.	4	0.07–0.18	4	0.13–0.28	4	0.18–0.34	4	0.24–0.49	4	0.36–0.57	4	0.47–0.93
BA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.
Volatile DBPs														
TCM	0	N.D.	4	0.24–0.39	4	1.0–1.4	4	1.7–2.1	4	5.5–6.5	4	8.4–11	4	14–20
BDCM	0	N.D.	0	N.D.	4	<0.03–0.50	4	0.31–0.62	4	1.5–2.3	4	2.2–3.0	4	3.2–5.5
DBCM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.05–0.10	4	<0.05–0.21	4	0.15–0.37
DCIM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	0.13–0.22	4	0.16–0.27	4	0.19–0.33
TCNM	0	N.D.	0	N.D.	4	0.16–0.41	4	0.23–0.81	4	1.7–2.3	4	2.5–3.4	4	2.4–3.7
DCAN	0	N.D.	0	N.D.	4	0.15–1.3	4	0.33–1.4	4	1.3–2.6	4	1.4–7.1	4	1.5–6.8
BCAN	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.15	4	<0.15	4	<0.15
HAAs														
MCAA	0	N.D.	4	0.55–0.72	3	0.67–0.78	3	0.43–0.60	4	0.77–1.8	4	0.72–2.2	4	1.0–2.4
MBAA	0	N.D.	2	<0.40	2	<0.40–0.50	2	<0.40	3	<0.40–0.54	3	<0.40–0.60	3	<0.40–0.64
DCAA	4	<0.10–0.28	4	0.80–2.5	4	1.1–2.8	4	0.30–1.1	4	1.0–3.1	4	1.8–4.3	4	2.6–5.4
MIAA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	0.36–0.59	4	0.40–0.66	4	0.46–0.70
TCAA	4	<0.10–1.3	4	0.82–5.3	4	0.88–5.5	4	0.80–5.2	4	2.8–9.7	4	3.7–11	4	4.5–13
BCAA	0	N.D.	2	0.60–1.2	4	<0.10–1.4	4	<0.10–0.70	4	0.40–1.2	4	0.51–1.7	4	0.80–2.2
DBAA	0	N.D.	1	<0.20	1	0.4	1	<0.20	2	<0.20–0.44	3	<0.20–0.50	3	0.30–0.53
BDCAA	0	N.D.	2	<0.30–0.70	3	<0.30–0.88	2	<0.30–0.58	3	<0.30–0.72	3	<0.30–0.97	3	<0.30–0.98

Table 2. Continuation

Autumn (November 2013)														
Aldehydes														
C1	4	0.34–0.63	4	0.39–1.9	4	1.3–2.0	4	0.57–1.2	4	1.7–3.2	4	1.8–3.4	4	1.7–3.1
C2	4	0.19–0.58	4	0.42–0.90	4	0.51–1.3	4	0.36–0.77	4	0.71–1.3	4	0.69–1.4	4	0.62–1.3
C3	4	0.04–0.07	4	0.10–0.18	4	0.15–0.31	4	0.07–0.15	4	0.12–0.28	4	0.13–0.26	4	0.14–0.25
C4	4	0.03–0.09	4	0.06–0.13	4	0.09–0.27	4	0.03–0.10	4	0.09–0.20	4	0.10–0.26	4	0.08–0.18
C5	3	<0.03–0.06	3	0.04–0.09	4	0.05–0.12	4	0.04–0.07	4	0.04–0.09	4	0.05–0.11	4	0.06–0.09
G	0	N.D.	4	0.20–0.33	4	0.25–0.40	4	0.34–0.64	4	0.54–0.82	4	0.68–0.98	4	0.83–1.5
MG	0	N.D.	4	0.04–0.09	4	0.08–0.14	4	0.10–0.18	4	0.12–0.25	4	0.16–0.29	4	0.23–0.42
BA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.
Volatile DBPs														
TCM	0	N.D.	4	0.14–0.25	4	0.53–0.62	4	0.71–1.5	4	3.8–4.5	4	4.3–5.8	4	5.5–6.3
BDCM	0	N.D.	0	N.D.	0	N.D.	4	0.23–0.44	4	0.95–1.6	4	1.2–2.1	4	1.5–2.6
DBCM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	0.22–0.33	4	0.28–0.39	4	0.33–0.65
DCIM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.07–0.16	4	0.11–0.17	4	0.10–0.16
TCNM	0	N.D.	0	N.D.	0	N.D.	4	<0.10	4	0.50–1.2	4	0.72–0.91	4	0.81–1.2
DCAN	0	N.D.	0	N.D.	4	<0.10–0.21	4	0.15–0.32	4	0.42–0.80	4	0.51–0.92	4	0.86–1.3
BCAN	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.15	4	0.16–0.70	4	0.25–0.43
HAAs														
MCAA	1	0.45	2	<0.40–0.79	2	<0.40–0.82	2	0.42–0.85	3	<0.40–1.1	3	<0.40–1.2	3	<0.40–1.2
MBAA	0	N.D.	3	<0.40–0.60	4	<0.40–0.68	3	<0.40–0.75	4	0.71–1.5	4	1.1–1.8	4	1.1–1.8
DCAA	4	0.18–0.91	4	0.65–3.5	4	0.75–3.8	4	0.47–1.7	4	1.0–4.6	4	1.8–5.5	4	2.3–6.6
MIAA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.10–0.31	4	<0.10–0.40	4	<0.10–0.38
TCAA	4	<0.10–0.27	4	0.27–1.8	4	0.40–2.1	4	0.37–2.2	4	1.3–4.9	4	1.7–6.5	4	1.9–6.3
BCAA	0	N.D.	4	<0.10–0.41	4	0.19–0.50	4	0.21–0.57	4	0.60–2.0	4	0.65–2.2	4	0.61–2.5
DBAA	0	N.D.	3	<0.20–0.48	3	<0.20–0.51	3	<0.20–0.50	4	0.26–1.1	4	0.29–1.5	4	0.33–1.7
BDCAA	0	N.D.	3	<0.30–1.1	3	<0.30–1.1	3	<0.30–1.3	4	<0.30–2.4	4	<0.30–2.8	4	<0.30–3.7

Table 2. Continuation

Winter (February 2014)														
Aldehydes														
C1	4	0.10–0.40	4	0.44–1.0	4	0.62–1.5	4	0.25–0.74	4	0.65–1.5	4	0.73–1.4	4	0.69–1.3
C2	4	0.15–0.39	4	0.27–0.49	4	0.35–0.71	4	0.24–0.50	4	0.45–0.89	4	0.48–0.90	4	0.46–0.88
C3	4	0.03–0.06	4	0.06–0.18	4	0.12–07	4	0.04–0.13	4	0.11–0.26	4	0.11–0.27	4	0.10–0.24
C4	4	0.03–0.07	4	0.05–0.09	4	0.07–0.17	4	0.03–0.10	4	0.05–0.14	4	0.07–0.16	4	0.07–0.15
C5	3	<0.03–0.05	3	0.04–0.08	4	0.05–0.10	4	0.04–0.06	4	0.05–0.11	4	0.04–0.09	4	0.04–0.10
G	0	N.D.	4	0.14–0.21	4	0.17–0.30	4	0.23–0.48	4	0.35–0.54	4	0.43–0.78	4	0.61–1.0
MG	0	N.D.	4	0.04–0.06	4	0.05–0.09	4	0.06–0.14	4	0.07–0.18	4	0.08–0.22	4	0.13–0.25
BA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.
Volatile DBPs														
TCM	0	N.D.	4	<0.03–0.22	4	0.33–0.55	4	0.55–1.0	4	2.1–3.2	4	2.8–4.2	4	3.5–4.6
BDCM	0	N.D.	0	N.D.	0	N.D.	4	0.15–0.36	4	0.87–1.3	4	1.0–1.5	4	1.2–1.8
DBCM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	0.10–0.15	4	0.12–0.23	4	0.18–0.32
DCIM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.07	4	<0.07–0.10	4	0.09–0.13
TCNM	0	N.D.	0	N.D.	0	N.D.	4	<0.10	4	0.47–1.1	4	0.53–1.1	4	0.76–1.3
DCAN	0	N.D.	0	N.D.	4	<0.10–0.19	4	0.12–0.31	4	0.44–0.85	4	0.59–0.88	4	0.82–1.1
BCAN	0	N.D.	0	N.D.	0	N.D.	0	N.D.	4	<0.15	4	<0.15	4	<0.15
HAAs														
MCAA	0	N.D.	0	N.D.	2	<0.40	2	<0.40–0.44	3	<0.40–0.66	3	<0.40–0.77	3	<0.40–0.78
MBAA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.
DCAA	0	N.D.	4	0.19–2.2	4	0.23–2.4	4	0.30–2.6	4	2.1–4.7	4	2.4–5.2	4	2.5–5.5
MIAA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	2	<0.10	2	0.12–0.22	2	0.15–0.24
TCAA	4	<0.10–0.94	4	0.25–1.9	4	0.31–2.0	4	0.50–2.2	4	1.1–3.9	4	1.2–4.2	4	1.2–4.3
BCAA	0	N.D.	4	<0.10–0.38	4	<0.10–0.42	4	<0.10–0.50	4	0.29–0.85	4	0.33–1.1	4	0.37–1.8
DBAA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	0	N.D.	2	<0.20	2	<0.20
BDCAA	0	N.D.	0	N.D.	1	<0.30	1	<0.30	4	<0.30–0.54	4	<0.30–0.55	4	<0.30–0.55

[a] Number of positive samples from a total of 4 each season, in triplicate.

[b] Minimum and maximum concentrations found.

[c] N.D., not detected.

[d] <LOQ.

C1 (Formaldehyde), C2 (Acetaldehyde), C3 (Propionaldehyde), C4 (Butyraldehyde), C5 (Valeraldehyde), G (Glyoxal), MG (Methylglyoxal), BA (Benzaldehyde), TCM (Trichloromethane), BDCM (Bromodichloromethane), DBCM (Dibromochloromethane), DCIM (Dichloroiodomethane), TCNM (Trichloronitromethane), DCAN (Dichloroacetonitrile), BCAN (Bromochloroacetonitrile), MCAA (Monochloroacetic acid), MBAA (Monobromoacetic acid), DCAA (Dichloroacetic acid), MIAA (Monoiodoacetic acid), TCAA (Trichloroacetic acid), BCAA (Bromochloroacetic acid), DBAA (Dibromoacetic acid), BDCAA (Bromodichloroacetic acid).

4. Conclusions

Changes in 46 DBPs from the raw water to the distribution network in a full-scale drinking water treatment plant (DWTP) using chlorine dioxide and chloramines as disinfectants was for the first time studied here. The targeted species included regulated (THMs and HAAs) and non-regulated DBPS (aldehydes, halonitromethanes and haloacetonitriles) that were determined by using accurate, sensitive methods previously developed by our group which afford quantitation at the trace level. Such a high sensitivity additionally facilitated detection of new species such as benzaldehyde and dichloroiodomethane at concentrations of 0.1–0.4 μg/L.

The results of a comprehensive sampling programme involving collection of water samples in the four seasons revealed the following: (i) The raw water was of a high quality as a result of no direct input of wastewater or recreational use being allowed. In fact, only 5 aliphatic aldehydes and 2 HAAs were detected, at concentrations below than 1 μg/L, and mainly as natural components of the result of pesticide degradation; (ii) Chlorine dioxide raised the levels of the 7 DBPs found in the raw water 2–6 times and caused the formation of 3 new aldehydes, 5 HAAs and TCM, mainly in the warmer seasons (spring and summer); (iii) The sedimentation step increased the concentrations of all species and led to the formation of 3 new volatile DBPs. However, passage through rapid sand filter shad the opposite effects; thus, it decreased the concentrations of 5 aliphatic aldehydes and 7 HAAs by 15–50%, especially in the warmer seasons, but increased those of 7 volatile DBPs by up to 70%, (iv) Chloramination further increased the concentrations of the 8 aldehydes and 7 HAAs by 100% and those of the 7 volatile DBPs up to 3.5 times, virtually in all seasons. Interestingly, monoiodoacetic acid, dibromochloromethane, dichloroiodomethane and bromochloroacetonitrile were detected in the water after this step. Therefore, chloramines are more influential on the presence of DBPs and changes in their concentrations and speciation than is chlorine dioxide, and (v) The 23 DBPs detected exhibited spatial changes along the network that were dependent on the residence time of the water. Thus, all species present at the DWTP exit except 5 aliphatic aldehydes were about 50% more concentrated than in the starting water —and THMs up to 350% more—, especially in the warmer seasons.

Acknowledgements

This work was funded in the frameworks of Projects CTQ2013–42701 (Spain's Ministry of Education) and P09–FQM–4732DGI (Andalusian Regional Government). Additional funding was also received from FEDER. Isabel Montesinos is also grateful for the award of a pre-doctoral grant for the conduct of Project P09–FQM–4732DGI and the authors would like to thank the staff at the municipal water treatment plant of Córdoba for their cooperation.

Appendix A. Supplementary material

Supplementary material pertaining to this paper can be found online.

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Supplementary Material

"Year-long evaluation of the presence of 46 disinfection by-products throughout a drinking water treatment plant"

I. Montesinos, M. Serrano, M.J. Cardador, M. Silva and M. Gallego*

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E–14071–Córdoba, Spain

The Supplementary Material includes analytical methods, limits of detection, linearity, precision and mass values used for detection of the 46 DBP (**Table S1**); DBPs concentrations (μg/L) from the DWTP samples collected in consecutive days in April 2013 (**Table S2**).

Analytical Methods

Aldehydes

Ten millilitres of water samples or aldehyde standards prepared in LC–MS Ultra-grade water containing between 0.01 and 200 µg/L of each aldehyde, 50 µL of 150 g/L of PFBHA aqueous solution and 20 µg/L of 1,2-dibromopropane (IS) were added to a 20 mL glass vial containing 5 g of anhydrous sodium hydrogen carbonate (saturated solution) for adjusting the pH (8.4) and the ionic strength. Then, a volume of 200 µL of *n*-hexane was added and the vial was immediately sealed and vortexed for 30 s for homogenisation purposes. The operating conditions for the HS autosampler were as follows: vial equilibration time, 20 min; oven temperature, 80 °C; vial pressurisation time, 30 s; loop fill time, 9 s; valve/loop temperature, 100 °C. The injector temperature was set at 200 °C and the source and quadrupole temperatures were maintained at 250 °C and 150 °C. The chromatographic temperature conditions were as follows: 40 °C, held 4 min; ramped to 200 °C at 5 °C/min and finally, increased to 250 °C at 20 °C/min and held for 1 min.

Volatile DBPs

Twelve millilitres of water samples or aqueous standard solutions at pH ~3.5 (adjusted with 20 µL of 0.1 M H_2SO_4) containing between 0.03 and 200 µg/L (THMs) and 0.10 and 100 µg/L (N-DBPs) of each compound and 5 µg/L of IS were added to a 20 mL glass vial with 6 g of Na_2SO_4 (saturated solution). Then, 250 µL of MTBE (as modifier) was added and the vial was immediately sealed and vortexed for 1 min for sample homogenisation. Finally, the vial was placed into the HS oven maintained at the same conditions as mentioned above for aldehyde determinations. The injector temperature was set at 170 °C and the source and quadrupole temperatures were maintained at 200 °C and 150 °C, respectively. The chromatographic temperature conditions were as follows: 40 °C for 5 min and then programmed to 80 °C at 5 °C/min, held for 2 min, and ramped at 40 °C/min to 180 °C, finally held for 3 min.

Haloacetic acids

Ten millilitres of water samples or standard solutions containing between 0.1 and 200 µg/L of each HAA, 5 µg/L of IS were placed in a 20 mL glass vial containing 5 g (3.5 M) of Na_2SO_4. Then, 125 µL of a 0.05 M concentration of an ion-pairing agent (TBA-HSO_4, 2.3 µmol as aqueous solution), 100 µL of derivatisation reagent (DMS, 1.1 mmol, i.e. high excess), and 150 µL of *n*-pentane were added sequentially. The vial was immediately sealed and vortexed for 3 min in order to carry out the liquid-liquid microextraction/methylation process. The operating conditions for the HS autosampler were as mentioned above excepting the oven temperature, 60 °C. The injector, source and quadrupole temperatures were maintained at 250, 230 and 150 °C, respectively. The chromatographic temperature conditions were as follows: 60 °C for 5 min and then raised at 5 °C/min to 95 °C, where was held for 3 min, ramped at 10 °C/min to 140 °C, and then up to 250 °C at 25 °C/min where was finally held for 3 min.

All sample analyses were carried out on an HP (Agilent Technologies, Palo Alto, CA) 6890 gas chromatograph-5973N mass selective detector equipped with a 7694 headspace autosampler. Helium was used both to pressurise the vial and to transfer the loop content to the injection port of the gas chromatograph, which was equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Sample injection was done in split mode (1:20 split ratio) for 1 min. Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 1 mL/min. Mass spectra (electron impact ionisation, 70 eV) and the ions selected for identification and quantification of DBPs (SIM mode) are listed in **Table S1**.

Table S1. Analytical characteristics of the three methods and *m/z* values used for MS detection

DBPs	**Linear range (µg/L)**	**LOD[a] (ng/L)**	**RSD[b] (%)**	***m/z*[c]**
Aldehydes				
Formaldehyde (C1)	0.01–50	2	5.2	**181**, 195, 225
Acetaldehyde (C2)	0.01–50	3	5.3	**181**, 209, 239
Propionaldehyde (C3)	0.02–50	6	5.7	**181**, 223,236
Butyraldehyde (C4)	0.02–50	7	5.8	**181**, 226, 239
Valeraldehyde (C5)	0.03–50	10	6.3	**181**, 207, 239
Glyoxal (G)	0.04–50	12	6.2	**181**, 418, 448
Methylglyoxal (MG)	0.03–50	8	5.9	**181**, 432, 462
Benzaldehyde (BA)	0.10–200	30	6.9	**181**, 271, 301
3-Methylbenzaldehyde (3-MBA)	0.20–200	60	7.3	91, **181**, 315
2-Ethylbenzaldehyde (2-EBA)	0.30–200	80	7.9	**181**, 132, 329
2,5-Dimethylbezaldehyde (2,5-DMBA)	0.30–200	80	8.0	77, **181**, 329
Trihalomethanes				
Trichloromethane (TCM)	0.03–50	10	4.7	47, **83**, 85
Bromodichloromethane (BDCM)	0.03–50	10	4.9	**83**, 85, 129
Dibromochloromethane (DBCM)	0.05–50	15	5.1	91, 127, **129**
Dichloroiodomethane (DCIM)	0.07–50	20	5.2	**83**, 85, 175
Tribromomethane (TBM)	0.07–50	20	4.9	171, **173**, 252
Bromochloroiodomethane (BCIM)	0.10–200	35	5.0	**127**, 129, 131
Dibromoiodomethane (DBIM)	0.15–200	50	5.2	**173**, 175, 127
Chlorodiiodomethane (CDIM)	0.15–200	50	5.0	127, **175**, 177
Bromodiiodomethane (BDIM)	0.30–200	100	5.2	127, **219**, 221
Triiodomethane (TIM)	0.30–200	100	5.3	127, 140, **267**
Halonitromethanes				
Chloronitromethane (CNM)	0.40–200	130	6.1	46, **49**, 51
Dichloronitromethane (DCNM)	0.10–200	40	5.4	46, **83**, 85
Trichloronitromethane (TCNM)	0.10–200	30	5.2	46, **117**, 119
Bromonitromethane (BNM)	0.45–200	140	6.3	46, **93**, 95
Bromochloronitromethane (BCNM)	0.20–200	60	5.4	46, 127, **129**
Dibromonitromethane (DBNM)	0.25–200	80	5.5	46, 171, **173**
Haloacetonitriles				
Chloroacetonitrile (CAN)	0.50–200	150	6.5	40, 48, **75**
Dichloroacetonitrile (DCAN)	0.10–200	30	5.3	**74**, 82, 84
Trichloroacetonitrile (TCAN)	0.10–200	30	5.5	73, **108**, 110
Bromoacetonitrile (BAN)	0.65–200	200	6.3	40, **119**, 121
Bromochloroacetonitrile (BCAN)	0.15–200	50	5.6	**74**, 76, 155
Dibromoacetonitrile (DBAN)	0.15–200	50	5.8	**118**, 120, 199

Table S1. Continuation

Haloacetic acids				
Monochloroacetic acid (MCAA)	0.40–200	130	9.5	**59,** 79, 108
Monobromoacetic acid (MBAA)	0.40–200	110	9.7	**59**, 93, 95
Dichloroacetic acid (DCAA)	0.10–200	20	6.3	**59**, 83, 85
Trichloroacetic acid (TCAA)	0.10–200	30	6.2	59, **117**, 119
Bromochloroacetic acid (BCAA)	0.10–200	20	5.8	59, 127, **129**
Dibromoacetic acid (DBAA)	0.20–200	50	6.8	59, 171, **173**
Bromodichloroacetic acid (BDCAA)	0.30–200	100	6.4	**59**, 161, 163
Dibromochloroacetic acid (DBCAA)	0.40–200	120	6.0	59, **207**, 209
Tribromoacetic acid (TBAA)	0.80–200	300	10.4	**59**, 251, 253
Monoiodoacetic acid (MIAA)	0.10–200	40	6.1	59, 141, **200**
Chloroiodoacetic acid (CIAA)	0.20–200	60	7.2	**107**, 127, 236
Bromoiodoacetic acid (BIAA)	0.60–200	160	6.8	127, **151**, 278
Diiodoacetic acid (DIAA)	0.30–200	80	7.1	127, **199**, 326

[a]Limits of detection

[b]Relative standard deviation. Values obtained for samples fortified with 5 µg/L of each DBPs.

[c]m/z values (base peaks for quantification are boldfaced); m/z for IS (1,2-dibromopropane): 42, **121**, 123.

