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Determination of halogenated mono-alcohols and diols in water by gas chromatography with electron-capture detection

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Abstract

We have developed an analytical method for the detection of halogenated alcohols in water with particular focus on 3-chloro-1,2-propanediol and 3-bromo-1,2-propanediol. In this method the target analytes are extracted from water, derivatized with heptafluorobutyric anhydride, and then analyzed with gas chromatography with electron-capture detection. The effects of water, pH and seawater constituents on the method were investigated. Method detection limits for a 5 ml aqueous sample ranged from 0.14 $\mu g\ l^{-1}$ for 2-bromo-1,3-propanediol to 1.7 $\mu g\ l^{-1}$ for 1,3-dichloro-2-propanol (1,3DCP).

Keywords: Water analysis; Alcohols; Diols; Halogenated compounds; Halohydrins; Chloropropanediols; Bromopropanediols

1. Introduction

Halohydrins are organic compounds composed of brominated (bromohydrins) and chlorinated (chlorohydrins) alcohols (e.g., mono-alcohols and diols). Chlorohydrins have been identified in hydrolyzed vegetable proteins (HVPs) [1], in food products containing HVPs [2], and in resins which are used to impart wet strength to paper products [3]. In addition several chlorohydrins have been identified in water treated with chlorine, chloroamine and combinations of ozone and chlorine [4,5], while bromohydrins have been identified in bromide-containing waters treated with ozone [5].

Two of the primary chlorohydrins identified in food products and paper are 3-chloro-1,2-propanediol (3CPD) and 1,3-dichloro-2-propanol (1,3DCP). The latter compound has also been identified in drinking water samples following chlorination [5]. These two compounds are of particular concern due to their associated toxicity: 3CPD is a male antifertility agent and a mutagen in bacterial assays [6], while 1,3DCP is a suspected carcinogen [7]. Although little is known about the toxicity of the halohydrins produced during the disinfection of drinking and wastewater, most halogen-containing organic compounds are thought to have adverse health effects [5].

Several methods have been developed for the analysis of chlorohydrins, primarily 3CPD, in extracts of food products. One technique involves the extraction of the samples into water, formation and extraction of boronic acid derivatives, and analysis by gas chromatography (GC) with various detectors. These methods are selective for diols and include *n*-butylboronic acid derivatization with GC–electron-capture detection (GC–ECD) [8] and phenylboronic acid derivatization with either GC–flame ionization

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detection (GC–FID) [9] or GC with mass spectrometry (GC–MS) [10]. A second technique, which is capable of detecting both diols and monohydroxy compounds, involves Extrelut solid-phase extraction, elution with ethyl acetate (EA), and analysis using either GC–MS in the selective ion mode [11] or GC with electrolytic conductivity detection [12]. Similar techniques involve Extrelut extraction, derivatization with heptafluorobutyrylimidazole (HFBI) and analysis using GC–ECD and GC–MS [1] or GC with ion trap tandem mass spectrometry (GC–ITMS) [2]. Although not an aqueous phase method, paper samples have been analyzed for 3CPD by extracting the sample with acetonitrile and derivatizing with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) followed by GC–MS analysis in the selective ion monitoring mode [3]. In addition, organic extracts of resins and solvents have been analyzed using BSTFA derivatization followed by GC–FID analysis [13]. Bromohydrins (primarily monohydroxy) in ozone-treated waters have been determined by a method that involves extraction with methyl *tert*.-butyl ether, concentration and then analysis by GC–MS and GC coupled to Fourier transform infrared spectrometry [5].

Halohydrins can be formed through a number of mechanisms including reaction of reactive halogens (e.g., halogen radicals, X_2, or HOX) with unsaturated compounds [5,14]. One goal of our research is to investigate the aqueous reactions of reactive halogen species with allyl alcohol (CH_2CHCH_2OH) to yield 3CPD, 3-bromo-1,2-propanediol (3BPD), and related monohydroxy and diol halohydrins. To characterize these reactions we needed a method capable of extracting small volumes of sample (~5 ml) quickly and efficiently, yet with low detection limits for both halogenated monohydroxy and diol compounds. The methods described above are not well suited for our purpose because they either: (1) can not achieve our desired detection limits; (2) will not extract both monohydroxy and diol halohydrins; (3) will not derivatize both monohydroxy and diol halohydrins; (4) utilize a derivatization reagent which is not compatible with all of our desired target analytes (e.g., Hamlet [2] reported that 3BPD was not stable in the presence of HFBI); (5) are work intensive and consume large amounts of extraction solvent (150–250 ml per sample); or (6) require the use of GC–MS or GC–ITMS instrumentation.

The method described in this paper uses a simple yet effective extraction procedure, in conjunction with a derivatizing reagent not utilized in the studies described above, to quantify both monohydroxy and diol halohydrins in water. In this method, halohydrins are extracted from water with ethyl acetate, derivatized with heptafluorobutyric anhydride (HFBA), and then analyzed with GC–ECD. Surrogate compounds are utilized to track the recovery of the halohydrins from water, and internal standards are used for quantification. Advantages of this method include: (1) the ability to extract and derivatize all monohydroxy and diol halohydrins tested (including 3BPD) quickly and easily with high extraction efficiencies; (2) low method detection limits for small sample volumes; (3) the use of small volumes of extraction solvents; and (4) the use of relatively inexpensive instrumentation.

