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Development of a Method for the Quantitation of Chloro-, Bromo-, and Iodoacetic Acids in Alcoholic Beverages

Maria Jose Cardador and Mercedes Gallego*

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

ABSTRACT: Chloroacetic, bromoacetic, and iodoacetic acids can be found in alcoholic beverages when they are used as preservatives/stabilizers or as disinfectants. As they are toxic components, their addition is not permitted under European Union and U.S. regulations. To date, no sensitive methods are available, and those proposed are very laborious. This paper describes a sensitive and straightforward method for the determination of the three monohalogenated acetic acids (m-HAAs) in wines and beers using static headspace extraction coupled with gas chromatography−mass spectrometry. Prior to extraction, the target analytes were esterified to increase their volatility, and all parameters related to the extraction/methylation process were optimized to achieve high efficiency (>90%). The study examined the influence both of the ethanol concentration on the headspace partitioning and of the primary acids present in wine on the derivatization reaction of the m-HAAs. The proposed method allows the determination of these compounds at microgram per liter levels in alcoholic beverages.

KEYWORDS: *static headspace, gas chromatography−mass spectrometry, monohalogenated acetic acids, wine and beer samples*

INTRODUCTION

Haloacetic acids (HAAs) are disinfection byproducts formed during the disinfection of water with chlorine or chloramine, from natural organic matter and/or bromide−iodide present in water.[1] These compounds exhibit toxicity and mutagenic and carcinogenic activities. On the basis of the induction of genomic DNA damage in hamster ovary cells, the iodinated HAAs are the most genotoxic and cytotoxic, followed by brominated and chlorinated analogues.[2−4] HAAs can enter the human body through different routes because drinking water is used not only for drinking but also for cooking, bathing, etc.[5] Although there are numerous halogenated acetic acid congeners, only monohalogenated acetic acids (m-HAAs) are not permitted under European Union[6] and U.S.[7] regulations in beverages and foods; in this sense the Association of Official Analytical Chemists has established an official method for the determination of chloroacetic acid in foods and beverages.[8] The characterization of the three m-HAAs arises from their suspected use as preservatives or stabilizers in wines and other alcoholic beverages because they have antimicrobial action.[9] In addition, solutions of these m-HAAs may also be used to clean wine tanks and barrels as disinfectants. In breweries and wine production plants, the beverage can become contaminated with m-HAA residues if the equipment is not rinsed adequately after treatment.[10]

The usual methods for the determination of m-HAAs in alcoholic beverages are based on colorimetric assays with two disadvantages, namely, they do not provide sufficient sensitivity to monitor these compounds in beverages, and they need to control the concentration of all halide ions because they are not specific to m-HAAs.[11,12] Later, procedures based on gas chromatography (GC) were reported in the literature due to their inherent advantages of high resolution, rapid separation, and low cost. However, these methods lack the sensitivity required for trace analysis.[13,14] The most recent method proposed for the determination of m-HAAs in wine by GC-electron capture detection goes back to 1991.[10] The method included acidification of the wine, solid phase extraction with a sorbent column (Extrelut, 20 g), elution with dichloromethane, evaporation of the extract (as the volume of eluent was 150 mL), redissolution in methanol/*n*-hexane, derivatization with BF_3/methanol, and heating at 70 °C for 1 h. Despite the numerous steps, the recoveries obtained were 93−112%. Such a method[10] has several drawbacks, such as being time-consuming (ca. 2 h per sample) and exhibiting low sensitivity, because the limits of detection (LODs) for the three m-HAAs ranged from 5 to 100 μg/L, which are similar to what is obtained by other GC methods.[13,14] With respect to unallowed use of such compounds, an exhaustive study of their stability in wines was carried out, which showed that the concentration of chloroacetic acid added to wine was unaffected for up to 3 months in storage in the dark at 30 °C, whereas <20% of both bromoacetic and iodoacetic acids remained unaffected under the same conditions.[10]

The quest for novel sample preparation procedures has led to the development of fast, simple, and solventless techniques. On this basis, the headspace (HS) technique generally offers straightforward sample preparation, automation, and good repeatability (coefficients of variation 4−12%) and is being considered as an interesting alternative in the analysis of complex matrices due to the absence of matrix effects.[15] In a previous work we satisfactorily employed static HS followed by GC-MS to determine the nine HAAs that contain chlorine or bromine in water samples,[16] where HAAs were extracted/derivatizated in situ to their respective methyl esters.

