Application of hollow fiber liquid phase microextraction for simultaneous determination of regulated and emerging iodinated trihalomethanes in drinking water

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Abstract

Trihalomethanes (THMs) are regulated disinfection by-products (DBPs) most commonly analyzed in quality control water supply due to their harmful effects on health. However, few data exist about the content of emerging iodo-trihalomethanes (I-THMs) which are present in drinking water at very low concentrations (in the order of ng L^{-1}). For this reason a two-phase hollow fiber liquid phase microextraction method for the simultaneous determination of four regulated trihalomethanes and six emerging iodo-trihalomethanes using GC-μECD and GC-MS with detection limits in the range of few ng L^{-1} has been developed. A central composite design was used to optimize conditions for simultaneous extraction. The best extraction recovery was obtained with 19.2 min at 27.1ºC and 900 rpm, without salt addition, using a supported hollow fiber membrane of 10.5 cm (0.6 mm id) and 1-octanol as acceptor phase. The limits of detection for the regulated THMs and I-THMs were 3-44 ng L^{-1} and 1-3 ng L^{-1} respectively. The calibration curves showed good linearity (r^2>0.995) and good repeatibility (3-22%). The relative recoveries in water were between 96.5% to 105.2%.

The method was applied for the simultaneous determination of trihalomethanes in supply water samples from seven water distribution systems (WDS) in the Huelva area, located at the southwest Spain, which use different water-treatment processes. The highest concentrations of I-THMs, particularly CHBrClI and $CHCl_2I$, were detected in water treated with advanced treatment process using pre-ozonation, however these compounds were not detected or decreased along distribution system. In the samples of treated water with conventional treatment, using pre-oxidation by permanganate and distribution network, $CHCl_2I$, CHBrClI, $CHClI_2$, $CHBrI_2$

and CHI_3 were detected at very low concentrations (1-18 ng L^{-1}). Finally, in water samples from underground origin without oxidation treatment, in which only disinfection with sodium hypochlorite was applied, I-THMs were not detected.

Keywords

Iodo-Trihalomethanes, Hollow fiber membrane, Gas chromatography, Disinfection by-products, Drinking water.

1. Introduction

Drinking water chlorination plays an important role in preventing pathogen contamination that causes water-borne diseases. However, chemical disinfection with chlorine leads to the formation of disinfection by products (DBPs) due to reaction with natural organic matter (NOM), which can cause potential health problems [1]. Epidemiologic investigations have demonstrated the association between exposure to DBPs present in drinking water and cancer (bladder [2], colon [3] stomach, pancreas, kidney and rectum) [4,5], as well as adverse birth outcomes [6,7]. THMs exposure may also contribute to increased risk of Hodgkin and non-Hodgkin lymphoma [8] and spontaneous abortion. Up to now, about 600–700 DBPs have been identified in drinking water [1], but only few DBPs have been evaluated for adverse effects. Therefore, only a limited number of regulations and guidelines have been established for DBPs, which include four trihalomethanes (THMs: chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) and five haloacetic acids (HAAs: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid and dibromoacetic acid) [9,10].

Iodinated DBPs (I-DBPs) include iodo-acids and iodo-trihalomethanes that have been identified in drinking water from ng L^{-1} to low µg L^{-1} levels. I-THMs have been described as powerful cytotoxics [11], and recent studies have demonstrated that iodinated DBPs had more cytotoxic and genotoxic effects than their brominated and chlorinated analogues, due to the stronger leaving potential of iodine atom. Iodoform (CHI_3) was considered the most cytotoxic compound, and chlorodiodomethane ($CHClI_2$) as the most genotoxic I-THMs species [12]. Additionally, mammalian cell toxicity results provided evidence for the toxicity of iodinated DBPs because iodoform (CHI_3) was 60 times and 146 times more cytotoxic than bromoform ($CHBr_3$) and chloroform ($CHCl_3$), respectively [13]. In addition to their toxicity, I-THMs have very low odor and taste thresholds, especially iodoform (CHI_3), which at concentrations above 0.1 µg L^{-1} water provides a characteristic medicinal smell [14].

Different analytical methods have been reported for analysis of regulated THMs in drinking water, but gas chromatography (GC) coupled with electron capture detection (ECD) or mass

spectrometry (MS) are widely used. ECD detector is generally more sensitive than mass spectrometry (MS) for halogenated organic compounds, altough MS detector is more selective to confirm the compounds detected in the samples [15].

Many of these methods study critically analyte extraction techniques or the introduction of the sample into the GC. Conventional liquid–liquid extraction (LLE) [16-19], purge and trap [20-24], direct aqueous injection (DAI) [25-27], static headspace (HS) [28-30], solid-phase microextraction (SPME) [31,32], solid-phase extraction [33], headspace solid-phase microextraction (HS-SPME) [34-37], liquid-phase microextraction (LPME) [38,39], headspace-liquid-phase microextraction (HS-LPME) [40,41], hollow fiber membrane (HF-LPME) [42,43] and dispersive liquid–liquid microextraction (DLLME) [44,45], have been used to monitor regulated THMs species in drinking water.

Low concentrations of I-THMs in water samples require inclusion of optimal pre-concentration step before the gas chromatography analysis, such as liquid–liquid extraction (LLE) [12,46,47]. An alternative method to extract and concentrate analytes from water samples is the use of solid-phase microextraction (SPME) [47,48,49].

Milton-Moreano et al. compared HS-SPME, HF-LPME and HS extraction for determination of trihalomethanes in drinking water by gas chromatography electron capture detector and mass spectrometry detection. As result, the HF-LPME–GC–ECD method proved to be the most sensitive for determination of regulated THMs compounds, with a very high concentration factor, low LOD, good accuracy and precision [43].

Due to the increased interest in monitoring the occurrence of I-DBPs in water, analytical methods for the simultaneous quantification of chloro, bromo and iodo-trihalomethanes are necessary. Recently S. Allard et al. developed a HS SPME-GC/MS method for simultaneous analysis of 10 THMs (4 regulated, chlorinated and brominated compounds and 6 unregulated iodinated ones) with detection limits ranging from 1 ng/L for iodoform to 20 ng L^{-1} for chloroform [50].