2. Experimental

2.1. Chemicals

The target analytes, surrogates and internal standards used in this work, along with their abbreviations, are listed in Table 1. 1,3DCP (98%), 3CPD (98%), 3BPD (98%), 2,3DBP (98%), 1,2PD (99%), 1,2BD (99%), 1,4DB2B (85%), 2,3DBBD (99%),

Table 1
Target analytes, surrogates, and internal standards

Target analytes	Surrogate compounds	Internal standards
1,3-Dichloro-2-propanol (1,3DCP)	1,2-Propanediol (1,2PD)	3-Fluoro-1,2-propanediol (3FPD)
3-Chloro-1,2-propanediol (3CPD)	1,2-Butanediol (1,2BD)	1,4-Dichloro-2-butanol (1,4DC2B)
3-Bromo-1,2-propanediol (3BPD)	2,2-Dichloro-1,3-propanediol (2,2DCPD)	
2-Bromo-1,3-propanediol (2BPD)	1,4-Dibromo-2-butanol (1,4DB2B)	
2,3-Dibromo-1-propanol (2,3DBP)	2,3-Dibromo-1,4-butanediol (2,3DBBD)	

3FPD (98%), and EA (99.8%, anhydrous) were obtained from Aldrich. 2,2DCPD (purity unknown; assumed 100% for calculations) was purchased from the Sigma–Aldrich Library of Rare Chemicals. 1,4DC2B (95%) was obtained from TCI America. Acetonitrile (Optima grade), hexane (Optima grade), sodium sulfate (ACS grade, 10–60 mesh), sodium tetraborate (ACS grade) and sodium bicarbonate (ACS grade) were obtained from Fisher. Sodium sulfite (99%) and sodium bisulfite (99%) were obtained from Sigma and HFBA (99.8%, 1-ml ampoules, stored in freezer at −16°C) was obtained from Supelco. 2BPD was synthesized according to the procedure of Masuda et al. [15], with some modifications, followed by silica gel column clean-up. The isolated reaction product was identified using proton and carbon-13 nuclear magnetic resonance (NMR), although the purity of the reaction product could not be unambiguously determined from this data. However, based upon the areas from GC–ECD analysis of the derivatized reaction product, the purity of the 2BPD appeared to be >95%. We have assumed that the purity of 2BPD is 100% for the calculations described in this work. Type I reagent grade water (Milli-Q, 18.2 MΩ cm) was obtained from a Millipore Milli-Q Plus water purification system.

2.2. Stock solutions

Individual concentrated stock solutions were prepared for each analyte by adding a known mass (approximately 20 mg) of neat compound to a 10-ml class A volumetric flask and diluting with acetonitrile. Three mix stock solutions were then prepared by adding known volumes of the appropriate concentrated stock solutions to a 10-ml class A volumetric flask and diluting with acetonitrile: (1) an internal standard mix stock (I.S. Mix) containing 3FPD and 1,4DC2B; (2) a surrogate mix stock (Surrogate Mix) containing 1,2PD, 1,2BD, 2,3DBBD, 2,2DCPD and 1,4DB2B; and (3) a target analyte mix (Target Analyte Mix) containing 1,3DCP, 3CPD, 3BPD, 2BPD and 2,3DBP. The concentrations of these mix stock solutions ranged from 4.22 to 13.1 μg ml^{-1}. A target analyte method detection mix (Target Analyte MDL Mix) was prepared in a manner analogous to the Target Analyte Mix, except at much lower concentrations, ranging from 13.8 to 171.4 ng ml^{-1}. Stock solutions were stable (<10% change in areas) for at least six weeks when stored at 4°C in 13-ml clear glass vials with screw-cap tops and PTFE-lined septa.

2.3. Sample extraction

A 5-ml volume of aqueous sample, 1.8 g of Na_2SO_4, 20 mg of $NaHSO_3$, and 40 μl of Surrogate Mix were added to a 13-ml glass vial, and the entire mixture was shaken by hand to dissolve the Na_2SO_4. If necessary, the sample was adjusted to pH 3–7 (see below). The sample was then extracted twice with 5±0.5 ml of anhydrous EA (transferred to the extraction vials using a 5-ml glass syringe with a 22 gauge needle) by shaking the sample vigorously by hand for 1 min. The two 5-ml EA extracts were transferred to a second 13-ml glass vial using a disposable glass Pasteur pipette and the extract was spiked with 25 μl of I.S. Mix. Difficulties were encountered with other brands of ethyl acetate. The use of Fisher (Optima and HPLC grade) and J.T. Baker (Ultra Resi-Analyzed Grade) EA led to chromatograms with large amounts of unknown peaks whose areas increased with EA storage time prior to use.

2.4. Sample concentration and drying

The EA extract was concentrated to approximately 50 μl by placing samples in a heating block at 65°C and evaporating the EA with a steady stream of N_2 (99.997%, Puritan-Bennett) supplied from a six-port needle evaporator (Supelco). Acetonitrile (100 μl) was then added to the concentrated EA extract and this mixture was transferred to a drying column. Each drying column consisted of a borosilicate glass Pasteur pipette (Fisher) plugged with pesticide-grade glass wool (Alltech) filled with approximately 3 g of anhydrous Na_2SO_4. The Na_2SO_4 was stored in a vacuum oven (100°C, 7800 Pa) for at least 24 h prior to use. After transferring the mixture, the sample vial was rinsed with 100 μl of acetonitrile, this rinse was added to the drying column, the sample was allowed to sit for 10 min in the column, and then the sample was eluted with 2 ml of acetonitrile into a third 13-ml sample vial.

2.5. Derivatization and hexane extraction

The derivatization technique was adapted from Moore et al. [16]. A 50-μl volume of HFBA was added to the sample vial containing the dried acetonitrile extract and the mixture was reacted in a heating block at 75°C for 30 min (the optimal time determined experimentally). The derivatized sample was then cooled at room temperature for ~5 min and placed in an ice bath. The sample was extracted by adding 5.0 ml of Milli-Q water, 2.0 ml of hexane and 3 ml of a saturated aqueous $NaHCO_3$ solution (made fresh daily), capping the vial, and shaking the sample by hand for 30 s. The aqueous layer was removed with a disposable glass Pasteur pipette, and the hexane layer was then washed twice with approximately 10 ml of a saturated aqueous $NaHCO_3$ solution and shaken by hand for 30 s each time. Using a disposable Pasteur pipette, the hexane extract was transferred to a 1.5-ml amber GC autosampler vial with screw-cap tops and PTFE-lined septum (National Scientific Company). Hexane extracts in these vials were stable for at least four weeks when stored at −16°C.

