Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1064 (2005) 97–106
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of odorous mixed chloro-bromoanisoles in water by solid-phase micro-extraction and gas chromatography–mass detection

Alfredo Díaz[a], Francesc Ventura[a,*], Mª. Teresa Galceran[b]

[a] *AGBAR, Aigües de Barcelona, Passeig de Sant Joan 39, 08009 Barcelona, Spain*
[b] *Dpt. Analytical Chemistry, University of Barcelona, Martí i Franqués 1–11, 08028 Barcelona, Spain*

Received 15 June 2004; received in revised form 26 November 2004; accepted 3 December 2004
Available online 25 December 2004

Abstract

A headspace–solid-phase micro-extraction (HS–SPME) and gas chromatography–mass spectrometry (GC–MS) method has been proposed for the simultaneous determination of odorous trihalogenated anisoles in water. Parameters affecting efficiency of HS–SPME procedure, such as the selection of the SPME coating, extraction time, temperature and ionic strength were optimized. The commercially available polydimethylsiloxane (PDMS 100 μm) fiber appears to be the most suitable for the simultaneous determination of these compounds. Run-to-run precision with relative standard deviations (R.S.D.s) between 5 and 15% were obtained for most of the compounds except for 2,5-dicloro-6-bromo-anisole, 2,3-dibromo-6-chloroanisole, pentachloro- and pentabromoanisole (>20%). The method was linear over two orders of magnitude, and detection limits were compound dependent and ranged from 0.03 ng/L for 2,4,6-trichloroanisole to 0.25 ng/L for 2,3-dibromo-6-chloroanisole. The HS–SPME–GC–MS procedure was tested using real samples and relatively good standard deviations were obtained when using *p*-iodoanisole as internal standard for quantification. This is the first time that the individual identification of odorous trihalogenated chloro-bromoanisoles has been reported, being HS–SPME–GC–MS a suitable method for simultaneous determination of these compounds in water at concentration levels below their odor limit of detection.

Keywords: Headspace solid-phase micro-extraction; Chloro-bromoanisoles; Water analysis; Odor; Gas chromatography–mass spectrometry

1. Introduction

Odors and tastes in drinking water are a matter of concern for water suppliers, and a frequent source of complaint by consumers, who mostly associate the presence of unpleasant odors and tastes with the possibility of health risks. The EEC Drinking Water Directive (European Council Directive 98/83 EEC) recommended that water intended for human consumption should include taste and odor parameters, and thus water companies have to carry out qualitative and/or quantitative determinations.

Chlorine and earthy, musty odors are the descriptions of taste and odors most frequently cited by consumers. At present, the major causes of earthy-musty odors are associated with algae metabolites such as geosmin and 2-methyl-*iso*-borneol (MIB), with odor thresholds in water of the 1–10 ng/L [1–5]. Recently, there has been much interest in those chlorinated anisoles that can impart a musty odor to water at even lower concentrations. A wide range of odor threshold values, 0.0007 ng/L [6] and 7 ng/L [2,7], has been reported for 2,3,6-trichloroanisole whereas for 2,4,6-trichloroanisole such values ranged from 0.03 ng/L [6] to 0.05–4 ng/L [8,9]. These compounds are probably formed by biomethylation of halogenated phenols coming from pesticides or wood preservatives such as 2,4,6-trichlorophenol or pentachlorophenol [10,11] or formed in water treatment processes, during transport through the distribution system or by microorganisms living in low chlorine disinfection areas of the water distribution system [12]. In addition, brominated anisoles can also be found in bromide-rich waters, for example, 2,4,6-tribromoanisole with an estimated odor

* Corresponding author. Fax: +34 93 342 3666.
E-mail address: fventura@agbar.es (F. Ventura).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.12.027

threshold of 0.03 ng/L has been identified as a source of odor in treated waters [9,13] and in wine [14]. Mixed chloro-bromo trihaloanisoles are also potential candidates for producing odors in waters containing low to medium bromide levels, although they have not been studied as they are not commercially available. The synthesis and determination of odor threshold concentration (OTC) values of several tri-chloro-bromo anisoles as well as their descriptors using the Flavor Profile Analysis (FPA) methodology [15,16] have been performed by our group and odor threshold values at the low ng/L level [17] have been obtained.

Different analytical strategies have been developed to determine organic compounds at these ultra-trace levels. For instance, closed-loop stripping analysis (CLSA) with large volume injection (LVI) has been applied to the determination of 2,4,6-tribromoanisole at the pg/L level [13]. Recently, new solventless techniques, such as stir bar sorptive extraction (SBSA) and solid-phase micro-extraction (SPME), have also been used for the analysis of odorous compounds. For instance, SBSA has successfully been applied to the analysis of trichloro- and tribromoanisoles [9,18] whereas SPME has been proposed for the simultaneous analysis of haloanisoles and halophenols [19], 2,4,6-trichloroanisole in cork [20,21], and other organic compounds causing odor events in water samples at low concentration levels [22,23].

The aim of this work is to develop an HS–SPME–GC–MS method for the determination of mixed halogenated anisoles at concentrations below or close to their odor threshold concentrations in water to control potential odor episodes in the water treatment plant.

2. Experimental

2.1. Chemicals and materials

Mixed trihaloanisoles (2,4-dibromo-6-chloro-; 2,4-dichloro-6-bromo-; 2,6-dichloro-4-bromo-; 2,6-dibromo-4-chloro-; 2,3-dichloro-6-bromo-; 2,3-dibromo-6-chloro-; 2,5-dichloro-6-bromo-; 2,5-dibromo-6-chloro-; 2,6-dichloro-3-bromo-; 2,6-dibromo-3-chloroanisole and 2,3,6-tribromoanisole) were synthesized. Purities were always higher than 90%. 2,4,6-Tribromo, 2,4,6,-trichloroanisole and pentachloroanisole were purchased from Sigma–Aldrich Chemie (Steinheim, Germany), whereas 2,3,6-trichloroanisole was from Ultra Scientific (North Kingstown, USA) Other reagents such as sodium chloride and the dechlorinating agent sodium thiosulfate, were obtained from Carlo Erba (Rodano, Italy) at a high purity (≥99%). The compound, *p*-iodoanisole (98%) used as internal standard was synthesized from commercially available *p*-iodophenol (Sigma–Aldrich Chemie, Steinheim, Germany) using dimethylsulfate (Merck, Darmstadt, Germany) in aqueous basic media. Methanol, hexane and ethyl acetate of residue analysis grade were supplied by J.T. Baker (Deventer, Holland), and acetone was supplied by Merck (Darmstadt, Germany). Water from the Milli-Q® water purification system (Millipore Corp., Beldford, MA, USA) was used.

SPME experiments were performed with a manual fiber holder supplied by Supelco (Bellefonte, PA, USA). Three commercially available fibers, poly(dimethylsiloxane), PDMS 100 μm; polyacrylate, PA, 85 μm; and StableFlex divinylbenzene-Carboxen-poly(dimethylsiloxane), DVB-CAR-PDMS, 50/30 μm from Supelco (Bellefonte, PA, USA) were tested. Before use, each fiber was conditioned in a heated GC split/splitless injection port under helium flow according to the manufacturer's instructions. Screw-capped vials (10 and 40 mL) sealed with a Teflon-lined silicone septum (Wheaton, Millville, NJ, USA) were used for storing standard solutions, as well as for sample derivatization and extraction in the HS–SPME procedure. The vials were previously cleaned by sonication with AP-13 Extran alkaline soap (Merck, Darmstadt, Germany) for 1 h, rinsed consecutively with (i) deionized water; (ii) nitric acid; (iii) again with deionized water; and (iv) acetone RS grade and dried at 50 °C overnight. Volumetric glassware was washed as described above, but was air-dried. Sodium chloride was cleaned (30 min sonication) with hexane:ethyl acetate solvent mixture (4:1), decanted and heated at 50 °C under low pressure to remove interfering organic substances.