Table S2. DBP concentrations (μg/L) from the DWTP samples collected in consecutive days (April 2013)

Compounds	**Sampling point**													
	Raw (1)		**Pre-oxidation (2)**		**Sedimentation (3)**		**Filtration (4)**		**Chloramination (5)**		**Distribution system 1 (6)**		**Distribution system 2 (7)**	
	n[a]	Min–Max[b]	n	Min–Max	n	Min–Max	n	Min–Max	n	Min–Max	n	Min–Max	n	Min–Max
Aldehydes														
C1	7	1.5–1.8	7	3.1–3.5	7	4.4–5.0	7	2.4–2.7	7	5.0–6.1	7	5.1–5.9	7	5.1–5.8
C2	7	080–1.4	7	1.8–2.9	7	2.1–3.3	7	1.0–2.1	7	3.2–4.1	7	3.4–4.5	7	3.5–4.3
C3	7	0.40–0.89	7	0.64–0.85	7	0.64–0.92	7	0.27–0.38	7	0.55–0.72	7	0.50–0.71	7	0.53–0.71
C4	7	0.09–0.18	7	0.26–0.35	7	0.43–0.54	7	0.12–0.20	7	0.26–0.40	7	0.27–0.42	7	0.29–0.43
C5	5	0.06–0.10	5	0.10–0.17	7	0.16–0.26	7	0.14–0.16	7	0.17–0.28	7	0.18–0.28	7	0.19–0.29
G	0	N.D.[c]	7	0.90–1.1	7	2.0–3.5	7	2.4–4.0	7	3.9–6.7	7	4.5–6.5	7	5.1–7.9
MG	0	N.D.	7	0.62–1.2	7	0.78–1.6	7	1.8–2.6	7	2.5–4.1	7	3.3–4.7	7	5.3–6.1
BA	0	N.D.	7	0.20–0.32	7	0.52–0.64	7	0.60–0.77	7	0.63–0.92	7	0.72–0.97	7	0.70–0.78
Volatile DBPs														
TCM	0	N.D.	7	0.29–0.50	7	1.3–1.7	7	1.8–2.0	7	6.5–7.3	7	7.5–8.3	7	9.2–10
BDCM	0	N.D.	0	N.D.	7	0.37–0.55	7	0.64–0.72	7	1.6–2.1	7	1.8–2.5	7	2.2–3.0
DBCM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	7	0.16–0.20	7	0.23–0.46	7	0.25–0.50
DCIM	0	N.D.	0	N.D.	0	N.D.	0	N.D.	7	<0.07[d]–0.14	7	0.09–0.13	7	0.09–0.14
TCNM	0	N.D.	0	N.D.	7	0.11–0.27	7	0.21–0.45	7	0.52–0.83	7	0.62–0.95	7	0.96–1.3
DCAN	0	N.D.	0	N.D.	7	0.14–0.20	7	0.18–0.26	7	0.50–0.72	7	0.66–0.97	7	0.82–1.2
BCAN	0	N.D.	0	N.D.	0	N.D.	0	N.D.	7	<0.15[d]	7	0.16–0.20	7	0.18–0.28
HAAs														
MCAA	1	<0.40	5	<0.40–0.55	5	<0.40–0.59	5	<0.40–0.61	6	0.88–1.2	7	0.90–1.3	7	0.92–1.3
MBAA	0	N.D.	2	<0.40–0.52	6	<0.40–0.55	6	<0.40–0.56	5	<0.40–0.91	5	<0.40–1.0	4	<0.40–1.1
DCAA	4	<0.10–0.31	7	2.3–3.5	7	2.4–3.9	7	1.4–3.0	7	3.1–6.3	7	4.9–7.8	7	5.1–7.8
MIAA	0	N.D.	0	N.D.	0	N.D.	0	N.D.	7	0.31–0.42	7	0.31–0.45	7	0.32–0.46
TCAA	5	<0.10–0.24	7	1.2–2.1	7	1.2–2.1	7	1.4–2.3	7	2.7–4.2	7	2.9–6.9	7	3.5–5.6
BCAA	0	N.D.	5	0.40–0.92	6	0.41–0.97	6	0.47–1.0	6	0.98–2.2	7	1.3–2.6	7	1.3–3.1
DBAA	0	N.D.	4	<0.20–0.25	5	<0.2–0.56	4	<0.2–0.69	5	0.22–1.2	5	0.23–1.3	6	0.29–1.2
BDCAA	0	N.D.	5	0.50–0.92	5	0.51–0.94	6	0.57–0.79	7	1.1–1.7	6	1.3–2.6	6	1.4–3.8

[a] Number of positive samples from a total of 7, in triplicate.

[b] Minimum and maximum concentrations found.

[c] N.D., not detected.

[d] <LOQ.

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How the inclusion of treated water in beverages influences the appearance of halogenated volatile organic compounds

Isabel Montesinos, Mercedes Gallego*

Department of Analytical Chemistry, Campus of Rabanales, University of Córdoba, E-14071, Córdoba, Spain

Journal of agricultural and food chemistry 62 (2014) 10240

Abstract

A simple, robust and reliable headspace gas chromatography method has been developed for the determination of 14 halogenated volatile organic compounds, including iodinated trihalomethanes (THMs), at ng/L levels in beverages. The main source of the presence of THMs in reconstituted fruit juices, nectars and soft drinks is the treated water included as an ingredient; the concentration and speciation depend on the volume and disinfection process of the treated water either from the distribution network or from water directly disinfected by the food factory. Chloroform appears at concentrations below 1 μg/L in natural juices and soft drinks prepared with mineral water due to contamination from the chlorinated sanitizers usually employed in the food industry. However, the beverages manufactured with treated water contain, in addition to chloroform, brominated THMs and dichloroiodomethane (detected in beverages for the first time) which can be used as indicators of the presence of treated water.

1. Introduction

The water used as drinking water or in a wide variety of industrial applications is frequently disinfected before use [1]. The employment of treated water in the food industry produces chemical contaminants in food and beverages, such as disinfection by-products (DBPs) and volatile organic compounds (VOCs) [2–4]. DBPs are formed during the disinfection of water, whereas VOCs can be present in untreated groundwater as a result of industrial pollution. These compounds are considered to be potentially carcinogenic and mutagenic, representing a real health risk for humans [5–7]. Because of that, Aggazzotti et al. have evaluated the exposure of pregnant women to DBPs through different media, one of them being bottled water-based beverages (i.e., juices, sodas, etc.) [8]. The most common DBPs in drinking water are four trihalomethanes (THMs), which are regulated by the U.S. Environmental Protection Agency (EPA) [9] or European Union (EU) [10] at 80 or 100 µg/L, respectively. With regard to VOCs, dichloromethane, 1,2-dichloroethane, and carbon tetrachloride are also regulated at 5 µg/L [9]. Furthermore, the World Health Organization has published a provisional guideline value of 0.4 µg/L for 1,2-dibromoethane [11]. To comply with current regulations, a large number of water utilities in the United States have changed chlorination for chloramination because the latter forms lower amounts of regulated THMs. Unfortunately, NH_2Cl forms its own suite of DBPs, including iodinated THMs [12–14]. These species have enhanced mammalian cell cytotoxicity and genotoxicity as compared to their brominated and chlorinated analogues [6], but no similar regulation for these compounds has been established to date. Thus, iodo-organic compounds should be considered when drinking-water exposure is evaluated.

The most significant pathway of exposure to DBPs and VOCs is the ingestion of drinking water. This can occur as direct ingestion or as a result of its inclusion in beverages and food. Consumption surveys indicate that approximately two-thirds of drinking water is ingested through other sources, for example, juices, soft drinks, coffee, soups, and infusions [15]. The types and levels of DBPs in beverages will depend on the disinfection process used to produce the treated water and the chemical constituents of the source water. In addition, VOCs can appear in food from other sources

such as the water used for production, wrapping materials, polluted air, and retained solvents used for the extraction of natural components [3]. Thus, international organizations FAO/WHO recommend the development of methods to determine DBPs in beverages and foods [16]. Despite toxic effects and potential human exposure to DBPs through food, information is scarce concerning their levels and they are mainly referred to as chloroform. The formation of THMs has been investigated using static headspace–gas chromatography (SHS–GC) during the preparation of 17 beverages (teas, coffees, concentrated juices, and chocolates) and 11 solid foods (vegetables, baby foods, starchy foods, and soups) using chlorinated drinking water [17]. However, these experiments are unrealistic because they use ultrapure solutions of water with high concentrations of chlorine instead of drinking water. By way of example, tea formed the highest chloroform levels (up to 67 μg/L), followed by coffee, rice, soups, vegetables, and baby food. Different methodologies have been used for the determination of THMs and VOCs in beverages and foods. Generally, EPA methods based on liquid-liquid extraction and GC have been used for this approach [18,19], although this technique is time-consuming and environmentally unfriendly. Headspace combined with GC has also been applied for the determination of THMs and VOCs in beverages and foods [4], but the method is laborious and requires drastic conditions (1 h in a 90 ºC water bath). Other HS–solid phase microextraction (SPME) [20] or purge-and-trap [21] GC methods require the dilution or centrifugation of beverages, which produces a loss of the species through volatilization. More recently, an HS–SPME method has been applied to determine four THMs in soft drinks [22] and beer [23]. In general, these methods require manual sample manipulation with low sensitivity because they are adapted from water methodologies.

The aims of this work were (i) to develop a solventless SHS–GC–mass spectrometric method with enough sensitivity to determine target analytes at nanogram per liter levels in beverages; (ii) to include six emerging iodinated THMs for the first time, in addition to the four common THMs and four VOCs regulated in treated water; and (iii) to discriminate the source of these species through treated water employed for beverage preparation or by contamination through the industrial process (i.e., washing, bottling line, etc.).

2. Material y methods

2.1. Chemicals and standards

The 14 species included in this study with their corresponding acronyms are indicated in **Table 1**. The majority of standards and the internal standard, 1,2-dibromopropane, were supplied from Sigma-Aldrich (Madrid, Spain). Iodinated THM (DCIM, BCIM, DBIM, CDIM, and BDIM) standards were supplied by Cansyn (Toronto, Canada). Methanol, sulphuric acid, and salts were supplied from Merck (Darmstadt, Germany). Stock standard solutions containing 1 g/L of each compound were prepared in methanol and stored in amber glass vials at −20 °C. Working-standard solutions were prepared on a daily basis by dilution at the μg/L level in mineral water (untreated water and free of DBPs).

2.2. Static headspace and gas chromatographic conditions

The experimental setup for the SHS–GC–MS determination of VOCs and THMs consisted of an SHS autosampler G1888 and an HP 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP 5973N mass selective detector. The operating conditions for the HS autosampler were as follows: vial equilibration time, 15 min; oven temperature, 80 °C; vial pressurization time, 30 s; loop fill time, 3 s; valve/loop temperature, 100 °C. Helium (6.0 grade purity, Air Liquid, Seville, Spain) was used both to pressurize vials and to drive the headspace formed to the injection port of the chromatograph via a transfer line at 110 °C. Injection was done in the split mode (split ratio 1:20) for 1 min with an inlet temperature of 250 °C; flow rate of carrier gas (He) was fixed at 1 mL/min. The gas chromatographic separation was achieved on an HP-5MS UI fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness) coated with a stationary phase consisting of 5% phenyl−95% methylpolysiloxane supplied by Agilent. Oven temperature was programmed as follows: 40 °C for 5 min, raised to 100 °C at 25 °C/min, held for 2 min, ramped at 30 °C/min to 250 °C, and finally held for 3 min. The MS was operated in the electron impact ionization mode at 70 eV, and ion source and quadrupole temperatures were set at 250 and 150 °C, respectively. Optimization experiments were conducted in total ion chromatography mode between m/z 25 and 400 at 3.5 scans/s. The ions selected for

identification and quantification of VOCs and THMs (SIM mode) are listed in **Table 1**; *m/z* values for 1,2-dibromopropane (IS) were 42, 121 (base peak), 123.

Table 1. Analytical characteristics for the HSH–GC–MS determination of halogenated VOCs and mass values used for MS detection

compound	LOD (ng/L)	linear range (μg/L)	precision RSD (%)[a]		*m/z*[b]
			intra-day	inter-day	
Dichloromethane (DCM)	50	0.17–100	5.1	5.9	**49**, 84, 86
1,2-Dichloroethane (1,2-DCE)	50	0.17–100	5.2	6.2	49, **62**, 64
Carbon tetrachloride (CTC)	8	0.03–50	4.6	5.4	82, **117**, 119
1,2-Dibromoethane (1,2-DBE)	35	0.12–100	4.8	5.6	27, **107**, 109
Trichloromethane (TCM)	10	0.03–50	4.7	5.5	47, **83**, 85
Bromodichloromethane (BDCM)	10	0.03–50	4.9	5.7	**83**, 85, 129
Dibromochloromethane (DBCM)	15	0.05–50	5.1	6.1	91, 127, **129**
Dichloroiodomethane (DCIM)	20	0.07–50	5.2	6.0	**83**, 85, 175
Tribromomethane (TBM)	20	0.07–50	4.9	5.7	171, **173**, 252
Bromochloroiodomethane (BCIM)	35	0.12–100	5.0	6.1	**127**, 129, 131
Dibromoiodomethane (DBIM)	50	0.17–100	5.2	6.1	**173**, 175, 127
Chlorodiiodomethane (CDIM)	50	0.17–100	5.0	6.0	127, **175**, 177
Bromodiiodomethane (BDIM)	100	0.30–100	5.2	6.0	127, **219**, 221
Triiodomethane (TIM)	100	0.30–100	5.3	6.2	127, 140, **267**

[a] RSD, relative standard deviation (n = 11) for 5 μg/L.

[b] *m/z* values (base peaks for quantification are boldfaced).

2.3. Sample preparation

The commercial beverages (soft drinks and juices) used in this study were purchased at local markets in Spain (one of the largest producers of fruits and derivatives in Europe). Several brands with different packaging types and different flavours were selected. In the laboratory, samples were stored refrigerated or at room temperature, depending on the manufacturer's instructions, and the seal of each bottle was broken right before its analysis. Freshly squeezed orange juice (blank) was used to optimize the analytical parameters of the method. For the preparation of the blank, fresh oranges were hand-peeled and homogenized with a laboratory squeezer. Food utensils were always washed with mineral water to ensure the prevention of DBP contamination.

2.4. Analytical procedure

Beverages were mixed in their own container for 1 min by manual shaking. Ten millilitres of beverage or standard solution containing between 0.03 and 100 µg/L of each target analytes and 5 µg/L of 1,2-dibromopropane (IS) was placed in a 20 mL glass vial containing 4 g of NaCl. For carbonated soft drink analyses, 300 µL of a 6 mol/L NaOH solution was also added to eliminate the carbonic acid present. Then, the vial was immediately sealed and vortexed for 1 min for mixing purposes and placed in the autosampler carousel. Samples were analyzed by SHS–GC–MS, using the operating conditions mentioned above.

3. Results and discussion

3.1. Evaluation of chemical parameters

First, the SHS variables were studied using 10 mL of freshly squeezed orange juice containing 10 µg/L of each VOC and THM, 5 µg/L of the IS, and 3 g of NaCl in a 20 mL glass vial. The most relevant parameters were oven temperature (60–80 °C) and vial equilibrationtime (5–20 min), providing the best results at 80 °C and 15 min, respectively. Other instrumental parameters did not present significant changes in the abundance signals for the compounds, and a pressurization time of 30 s and a venting time of 12 s were selected as the working values.

Salting-out increases the ionic strength of the aqueous solution; this can decrease the solubility of organic species, improving their distribution from the aqueous solution to the headspace. Hence, the addition of NaCl was studied between 0 and 5 g. The analytical signal of the compounds increased with increases in the amounts of salt up to 4 g; therefore, this amount was selected per 10 mL of sample. The effect of the sample pH on the extraction of the 14 species was studied in the 2.5–9.0 range by adjusting the fortified juice with diluted H_2SO_4 or NaOH as required. As the peak area ratio of all species remained constant throughout this range, beverage samples were analyzed without pH adjustment. Fruit juices did not present any problem for their analyses by SHS, but carbonated soft drinks can have certain problems due to the presence of CO_2. When a soft drink bottle is opened, the pressure is reduced to atmospheric pressure, causing decomposition of the carbonic acid, releasing CO_2. This loss of CO_2 originates a sweep of the most volatile compounds into the atmosphere. Moreover, the CO_2 could be competing with the analytes for the headspace in the vial. To solve this problem, different procedures have been proposed, such as nitrogen bubbling, agitation, or ultrasonication [24]. However, these decarbonation methods can remove the volatile compounds from the matrix, so the best alternative can be the addition of NaOH to eliminate carbonic acid, which does not involve the volatilization of the analytes. With the wide pH range of the proposed method (2.5–9.0) taken into account, the addition of NaOH up to pH 8–9 is possible. For this purpose, the recovery of the 14 species was studied by adding various volumes (0–350 µL) of a 6 mol/L NaOH solution to 10 mL of a representative carbonated soda. As can be seen in **Figure 1** (for nine representative analytes), the relative peak areas were extremely low for the most volatile compounds (DCM, 1,2-DCE, CTC, and TCM) without NaOH, whereas the addition of NaOH solution to the carbonated sample reduced the loss of these compounds. The recovery (versus an aqueous standard solution) for all analytes improved as the volume of NaOH increased to 300 µL (pH ~8.4). This improvement was more noticeable for the most volatile analytes, which underwent an increase of ~90% as compared to the aqueous standard. For 1,2-DBE, BDCM, DBCM, DCIM, and TBM, recoveries increased ~40–50%, whereas for the majority of iodinated THMs (BCIM, CDIM, DBIM, BDIM, and TIM), this increase was only ~10–20%. The final volume selected was 300 µL of a 6

mol/L NaOH solution added to 10 mL of carbonated soft drink because it provided a sample pH of ~8.4 (the prevalent species is HCO_3^-; pK_a= 6.1).

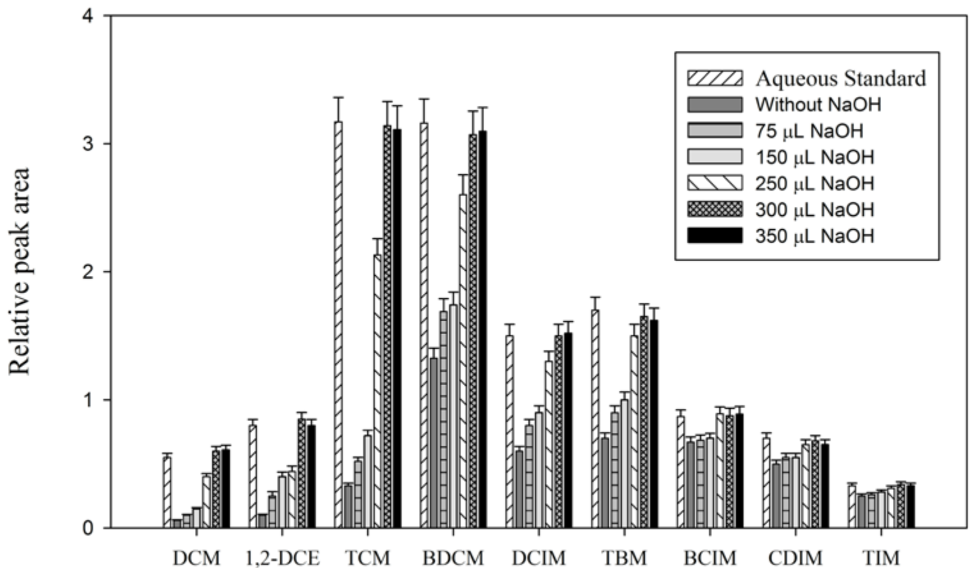

Figure 1. Influence of the addition of variable volumes of a 6 mol/L NaOH solution to 10 mL of fortified natural orange juice on the recoveries of nine representative analytes versus an aqueous standard. Concentration: 10 µg/L of each compound. Error bars are the standard deviation for five measurements.

Finally, the efficiency percentage of the whole process for the 14 compounds in a fresh orange juice (blank) was calculated in quintuplicate by a second SHS extraction of the remaining liquid phase to check the absence of any compound. For this purpose, 10 mL of the blank was fortified with 10 µg/L of each compound and salt according to the procedure. The average yield of the whole analytical process was ≥95% for all analytes, showing that the method is adequate for the determination of these species.

3.2. Sensitivity and validation of the method

Linear range, analyte detectability, and precision of the proposed method were studied under optimal experimental conditions (see **Table 1**). Calibration curves were constructed by using fresh orange juice fortified with 0.03–100 µg/L of each compound and processed as described under Analytical procedure. The equations for the standard curves were obtained by plotting the analyte to internal standard peak area ratios against the amount of the analytes. Regression coefficients were >0.995 in all cases. Limits of detection (LODs) were determined as the analyte concentration that provides a chromatographic peak equal to 3 times the regression standard deviation, $S_{y/x}$, divided by the slope of each calibration graph. The lower limit of the linear range corresponds to the limit of quantification, which is 3.3 × LOD [25]. The precision was calculated by measuring 11 fresh orange juices on the same day and different days. The results obtained were satisfactory, with average RSD values ranging from 5.0 ± 0.2 to 5.9 ± 0.3% for intra- and interdays, respectively, which indicates that the method is absolutely repeatable and reproducible.

In the bibliography, different solventless techniques have been employed for the determination of several VOCs and THMs in beverages and foods. All of these methodologies have been proposed only for the four common THMs and do not reach the sensitivity of the proposed method [4,17–23]. In addition, some of these methodologies involve the manipulation of the beverages by dilution [20]/centrifugation [21]/heating in water baths [4], etc., with the consequent loss of volatile compounds. More recently, the four THMs have been determined in soft drinks [21] and beers [22], but the method is ca. 10 times less sensitive than the present alternative.

To validate the proposed method, a recovery study was conducted by analyzing several representative soft drinks and fruit juices fortified with 5 and 20 µg/L of each compound (n = 5). The analytical responses (taking into account the genuine concentration of the analytes) from all samples were compared to similar standard additions to mineral water. Good recoveries (93–95% for apple, 94–96% for pineapple, 92–96% for orange, and 90–92% for peach juices; 94–96% for tonic; and 95–97% for soda) were obtained for all compounds.

3.3. Analysis of beverage samples

VOCs and THMs can occur in beverages through multiple pathways, namely, (i) inclusion of drinking water as an ingredient in the production of the beverages; (ii) accumulation and sorption from retained water in beverage packaging and wrapping that had been disinfected/washed with chlorinated sanitizers; (iii) contamination by contact with cleaners/disinfectants used in beverage processing equipment, rinses with water in beverage processing, storage, and/or marketing; and (iv) formation during beverage preparation due to reactions between residual chlorine and precursors present in food, for example, carbohydrates, lipids, and proteins. The samples selected for analysis, soft drinks and fruit juices, are products that contain water as an ingredient and that are frequently consumed every day.

The proposed SHS–GC–MS method was applied to determine 4 VOCs and 10 THMs in 100 types of samples: 40 soft drinks (teas, isotonics, fruit beverages, tonics and sodas) and 60 fruit juices (natural juices 100%, reconstituted juices and nectars). All samples gave positive results containing up to five THMs including DCIM, in different distributions and levels; none of the four VOCs studied were present in these beverages. **Tables 2 and 3** list the concentrations of the THMs found at detectable concentrations in soft drinks (ca. 90% water) and fruit juices (up to 70% water), respectively. The analytes not shown were either not contained or present at levels below their LODs. The species TCM, BDCM, and DBCM were present in practically all samples and at higher concentrations, whereas TBM and DCIM were found in lower proportions. This could be explained because the water used in food applications is frequently disinfected with chlorine due to its economic impact and simple use.

Table 2 lists the levels of each THM found in soft drinks as well as the total concentration of trihalomethanes (TTHMs).