The objectives of the present study were to (i) develop a sensitive/selective GC-MS method for the determination of the

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three m-HAAs in wine and beer samples, (ii) simplify the sample treatment by using a solventless technique such as HS, and (iii) study the influence of both the ethanol concentration on headspace partitioning and of the primary acids present in wine on the derivatization reaction of the m-HAAs.

MATERIALS AND METHODS

Chemicals and Standards. All chemicals and solvents were of analytical grade or better. Chloroacetic, bromoacetic, iodoacetic, and dichloroacetic (internal standard, IS) acids, methyl chloroacetate, and methyl bromoacetate were purchased from Sigma-Aldrich (Madrid, Spain). The derivatization reagent, dimethyl sulfate (DMS), the ion-pairing agent, tetrabutylammonium hydrogen sulfate (TBA-HSO_4), and anhydrous sodium sulfate were supplied by Fluka (Madrid, Spain). The solvents, *n*-pentane, methyl *tert*-butyl ether (MTBE), and ethanol, were purchased from Merck (Darmstadt, Germany).

Individual stock solutions of each halogenated acid (1 g/L) were prepared in MTBE and stored in amber glass vials at −20 °C. More dilute individual or mixed solutions were prepared in 5 mL of MTBE and used for the fortification of the wine samples. Standard solutions were prepared daily or weekly depending on its concentration.

Instruments. Gas chromatographic analyses were performed with an HP 6890N gas chromatograph equipped with an HP 7694N headspace autosampler and connected to an HP 5973N mass spectrometer (Agilent Technologies, Palo Alto, CA). 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, 30 min; oven temperature, 60 °C; vial pressurization time, 30 s; venting time, 12 s; loop fill time, 3 s; valve/loop temperature, 90 °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 pressure flow) and to drive the headspace formed to the injection port of the chromatograph via a transfer line at 100 °C (2.0 psi of pressure flow). Injection was done in the split mode (split ratio 1:20) for 1 min. Compounds were separated using a cross-linked HP-5MS [(5%) phenyl-(95%) methylpolysiloxane] capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W). Oven temperature was programmed as follows: 40 °C for 3 min, heated at 20 °C/min to 60 °C and held there for 3 min, and finally raised to 200 °C at 20 °C/min and held for 3 min. The chromatographic run was complete in 17 min. Helium carrier gas was passed at a constant rate of 1 mL/min. The injector, source, and quadrupole temperatures were maintained at 250, 230, and 150 °C, respectively. The MS was operated in electron impact (EI^+) ionization mode, using electron energy of 70 eV and a solvent delay of 4 min. Optimization experiments were conducted in total ion chromatography (TIC) mode between *m*/*z* 50 and 210 at 3.5 scans/s; *m*/*z* ratios lower than 50 were not monitored to avoid the interference related to the ethanol according to its abundance in alcoholic beverages. Quantification of the three m-HAA methyl esters was performed in selected ion monitoring (SIM) mode, and three different acquisition windows were defined, taking into account the retention times and the suitable fragments of m-HAA methyl esters, which are included along with the analytical figures of merit on the proposed method. All of the scans were performed in high-resolution mode and with a dwell time of 100 ms. Total ion current chromatograms were acquired and processed using MSD ChemStation G1701DA D.01.02 Standalone data analysis software (Agilent Technologies).