Stock standard solutions of each halogenated anisole metabolite (1000 μg/mL) were prepared by weight in isooctane. Standard mixtures were prepared weekly or daily in acetone, depending on their concentration. All solutions were stored in the dark at 4 °C until use. For the optimization of the SPME procedure, Milli-Q® water spiked samples each containing 33 ng/L of the studied compounds, were prepared by adding 10 μL of a standard mixture of 100 ng/mL into 30-mL Milli-Q® water and placed in a 40-mL screw capped vial.

2.2. Sample collection

Llobregat River raw water (N.E. Spain, Barcelona S.) entering two water treatment plants, Sant Joan Despi (SJD) and Abrera-WTPs, located respectively near the mouth and upstream of the Llobregat river course and from several distribution reservoirs along the distribution system were analyzed using the HS–SPME method. Moreover, four tap water samples corresponding to different sources of the Barcelona water distribution system, Abrera and Sant Joan Despi WTPs (*Abrera and SJD*); Cardedeu WTP (*Ter*) located in Ter River (N.E. Spain, Barcelona N.) and Llobregat blended water from Abrera- and SJD-WTPs (*Llobregat*) were also analyzed. Additionally, a wastewater effluent treated with hypobromous acid exhibiting a strong odor was also analyzed. The samples were collected in 1-L amber glass bottles with PTFE-faced septa and polypropylene screw caps and stored at 4 °C. All analyses were performed within 2 days of sampling. Sodium thiosulfate (2 mL, 0.1 M) was added as a dechlorinating agent to tap water samples.

2.3. Synthesis of mixed halogenated anisoles

The first step in obtaining these compounds was the synthesis of the corresponding phenols, followed by methylation with dimethylsulfate (DMS) in aqueous basic media to produce the desired anisoles (average 60% yield) was performed. Depending on the halogen position, different synthetic procedures were used to obtain the phenolic precursors: (i) for 2,4,6-trihaloanisoles, direct bromination of the aromatic ring by aromatic electrophilic substitution was performed using the corresponding commercially chlorinated phenols; (ii) for 2,3,6-trihaloanisoles, the synthesis was basically the same but using available phenols halogenated in meta-position and favoring the reaction in *ortho*-position [31]. Both schemes are displayed in Fig. 1.

2.3.1. 2,4,6-Trihalophenols

Three grams (0.019 mol) of 2,4- or 2,6-dichlorophenol and 9 g of bromine (3 ml; 0.056 mol) were mixed and stirred in methylene chloride for 6 h at room temperature. The resulting solution was quenched with 2 ml of $Na_2S_2O_5$ (0.5 M). The organic phase was washed twice with 150 mL of a saturated NaCl solution, dried and evaporated in a rotary vacuum evaporator. The resulting product was purified by chromatography using a silica gel column (20 cm × 5 cm; 70–230 mesh, Fluka) and hexane/ethyl acetate, 20/1 (v/v) as elution solvent. The eluate was then evaporated and 4.7 g of the expected product 2,4-diCl-6Br (or 2,6-diCl-4Br phenol; 90% yield both) was obtained.

The same procedure was performed to obtain 2,6-diBr-4-Cl and 2,4-diBr-6-Cl-phenols. In this case, 2-Cl-4Br- and 2-Br-4-Cl-phenols (1.1 g; 0.053 mol) and 3 g of bromine (approximately 1 ml; 0.018 mol) were used. The expected products were obtained (1.4 g, 95% yield). The purity (>95%) of the compounds was established by ^{1}H NMR and GC–MS.

2.3.2. 2,3,6-Trihalophenols

2,3,6-triBr-; 2,5-diCl-6-Br-; 2,3-diCl-6-Br-; and 2,6-diBr-3Cl-phenols were synthesized following the procedure described by Pearson et al. [24] with minor modifications. Briefly, into a stirring solution of *t*-butylamine (3.65 g; 0.05 mol) in 30 ml of toluene at −20 °C, bromine (3.84 g; 0.020 mol) was added drop wise over 2 min. The resulting solution was cooled at −70 °C and the corresponding 2,5-diCl-; 2,3-diCl-; 3-Br-; or 3-Cl-phenols (2 g; 0.01–0.015 mol) were then added and stirred for 6 h in dry conditions. The resulting solution was quenched with 2 ml of $Na_2S_2O_5$ (0.5 M) and purified as described above. The expected products 2,3-diCl-6-Br-; 2,5-diCl-6-Br-; 2,6-diBr-3Cl- and 2,3,6-triBr-phenols were obtained (average yield = 40%). The latter also gave 2,3-diBr- and 2,5-diBr-phenols as byproducts. Purity (>90%) of the compounds was established by means of ^{1}H NMR and GC–MS.

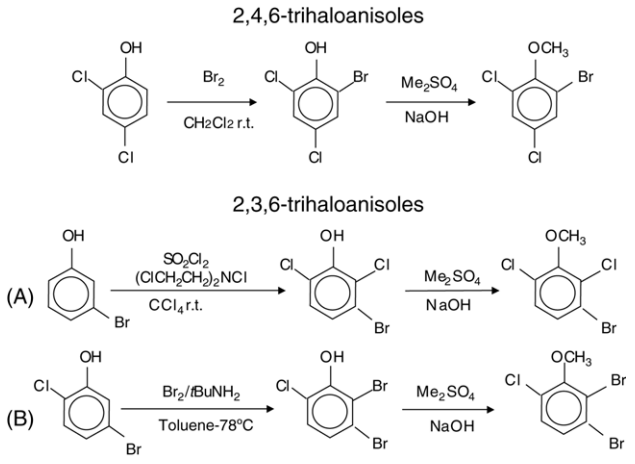

Fig. 1. Scheme of reactions to synthesize the target compounds.

2,5-diBr-6-Cl-phenol was synthesized following the procedure described by Smith and Butters [25] with minor modifications. Into a stirring solution of *N*-chloro-bis(2-chloroethyl)amine freshly prepared from the commercially available bis(2-chloroethyl)amine hydrochloride (4.13 g; 0.024 mol) in 10 ml of CCl_4, sulfurylchloride (1.56 g; 0.011 mol) was added drop wise over 2 min at room temperature. The resulting solution was added into a mixture of 2,5-diBr-phenol (1 g; 0.004 mol) and silica gel (6 g; 70–230 mesh) in 20 ml of CCl_4 and stirred overnight. The resulting solution was quenched and purified as described, rendering the desired 2,5-diBr-6-Cl-phenol (<40% yield). Its purity (85%) was established by means of ^{1}H NMR and GC–MS. The 2,5-diBr-4Cl-phenol and polychlorination products were determined as byproducts of the reaction.