Table 2. Concentrations[a] of THMs (μg/L) found in soft drinks

Sample	Flavour	TCM	BDCM	DBCM	DCIM	TBM	TTHMs[b]
Tea 1	Lemon	1.1 ± 0.1	0.51 ± 0.03	0.23 ± 0.01	ND[c]	<0.07[d]	1.8 ± 0.1
Tea 2	Peach	27 ± 2	3.1 ± 0.2	0.24 ± 0.01	ND	ND	30 ± 2
Tea 3	Passion Fruit	14 ± 1	3.5 ± 0.2	0.73 ± 0.04	ND	<0.07	18 ± 1
Isotonic 1	Lemon	23 ± 1	4.6 ± 0.3	0.76 ± 0.04	ND	ND	28 ± 2
Isotonic 2	Lemon	1.8 ± 0.1	0.18 ± 0.01	0.08 ± 0.01	ND	<0.07	2.1 ± 0.1
Isotonic 3	-	27 ± 2	3.4 ± 0.2	0.27 ± 0.02	ND	<0.07	31 ± 2
Isotonic 4	-	31 ± 2	3.4 ± 0.2	0.26 ± 0.02	<0.07	<0.07	35 ± 2
Fruit beverage 1	Lemon	7.1 ± 0.4	0.70 ± 0.04	0.11 ± 0.01	ND	ND	7.9 ± 0.5
Fruit beverage 2	Apple	21 ± 1	1.3 ± 0.1	0.10 ± 0.01	ND	ND	22 ± 1
Fruit beverage 3	Lemon	5.1 ± 0.3	0.44 ± 0.03	<0.05	ND	ND	5.5 ± 0.3
Fruit beverage 4	Orange	6.8 ± 0.4	1.1 ± 0.1	0.11 ± 0.01	ND	<0.07	8.0 ± 0.5
Fruit beverage 5	Orange	14 ± 1	1.2 ± 0.1	0.13 ± 0.01	ND	ND	15 ± 1
Fruit beverage 6	Pineapple	23 ± 1	1.4 ± 0.1	0.20 ± 0.01	ND	ND	25 ± 1
Tonic 1	-	20 ± 1	1.2 ± 0.1	0.18 ± 0.01	ND	ND	21 ± 1
Tonic 2	-	3.8 ± 0.2	0.39 ± 0.02	<0.05	ND	ND	4.2 ± 0.2
Tonic 3	-	16 ± 1	2.1 ± 0.1	0.32 ± 0.02	ND	ND	18 ± 1
Soda 1	-	35 ± 2	2.0 ± 0.1	0.25 ± 0.01	ND	ND	37 ± 2
Soda 2	Lemon	20 ± 1	4.5 ± 0.3	0.82 ± 0.05	0.11 ± 0.01	<0.07	25 ± 1
Soda 3	Lemon	7.6 ± 0.4	0.62 ± 0.04	0.07 ± 0.01	ND	ND	8.3 ± 0.5
Soda 4	Lemon	9.6 ± 0.6	0.70 ± 0.04	<0.05	ND	ND	10 ± 1
Soda 5	Lemon	31 ± 2	1.5 ± 0.1	0.12 ± 0.01	ND	ND	33 ± 2
Soda 6	Lemon	28 ± 2	1.4 ± 0.1	0.19 ± 0.01	ND	ND	30 ± 2
Soda 7	Orange	1.0 ± 0.1	0.22 ± 0.01	0.13 ± 0.01	ND	<0.07	1.4 ± 0.1
Soda 8	Orange	22 ± 1	3.4 ± 0.2	0.55 ± 0.03	ND	<0.07	26 ± 2
Soda 9	Orange	3.9 ± 0.2	0.31 ± 0.02	<0.05	ND	<0.07	4.2 ± 0.2
Soda 10	Orange	31 ± 2	1.5 ± 0.1	0.12 ± 0.01	ND	ND	33 ± 2
Soda 11	Orange	24 ± 1	1.3 ± 0.1	0.10 ± 0.01	ND	ND	25 ± 1
Soda 12	Cola	6.5 ± 0.4	0.68 ± 0.04	0.08 ± 0.01	ND	ND	7.3 ± 0.4
Soda 13	Cola	5.4 ± 0.3	0.45 ± 0.03	0.06 ± 0.01	ND	ND	5.9 ± 0.3
Soda 14	Cola	5.7 ± 0.3	0.44 ± 0.03	<0.05	ND	ND	6.1 ± 0.4
Soda 15	Cola	27 ± 2	4.5 ± 0.3	0.80 ± 0.05	0.10 ± 0.01	<0.07	32 ± 2
Soda 16	Cola	7.4 ± 0.4	0.63 ± 0.04	0.07 ± 0.01	ND	<0.07	8.1 ± 0.5
Soda 17	Cola	28 ± 2	4.9 ± 0.3	0.92 ± 0.05	<0.07	<0.07	34 ± 2
Soda 18	Cola	43 ± 3	7.8 ± 0.5	1.3 ± 0.1	0.13 ± 0.01	0.17 ± 0.01	52 ± 3
Soda 19	Cola	16 ± 1	1.0 ± 0.1	0.08 ± 0.01	ND	ND	17 ± 1
Soda 20	Cola	28 ± 2	6.3 ± 0.4	1.3 ± 0.1	0.13 ± 0.01	<0.07	36 ± 2
Soda 21	Cola	1.6 ± 0.1	0.09 ± 0.01	0.06 ± 0.01	ND	ND	1.8 ± 0.1
Soda 22	Cola	31 ± 2	6.4 ± 0.4	1.2 ± 0.1	<0.07	<0.07	39 ± 2
Soda 23	Cola	14 ± 1	0.90 ± 0.05	0.08 ± 0.01	ND	<0.07	15 ± 1
Soda 24	Cola	34 ± 2	7.1 ± 0.4	0.52 ± 0.03	<0.07	<0.07	42 ± 2

[a]±Standard deviation, n=5. [b]Total THMs. [c]Not detected. [d]<LOQ.

There are two levels of TTHM concentrations: soft drinks A for concentrations <15 µg/L (n = 15; average value of 5.5 ± 2.8 µg/L) and soft drinks B containing ≥15 µg/L (n = 25; average value of 28.8 ± 9.1 µg/L). From **Table 2** it can be concluded that (i) there were no significant differences in either TTHM concentrations in each kind of soft drink (tea, tonic, soda, etc.) or in the species of THMs; (ii) there were no differences between flavors (lemon, orange, cola, etc.) in the same kind of soft drinks; and (iii) the presence of THMs in these beverages should be ascribed to the type of treated water employed in their preparation. One factor that influenced the TTHM concentrations was the factory that bottled the samples. Each factory has a different source of water, and the levels of THMs depend on the disinfection process. However, in this case, no differences were found between TTHM concentrations, presumably because the samples manufactured came from only four different factories, so there was not great variability. By way of example, tea 2 and soda 7 contain 30 and 1.4 µg/L, respectively, and both were manufactured in the same factory. Thus, the differences between the two groups of soft drinks (A and B) could be associated with the type of treated water used in their manufacturing at each moment. No sample exceeded the limits established for the concentration of TTHMs, so the soft drinks were prepared with water from the distribution network or treated water prepared in the same factory following EU normative [10]. This has been corroborated by analyzing five soft drinks elaborated with mineral water (free of DBPs), which was negative for the species studied or contained chloroform only at very low levels (<1 µg/L). **Figure 2** shows the chromatogram for soda 18, which shows the peaks corresponding to the five species of THMs detected. After a retention time of 9 min, some peaks appeared in the chromatogram that correspond to flavors or other volatile compounds present in the beverage; these compounds did not interfere with the target analytes, so there was no matrix interference.

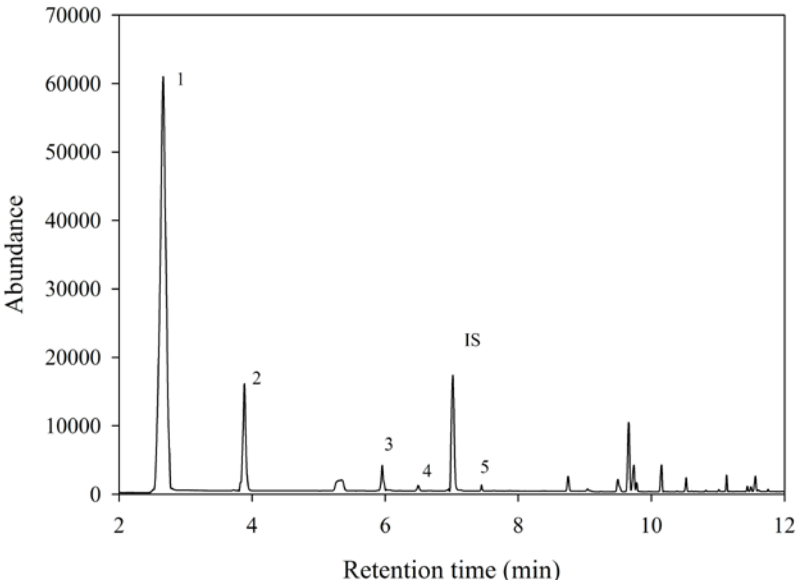

Figure 2. GC-MS chromatogram in SIM mode obtained in the analysis of soda 18. Peaks: (1) TCM; (2) BDCM; (3) DBCM; (4) DCIM; (5) TBM; (IS) internal standard. For compound abbreviations, see Table 1.

Table 3 shows the results obtained in the analysis of 60 fruit juices and nectars. In this case, only the four common THMs were found in these samples. DCIM was not detected because its level is associated with the amount of treated water in the beverage, and fruit juices contain a lower volume of water than soft drinks. The average TTHM concentrations in 100% natural juices were always <1 µg/L, and the predominant species was chloroform (TCM) followed by BDCM. As these juices do not contain treated water, the presence of these species at such a low level can be ascribed to contamination from the use of chlorinated sanitizers in the industry. In contrast, reconstituted juices can contain up to four THMs, and there are two distinctive groups as in soft drinks; reconstituted juices A with TTHM concentrations <1 µg/L and reconstituted juices B containing >2 µg/L. The average value for groups A and B were 0.5 ± 0.2 (n = 10) and 6.2 ± 3.6 (n = 11) µg/L, respectively. Moreover, the kind of fruit was not a relevant parameter because the same fruit was present in both groups. On the other hand, the average values of reconstituted juices A were similar to those of natural juice, but there was a significant difference because brominated species were present in reconstituted juices due only to the

addition of treated water. With regard to nectar (ca. 50–75% of water), the average TTHM concentration was higher than that obtained in reconstituted juices, depending on the higher water volume [26]. Hence, there were noticeable variations in concentrations of TTHMs for natural juices, 100%, reconstituted juices, nectars, and soft drinks with average values of 0.47, 3.5, 8.3, and 20 μg/L, respectively, according to the volume of treated water included.

Table 3. Concentrations[a] of THMs (μg/L) found in fruit juices

Sample	Fruit	Factory	TCM	BDCM	DBCM	TBM	TTHMs[b]
Natural juice 1	Apple	A	0.20 ± 0.01	ND[c]	ND	ND	0.20 ± 0.01
Natural juice 2	Pineapple	A	0.39 ± 0.02	0.22 ± 0.01	ND	ND	0.61 ± 0.03
Natural juice 3	Orange	A	0.27 ± 0.02	ND	ND	ND	0.27 ± 0.02
Natural juice 4	Orange	B	0.48 ± 0.03	0.11 ± 0.01	ND	ND	0.59 ± 0.03
Natural juice 5	Orange	C	0.60 ± 0.03	0.23 ± 0.01	ND	ND	0.83 ± 0.05
Natural juice 6	Orange	D	0.33 ± 0.02	ND	ND	ND	0.33 ± 0.02
Natural juice 7	Orange	E	0.52 ± 0.03	ND	ND	ND	0.52 ± 0.03
Reconstituted juice 1	Apple	F	0.36 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	<0.07[d]	0.48 ± 0.03
Reconstituted juice 2	Apple	G	0.92 ± 0.05	1.6 ± 0.1	1.4 ± 0.1	0.26 ± 0.02	4.2 ± 0.2
Reconstituted juice 3	Pineapple	H	3.6 ± 0.2	3.3 ± 0.2	2.9 ± 0.2	0.40 ± 0.02	10 ± 1
Reconstituted juice 4	Orange	D	ND	ND	0.07 ± 0.01	0.12 ± 0.01	0.19 ± 0.01
Reconstituted juice 5	Orange	H	3.0 ± 0.2	3.7 ± 0.2	3.3 ± 0.2	0.50 ± 0.03	11 ± 1
Reconstituted juice 6	Orange	I	0.45 ± 0.03	ND	0.08 ± 0.01	0.13 ± 0.01	0.66 ± 0.04
Reconstituted juice 7	Peach	H	1.8 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	0.22 ± 0.01	5.1 ± 0.3
Reconstituted juice 8	Peach	J	3.1 ± 0.2	0.27 ± 0.02	ND	ND	3.4 ± 0.2
Reconstituted juice 9	Grape	D	0.56 ± 0.03	0.18 ± 0.01	<0.05	ND	0.74 ± 0.04
Reconstituted juice 10	Cranberry	A	0.32 ± 0.02	0.25 ± 0.01	0.21 ± 0.01	ND	0.78 ± 0.04
Reconstituted juice 11	Mix	A	0.38 ± 0.02	0.05 ± 0.01	<0.05	ND	0.43 ± 0.03
Reconstituted juice 12	Mix	A	0.37 ± 0.02	ND	0.16 ± 0.01	0.29 ± 0.02	0.82 ± 0.05
Reconstituted juice 13	Mix	A	0.72 ± 0.04	3.7 ± 0.2	ND	ND	4.4 ± 0.3
Reconstituted juice 14	Mix	C	0.39 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	<0.07	0.52 ± 0.03
Reconstituted juice 15	Mix	C	0.27 ± 0.02	<0.03	<0.05	<0.07	0.27 ± 0.02
Reconstituted juice 16	Mix	D	10 ± 1	2.2 ± 0.1	0.62 ± 0.04	ND	13 ± 1
Reconstituted juice 17	Mix	D	0.23 ± 0.01	ND	<0.05	ND	0.23 ± 0.01
Reconstituted juice 18	Mix	D	2.2 ± 0.1	ND	ND	ND	2.2 ± 0.1
Reconstituted juice 19	Mix	H	2.0 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	0.10 ± 0.01	4.9 ± 0.3
Reconstituted juice 20	Mix	J	6.0 ± 0.3	0.92 ± 0.05	0.16 ± 0.01	ND	7.1 ± 0.4
Reconstituted juice 21	Mix	K	1.9 ± 0.1	0.30 ± 0.02	0.15 ± 0.01	0.11 ± 0.01	2.5 ± 0.1

Table 3. Continuation

Nectar 1	Apple	A	0.93 ± 0.05	0.30 ± 0.02	0.16 ± 0.01	0.10 ± 0.01	1.5 ± 0.1
Nectar 2	Apple	C	0.35 ± 0.02	0.24 ± 0.01	0.18 ± 0.01	0.13 ± 0.01	0.90 ± 0.05
Nectar 3	Apple	C	6.3 ± 0.4	2.1 ± 0.1	0.52 ± 0.03	0.18 ± 0.01	9.1 ± 0.5
Nectar 4	Apple	J	4.5 ± 0.3	1.1 ± 0.1	0.19 ± 0.01	0.10 ± 0.01	5.9 ± 0.3
Nectar 5	Pineapple	A	3.2 ± 0.2	2.6 ± 0.2	2.8 ± 0.2	0.51 ± 0.03	9.1 ± 0.5
Nectar 6	Pineapple	B	2.2 ± 0.1	0.21 ± 0.01	ND	ND	2.4 ± 0.1
Nectar 7	Pineapple	F	0.38 ± 0.02	ND	ND	ND	0.38 ± 0.02
Nectar 8	Pineapple	J	12 ± 1	1.6 ± 0.1	0.24 ± 0.01	ND	14 ± 1
Nectar 9	Pineapple	J	8.5 ± 0.5	1.0 ± 0.1	0.12 ± 0.01	ND	9.6 ± 0.6
Nectar 10	Pineapple	L	32 ± 2	5.3 ± 0.3	0.96 ± 0.06	ND	38 ± 2
Nectar 11	Pineapple	L	16 ± 1	3.3 ± 0.2	0.60 ± 0.03	ND	20 ± 1
Nectar 12	Orange	B	5.8 ± 0.3	0.86 ± 0.05	0.12 ± 0.01	ND	6.8 ± 0.4
Nectar 13	Orange	B	2.1 ± 0.1	0.43 ± 0.03	0.11 ± 0.01	ND	2.6 ± 0.2
Nectar 14	Orange	C	0.26 ± 0.02	0.12 ± 0.01	0.17 ± 0.01	0.39 ± 0.02	0.94 ± 0.05
Nectar 15	Orange	C	18 ± 1	4.8 ± 0.3	0.86 ± 0.05	ND	24 ± 1
Nectar 16	Orange	D	0.22 ± 0.01	0.19 ± 0.01	0.25 ± 0.01	0.36 ± 0.02	1.0 ± 0.1
Nectar 17	Orange	G	5.9 ± 0.3	4.6 ± 0.3	2.9 ± 0.2	0.31 ± 0.02	14 ± 1
Nectar 18	Orange	I	1.3 ± 0.1	0.82 ± 0.05	0.89 ± 0.05	0.46 ± 0.03	3.5 ± 0.2
Nectar 19	Orange	J	4.0 ± 0.2	0.93 ± 0.05	0.17 ± 0.01	0.12 ± 0.01	5.2 ± 0.3
Nectar 20	Orange	K	3.3 ± 0.2	0.41 ± 0.02	ND	ND	3.7 ± 0.2
Nectar 21	Orange	L	17 ± 1	3.8 ± 0.2	0.78 ± 0.04	ND	22 ± 1
Nectar 22	Peach	G	1.5 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	<0.07	3.9 ± 0.2
Nectar 23	Peach	L	16 ± 1	2.9 ± 0.2	0.61 ± 0.03	ND	20 ± 1
Nectar 24	Peach	L	9.6 ± 0.6	2.0 ± 0.1	0.45 ± 0.03	ND	12 ± 1
Nectar 25	Pear	G	2.6 ± 0.2	2.5 ± 0.1	1.9 ± 0.1	0.27 ± 0.02	7.3 ± 0.4
Nectar 26	Grapefruit	G	2.4 ± 0.1	2.2 ± 0.1	1.7 ± 0.1	0.20 ± 0.01	6.5 ± 0.4
Nectar 27	Grapefruit	J	3.9 ± 0.2	0.64 ± 0.04	0.19 ± 0.01	ND	4.7 ± 0.3
Nectar 28	Guava	A	2.9 ± 0.2	1.5 ± 0.1	0.97 ± 0.06	0.14 ± 0.01	5.5 ± 0.3
Nectar 29	Mango	C	0.67 ± 0.04	0.25 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	1.4 ± 0.1
Nectar 30	Passion Fruit	A	4.8 ± 0.3	2.3 ± 0.1	1.1 ± 0.1	0.16 ± 0.01	8.4 ± 0.5
Nectar 31	Mix	C	0.12 ± 0.01	0.17 ± 0.01	0.25 ± 0.01	0.38 ± 0.02	0.92 ± 0.05
Nectar 32	Mix	D	ND	ND	0.16 ± 0.01	0.29 ± 0.02	0.45 ± 0.03

[a]±Standard deviation, n=5. [b]Total THMs. [c]Not detected. [d]<LOQ.

Figure 3 shows the distribution of the total trihalomethane concentrations as box plots in the beverages studied. The bottom and top of the box correspond to the 25th and 75th percentiles, whereas the band (median) inside the box is related to the 50th. The ends of the whiskers represent the lowest and highest observations, and the spacing between the different parts of the box give an indication of the degree of spread and skewedness in the data.

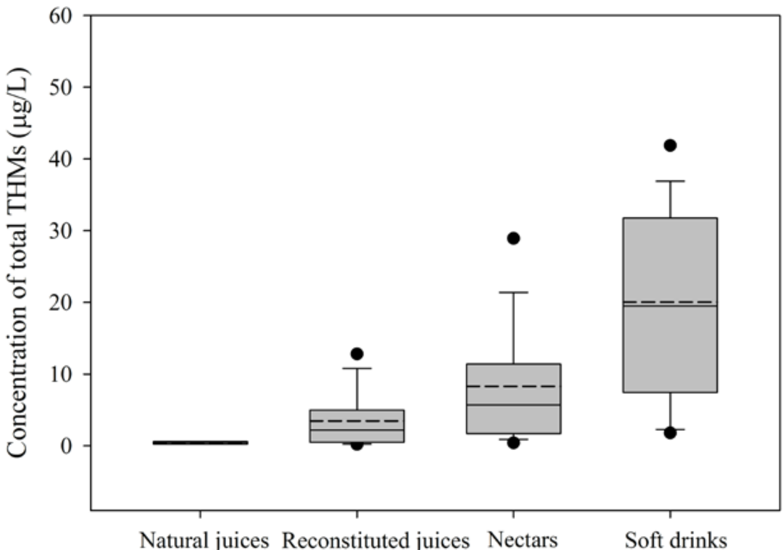

Figure 3. Representation of the total trihalomethane concentrations distributed as box plots in the beverages. The box plots indicate the mean concentration (dashed line) and the 10th, 25th, 50th, 75th, and 90th percentiles (points denote the 5th and 95th percentiles).

There are again two different groups, nectars A with TTHM concentrations <6 µg/L (n = 17; average value of 2.6 ± 1.9 µg/L) and nectars B with concentrations >6 µg/L (n = 15; average value of 14.7 ± 8.7 µg/L). As occurred in the previous case, these variations cannot be attributed to the kind of fruit but rather to the difference between the qualities of the treated water used in their production. Moreover, this deduction was consolidated by comparison to the same product (nectar) manufactured in different bottling factories (n = 12) shown in **Table 3** following a word code. Thus, the average TTHM concentrations in nectars produced by A (nectars 1, 5, 28 and 30) or L (nectars 10, 11, 21, 23 and 24) bottling factories were 6.1 or 22.2 µg/L, respectively. Thus, in our study, the juices from factory L always provided the highest concentrations of THMs. These variations in the concentration of THMs in the same product can be explained on the basis of the type of treated water employed at each moment. **Figure 4** shows the chromatograms obtained from the analysis of natural juice 7 (A), reconstituted juice 2 (B), and nectar 21 (C).

