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Determination of haloacetic acids in human urine by headspace gas chromatography–mass spectrometry

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ABSTRACT

Haloacetic acids (HAAs) are water disinfection byproducts (DBPs) formed by the reaction of chlorine oxidizing compounds with natural organic matter in water containing bromine. HAAs are second to trihalomethanes as the most commonly detected DBPs in surface drinking water and swimming pools. After oral exposure (drinking, showering, bathing and swimming), HAAs are rapidly absorbed from the gastrointestinal tract and excreted in urine. Typical methods used to determine these compounds in urine (mainly from rodents) only deal with one or two HAAs and their sensitivity is inadequate to determine HAA levels in human urine, even those manual sample preparation protocols which are complex, costly, and neither handy nor amenable to automation. In the present communication, we report on a sensitive and straightforward method to determine the nine HAAs in human urine using static headspace (HS) coupled with GC–MS. Important parameters controlling derivatisation and HS extraction were optimised to obtain the highest sensitivity: 120 μl of dimethylsulphate and 100 μl of tetrabutylammonium hydrogen sulphate (derivatisation regents) were selected, along with an excess of Na_2SO_4 (6 g per 12 ml of urine), an oven temperature of 70 °C and an equilibration time of 20 min. The method developed renders an efficient tool for the precise and sensitive determination of the nine HAAs in human urine (RSDs ranging from 6 to 11%, whereas LODs ranged from 0.01 to 0.1 μg/l). The method was applied in the determination of HAAs in urine from swimmers in an indoor swimming pool, as well as in that of non-swimmers. HAAs were not detected in the urine samples from non-swimmers and those of volunteers before their swims; therefore, the concentrations found after exposure were directly related to the swimming activity. The amounts of MCAA, DCAA and TCAA excreted from all swimmers are related to the highest levels in the swimming pool water.

1. Introduction

Chemical disinfectants are effective for killing harmful microorganisms in drinking water, but they also oxidize organic matter forming disinfection byproducts (DBPs). The major DBP fraction corresponds to the volatile trihalomethanes and the non-volatile haloacetic acids (HAAs), regulated in drinking water by the U.S. EPA, with the maximum aggregate contaminant level established as 60 μg/l [1,2]. HAAs either proven to be a health risk and their effects on mammals have been studied extensively [3–6]. HAAs can enter the human body through different routes since drinking water, apart from drinking, is also used for cooking, showering, bathing, and similar activities [7]. Ingestion of swimming pool water during swimming is expected to be an important route of exposure because in a swimming pool, HAAs may reach higher concentrations (1–800 μg/l) than those normally found in drinking water (0–100 μg/l) [5,8]. On the other hand monochloroacetic, dichloroacetic and trichloroacetic acids have also been detected in urine as metabolites of trichloroethylene, trichloroetanol and chloral hydrate, which have been used extensively as industrial solvents and extractants as well as therapeutic agents and anaesthetics [9,10].

Few methods have been published to determine HAAs in urine and they generally lack the sensitivity required for their detection at trace levels. Dichloroacetic and trichloroacetic (TCAA) acids have previously been measured in urine by using liquid chromatography [10,11], although gas chromatography (GC) is the most widely used technique due to its inherent advantages. GC determination requires a preliminary derivatisation step due to the low volatility and high polarity of these compounds; several of the derivatising reagents employed have been dimethylsulphate [9], acid–alcohol [12–15], BF_3–methanol [16] or pentafluorobenzyl bromide [17]. These methods are characterised by numerous separation steps such as: centrifugation of the urine sample, acidification and extraction with methyl *tert*-buthyl ether (MTBE), centrifugation, evaporation and finally derivatisation. Only two methods have been

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developed to determine the nine HAAs in urine; in both cases the above-mentioned separation steps are required [14,17]. The derivatisation of the HAAs to their methyl esters using sulphuric acid and methanol (80 °C, 20 min) after evaporation, followed by headspace solid-phase microextraction with GC and capture detection, [14] provides detection limits (LODs) in the range of 1–16 μg/l. This method has several drawbacks such as inadequate sensitivity to determine HAAs in human urine (the experiments are carried out with rat urine using intentional high levels of the toxic), multiple sample manipulations (which increase its uncertainty), damage of the fiber during the absorption step by methanol and the heating of the sample which favours the degradation of HAAs to trihalomethanes [14]. There is only one method to determine the nine HAAs at ng/l levels (LODs, 25–1000 ng/l) in human urine, which is using GC/ion trap mass spectrometry (negative ion chemical ionization mode). Samples are also acidified, extracted and evaporated before derivatisation with pentafluorobenzyl (PFB) bromide by sonication (2.5 h, 45 °C) [17]. The method is not only labour-intensive and time consuming but also trihalogenated HAAs might initially form unstable PFB derivatives that quickly decarboxilate to PFB-ethane; moreover the high sensitivity achieved is related to the high response of the detection instrument for PFB derivatives [17].