In this work a new analytical method for the simultaneous determination of the ten trihalomethanes present drinking water based on supported liquid hollow fiber membrane microextraction (HF-LPME) and GC-μECD was developed. In addition confirmation of compounds in the samples was performed by GC-MS. A remarkable advantage of this chromatographic approach is the combination of the sensitivity of electron capture detector with the selectivity of the mass spectrometer to get low detection limits and unequivocal identification of the analytes. The developed

method is simple, reproducible and cheap, reaching low detection limits that makes the approach suitable for routine laboratories control in water distribution systems.

2. Experimental. Material and methods

2.1. Chemicals and solutions

All reagents used were of highest purity, iodoform (CHI_3), 1,2 dibromopropane (used as internal standard SI) and THMs mixture 100 µg/µL of each compound: chloroform ($CHCl_3$), bromodicloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$), and bromoform ($CHBr_3$), were purchased from Sigma-Aldrich (Steinhein Germany). Iodo-trihalomethanes (I-THMs): bromodiiodomethane ($CHBrI_2$ 90%), bromochloroiodomethane (CHBrClI 95%), chlorodiiodomethane ($CHClI_2$ 95%), dibromoiodomethane ($CHBr_2I$ 95%) and dichloroiodomethane ($CHCl_2I$ 95%) were purchased from Orchid Cellmark (New Westminster, BC, Canada). Methanol, n-hexane, toluene, acetonitrile and acetone were purchased from Teknokroma (Barcelona, Spain). n-Octanol was supplied from Merck (Barcelona, Spain).

Accurel Q 3/2 polypropylene hollow fiber membranes with an inner diameter of 600 µm, 200 µm of wall thickness and 0.2 µm pore size was obtained from Wuppertal (Germany). Ultrapure water (18MΩ cm) was obtained from a Milli-Q water-purification system (Millipore, Watford, UK) and was used for spiked samples and blanks preparation.

Standard stock, intermediate and work solutions of each regulated THMs (chloroform, bromodicloromethane, dibromochloromethane and bromoform) were prepared in methanol at 20 mg L^{-1}, 200 µg L^{-1} and 5 µg L^{-1}, respectively. Standard stock, intermediate and work solutions of each I-THMs (iodoform, bromodiiodomethane, bromochloroiodomethane, chlorodiiodomethane, dibromoiodomethane and dichloroiodomethane) were prepared in methanol at 10 mg L^{-1}, 10 µg L^{-1} and 1µg L^{-1}, respectively. Stock and intermediate solutions were stored into the dark at -20 ºC for a maximum of one month. Work standard solutions were prepared daily in ultra pure water. The internal standard (IS) solution (1,2-dibromopropane) was prepared in methanol at a concentration of 5 mg L^{-1} to achieve a final concentration of 5 µg L^{-1}.

2.2. Sample collection

Water samples were taken in twelve sampling points from seven different water distribution systems. The sampling points were defined in finished water reservoirs of six water treatment plants (S1, S2, S3, S4, S5 and S6), in two water reservoirs of underground origin and disinfection treatment (S7 and S8), and four water reservoirs of the corresponding distribution systems (S2.1, S3.1, S3.2 and S4.1).

Water samples include five finished water from WTPs with conventional treatment: Aljaraque (S1), Lepe (S2), Riotinto (S4), Encinasola (S5) and Cumbres San Bartolomé (S6), one sample of finished water from Lepe WTP with advanced treatment (S3), two treated water from underground origen with disinfection treatment: Jabugo (S7) and Hinojales (S8) reservoirs, and four samples water from reservoirs of corresponding distribution systems: Ayamonte (S2.1 and S3.1), Isla Canela (S3.2) and Fuente la Corcha (S4.1). Sampling program with the characteristics of the water distribution systems and sample points are shown in Fig. S1 and Table S1 in the Supplementary Information.

Samples were collected from the tap of each sample point. Before collecting tap water samples, the tap was opened for 5 min to assure the representativeness of the sample. The samples were collected into 125 mL amber glass bottles with teflon-lined screw caps, containing 1.5 mL of sodium thiosulfate solution 0.1 M to eliminate any residual chlorine and to prevent additional DBP formation during trasportation to the laboratory. Samples were stored at 4 °C until analysis.

2.3. Hollow-fiber liquid phase microextraction (HF-LPME)

The configuration of the supported hollow fiber membrane liquid microextraction system is based on other described previously elsewere [42, 51-53], which is illustrated in Fig. 1. The hollow fiber was cut into pieces of 10.5 cm length and cleaned with acetone to remove any possible contaminant. The fiber was removed and allowed to be completely dry. To carry out the extraction, one end of the cleaned fiber was attached to a medical syringe needle (inner diameter of 0.7 mm x 50 mm). Then, 1 mL of solvent was passed though the fiber to ensure that the lumen of membrane was filled with the organic phase. The other end of the fiber was joined to another medical syringe needle that were connected to the syringe body, acting as a cap to prevent the solvent loss during extraction. After that, the U-shaped fiber system was immersed into an extracting organic solvent for around two minutes to impregnate the fiber pores with the organic solvent. The U-shaped fiber was placed into the aqueous sample (20 mL) for extraction. Then the fiber was removed from the donor solution and dried with a piece of paper to eliminate trace of water. One end of the fiber was detached from the medical syringe needle and the extracting solvent was flushed by an air blow from the syringe into a microvial.The extract obtained was collected into a 1.5 mL vial attached to a 250 µL insert. A total of 4 µL of the organic extract was transferred to another GC vial containing 16 µL of n-hexane (extract:n-hexane ratio 20:80). Finally, 1 µL of this solution was injected into the GC-µECD. The extraction was performed during 19.2 min at 27.1 °C and 900 rpm.

2.4. Gas chromatographic analysis

The extracts were analysed in a Agilent 6890N gas chromatograph system with a ^{63}Ni microelectron capture detector (GC-µECD) (Hewlett Packard, Wilmington, DE, USA). Initially the

chromatographic conditions were optimized to achieve the highest peak areas, good chromatographic resolution and suitable integration. A DB-5 chromatographic column (30m x 0.25mm x 0.25µm J&C Scientific) was used. Helium (99.999 %) was used as carrier gas with a flow rate of 1.2 ml/min. The injector was operated in the split mode at 1:10 ratio. The injector temperature was 180 ºC. The oven temperature was optimised and programmed at 35 ºC for 12 min, subsequently increased to 200 ºC at 6 ºC/min and hold for 1 min, with a total time run of 40.5 minutes. The detector temperature was held at 280 ºC. Nitrogen (99.999 %) was used as a makeup gas at 60 ml/min. ChemStation software package (version A0903) was used for data acquisition and evaluation. A good separation was achieved under conditions described. A typical chromatogram showing the separation of all the analytes with direct injection is shown in Fig. 3.