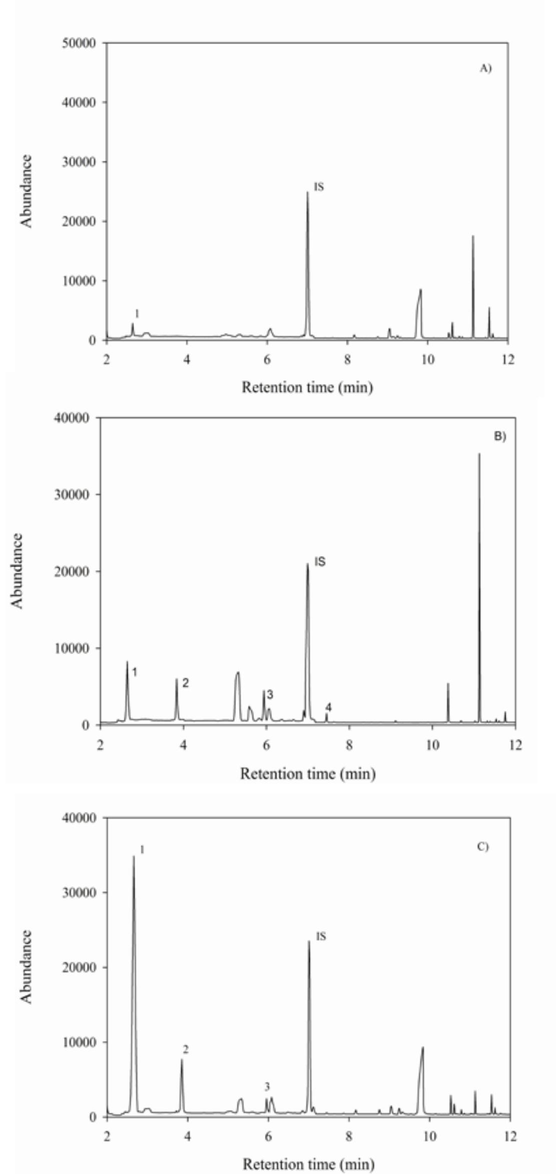

Figure 4. GC–MS chromatograms in SIM mode obtained in the analysis of natural juice 7 (A), reconstituted juice 2 (B), and nectar 21 (C) samples. Peaks: (1) TCM; (2) BDCM; (3) DBCM; (4) TBM; (IS) internal standard. For compound abbreviations, see Table 1.

Finally, for comparison purposes, several reconstituted juices of various flavors were analyzed from different European countries. As can be seen in **Table 4**, no sample presented TTHM concentrations above the levels allowed by EPA or EU for drinking water (80 or 100 μg/L) [9,10]. Once again, two differentiated groups existed: one for TTHM concentrations of 0.4 ± 0.2 μg/L (n = 8) and the other for 9.9 ± 8.2 μg/L (n = 6), similar to Spanish juices.

Table 4. THMs found in reconstituted fruit juices from several european countries (±SD, μg/L, n = 5)

Country (favlour)	Compounds				
	TCM	BDCM	DBCM	TBM	TTHMs[a]
France (apple)	ND[b]	ND	0.13 ± 0.01	0.09 ± 0.01	0.22 ± 0.01
France (orange)	0.77 ± 0.04	ND	ND	ND	0.77 ± 0.04
Poland (orange)	0.36 ± 0.02	ND	ND	ND	0.36 ± 0.02
Poland (orange)	0.25 ± 0.01	ND	ND	ND	0.25 ± 0.02
Belgium (orange)	0.35 ± 0.02	ND	ND	ND	0.35 ± 0.02
Belgium (Orange)	0.90 ± 0.05	0.21 ± 0.01	ND	ND	1.1 ± 0.1
Belgium (Orange)	18 ± 1	0.14 ± 0.01	0.08 ± 0.01	<0.07	18 ± 1
Belgium (mix)	17 ± 1	1.3 ± 0.1	0.09 ± 0.01	ND	18 ± 1
Belgium (mix)	0.61 ± 0.03	ND	ND	ND	0.61 ± 0.03
Belgium (cranberry)	14 ± 1	1.1± 0.1	0.44 ± 0.03	<0.07	16 ± 1
Portugal (apple)	3.6 ± 0.2	1.1 ± 0.1	ND	ND	4.7 ± 0.3
Portugal (mix)	0.26 ± 0.02	0.12 ± 0.01	<0.05[c]	ND	0.38 ± 0.02
Portugal (mix)	1.8 ± 0.1	ND	ND	ND	1.8 ± 0.1
Portugal (peach)	0.45 ± 0.03	0.18 ± 0.01	ND	ND	0.63 ± 0.04

[a]Total THMs. [b]Not detected. [c]<LOQ.

This study has revealed that chloroform can appear at concentrations <1 μg/L in beverages without treated water as a consequence of contamination due to the chlorinated sanitizers usually employed in the food industry. The treated water included as an ingredient is the main source of the presence of THMs, and the concentration and speciation of these compounds depend on the volume of treated water as well as the water-quality employed. Moreover, brominated THMs and one iodinated one (DCIM) appeared in these samples, which can be used as an indicator of the presence of treated water. The proposed method could be adopted by public laboratories to perform routine controls of VOCs and THMs in beverages.

On the other hand, if it is estimated that a person can consume 2 L per day of drinking water (this includes drinking water consumed in the form of juices and other beverages containing tap water) [27] and taking into account that the most contaminated sample (soda 18 in **Table 2**) contained 52 μg/L of total THMs, a person could ingest >100 μg/L of THMs each day (MCL 80–100 μg/L) [9,10]. This problem is aggravated because the default assumption of 2 L per day is not always appropriate or conservative with respect to populations, climates, and physical activity; thus, variations between 3.8 and 4.8 L have been referenced [28]. On the other hand, although chlorinated THMs were the common THMs present in beverages, some samples contained high concentrations of brominated compounds (reconstituted juice 5 and nectar 5 in **Table 3**) and one iodinated compound (sodas 18 and 20 in **Table 2**), these compounds being more cytotoxic and genotoxic when compared to their chlorinated analogues. In our opinion, the contribution of human exposure through beverages such as fruit juices and soft drinks per day is significant, taking into account that there are other sources of exposure through foods during the day. Thus, it is deemed acceptable to include these compounds as emergent pollutants in beverages because they have been already established as such for drinking water in several countries.

Funding

This work was funded within the frameworks of Projects CTQ2013–42701 (Spain's Ministry of Education) and P09–FQM–4732DGI (Andalusian Regional Government). FEDER provided additional funding. I.M. is grateful for the award of a predoctoral grant from Project P09–FQM–4732DGI.

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CAPÍTULO 6

Graphene-coated cotton fibers as a material for the extraction of multi-class pesticide residues from natural water and their determination by gas chromatography-mass spectrometry

Con el objeto de adquirir nuevos conocimientos en las técnicas de miniaturización de preparación de la muestra, se realizó una estancia breve de 4 meses en el Departamento de Química de la Universidad de Ioannina (Grecia). En el desarrollo de la estancia se han adquirido conocimientos acerca de los procedimientos de preparación y estudio de nanomateriales funcionalizados así como sus aplicaciones analíticas para propósitos de microextracción.

La realización de esta estancia ha permitido también cumplir uno de los requisitos necesarios para poder optar a la Mención de Doctorado Internacional.

Graphene-coated cotton fibers as a material for the extraction of multi-class pesticide residues from natural water and their determination by gas chromatography-mass spectrometry

1.Introduction

Organic pesticides, including organochlorine, organophosphorus, carbamates, pyrethroids etc., are the main types of pesticides widely used in agriculture. Due to their high toxicity to human body, European Union has set a maximum concentration of 0.5 μg/L for total pesticides in drinking water and the individual concentration less than 0.1 μg/L, for single pesticides [1]. Gas chromatography (GC) equipped with different detectors, such as flame ionization, flame photometric, electron capture, nitrogen phosphorus and mass spectrometry (MS) is one of the most widely used techniques for their analysis owing to its excellent analytical.

Suitable sample pretreatment techniques are often required prior to GC analysis. The selection of an extraction technique is made on the basis of the sample matrix, concentration and type of analytes in the sample, speed of extraction, simplicity and flexibility of the method development and ruggedness of the method [2]. Organic compounds occurring in aqueous sample matrices can be treated by various extraction and enrichment methods. Liquid-liquid extraction (LLE) is the most conventional sample pretreatment technique for the determination of pesticides [3]. Many of the problems associated with LLE, such as incomplete phase separation, less-than-quantitative recoveries and disposal of large quantities of organic solvents have been circumvented by using solid-phase extraction (SPE) [4,5]. However, they both require large volumes of sample and reagents and are time consuming.

In recent years, sample pretreatment techniques have been directed toward simple, miniaturized and environmentally friendly methods and some solventless or solvent-free sample pretreatment techniques have appeared, including liquid-phase microextraction [6,7], solid-phase microextraction [8–11] and stir-bar soptive extraction [12,13]. The SPE remains one of the most widely used preconcentration techniques mainly due to the variety of different materials employed as sorbents [14]. Solid-phase materials useful for extraction, concentration and cleanup are available in a wide variety of chemistries, adsorbents and sizes; their characteristics control the analytical parameters such as selectivity, affinity and capacity. For this reason, different methods have been used to modify the classical SPE material (such as silica and polymer) towards increasing the selectivity. Scientists have widely modified classical SPE materials and a few reports have been published on the immobilization of material surfaces [15]. SPE is most commonly used for the extraction of liquid samples and especially for that of semivolatile or nonvolatile analytes, but also solid samples can be pre-extracted into solvents. In addition, several sorbents have been developed and SPE has become a pretreatment technique of choice for the analysis of pesticides [16–18].

Carbon-based materials constitute a particular kind of organic materials that have come of age as trapping sorbents for separating organic compounds [19]. Graphene (GR), which is a new type of carbon nanomaterial with one single atomic layer of graphite, consists of a one-atom-thick planar sheet of sp^2 bonded carbon atoms [20]. It can adsorb and desorb a variety of molecules and ions and can interact through hydrophobic and π-π interactions with organic molecules [21] like chlorophenols [22], organophosphate pesticides [23], aromatic pollutants [24], etc. However, its direct use as sorbent in SPE can easily form irreversible agglomerates or GR sheets, which may escape from the cartridge due to their polydispersity. On the other hand, GR sheets are relatively soft and flexible and can be attached onto a support more easily than carbon nanotubes and fullerenes. In addition, particular magnetic forms of GR have been developed by researchers, with many applications [25,26].

Recently, we functionalized cotton fibers with graphene and aminosilica nanoparticles and we employed the resulting cotton-supported graphene-aminosilica as a novel material in an easily applicable extraction mode for polycyclic aromatic hydrocarbons (PAHs), phthalates, musks, phenolic endocrine disrupters and haloacetic acids [27]. Cotton microfibrils are amenable to modifications through physical (sorption) and chemical processes since they are made of poly–D–glucose chains. Hoefnagels *et al.* have reported biomimetic superhydrophobic and highly oleophobic cotton textiles [28]. Microcolumns filled with cotton have also been applied to the retention of synthetic colorants [29] and enrichment of PAHs [30]. In this work, relying on the hydrophilic character of cotton fibers and hydrophobic and π-stacking behavior of GR, we followed a simple functionalization procedure to synthesize a cotton-GR based extraction material, which could effectively be employed for the extraction of pesticides from environmental water. The cotton-fiber matrix consisting of a web of GR-coated cotton microfibrils was proven to be a successful material for the extraction of multi-class pesticide residues. After extraction, the material is collected easily and pesticides are eluted and subsequently injected into a gas chromatograph.

2. Experimental

2.1. Chemicals and solutions

Pure hydrophilic, non-sterile cotton pads were purchased from a local pharmacy. Graphite powder (purity 99.9%) was purchased from Aldrich (Sigma-Aldrich Ltd., Greece). Hydrazine for synthesis (about 100%) was supplied from Merck (Merck Chemicals, Darmstadt, Germany). Twenty analytical standards of pesticides (PESTANAL) were obtained from Fluka (Basel, Switzerland). The organic solvents *n*-hexane and ethyl acetate obtained from Labscan (Labscan, Dublin, Ireland) were of GC grade.

2.2. Instrumentation-chromatographic conditions

Analyses were carried out on a Shimadzu GC–17A gas chromatograph equipped with a QP 5000 mass spectrometer (Kyoto,

Japan). The selective ion monitoring mode was adopted for the determination of the analytes grouping the fragment ions.

Helium (purity ≥ 99.999%) was used as the carrier gas, at a flow rate of 1.0 mL/min. The injection volume was 1 µL, injected in splitless mode, hold for 60 sec and then purge, at a split ratio of 1:50. The 2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl was used as internal standard (IS). The separations were performed on a Supelco MDN-5 fused–silica capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness). The GC conditions were set as follows: injector temperature, 250 °C; initial oven temperature, 50 °C for 2 min, programmed to 290 °C at 10 °C/min and then maintained at 290 °C for 5 min; transfer line temperature, 290 °C.

2.3. Synthesis of graphene oxide

Graphite powder was oxidized with a mixture of acids and $KMnO_4$, as described by Jabeen*et al.* [31], with modifications [25]. Briefly, a mixture of 98% H_2SO_4 – 85% H_3PO_4 (120:13 mL) was added to a blend of graphite powder (1.0 g) and $KMnO_4$ (6.0 g). The temperature was maintained at 50 °C under stirring, for 24 h. Then, it was cooled to room temperature and poured into cool water (130 mL) containing 30% H_2O_2 (6 mL), in an ice bath. The supernatant was decanted away after settling overnight and the remaining solid was stirred overnight, after the addition of 37% HCl (60 mL). The mixture was centrifuged for 10 min at 4000 rpm and washed several times with double distilled water (DDW) till the pH of solution was almost neutral. Finally, the solid was washed with pure ethanol (3×25 mL) followed by centrifugation. Ethanol was removed by rotary vacuum evaporation and dried in vacuum oven to obtain graphene oxide (GO).

2.4. Functionalization of cotton fibers with GO

A GO 'ink'-dispersion was prepared by bath ultrasonication (120 W) of 100 mg of GO in 50 mL of DDW for 10 min, followed by probe-ultrasonication at 250 W (MRC Scientific Instruments, Holon, Israel), for 30 min. The coating of cotton fibers with GO nanostructures was achieved by successive immersions of a cotton piece under ultrasonication, followed by drying steps. Briefly, 0.50 g of cotton was rinsed with acetone and plenty of water. The cotton pad was immersed in the GO ink under bath

ultrasonication, for 10 min. After that, the GO-coated cotton was taken out of the solution and left drain on a net, for 10 min. The resulting material was placed in an oven at 120 °C, for 20 min to complete the attachment. The coating process was repeated until the complete consumption of the aqueous dispersion of GO in order to increase the GO loading on the cotton. Finally, the functionalized cotton was washed with DDW for the removal of unretained GO and dried overnight, at 100 °C. The mass of the GO was obtained from the mass difference before and after the immersion and drying of the cotton pad.

2.5. Cotton-supported GR

The reduction to cotton-GR was achieved by refluxing the cotton-GO in 80 mL of DDW, which contained 500 µL of hydrazine at 90 °C, for 24 h. The final material was rinsed with plenty of water to remove minute amounts of GR and dried at 60 °C, overnight.

Precaution: The use of hydrazine requires particular care because it is both highly toxic and potentially explosive.

2.6. Extraction procedure

Extractions were carried out as follows: Seventy five mg of the functionalized cotton was cut and preconditioned by dipping it successively in methanol (once) and DDW (twice), for few seconds. Then, it was placed in a glass beaker containing 200 mL of an aqueous solution of the target pesticides already adjusted to pH 6, for extraction. The solution was stirred during extraction and the cotton was allowed to tumble freely during extraction. After an extraction time of 20 min, the functionalized cotton piece was removed with tweezers and placed in a 5-mL micropipette tip. The cotton was washed with 2 mL of DDW and dried with nitrogen, until complete removal of moisture. The cotton was removed and elution was carried out with 2 mL of ethyl acetate/hexane 1:1 containing the IS, under ultrasonication, for 2 min. Among extractions, the cotton was squeezed in order to collect the extracts in vials, which were then condensed to 200 µL, under a nitrogen stream. Finally, the condensed organic eluant was directly injected into the GC for analysis.

3. Results and discussion

3.1. Synthesis of the GR-coated cotton fibers

The microfibers of cotton were coated using a multiple immersion-drying process in a well-dispersed GO 'ink', where cellulose fibers form hydrogen bonds with the hydroxyl groups of GO [32]. Repeating this simple immersion-drying process, a densely coated cotton-GO was fabricated and the white color of the cotton changed into blackish brown confirming the uniform coating of microfibers by GO. The cotton-GO was transformed to the cotton-GR through reduction with hydrazine. The reduction was confirmed by obtaining FT-IR spectra before and after reduction of bare GO, following the same reduction conditions (data not shown). After coating and reduction, the amount of GR on the fibers was calculated to be 140 mg/g of cotton, using the weighing method. The overall procedure of cotton modification and functionalized cotton-GR based extraction procedure is illustrated in **Figure 1**.

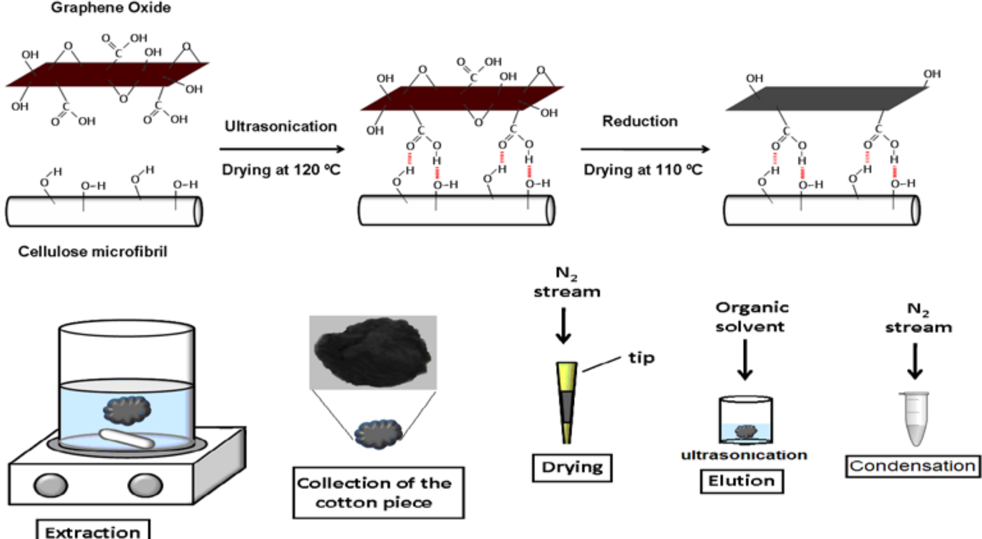

Figure 1. Schematic illustration of the preparation of functionalized cotton-GR and the overall extraction procedure.

3.2. Effect of experimental conditions on the extraction efficiency of cotton-GR

Based on the hydrophobic and π–π properties of GR and the hydrophilic character of cotton fibers, a pesticide residue method was developed to assess its suitability as an extraction material. The high to moderate log*P* values of the multi-class neutral and ionizable pesticides are indicative of their hydrophobic character.

Different experimental parameters, which affect the extraction efficiency including pH of extraction, stirring rate, extraction time, ionic strength, presence of humic acid, sample volume and amount of sorbent and elution conditions were investigated and optimized in sequence, using 20 mL of aqueous solution spiked with 1.0–2.0 μg/L of pesticides. Relevant graphs will be presented, which account for the behavior of representative pesticides selected on the basis of their log*P*, pK_a and class pesticides they fall in. The chromatographic peak areas of the individual compounds were compared with pure standards prepared in organic solvents to appraise the extraction efficiency. The most abundant and characteristic ions in the spectra were selected for quantification and confirmation purposes. Two fragment ions were monitored for each compound, in order to maximize the detector signal.

3.2.1. Effect of sample pH

The effect of sample pH was investigated at pH 2.5, 6.0 and 9.5 by adjusting it with 0.1 M HCl and 0.1 M NaOH. Uniformly coated fibers with GR do not possess ionized groups, except for some residual oxygen-containing groups on GR, which can participate in hydrogen bonding. The results portrayed in **Figure 2** show no obvious variations in the extraction efficiency, as a function of pH, for all the pesticide groups studied. For triazine herbicides, the pK_a values are close to 1.6 and are very weak bases. The fact that sample pH do not affect their extraction yield demonstrated that hydrophilic (hydrogen-bond) interactions between analytes and hydrophilic parts of sorbent may play an important role in addition to the hydrophobic interactions. For the rest of target pesticides, it is reasonable that sample pH do not affect the extraction yield since they are neutral and

the major interactions expected to drive extraction into cotton-GR are hydrophobic. Moreover, taking into account that many of the target-pesticides have aromatic rings in their structure an increased extraction yield is due to their ability to establish with pesticides π-π interactions. As a result, it is unnecessary to adjust the pH of the sample solution, but close to 6 is more preferable.

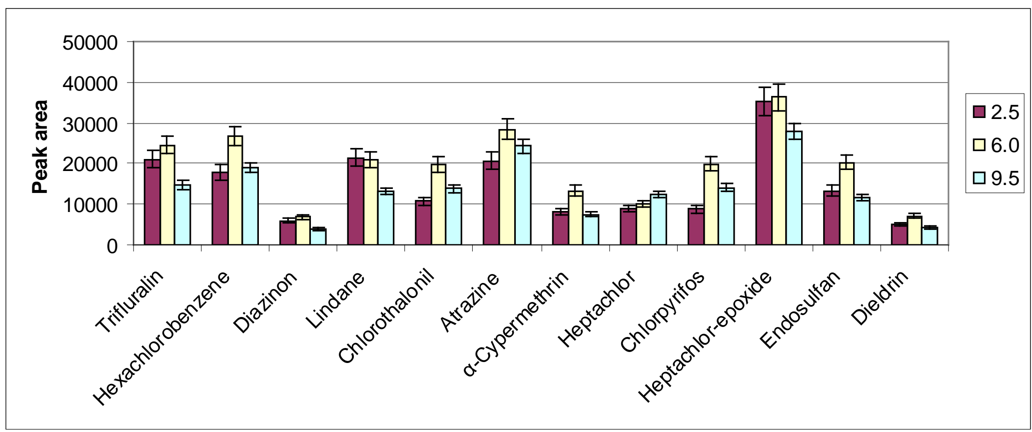

Figure 2. Influence of the sample pH on the extraction efficiency of twelve representative pesticides. Error bars are the standard deviation for three measurements.

3.2.2. Stirring rate and extraction time

The influence of mass-transfer rate on the extraction was ascertained by varying the stirring rate in the range 50–400 rpm, under otherwise constant conditions. The experimental results indicated that the extraction efficiency was increased with the increase in stirring rate acquiring a plateau at around 300 rpm, for an extraction time of 20 min. Shorter extraction time, at 300 and 400 rpm, causes the extraction efficiencies to deteriorate while higher do not improve upon. Stirring can accelerate molecular mass-transfer rate and reduce the time to reach the thermodynamic equilibrium permitting continuous exposure of the modified cotton fibrils coating to “fresh” sample solution. However, too high stirring rate loosens the network of microfibers rendering their collection

troublesome, after the completion of extraction. Although a single stirring rate cannot account for the behavior of all the target pesticides, their extraction yield can be reconciled at a stirring rate of 300 rpm for 20 min.

3.2.3. Sample volume and amount of sorbent

The responses were obtained for sample volumes between 20 and 200 mL, spiked with the same amount of pesticides. Highest signal intensity was obtained with extraction volumes between 100 and 200 mL and a tendency to decline above it, for most of the pesticides. It is evident that the higher the analyte concentration in water sample – or the same absolute amount of analyte is present in a lower volume – the larger the analyte mass sorbed on the cotton-GR. In contrast, for sample volumes between 20 and 200 mL, at constant concentration of pesticides, the highest response was acquired employing the highest sample volume. As the sample volume is not a limiting factor in the analysis and the target analytes are usually at low concentrations, an extraction procedure with 200 mL of sample is recommended.