Wine and Beer Samples. Wine and beer samples were purchased at local supermarkets in Spain. In the laboratory, the samples were kept cold (4 °C), stored in the darkness until analysis, and the seal of each bottle was broken before its analysis. Beer and rosé wine samples were degassed in an ultrasonic bath for 15 min to avoid interference from CO_2 in the HS of the vial. For beverages containing >15% (v/v) alcohol (viz., spirituous beverages), the samples were diluted with mineral water (the only nondisinfected water and therefore free of HAAs).

Uncontaminated white wine matrices (blank) were used for the optimization of the method, to check the existence of a matrix effect, and to determine their analytical characteristics.

Analytical Procedure. Ten milliliters of wine or beer sample containing between 0.3 and 500 μg/L of each m-HAAs was placed in a 20 mL glass vial with 5 g of Na_2SO_4. Then, 50 μL of a 1 mol/L H_2SO_4 (sample pH ∼2), 150 μL of a 1.0 mol/L TBA-HSO_4 (ion-pairing agent), 500 μL of DMS (derivatization reagent), 10 μL of 20 μg/mL of dichloroacetic acid (IS, 20 μg/L), and 300 μL of *n*-pentane (organic modifier) were added sequentially. The vial was immediately sealed and vortexed for 3 min for homogenization purposes and then placed in the autosampler carousel from which the robotic arm took each and introduced it in the HS oven. Samples were analyzed by HS-GC-MS, using the operating conditions mentioned above.

RESULTS AND DISCUSSION

Optimization of Chemical Variables. Chromatography of halogenated acetic acids in GC systems is difficult because of their low volatility and high polarity; thus, these acids inevitably require a previous derivatization step. In a previous work, we developed a straightforward method for the determination of the nine chlorinated and brominated haloacetic acids at nanogram per liter levels in water samples,[16] after simultaneous extraction/derivatization by HS-GC-MS. In this study, we demonstrate that the presence of 150 μL of *n*-pentane increases the derivatization yields of methyl haloacetates. This method

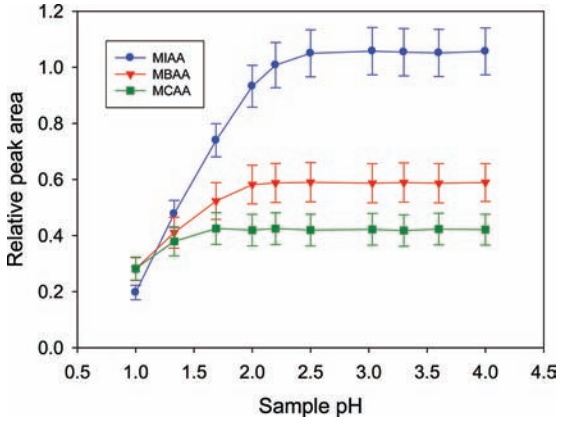

Figure 1. Influence of the sample pH on the extraction/derivatization of the three m-HAAs.

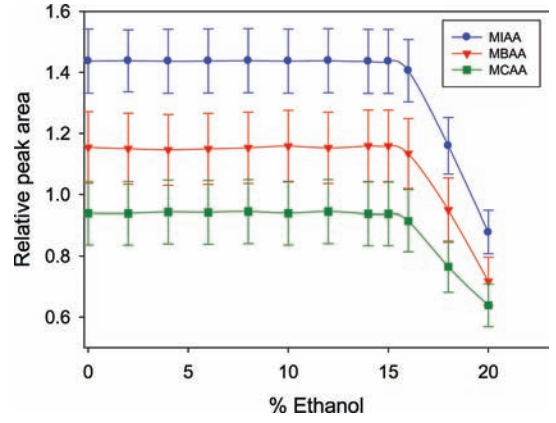

Figure 2. Effect of the percentage of ethanol in the sample on the extraction/derivatization of the three m-HAAs.