The extracts were simultaneously analysed in a Varian CP 3800 GC gas chromatograph coupled to an ion trap mass spectrometer detector Varian Saturn 2000 MS (Varian, Sunnyvale, CA, USA). The analytical column was a VF-5ms 30m x 0.25mm x 0.25 µm film thickness (Varian Iberica). The oven temperature conditions were the same as those used in GC-µECD: 35 ºC for 12 minutes, increased to 200 ºC at a rate of 6 ºC/min and held for 1 min, with a total run time of 40.5 min. The injector port temperature was set at 220 ºC. Helium was used as carrier gas at a constant flow rate of 1.2 mL min^{-1}. The effectiveness of selected GC-MS conditions were tested, considering sensitivity, baseline separation of analytes and peak shapes. Optimum chromatographic conditions allow a good separation of the ten trihalomethanes and the IS.

For mass spectrometry detection, ionization was carried out by electronic impact (EI) with a voltage of 70 eV, using full scan mode in the m/z range of 35-650, with an ion source temperature of 220 ºC and transference line temperature of 280 ºC. For the analysis, 1 µL of sample was injected in splitless mode. The identification of the chloro, bromo and iodo halomethanes was based on comparison with corresponding standard according to their retention times and mass spectra characteristics, complementarily, searching on NIST Mass Spectral Library was performed. For each compound several monitoring ions were selected (Table 1). The mass to charge ions were used for confirmation.

2.5. Method optimization

To find the most favorable conditions for HF-LPME for the simultaneous analysis of 10 THMs (THM10) in water samples, a preliminary screening of the critical variables by univariate optimization was carried out setting the following initial conditions: temperature (30 °C), HF length (7 cm), direct extraction mode, immersion time (2 min), salt addition (0 %), extraction time (25 min), stirring speed (700 rpm), sample volume (20 ml). In a first step, univariate analysis for the selection of extraction

solvent, extraction mode and optimization of operation extraction variables such as immersion time, sample volume, stirring speed and addition of salt was applied. Then in a second step, a second-order rotable Central composite design (RCCD) was applied to optimize the most significant operation variables, extraction time and fiber length. The experiments were performed with ultrapure water spiked with 1 μg L^{-1} of each analyte. All results were expressed as mean values of two replicates.

3. Results and discussion.

In this study we used a hollow fiber device for simultaneous extraction of ten trihalomethanes, in order to check the advantages over other extraction techniques, such as reproducibility, absence of sample carryover (due to the disposable nature of the membranes), simplicity, low analytical time, low cost, high analyte enrichments, high throughput and large pH tolerance range.

3.1 Selection of organic solvent

The extracting solvent in LPME should be immiscible with water and strongly immobilized in the pores of the hollow fiber to prevent leakage, it should provide high partition coefficients for the target analytes to ensure efficient extraction, and low volatility to avoid evaporation during extraction. In addition, it should have a suitable low viscosity to ensure high diffusion coefficients and should be selective towards the analytes of interest [54]. Based on these considerations, five organic solvents were tested to extract the analytes (1-octanol, n-hexane, ethyl acetate, dihexyl ether and toluene). As can be seen in Fig. 2, 1-octanol and n-hexane gave the best results. The experiments showed that n-hexane was lost during the extraction to a larger extent than 1-octanol. Consequently 1-octanol was chosen for further experiments. It was verified that an immersion time of 2 minutes was sufficient to impregnate the pores of the HF because longer times do not increase the extraction efficiency.

Although extractions with 1-octanol perform the best result the quantification by GC showed poorly resolved chromatographic peaks, especially for $CHBrI_2$. To optimize the resolution of chromatographic peaks, several mixtures of acceptor solvent with other ones were tested, obtaining improvements in the resolution but with reduced peak areas. Very good results were obtained by diluting the extract obtained with n-hexane at ratio 20:80 (extract: n-hexane) Fig. 3.

3.2 Extraction mode

Direct and headspace extractions were compared using liquid hollow fiber membranes with 1 μg L^{-1} spiked THM10 water samples using 1-octanol as extracting solvent. In Fig.2 can be seen that extraction is higher using direct extraction mode that was chosen for further experiments.

3.3. Stirring speed

The stirring speed agitation accelerate the mass transfer of extracted analytes from the sample to the organic solvent and reduce the time to reach equilibrium. To estimate the influence of this variable, several values were tested from 0 to 1500 rpm. The Fig. 4 shows that relative area of the analytes increased with the stirring speed, although above 1500 rpm decreased. The extraction of regulated THMs is favored by low stirring speed, obtaining the best results in the range of 0 to 200 rpm. However, the best results of I-THMs were obtained with speeds between 500 and 900 rpm. Therefore, a compromise solution was adopted weighting the values obtained with a speed of 900 rpm.

3.4. Salt addition

Salt addition to the sample can reduce the amount of water available to dissolve the analytes due to the formation of hydration spheres around the ionic salt molecules and can improve the extraction efficiency of target analytes into the organic phase. In addition, salt can prevent loss of solvent from HF [55-58]. Different percentages of NaCl and $NaSO_4$ from 0% to 20% (w/v) were studied; the best results were obtained with $NaSO_4$.

As shown in Fig. 5 the addition of salt (20%) increased peak areas of the regulated THMs, especially TCM and BDCM (Fig.5A), however did not improve peak areas I-THMs (Fig. 5B). The weighted means (Fig. 5W) confirm that the addition of salt at about 20% did not improve the simultaneous extraction of THM10. According to this salt was not used in subsequent experiments.

3.5. Experimental design. Multivariate optimization

A rotable second-order central composite design (RCCD) was applied, using MINITAB® Release 15 Statistical Software (State College, PA, USA) [59] and some home-made programs written in QUICKBASIC, to study the effect of three independent variables: extraction time (*tm*), temperature of the extraction (*T*) and HF length (*L*).