2.6. Calibration standards

To prepare a matrix similar to that of actual samples, a mixture containing 5.0 ml of Milli-Q water, 1.8 g of Na_2SO_4, and 20 mg of $NaHSO_3$ was extracted twice with 5.0 ml of EA. These two water-saturated EA extracts were combined in a 13-ml glass vial and spiked with equal volumes (5–80 μl) of Target Analyte Mix and Surrogate Mix and 25 μl of I.S. Mix. Each calibration standard was then concentrated to 50 μl, dried, derivatized, and extracted into hexane as described above. This yielded a five-point calibration curve with a range of approximately 0.01 to 0.18 μg ml^{-1} for all target analytes and surrogates (Table 2). The internal standards 3FPD and 1,4DC2B were present at 0.062 and 0.138 μg ml^{-1}, respectively, in each standard.

2.7. Controls and blanks

Three types of controls were used: (1) water-saturated EA controls (prepared in a manner analogous to the calibration standards) as a check for the calibration curve; (2) spiked-water controls (prepared by spiking known amounts of Target Analyte Mix, 40 μl of Surrogate Mix, 1.8 g of Na_2SO_4, and 20 mg of $NaHSO_3$ into 5.0 ml of Milli-Q) to determine extraction efficiencies; and (3) matrix spikes (prepared in a manner analogous to spiked-water controls using sample matrix, e.g., seawater) as a check for possible matrix effects. These controls were processed and prepared for GC analysis as described above. To determine the method background concentrations of target analytes, method blanks were prepared in the same way as spiked-water controls, except no target analytes were added.

Table 2
Regression parameters for calibration data

Analyte	Calibration range (μg ml^{-1})	No. of data points	Slope (S.E.)	Intercept (S.E.)	S.E.	r
Target analytes						
1,3DCP	0.012–0.184	5	20.6 (0.85)	−0.119 (0.090)	0.1040	0.9983
3CPD	0.011–0.169	5	11.1 (0.07)	0.045 (0.010)	0.0075	0.9999
3BPD	0.012–0.185	5	8.32 (0.030)	0.0019 (0.0032)	0.0037	0.9999
2BPD	0.011–0.176	5	3.81 (0.070)	−0.0042 (0.0071)	0.0082	0.9997
2,3DBP	0.011–0.169	5	7.44 (0.025)	0.008 (0.002)	0.0028	0.9999
Surrogate compounds						
1,2PD	0.023–0.180	4	12.3 (0.370)	0.106 (0.044)	0.0359	0.9995
1,2BD	0.011–0.180	5	5.42 (0.075)	0.013 (0.008)	0.0090	0.9998
2,2DCPD	0.011–0.173	5	12.9 (0.093)	0.081 (0.009)	0.0108	0.9999
1,4DB2B	0.033–0.526	5	2.10 (0.020)	0.044 (0.006)	0.0071	0.9999
2,3DBBD	0.024–0.379	5	1.53 (0.037)	0.018 (0.008)	0.0094	0.9994

2.8. Gas chromatography with electron-capture detection

A Hewlett-Packard (HP) 5890 gas chromatographic system with split/splitless injector was used in this work. The GC system was equipped with a HP 7673A autosampler, a HP 3396A integrator, and a HP 19233 nickel-63 electron capture detector. The analytical column used was a 30 m×0.25 mm I.D. DB5-MS fused-silica capillary column with a phase thickness of 1.0 μm (J&W Scientific). The injector and ECD temperatures were set at 250°C and 300°C, respectively. Helium (99.999%, Puritan-Bennett) at 1 ml min^{-1} was used for the carrier gas and nitrogen (99.999%, Matheson) at 42 ml min^{-1} was used as the ECD makeup gas. The split and septum purge flows were set to 30 ml min^{-1} and 1 ml min^{-1}, respectively. All gas lines were outfitted with appropriate oxygen, water and hydrocarbon scrubbers. Hexane extracts (1 μl) were injected in the splitless mode with the vent opened after 0.75 min. The following oven temperature program was used: start at 40°C and hold for 17 min, ramp at 1°C min^{-1} to 65°C and hold for 1 min, ramp at 2°C min^{-1} to 112°C and hold for 2 min, ramp at 30°C min^{-1} to 280°C and then hold for 5 min. The total run time was 79.1 min.

3. Results and discussion

3.1. Chromatography

As shown in Fig. 1, the thick phase DB-5MS column, in conjunction with the chosen temperature program, yielded baseline resolution for the target analytes, surrogates, and internal standards while also separating the early eluting unknown peaks resulting from the HFBA derivatization. Several other columns (including 30 m×0.25 mm I.D., 0.25 μm phase thickness DB-5MS, DB-XLB, DB-35MS