The static headspace (HS) technique arises as a simple and fast technique that allows *in situ* derivatisation/extraction of the compounds, which greatly simplifies sample treatment. However, to our knowledge, only three methods have been reported about the determination of TCAA by static HS: one is related to the determination of TCAA after its derivatisation with dimethylsulphate (4 h, 60 °C) by GC–ECD (LOD, 100 μg/l) [18], and the others have to do with the evaluation of the thermal decarboxylation of TCAA to chloroform (1.5 h, 90 °C) providing LOD of 2 mg/l [19] or 20 μg/l [20] by manual or automatic alternative HS, respectively. In all cases the sensitivity is inadequate to quantify TCAA in human urine and, in addition, it is difficult to discriminate between the amount of TCAA and chloroform (both DBPs) present in the native sample. Dynamic HS (purge and trap, P&T) CG–MS has also been used to determine TCAA in human urine, providing a low sensitivity (LOD, 3 μg/l); the derivatising reagent is 600 μl of sulphuric acid–methanol per 300 μl of urine using the soil module of a modified P&T autosampler [15].

From the foregoing, the aims of this work are to: (i) reduce the time of sample treatment (omitting extraction, centrifugation, evaporation, etc.) (ii) employ aqueous derivatising reagents, (iii) minimise the degradation of the HAAs during the whole procedure, and (iv) obtain enough sensitivity to determine HAAs at ng/l levels in urine samples. The proposed HS–GC–MS method consists of a fast derivatisation stage in which an aliquot of *n*-pentane (200 μl per 12 ml of urine) favours the evaporation of methyl esters under soft conditions (*viz*. 70 °C, 20 min) during automatic HS extraction. For the first time a rigorous study has been carried out on the absorption of HAAs in swimmers after bathing.

2. Experimental

2.1. Chemicals and standards

Monochloroacetic (MCAA), monobromoacetic (MBAA), dichloroacetic (DCAA), dibromoacetic (DBAA), bromochloroacetic (BCAA), trichloroacetic (TCAA), tribromoacetic (TBAA), bromodichloroacetic (BDCAA) and dibromochloroacetic (DBCAA) acids, as well as their respective methyl esters and 1,2-dibromopropane (internal standard, IS) were purchased from Sigma–Aldrich (Madrid, Spain). Anhydrous sodium sulphate, dimethylsulphate (DMS) and tetrabutylammonium hydrogen sulphate (TBA–HSO_4) were supplied by Fluka (Madrid, Spain). The solvents *n*-pentane and methyl *tert*-butyl ether (MTBE) were obtained from Merck (Darmstadt, Germany). All products were handled using efficient fume hoods and wearing protective gloves. Individual stock solutions of haloacetic acids and their methyl esters were prepared at concentrations of 1 g/l in MTBE and stored in amber glass vials at −20 °C. More dilute individual or cumulative solutions were prepared in 5 ml of MTBE. Working standard solutions were prepared in mineral water (free of DBPs) at the microgram-per-liter level.

2.2. Gas chromatographic system and conditions

Sample analyses were performed with an HP 6890/5973N GC/MS instrument (Agilent Technologies, Palo Alto, CA, USA) coupled to an HS autosampler (HP 7694). The autosampler was equipped with a tray for 44 consecutive samples, an oven capable of holding six glass vials, where the headspace was generated, and a sampling system comprising a stainless steel needle, a six-port-injection valve with a 3 ml loop, and two solenoid valves (for pressurization and venting). The operating conditions for the HS autosampler were as follows: vial equilibration time, 20 min; oven temperature, 70 °C; vial pressurization time, 30 s; loop temperature, 95 °C; loop fill time, 12 s; transfer line temperature, 105 °C. Helium (6.0 grade purity, Air Liquid, Seville, Spain), regulated with a digital pressure and flow controller, was used both to pressurize vials (18 psi of flow pressure) and drive the formed headspace to the injection port of the gas chromatograph, which was equipped with a crosslinked HP-5MS [(5%)-phenyl-(95%) methylpolysiloxane] capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The chromatographic oven temperature program was as follows: 40 °C, hold 3 min, 20 °C/min to 60 °C with a 3 min hold, then 5 °C/min to 100 °C and finally 25 °C/min to 250 °C and hold for 3 min. The chromatographic run was complete in 24 min. A solvent delay of 3 min was set to protect the filament from oxidation. Injection was done in the split mode (1:20 split ratio) for 1 min, with an inlet temperature of 250 °C. Mass spectra were obtained at 70 eV in the electron impact ionization mode. The source and quadrupole temperatures were maintained at 230 °C and 150 °C, respectively. Optimization experiments were conducted in total ion chromatogram (TIC) mode between *m*/*z* 45 and 255 at 3.5 scans/s. Quantification of HAA methyl esters was performed in selected ion monitoring (SIM) mode, and five different acquisition windows were defined taking into account the retention times and suitable fragments of HAA methyl esters (the base peaks used for quantification are boldfaced): **59**, 79, 108 (MCAA); **59**, 93, 95 (MBAA); **59**, 83 85 (DCAA); 59, **117**, 119 (TCAA); 59, 127, **129** (BCAA); 59, 171, **173** (DBAA); **59**, 161, 163 (BDCAA); 59, **207**, 209 (CDBAA); **59**, 251, 253 (TBAA); 42, 123, **121** (1,2-dibromopropane, the internal standard). All the scans were performed in high resolution mode and with a dwell time of 100 ms. Total ion chromatograms were acquired and processed using G1701DA (rev. D.01.02) MSD Productivity ChemStation software (Agilent Technologies) on a Pentium IV computer that was also used to control the whole system.