Table 1. Analytical Figures of Merit of the Proposed HS-GC-MS Method for the Determination of the Three m-HAAs in Wine and Beer Samples

compound	retention time (min)	m/z[a]	linearity range (μg/L)	LOD (μg/L)	RSD (%)	uncertainty[b] (μg/L)
chloroacetic acid	5.04	**59**, 79, 108	0.8−500	0.25	9.8	9 ± 1
bromoacetic acid	6.55	**59**, 93, 95	0.7−500	0.22	10	11 ± 1
iodoacetic acid	8.64	59, 141, **200**	0.3−500	0.10	7.2	10 ± 1

[a]The peaks used for quantification are boldfaced; m/z for IS (dichloroacetic acid): **59**, 83, 85. [b]Uncertainty of the whole process expressed as $X \pm U$ (n = 12).

was initially adopted, but the inclusion of iodoacetic acid entailed checking that the GC conditions and variables influencing the extraction/derivatization of the three m-HAAs also took into account the different matrices (alcoholic beverages). For the optimization of the variables, 10 mL of white wine (blank) spiked with 50 μg/L of each m-HAA, 125 μL of a 0.5 mol/L TBA-HSO_4 solution, and 100 μL of pure DMS (derivatization reagents), 20 μg/L of dichloroacetic acid (IS), and 150 μL of n-pentane were added in 20 mL glass vials containing 5 g of Na_2SO_4, according to the reagent concentrations used for water analysis.[16]

Tartaric acid is the most significant part of the acid fraction of the wines (which is related to the pH of the wines) as well as citric and malic acids, which are also found in high amounts (primary acids). Taking into account that target analytes can be found at nanogram or microgram per liter levels, their derivatization to methyl esters can be hindered because the primary acids of the wine (present at gram per liter levels) can also react with the derivatization reagents. To diminish the competition of primary acids, the first chemical variable studied was the sample pH because it is the factor related to the protonation of all acids in the wine, which also affects the derivatization efficiency of the m-HAAs. The influence of the sample pH was studied in the acid region from pH 1.0 (the pK_a values for chloroacetic, bromoacetic, and iodoacetic acids were 2.6, 2.7, and 3.2, respectively) to ∼4.0. The composition of the headspace was markedly affected by the sample pH as can be seen in Figure 1. The relative peak areas for all m-HAAs increase as the sample pH does, up to 2.0 or 2.5 for chloroacetic, bromoacetic, and iodoacetic acids, respectively, remaining constant afterward. The optimal sample pH was selected in accordance with two criteria, namely, (i) the control of the chloroacetic acid, because it is the only m-HAA that remained stable in wines for at least 3 months and therefore is the one most commonly found in alcoholic samples,[10] and (ii) the selectivity of the method, taking into account that the primary acids of the wine (present at gram per liter levels) can also be derivatized with DMS, hindering the derivatization of the analytes (present at microgram per liter levels); thus, the former prevail, because they are at a higher concentration. Following these premises, a sample pH of 2 was selected as a compromise, which was obtained by the addition of 50 μL of 1 mol/L H_2SO_4 (pH ∼2) to 10 mL of the wine sample. The pK_a values of all the primary acids of the wine are >3;[17] thus, at pH 2 they exist mainly as protonated acids, and therefore their competition in the derivatization reaction (as anion) was minimized, whereas that of the target analytes was favored.

