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Matrix effects in the gas chromatographic–mass spectrometric determination of brominated analogues of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone

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Abstract

Brominated analogues (BMXs) of the strong drinking water mutagen MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone) were found to be subject to strong matrix induced chromatographic response enhancement effects. We evaluated different ways to reduce errors in quantification including comparison of gas chromatographic inlet systems, improved clean up of sample extracts, and preparation of calibration standards in the sample matrix. The best quantitative accuracy and long term performance were achieved when the calibration standards were prepared in sample matrix, samples were cleaned up with C_{18}-resin in conjunction with solid phase extraction (SPE) with Oasis HLB cartridges, and gas chromatography with PTV splitless injection was used. This method enables the determination of MX and BMXs from 500 ml water sample with quantitation limits of 1 ng/l or less.
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Keywords: Matrix effects; 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; MX; Pesticides

1. Introduction

Matrix induced gas chromatographic response enhancement is a well known phenomenon in the analysis of pesticide residues present in different food commodities. It was first systematically studied by Earney et al. (1993), who demonstrated that matrix components, when injected together with the analytes, could protect organophosphorous compounds from adsorption and decomposition in the hot injection liner permitting more complete transfer of analytes from injector to column [1]. This results in an overestimation of the analytical results when one uses calibration standards prepared in organic solvents. Matrix effects are attributable to a variety of factors, which have different impacts on the analyte peak size and shape. Active sites in the GC inlet liner and column tend to adsorb and/or degrade analytes resulting in smaller peak sizes and/or tailing peaks. The injection temperature and the interaction time with liner (depending on carrier gas flow rate, pressure, injection volume) also have an impact on the matrix effect. Compounds prone to matrix effects are either thermolabile or rather polar and typically capable of hydrogen bonding. Many pesticides possess susceptible functional groups e.g. organophosphates, carbamates, hydroxy compounds, amino compounds, and imidazoles [2,3]. In addition to these polar, hydrogen bonding compounds, it has been shown, that lipophilic, non-polar brominated flame retardants also undergo matrix effects [4].

Different means have been proposed to reduce or compensate for matrix effects in pesticide analysis. Gonzáles et al. used correction functions to mathematically account for these effects to enable quantification using standards made up in organic solvents. Unfortunately these functions were crucially dependent on the stability of the entire analytical process, e.g. the use of a different GC system could well lead to varying results [5]. Attempts have also been made to reduce or eliminate matrix effects in vegetable and fruit samples by extensive sample purification procedures. Different combinations of solid phase extraction (SPE) cartridges were investigated [6]. A graphitized carbon black (GCB)

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cartridge, though effectively removing color from sample extracts, had little impact on matrix effect. Weak anion exchange cartridges alone or in combination with GCB were effective in reducing matrix effects, but complete elimination was still not achieved in that study. Obviously, many of the matrix components causing matrix effects behave very similarly to analytes in the clean up step, and therefore it is not possible to remove them from the sample extract without also removing the analytes. Adequate sample clean up is always desirable to reduce GC contamination and the need for system maintenance. The long term stability and matrix effects of different injection methods have also been compared. Three methods were compared: on-column, pulsed splitless, PTV solvent split, and PTV splitless injections. It was found that PTV solvent split was the most effective in reducing matrix effect and this technique also possessed the best long-term stability [7]. Calibration using standards prepared in sample matrix can compensate for these effects and is a recommended choice [1,8], but also suffers from some drawbacks such as the problems of obtaining a closely matching blank matrix for every sample type, increased instrumentation maintenance, and additional workload [3]. A very elegant approach to solving matrix effects in the analysis of pesticides has been proposed recently, where “analyte protectants” are added to both sample extracts and calibration standards in organic solvents. This approach extended all of the benefits of matrix effect to calibration standards in organic solvents with the least detrimental impacts, i.e. better quantification of real samples, large peak areas and good peak shapes, less GC maintenance, and low cost. The main limitation of this approach was that the best protectants were very polar (containing multiple hydrogen bonds), thus requiring the use of relatively polar solvents (like acetonitrile) with some water added to the final extracts, which limited their application range. Also, the long term stability of the chromatographic system has yet to be established [2].