2.3. Sampling

Urine samples were kindly supplied by swimmers of an indoor swimming pool as well as other subjects who had not entered a swimming for a period of 48 h. This study was carried out during the winter of 2009 for 4 months, when the temperatures ranged from 1 to 15 °C and, therefore, it was impossible for the swimmers to use the outdoor swimming pools. The volunteers were healthy adults and they did not consume any water or drinks that contained chlorinated water during the experiment, they consumed only min-

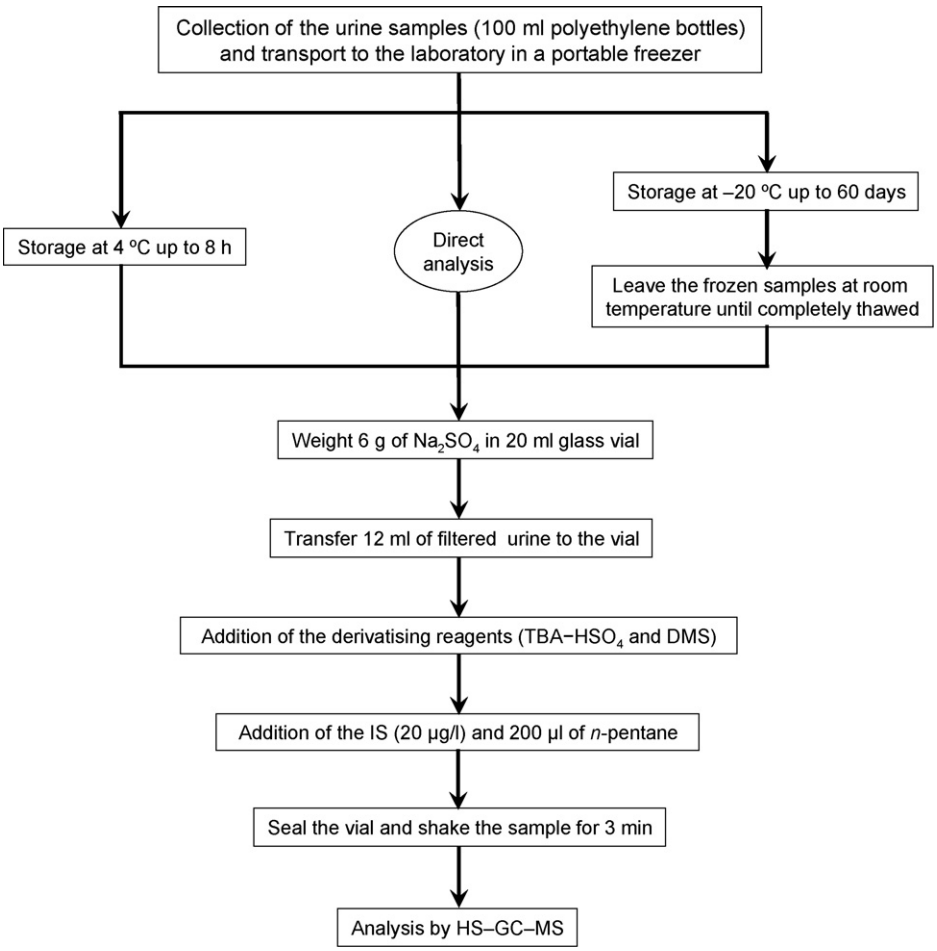

Fig. 1. Flow diagram representing the whole protocol carried out in the determination of HAAs in urine.

eral water (free of DBPs). In addition, the subject avoided activities such as visiting a dry cleaning store or industries (which can contain solvents in the indoor that are metabolised to HAAs), which might influence urinary HAAs excretions during the 24 h prior to the swimming activity. Individuals were informed of the research study and agreed to participate in the study and gave permission for the analysis of their urine. In the present study we also obtained the permission of the managers of the swimming pool subject.