The method is based on the liquid−liquid microextraction/methylation of the m-HAAs, according to a mechanism previously proposed for other haloacetic acids.[16] Accordingly, m-HAAs (XCH_2−COOH) are converted in situ into the corresponding anion in aqueous medium, producing an ion pair with TBA-HSO_4 (R_4−N^+ HSO_4^-), XCH_2−COO^- N^+−R_4 at pH 2, which can cross the liquid−liquid interface due to the lipophilicity of the tetrabutylammonium cation, diffusing into the organic phase (n-pentane). Next, it reacts with DMS, $(CH_3)_2SO_4$, to produce methyl haloacetates (XCH_2−COO−CH_3) in the organic phase, and the free cation is then transferred to the aqueous phase. Finally, the esters and the organic phase were completely volatilized. Therefore, the amounts of DMS and TBA-HSO_4 are critical because the primary acids of the wine can also react with derivatization reagents in detriment of the m-HAA derivatization. The effect of DMS volume on the derivatization of the m-HAAs was studied from 100 to 600 μL. The derivatization reaction of the m-HAAs was completed above 400 μL of DMS. However, the excess of DMS was extracted in n-pentane and volatilized appearing in the chromatogram (the band of DMS overlaps with the iodoacetic acid peak for concentrations >500 μL). To ensure complete derivatization of the analytes without too much excess, 500 μL was chosen as the optimal volume. Volumes between 50 and 200 μL of a 1.0 mol/L TBA-HSO_4 solution were assayed in the reaction; the analytical signals of the three m-HAAs increased drastically on increasing volume up to 125 μL, above which they remained constant. As TBA-HSO_4 acts as catalyst for the reaction, an excess of the 150 μL was selected as the optimal volume. The last parameter tested was the volume of n-pentane because, as described above, the addition of an organic modifier is essential for the methylation of m-HAAs because it occurs in an organic medium. The addition of 300 μL of n-pentane provided the best results in terms of peak area for the three m-HAAs. A previous work of ours established that the presence of Na_2SO_4 favored the extraction/derivatization of the HAAs as well as the volatilization of the organic phase.[16] Hence, the addition of Na_2SO_4 was studied between 0 and 6 g. The analytical signal of the m-HAAs increased as the amounts of salt increased to 5 g, which was therefore the amount selected per 10 mL of wine (saturated solution).

Finally, the yield of the extraction/derivatization reaction in the proposed method was evaluated; the acids studied were those for which ester standards were available (chloroacetic and bromoacetic acids) because the standard of methyl iodoacetate is not commercialized. For this purpose, 10 mL of white wine (blank) was fortified with both monohalogenated acetic acids, derivatization reagents, 300 μL of n-pentane, and salt according to the procedure or with only both esters (methyl chloroacetate and methyl bromoacetate), the n-pentane, and salt, using the same molar concentrations of each m-HAA as those of their respective methyl ester standards (50 μg/L of each compound, ∼5 nmol of each). The average yield (n = 5) of the whole analytical process was >90% for bromoacetic acid and >95% for chloroacetic acid.

Optimization of HS Conditions. The optimum conditions for HS generation were established by examining the influence of each individual parameter. The oven temperature and the

Table 2. Percent Recovery (± SD, n = 5) of the Three m-HAAs Added to Wine and Beer Samples

compound	white wine (11.5)[a]			red wine (13)[a]			rosé wine (12)[a]			barley beer (4.5)[a]			wheat beer (5.2)[a]		
	10 μg/L	50 μg/L	400 μg/L	10 μg/L	50 μg/L	400 μg/L	10 μg/L	50 μg/L	400 μg/L	10 μg/L	50 μg/L	400 μg/L	10 μg/L	50 μg/L	400 μg/L
chloroacetic acid	91 ± 11	92 ± 10	94 ± 10	87 ± 12	91 ± 11	91 ± 11	92 ± 11	94 ± 10	97 ± 11	89 ± 11	91 ± 10	94 ± 10	90 ± 11	91 ± 10	93 ± 10
bromoacetic acid	92 ± 11	94 ± 10	94 ± 10	89 ± 12	92 ± 11	93 ± 11	91 ± 11	92 ± 10	94 ± 10	91 ± 11	92 ± 10	94 ± 10	90 ± 11	93 ± 10	96 ± 11
iodoacetic acid	94 ± 8	95 ± 8	97 ± 8	91 ± 9	92 ± 8	94 ± 8	95 ± 9	97 ± 8	98 ± 8	93 ± 8	95 ± 8	96 ± 8	94 ± 8	94 ± 8	97 ± 8

[a]Ethanol (%).