Under the above conditions, 20 mg of sorbent are not effective for the extraction of pesticides while 75 mg are in reasonable excess for the extraction of even higher concentrations. This amount of cotton-GR corresponds to 10.5 mg of pure GR.

3.2.4. Ionic strength and humic acids

The effect of ionic strength was evaluated by adding to the sample solution two sodium salts, namely: NaCl and Na_2SO_4, at different concentrations of 50 and 100 g/L. The highest ionic strength of NaCl (100 g/L, corresponding to 2.12 M) augments the signal intensities for the more polar atrazine and simazine by ~25%. It seems that the addition of NaCl affects the activity coefficients of the analytes considered, increasing the concentration of water-soluble compounds sorbed onto cotton-GR. On the other hand, for the more apolar pesticides lower extraction efficiencies were noticed especially using Na_2SO_4 or no statistically significant changes (at 95% confidence level) were monitored.

As far as the presence of humic acids is concerned, no obvious difference in the response was observed for most of the pesticides up to 15 mg/L except for dimethoate and to a lesser degree for atrazine, simazine and trifluralin show an increase in the extraction efficiency up to 8% probably due to a favorable interaction (hydrogen bonding) with the carboxyl, hydroxyl and amino-groups of humic acids, which may form aggregates on the GR of the sorbent [25].

3.2.5. Elution conditions

n-Hexane and ethyl acetate as non-polar and moderately polar solvents, respectively as well as their mixture of 1:1 were tested for the elution of target pesticides. The results showed in **Figure 3** indicated that 2 mL *n*-hexane:ethyl acetate (1:1) can fairly elute the extracted herbicides in contrast to *n*-hexane and ethyl acetate, which individually are not proper for the whole range of the target pesticides. Most importantly, it was found that ultrasonication for 2 min during elution has a significant bearing on the eluting efficiency. In addition, a drying step before elution was essential for the desorption of pesticides, as the presence of small amounts of water hampers the approach of the organic solvent to the sorbent.

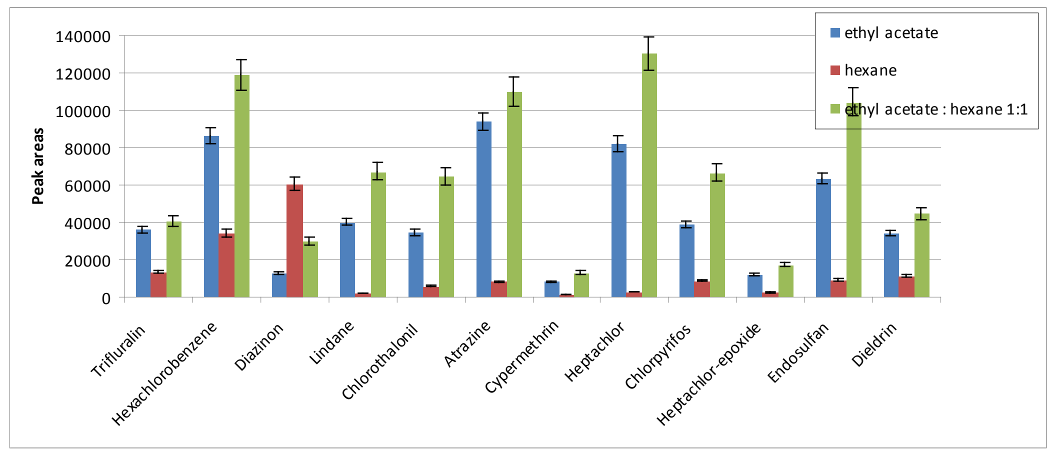

Figure 3. Influence of the eluent (ethyl acetate and/or *n*-hexane) on the extraction of twelve representative pesticides. Error bars are the standard deviation for three measurements.

3.3. Stability of studied pesticides in cotton-GR

The recoveries of the studied pesticides, at the three different storage conditions in the dark were compared with the data after their prompt analysis. Clearly, the storage of the cotton-GR for 2 days at 20 ºC showed remarkable decrease in relative recoveries for most of the target pesticides. Some of them were not detected due probably to degradation or complete evaporation. After 5 days of storage at 6 ºC the relative recoveries are obviously higher than at 20 ºC and serious loss was not visible. As long as water samples have to be extracted immediately after sampling, shipping to the lab of all cotton-GR pieces used for the extraction of water samples and the matrix match calibration samples can be facilitated under cooling. Freezing at −10 ºC for 5 days after extraction on cotton fibers, appeared as the best storage conditions, as no loss of the pesticides sorbed on the cotton fibers was observed. In addition, the storage conditions seemed to have less impact on the variability of the recoveries. Therefore, cotton-GR loaded with pesticides can tolerate an additional storage step at −10 ºC, before analysis by GC–MS.

3.4. Preparation reproducibility of cotton-GR

The preparation reproducibility of GR-coated cotton was investigated employing four functionalized cottons prepared under identical conditions from different batches for the extraction of pesticides from aqueous solutions, each containing the entire range of target pesticides. The reproducibility of the peak areas obtained for the preparation of GR-coated cotton expressed as the relative standard deviations (RSDs) ranged from 6 to 9%. The procedure for the preparation of cotton-GR led to fairly reproducible results and hence it is suitable for the preparation of sorbent batches.

3.5. Evaluation of the GR-cotton–GC-MS method

The performance and reliability of the method proposed was evaluated under optimum conditions in terms of linearity, limits of quantification (LOQ), accuracy and repeatability. The analytical procedure was operated under the optimal conditions mentioned above. The accuracy and precision of the developed method were assessed at two spiking levels, corresponding to LOQ and three times the LOQ limits for each compound. Accuracy is expressed as analyte recovery i.e. percent closeness between the calculated and the theoretical concentrations of a spiked tap water sample for each analyte, whereas precision was calculated as RSD % of three replicates (**Table 1**). The analysis revealed that the tap water sample was free from contamination from the target analytes. Recoveries ranged from 83 to 107%, while the calculated RSDs ranged from 3 to 8% (data obtaining with the same batches). As shown in **Table 1**, there were no differences in the recoveries between the samples with different spiked levels. Among these twenty pesticides, lowest recovery was noticed for metolachlor (83%), but still it meets the requirement of USEPA (recovery: 70–130%).

To demonstrate the applicability of GR-cotton based procedure for routine analysis, the developed method was applied to the determination of pesticides in a lake water sample. Three pesticides, i.e. atrazine, diazinon and malathion were detected and their concentrations were 0.15, 0.44 and 0.19 µg/L, respectively. The spiked recoveries of the three pesticides at the measured concentration levels were from 95 to 97%.

Table 1. Validation parameters obtained for the target pesticides after extraction with cotton-GR and determination with GC-MS

Pesticide	LOQ (μg/L)	Linear range (μg/L)	Fortification level at LOQ		Fortification level at 3´LOQ	
			Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
atrazine	0.04	0.04–10	87	4	94	4
simazine	0.03	0.03–10	94	3	96	4
trifluralin	0.04	0.09–25	84	6	89	8
hexachlorobenzene	0.09	0.04–10	91	5	96	4
lindane	0.02	0.02–10	89	5	94	5
heptachlor	0.07	0.07–20	90	4	92	4
heptachlor epoxide	0.04	0.04–10	79	3	91	4
endosulfan	0.03	0.03–10	91	6	98	6
dieldrin	0.09	0.09–20	96	7	98	7
chlorothalonil	0.07	0.07–20	89	6	90	5
dimethoate	0.04	0.04–10	94	3	95	3
diazinon	0.02	0.02–10	94	4	96	3
parathion methyl	0.02	0.02–10	97	5	98	5
pirimiphos methyl	0.03	0.03–10	90	6	92	6
malathion	0.03	0.03–10	91	8	96	7
chlorpyrifos	0.03	0.03–10	103	6	106	6
alachlor	0.09	0.09–20	97	3	107	2
metolachlor	0.08	0.08–20	83	6	89	7
α-cypermethrin	0.05	0.05–10	107	4	99	4
τ-fluvalinate	0.06	0.06–20	94	5	96	6

Acknowledgements

Isabel Montesinos is grateful for the award of a pre-doctoral grant from Project P09-FQM-4732DGI and for the financial support for obtain the international doctorate mention through eidA3 and ceiA3.

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CAPÍTULO 7

Resultados y discusión

En este Capítulo de la Memoria se presenta un resumen de los resultados más relevantes obtenidos dentro de los objetivos e hitos marcados en la misma. Para ello los resultados experimentales se han dividido en 3 bloques. En el primer bloque se exponen los analitos diana, las muestras, así como los estudios para la conservación de las muestras y condiciones cromatográficas para ciertos compuestos. En el segundo bloque se discuten las ventajas e inconvenientes de los métodos de extracción desarrollados para la determinación de las distintas familias de DBPs. Finalmente, en el último bloque se incluyen los aspectos más relevantes en relación a las aplicaciones de las metodologías desarrolladas.

En este contexto, las aportaciones más relevantes han sido el desarrollo de metodologías específicas para la determinación conjunta de estas especies, así como la determinación de THMs en bebidas, debido a la ausencia de métodos e información referente a la presencia de estos compuestos en matrices como son los refrescos y zumos de frutas.

1. Estabilidad de analitos y muestras

1.1. Analitos

Los analitos abordados como especies de estudio a lo largo del desarrollo de la Tesis han sido varios. Se trata de subproductos de desinfección del agua y se han seleccionado entre ellos los trihalometanos (THMs), halonitrometanos (HNMs) y haloacetonitrilos (HANs). Los THMs son los compuestos más importantes y más abundantes de la fracción volátil. En el caso de los HNMs y HANs, son compuestos de la fracción volátil pertenecientes a la parte nitrogenada de los DBPs y que son potencialmente más cito- y genotóxicos que los THMs. Además, al final de la Tesis Doctoral se aborda el estudio de THMs yodados emergentes, que constituyen un nuevo grupo de DBPs más tóxicos que sus homólogos clorados y bromados.

1.2. Muestras

Las muestras incluidas en el desarrollo experimental de esta Memoria se pueden dividir en dos grupos perfectamente diferenciados. Por un lado las muestras de aguas tratadas (de grifo y de piscina) y aguas

medioambientales (ríos, lagos y estanques), que se tratan en los Capítulos 3, 4, 5 y 6; por otro lado las muestras de bebidas como son refrescos y zumos de frutas, que se abordan en el Capítulo 5.

1.3. Conservación de la muestra

1.3.1. Aguas tratadas y medioambientales

Los analitos determinados (THMs, HNMs y HANs) aparecen en las aguas como subproductos generados por la reacción de la materia orgánica con el desinfectante usado durante el tratamiento de potabilización. Teniendo en cuenta la volatilidad de los analitos estudiados, la toma y conservación de la muestra es clave para la obtención de resultados fiables. Por este motivo, todas las muestras de agua fueron recogidas en botes de vidrio ámbar, sin dejar espacio de cabeza ni burbujas, cerradas herméticamente y transportadas al laboratorio en una nevera donde se conservaron entre 0 y 4 ºC hasta su análisis, periodo que nunca fue mayor de 2 días. Si el tiempo entre la toma de la muestra y su análisis excede estos 2 días, se conservan a –20 ºC durante dos semanas.

Además, los desinfectantes usados para el tratamiento de potabilización del agua generan DBPs durante su almacenamiento, a menos que se enmascaren usando agentes de decloración. Los THMs han sido preservados usando NH_4Cl, ácido ascórbico o Na_2SO_3, sin embargo en el caso de los HNMs (mayoritariamente centrados en el TCNM) hay varias controversias descritas en la bibliografía. El método oficial de la EPA 551.1 para compuestos halogenados orgánicos volátiles propone NH_4Cl como agente declorante, sin embargo un estudio reciente ha demostrado que con esta sal, el cloro libre residual evoluciona hacia cloraminas formando nuevos DBPs como ácidos haloacéticos. Otras investigaciones, centradas en estos compuestos halogenados orgánicos volátiles, han recomendado $(NH_4)_2SO_4$, $Na_2S_2O_3$ o ácido ascórbico. Más recientemente, la EPA recomienda dos tipos de sales en el caso de los HNMs: acido ascórbico a pH 3.5 para 6 HNMs y NH_4Cl para los otros, lo que complica bastante el muestreo.

Para aclarar estas discrepancias se ha realizado un estudio de la influencia de varios agentes de decloración en la estabilidad de los HNMs.

En un primer estudio se adicionó diversas sales a las muestras para su conservación: Na_2SO_3, $(NH_4)_2SO_4$, $Na_2S_2O_3$ y ácido ascórbico; y en un segundo estudio se optó por acidificar las muestras (pH ~3.4). Tanto Na_2SO_3, $Na_2S_2O_3$ y ácido ascórbico descomponen los HNMs a nitrometano o metilamina, la descomposición fue el resultado de una deshalogenación reductiva debida al carácter reductor de estas sales. Este efecto es más marcado para las especies bromadas debido a su mayor estado de oxidación. Solo $(NH_4)_2SO_4$ mantiene la estabilidad de los HNMs aunque solo durante un día, mientras que la acidificación de la muestra a un pH ~3.4 los mantiene estables durante dos días. En el caso de los THMs y HANs su estabilidad también esta favorecida con el ajuste a un pH ácido. A pH ácido además se evita la hidrólisis de HANs y simplifica una etapa en el método analítico, ya que estos compuestos son extraídos mejor a pH ácido.

1.3.2. Bebidas (refrescos y zumos de frutas)

Los DBPs pueden aparecer en las bebidas debido al uso de agua tratada como un ingrediente más del producto elaborado o a la desinfección con productos clorados de la línea de preparación y envasado de la bebida, con lo que queda retenida agua que puede ser una fuente de contaminantes. Las bebidas (refrescos y zumos de frutas) se adquirieron en supermercados españoles y de otros países europeos. Las bebidas españolas incluyen diferentes marcas, embalajes y sabores. Todas las muestras se conservaron en su embase original, se almacenaron según las indicaciones del etiquetado, se analizaron antes de su fecha de caducidad, y el sello de cada producto se abrió justo antes de sus análisis. La preparación del blanco se llevó a cabo con un zumo de naranja recién exprimido, donde todos los utensilios de preparación fueron lavados con agua mineral para asegurar que no hubiera DBPs. Un aspecto clave en los refrescos carbonatados es el CO_2, ya que al abrir el envase puede arrastrar también a los DBPs volátiles originándose pérdidas. Además, el CO_2 en el vial de HS compite con los analitos por el espacio de cabeza, lo que puede disminuir la eficacia de la extracción. La opción más frecuente es la desgasificación de las bebidas por agitación, antes de proceder a la determinación de especies. En este caso no fue posible debido a la volatilidad de los analitos. De los estudios realizados la opción más simple fue la neutralización de la acidez de la muestra con alícuotas de NaOH 6 M

(300 µL para 10 mL de refresco) habida cuenta que los analitos diana se extraen en un amplio intervalo de pH (2.5–9.0).

1.4. Condiciones cromatográficas

La primera técnica analítica que se utiliza para la determinación de los DBPs es la cromatografía de gases-espectrometría de masas. Algunos compuestos que poseen estructuras de trihalometilos se pueden descomponer parcialmente a las temperaturas elevadas del cromatógrafo y/o en el espectrómetro de masas. Existe una amplia bibliografía sobre la estabilidad térmica de los THMs no ocurriendo lo mismo para los HNMs. Los HNMs son térmicamente inestables y pueden descomponerse a las temperaturas usuales en cromatografía de gases durante su determinación. Las temperaturas del inyector (150–250 ºC) y del espectrómetro de masas (200–250 ºC) se variaron para estudiar la posible descomposición de los 9 HNMs. En el caso del espectrómetro de masas ninguno de estos compuestos se descompone a las temperaturas estudiadas, sin embargo en el inyector si se produce dicha descomposición en ciertos compuestos. Las especies mono- y dihalogenadas permanecen estables en todo el intervalo estudiado, mientras los trihalonitrometanos se descomponen a partir de los 170 ºC, llegando a ser de hasta un 45% para 250 ºC. Los mayores productos de descomposición son los haloformos (como son los THMs), los cuales se forman probablemente por la abstracción de hidrógeno a partir de los disolventes usados en la preparación de los estándares en la inyección debido a la presencia de radicales trihalometilo. Este hecho dificulta la identificación y cuantificación de otros DBPs también presentes en el agua, como son los THMs, ya que podría haber una sobreestimación de los mismos. Finalmente, se ha seleccionado 170 ºC para el inyector y 200 ºC para la fuente de ionización del espectrómetro de masas, con el fin de evitar/minimizar la descomposición de los HNMs.

2. Desarrollo de metodologías para la determinación de DBPs volátiles

A lo largo de la presente Memoria las técnicas de extracción han jugado un papel fundamental, principalmente para la mejora de la sensibilidad y la selectividad de las determinaciones llevadas a cabo. La sensibilidad puede ser un parámetro crítico cuando se trabaja con compuestos a baja concentración como es el caso de la mayoría de DBPs estudiados en esta Memoria. Durante el desarrollo experimental se han empleado varias técnicas de extracción que serán discutidas con detenimiento a continuación. De todas las metodologías desarrolladas, la técnica de espacio de cabeza estático, ha sido la más empleada a lo largo del desarrollo de esta Tesis y será por tanto, discutida en mayor profundidad.

2.1. Métodos de extracción

2.1.1. Extracción en una gota de disolvente (SDME)

La LLE ha sido la técnica más ampliamente utilizada para llevar a cabo la extracción de compuestos volátiles como son THMs, HNMs y HANs. Sin embargo, el uso de esta técnica implica un consumo elevado de disolventes orgánicos y un gran coste. Siguiendo los principios de una "Química verde" se han diseñado las técnicas de LPME con vistas a simplificar los procedimientos y reducir el volumen de extractante, no sólo desde el punto de vista económico sino también medioambiental.

Dentro de las distintas modalidades de LPME, se escogió para ensayar la SDME en el modo de espacio de cabeza por el carácter volátil de las 9 especies halogenadas de HNMs. En la **Figura 1**, se observa un diagrama esquemático del sistema de extracción.

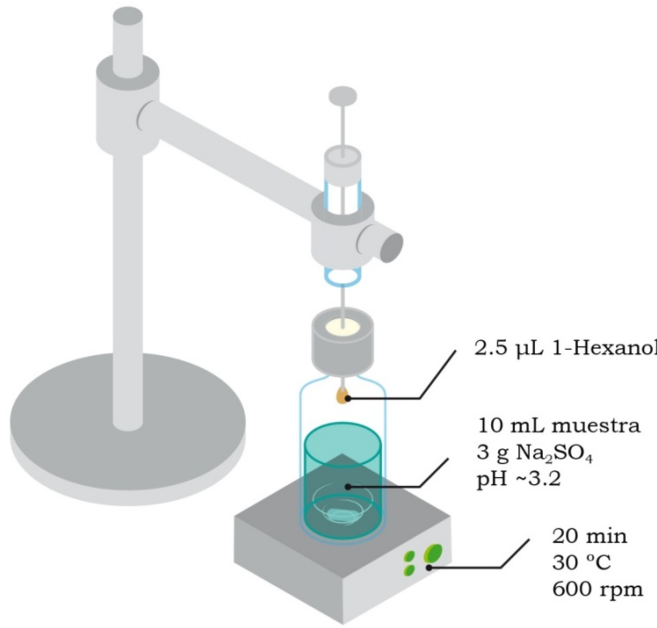

Figura 1. Diagrama esquemático de la extracción SDME.

Una etapa crucial en esta técnica es la selección del disolvente orgánico más adecuado, el cual debe tener una baja volatilidad para prevenir pérdidas por volatilización durante la extracción. Se ensayaron distintos disolventes (1-hexanol, 1-octanol, decano y o-xileno), de los cuales el 1-hexanol seguido del 1-octanol, fueron los disolventes que proporcionaron la mayor eficacia en la extracción de las 9 especies (aunque el 1-octanol no extrae tribromonitrometano). Finalmente se seleccionó 1-hexanol como extractante, ya que además de ser el que mayor número de compuestos extrajo, también tiene un punto de ebullición más bajo (~160 °C) que 1-octanol (~200 °C), y por tanto no es necesario emplear altas temperaturas para su volatilización ya que favorecería la descomposición de los HNMs como se ha descrito en el punto 1.4. de este Capítulo. En la optimización del volumen de gota hay que tener en cuenta que volúmenes superiores a 2.5 µL dificulta su manipulación, lo que origina resultados

menos fiables. Considerando este factor se seleccionó 2.5 µL de 1-hexanol, que finalmente se traduce en un volumen de extracto de 2 µL. Este extracto se inyecta en el cromatógrafo por lo que no quedan residuos. Los valores seleccionados de las demás variables se muestran en la **Tabla 1**. Una de las más críticas es el pH de la muestra, ya que la única información disponible en la bibliografía para HNMs es sobre el TBNM el cual requiere un pH entre 3.5 y 4.0 para minimizar su hidrólisis en medio básico; ya que los métodos oficiales se han orientado a la determinación de TCNM empleando un intervalo de pH de 4.8–5.5. El resultado del estudio del pH para la extracción de los 9 HNMs pone de manifiesto que la extracción debe ser llevada a cabo a pH ácido (3.0–3.5) para minimizar su hidrólisis. Por otra parte, la adición de sal a la muestra para aumentar la fuerza iónica favorece la extracción de los compuestos, siendo el Na_2SO_4 el que mejores resultados proporcionó. En el caso de las variables físicas hay que tener en cuenta muchos factores, ya que tiempos de extracción superiores a 40 min puede hacer que parte de la gota se evapore, así como temperaturas superiores a los 35 ºC. La opción que más favorecía la extracción de estos compuestos para evitar la pérdida del extractante fue calentar la muestra a 30 ºC y exponer la gota al espacio de cabeza durante 20 min.

La eficiencia de la extracción en SDME en relación a LLE convencional con una relación de fases 1:1 fue aproximadamente del 20% para los trihalonitrometanos (TCNM, BDCNM, DBCNM y TBNM), 10 % para DCNM y BCNM, y del 3% para CNM, BNM y DBNM. Los resultados obtenidos muestran que los trihalonitrometanos fueron favorablemente extraídos probablemente por su menor polaridad. La baja eficiencia de extracción que caracteriza a las técnicas de microextración en comparación con la LLE convencional se debe a que no son exhaustivas, debido a que los volúmenes de fase orgánica son extraordinariamente pequeños en relación al volumen de fase acuosa usada.