The CCD consists in a 2^k (k = number of variables) full factorial design augmented with $2k$ vertices of a cross-polytope (star points) positioned on the coordinate axes of the factorial space and C (number of replicates) points at the center of the design, (0,0,…0). For three factors, the resulting points are $(\pm\alpha,0,0)$, $(0,\pm\alpha,0)$ and $(0,0,\pm\alpha)$, where α is the distance (star arm) from the center of the design to a star point. This implies performing $2^k + 2k + C$ experiments. We applied a rotable CCD variant method [60,61] in which the variance of the predicted response values depends only on the distance from the center of the design and $\alpha = 2^{k/4}$ (k = number of variables). Each variable has five coded levels ($-\alpha$, −1, 0, 1, α). Star arms $-\alpha$ and α are the minimum and maximum value of the factor in the

common working range. The experimental design RCCD for K = 3 and C = 3 requires 17 random trials to avoid systematic errors (Table S2).

The response (relative areas peak) of the dix trihalomethanes obtained in the different experiments (RCCD) carried out by HF-LPME for the optimization is shown in Table 2. Results for the experimental design, a multilinear regression was applied obtaining reduced models of polynomial equations representing the relationship between variables and responses Table 3. A significant assay for the regression coefficients was performed based on the Student's "t-test", once the standard deviation of the regression coefficients b_k, S (b_k) is known. The significance of the effect is demonstrated when the absolute value of $t_k = |b_k| / S(b_k)$ is found to be greater than the critical tabulated value t critic (υ, P) for the υ degrees of freedom corresponding to the tested regression model (υ = number of run – number of coefficients to be estimated) with a P confidence level of 95% (p-value > 0.05). In all simplified models obtained for the different analytes a significant positive effect of the three variables studied were observed; therefore conducted to optimize the response by simultaneous maximization of peak areas of all the analytes studied by determining the Combined Response. Combined Response is a response variable created as a weighed sum of several responses according to the following equation:

$$COMB = \sum_i \left\{ Kw_i \left| \frac{Y_i - G_i}{R_i} \right| \right\}$$

where w_i is a user chosen weight, generally set as unity, Y_i is each response to be optimized and R_i the range of Y_i. When the responses in the combined response are to be maximized, then k = 1 and G_i = minimum (Y_i − ½ R_i). Using a home-made routine algorithm, a random search of the surface grid COMB (tm, T, L) is performed. The maximum of the COMB surface is the simultaneous optimum. This optimization method has been applied in several studies, with good results [62,63]. The COMBINED 3D and contour plot showing surface shape against the axis temperature, time and length is plotted optimum point is displayed in Fig. S2. The optimum value was reached at the following coordinates: time (tm) = -0.58, temperature (T) = -0.58 and length (L)= 1.3. The optimum values of the variables resulted to be: 19.2 minutes of extraction time, 27.1 °C of extraction temperature and 10.5 cm of hollow fiber length.

3.6. Validation of the method

To evaluate the practical applicability of the developed HF LPME-GC/μECD method to drinking water analysis, the linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability (intra-day), reproducibility (inter-day), recovery extraction, and enrichment factor were determined. The linearity was obtained in the range 0.1 to 45 μg L^{-1} for regulated THMs and 0.010 to 4 μg L^{-1} for

I-THMs, considering that I-THMs concentrations in water supply are usually in the range from ng^{-1} to low μg^{-1} (Table S3).

The linearity was examined by plotting calibration curves of the relative area (ratio of the peak areas of the analyte to the peak areas of the internal standard) versus the concentration of each analyte. Overall, linearity was very good along the whole evaluated range with the determination coefficients (R^2) ranging from 0.995 to 0.999.

The detection (LOD) and quantification (LOQ) limits, defined as the minimum concentration providing chromatographic signals 3 times (S/N=3) or 10 times (S/N=10) higher than background noise were obtained in the range 100-1000 ng L^{-1} for regulated THMs and 10-100 ng L^{-1} for I-THMs. The LOD values obtained ranged from 1 ng L^{-1} for CHI_3 to 44 ng L^{-1} for $CHCl_3$ and from 3 ng L^{-1} for CHI_3 to 65 ng L^{-1} for $CHCl_3$ for LOQ. LOD results obtained with the new method are very satisfactory, as can be seen in the comparison table (Table 4).

The precision of the proposed method was evaluated by determination of the repeatability and reproducibility analyzing water samples spiked with 1 µg L^{-1} and 0.1 µg L^{-1} of each trihalomethane. The repeatability was studied by analyzing five water samples in one day. The repeatability values (RSD %) were very good ranging from 6 to 22 % for regulated THMs and from 3 to 12 % for I-THMs. The reproducibility refers to the analysis of 12 samples over 3 different days. The reproducibility (RSD %) was very good ranging from 10 to 18 % for regulated THMs and from 6 to 12 % for I-THMs.

The extraction recovery was calculated using a tap water fortified at two different concentrations (5 and 20 µg L^{-1} for regulated THMs, 0.02 and 0.1 µg L^{-1} for I-THMs) of each target analyte. These concentrations were selected according to their usual concentration in drinking water; all experiments were carried out in quintuplicate (n=5). Results show that the recoveries for regulated THMs were from 97.8 % to 105.2 % and for I-THMs from 96.5 % to 104 % at the spiked levels, with relative standard deviation in the range of 4-10 %.

The results of method validation are shown in Table 1, which demonstrate the good applicability of the developed method for simultaneous determination of 10 THMs in water samples.

Only the method from S. Allard et al. [50] based on HS-SPME-GC-MS allows the simultaneous analysis of regulated and emerging (iodinated) trihalomethanes (Table 4), however, present work improves the extraction recovery and limits of detection are lower, which reinforces the interest of use HF-LPME to trihalomethanes analysis in waters.

4. Application to water distribution system

The formation mechanisms of regulated THMs, their health effects and treatment technologies have been widely studied; however there are few published studies on I-THMs in spite of their demonstrated negative health effects. Formation of I-THMs are affected by the matrix of water source (especially the presence of COT, Br^-, I^- and pH value), the treatment process and environmental and operational variables (temperature, contact time, disinfectant dose). The presence of I-THMs in water supplies is mainly associated to chloramination treatment, but also to oxidation by ozone, chlorine, potassium permanganate, and chlorine dioxide (Table S3).