vial equilibration time are the most influential parameters because both variables affect the extraction/derivatization of the m-HAAs. Thus, the effect of these parameters was evaluated from 50 to 80 °C and from 20 to 50 min, respectively. The highest peak areas for the three m-HAAs were achieved at an oven temperature of 60 °C and a vial equilibration time of 30 min, so these settings were selected. The analytical signal decreased at higher oven temperatures (20%) and equilibration times (10%), due to the evaporation of a significant amount of volatile compounds (the peaks of which were visualized in the chromatogram) from the wine, which caused saturation of the headspace, and/or because they overlap with the m-HAAs peaks in the chromatogram (mainly with chloroacetic and bromoacetic methyl esters). Other instrumental parameters that affected the sensitivity of the method were the pressurization time and the venting time of the vials, which were also studied from 15 to 45 s and from 12 to 20 s, respectively. There were no significant changes in the abundance signals for the three m-HAAs at a pressurization time above 30 s and a venting time above 12 s, which were selected as the working values.

Influence of the Sample Matrix. To ensure the applicability of the optimized HS-GC-MS method on alcoholic beverages (wines and beers) that contain variable amounts of ethanol, a rigorous study of this possible interference was done by taking into account its high volatility.[18] Initially, such an influence was examined by using standard solutions prepared in water−ethanol medium containing 50 μg/L of each m-HAA and variable proportions of ethanol between 0 and 20%. As can be seen in Figure 2, ethanol had no effect on extraction/derivatization in proportions up to 15% for all m-HAAs. Higher ethanol concentrations, however, resulted in dramatically decreased analytical signals. This can be ascribed to the volatilization of ethanol, which competes with the esterified analytes for headspace in the vials prevailing of the higher concentration, which hinders the volatilization of the analytes. Because the ethanol content in the selected beverages ranged from 4% for beer to 14% for wine, no problem in the determination of m-HAAs could be found. Nevertheless to apply the method to spirituous drinks (whiskey, rum, gin, vodka, etc.), the beverages would have to be diluted 3−4 times with mineral water before analysis.

As described above, m-HAAs can be present in alcoholic beverages at nanogram or microgram per liter levels, whereas the primary acids of the wine are found at gram per liter levels (6, 4, and 1.5 g/L of tartaric, citric, and malic acids, respectively).[17] These acids can interfere in the derivatization of m-HAAs because they also compete for derivatization reagents. The study was carried out by adding known concentrations of the possible interfering compounds individually to a standard solution containing 50 μg/L of each m-HAA in mineral water (free of haloacetic acids). A compound is considered to be an interferent when it has a relative error higher than the standard deviation of the method.[19] The results reveal (for a relative error of 15%) that none of the primary acids compete with the analytes up to concentrations twice that found in wines (10, 8, and 3 g/L of of tartaric, citric, and malic acids, respectively). Primary acids did not compete in the determination of the three m-HAAs because at the sample pH (~2) only ~5% of the primary acids ($pK_a \sim 3$) were dissociated like anion, and therefore their derivatization was minimized; in addition, to ensure the derivatization of the three m-HAA, an excess of the derivatizing reagent was used. In conclusion, no

significant effect from the sample matrix was detected, and therefore the method proposed to determine the three m-HAAs in alcoholic beverages can be quantified with standards prepared in mineral water; for beverages containing >15% alcohol (viz., spirituous beverages) a dilution with mineral water was required.

Validation of the Method. Three organic compounds, 1,2-dibromopropane (usually employed as IS for the determination of haloacetic acids in water) and trichloroacetic and dichloroacetic acids (the presence of which in wines is impossible either as a component or as a contaminant) were assessed as IS to be added to the sample; the best result was obtained for dichloroacetic acid because it did not overlap with any analytes (as occurred with trichloroacetic and iodoacetic acids). Validation was needed to demonstrate that the analytical method complied with established criteria for different performance characteristics.[20] Table 1 summarizes the figures of merit in the calibration curves for the three m-HAAs. The calibration curves for the three m-HAAs were constructed by plotting the analyte to the internal peak area against the analyte concentration. The linearity of the chromatographic method was satisfactory in the range of concentrations between 0.3 and 500 μg/L with regression coefficients >0.995. The LOD was calculated as 3 times the standard deviation of background noise divided by the slope of each calibration graph. The repeatability of the HS-GC-MS method as RSD for a standard mixture containing a 20 μg/L concentration of each m-HAAs ranged from 7 to 10% (n = 11, within day). To calculate the uncertainty of the whole method, 12 wine samples containing 10 μg/L of each m-HAAs were subjected to all of the process: fortification, storage at 4 °C for 24 h, extraction/derivatization, and analysis. The uncertainty values were calculated on the basis of the equation $U = t \times s/\sqrt{n}$ (where U is the uncertainty, t the statistical parameter, s the standard deviation, and n the number of measures).[21] Table 1 summarizes the specific uncertainty for each m-HAAs for a probability imposed at a 95% confidence level (K = 2).