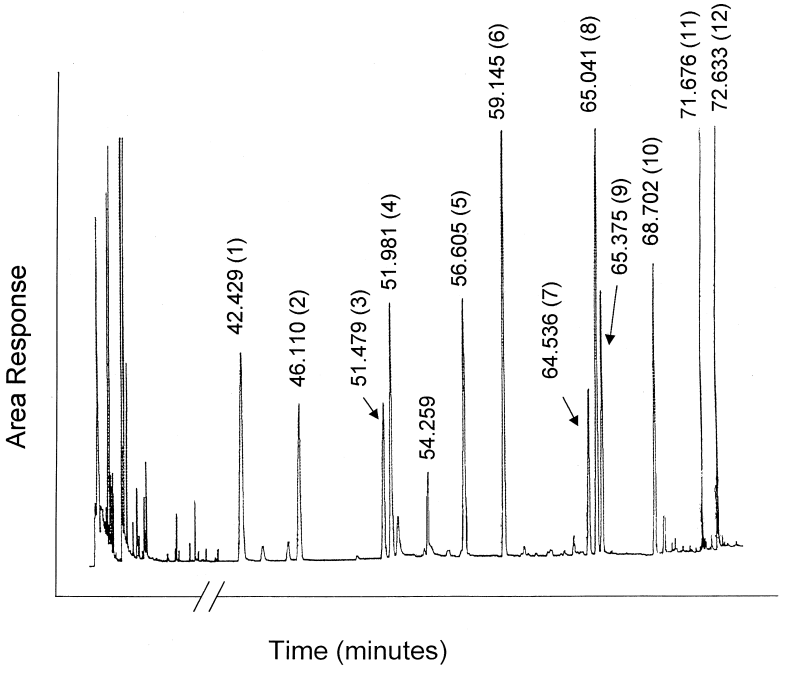

Fig. 1. Gas chromatogram for a high-range calibration standard (target analyte concentrations=0.169 to 0.185 μg ml^{-1}). Key to compounds: 1=1,2PD, 2=3FPD, 3=1,2BD, 4=1,3DCP, 5=2,2DCPD, 6=3CPD, 7=1,4DC2B, 8=3BPD, 9=2BPD, 10=2,3DBP, 11=1,4DB2B and 12=2,3DBBD. Retention times are given for each known peak. Unlabeled peaks are unknown except for the peak at 54.259 min (4-chloro-1-butanol).

and DB-1 columns) and temperature programs were also tested but these gave poorer resolution.

Injector liners from HP (5181-3316) and SGE (092002 and 092010) were also evaluated as part of this work. Chromatograms produced with SGE liners gave peak areas ~50% lower than those with the HP liner, apparently because of the silanized glass wool in the SGE liners. Thus HP liners were used here. In addition, the effect of ECD temperature on analyte response was investigated at 280, 300 and 320°C. While the responses for the target analytes and surrogates increased with ECD temperature, these changes were small (e.g., responses at 320°C were only 3 to 9% higher than at 300°C) and 300°C was selected as the ECD temperature.

3.2. Calibration

Calibration plots were prepared by plotting the response ratio (defined as the analyte area divided by the internal standard area) as a function of analyte concentration ($\mu g\ ml^{-1}$). Internal standards were selected based upon their structural similarities to the target analytes: 3FPD was used as the internal standard for 2,2DCPD, 3CPD, 3BPD, 2,3DBBD and 2BPD, while 1,4DC2B was used for 1,4DB2B, 1,3DCP and 2,3DBP. As shown in Table 2, calibration curves were linear over the tested range.

Calibration curves for the target analytes were stable for at least six weeks, based on the analysis of 10 separate water-saturated EA controls analyzed over this period. For all target analytes, the relative standard deviation (RSD) expressed as a percent of the response ratios for the 10 samples was $<5\%$ while response ratios within individual controls were within $\pm 10\%$ of the expected value. The curves for the surrogates were also stable for six weeks, although variabilities for 1,2BD, 1,4DB2B and 2,3DBBD were somewhat higher (RSD values of 11.4, 11.8 and 10.0%, respectively).

3.3. Sample extraction

As shown in Fig. 2, the efficiencies with which target analytes and surrogates were extracted from water generally increased with the amount of sodium sulfate added to the water prior to extraction. This effect was most pronounced for the diols, where saturating the water with Na_2SO_4 (i.e., 1.8 g) led to a 30–40% increase in extraction efficiencies (Fig. 2). The extraction efficiencies for all monohydroxy compounds (data not shown) and two diol surrogates

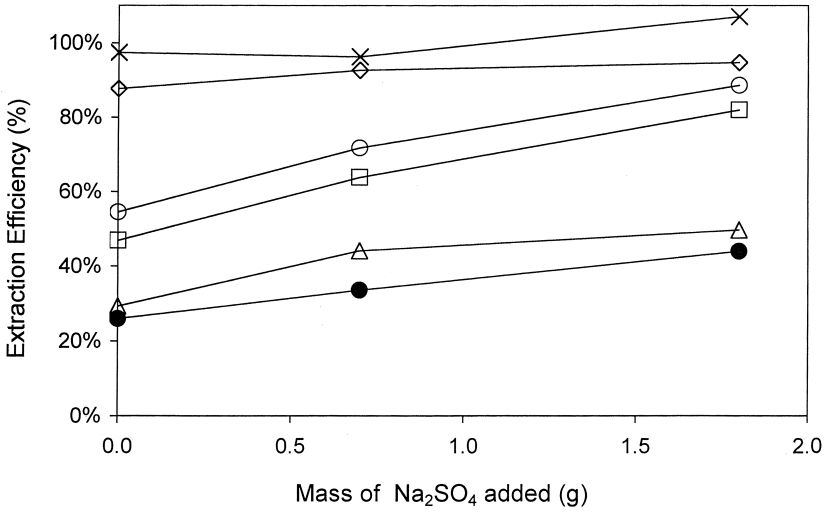

Fig. 2. Extraction efficiencies for diol target analytes and surrogate compounds as a function of the amount of Na_2SO_4 added prior to extraction. Efficiencies were calculated based on response ratios relative to a wet EA control (defined as 100%). Key: 2,3DBBD (×); 2,2DCPD (◇); 3BPD (○); 3CPD (□); 1,2BD (△); 1,2PD (●). The extraction efficiencies for 1,3DCP, 2,3DBP, and 1,4DB2B were >98% (Table 3) and independent of added Na_2SO_4 (data not shown).