Samples were collected 15–30 min before and after bath activity in sterilized polyethylene bottles of 100 ml (with hermetical close). The swimmers dried themselves properly before urinating and samples were collected in an area separated from the site of exposure in order to avoid the risk of contamination. Urine samples were transported to the laboratory in a portable freezer and immediately analysed after collection or stored at 4 °C up to 8 h. When the time between sample collection and analysis exceeded 8 h, samples were stored at −20 °C up to 60 days as maximum to avoid storage degradation. The frozen samples were left at room temperature until completely thawed. After gentle mixing, urine was transferred into the analysis vials and analysed in quintuplicate ($n = 5$). The whole procedure followed in this work is schematically shown in Fig. 1. Water samples were collected in amber glass bottles containing 1.6 g of ammonium sulphate as quenching reagent of residual chlorine, stored at 4 °C and analysed within 2 days of collection. Two samples were collected each day and both analysed in quintuplicate ($n = 10$).

Urine samples were filtered through a 0.45 μm cellulose filter (Millipore Ibérica, Spain). Samples were placed in 20 ml glass flat bottomed vials, with 20 mm PTFE-silicone septum caps and a crimped aluminium closure (Supelco, Madrid, Spain). Vials and septa were heated at 100 and 70 °C, respectively, overnight prior to use.

2.4. Analytical procedure

As depicted in Fig. 1, 12 ml of filtered urine or blank urine sample containing between 0.1 and 300 μg/l of each HAA were placed in a 20 ml glass vial containing 6 g of Na_2SO_4 to saturate the sample. Then, 100 μl of a 0.5 M concentration of the ion-pairing agent (TBA–HSO_4), 120 μl of derivatisation reagent (DMS, 1.3 mmol, i.e., high excess), 1,2-dibromopropane (IS) at a level of 20 and 200 μl of *n*-pentane were added sequentially. The vial was immediately sealed and stirred in a Vortex mixer for 3 min, and then placed in the autosampler carrousel. Samples were analysed by HS–GC–MS, using the operating conditions as above mentioned. The HAA concentrations were calculated by relating to previously created calibration curves, where the peak area ratios (sample/IS) were plotted as a function of the sample concentration.

3. Results and discussion

3.1. Optimization of the chemical variables

In a previous work, we developed a method for the determination of the nine HAAs at ng/l level in water after simultaneous extraction/derivatisation by HS–GC–MS [8]. In this paper, we demonstrate that the presence of *n*-pentane aliquots increases the derivatisation yields of methyl haloacetates as well as min-

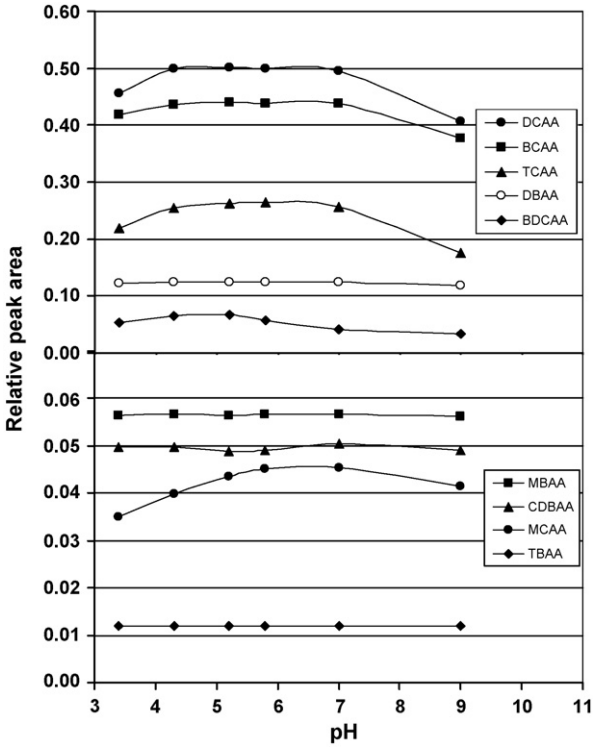

Fig. 2. Influence of the pH on the generation headspace process of HAAs from urine samples.

imising their degradation to trihalomethanes. For urine samples, preliminary experiments were carried out using the same GC–MS conditions [8] but the chemicals and HS conditions must again be optimised taking into account the different matrices.