Finally, to establish the overall effect of the matrix of wines and beers, several calibration curves for the three m-HAAs were constructed using different alcoholic beverages. The repeatability of the curve slope was calculated from three replicates prepared in different matrices (white, red, and rosé wines or barley and wheat beers); the alcoholic grade of the beer and wine samples ranged between 4 and 14% in all instances. The averages of the slopes (expressed as signal: μg/L) of the three m-HAAs were $(9 \pm 1) \times 10^{-4}$, $(8 \pm 1) \times 10^{-4}$, $(10 \pm 1) \times 10^{-4}$, $(9 \pm 1) \times 10^{-4}$, and $(10 \pm 1) \times 10^{-4}$ for white, red, and rosé wines or barley and wheat beers, respectively. The average sensitivities of the method (shown as the slope of the calibration curves) for the three m-HAAs were similar, independent of the type of alcoholic beverage, which demonstrated that there was no effect of the matrix or it was negligible.

Analysis of Wine and Beer Samples. The proposed HS-GC-MS method was applied for the determination of m-HAAs in various types of Spanish wines (white, red, and rosé) and in beers of different origins (five countries) produced from barley or wheat and with various amounts of alcohol (0−8%). Samples were analyzed in triplicate by using the analytical procedure described under Materials and Methods. As could be expected, no positive sample was found in the 25 wines and 30 beers tested, because these acids are not permitted in alcoholic beverages. Therefore, to test the reliability of the proposed method, the recovery of the three m-HAAs was assessed after they were added to three wines of different types and two beers produced from barley or wheat. Although the samples contained alcohol, it did not interfere, as its content was below 15%, and therefore none of the samples were diluted with mineral water. The recovery study was carried out by performing three standard additions (10, 50, and 400 μg/L) to 10 mL of each sample. To facilitate potential analyte interaction with the sample matrix, all spiked samples were left to stand for 24 h before analysis. Each sample was analyzed in quintuplicate, and the results obtained are listed in Table 2. All compounds were accurately identified, and the average recoveries for all samples (range = 91 and 95% for red and rosé wines, respectively) were acceptable in all instances.

In conclusion, in the absence of a comprehensive method to determine the three m-HAAs at nanogram per liter levels by GC in alcoholic beverages, the proposed HS-GC-MS method may be an appropriate procedure for the simultaneous determination of these compounds (not permitted in beverages and foods). The aims have been fully achieved, so this method can be used to control these contaminants in wines and beers as well as in other applications such as spirituous drinks (if they are diluted by water).

AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +34-957-218-614. E-mail: mercedes.gallego@uco.es.

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ABBREVIATIONS USED

DMS, dimethyl sulfate; HS-GC-MS, headspace−gas chromatography−mass spectrometry; IS, internal standard; LOD, limit of detection; m-HAAs, monohalogenated acetic acids; MTBE, methyl *tert*-butyl ether; RSD, relative standard deviation; SIM, selected ion monitoring; TBA-HSO_4, tetrabutylammonium hydrogen sulfate.

REFERENCES

(1) Richardson, S. D. Environmental mass spectrometry: emerging contaminants and current issues. *Anal. Chem.* **2010**, *82*, 4742−4774.

(2) Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; DeMarini, D. M. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection byproducts in drinking water: a review and roadmap for research. *Mutat. Res.* **2007**, *636*, 178−242.