2,3-diBr-6-Cl-phenol was synthesized following the procedure described by Watson [26] with minor modifications, 2,3-diBr phenol (0.7 g; 0.003 mol) was dissolved in 30 ml of CCl_4 and heated to reflux (80 °C). A solution of *tert*-butyl hypochlorite (1 g; 0.010 mol), freshly prepared according to the method described by Mintz and Walling [27] was added drop wise over 2 h. The resulting solution was quenched and purified as explained above, rendering the desired 2,3-diBr-6Cl-phenol (40% yield). Its purity (90%) was established by means of ^{1}H NMR and GC–MS.

2,6-diCl-3-Br-phenol was synthesized following two steps. First, 3-Br-phenol (2 g; 0.01 mol) was dissolved in 30 ml of CCl_4 and heated to reflux (80 °C). A solution of *tert*-butyl hypochlorite (2 g; 0.020 mol) was added drop wise over 2 h. This solution was quenched and purified as explained above, rendering the desired 3-Br-6-Cl-phenol (40% yield) and other byproducts. The obtained product (1 g) was then dissolved in 20 ml of CCl_4 and silica gel (6 g; 70–230 mesh). A solution of *N*-chloro-bis(2-chloroethyl)amine (4.13 g; 0.024 mol) freshly prepared and sulfurylchloride (1.56 g; 1 ml; 0.011 mol) was added dropwise to the mixture over 2 min and stirred overnight. After purification, the expected 2,6-diCl-3Br-phenol (<40% yield) was obtained and its purity (95%) was established by ^{1}H NMR and GC–MS.

2.4. HS–SPME procedure

The water sample (30 mL) was placed in a 40-mL screw-cap glass vial containing a 10 mm × 5 mm Teflon-coated stir bar and sodium chloride (6.5 g); the vial was closed and 5 μL of the internal standard, *p*-iodoanisole (1 ng/mL) in acetone was added through the septum. Then, the vial was clamped into a thermostatic water bath at 60 °C, which was placed on a hot plate/stirrer. A PDMS (100 μm) fiber was exposed to the headspace above the aqueous solution for 60 min Magnetic stirring at 900 rpm was applied during extraction. Finally, the fiber was desorbed in the injection port of the gas chromatograph for 1 min at 270 °C. Possible carryover was prevented by keeping the fiber in the injector for an additional time (∼10 min) with the injector in the split mode (purge on). Moreover, blanks were run periodically during the analysis to confirm the absence of memory effects.

2.5. Instrumentation

The optimization of the SPME procedure was carried out on a GC-8060 Fisons Instruments (Milan, Italy) capillary gas chromatograph equipped with an ECD detector. Quality parameters and quantitation of samples by HS–SPME were performed on a GC Fisons 8060 capillary gas chromatograph coupled to a Fisons MD 800 GC–MS quadrupole mass spectrometer (Milan, Italy). Separations were conducted on a DB-5 MS fused-silica capillary column, 30 m × 0.25 mm i.d. × 0.25 μm (J&W Scientific, Folsom, CA), with helium as carrier gas (70 kPa; 100 kPa N_2 for ECD make-up). The column was held at 30 °C for 3 min, ramped at 10 °C/min to 130 °C, next ramped at 15 °C/min to 250 °C and finally at 20 °C/min to 285 °C, where it was held for 7 min splitless injection at 270 °C in a SPME liner was used.

The quadrupole mass spectrometer was operated in electron ionization (EI) positive-mode. For EI experiments, instrumental parameters were set at the following values: filament emission current of 750 μA and electron multiplier voltage of 450 V, using perfluorotributylamine (FC-43) as reference. The transfer line and the source temperature were maintained at 290 °C and 200 °C, respectively. The instrument was operated in SIR mode at 0.08 s/scan with an ionization time of 100 ms. Different selected ions were monitored for the identification and quantification of each compound. The following ions were chosen (in italics the selected ion for quantification, the others were used as qualifier ions): *m/z* *212* and 195/197 for 2,4,6- and 2,3,6-trichloroanisoles; *m/z* *241*/256 for 2,4-diCl-6-Br-, 2,6-diCl-4-Br-, 2,5-diCl-6-Br- and 2,6-diCl-3-Br-anisoles; *m/z* 285/*300* for 2,6-diBr-4-Cl-, and 2,6-diBr-3-Cl-anisoles; *m/z* 344/*346* for 2,4,6- and 2,3,6-tribromoanisoles. Additionally, pentachloroanisole (*m/z* *237* and 265/280); pentabromoanisole (*m/z* *220* and 487/489) and *m/z* 219/*234* of the internal standard, *p*-iodoanisole were also selected. Once the optimum conditions were established (see Fig. 2) different acquisition windows during each chromatographic run were applied using a narrow mass range. Masslab version 1.4 software was used for data acquisition.

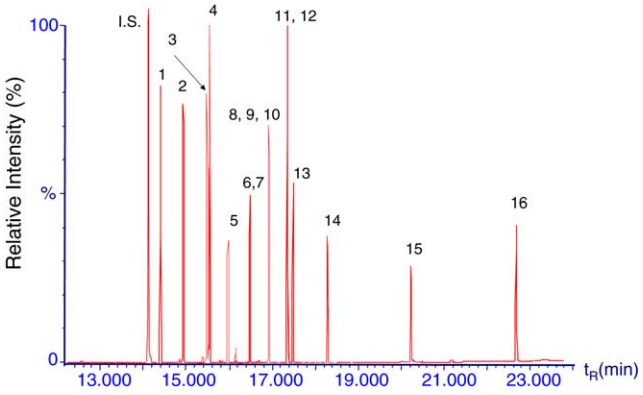

Fig. 2. TIC chromatogram of trihaloanisoles: (1) 2,4,6-triCl; (2) 2,3,6-triCl; (3) 2,4-diCl-6Br; (4) 2,6-diCl-4Br; (5) 2,5-diCl-6Br; (6) 2,6-diBr-4Cl; (7) 2,4-diBr-6Cl; (8) 2,5-diBr-6Cl; (9) 2,6-diBr-3Cl; (10) 2,3-diBr-6Cl; (11) 2,3-diCl-6Br; (12) 2,6-diCl-3Br; (13) 2,4,6-triBr; (14) 2,3,6-triBr; (15) pentachloroanisole; (16) pentabromoanisole. Conditions described in the text.

3. Results and discussion

The objective of this work was to develop a method for the simultaneous quantification of mixed halogenated anisoles in water at concentrations close to or under their odor threshold. Head space SPME was used instead of direct immersion in order to minimize the potential matrix effect at these low concentration levels, even in treated water samples, and taking advantage of the relatively low polarity and relatively high volatility of these compounds compared to halogenated phenols [28,29].

Several capillary chromatographic columns (i.e. DB-5MS, DB-17, DB-1701 and CP-Sil 8 CB), different film thickness (i.e. DB-5MS 0.25 μm and 1 μm) and temperature ramps were used to optimize the chromatographic separation of the compounds of interest. However, even at the best chromatographic conditions, some trihalogenated anisoles could not be separated. Thus, using the DB-5 MS 0.25 μm column at the conditions given in Section 2, the 2,4-diBr-6-Cl- and 2,6-diBr-4-Cl-coeluted as well as 2,3-diCl-6-Br- and 2,6-diCl-3-Br. Moreover, the three compounds, 2,3-diBr-6-Cl-, 2,6-diBr-3-Cl- and 2,5-diBr-6-Cl-anisoles, also eluted as a single peak. These compounds gave, as expected, similar mass spectra with the same characteristic *m/z* peaks and, therefore, MS separation was not possible. Quantification of unresolved peaks in real world samples was performed using a single compound as a reference for each group (2,6-di-Br-4-C1; 2,6-diCl-3-Br and 2,6-diBr-3-Cl, respectively). In relation to the elution order, it can be mentioned that the 2,4,6-mixed chloro-bromoanisoles were less retained than their corresponding 2,3,6-homologues. However, whereas 2,3,6-dichloro-bromoanisoles showed higher retention times than the 2,3,6-dibromochloroanisoles, the

2,4,6-homologues showed a different behavior eluting the diclhoro-bromo anisoles at lower retention times.