In mammals, haloacetic acids can be directly determined in urine since they are excreted unchanged or as metabolites of other compounds, which simplifies the analytical procedure although the derivatisation step is mandatory. In order to obtain the best sensitivity of the method, the first variable studied was the sample volume and therefore uncontaminated urine was used as the blank. To ensure that the autosampler needle will not come into contact with the urine sample during the sampling time, 12 ml of urine was taken as the highest value (when salt was added to 20 ml vials containing 12 ml urine, the volume was increased to ~15 ml). The influence of the sample volume was examined from 8 to 12 ml (in 20 ml vials) using the blank urine fortified with 20 μg/l of each methyl ester standard and 6 g of Na_2SO_4. The signal abundance increases on increasing the sample volume up to 12 ml, probably as the result of the increasing HAA ester concentrations in the headspace. Therefore, a sample volume of 12 ml (in 20 ml vials) was adopted.

For the study of the chemical variables, 12 ml of the blank urine was prepared containing 20 μg/l of each HAA, 5 g of Na_2SO_4, 125 μl of a 0.05 M TBA–HSO_4 solution and 100 μL of pure DMS (derivatisation reagents), 20 μg/l of internal standard (1,2-dibromopropane) and 150 μl of n-pentane in 20 ml glass vials according to the reagent concentration used for water analysis [8]. The generation of the headspace is markedly affected by the sample pH; this variable was studied over the 3–9 range, using a spiked blank urine. As can be seen in Fig. 2, The sample pH influences in a minor extension to four HAAs (MCAA, DCAA, TCAA and BDCAA) than to the other five remaining ones that did not show any influence Since the HAAs commonly found in the real urine sample are MCAA, DCAA and TCAA, a pH value of 5.5–6.5 was selected as optimal. This range of pH was obtained by the addition of the derivatising reagents to the urine sample, thus for simplicity, adjustment of the pH was not applied whereby the aqueous standard should be adjusted to pH 5.5–6.5 with diluted NaOH. The addition of salts may result in the change of the vapour and partial pressure, solubility, thermal conductivity, density, surface tension, etc. of each compound. These changes, if they occur, will result in the variation of the vapour/liquid equilibrium system [21]. Hence, the addition of Na_2SO_4 was studied between 0 and 6 g (amount that provides the saturation of the sample). The abundance signal increased for the nine HAAs as the amounts of salt increased. A concentration of 6 g of Na_2SO_4 per 12 ml of urine (saturated solution) was selected as the working value in order to homogenize urine samples since they contain different values of ionic strength [22]. The effect of the concentration of the derivatising reagent was studied using amounts of pure dimethylsulphate (DMS) between 50 and 200 μl. The reaction yield increased as the volume rose to 110 μl, above which it remained constant, but above 130 μl the excess of DMS was extracted in *n*-pentane and volatilised, appearing in the chromatogram. The derivatisation of HAAs is implemented in the presence of tetrabutylammonium hydrogen sulphate (TBA–HSO_4) as an ion-pairing agent; volumes between 75 and 150 μl of a 0.5 M TBA–HSO_4 solution were assayed in the reaction. The analytical signals for all HAAs increased drastically on increasing TBA–HSO_4 solution volume up to 90 μl, above which it remained constant; in order to ensure complete derivatisation of the HAAs without too much excess, 120 μl (1.3 mmol) of pure DMS and 100 μl of 0.5 M TBA–HSO_4 were chosen for the optimal derivatising volume. The last parameter tested was the volume of *n*-pentane since, as mentioned above, the addition of an organic modifier increases the derivatisation yields of methyl haloacetates and also minimises their degradation to THMs [8]. The addition of 200 μl of *n*-pentane provided the best results in terms of peak area for all compounds. This can be ascribed to the fact that in the presence of *n*-pentane the most unstable HAA methyl esters (BDCAA, CDBAA and TBAA) are stabilized in the organic medium hindering their hydrolysis and decarboxylation to their respective THM. The optimal amounts of each variable in urine samples are higher than those found for the determination of haloacetic acids in water due to the matrix effect.

Urine samples require filtering through 0.45 μm cellulose filters before analysis because the derivatisation and extraction process are favoured; so, adsorption of HAAs onto the cellulose material and particles present in the samples was checked before-hand. To this end, recovery studies of HAAs added to urine samples before and after filtration through these filters were conducted and the results were found to be close to 100%. Therefore, the filtering material and particles present in the samples did not adsorb HAAs.