Tabla 1. Condiciones experimentales empleadas para el análisis de muestras de agua por distintas metodologías

Variable	Valor seleccionado		
	SDME	MLLE/LVI-PTV	HS
Extractante (µL)	1-Hexanol (2.5)	Acetato de etilo (200)	–
Na_2SO_4 (g)	3	3	6
pH	3.2	3.4	3.5
Volumen muestra (mL)	10	9	12
Modificador orgánico (µL)	–	–	MTBE (250)
Tiempo de extracción (min)	20	1	20
Temperatura (ºC)	30	Ambiente	80
Agitación (rpm)	Magnética (600)	Vortex	Mecánica

2.1.2. Microextracción líquido-líquido

La microextracción líquido–líquido (MLLE) se basa en la miniaturización de la LLE convencional, reduciendo tanto como sea posible el volumen de extractante. Con el empleo de esta modalidad buscamos conseguir una extracción exhaustiva y empleo de extractantes communes a diferencia de la SDME ensayada anteriormente. Así, la MLLE es exhaustiva, se alcanza el equilibrio entre ambas fases y se pueden emplear disolventes orgánicos convencionales (*n*-hexano, acetato de etilo, MTBE, etc.) con buenas prestaciones cromatográficas a diferencia de los generalmente empleados en otras modalidades de LPME (1-octanol, 1-hexanol, decano, o-xileno, etc.).

En este método se aborda la determinación conjunta de 4 THMs y 9 HNMs. En la **Figura 2** se observa un diagrama esquemático del sistema de extracción.

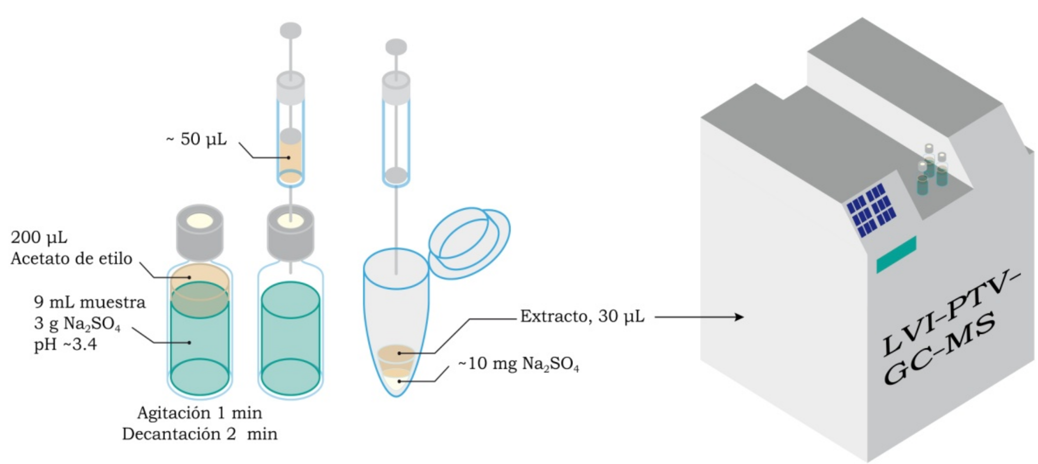

Figura 2. Diagrama esquemático de la extracción MLLE.

Una de las variables más importantes, dentro de las técnicas LPME es la selección del disolvente ya que es primordial que tenga una alta afinidad por los analitos diana y una baja solubilidad en agua. En MLLE es importante que el extractante sea volátil y tenga buenas prestaciones cromatográficas al igual que en LLE convencional. Esta modalidad de microextracción se ha combinado con un inyector de elevados volúmenes y temperatura programable (LVI–PTV) en el modo *solvent vent*. En este caso el PTV se programa de modo que en el momento de inyección de muestra, el *liner* esté a una temperatura inferior al punto de ebullición de los analitos y la válvula de desecho abierta. Como consecuencia, el disolvente se elimina a través de dicha válvula mientras los analitos, de mayor punto de ebullición, permanecen condensados en el *liner*. Una vez eliminado el disolvente, la válvula de desecho se cierra y los analitos son transferidos a la columna mediante un rápido calentamiento del *liner*. Esta modalidad permite inyectar elevados volúmenes de muestra, ya que el disolvente se elimina antes de su entrada a la columna cromatográfica.

A diferencia de las características de los extractantes empleados en SDME, en MLLE se pueden emplear extractantes usuales en LLE para cromatografía de gases. Dentro de los disolventes orgánicos estudiados

(acetato de etilo, *n*-hexano y MTBE), el acetato de etilo fue el que mayor rendimiento proporcionó para el conjunto de compuestos estudiados. *n*-Hexano extrae mejor a los HNMs menos polares, pero no a los más polares como son los monohalonitrometanos (CNM y BNM). Por otra parte, el MTBE dificulta la recogida del extracto, debido a su mayor solubilidad en agua. Otra variable interesante a estudiar fue la agitación, se ensayó tanto manual, magnética, ultrasónica como mecánica (vortex). Las agitaciones magnética y ultrasónica no proporcionaron buenos resultados seguramente porque dificultan la solubilización de la sal, la cual es importante para obtener una buena extracción y separación de fases. La agitación escogida fue la mecánica utilizando un vortex ya que proporciona resultados más reproducibles que la manual. Los valores óptimos de las demás variables se muestran en la **Tabla 1**. El método presenta la ventaja adicional de que todo el extracto recogido (30 μL) se inyecta en el LVI–PTV obteniéndose una mejora sustancial de la sensibilidad en comparación con otros inyectores convencionales que solo inyectan entre 1 y 2 μL de extracto. El método propuesto no genera residuos en consonancia con la tendencia actual de la "Química Verde".

La eficiencia de la extracción del proceso de MLLE en relación a la LLE convencional fue del ~85%, la cual es muy favorable teniendo en cuenta la relación muestra acuosa/extractante (~40) empleada en el proceso miniaturizado.

2.1.3. Espacio de cabeza estático

La técnica de espacio de cabeza ha sido la más aplicada en la presente Memoria, como se indica en el Capítulo 4 en el análisis de muestras de agua y en el Capítulo 5 en muestras de una planta potabilizadora y de bebidas (refrescos y zumos de frutas). En la **Figura 3**, se observa un esquema del módulo de espacio de cabeza utilizado en las metodologías propuestas en esta Memoria.

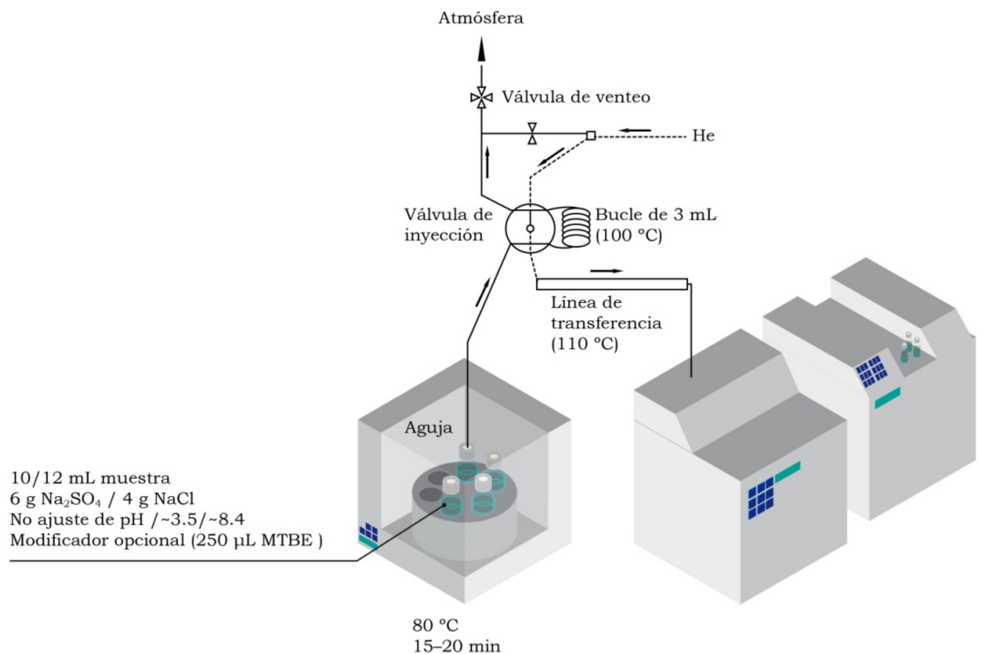

Figura 3. Diagrama esquemático del módulo de espacio de cabeza.

El procedimiento consiste en introducir el vial sellado con la muestra acuosa y reactivos en el horno, en él se calienta el vial con una agitación mecánica durante unos minutos hasta que se alcanza el equilibrio. La fase gaseosa de la muestra, una vez enriquecida con los analitos, es presurizada introduciendo helio en el vial. Posteriormente, se abre la válvula de venteo y la diferencia de presión entre el interior del vial y la presión atmosférica hace que el espacio de cabeza de la muestra salga y llene el bucle de 3 mL conectado a la válvula de inyección de 6 vías. Finalmente, una corriente de helio arrastra el contenido del bucle hacia la columna cromatográfica.

En el desarrollo de las metodologías usando esta técnica se ha estudiado la influencia de una serie de variables que influyen en la exactitud de los resultados. Dichas variables son de dos tipos: i) instrumentales, basadas en parámetros de la propia instrumentación empleada (temperatura del horno o tiempo de equilibración), y ii) químicas, basadas en parámetros de la propia muestra (cantidad de muestra, adición

de sal, pH de la muestra, y agentes modificadores como disolventes orgánicos que favorecen la volatilización de los compuestos). El valor seleccionado para cada variable se estableció en base a un proceso de optimización del sistema en el cual se tuvieron en cuenta criterios de sensibilidad, selectividad, precisión y rapidez.

En la **Tabla 2** se enumeran las variables instrumentales y químicas estudiadas en la metodología HS a lo largo del desarrollo experimental de esta Memoria, con el fin de poder ser comentadas a continuación con más detalle.

Tabla 2. Variables instrumentales y químicas del HS empleadas para cada tipo de muestra

Variable	Muestra		
	Agua	Zumo de frutas	Refresco sin/con CO_2
Tiempo de extracción (min)	20	15	15
Temperatura (ºC)	80	80	80
Volumen muestra (mL)	12	10	10
Sal (g)	Na_2SO_4 (6)	NaCl (4)	NaCl (4)
pH	3.5	sin ajuste	sin ajuste/8.4
Modificador (μL)	MTBE (250)	Ninguno	Ninguno
Especies	9 HNMs, 6 HANs y 4 THMs	10 THMs y 4 VOCs	10 THMs y 4 VOCs

i) Variables instrumentales

La temperatura del horno y el tiempo de equilibración tienen una gran influencia en la eficiencia de la extracción, ya que tienen un impacto directo en la concentración de los analitos en el espacio de cabeza del vial. En primer lugar se observa en la **Tabla 2** que tiempos menores de

equilibración son necesarios en las muestras de bebida incluso siendo muestras más complejas que las de agua, esto es debido a que los analitos determinados en estas muestras solo incluyen THMs y VOCs (diclorometano, 1,2-dicloroetano, tetracloruro de carbono y 1,2-dibromoetano) los cuales son más volátiles. Por el contrario, se requiere más tiempo cuando se incluyen especies nitrogenadas ya que son menos volátiles, además estas familias incluyen especies monohalogenadas, las cuales poseen mayor polaridad y solubilidad en agua, por lo que no se ven tan favorecidas a pasar al espacio de cabeza como lo hacen las especies trihalogenadas que son menos polares. En el caso de la temperatura, a mayores valores de este parámetro mayor volatilización de los compuestos se obtiene, lo que resulta que para todas las muestras se utilice el valor máximo de 80 ºC (mayores valores no pueden ser estudiados para minimizar la evaporación del agua).

El proceso de transporte del espacio de cabeza enriquecido con los analitos desde el vial a la válvula de inyección tiene lugar en dos etapas: presurización del vial y llenado del bucle por venteo del vial. No obstante los tiempos de presurización y de venteo no afectaron de manera significativa a ninguno de los analitos estudiados. Finalmente se seleccionó un tiempo de presurización de 30 s y un tiempo de venteo de 12 s para todos los tipos de muestras.

ii) Variables químicas

En el caso del volumen de muestra, se debe diferenciar entre muestras de agua y muestras de bebida (zumos de frutas y refrescos). En la técnica de espacio de cabeza estático lo habitual es trabajar con un relación de volúmenes de muestra:espacio de cabeza igual a 1:1. En el caso de las muestras de agua, muestras muy limpias, se empleó un volumen de 12 mL (en un vial de 20 mL) para conseguir una mayor sensibilidad en las metodologías desarrolladas. Por el contrario en el análisis de zumos de frutas y refrescos se utilizó un volumen de 10 mL ya que al ser muestras más complejas pueden producir espumas que podían afectar a la aguja del espacio de cabeza durante la extracción y/o poseen muchos aromas en la matriz que pueden competir por el espacio de cabeza, saturando la fase gaseosa.

La adición de sales aumenta la fuerza iónica de la disolución acuosa, esto puede disminuir la solubilidad de las especies orgánicas, mejorando su distribución entre la disolución acuosa y el espacio de cabeza. A su vez, minimiza la evaporación de agua al aumentar la actividad de la disolución y por lo tanto reduce la entrada de agua en el cromatógrafo de gases y en el detector. La cantidad de sal empleada será tanto mayor cuanto mayor sea la solubilidad del analito en la disolución y cuanto mayor sea la polaridad del mismo. Así, compuestos monohalogenados (CNM, BNM, CAN y BAN) necesitan una mayor cantidad de sal para su extracción mientras que los compuestos trihalogenados de menor polaridad y solubilidad en agua, requieren menor cantidad de sal. Así disoluciones saturadas fueron seleccionadas para la extracción de estos compuestos siendo 6 g de Na_2SO_4 o 4 g de NaCl para aguas o zumos de frutas y refrescos, respectivamente.

Otro parámetro estudiado ha sido la influencia del pH de la muestra, este puede influenciar la generación del espacio de cabeza. En el caso de los N-DBPs estos se encuentran más favorecidos a pH ácido (2.6–4.1), ya que disminuye su hidrólisis catalizada en medio básico, mientras que los THMs y VOCs no se ven influenciados por el pH. Finalmente, se seleccionó un pH ~3.5 en las muestras de agua para favorecer la extracción de los N-DBPs. En el caso de las bebidas, teniendo en cuenta que solo se han analizado THMs y VOCs no fue necesario el ajuste del pH de la muestra. Sin embargo en las bebidas carbonatadas puede haber ciertos problemas debido a la presencia de CO_2. Cuando se abre una bebida carbonatada, la presión se reduce a la presión atmosférica, provocando la descomposición del ácido carbónico y liberando CO_2. Esta pérdida de CO_2 origina un arrastre de los compuestos más volátiles a la atmósfera. Además, el CO_2 puede competir con los analitos por el espacio de cabeza dentro del vial. Para resolver este problema, se ha propuesto la adición de 300 µl de NaOH (6 M) que añadidos a 10 mL de muestra proporciona un pH de ~8.4 (la especie prevalente es el HCO_3^-; pk_a=6.1).

Se estudió la utilización de un disolvente orgánico como modificador para favorecer la volatilización de los analitos ya que actúa como extractante. El MTBE fue el disolvente que mas favoreció a los N-DBPs extrayendo los compuestos alrededor de un 50–70% más que sin el uso de un modificador. Este incremento fue más pronunciado en los compuestos

bromados seguramente debido a su menor volatilidad. En los THMs el efecto fue insignificante posiblemente debido al carácter apolar y volátil de estos compuestos en comparación con los otros. Finalmente, se seleccionó 250 µL de MTBE en muestras de agua, mientras que en bebidas no es necesario ya que solo se determinan VOCs y THMs simplificándose el método.

La eficiencia de la extracción proporcionada por el método depende del tipo de especie, siendo alrededor del 95% para las especies trihalogenadas (VOCs, THMs, TCNM y TCAN), del 85% para las dihalogenadas (DCNM, BCNM, DBNM, DCAN, BCAN y DBAN) y del 60% para las especies monohalogenadas (CNM, BNM, CAN y BAN). La menor eficiencia de las especies monohalogenadas se debe a su alta polaridad y solubilidad en agua en comparación con las otras especies. De todos modos, como los analitos comúnmente detectados en estas muestras son los tri- y dihalogenados, este dato no es muy relevante.

2.2. Técnica de separación/detección: GC–MS

Dado que los compuestos objeto de estudio de esta Memoria son compuestos orgánicos volátiles, la separación de los mismos se ha realizado empleando la cromatografía de gases.

La temperatura del bucle y de la interfase en el caso de la técnica de espacio de cabeza se mantuvo a 100 y 110 ºC, respectivamente en todas las metodologías. Las técnicas de microextracción se han llevado a cabo recogiendo los extractos (SDME) e inyectando 2 µL en un inyector convencional (modo *split*, 1:20), o 30 µL en un inyector LVI–PTV. En todas las metodologías se usó el inyector a una temperatura de 170 ºC para minimizar/evitar la descomposición de ciertos analitos como se ha explicado en el punto 1.4 de este Capítulo, a excepción del uso a 250 ºC en el método desarrollado para THMs yodados en bebidas en el cual no se incluyen los compuestos termolábiles.

Las columnas cromatográficas empleadas fueron convencionales no polares (TRB-5, SLB-5MS, HP-5MS o HP-5MS UI) con una fase estacionaria compuesta por 5%-fenil–95%-metilpolisiloxano. El programa de temperatura utilizado en cada método varió en función de los analitos a

separar, oscilando el intervalo de temperaturas entre 40 y 180 ºC en todos los casos a excepción del método que incluye los THMs yodados que varió entre 40 y 250 ºC. El gas portador empleado fue helio a un caudal de 1 mL/min.

Por otro lado para la detección de este tipo de compuestos, la espectrometría de masas con analizador cuadrupolar ha sido la técnica utilizada a lo largo de todo el desarrollo experimental de la presente Memoria. La temperatura de la fuente de ionización se mantuvo entre 200 y 250 ºC según las especies determinadas. El voltaje aplicado en la fuente de ionización para la fragmentación de las moléculas fue de 70 eV, y el vacio mantenido durante todos los trabajos fue de 30 a 40 mTorr. La optimización de los experimentos se realizó en la modalidad *full scan* realizando barridos desde la relación *m/z* 29 hasta la relación *m/z* 300, a excepción de la metodología que incluye otros compuestos orgánicos volátiles halogenados y THMs yodados que se realizó desde la relación *m/z* 25 hasta la relación *m/z* 400. Una vez que los analitos fueron identificados y sus tiempos de retención establecidos, para aumentar la sensibilidad del método se usó el modo SIM (selected ion monitoring). En este modo el espectrómetro de masas no realiza barridos a lo largo de un intervalo consecutivo de relaciones *m/z*, sino que sólo registra una serie de iones o relaciones *m/z* de interés que han sido seleccionadas previamente. De este modo como sólo se registra un número muy reducido de iones, el tiempo durante el que se registra cada ión aumenta considerablemente aumentando a su vez la sensibilidad del método. En modo SIM, se utiliza habitualmente 3 relaciones *m/z* características de cada analito, en esta Memoria se han seleccionado iones característicos como pueden ser el ion 46 común en los halonitrometanos, que corresponde al fragmento NO_2 y en la mayoría de los otros compuestos iones característicos de la existencia de isótopos de los halógenos con dos unidades más de masas.

2.3. Estudio comparativo de las metodologías desarrolladas

A continuación se realiza una comparación entre los métodos descritos anteriormente, en la **Tabla 3** se muestran los valores medios de los parámetros analíticos más relevantes de las metodologías propuestas, como son los LODs y RSD medios de las especies determinadas.

Tabla 3. Características analíticas (valores medios) de las metodologías desarrolladas en el análisis de agua

Método	LOD (µg/L)	RSD (%)	Especies
SDME	0.5	8.2	9 HNMs
MLLE/LVI–PTV	0.06	6.0	9 HNMs + 4 THMs
HS	0.1	5.8	9 HNMs + 4 THMs + 6 HANs

Como se observa en la **Tabla 3**, los métodos de HS y MLLE/LVI–PTV proporcionaron los LODs medios más bajos. Aunque la alta sensibilidad en la metodología MLLE se debe a la inyección de elevados volúmenes de extracto (30 µL) por lo que no es realmente comparable. En el caso de la precisión del método, los valores de RSDs proporcionados son muy similares, aunque ligeramente superior en la modalidad SDME debido a la dificultad de trabajar con una gota de extractante. La metodología de HS no necesita etapa de preconcentración lo que permite acortar el tiempo de análisis y simplificar el procedimiento analítico, además es en la que mayor número de analitos se ha estudiado. Por ello la modalidad HS se plantea como una alternativa simple, rápida, robusta y automática, siendo por tanto la más empleada en la presente Memoria.

3. Aplicaciones

3.1. Determinación de DBPs volátiles en aguas tratadas y ambientales

A lo largo de esta Memoria se han desarrollado distintos métodos, expuestos en los Capítulos 3 y 4, para la determinación de DBPs volátiles en muestras de aguas, fundamentalmente tratadas. En el desarrollo de nuevas metodologías es necesario asegurar la trazabilidad de los

resultados, para ello los métodos fueron validados con un método normalizado, ya que no existen materiales de referencia certificados. Por ello, en todos los casos los métodos desarrollados se validaron con el método de la U.S. EPA 551.1, que incluye a 9 de los analitos estudiados en esta Memoria.

Las metodologías propuestas en los Capítulos 3 y 4 se han evaluado mediante el análisis de muestras de grifo y de piscina. El método HS se ha empleado además para determinar HNMs en muestras ambientales de ríos, estanques y lagos, aunque las muestras dieron resultados negativos en todos los casos. Los resultados derivados del análisis de otros tipos de muestras de agua a lo largo de la Tesis Doctoral se muestran en la **Tabla 4**. La influencia de algunos parámetros cualitativos del agua y del tipo de desinfectante usado ha sido estudiada para ver su efecto sobre la concentración y especiación de DBPs volátiles. Los resultados demuestran que se pueden encontrar entre 6 o 10 especies a niveles de µg/L dependiendo del contenido de materia orgánica, concentración de bromuro y tratamiento de desinfección empleado.

Se estudiaron muestras de aguas con diferentes contenidos de materia orgánica (utilizando el parámetro de oxidabilidad al permanganato), obteniéndose una concentración total de DBPs volátiles entre 6 y 42 µg/L para valores de oxidabilidad de entre 0.1 y 2.0 mg O_2/L, respectivamente. Lo que demuestra que la concentración de DBPs está directamente relacionada con la cantidad de materia orgánica presente en el agua. En relación al tratamiento de desinfección, como se observa en la **Tabla 4**, la concentración de THMs no se ve influenciada por el tipo de desinfectante; en cambio para el caso de N-DBPs si es un parámetro influyente. Se observa que las concentraciones de TCNM se incrementan cuando se incluye cloraminas como tratamiento de desinfección, debido seguramente a la contribución de nitrógeno por parte de las cloraminas. Este incremento es más acusado cuando se emplea O_3, donde las concentraciones se duplican y se forma DCNM. Por otro lado, las concentraciones de HANs (DCAN y BCAN) son más elevadas en el caso del empleo de Cl_2/NH_2Cl debido como hemos dicho anteriormente al aporte de nitrógeno por parte del desinfectante y que el uso de ClO_2 y O_3 antes de la cloración reduce su concentración. Finalmente destacar que la aparición de los compuestos

bromados como son el TBM, DBNM y DBAN, así como el aumento de concentración del DBCM y del BCAN en las aguas desinfectadas con ClO_2/Cl_2 no son debido al desinfectante, sino a que estas aguas contenían una mayor concentración de bromuro. La concentración de bromuro influencia la distribución de las especies cloradas/bromadas, siendo la concentración de los DBPs bromados alrededor de un 70% mayor en estas aguas.