3.1. Optimization of the HS–SPME conditions

Different commercially available fibers were evaluated to obtain high sensitivity and selectivity for HS–SPME method. Three fibers were tested: polyacrylate, PA, 85 μm; polydimethylsiloxane, PDMS, 100 μm and Stable-Flex divinylbenzene-Carboxen-polydimethylsiloxane, DVB-CAR-PDMS, 50/30 μm. SPME conditions are described in Section 2. Ultrapure water spiked with mixed haloanisoles (33 ng/L each) was analyzed twice with each fiber by HS–SPME. To ensure the extraction of a large amount of compound, a long extraction time (60 min) was applied. Desorption temperature and desorption time for all tested fibers was 250 °C and 5 min. No carryover on a second desorption was found for any of the fibers, indicating complete removal of analytes at this time/temperature. The relative responses obtained for selected compounds using the different fibers are displayed in Fig. 3 as an example.

The three tested fibers gave good responses for these compounds in HS–SPME. Haloanisoles have a high affinity for all fibers tested independently of fiber polarity showing that physical constants such as high partition constant octanol–water (K_{ow}) [30] are more important than fiber polarity or volatility of compounds. As small differences for almost all compounds were observed for different fibers; PDMS 100 μm was chosen because. In addition to its high efficiency for the less volatile compound (pentabromoanisole), the chromatograms showed less interferences. So, this fiber was finally selected for the simultaneous analysis of mixed halogenated anisoles in water.

The extraction temperature profiles of halogenated anisoles were then studied. Four different temperatures starting from room temperature to 65 °C were examined. The profiles obtained (see Fig. 4) for all compounds showed the highest intensities at 50 °C for chlorinated compounds and 65 °C for the less volatile ones, such as pentachloro- and pentabromo-anisoles. A slight decrease at 65 °C was observed for the more volatile chlorinated and mixed chloro-

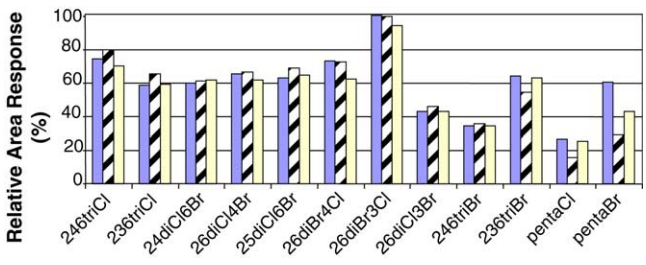

Fig. 3. Extraction efficiency of three SPME fibers using the HS–SPME–GC–MS procedure. Milli-Q® water containing 33 ng/L of halogenated anisoles; with 6.5 g of NaCl added; extraction time, 60 min; extraction temperature, 65 °C and stirring rate 1200 rpm: (■) PDMS 100 μm; (▨) CAR/DVB/PDMS; (□) polyacrylate.

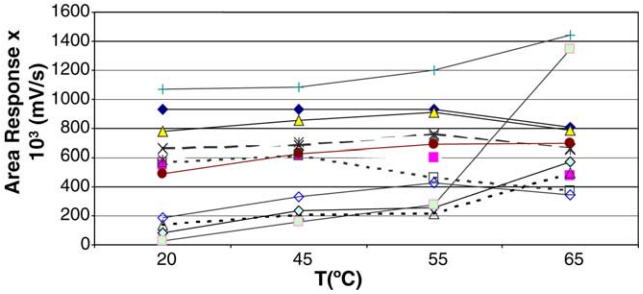

Fig. 4. Temperature profiles for HS–SPME of halogenated anisoles using the PDMS 100 μm fiber. Conditions as in Fig. 3. Compound identification: (♦) 246triCl; (■) 236triCl; (▲) 24diCl6Br; (×) 26diCl4Br; (✳) 25diCl6Br; (●) 26diBr4Cl; (|) 26Br3Cl; (◇) 26diCl3Br; (△) 246triBr (◊) 236triBr; (▫) pentaCl; (□) pentaBr.

bromo compounds and therefore, 60 °C was chosen as the optimum temperature for all subsequent analyses. The effect of ionic strength was also tested. An enhancement on the responses was obtained when sodium chloride concentration was increased to 3.7 M (6.5 g). As no increase on area response was observed at higher concentrations, this value was used for subsequent studies.

The extraction time profiles of halogenated anisoles were studied from 30 min up to 90 min, under the conditions described in Section 2. The equilibrium time is reached when a further increase of the extraction time does not result in a significant increase in the detector response, which was established as 1 hour at 60 °C. The effect of the stirring rate on the responses was also tested between 700 rpm and 1200 rpm. Based on extraction efficiency, similar responses were obtained for all compounds. As a compromise between analysis time and precision, which is a critical data in the quality parameters of the method, 900 rpm was selected.

Three desorption temperatures, 230 °C, 250 °C and 270 °C, within the recommended PDMS 100 μm fiber operating range, were evaluated for a desorption time from 1 to 5 min. Results showed that optimum temperature was 270 °C. All compounds were quantitatively desorbed from the PDMS 100 μm fiber in 1 min at 270 °C. In summary, for optimum sampling of odorous mixed trihaloanisoles from water by HS–SPME, 6.5 g of NaCl was added to 30 mL of water. Then the sample was maintained at 60 °C stirred at 900 rpm and the PDMS 100 μm fiber was exposed to the headspace for 1 h. The optimum desorption conditions in the GC injection port were 270 °C for 1 min.

3.2. Estimation of quality parameters

Quality parameters of the HS–SPME–GC–MS method were evaluated using the optimized conditions. To increase precision, a careful selection of internal standards for HS–SPME–GC–MS was carried out and *p*-iodoanisole, with a solubility, polarity and volatility in the same order as the analytes, was selected. The estimated log *P* value [31] of *p*-iodoanisole (3.24) is close to the experimental log *P* values

of 2,3,6-trichloroanisole (3.64) and 2,4,6-trichloroanisole (4.11) and the boiling points of the three compounds lie in the same range: 237 °C (*p*-iodoanisole), 227 °C (2,3,6-trichloroanisole) and 241 °C (2,4,6-trichloroanisole). Thus, *p*-iodoanisole can be reasonably suitable as internal standard if available deuterated standards are not used.