3.2. Optimization of instrumental variables

The instrumental variables of the headspace autosampler were also studied and verify that oven temperature and vial equilibration time are the most influential parameters since both variables affect the derivatisation and extraction as well as the stability of the HAAs. The effect of the oven temperature and vial equilibration time was studied from 50 to 90 °C and 10 to 40 min, respectively. The peak areas increased for MCAA, MBAA, DCAA, TCAA, BCAA, DBAA and BDCAA as the oven temperature and equilibration time rise to 70 °C and 20 min, respectively. However, at this temperature some degradation of the CDBAA and TBAA to their respective trihalomethanes was observed in the chromatogram. Since both compounds were usually undetected in drinking and swimming waters as well as in the urine of people exposed (the major compounds are MCAA, DCAA and TCAA), an oven temperature of 70 °C and a vial equilibration time of 20 min were selected as a compromise. The oven used was one capable of simultaneously holding six glass vials, where the headspace was generated. Once the headspace was generated

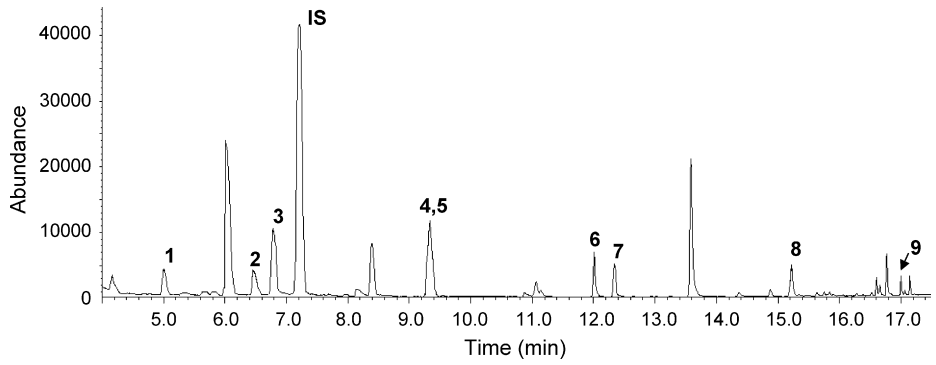

Fig. 3. GC–MS chromatogram in SIM mode for urine sample spiked with 5 μg/l of each HAA. Peak identification: MCAA (1); MBAA (2); DCAA (3); TCAA (4); BCAA (5); DBAA (6); BDCAA (7); CDBAA (8); TBAA (9); 1,2-dibromopropane (IS).

and enriched with HAA methyl esters, its individual injection into the mass spectrometer through the interface was carried out in two steps, namely: vial pressurization and the filling of the 3-ml injection valve loop by venting the vial. Pressurization times between 15 and 45 s and venting time above 12 s caused negligible changes in the abundance signals for all HAAs and thus a pressurization time of 30 s and a venting time of 12 s were selected as the working value.

3.3. Study of HAA stability in urine

The critical steps in the analysis of biological samples are the collection, storage and handling of samples, during which contamination, loss and/or degradation of analytes may occur. A thorough study has been performed concerning the stability of the nine HAAs in two groups of urine specimens from unexposed volunteers (free of HAAs) and exposed swimmers. Urine specimens from unexposed people were spiked with microliters of HAAs in MTBE in order to obtain a final concentration of 5 μg/l of each compound in the urine sample. The time of urine spike/collection was recorded on the label of the urine containers. Spiked urine samples and urines from exposed people were stored in 100 ml polyethylene bottles at 4 or at −20 °C and analysed between 0 and 60 days after storage using the procedure described in Section 2.4. The results obtained show that, in all instances, negligible differences in stability were found between spiked urine samples and urine from exposed volunteers (which only contained DCAA and TCAA). Concentrations of five HAAs (DCAA, TCAA, BCAA, DBAA, BDCAA) of spiked urine samples (DCAA and TCAA for urine from exposed people) stored at 4 °C remained constant for up to 4 days, after which their concentrations decreased slightly as the storage time is increased up to 14 days, probably due to the fact that they were degraded. There were significant differences for the other compounds, thus MCAA and MBAA remained constant for 2 days whereas TBAA and CDBAA only for 8 h since they were degraded to their respective THMs. In order to determine the nine HAAs in the urine samples, the storage at 4 °C was maintained only 8 h. On the other hand, small fluctuations around the mean value of the HAA concentrations with time were observed in all the urine samples stored at −20 °C for the 60 days of the study. Based on these results, urine samples were collected in polyethylene bottles, transported to the laboratory in a portable freezer and stored at 4 °C up to 8 h or at −20 °C up to 60 days before their analysis.