The method developed was applied to the simultaneous determination of trihalomethanes in seven water distribution systems in the Huelva area (southwest Spain) from May to August of 2014. Sample points were selected to evaluate the formation of the regulated and emerging trihalomethanes under different conditions of water treatment process, raw water source and distribution system.

Water samples analyzed had different origins: Surface water of Chanza reservoir, treated in Aljaraque WTP using conventional treatment and Lepe WTP (with both conventional treatment and advanced treatment process), surface water of small reservoirs (Jarrama, Encinasola and CSBartolomé) with conventional treatment, and water from underground sources with disinfection treatment (Jabugo and Hinojales). Samples of water distribution systems of Lepe and Rio Tinto in sampling point located to distance of 2-45 km from the WTP, and water retention times between 23 and 73 hours were taken. Sample collection, configurations and features of the seven distribution system studied are shown in Fig. S1 and Table S1. Samples were analyzed in triplicate. The results obtained are shown in Table 5.

Concentrations of regulated THMs in water from WTP fed with surface water (S1, S2, S3, S4, S6) ranged from 36.2 and 26.4 μg L^{-1}. Minor concentrations of regulated THMs (2.4-9.4 μg L^{-1}) were observed in groundwater WTPs (S7, S8) or mixture (S5). When water is oxidized with potassium permanganate, chloroform concentration was higher (41-72 % of total regulated THMs), however when ozonation was used, concentration of brominated and chlorobrominated trihalomethanes were higher than chlorinated ones, accounting for 73 % of the total regulated THMs. The bromoform concentration was higher in waters oxidized with ozone (5.9 μg L^{-1}) than that with permanganate (1.2 μg L^{-1}). Regulated THMs concentrations increase in the distribution systems, affected by rechlorination processes and increase with contact time.

In samples oxidized with potassium permanganate (S1, S2, S4, S5, S6) the I-THMs were detected at low concentrations (∑ I-THM ranged 17 to 4 ng L^{-1}). The species identified were $CHCl_2I$ (3-18 ng L^{-1}), $CHClI_2$ (8-12 ng L^{-1}), $CHBrI_2$ (2-6 ng L^{-1}), CHBrClI (4-7 ng L^{-1}) and CHI_3 (1-6 ng L^{-1}). The $CHBr_2I$ was not detected in any sample. The behavior of iodinated species in the distribution system is different to regulated THMs, a slight increase in the concentration of $CHCl_2I$ was observed in samples from reservoirs, probably due to rechlorination treatment. The CHI_3 were not detected in reservoir

samples, since increased contact time favors the oxidation of iodide to iodate. The highest concentration of I-THMs (Σ I-THM: 126.9 ng L^{-1}) was obtained in S3 that uses a combination of ozone and granular activate carbon filters treatment. Only two of the six possible iodinated compounds were identified in this sample, $CHCl_2I$ (43.0 ng L^{-1}) and CHBrClI (83.9 ng L^{-1}). However, only $CHCl_2I$ was identified in the distribution system, with decreasing concentrations depending on contact time and distance from WTP to distribution reservoir.

Finally, in the two samples from water supply of underground origin (S7 and S8) I-THMs were not detected.

5. Conclusion

A new analytical method based on HF-LPME and GC-μECD for the simultaneous determination of ten trihalomethanes (regulated and emergent) in drinking water was studied for the first time. The different parameters affecting the HF-LPME extraction were optimized with a rotable second-order central composite multivariate design (RCCD). The analytical performance of the optimized procedure was evaluated and good linearity, reproducibility and accuracy was obtained together to low detection and quantification limits. The method allows fast extraction and high pre-concentration of analytes, reduction of organic solvent, elimination of carry-over effects using disposable fibers and high applicability to different water matrices The analytical approach is applicable to trace analysis (at ng L^{-1} levels) of conventional and emerging trihalomethanes in drinking water reservoir and WTPs.

Acknowledgements

The authors wish to express their appreciation to the plant managers GIAHSA and employees for their help and cooperation in facilitating the collection of water samples during this study. This work was supported by the project CTM2012-38720-C03-01 from the Spanish Ministry of Economy and Competitiveness (MINECO), and by project P012-FQM-0442 from the Regional Ministry of Economy, Innovation, Science and Employment (Andalusian Government, Spain).

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Figure captions

Fig. 1 Extraction device for HF-LPME

Fig. 2 Chromatographic peak area (average n=2) for the selection of organic solvent and extraction mode. (A) Regulated THMs: $CHCl_3$ (TCM), $CHCl_2Br$ (BDCM), $CHClBr_2$ (BDCM) and $CHBr_3$ (TBM). (B) Unregulated I-THMs: $CHCl_2I$ (DCIM), CHBrClI (BCIM), $CHBr_2I$ (DBIM), $CHClI_2$ (CDIM), $CHBrI_2$ (BDIM) and CHI_3 (IF), using three extraction solvents: 1-Octanol (OCT), n-Hexane (HEX) and Heptane (HEP), and two extraction mode: Direct extraction (DE) and head space (HS). (W) Weighted peak area from the extraction of regulated THM (THM4), unregulated I-THM (I-THM), and total THMs (THM10)

Fig. 3 GC-μECD chromatogram obtained from a standard solution at 1 μg L^{-1} of each regulated THM and 0.1 μg L^{-1} of each I-THM, using HF-LPME

Fig. 4 Influence of stirring speed in the extraction of THMs: (A) regulated THMs; (B) emerging I-THMs; (C) total THMs

Fig. 5 Effect of the addition of sodium sulfate on the chromatographic peak area: (A) regulated THM; (B) unregulated iodo-THM

Tables.