In addition, this compound has a suitable retention time and a response similar to the studied compounds working at the optimized conditions. Moreover, it is not currently found in environmental samples and it was not observed in the samples analyzed. Run-to-run precision of the method was determined using this internal standard and analyzing consecutively, on the same day, three replicates of Milli-Q® water spiked at a concentration 0.30 ng/L. R.S.D. values ranged from 3.8% (2,3,6-triCl-anisole) to 24.6% (2,3-diBr-6-Cl-anisole). For day-to-day precision three replicates were analyzed on three different days. Results are given in Table 1. All compounds gave R.S.D. values for run-to-run precision ranging from 17.9% (2,3,6-triCl-anisole) to 39% (2,3-diBr-6-Cl-anisole), the same as the compounds cited above. These values are acceptable, taking into account the very low concentration level spiked (0.30 ng/L), which is one-third of the odor threshold concentration obtained for these compounds [17]. The linearity of the optimized HS–SPME–GC–MS method was examined over the range 0.05–3 ng/L, expressed as the initial concentration of halogenated anisoles in water. This range agreed with environmental levels of the haloanisoles in water found in the literature [9,13], and it is a desirable range to evaluate possible odor events at the low concentration levels achieved by these compounds in a water distribution system. Linearity curves were obtained by plotting the area of each halogenated anisole relative to that of the internal standard—*p*-iodoanisole—(A/A_{is}) versus the concentration of each compound. The trichlorinated compounds and pentabromoanisole were linear over the range studied; the tribrominated species and 2,6-diBr-4-Cl-anisole were linear from 0.1 ng/L to 3 ng/L, whereas for the rest of anisoles the linearity was from 0.2 ng/L to 3 ng/L. Limits of detection (LODs) defined as the concentration which gives an area equal to the blank plus three standard deviations were determined. The area and the standard deviation of the blank were estimated from the calibration curve along the ranges studied. The limits of detection ranged from 0.03 ng/L for the most volatile compound (2,4,6-trichloroanisole) to 0.25 ng/L for 2,3-diBr-6-Cl-anisole. Data is given in Table 1 and the values obtained are at least two-fold lower than the experimental odor threshold concentration obtained for all compounds (1–30 ng/L) [17]. LODs for the studied compounds using this procedure as well as R.S.D.% values obtained, were similar or slightly higher than the values reported for 2,4,6-tribromoanisole using CLSA–LVI–GC–MS (0.015 ng/L ± 15%) [13], which is more complex and expensive and for stir-bar sorptive extraction SBSE–thermal desorption–GC–MS (0.022 ng/L ± 3%) [18].

To examine the feasibility of the HS–SPME method, three replicates of a free chlorine treated water sample spiked at 0.30 ng/L level were analysed. For some compounds this value lies between the LOD and LOQ but it has been used with the objective of studying the applicability of the method at these low concentration levels. The mean, bias and R.S.D.%

Table 1
Characterization, purity and quality parameters using the HS–SPME–GC–MS method of mixed haloanisoles

Trihaloanisole	*m/z* (%) (EI mode)	Purity[a] (%)	R^2 (linearity)[b]	LOD (ng/L)[c]	Run-to-run[d,e]	Day-to-day[f,g]
2,4,6-triCl	195 (100), 212 (70), 167 (47), 97 (25), 109 (20)	99	0.9989	0.03	4.9	23.7
2,3,6-triCl	212 (100), 169 (73), 197 (67), 97 (30), 109 (22)	98	0.9920	0.04	3.8	17.9
2,4-diCl-6-Br	241 (100), 256 (88), 213 (45), 97 (35), 109 (20)	99	0.9915	0.07	8.1	26.5
2,6-diCl-4-Br	241 (100), 256 (89), 213 (40), 97 (37), 74 (18)	90	0.9876	0.09	14.7	28.7
2,5-diCl-6-Br	256 (100), 241 (50), 213 (50), 97 (27), 109 (15)	99	0.9949	0.20	23.4	30.6
2,4-diBr-6-Cl	285 (100), 300 (90), 257 (40), 97 (30), 62 (22)	99	0.9942	0.20	9.8	19.9
2,6-diBr-4-Cl	300 (100), 285 (90), 257 (40), 97 (32), 62 (25)	98	0.9894	0.14	9.9	26.6
2,3-diBr-6-Cl	300 (100), 302 (70), 257 (52), 285 (45), 62 (21)	90	0.9910	0.25	24.6	39.0
2,6-diBr-3-Cl	300 (100), 285 (45), 257 (44), 97 (28), 62 (22)	85	0.9971	0.12	12.5	35.2
2,6-diCl-3-Br	256 (100), 241 (70), 213 (48), 97 (33), 74 (15)	80	0.9941	0.13	15.9	30.7
2,3-diCl-6-Br	256 (100), 241 (73), 254 (65), 97 (43), 109 (20)	98	0.9972	0.14	11.2	22.3
2,4,6-triBr	344 (100), 346 (95), 329 (83), 62 (55), 141 (37)	95	0.9961	0.09	12.5	31.1
2,3,6-triBr	344 (100), 346 (95), 303 (40), 62 (41), 329 (39)	99	0.9889	0.09	15.3	37.5
PentaCl	265 (100), 280 (90), 237 (80), 165 (40), 130 (30)	98	0.9868	0.15	21.0	34.3
PentaBr	502 (100), 459 (55), 299 (55), 487 (50), 220 (45)	98	0.9987	0.03	21.3	37.7

[a] Determined by means of ^{1}H NMR and GC–MS.
[b] Linearity range plotted from 0.025 ng/L to 3 ng/L for trichloroanisoles; 0.10 ng/L to 3 ng/L for 2,6-diBr-4-Cl-anisole, tribromoanisoles and pentahaloanisoles and 0.20 ng/L to 3 ng/L for 2,5-diCl-6-Br-, 2,6-diCl-3-Br- and 2,6-diBr-3-Cl-anisoles.
[c] Limit of detection.
[d] Precision expressed as R.S.D.s (%).
[e] Milli-Q® water spiked at: 0.30 ng/L.
[f] $n = 3$.
[g] $n = 3$ replicates × 3 days.

Table 2
Feasibility of HS–SPME–GC–MS method[a]

Trihaloanisole	Treated water (spiked at 0.30 ng/L)		
	Mean (ng/L)	R.S.D. (%), $n = 3$	Bias
2,4,6-triCl	0.22	8.8	−0.08
2,3,6-triCl	0.31	10.9	0.01
2,4-diCl-6-Br	0.24	29.8	−0.06
2,6-diCl-4-Br	0.35	32.4	0.05
2,5-diCl-6-Br	0.28	25.0	−0.02
2,4-diBr-6-Cl	0.30	12.7	0.00
2,6-diBr-4-Cl	0.18	35.0	−0.12
2,3-diBr-6-Cl	0.27	29.4	−0.03
2,6-diBr-3-Cl	0.24	32.4	−0.06
2,6-diCl-3-Br	0.22	35.6	−0.08
2,3-diCl-6-Br	0.31	11.3	0.01
2,4,6-triBr	0.24	20.0	−0.06
2,3,6-triBr	0.31	25.7	0.01
PentaCl	0.32	30.1	0.02
PentaBr	0.26	30.4	−0.04

[a] I.S.: *p*-iodoanisole.

values obtained are given in Table 2. The deviations of the mean from the true value fall in the range considered acceptable at this very low concentration level (−50% to +20%) [32] and as a consequence the method can be used for the analysis of 'real world' water samples.