3.4. Validation of the method

After optimization of the HS–GC–MS method, the next step was research on the application of the method in human urine samples. The mass spectrometer was set in SIM mode to reach higher sensitivity and selectivity. The ions monitored to identify and quantify each analyte are listed in Section 2.2. For quantification, calibration curves were constructed by analysing 12 ml blank urine spiked with the nine HAAs at eleven different concentrations ranging from 0.1 to 300 μg/l. Figures of merit from the proposed method, listed in Table 1, show: detection limits (LOD, expressed as three times the regression standard deviation divided by the slope of calibration graphs); linear ranges (the limit of quantification for each compound was taken as the lowest concentration in the linear range); and precision (calculated as RSD percentage), obtained through measuring eleven samples containing 2 μg/l of each HAA expressed as within-day and between-day (four different days). The LODs ranged from 0.01 to 0.1 μg/l for all compounds except for TBAA (0.4 μg/l) due to its lower GC signal. In order to assess the chromatographic resolution and efficiency of the analytical procedure, Fig. 3 shows an HS–GC–MS chromatogram corresponding to a blank urine spiked with 5 μg/l of the nine HAAs.

A recovery study was conducted in order to validate the method proposed for urine samples. Five urine samples from unexposed individuals (free of HAAs) were spiked with HAAs at two concentration levels (2 and 20 μg/l) and analysed in quintuplicate. The concentrations in the spiked urine samples were compared to those obtained from aqueous standards prepared like the urine samples but adjusting their final pH to values similar to the urine sample ones (5.5–6.5) with diluted NaOH. All compounds were found to have average recoveries between 92 and 95% for low and high amounts, respectively (88 and 92% for TBAA probably because its partial degradation to bromoform increases in urine with respect to the water sample). Therefore, no significant matrix effect was observed in the determination of HAAs in urine samples. Compared to previous results, the recoveries of the proposed HS–GC–MS method (90–95%; nine HAAs) are similar to those obtained by the HS–SPME–GC–ECD method (86–110%; DCAA and TCAA) [14] and higher than what is found using GC/ion trap mass spectrometry (77–104%; nine HAAs) [17].

The quality control for swimming water and urine samples was evaluated by checking several parameters. The same amount of 1,2-dibromopropane solution in MTBE was spiked into each sample as an internal standard to check the efficiency of the HAA extractions from water/urine samples. A field water/urine blank was analysed with each set of samples to confirm that no contamination occurred. Calibration was performed by the external standard method using eleven different concentration standards. The retention times of the prepared methyl ester derivatives of HAAs were confirmed by comparing their GC peaks to the peaks of commercially available methyl haloacetates. An external standard solution with known HAA concentrations was analysed with each set of water/urine samples after every tenth sample, to check the validity of the calibration curves. If the observed concentrations of the external standard differed from the expected value by

Table 1
Analytical figures of merit of the determination of HAAs.

Compound	LOD (μg/l)	Linear range (μg/l)	RSD, $n = 11$ (%)	
			Within-day	Between-day
MCAA	0.11	0.4–300	9.5	10.4
MBAA	0.09	0.4–300	9.8	10.7
DCAA	0.01	0.1–300	5.8	6.7
TCAA	0.02	0.1–300	6.5	7.3
BCAA	0.01	0.1–300	6.6	7.5
DBAA	0.04	0.2–300	6.7	7.5
BDCAA	0.08	0.3–300	6.2	7.3
CDBAA	0.10	0.4–300	6.8	7.9
TBAA	0.40	1–300	11.5	12.6

Table 2
HAA concentrations[a] (μg/l) found in swimming pool water and in swimmers' urine after swimming 1 h.

	MCAA		DCAA		TCAA	
	Swimming pool water	Urine	Swimming pool water	Urine	Swimming pool water	Urine
Swimmer 1	17 ± 2	ND	65 ± 4	1.5 ± 0.1	21 ± 1	0.7 ± 0.1
Swimmer 2	17 ± 2	ND	65 ± 4	1.4 ± 0.1	21 ± 1	0.7 ± 0.1
Swimmer 3	26 ± 2	0.5 ± 0.1	138 ± 8	3.5 ± 0.3	32 ± 2	1.5 ± 0.1
Swimmer 4	26 ± 2	0.6 ± 0.1	138 ± 8	4.6 ± 0.3	32 ± 2	2.3 ± 0.2
Swimmer 5	24 ± 2	0.5 ± 0.1	127 ± 7	4.3 ± 0.3	28 ± 2	2.1 ± 0.2
Swimmer 6	24 ± 2	0.4 ± 0.1	127 ± 7	3.1 ± 0.2	28 ± 2	1.4 ± 0.1
Swimmer 7	19 ± 2	ND	80 ± 6	1.8 ± 0.1	22 ± 1	0.6 ± 0.1
Swimmer 8	19 ± 2	ND	80 ± 6	2.3 ± 0.2	22 ± 1	0.8 ± 0.1
Swimmer 9	22 ± 2	0.4 ± 0.1	117 ± 8	3.9 ± 0.3	26 ± 2	1.3 ± 0.1
Swimmer 10	22 ± 2	0.4 ± 0.1	117 ± 8	3.1 ± 0.2	26 ± 2	1.5 ± 0.1
Swimmer 11	19 ± 2	ND	109 ± 7	3.8 ± 0.3	25 ± 2	1.1 ± 0.1
Swimmer 12	19 ± 2	ND	109 ± 7	2.5 ± 0.2	25 ± 2	0.9 ± 0.1