Table 1 Method validation

Analyte	m/z ions	E_e	Range (μgL^{-1})	R^2	LOD (ngL^{-1})	LOQ (ngL^{-1})	Repeatibility (% n=5)		Reproductibility (% n=12)	
							100ngL^{-1}	1 μg L^{-1}	100 ngL^{-1}	1 μgL^{-1}
$CHCl_3$	83+85	21	0.1-45	0.999	44	65	22	9	18	7
$CHBrCl_2$	129+85+127	32	0.1-45	0.999	3	5	12	6	13	3
$CHClBr_2$	129+127	52	0.1-45	0.999	7	13	7	4	10	4
$CHBr_3$	173+171	56	0.1-45	0.999	3	6	6	4	10	3
$CHCl_2I$	175+83	618	0.010-4	0.995	1	4	7	3	7	5
CHBrClI	256+129+131	311	0.010-4	0.998	3	12	3	2	8	7
$CHBr_2I$	302+219	469	0.010-4	0.996	3	10	5	3	6	6

$CHClI_2$	175+302	574	0.010-4	0.996	1	4	6	6	8	7
$CHBrI_2$	219+348	789	0.010-4	0.999	1	3	10	8	12	10
CHI_3	394+267	1016	0.010-4	0.999	1	4	12	10	10	10

E_e = Enrichment factor = Ratio of the concentration of analyte in the aceptor phase (c_a) divided by the initial concentration in the sample before extraction (c_d). $E_e = c_a / c_d$

m/z ions: mass to change ratio of specific fragments used for selected-ion monitoring MS analysis.

Table 2 Relative response of peak area in RCCD experiment

	$CHCl_3$	$CHBrCl_2$	$CHClBr_2$	$CHCl_2I$	$CHBr_3$	CHBrClI	$CHBr_2I$	$CHClI_2$	$CHBrI_2$	CHI_3
C1	207	473	951	2236	824	1602	2286	8410	10613	17500
C2	188	353	581	1595	512	1127	1517	4960	6764	11483
C3	113	212	349	958	308	677	911	2892	4018	6830
C4	243	457	750	2060	661	1456	1960	6421	8982	17924
C5	525	987	1622	4455	1430	3150	4238	17159	24698	46660
C6	337	634	1042	2861	919	2023	2722	10229	14912	28041
C7	421	793	1303	3578	1149	2529	3404	9572	12551	23185
C8	290	546	898	2465	791	1743	2345	7572	10424	19577
C9	278	523	859	2359	757	1668	2244	6468	8219	11892
C10	237	446	734	2014	647	1424	1916	5438	7394	12940
C11	348	656	1077	2958	950	2091	2814	7452	8666	15857
C12	307	578	950	2609	837	1844	2482	7262	8770	13757
C13	226	425	698	1917	616	1356	1824	5313	6678	9830
C14	560	1054	1732	4756	1527	3363	4525	15625	22057	40425
C15	557	1045	1670	3501	1225	2463	2909	9992	11809	18376
C16	537	890	1566	3547	1244	2797	3182	9836	11356	17334
C17	504	964	1570	3776	1317	2835	3691	9842	11550	17900

Table 3 Reduced models

Model Equation	R^2 (%)	p
$CHCl_3 = 533.96 – 20.23*tm – 18.92*T + 101.44*L – 102.36*tm^2 – 77.52*T^2$ -54.47*L2 + 25.68 tm*T – 53.68*tm*L	98.40	0.000
$CHBrCl_2 = 968.52 – 44.22*tm + 184.79*L – 178.1*tm^2$ -131.36*T^2 – 87.97*L^2 + 58.81*tm*T – 90.55*tm*L	97.03	0.000
$CHClBr_2 = 1604.08 – 85.39*tm – 81.34*T + 290.85*L – 292.65*tm^2 – 215.86*T^2$ -144.57*L^2 + 118.38*tm*T – 127.01*tm*L	96.56	0.000
$CHCl_2I$= 3620.33 – 206.9*tm – 195.77*T + 826.36*L – 545.13*tm^2 – 334.26*T^2 -277.98*tm*T – 395.93*tm*L	96.7	0.002
$CHBr_3 = 1264.49 – 74.2*tm$ - 70,63*T + 257.50*L – 207.27*tm^2 – 139.58*T^2 -76,73*L^2 + 102.52*tm*T - 113,82*tm*L	96.01	0.001
CHBrClI = 2707.35 – 147.86*tm + 582.62*L – 435.01*tm^2 – 285.92*T^2 +199.23*tm*T -277.2*tm*L	96.22	0.001
$CHBr_2I$= 3272.02 + 774.51*L - 455.85*tm^2 - 255.23*T^2 - 356.78tm*L	94.04	0.016
$CHClI_2$=9851,7 - 1070.6*T + 2869.7*L - 1259.9*tm^2 + 1488.6*tm*T	93.3	0.043
$CHBrI_2$= 11481.4 -1525.7*T + 4252.3*L + 2059*tm*T	92.2	0.001
$CHI_3 = 17613$ - 2906.9*T + 8433.9*L +3448.4*L^2 + 4015.2*tm*T	92.7	0.025

Equation model: $y = \beta_0 + \Sigma\beta_0 X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j$; response: peak area of each THM. Variable: tm (extraction time), T (extraction temperature ºC), L (HF length).

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584 Table 4 Comparison of detection limits and recoveries of developed method with others previously reported

		Regulated THM		I-THMs		
Instrumental Method	Extraction procedure	Recovery %	LOD µg L^{-1}	Recovery %	LOD µg L^{-1}	Ref.
LLE-GC-ECD	2 ml glass-distilled; n-hexane	103.0-111.0	0.025-0.05			[18]
LLE-GC-ECD	2 ml MTBE; 6gr NaSO4	87.6-112.8	0.010-0.007			[19]
LLE-GC-MS	2 ml MBTE; 6gr NaSO4	87.6-112.8	0.010-0.030			[19]
HS-LPME-GC-ECD	1-Octanol. 15ml, HS, 40ml vial, 10min. extract., 800rpm, 20ºC. 0.3gr/ml NaCl	108.4-112	0.15-0.40			[40]
LPME-GC-ECD	n-hexane,t: 5min, stirring rate 600rpm, T: 25ºC, NaCl 3M	73.0-78.0	0.23-0.45			[39]
HF-LPME-GC-ECD	Accurel Q 3/2 PP HF, ø600µm; e:200µm; 35ºC, no stirring, no salt, 30 min., long: 8.5cm. 1-octanol	98.0-105.0	0.010-0.200			[42]
HS-SPME-GC-µECD	PDMS, T: 30-45ºC, t: 20-40min. Desorpcion T: 200-300ºC,t:2-6min.	74.7-120.9	0.059-0.317			[43]
HF-LPME-GC-ECD	Accurel PP ø330µm; e:150µm; L:110mm, T:25-50ºC, t:5-20min; 0-10%salt; 1-octanol.	80.3-104.2	0.018-0.049			[43]
DLLME-GC-ECD	0.5ml acetone (dispersor solvent) whit 20 mL carbon disulfide (extraction solvent)	95.0-107.8	0.05-0.040			[44]
LLE-GC-ECD	2 ml glass-distilled; MTBE			100.0-104.0	0.010-0.030	[47]
HS-SPME-GC-ECD	65 µm Carbowax-divinylbenzene fiber.			83.3-90.0	0.0012-0.030	[48]
HS-SPME-GC-MS	CAR/PDMS/DVB,t 30min, T 40ºC			80.9-93.0	0.002-0.070	[49]
HS-SPME-GC-MS	CAR/PDMS/DVB,t 15min, T 70ºC	84.0-103.0	0.003-0.020	84.0-103.0	0.003-0.008	[50]
HF-LPME-GC-ECD	Accurel Q 3/2 PP HF Ø600µm; e:200µm, 27.1ºC, 900rpm, no salt, 19.2 min., 10.5cm. 1-octanol.	97.8-105.2	0.003-0.044	96.5-104.0	0.001-0.003	This work