3.3. Analysis of water samples

River water samples from the Llobregat river entering the Sant Joan Despí (SJD) and Abrera-WTPs were analyzed using the optimized HS–SPME method. Moreover, four tap water samples (Abrera, SJD, Llobregat and Ter) which are the main types of treated water in the Barcelona area and six samples collected in reservoirs along the distribution system were also analysed. As an example, the single-ion chromatogram of halogenated compounds from a river water sample entering the SJD water treatment plant is depicted in Fig. 5, where it can be observed that HS–SPME–GC–MS is a highly selective method for the analysis of halogenated anisoles. The results obtained are given in Tables 3 and 4. For the unresolved group of compounds (i.e. 2,4-diBr-6-Cl- and 2,6-diBr-4-Cl-anisoles or 2,3-diBr-6-Cl-, 2,6-diBr-3-Cl- and 2,5-diBr-6-Cl-anisoles or 2,3-diCl-6-Br- and 2,6-diCl-3-Br-anisoles), the concentration of coeluting compounds as a sum was estimated using the linear regression of the most sensitive compound of each group. Thus, the first group was quantified using 2,6-diBr-4-Cl-anisole as a reference compound whereas 2,6-diBr-3-Cl- and 2,6-diCl-3-Br-anisoles were selected for the second and third groups, respectively.

All studied compounds were found in Llobregat river water entering SJD-WTP, except pentabromoanisole. The

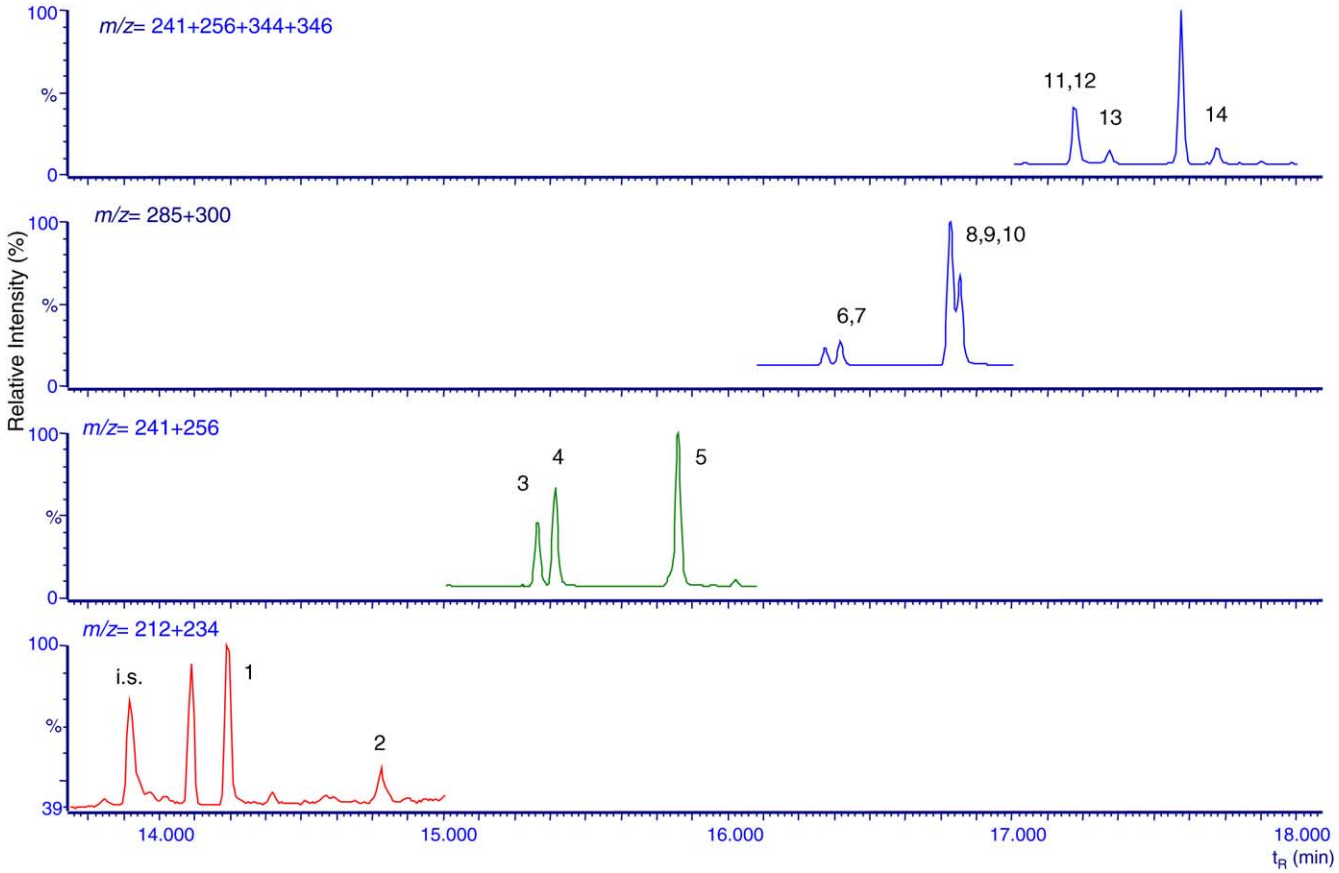

Fig. 5. HS–SPME single-ion chromatograms of haloanisoles from a river water sample entering SJD water treatment plant. Acronyms as in Fig. 2.

Table 3
Concentrations of mixed halogenated haloanisoles in Barcelona tap water and river water samples from different sources by HS–SPME–GC–MS method[a]

River water (ng/L)		Second step of SJD-WTP	Tap water (ng/L)			
Trihaloanisole	SJD	Sand filtered water (chlorinated)	SJD treated[b]	Abrera treated[c]	Llob. distrib.[d]	Ter distrib.[e]
2,4,6-triCl	0.12	*0.04*	*0.03*	0.29	0.22	<LOD
2,3,6-triCl	0.04	*0.04*	<LOD	0.89	*0.04*	<LOD
2,4-diCl-6-Br	0.26	*0.07*	*0.04*	0.29	*0.07*	0.14
2,6-diCl-4-Br	0.46	*0.09*	<LOD	0.35	*0.09*	*0.13*
2,5-diCl-6-Br	0.56	*0.20*	*0.20*	4.46	*0.20*	*0.20*
2,4-diBr-6-Cl; 2,6-diBr-4-Cl[f]	*0.14*	<LOD	<LOD	0.29	<LOD	<LOD
2,3-diBr-6-Cl; 2,6-diBr-3-Cl; 2,5-diBr-6-Cl[f]	*0.12*	<LOD	<LOD	0.18	<LOD	<LOD
2,3-diCl-6-Br; 2,6-diCl-3-Br[f]	1.49	*0.13*	*0.13*	0.45	*0.13*	*0.26*
2,4,6-triBr	*0.09*	<LOD	<LOD	0.53	<LOD	<LOD
2,3,6-triBr	0.48	0.59	*0.16*	0.60	*0.13*	*0.44*
Sum (ng/L)	3.61	1.35	0.47	8.13	1.03	1.20
PentaCl	0.28	0.72	<LOD	4.42	<LOD	<LOD
PentaBr	<LOD	*0.03*	<LOD	1.40	*0.09*	<LOD

LOD: limit of detection; estimated values between limit of detection and quantification are in italics.
[a] I.S.: *p*-iodoanisole.
[b] SJD treated: treated water leaving SJD-WTP.
[c] Abrera treated: treated water leaving Abrera-WTP.
[d] Llob. distrib.: blended water from SJD and Abrera-WTPs with grounwater.
[e] Ter distrib.: treated water from Ter river.
[f] Estimated values using the linear regression of 2,6-diBr-4-Cl; 2,6-diBr-3-Cl and 2,6-diCl-3-Br compounds, respectively, as a reference for each group.