(2,3DBBD and 2,2DCPD; Fig. 2) were high in the absence of Na_2SO_4 and were only slightly enhanced by the addition of sodium sulfate.

In test samples containing bromide and allyl alcohol, we found that 3BPD and 2BPD could be generated during extraction, presumably as a result of bromide oxidation. Analogous reactions might also occur in aqueous samples containing bromide and dissolved organic matter (e.g., in drinking water or wastewater). In our tests, adding 20 mg of $NaHSO_3$ to the aqueous sample prior to extraction prevented the production of 3BPD and 2BPD but had no effect on target analytes and surrogates already present in the aqueous samples.

The extraction efficiencies for the target analytes using our optimal method (i.e., with 1.8 g Na_2SO_4 and 20 mg $NaHSO_3$) were determined by preparing spiked-water controls at levels that, once extracted, would yield concentrations that fell at the high and low ends of the calibration range. Surrogate recoveries were also determined, but only at the concentration at which they would be added to actual samples (see Experimental section). The extraction efficiencies were calculated by dividing the response ratios obtained from the spiked-water control by those obtained from water-saturated EA controls prepared on the same day at the same concentration. As shown in Table 3, the extraction efficiencies are high for both the monohydroxy and diol halohydrins, especially when considering the simplicity of the extraction technique. Although the extraction efficiencies for the diols were ~10% lower than those obtained from other extraction techniques [1,2], as reflected by the low standard deviations (Table 3), the precision of the extraction technique is very good.

Table 3
Extraction efficiencies in Na_2SO_4-saturated water

Analyte	Number of samples	Extraction efficiency (%) (mean±SD)
Target analytes		
1,3DCP	20	98.5±9.9
3CPD	20	82.1±2.9
3BPD	20	88.4±3.8
2BPD	20	88.1±4.2
2,3DBP	20	99.2±3.2
Surrogate compounds		
1,2PD	13	49.1±5.8
1,2BD	13	69.8±11.2
2,2DCPD	13	97.7±3.5
1,4DB2B	20	98.6±4.2
2,3DBBD	20	98.9±6.1

3.4. Effect of water on HFBA derivatization

During the extraction step, the EA becomes saturated with water which, as discussed below, can inhibit derivatization. To test the effect of water during the derivatization step, acetonitrile controls spiked with 0.0 to 5.0 μl of Milli-Q water were derivatized without a drying step. As shown in Fig. 3a, 3.0 μl of water caused a significant reduction in peak area for all compounds except for 2BPD (Fig. 3a) and 2,3DBBD (data not shown), while 4.0 μl of water was enough to drop the areas of all compounds to near (or below) the method detection limits. For all compounds, the effects associated with ≤2 μl of water were corrected for by using the appropriate internal standard (Fig. 3b).

Because the derivatization step is sensitive to water, samples must first be dried. The drying steps described in the Experimental section (concentration under N_2 followed by a column of Na_2SO_4) were an effective and reproducible means for sample drying and caused only minor (<10%) losses in analytes. Several other drying agents were tested, including $MgSO_4$, CaCl, molecular sieve 5A, $CaSO_4$, and CaO, but the use of these drying agents resulted in lower peak areas, presumably due to the loss of analytes to the drying agents.

3.5. Matrix effects: pH

In order to determine if the pH of the aqueous sample has an effect upon the final analyte areas, spiked-water controls at various pH values (1.13–9.22) were taken through the method. For each control, the pH values of the aqueous solutions were adjusted (using H_2SO_4, Na_2SO_3 or $NaHSO_3$), the analytes were added, and the samples were immediately extracted. As illustrated in Fig. 4a, areas for most compounds were lower at either pH values <2 or >8, although a few compounds were stable throughout the entire pH range. For nearly all of the compounds, the internal standards corrected for these