Tabla 4. Análisis de muestras de aguas tratadas

Muestra (proceso de desinfección)	n[a]	Analitos			
		Trihalometanos (µg/L)			
		TCM	BDCM	DBCM	TBM
Grifo (Cl_2)	6	3.2–36[b]	1.8–13	1.3–3.1	N.D.[c]
Grifo (ClO_2/Cl_2)	9	2.1–21	1.6–12	1.0–19	N.D.–8.8
Grifo (Cl_2/NH_2Cl)	3	8.7–17	5.9–10	2.5–3.8	N.D.
Grifo (O_3/Cl_2)	3	19–22	9.5–12	3.2–4.4	N.D.
Piscina (Cl_2)	2	56,68	4.5,16	N.D.	N.D.
Piscina (Br_2)	1	6.6	3.6	5.7	11
		Halonitrometanos (µg/L)			
		TCNM	DCNM	DBNM	
Grifo (Cl_2)	30	<LOQ–3.0	N.D.	N.D.	
Grifo (ClO_2/Cl_2)	9	N.D.–<LOQ	N.D.	<LOQ–1.7	
Grifo (Cl_2/NH_2Cl)	3	0.9–1.8	N.D.	N.D.	
Grifo (O_3/Cl_2)	6	3.5–4.3	N.D.–1.5	N.D.	
Piscina (Cl_2)	13	0.4–2.4	N.D.	N.D.	
Piscina (Br_2)	1	0.5	N.D.	N.D.	
		Haloacetonitrilos (µg/L)			
		DCAN	BCAN	DBAN	
Grifo (ClO_2/Cl_2)	9	0.4–2.1	0.2–6.3	N.D.–1.6	
Grifo (Cl_2/NH_2Cl)	3	3.3–4.3	0.6–0.9	N.D.	
Grifo (O_3/Cl_2)	3	1.1–1.6	0.2–0.5	N.D.	

[a]n, número de muestras analizadas. [b]Concentración mínima y máxima encontrada. [c]N.D., no detectado.

Los niveles encontrados en piscina para THMs fueron mayores que en agua de grifo debido a las mayores dosis de cloro empleadas y al aporte extra de materia orgánica de los bañistas u otras fuentes. Además se puede observar que la especiación de estos compuestos depende de si la piscina se clora o broma, siendo las especies predominantes el cloroformo o el bromoformo, respectivamente. Sorprendentemente, la concentración de TCNM encontrada en piscinas es similar a la que se encuentra en grifo, esto puede ser debido a la baja reactividad del cloro con la materia orgánica natural para producir HNMs tal como se describe en la bibliografía.

3.2. Control de DBPs volátiles en una planta de potabilización

La aparición de DBPs en las aguas tratadas varía de acuerdo a la calidad del agua de origen (precursores orgánicos e inorgánicos y parámetros cualitativos) y las operaciones llevadas a cabo en la planta de tratamiento (dosis de desinfectante, tiempo de reacción entre los precursores y el tipo de desinfectante). Se ha evaluado la formación/eliminación de DBPs volátiles en diferentes puntos de una planta de tratamiento de agua potable (que emplea dióxido de cloro y cloraminas como desinfectantes) y en su red de distribución. Así, se ha podido establecer la incidencia y perfiles de distribución de estos compuestos desde el agua bruta hasta la red. Además, se ha realizado el estudio a lo largo del año para estudiar los efectos de los cambios estacionales en la concentración de estos DBPs. Para ello se han recogido muestras en siete puntos distintos en cada estación midiendo paralelamente los parámetros cualitativos del agua. El análisis de las muestras de agua se llevó a cabo por HS–GC–MS y las especies incluidas fueron 10 THMs (incluyendo 6 especies yodadas), 6 HNMs y 6 HANs. La **Figura 4** muestra el esquema de tratamiento de la planta de potabilización y la localización de los 7 puntos de recogida de las muestras (el periodo de estudio se realizó entre mayo de 2013 y febrero de 2014).

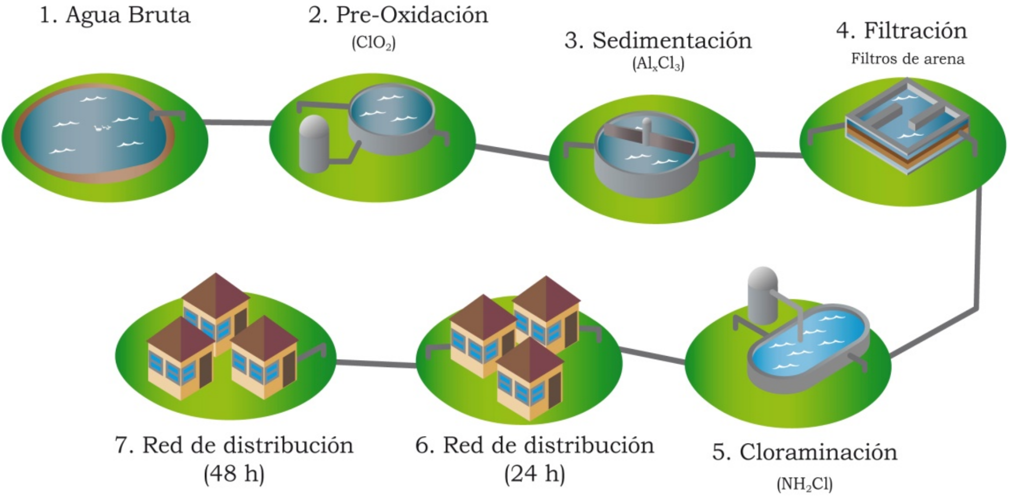

Figura 4. Representación esquemática de los puntos de muestreo de la planta de potabilización y red de distribución.

Con vistas a establecer el número de muestras representativas dentro de cada estación, se realizó inicialmente un estudio de la variabilidad entre días secuenciales. Para ello se analizaron muestras de los diferentes puntos de muestreo durante siete días consecutivos en primavera (abril de 2013) por tratarse de una estación intermedia desde el punto de vista climatológico. Las concentraciones de DBPs variaron poco dentro de una semana, por lo que el seguimiento de DBPs en el agua durante un mes se puede realizar muestreando un día cada semana debido a su representatividad. En la **Tabla 5** se recopilan los datos de las concentraciones de DBPs en las diferentes etapas de la planta de potabilización durante mayo de 2013. Como se observa ningún compuesto se detectó en el agua bruta lo que reafirma que estos subproductos provienen exclusivamente del proceso de desinfección. En la etapa de pre-oxidación solo cloroformo aparece, probablemente porque su formación es más rápida que la de las otras especies o porque requiere menor dosis de desinfectante o materia orgánica para su formación.

Tabla 5. Concentración de DBPs volátiles en la planta de potabilización en mayo de 2013

Puntos de muestreo	Analitos						
	TCM	BDCM	DBCM	DCIM	TCNM	DCAN	BCAN
Agua bruta	N.D.[a]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Pre-oxidación	0.32–0.60[b]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sedimentación	1.2–1.7	0.42–0.60	N.D.	N.D.	0.15–0.37	0.12–0.22	N.D.
Filtración	2.1–2.6	0.69–0.84	N.D.	N.D.	0.18–0.73	0.14–0.36	N.D.
Cloraminación	7.3–8.6	1.9–2.5	0.23–0.48	0.12–0.24	0.62–1.3	0.37–1.2	<0.15[c]
Sistema de distribución (24 h)	8.5–9.4	2.2–2.9	0.32–0.62	0.14–0.25	0.68–1.4	0.45–1.5	0.17–0.21
Sistema de distribución (48 h)	10–12	2.5–3.4	0.41–0.82	0.14–0.26	1.0–2.4	0.86–1.3	0.18–0.31

[a]N.D. no detectado, [b]Concentración mínima y máxima encontrada, [c]<LOQ.

En la etapa de sedimentación se forman el BDCM, TCNM y DCAN, mientras la concentración de TCM aumenta unas 3.5 veces debido a que el dióxido de cloro sigue reaccionando con la materia orgánica; en la etapa de filtración aumentan estos compuestos alrededor de un 40–70%, lo cual está de acuerdo con la bibliografía de que los filtros de arena no eliminan estos compuestos. En la segunda etapa de desinfección con cloraminas aumentan aún más las concentraciones de los analitos ya presentes en el agua alrededor de 2.5–3.5 veces con respecto a la etapa anterior, además tres nuevas especies se forman (DBCM, DCIM y BCAN). La formación de estos nuevos compuestos en esta etapa puede deberse a que necesitan una mayor cantidad de desinfectante (2.2 mg/L de cloraminas frente a 1.0 mg/L de dióxido de cloro) para su formación o el uso de cloraminas como desinfectante ya que implica otras reacciones de sustitución como en el caso de los compuestos yodados y nitrogenados como ha sido descrito en la

bibliografía. En las etapas que corresponden a la red de distribución, todas las concentraciones aumentan con el tiempo excluyendo DCIM que se mantiene constante.

En la **Tabla 6** se muestran los datos de las concentraciones de DBPs a la salida de la planta de potabilización y en la red de distribución en las cuatro estaciones del año. Se puede observar que todas las especies (excluyendo al DCIM que se encuentra a muy baja concentración) aumentan a las 24 h en la red de distribución alrededor de un 45–80% en verano y un 10–30% en el resto de estaciones; esto es debido a que las altas temperaturas favorecen las reacciones de formación de estos compuestos. Por otra parte, a las 48 h en la red de distribución, las concentraciones de los THMs aumentan en verano alrededor de un 70–85% y en las otras estaciones alrededor de un 20–40%, mientras en el caso de los N-DBPs también se incrementan pero en un menor porcentaje en verano siendo alrededor de un 10% y de un 20–30% en las otras estaciones. Se puede concluir que los DBPs volátiles incrementan sus concentraciones al aumentar la temperatura y con el tiempo en la red de distribución como consecuencia de que el desinfectante residual sigue reaccionando con la materia orgánica presente en el agua. Otra observación en el agua a la salida de la planta de potabilización, es que las concentraciones de los DBPs volátiles son diferentes en función de la estación del año. Así la concentración total media de THMs es mayor en primavera (10 µg/L), seguida del verano (8 µg/L), otoño (6 µg/L) e invierno (4 µg/L). Estos resultados concuerdan con los valores de la oxidabilidad al permanganato (relacionada con la materia orgánica), que es mayor en el agua bruta en primavera (5.7 mg O_2/L) que en invierno (3.5 mg O_2/L). Por lo tanto, la oxidabilidad al permanganato puede usarse como indicador en la formación de THMs.En el caso de los N-DBPs es diferente, así la concentración total media es mayor en verano (4 µg/L) que en primavera (2 µg/L); la diferencia puede deberse a la mayor temperatura en verano en relación con las otras estaciones. La presencia de los DBPs bromado es importante debido a su mayor toxicidad, como se observa en la **Tabla 6**, los DBPs bromados (DBCM y BCAN) se encuentran en mayores concentraciones en primavera y otoño debido a que la concentración de bromuro es mayor en estas estaciones (0.06 y 0.04 mg/L, respectivamente) que en las otras (<0.01 mg/L).

Tabla 6. Concentración de DBPs volátiles en aguas potables después de la cloraminación y en diferentes puntos de la red de distribución (24 y 48 h) en cada estación (2013–2014)

Puntos de muestreo	Analitos	Primavera	Verano	Otoño	Invierno
Cloraminación	TCM	7.3–8.6[a]	5.5–6.5	3.8–4.5	2.1–3.2
	BDCM	1.9–2.5	1.5–2.3	0.95–1.6	0.87–1.3
	DBCM	0.23–0.48	<0.05[b]–0.10	0.22–0.33	0.10–0.15
	DCIM	0.12–0.24	0.13–0.22	<0.07–0.16	<0.07
	TCNM	0.62–1.3	1.7–2.3	0.50–1.2	0.47–1.1
	DCAN	0.37–1.2	1.3–2.6	0.42–0.80	0.44–0.85
	BCAN	<0.15	<0.15	<0.15	<0.15
Sistema de distribución (24 h)	TCM	8.5–9.4	8.4–11	4.3–5.8	2.8–4.2
	BDCM	2.2–2.9	2.2–3.0	1.2–2.1	1.0–1.5
	DBCM	0.32–0.62	<0.05–0.21	0.28–0.39	0.12–0.23
	DCIM	0.14–0.25	0.16–0.27	0.11–0.17	<0.07–0.10
	TCNM	0.68–1.4	2.5–3.4	0.72–0.91	0.53–1.1
	DCAN	0.45–1.5	1.4–7.1	0.51–0.92	0.59–0.88
	BCAN	0.17–0.21	<0.15	0.16–0.70	<0.15
Sistema de distribución (48 h)	TCM	10–12	14–20	5.5–6.3	3.5–4.6
	BDCM	2.5–3.4	3.2–5.5	1.5–2.6	1.2–1.8
	DBCM	0.41–0.82	0.15–0.37	0.33–0.65	0.18–0.32
	DCIM	0.14–0.26	0.19–0.33	0.10–0.16	0.09–0.13
	TCNM	1.0–2.4	2.4–3.7	0.81–1.2	0.76–1.3
	DCAN	0.86–1.3	1.5–6.8	0.86–1.3	0.82–1.1
	BCAN	0.18–0.31	<0.15	0.25–0.43	<0.15

[a]Concentración mínima y máxima encontrada, [b]<LOQ.

3.3. Determinación de THMs (clorados, bromados y yodados) y VOCs en bebidas (refrescos y zumos de frutas)

En esta última parte del Capítulo se discuten los aspectos más relevantes sobre el estudio de 10 THMs y 4 VOCs (diclorometano, 1,2-dicloroetano, tetracloruro de carbono y 1,2-dibromoetano) en bebidas (refrescos y zumos de frutas). Tanto los 4 THMs comunes como los VOCs están regulados en aguas potables y por lo tanto pueden estar presentes en estas bebidas. La metodología empleada para la determinación de estos compuestos en estas matrices se ha descrito en el Capítulo 5 de esta Memoria.

El agua usada como agua de bebida o la usada en un amplio rango de aplicaciones industriales alimentarias es frecuentemente desinfectada antes de su uso. El empleo de esta agua tratada en la industria alimentaria aporta contaminantes químicos a las comidas y bebidas, como son los DBPs y algunos VOCs. Los DBPs se forman durante la desinfección del agua por reacción del desinfectante con los precursores orgánicos e inorgánicos presentes en la misma, mientras que los VOCs pueden estar presentes en las aguas subterráneas como resultado de la polución industrial. Estos compuestos en las bebidas pueden aparecer por diferentes vías, (a) inclusión de agua potable como ingrediente en la producción de éstas; (b) acumulación y sorción del agua retenida en los envases que se han desinfectado/lavado; (c) contaminación por contacto con limpiadores/desinfectantes clorados utilizados en la línea de procesamiento de la bebida, en el almacenamiento y/o comercialización; y (d) formación durante la preparación de la bebida debido a reacciones entre el cloro residual del agua y precursores presentes en el alimento, como son los hidratos de carbono, lípidos y proteínas.

En el Capítulo 5 se ha descrito el primer método desarrollado hasta la fecha para la determinación de THMs yodados además de los 4 THMs comunes y 4 VOCS en bebidas. El método se caracteriza por una elevada sensibilidad ya que presenta LODs entre 8 y 100 ng/L. La validación del método para diferentes muestras se ha evaluado mediante estudios de recuperación en muestras fortificadas a dos niveles de concentración. Se estudiaron muestras de distinta naturaleza: refrescos (tónicas y bebidas

carbonatadas) y zumos de frutas (manzana, piña, naranja y melocotón). Los porcentajes de recuperación medios están comprendidos entre el 90 y 97%.

El método propuesto se aplicó a 100 tipos de muestras: 40 refrescos (té, isotónica, bebida de fruta, tónica y bebida carbonatada) y 60 zumos de frutas (zumos naturales 100%, zumos reconstituidos y néctares). No se detectó ninguno de los VOCs estudiados ya que no estaban presentes o se encontraban a concentraciones inferiores a su LOD. Todas las muestras dieron positivas conteniendo hasta 5 THMs (por primera vez se detectó dicloroiodometano, DCIM), en diferentes distribuciones y concentraciones. Las especies TCM, BDCM se detectaron en prácticamente todas las muestras y a mayores concentraciones que los otros DBPs, debido seguramente a que el agua utilizada en estas aplicaciones alimentarias es habitualmente desinfectada con cloro debido a su menor precio y sencillo empleo. En la **Tabla 7** se muestran los resultados obtenidos en el análisis de 40 refrescos y 60 zumos de frutas. Las muestras de refrescos se pueden agrupar en dos grupos de acuerdo a las concentraciones totales media de THMs. El grupo A con una concentración <15 μg/L (n=15, valor medio de 5.5 ± 2.8 μg/L) y el grupo B con una concentración ≥15 μg/L (n=25, valor medio de 28.9 ± 9.1 μg/L). Se concluye del estudio de todas estas muestras, que la concentración no depende del tipo de refresco (té, tónica, bebida carbonatada, etc.), ni del tipo de sabor (limón, naranja, cola, etc.) y que la presencia de THMs se debe al tipo de agua empleada en su preparación en cada momento.

Tabla 7. Concentraciones de THMs (µg/L) encontradas en refrescos y zumos de frutas

Muestra (n[a])	TCM	BDCM	DBCM	DCIM	TBM	TTHMs[e]
Té (3)	1.1–27[b]	0.51–3.5	0.23–0.73	N.D.[c]	N.D.–<0.07[d]	1.8–30
Isotónica (4)	1.8–31	0.18–4.6	0.08–0.76	N.D.–<0.07	N.D.–<0.07	2.1–35
Bebida de fruta (6)	5.1–23	0.44–1.4	<0.05–0.20	N.D.	N.D.–<0.07	5.5–25
Tónica (3)	3.8–20	0.39–2.1	<0.05–0.32	N.D.	N.D.	4.2–21
Bebida Carbonatada (24)	1.0–35	0.09–7.8	0.05–1.3	N.D.–0.13	N.D.–0.17	1.4–52
Zumo natural 100% (7)	0.20–0.60	N.D.–0.23	N.D.	N.D.	N.D.	0.20–0.83
Zumo reconstituido (21)	N.D.–10	N.D.–3.7	N.D.–3.3	N.D.	N.D.–0.50	0.19–13
Néctar (32)	N.D.–18	N.D.–5.3	N.D.–2.9	N.D.	N.D.–0.51	0.38–38

[a]número de muestras analizadas, [b]Concentración mínima y máxima encontrada, [c]N.D. no detectado, [d]<LOQ, [e]concentraciones totales media de THMs.

Los zumos de frutas también se pueden clasificar en dos grupos distintos de acuerdo a los niveles de las concentraciones totales de THMs. En zumos reconstituidos, el grupo A contiene <1 µg/L (n=10, valor medio de 0.5 ± 0.2 µg/L) y el grupo B contiene >2 µg/L (n=11, valor medio de 6.2 ± 3.6 µg/L). Con respecto a los néctares, el grupo A contiene concentraciones totales medias de THMs <6 µg/L (n=17, valor medio de 2.6 ± 1.9 µg/L) y el grupo B >6 µg/L (n=15, valor medio de 14.7 ± 8.7 µg/L). El estudio concluye que las concentraciones totales de THMs no depende del tipo de fruta, pero si del contenido de agua. Así, las concentraciones totales medias de THMs para zumos naturales 100%, zumos reconstituidos, néctares y refrescos son de 0.47, 3.5, 8.3 y 20 µg/L, respectivamente, poniendo de manifiesto que dependen del volumen de agua tratada incluida

en su elaboración. Así el DCIM presente a muy bajas concentraciones en el agua tratada solo se detectó en los refrescos lo cual se adscribe a su mayor contenido de agua tratada para su elaboración en comparación con los otros productos.

Finalmente para corroborar que la concentración total de THMs presente en las bebidas se relaciona con la concentración existente en el agua potable empleada en su elaboración, se han agrupado diferentes bebidas por fabricante. Como modelo se ha escogido los néctares por su mayor contenido en agua (50–75%). Como se observa en la **Tabla 8** donde están representados los valores medios de la concentración total de THMs con su respectiva empresa codificada (A–J). La concentración media de la concentración total de THMs en los néctares producidos por las empresas donde el número de muestras es mayor (A, B, C, F y H) varió entre 6 y 8 μg/L para las empresas A, B, C y H, mientras que el valor es de 22.4 μg/L para la empresa F. Así los néctares de la empresa F siempre contenían mayores concentraciones de THMs, esto se puede explicar en base al tipo de agua tratada empleada por dicha empresa para la fabricación del producto. Es importante resaltar que en ningún caso estas bebidas contenían concentraciones totales de THMs superiores a los máximos establecidos en Europa para agua potables (100 μg/L).

Tabla 8. Concentraciones medias totales (µg/L) de THMs en néctares de distintas empresas

Empresa	n[a]	TTHMs[b]
A	4	6.1
B	6	6.2
C	5	7.9
D	3	3.9
E	1	0.38
F	5	22.4
G	2	0.73
H	4	7.9
I	1	3.5
J	1	3.7

[a]n, número de muestras; [b]TTHMs, concentración total de THMs.

Estos compuestos no solo aparecen por la inclusión de agua tratada como parte de un ingrediente de la bebida sino que pueden aparecer debido a la desinfección con productos clorados de la línea de preparación y envasado de la bebida, con lo que pueden quedar alícuotas de agua que podrían ser una fuente de contaminantes de THMs. Para aclarar esta cuestión se han analizado zumos naturales 100% exprimidos (bebidas que no poseen agua tratada como ingrediente) y refrescos elaborados solo con agua mineral (no contienen DBPs) en los que sin embargo se han detectado trihalometanos clorados aunque a muy bajas concentraciones (<1 µg/L). Hay que recordar que como se ha indicado en la **Tabla 7**, los otros tipos de bebidas preparadas con agua potable contienen además de trihalometanos

clorados, THMs bromados y dicloroidometano que pueden utilizarse como indicadores de la presencia de agua tratada. Con lo que se concluye que: (a) la fuente principal de la presencia de THMs en zumos de frutas reconstituidos, néctares y refrescos es el agua tratada que se incluye como ingrediente; (b) la concentración y la especiación dependen del volumen de agua añadido y del proceso de desinfección de esa agua; (c) los zumos naturales 100% pueden contener THMs clorados procedentes del lavado de los envases aunque a niveles traza <1 μg/L.

Finalmente, se analizaron zumos reconstituidos de diferentes frutas procedentes de otros países Europeos (Francia, Polonia, Bélgica y Portugal). También existen dos grupos bien diferenciados, uno con una concentración media total de THMs de 0.4 ± 0.2 μg/L y el otro de 9.9 ± 8.2 μg/L, similar a los zumos españoles. Por lo tanto se puede concluir que no existe diferencias en estas bebidas entre países europeos.