highest concentrations among these low levels corresponded to the chlorinated compounds, 2,6-diCl-3-Br-/2,3-diCl-6-Br-anisoles being the most abundant species. For most of the anisoles, the concentration levels found were lower than 1 ng/L. Nevertheless, a total amount of trihaloanisoles of 3.6 ng/L was obtained, which is close to the average odor threshold concentration for the studied trihalogenated compounds [17]. The presence of these compounds in the water entering SJD-WTP can be due to the effluents of waste water treatment plants containing haloanisoles which are discharged into the river, and also to the biomethylation of halogenated phenols coming from natural origin or from industrial applications (i.e. pesticides, wood preservatives, etc.). In contrast, the analysis of Llobregat River water in its upper course, entering Abrera-WTP (35 km upstream SJD-WTP) did not show any detectable concentration of these compounds. The presence of high bromide levels in the upper course of the river where Abrera-WTP is located is probably

Table 4
Concentrations of mixed halogenated haloanisoles in Barcelona distribution reservoirs from different origins (Llobregat (1) and Ter (2)) by HS–SPME–GC–MS method[a]

Trihaloanisole	Distribution tanks (ng/L)					
	Cerdanyola–Montbrit (2)[b]	Trinitat (2)[b]	Guinardo (2)[b]	Las Lurdes (1)[b]	Esplugues (1)[b]	SJD (1)[c]
2,4,6-triCl	*0.03*	*0.03*	<LOD	<LOD	<LOD	<LOD
2,3,6-triCl	*0.04*	*0.04*	<LOD	<LOD	<LOD	<LOD
2,4-diCl-6-Br	<LOD	*0.20*	*0.11*	*0.13*	<LOD	<LOD
2,6-diCl-4-Br	<LOD	*0.20*	*0.12*	*0.12*	<LOD	<LOD
2,5-diCl-6-Br	<LOD	*0.20*	<LOD	0.49	1.55	<LOD
2,4-diBr-6-Cl; 2,6-diBr-4-Cl[d]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2,3-diBr-6-Cl; 2,6-diBr-3-Cl[d]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2,3-diCl-6-Br; 2,6-diCl-3-Br[d]	0.87	0.31	0.56	0.47	1.53	<LOD
2,4,6-triBr	*0.25*	*0.09*	*0.22*	*0.21*	0.36	<LOD
2,3,6-triBr	*0.20*	<LOD	<LOD	0.55	1.14	<LOD
Sum (ng/L)	1.21	0.71	1.01	1.97	4.58	<LOD
PentaCl	1.01	0.78	371	0.78	6.52	0.69
PentaBr	24.06	4.21	4.62	6.93	124.5	<LOD

LOD: limit of detection; estimated values between limit of detection and quantification are in italics. In all cases $Cl_2 < 0.9$ mg/L.
[a] I.S.: *p*-iodoanisole.
[b] Distribution reservoirs with blended water from SJD- and Abrera-WTP (2) or Ter WTP (1).
[c] Distribution reservoir from SJD-WTP.
[d] Estimated values using the linear regression of 2,6-diBr-4-Cl; 2,6-diBr-3-Cl and 2,6-diCl-3-Br compounds, respectively, as references for each group.

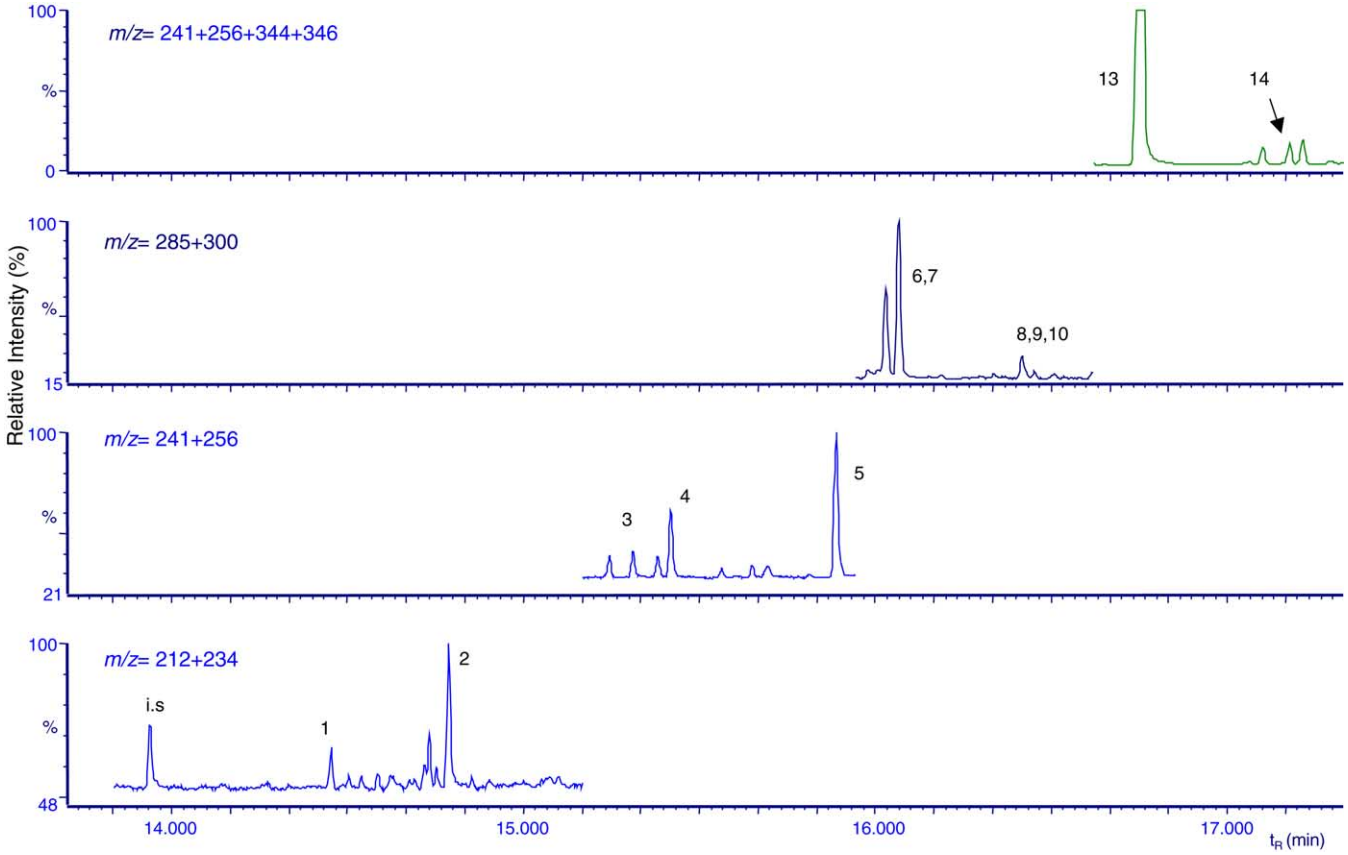

Fig. 6. HS–SPME single-ion chromatograms of haloanisoles from a wastewater effluent sample treated with hypobromous acid. Acronyms as in Fig. 2.

the origin of mixed haloanisoles found in Abrera treated water (see Table 3). In contrast, in SJD-WTP treated water, haloanisoles were found near their quantification limits and far from their odor threshold concentrations. Formation of higher molecular weight compounds such as pentachloroanisole in the second step of water treatment (after prechlorination) was observed. Both Ter distribution water and Llobregat tap water, coming from blending of SJD treated water and Abrera treated water, presented low levels of these compounds, which were always lower than their odor threshold concentration. Moreover, six distribution reservoirs located along distribution system were analysed (see Table 4), and the results obtained prove that the formation of these compounds in the distribution system is not negligible. The levels measured were higher in distribution reservoirs of water from Llobregat River origin than from Ter River origin. Moreover, anisoles concentration was higher in water from big distribution reservoirs than from small ones, probably due to the longer storage time of the water. Concentrations were in some cases near or even higher than the average OTC of these compounds. For instance, pentabromoanisole, which was detected in Esplugues reservoir has an OTC = 40 ng/L [17].