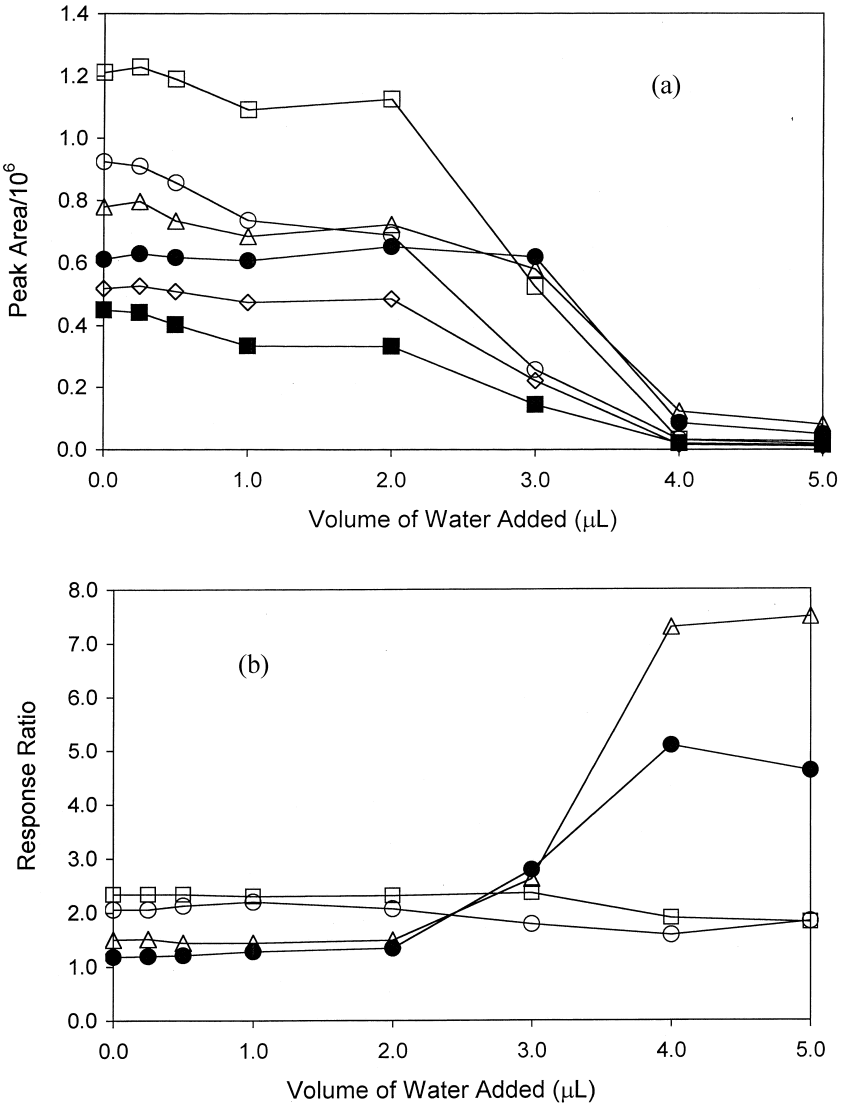

Fig. 3. Selected areas (a) and response ratios (b) as a function of the amount of water added to acetonitrile controls prior to derivatization. Key: 3CPD (□); 1,3DCP (○); 1,2PD (△); 2BPD (●); 3FPD (◇); and 1,4DC2B (■). The controls consisted of 2.0 ml of acetonitrile containing 25 μl of I.S. Mix, 40 μl of Surrogate Mix, 80 μl of Target Analyte Mix, and 0–5 μl of Milli-Q water. Analytes not shown exhibited trends similar to the compounds in the figure as follows: 2,3DBBD and 2,3DBP (not shown; similar to 2BPD); 1,2BD and 2,2DCPD (1,2PD); 3BPD (3CPD); and 1,4DB2B (1,4DC2B).

effects at pH>2 (Fig. 4b). However, because the response ratios for some compounds were only stable at pH≥3 (e.g., 2,3DBP and 2BPD) or pH≤7 (e.g., 3BPD), samples should be adjusted to within this range prior to extraction. The fact that both internal standards showed reductions in areas at pH<2 indicates that at least some of the reduction in analyte areas occurs after the extraction step. Other researchers have avoided using 3FPD as an internal standard for 3CPD due to these losses at low pH [2]. However, as shown in Fig. 4b, 3FPD can be used as the I.S. for 3CPD if the sample is adjusted to pH>2 prior to extraction.

To determine the aqueous stabilities of target

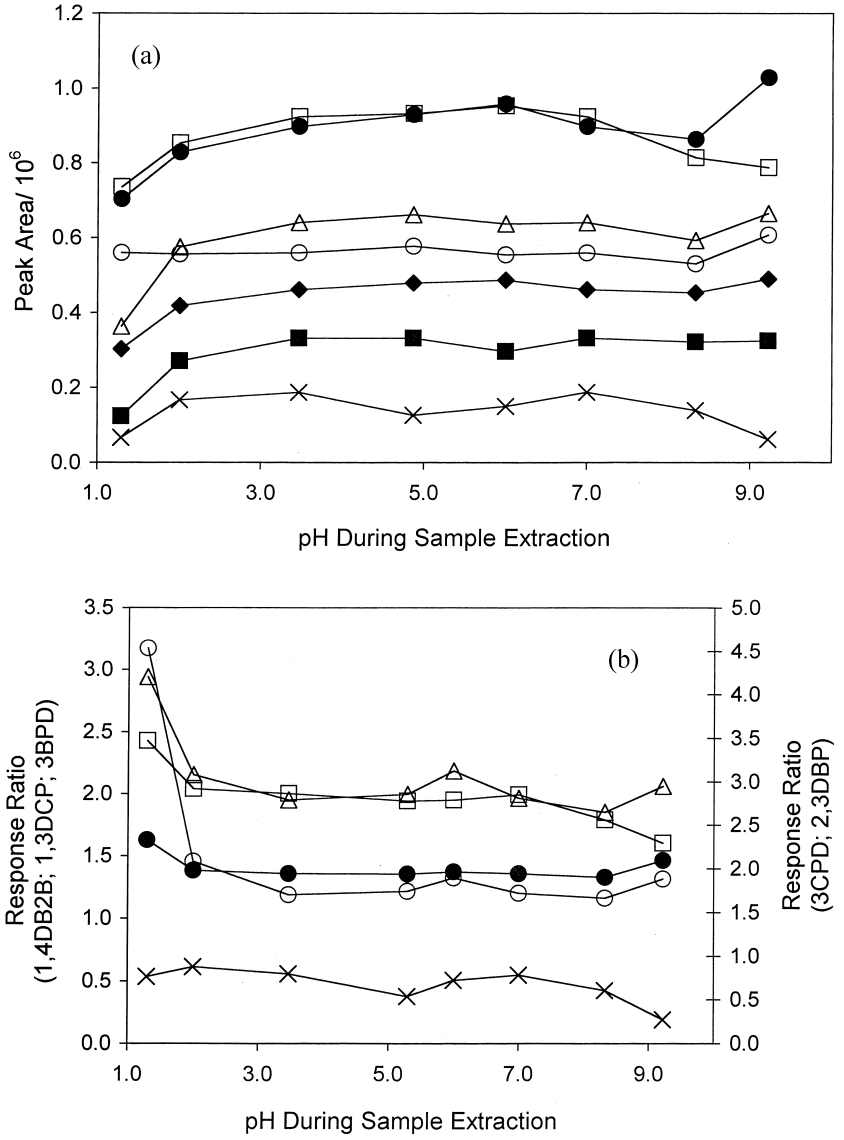

Fig. 4. Selected areas (a) and response ratios (b) as a function of pH during sample extraction. Key: 3BPD (□); 3CPD (●); 2,3DBP (○); 1,3DCP (△); 3FPD (♦); 1,4DC2B (■); and 1,4DB2B (×). Analytes not shown exhibited trends similar to the compounds in the figure as follows: 1,2BD and 1,2PD (not shown; similar to 3FPD); 2BPD, 2,3DBBD, and 2,2DCPD (2,3DBP).