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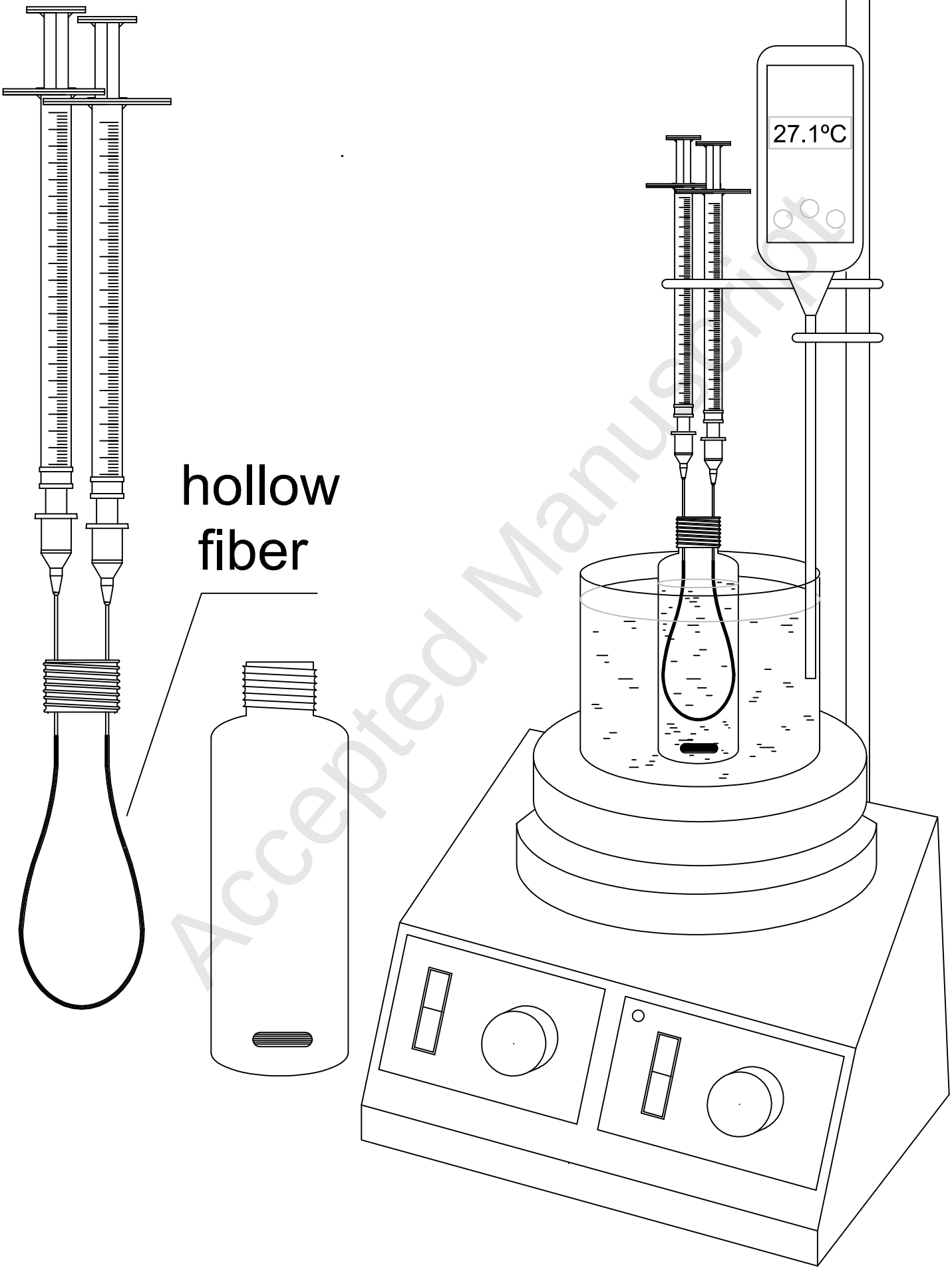
588 Table 5. Presence of 10 THMs (n=3) in different water distribution systems from Huelva province (southwest Spain).

Nº	$CHCl_3$ (μgL^{-1})	$CHBrCl_2$ (μgL^{-1})	$CHBr_2Cl$ (μgL^{-1})	$CHBr_3$ (μgL^{-1})	∑*THMs* (μgL^{-1})	$CHCl_2I$ (ngL^{-1})	CHBrClI (ngL^{-1})	$CHBr_2I$ (ngL^{-1})	$CHClI_2$ (ngL^{-1})	$CHBrI_2$ (ngL^{-1})	CHI_3 (ngL^{-1})	∑ *I-THM* (ngL^{-1})
S1	12.2	9.3	5.5	0.8	*27.8*	18	<LOD	<LOD	12	6	1	*37*
S2	11.8	8.7	6.8	1.2	*28.5*	9	7	< LOD	9	< LOD	2	*27*
S2.1	25.1	18.7	9.3	1.3	*54.4*	16	6	< LOD	12	5	< LOD	*39*
S3	3.9	12.0	16.4	5.9	*36.2*	43.0	83.9	< LOD	< LOD	< LOD	< LOD	*126.9*
S3.1	26.4	18.7	19.6	5.6	*70.3*	20.8	< LOD	< LOD	< LOD	< LOD	< LOD	*20.8*
S3.2	30.8	25.4	20.6	5.4	*82.2*	11.7	< LOD	< LOD	< LOD	< LOD	< LOD	*11.7*
S4	19.9	7.3	1.8	0.2	*29.2*	15	6	< LOD	12	5	< LOD	*38*
S4.1	40.7	14.1	4.7	0.6	*60.1*	18	4	< LOD	12	5	6	*45*
S5	2.0	0.1	0.1	0.2	*2.4*	7	4	< LOD	8	2	< LOD	*21*
S6	18.9	5.6	1.7	0.2	*26.4*	3	< LOD	< LOD	9	5	< LOD	*17*
S7	4.3	0.6	1.4	2.1	*8.4*	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< *LOD*
S8	3.9	1.2	2.1	2.2	*9.4*	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< *LOD*