Finally, the method was also applied to a case study of wastewater treated with hypobromous acid from an industrial process which presented an intense earthy-musty odor. Geosmin and 2-methyl-isoborneol, which are the compounds that usually produce earthy-musty odors, were not found in detectable amounts. However, the presence of 2,4,6-triBr-anisole at 5 μg/L level can explain the characteristic earthy-musty odor of the sample. Other halogenated anisoles such as 2,6-diBr-4-Cl-; 2,4-diBr-6-Cl-anisole (4 ng/L) or 2,3,6-triBr-anisole (10 ng/L) were found at levels lower than their odor threshold concentrations (see Fig. 6).

4. Conclusions

The feasibility of HS–SPME–GC–MS for the analysis of odorous halogenated anisoles in water at a concentration near their odor threshold levels has been demonstrated. The PDMS coating was found to be the most effective for the analysis of halogenated anisoles. Maximum responses were obtained using 30-mL water samples salted with sodium chloride and set at an equilibration time of 60 min at 60 °C. HS–SPME in conjunction with GC–MS gave acceptable precision working at these low concentration levels. It was linear over two orders of magnitude, and the detection limits were at the low sub ng/L level. This inexpensive method avoids the use of organic solvents, is easy to use and requires less analysis time than the alternative CLSA–LVI–GC–MS or SBSE–TD–GC–MS methods currently used for the analysis of halogenated anisoles in

aqueous matrixes at their odor threshold concentration levels. Speciation of these compounds was performed for river water, treated water; water from distribution reservoirs of Barcelona and treated wastewater. From the results obtained, it can be concluded that although the background levels found in treated water do not cause odor problems, the very low odor threshold concentration of halogenated anisoles recommends their routine control to prevent taste and odor events.

Acknowledgements

This study was supported by Fundació AGBAR (Projecte Gustos i Olors). A. Diaz acknowledges a Ph.D. fellowship from Fundació Agbar.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.chroma.2004.12.027.

References

[1] A.A. Rosen, C.I. Masnni, R.S. Safferman, Water Treat. Exam. 19 (1970) 106.
[2] M.J. McGuire, S.W. Krasner, C.J. Hwang, G. Izaguirre, J. AWWA (1981) 530.
[3] G.A. Burlingame, R.M. Dann, G.L. Brock, J. AWWA (1986) 56.
[4] M. Pirzabari, H.S. Borow, S. Graig, V. Ravindran, M.J. McGuire, Water Sci. Technol. 25 (2) (1992) 81.
[5] J. Romero, F. Ventura, Tecnologia del Agua (1999) 25.
[6] R.F. Curtis, D.G. Land, M.N. Griffiths, D. Gee, M. Robinson, J.L. Peel, C. Dennis, J.M. Gee, Nature 235 (1972) 223.
[7] D.G. Guadagni, R.G. Buttery, J. Food Sci. (1978) 1346.
[8] UKWIR, Formation and occurrence of bromophenols, iodophenols, bromoanisoles and iodoanisoles in drinking water: an investigation of taste and odor potential, Report DW-05/13, UK Water Industry Research Limited, London, 1996.
[9] D. Benanou, F. Acobas, M.R. de Roubin, F. David, P. Sandra, Anal. Bioanal. Chem. 376 (2003) 69.
[10] J.W. Moore, S. Ramamoorthy, Organic Chemicals in Natural Waters. Applied Monitoring and Impact Assessment, Springer-Verlag, New York, 1984, p. 141.
[11] O. Jauregui, M.T. Galceran, in: W. Kleibohmer (Ed.), Environmental Analysis. Handbook of Analytical Separations, vol. 3, Elsevier, The Netherlands, 2001, p. 175.
[12] I.H. Suffet, C. Anselme, J. Mallevialle (Eds.), Advances in Taste and Odor Treatment and Control, AWWA Research Foundation and Lyonnaise des Eaux, Denver, CO, 1995.
[13] L. Malleret, A. Bruchet, M.-C. Hennion, Anal. Chem. 73 (2001) 1485.
[14] P. Chatonnet, S. Bonnet, S. Boutou, M.D. Labadie, J. Agric. Food Chem. 52 (2004) 1255.
[15] I.H. Suffet, J. Malleviale, Water Sci. Technol. 20 (8–9) (1988) 1.
[16] APHA, Section 2170: Flavor Profile Analysis. In Standard Methods for the Examination of Water and Wastewater, 20th ed. (Suppl.), American Public Health Association, Washington, DC, 1998.
[17] A. Díaz, C. Fabrellas, M^{a}.T. Galceran, F. Ventura, J. Agric. Food Chem. (2004), accepted for publication.
[18] O. Ochiai, K. Sasamoto, M. Takino, S. Yamashita, S. Dhaishima, A. Heiden, A. Hoffman, Analyst 126 (2001) 1652.
[19] L. Malleret, J. Dugay, A. Bruchet, M.-C. Hennion, J. Chromatogr. A 999 (2003) 135.
[20] C. Fischer, U. Fischer, J. Agric. Food Chem. 45 (1997) 1995.
[21] F. Bianchi, M. Careri, A. Mangia, M. Musci, J. Sep. Sci. 26 (2003) 369.
[22] B. Cancho, F. Ventura, M.T. Galceran, J. Chromatogr. A 841 (1999) 197.
[23] A. Diaz, F. Ventura, M.T. Galceran, J. Chromatogr. A 1034 (2004) 175.
[24] D.E. Pearson, R.D. Wysong, C.V. Breder, J. Am. Chem. Soc. 32 (1967) 2358.
[25] K. Smith, M. Butters, Tetrahedrom Lett. 29 (11) (1988) 1319.
[26] D.W. Watson, J. Org. Chem. 39 (1974) 1160.
[27] M.J. Mintz, C. Walling, Org. Synth. 49 (1969) 9.
[28] K.D. Buccholz, J. Pawliszyn, Anal. Chem. 66 (1994) 160.
[29] M. Moder, S. Schrader, U. Franck, P. Popp, Fresenius J. Anal. Chem. 357 (1997) 326.
[30] O. Pfeifer, U. Lohmann, K. Ballschmiter, Fresenius J. Anal. Chem. 371 (2001) 598.
[31] Syracuse Research Corporation (http://www.syrres.com), KowWin program, 2004.
[32] European Commission, Commission Directive 2002/657/EC of 12 August 2002, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Brussels, 2002.