analytes and surrogates as a function of time and pH, water-spiked controls at pH 1, 5, 8 and 9 were tested after being stored for 0–15 days at 4°C. Aqueous solutions containing analytes and surrogates were pH-adjusted (using H_2SO_4, borate or borate-NaOH; final borate concentration of 50 μM), refrigerated at 4°C in 13-ml glass vials, and extracted after 0, 3, 8 and 15 days. Prior to extraction the pH of the sample was checked and the sample was adjusted with sodium sulfite and sodium bisulfite to a pH between 3 and 7. For all samples, the pH remained constant during the storage time. Except for three compounds, all target analytes and surrogates were stable for 15 days at pH 1, 5, 8 and 9. The three exceptions (3BPD, 1,3DCP and 1,4DB2B) were stable at pH 1, 5 and 8, but labile to varying degrees at pH 9:

1,4DB2B was undetected after three days; 80–90% of 3BPD was lost after 3–15 days; and 25% of 1,3DCP had disappeared after 15 days. The loss of these three compounds at high pH is likely due to the base-promoted dehydrohalogenation of vicinal halohydrins to form epoxides [17]. Based on these results, aqueous samples can be stored for at least 15 days at 4°C as long as the pH is adjusted to ≤8.

3.6. Matrix effects: seawater and borate

Because we intend to conduct kinetic experiments with allyl alcohol in seawater, tests were conducted to examine possible seawater matrix effects using samples collected at the UC Davis Marine Laboratory at Bodega Bay, CA, USA. Four 5.0-ml seawater samples were spiked with 80 μl of Target Analyte Mix and 40 μl of Surrogate Mix. The initial pH of the seawater was 8.1 and after the addition of the sodium sulfate and sodium bisulfite the pH was approximately 3.6. As shown in Table 4, the absolute areas of the diols were 3 to 60% lower in the seawater samples compared to spiked-water controls. However, the seawater matrix spike recovery values (Table 4) were very close to the extraction efficiencies for spiked water controls shown in Table 2 for all diol target analytes except 2BPD. This indicates that 3FPD worked well as an internal standard to correct for seawater matrix effects for these diol target analytes. For 2BPD and the remaining diols (1,2PD, 1,2BD, 2,2DCPD and 2,3DBBD) the seawater matrix spike recoveries were significantly (>10%) different from the water extraction efficiencies, indicating that the compounds encounter matrix effects in seawater that are not corrected for by the internal standard (3FPD). However, because the precision of the response ratios was very good for these compounds (as well as all others; Table 4) they can be quantified using extraction efficiencies derived from seawater matrix spikes. For the monohydroxy compounds, both the areas and recoveries from seawater (Table 4) were very similar to those

Table 4
Seawater matrix effects

Analyte	Average areas[a]			Matrix spike recovery[b] (%)	RSD (%)[c]
	Milli-Q (MQ)	Seawater (SW)	SW/MQ		
Internal standards					
3FPD	447 715	267 986	0.60	–	3.7
1,4DC2B	340 231	367 731	1.08	–	4.1
Target analytes					
1,3DCP	641 014	676 795	1.06	92.9	2.3
3CPD	870 508	547 539	0.63	86.1	2.4
3BPD	903 854	543 190	0.60	88.5	3.2
2BPD	461 021	205 004	0.44	68.6	4.9
2,3DBP	555 818	608 929	1.10	96.5	3.5
Surrogate compounds					
1,2PD	362 621	257 690	0.71	60.4	2.3
1,2BD	206 585	82 881	0.40	48.8	2.4
2,2DCPD	558 856	502 505	0.90	146	3.2
1,4DB2B	197 290	235 301	1.19	107	4.9
2,3DBBD	438 005	426 298	0.97	114	3.5

[a] Average area for 10 spiked-water controls (Milli-Q) and four seawater matrix spikes prepared at the same concentration. The column labeled SW/MQ represents the ratio of the analyte areas obtained from seawater and Milli-Q, respectively.

[b] Matrix spike recoveries of analytes from seawater, calculated as the response ratios in seawater divided by the response ratios in water-saturated EA controls prepared on the same day at the same concentration.

[c] Percent relative standard deviation of areas (for internal standards) or response ratios (for target analytes and surrogate compounds) in the four seawater matrix spikes.

observed in Milli-Q water (Table 2) and therefore, the monohydroxy compounds do not demonstrate a significant matrix affect in seawater.

A likely reason for the reduced diol areas in the seawater matrix is the presence of borate. Borate can react with diols (especially 1,2 diols) to form cyclic borate esters [18] and is present in seawater at a concentration of ~400 μ*M* [19]. To test the effect of borate, spiked-water controls containing 50, 500 and 1000 μ*M* sodium borate were prepared and analyzed. The addition of 50 μ*M* borate resulted in area reductions of~20% for all diols (except for 2,3DBBD, which was largely unaffected), while 500 and 1000 μ*M* borate reduced areas by ~80–90% for all diols (except 2,3DBBD, which again was largely unaffected). At borate concentrations ≤50 μ*M*, the diol internal standard (3FPD) was capable of correcting for the borate effect. All monohydroxy compounds were stable at borate concentrations ≤50 μ*M*, but at 500 and 1000 μ*M* areas for these compounds were reduced by ~50% (except 2,3DBP which was unaffected throughout the tested borate range). The monohydroxy internal standard 1,4DC2B was capable of correcting for the borate effect out to 1000 μ*M* for all compounds except 2,3DBP which was stable to 50 μ*M*. Since both internal standards were affected by the addition of borate, it appears that the borate is being extracted into the EA and that reactions leading to the loss of the analytes occurred after the extraction step.