Este estudio permite evaluar la exposición diaria a los THMs si tenemos en cuenta que una persona puede consumir 2 L de agua tratada al día (esto incluye además la bebida consumida en forma de zumos y otras bebidas que contienen agua tratada). Si se tiene en cuenta que la muestra de refresco más contaminada contenía 52 μg/L del total de THMs, una persona podría ingerir más de 100 μg de THMs totales por día. Además este problema se agravaría si tenemos en cuenta que 2 L al día no es una cantidad representativa, ya que depende de la población, clima y actividad física, lo que puede variar la media de litros consumidos diarios entre 3.8 y 4.8. Por otra parte, aunque los THMs clorados son los compuestos más comunes presentes en las muestras, algunas muestras contenían concentraciones significativas de compuestos bromados y yodados, siendo estos compuestos más citotóxicos y genotóxicos que sus análogos clorados. En nuestra opinión esta exposición es significativa teniendo en cuenta que hay otras fuentes de exposición como es a través de la comida. Por lo tanto sería conveniente que se controlara la concentración de THMs en bebidas al igual que se hace en aguas potables, y establecer niveles máximos de concentraciones similares a los del agua potable (100 μg/L).

CONCLUSIONES

CONCLUSIONS

A lo largo de esta Memoria se ha seguido una línea de investigación centrada en la determinación de DBPs volátiles en aguas. Las innovaciones principalmente se han centrado en el desarrollo de metodologías rápidas para la determinación de N-DBPs (HNMs y HANs) debido a la inexistencia de métodos en la bibliografía, junto a otros DBPs comunes como son los THMs pero con la novedad de incluir especies yodadas. Como colofón a estas metodologías cabe resaltar el estudio de la presencia de THMs en bebidas refrescantes y zumos de frutas como marcadores de la presencia de agua tratada en las mismas. A continuación se presentarán, de manera resumida, los resultados más relevantes obtenidos en esta Tesis Doctoral:

1. Se ha estudiado las condiciones cromatográficas para evitar/minimizar la descomposición de los HNMs durante su calentamiento en el puerto de inyección del GC y en la fuente de ionización del espectrómetro de masas. El estudio revela que temperaturas entre 200 y 250 ºC en la fuente de ionización no produce la descomposición de ningún HNM, sin embargo temperaturas superiores a 170 ºC en el inyector no son validas para evitar la descomposición de las especies trihalogenadas de los HNMs.

2. Se ha desarrollado el primer método para la determinación de los 9 HNMs clorados y bromados en aguas basado en HS–SDME/GC–MS. Esta técnica de microextracción, ofrece la ventaja de que todo el extracto (~2 μL) es inyectado en el GC sin producir residuos, en consonancia con la tendencia actual de una “Química Verde”.

3. En una segunda etapa, se ha desarrollado un método rápido, simple, robusto y sensible para la determinación de las mismas especies en aguas potables por HS–GC–MS que permite la volatilización de los analitos en una sola etapa simplificando de esta manera el tratamiento de la muestra. Se han estudiado diferentes variables implicadas en la volatilización de estas especies, siendo la adición de alícuotas de MTBE como modificador orgánico la que incrementó el rendimiento de la volatilización en aproximadamente 4 veces en relación a su omisión. Además se pone de manifiesto por primera vez que no existen diferencias significativas en la concentración de

tricloronitrometano (el único HNM encontrado en las aguas tratadas) entre aguas de grifo y de piscina, a pesar de que la concentración de cloro residual libre y de materia orgánica es superior en la de piscina. Esto se debe a que la formación de HNMs no es muy dependiente de la materia orgánica presente en estas piscinas sino de otros precursores nitrogenados.

4. Se ha realizado un estudio riguroso de la estabilidad de los 9 HNMs en presencia de varias sales como agentes de decloración, siendo el $(NH_4)_2SO_4$ la que proporcionó una ligera ventaja. Por primera vez se demuestra que no son sales la mejor vía de conservación de aguas potables para la determinación de DBPs nitrogenados sino que es la acidificación de la muestra a pH 3.0–3.5 en el momento de la recogida, lo que asegura su preservación durante 48 horas a 4 °C.

5. Se ha diseñado un nuevo método de microextracción líquida (MLLE) en el que los problemas inherentes a las técnicas de microextracción con gota se soslayan. El método propuesto incluye además de los 9 HNMs los 4 THMs comunes. Mediante la miniaturización de la LLE convencional se obtienen rendimientos de extracción del 76% y con la importante novedad de que se emplean extractantes convencionales (*n*-hexano, acetato de etilo o MTBE). Casi todo el extracto obtenido (30 μL) se inyecta en un LVI–PTV–GC–MS lo que proporciona dos ventajas importantes frente a la inyección convencional (1–2 μL): mayor sensibilidad y apenas residuos, en consonancia con una “Química Verde”.

6. Se ha extendido el método más simple y robusto (HS–GC–MS) a nuevas especies nitrogenadas (HANs). La metodología para la determinación de 4 THMs, 6 HNMs y 6 HANs se ha usado para la evaluación del impacto de algunos parámetros cualitativos del agua potable y procesos de desinfección en la formación y especiación de estos compuestos. Los resultados sugirieron que la concentración de DBPs está directamente relacionada con la cantidad de materia orgánica (contrastada con el parámetro de oxidabilidad al permanganato), así como la concentración de bromuro influencia la

distribución de las especies cloradas/bromadas, siendo la concentración de los DBPs bromados alrededor de un 70% mayor en aguas tratadas que contienen una concentración alta de bromuro. El tipo de desinfectante también está relacionado con la formación y especiación de los DBPs, la concentración de THMs no se ve influenciada por los tipos de desinfectantes estudiados en esta Memoria; en cambio para el caso de N-DBPs si es un parámetro influyente. Las concentraciones de TCNM se incrementaron cuando se utilizó cloraminas como desinfectante; este incremento es más acusado cuando se emplea ozono, donde las concentraciones se duplican y se forma además DCNM. Por otro lado, las concentraciones de HANs (DCAN y BCAN) son más elevadas en el caso del empleo de cloraminas que con el uso de dióxido de cloro u ozono que reduce su concentración.

7. Se han comparado todos los resultados obtenidos por los nuevos métodos desarrollados a lo largo de la Memoria para la determinación de DBPs volátiles, con aquellos obtenidos por un método bien establecido por la USEPA (método EPA 551.1) que emplea LLE aunque propuesto para la determinación de 4 THMs, TCNM y 4 HANs en aguas potables. Las conclusiones más relevantes de este estudio comparativo demuestra las ventajas de las metodologías propuestas frente al método EPA en términos de: a) incrementos de 2 a 5 veces de la sensibilidad y b) menor consumo de reactivos y disolventes. Otras características analíticas como las recuperaciones (>90%) y la precisión (~6–7%) fueron similares.

Finalmente, los métodos por HS–GC–MS resultaron ser los más simples, robustos y sensibles, y por ello se aplicaron a la determinación de THMs (incluyendo nuevas especies yodadas), HNMs y HANs en aguas y bebidas.

8. Se ha evaluado la distribución de 10 THMs, 6 HNMs y 6 HANs a lo largo de los distintos procesos de una planta de potabilización (que emplea dióxido de cloro y cloraminas como desinfectantes) y en la red de distribución durante las cuatro estaciones del año. De este estudio se obtienen las siguientes conclusiones: a) ningún compuesto se detectó en el agua bruta lo que reafirma que estos subproductos provienen exclusivamente del proceso de desinfección; b) en la etapa de pre-oxidación con dióxido de cloro solo se detecta TCM; c) después de la sedimentación se forman BDCM, TCNM y DCAN, mientras el TCM aumenta su concentración ~3.5 veces; d) en la etapa de filtración aumentan las concentraciones de estos compuestos alrededor de un 40–70%; e) después de la segunda etapa de desinfección con cloraminas, los analitos anteriormente detectados incrementan sus concentraciones alrededor de 2.5–3.5 veces más y se forman tres nuevas especies como son el DBCM, DCIM y BCAN; f) todos los compuestos aumentan su concentración a lo largo de la red de distribución, dado que el desinfectante residual sigue reaccionando con la materia orgánica a lo largo del tiempo (excepto para el DCIM que se mantiene constante) con mayor énfasis en las estaciones calurosas; y g) los DBPs bromados (DBCM y BCAN) se encontraron a mayores concentraciones en primavera y otoño dada la mayor concentración de bromuros presentes en estas estaciones. Por otra parte se ha contado con la colaboración de otros becarios del grupo de investigación para evaluar sistemáticamente además de los DBPs volátiles diana de esta Memoria, otros DBPs como los ácidos haloacéticos y aldehídos alifáticos y aromáticos.

9. Se ha estudiado la posible aparición de varias especies orgánicas volátiles (10 THMs y 4 VOCs), que están limitadas en aguas potables, en bebidas de amplio consumo. Las bebidas seleccionadas han sido refrescos y zumos de frutas por incluir agua potable en su elaboración o entrar en contacto con dicha agua durante el proceso de elaboración. Este estudio ha puesto de manifiesto que el cloroformo puede aparecer a concentraciones inferiores a 1 μg/L en bebidas (refrescos preparados con agua mineral o zumos naturales 100% exprimidos) que no contienen agua tratada, como

consecuencia de la desinfección con productos clorados de la línea de preparación y envasado de la bebida, donde pueden quedar alícuotas de agua que podrían ser una fuente de contaminación. Las bebidas preparadas con diferentes cantidades de agua potable (refrescos, zumos reconstituidos y néctares), contienen además de trihalometanos clorados, THMs bromados y dicloroidometano (detectado en refrescos por primera vez), que pueden utilizarse como marcadores de la presencia de agua tratada en estas muestras. Además se ha demostrado que la principal fuente de la presencia de THMs en este tipo de bebidas es la inclusión de agua tratada como ingrediente en la elaboración del producto. La concentración y especiación de los compuestos depende del volumen de agua añadido y de la fuente de agua empleada.

All through this Report a line of investigation has been followed focused on the determination of volatile DBPs in water. Innovations have been mainly aimed to the development of fast methodologies for the determination of N-DBPs (HNMs and HANs), because of the lack of them in the bibliography along with other common DBPs such as THMs but with the inclusion of iodine species as a novelty. It is worth highlighting the study of the presence of THMs in soft drinks and fruit juices as indicators of the presence of treated water into them. Following, a summary with the most relevant results obtained in this Doctoral Thesis is presented:

1. The chromatographic conditions to avoid/minimize the decomposition of HNMs during heating in the injection port of GC and ion source temperature of the mass spectrometer have been studied. The study shows that temperatures between 200 and 250 °C in the ion source do not produce any decomposition of HNM, however injector port temperatures above 170 °C should not be used for preventing the decomposition of trihalogenated species of HNMs.

2. The first method for the determination of 9 chlorinated and brominated HNMs in water based on HS–SDME/GC–MS has been developed. This microextraction technique has the advantage of injecting the whole extract (~2 µL) into the GC without residues, following the trend of "Green Chemistry".

3. In a second stage, a fast, simple, robust and sensitive method for the determination of the same species in drinking water by HS–GC–MS has been developed. Moreover, this method allows the volatilization of the analytes in one step simplifying the sample treatment. Different variables involved in the volatilization of these species were studied, in that way, the addition of aliquots of MTBE as organic modifier increased the yield of the volatilization approximately 4 times in relation to without modifier. Furthermore, for the first time it has been shown that there are no significant differences in the concentration of trichloronitromethane (the only HNM found in treated water) between tap and pool water, despite the fact that the concentration of free residual chlorine and organic matter are higher

in pool water. The formation of HNMs does not depend largely on the organic matter of these pools but other nitrogen precursors.

4. A rigorous study of the stability of the 9 HNMs in presence of various dechlorinating agents has been performed, being $(NH_4)_2SO_4$ the best option. For the first time it has been demonstrated that salts are not the best option of preserving drinking water for the determination of nitrogen DBPs, but acidifying the sample at pH 3.0–3.5 at the time of collection is recommended in order to ensure its preservation for 48 hours at 4 °C.

5. A new liquid microextraction (MLLE) method, which overcomes the problems of the single drop microextraction techniques, has been developed. The proposed method includes the common 4 THMs in addition the 9 HNMs. The microextraction of the conventional LLE has provided extraction yields of 76% with the novelty of using conventional extractants (*n*-hexane, ethyl acetate or MTBE). Almost all the obtained extract (30 μL) is injected into a LVI–PTV–GC–MS, which provides two main advantages compared with the conventional injection (1–2 μL): higher sensitivity and without residues in accordance with the “Green Chemistry”.

6. The most simple and robust method (HS–GC–MS) has been used for the determination of new nitrogen species (HANs). The methodology for the determination of 4 THMs, 6 HNMs and 6 HANs has been used for evaluating the impact of some water quality parameters in drinking water and different disinfection process in the formation and speciation of these compounds. The results suggested that the concentration of DBPs is directly related to the amount of organic matter (contrasted by permanganate oxidability parameter), and that bromide concentration influenced the distribution of the chlorinated/brominated species, being the brominated DBPs concentrations more than 70% in treated water containing a high bromide concentration. The type of disinfectant is also related to the occurrence and speciation of DBPs, as with N-DBPs and in contrast to THMs, whose concentrations were not influenced by the

disinfectants studied in this Report. TCNM concentrations increased when chloramines were used as disinfectant, but the increase was higher with ozone, increasing two times the concentrations of the previous detected analyte and DCNM was detected. Furthermore, HANs concentrations (DCAN and BCAN) were higher using chloramines than chlorine dioxide or ozone.

7. Every results obtained by the developed methods along the Report for the determination of volatile DBPs have been compared with the proposed USEPA method (EPA method 551.1), which uses LLE for the determination of 4 THMs, TCNM and 4 HANs in drinking water. The main conclusions of this comparative study demonstrate some advantages of the proposed methodologies against EPA method: a) increase of the sensitivity from 2 to 5 times and b) reduction in the consumption of reagents and solvents. Others analytical characteristics, such as recoveries (>90%) and precision (~ 6–7%), were similar.

Finally, HS–GC–MS methods were the simplest, robust and sensitive ones, and therefore they were used for the determination of THMs (including new iodine species), HNMs and HANs in water and beverage.

8. The distribution of 10 THMs, 6 HNMs and 6 HANs along different processes in a water treatment plant (which uses chlorine dioxide and chloramines as disinfectants) and distribution network during the four seasons have been evaluated. The following conclusion were obtained from this study: a) no compound was detected in the raw water, and therefore the presence of these compounds in the treated water can be exclusively ascribed to the process of disinfection; b) only TCM is detected in the pre-oxidation step with chlorine dioxide; c) BDCM, TCNM and DCAN are formed after sedimentation, while TCM increased its concentration ~3.5 times, d) the filtration step increased the concentrations of these compounds approximately 40–70%; e) after the second disinfection step with chloramines, the

analytes previously detected increased their concentrations 2.5–3.5 times and three new species, such as DBCM, DCIM and BCAN, were formed; f) all compounds increased their concentration throughout the distribution network since the residual disinfectant will continue react with organic matter over time (excepting DCIM which is constant), being that increase more remarkable in warm seasons; and g) brominated DBPs (DBCM and BCAN) were found at higher concentrations in spring and autumn due to the higher concentration of bromide in these seasons. Moreover, the collaboration of other PhD students from our research group has been necessary for the systematically evaluation of other DBPs such as haloacetic acids and aliphatic and aromatic aldehydes jointly with volatile DBPs of this Report.

9. The possible occurrence of several volatile organic species (10 THMs and 4 VOCs), which are limited in drinking water, has been studied in beverages that are widely consumed. Soft drinks and fruit juices were selected since they include drinking water along their elaboration or they are in contact with this water during the elaboration process. This study has shown that chloroform can appear at concentrations below 1 μg/L in beverages without having treated water as ingredient (soft drinks prepared with mineral water or natural juices 100% squeezed) due to a contamination by contact with chlorinated sanitizers used in beverage processing line and packaging, being some aliquots of water enough as a source of contamination. Beverages manufactured with different amounts of water (soft drinks, reconstituted juices and nectars) contain, in addition to chlorinated THMs, brominated THMs and dichloroiodomethane (detected in soft drinks for the first time), which can be used as indicators of the presence of treated water. Moreover, the treated water included as an ingredient in the elaboration is the main source of the presence of THMs in these beverages. The concentration and speciation depends on the volume of water and water source employed.

ANEXO

Producción científica derivada de la Tesis Doctoral

Artículos científicos:

Determination of halonitromethanes in treated water.
I. Montesinos, M.J. Cardador y M. Gallego.
Journal of Chromatography A 1218 (2011) 2497.
Índice de impacto: 4.531 (sexta posición de las revistas en el área de Química Analítica según el Journal Citation Report 2011).

Headspace gas chromatography–mass spectrometry for rapid determination of halonitromethanes in tap and swimming pool water.
I. Montesinos y M. Gallego.
Analytical and Bioanalytical Chemistry 402 (2012) 2315.
Índice de impacto: 3.659 (novena posición de las revistas en el área de Química Analítica según el Journal Citation Report 2012).

Solvent-minimized extraction for determining halonitromethanes and trihalomethanes in water.
I. Montesinos y M. Gallego.
Journal of Chromatography A 1248 (2012) 1.
Índice de impacto: 4.612 (sexta posición de las revistas en el área de Química Analítica según el Journal Citation Report 2012).

Speciation of common volatile halogenated disinfection by-products in tap water under different oxidising agents.
I. Montesinos y M. Gallego.
Journal of Chromatography A 1310 (2013) 113.
Índice de impacto: 4.258 (sexta posición de las revistas en el área de Química Analítica según el Journal Citation Report 2013).

How the inclusion of treated water in beverages influences the appearance of halogenated volatile organic compounds.
I. Montesinos y M. Gallego.
Journal of Agricultural and Food Chemistry 62 (2014) 10240.
Índice de impacto: 3.107 (segunda posición de las revistas en el área de Agricultura, multidisciplinario según el Journal Citation Report 2013).

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Year-long evaluation of the presence of 46 disinfection by-products throughout a drinking water treatment plant.
I. Montesinos, M. Serrano, M.J. Cardador, M. Silva y M. Gallego.
Enviado a Science of the Total Environment.

Graphene-coated cotton fibres as a material for the extraction of multi-class pesticide residues from natural water and their determination by gas chromatography-mass spectrometry.
En redacción.

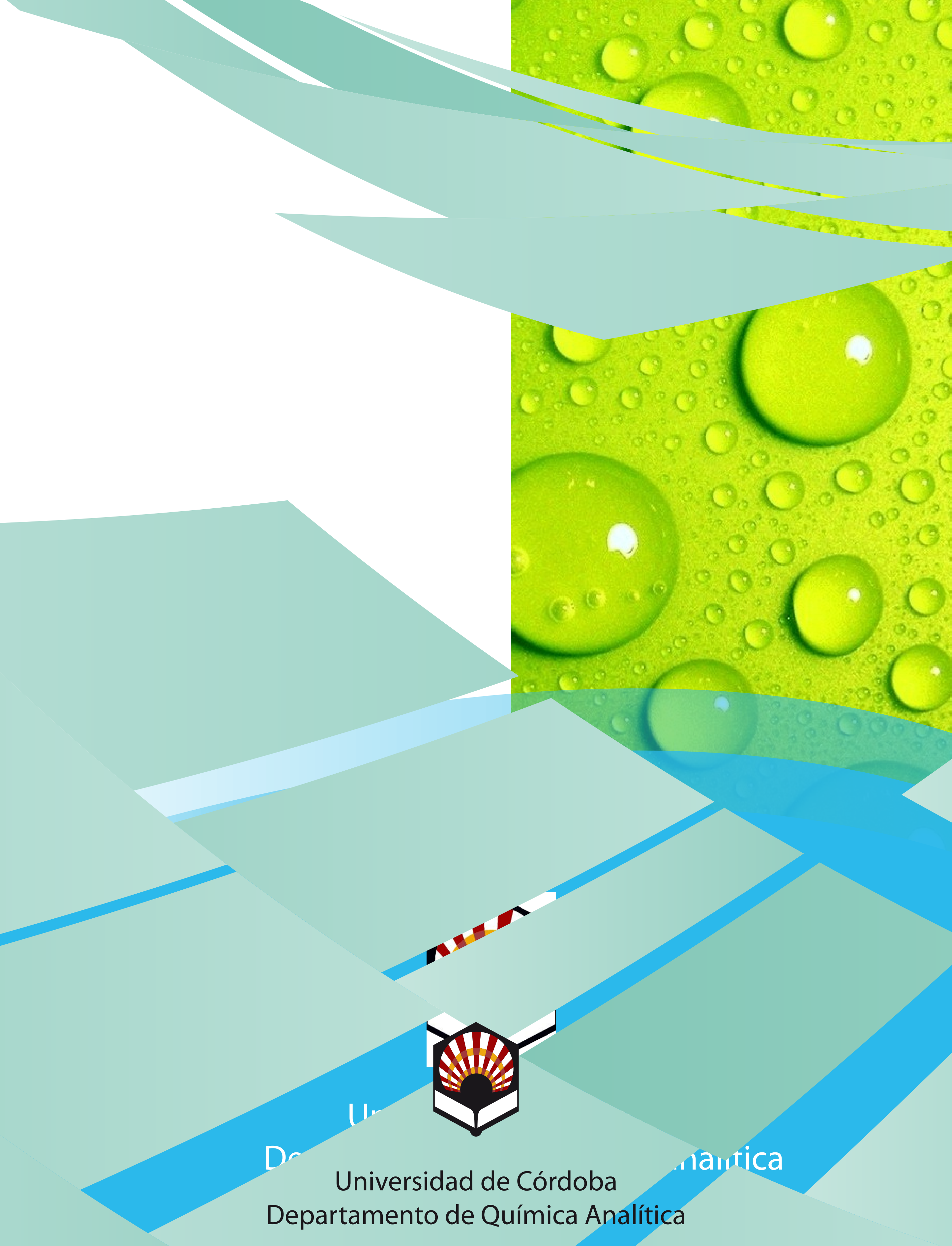
Comunicaciones a congresos:

V Reunión de la Sociedad Española de Espectrometría de Masas (SEEM). Málaga, 2011.
Headspace gas chromatography–mass spectrometry for rapid determination of halonitromethanes in tap and swimming pool water.
I. Montesinos y M. Gallego.
Comunicación en póster.

XIII Reunión del Grupo Regional Andaluz de la Sociedad Española de Química Analítica (GRASEQA). Málaga, 2012.
Determination of halonitromethanes in treated water.
I. Montesinos y M. Gallego.
Comunicación flash y póster.

XVIII Reunión de la Sociedad Española de Química Analítica (SEQA). Úbeda, 2013.
Solvent-minimized extraction for determining halonitromethanes and trihalomethanes in water.
I. Montesinos y M. Gallego.
Comunicación en póster.

XXX International Symposium on Chromatography. Salzburgo, 2014.
Speciation of common volatile halogenated disinfection by-products in tap water under different oxidising agents.
I. Montesinos y M. Gallego.
Comunicación en póster.


Universidad de Córdoba
Departamento de Química Analítica