(3) Attene-Ramos, M. S.; Wagner, E. D.; Plewa, M. J. Comparative human cell toxicogenomic analysis of monohaloacetic acid drinking water disinfection byproducts. *Environ. Sci. Technol.* **2010**, *44*, 7206−7212.

(4) Pals, J. A.; Ang, J. A.; Wagner, E. D.; Plewa, M. J. Biological mechanism for the toxicity of haloacetic acid drinking water disinfection byproducts. *Environ. Sci. Technol.* **2011**, *45*, 5791−5797.

(5) Cardador, M. J.; Gallego, M. Haloacetic acids in swimming pool: Swimmers and worker exposure. *Environ. Sci. Technol.* **2011**, *45*, 5783−5790.

(6) Regulation No. 8222/87 on the common organization of the market in wine. *Off. J. Eur. Communities* **1987**, *L 84*, 1−58.

(7) Title 21: Food and drugs, Vol. 3, Chapter 3, Part 189 relating to substances prohibited from use in human food. *Code Fed. Regul.* **2009**, 593−600.
(8) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13th ed.; Washington, DC, 1980; Sections 20.067−20.072.
(9) Dickens, F. Interaction of haloacetates and SH compounds. The reaction of haloacetic acids with glutathione and cysteine. The mechanism of iodoacetate poisoning of glyoxalase. *Biochem. J.* **1933**, *27*, 1141−1151.
(10) Willetts, P.; Dennis, M. J.; Massey, R. C. Measurement of haloacetic acids and their decomposition in wine by capillary gas chromatography. *Food Addit. Contam.* **1991**, *8*, 119−124.
(11) Guyot, A. M.; Balatre, P. Hydrolyse de ĺacide monobromacetique et de son ester ethylique dans les boissons. *Ann. Falsif. Exp. Chim.* **1968**, *61*, 346−359.
(12) Mergenthaler, E. Monobromoacetic acid determination. *Z. Lebensm. Unters.− Forsch.* **1962**, *119*, 144−155.
(13) Fuerst, P.; Krueger, C.; Habersaat, K.; Groebel, W. Halogenated carboxylic acids in beverages. Gas chromatography determination and confirmation by gas chromatography/mass spectrometry with negative chemical ionization. *Z. Lebensm. Unters.− Forsch* **1987**, *185*, 17−20.
(14) Gilsbach, W. Gas chromatography determination of monohaloacetic acids in beer and wine-containing drinks. *Dtsch. Lebensm. Rundsch.* **1986**, *82*, 107−111.
(15) Snow, N. H.; Bullock, G. P. Novel techniques for enhancing sensitivity in static headspace extraction-gas chromatography. *J. Chromatogr., A* **2010**, *1217*, 2726−2735.
(16) Cardador, M. J.; Serrano, A.; Gallego, M. Simultaneous liquid-liquid microextraction/methylation for the determination of haloacetic acids in drinking waters by headspace gas chromatography. *J. Chromatogr., A* **2008**, *1209*, 61−69.
(17) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. Handbook of Enology Vol. 2: The Chemistry of Wine and Stabilization and Treatments. In *Organic Acids in Wine*, 2nd ed.; Wiley: West Sussex, U.K., 2006; pp 3−50.
(18) Robinson, A. L.; Ebeler, S. E.; Heymann, H.; Boss, P. K.; Solomon, P. S.; Trengove, R. D. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *J. Agric. Food Chem.* **2009**, *57*, 10313−10322.
(19) Michałowski, J.; Hałaburda, P.; Kojło, A. Determination of humic acid in natural waters by flow injection analysis with chemiluminescence detection. *Anal. Chim. Acta* **2001**, *438*, 143−148.
(20) Taverniers, I.; De Loose, M.; Van Bockstaele, E. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends Anal. Chem.* **2004**, *23*, 535−552.
(21) Desimoni, E.; Brunetti, B. Uncertainty of measurement and conformity assessment: a review. *Anal. Bioanal. Chem.* **2011**, *400*, 1729−1741.