Based upon these borate experiments, and the fact that seawater contains ~400 μ*M* borate (see above), there should have been a more dramatic reduction in the diol and monohydroxy areas for the seawater spiked samples. Borate in seawater might have had less of an effect because it was complexed with cations (e.g., Ref. [19]) or because the chemical speciation of the seawater borate might have been different than the tetraborate used in our control experiments.

3.7. Method detection limits

Method detection limits (MDLs), defined as the concentration which after being processed through the entire method would produce a signal with 99% probability that it is different from the blank, were determined as outlined by the American Public Health Association et al. [20]. In order to determine the MDLs, seven MDL control samples (prepared in the same fashion as spiked water controls but spiked with 50 μl of Target Analyte MDL Mix) and 12 method blanks were prepared and analyzed. As shown in Table 5, method detection limits for the brominated target analytes were 0.14–0.25 μg l^{-1} while MDLs for the chlorinated compounds were ~4–8-times higher because of higher levels of background contamination or coeluting peaks. The accuracy and precision for the method at the MDL is shown in Table 5 as the extraction efficiency and RSD. The extraction efficiencies for all target analytes (except 3BPD) at the MDL (Table 5) were within ±20% (+24% for 3BPD) of the average extraction efficiencies shown in Table 3. This demonstrates that even at extremely low concentrations, the method is capable of accurately quantifying the target analytes. In addition, the RSDs from the seven MDL samples (Table 5) were <10% for all target

Table 5
Method detection limits

Target analyte	Average blank area	Average MDL area	Extraction efficiency of MDL[a] controls		MDL concentration (μg l^{-1})
			Recovery (%)	RSD (%)	
1,3DCP	3790	32 255	111	5.5	1.7
3CPD	2587	15 159	79.5	4.7	0.73
3BPD	437	2899	113	19	0.17
2BPD	172	855	83.2	4.7	0.14
2,3DBP	360	2455	120	9.8	0.25

[a] The recovery of the MDL controls was calculated as the response ratio of the MDL control divided by the average response ratio of water-saturated EA controls prepared at the same concentration on the same day. Also shown is the RSD (%) of the percent recovery values for the seven MDL controls.

analytes except 3BPD (<20%), indicating that the method is reproducible at low concentrations.

Few reported MDLs exist for halohydrins from any matrix, and those that do exist are primarily for 3CPD. Our reported MDLs for 3CPD are ~140–280-times lower than other values reported for aqueous samples [8,9]. Other methods for 3CPD involve the extraction of food products. If assumptions are made concerning the volume of sample extracted for these methods (i.e., 8 g of aqueous food extract is equivalent to 8 ml of water), our MDL values are ~2–27-times lower than those reported by other investigators [1,2]. However, it should be noted that these latter methods involve the extraction of food products or resins which are very complicated matrices and most likely result in higher background contamination in method blanks (which increases the MDL) compared to our sample matrix (Milli-Q water).

3.8. Effectiveness of surrogates

Surrogates were selected based upon structural similarities to the target analytes. They were used to qualitatively identify any problems that occurred prior to or during the extraction and were not employed quantitatively as correction factors. Based on our tests, only two variables – the amount of Na_2SO_4 used during the extraction and the pH of the samples during storage – could be classified as extraction effects. The other variables tested (presence of water, sample pH, and presence of borate) appeared to affect the derivatization process and were corrected for by the internal standards.

Extraction efficiencies for all the diol target analytes were affected by the amount of Na_2SO_4 present during the extraction, as shown previously (Fig. 2). Both 1,2PD and 1,2BD showed the same trend as the diols (although their extraction efficiencies were lower), indicating that these two compounds would be good surrogates for this or similar effects. Extraction efficiencies for the monohydroxy target analytes, and for the other potential surrogates (2,2DCPD; 1,4DB2B; 2,3DBBD) were high and unaffected by Na_2SO_4 (Fig. 2). Therefore, although no monohydroxy surrogate is needed to check for Na_2SO_4-saturation of the aqueous sample, either 2,2DCPD or 2,3DBBD can be used as a check for accidental sample loss during processing or, possibly, for as-yet-unidentified matrix effects. Based on the pH experiments, a surrogate is also needed to check for dehydrohalogenation of 3BPD and 1,3DCP at high pH values. As discussed previously, 1,4DB2B was quickly lost at high pH, indicating that it would be a useful surrogate for base-catalyzed dehydrohalogenation.

4. Conclusions

We have developed an analytical method for the extraction and quantification of monohydroxy and diol halohydrins from aqueous solution. The extraction technique is short, simple and capable of extracting low volumes of sample (~5 ml) with relatively small volumes of extracting solvent (~10 ml) yet maintains very low method detection limits. Based upon the recovery data for samples with concentrations ranging from the high end of the calibration range to the MDLs, the overall method appears to be very accurate and precise. We have also shown that HFBA is a very good derivatization reagent for both monohydroxy and diol halohydrins, including 3BPD. Unlike methods which utilize heptafluorobutyrylimidazole (see above), we saw no evidence that 3BPD is unstable in the presence of HFBA. This finding will allow other researchers to utilize 3BPD as a possible surrogate or internal standard in the analysis of food products for 3CPD. Finally, this method utilizes GC–ECD analysis which is relatively inexpensive, and may be more appealing to researchers who do not have access (physically or financially) to more expensive alternatives (e.g., GC–MS; GC–ITMS).

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