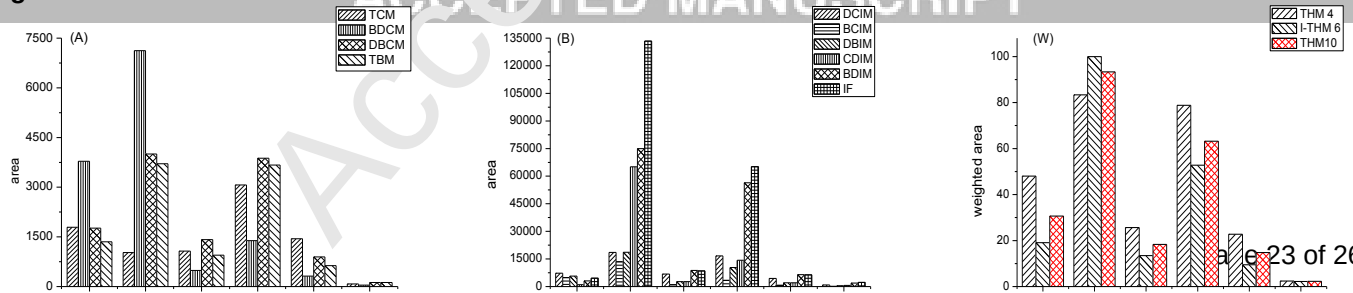
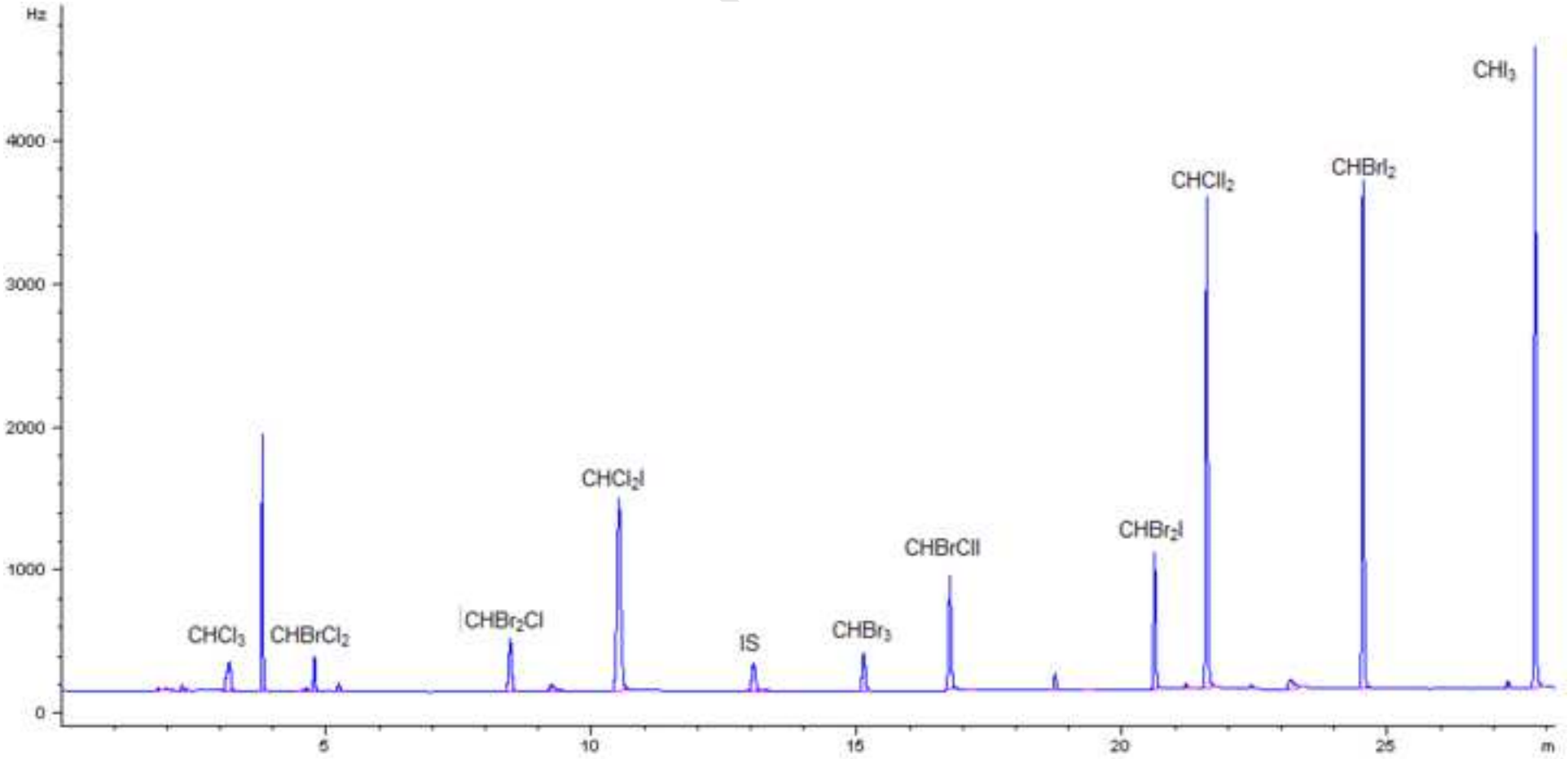


Fig 3


Hz
4000
3000
2000
1000
0
5
10
15
20
25
m
CHCl3
CHBrCl2
CHBr2Cl
CHCl2I
IS
CHBr3
CHBrClI
CHBr2I
CHClI2
CHBrI2
CHI3