ND: not detected.
[a] ±Standard deviation, $n = 10$ for water and $n = 5$ for urine.

more than ±15%, a new calibration curve was prepared for each compound.

3.5. Analysis of urine samples

Several authors have reported that after oral exposure, DCAA and TCAA are rapidly absorbed from the gastrointestinal tract and excreted in urine (mainly through experiments in rodents). DCAA are excreted in humans between 20 and 36 min after an intravenous administration of sodium dichloroacetate [23] and more than 50% of the dose administered is recovered in rat urine, unchanged [24]. With regard to TCAA, several experiments have been carried out in workers exposed to trichloroethylene since it is mainly metabolised into TCAA; in this case the sampling time is critical because the metabolite is excreted, from 50 until 120 h after exposure, over the range 0.5 and 90 mg/l [15,16,19,20]. There is very little information in the literature about direct ingestion of TCAA in humans [12] and nothing about MCAA since its concentration in urine is too low to detect. Dermal exposure to DCAA has been researched in four persons during a swimming activity by measuring pool water concentration (600 μg/l) and urinary excretion (3% of the dermal dose) [12]. The present study has evaluated for the first time exposure in indoor swimming pools and swimmers' uptake by selecting the urinary excretion of these compounds as biomarkers. Twenty subjects, including 12 swimmers and eight non-swimmers, have participated in this study. Samples were collected as described in Section 2.3 and analysed immediately or stored at 4 or at −20 °C until their analysis. Samples were prepared as explained in Section 2.4. The optimum time for urine collection was studied in five swimmers by collecting spot samples from all their voids before exposure, right after exposure, at intervals of 30 min within 2 h after exposure and using 1 h intervals from then on until the end of the day of study. During this study volunteers only consumed mineral water (after each void), which was HAA-free. In all instances, the highest concentrations of HAAs detected in urine were found 15–30 min after a swim, so sampling was done in this interval before and after the bathing activities. The average time of the swims was 1 h approximately. HAAs removed in unchanged form in urine were thoroughly excreted 3–4 h after the end of exposure. In all cases (twenty subjects), HAAs were undetected in the urine samples from non-swimmers and of swimmers before their swim so that the concentrations found after exposure were directly related to the swimming activity. After the swim, only MCAA, DCAA and TCAA were detectable in the samples collected (if other HAA compounds were present, they are found at concentrations lower than their detection limits). Table 2 lists the results obtained for the twelve swimmers after 1 h swimming and the concentrations found in the water of the swimming pool at the same time. As can be seen in this Table, MCAA was detected in six subjects in accord with the highest concentration in the swimming pool water those days. The HAA found at the highest concentration in urine was DCAA, in accord with its highest concentration in the water. On the other hand, it is surprising to find a high concentration of TCAA taking into account that its concentration in water is similar to that of MCAA and this may be due to the fact that the later is metabolised through glutathione conjugation [24]. All subjects' excreted amounts of MCAA, DCAA and TCAA are related to the highest levels in the swimming pool water, thus there is a simple linear relationship between the data obtained (water and urine) with correlation coefficients over 0.75.

4. Conclusions

The methodology proposed renders an efficient, cost effective and simple preparation process for the determination of HAAs in human urine. Although blood is generally accepted as the most appropriate sample for toxic metabolism studies, urine is also a very useful specimen to monitor excretion of metabolites known to be

toxic. HS in combination with GC–MS afford a wide linear rage, and satisfactory detection sensitivity and precision, thus enabling application for bioanalytical purposes. Several parameters have been used to validate this method, namely: linearity, accuracy, precision, sensitivity, ruggedness and selectivity. Compared to conventional methods, our protocol has the advantage of not needing time-consuming procedures (such as extraction, centrifugation, acidification, drying and reconstitution). Until now, no data has been published concerning the absorption of haloacetic acids in swimmers, probably due to the fact that these compounds are only present in urine at very low concentrations and the methods described are not sensitive enough to detect these compounds at ng/l levels.

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