If raw water contains bromide ions, brominated analogues (BMXs) of strong drinking water mutagen, MX, (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone) can be formed during the chlorination of drinking water in addition to MX [9]. BMXs were ranked as high-priority disinfection byproducts (DBSs) in a recent US nationwide DBP occurrence study, increasing the need for their accurate quantification [10]. In the brominated analogues of MX, one to three chlorine atoms in MX are replaced with bromine. The structures of MX and BMXs are shown in Fig. 1. The mutagenic potential of these compounds is similar to that measured for MX [11–13]. Analysis of MX has traditionally been carried out by adsorption onto XAD resin followed by methylation and measurement by GC–MS [14,15]. There are only a few published studies on BMXs, most of which employ a similar analytical protocol [9,11,16]. Liquid–liquid extraction, metlylation and analysis with GC–ECD has also been used [17]. Derivatization methods other than methylation for MX determination have been proposed. Nawrocki et al. found

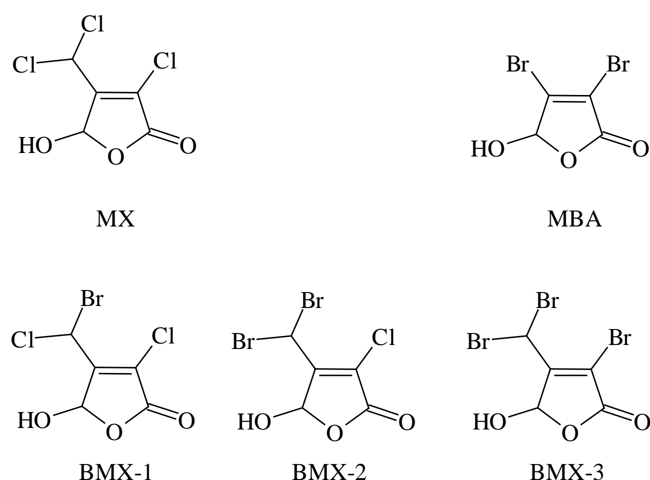

Fig. 1. Molecular structures of MX, BMX-1, BMX-2, BMX-3, and internal standard MBA.

that propylation with 2-propanol gave significantly better responses in MS-detection than methylation [18]. Rezemini et al. developed a very interesting approach to MX analysis including sample clean up with C_{18}-cartridge, liquid–liquid extraction and derivatization in the GC injection port liner with bis(trimethylsilyl)trifluoroacetamide (BSTFA) to form a trimethylsilyl derivative [19].

No systematic studies on the matrix effects of MX or BMXs have been carried out thus far. This phenomenon was briefly touched on by Suzuki and Nakanishi (although not referred to as a “matrix effect”), who corrected their MX and BMX results from real water samples with recovery results from corresponding spiked sample data [9]. This paper describes some improvements to the existing analytical methods for MX and BMXs and provide a detailed evaluation of the matrix effects that frequently occur in the analysis of BMXs. Different solutions to overcome this phenomenon are also given.

2. Experimental

2.1. Reagents and standard solutions

MX (99%) was purchased from Radian International (Austin, Texas, USA, Cat.No.: CSQ-1775). Brominated MX-analogues were synthesized using the method described by Lloveras et al. [20] and kindly donated by Professor Angel Messeguer (CSIC, Barcelona, Spain). Mucobromic acid (MBA) (≥99%) used as an internal standard was obtained from Fluka (Buchs, Switzerland, Cat.No. 69980) and PCB-30 (2,4,6-trichlorobiphenyl) used as a syringe standard from Dr. Ehrenstorfer (Augsburg, Germany, Cat.No. C200030). Acetone, ethyl acetate, methanol, 2-propanol, H_2SO_4 (95–97%) and HCl (36–38%) were all supplied from J.T. Baker (Deventer, The Netherlands). All solvents used were of HPLC or analytical grade purity.

Primary stock standard solutions of MX (0.5 mg/ml), BMX-2 (0.5 mg/ml), BMX-3 (0.5 mg/ml) and a mixture of BMX-1 and BMX-2 (1.2 mg/ml in total) were prepared by weight in ethyl acetate. A mixed standard solution of MX and BMXs was prepared at a concentration of 5 μg/compound/ml in ethyl acetate. All standard solutions were stored in the dark at −18 °C. A fresh working solution of MX and BMXs (50 ng/compound/ml) and a fresh internal standard solution of MBA (500 ng/ml) were prepared in ethyl acetate each analytical day. The working solution of PCB-30 (100 ng/ml) was made in toluene and stored at 4 °C.

2.2. Sample and standard preparation

Fresh tap water was used in every experiment for spiked samples and matrix calibration. Blank matrix for matrix matched calibration was prepared from City A tap water by adjusting the pH to 12 with NaOH and leaving at the room temperature and under laboratory irradiation for 3 days. Matrix standards and water samples were prepared similarly. First 500 ml of water sample was acidified to pH 2 with hydrochloric acid and then mucobromic acid internal standard (50 ng) was added. Four levels of matrix calibration solutions were prepared by adding 0, 2.5, 12.5, and 22.5 ng of each compound to 500 ml of acidified blank matrix. All samples were then pumped at 10.5 ml/min with Ismatec IP 12 tubing pump (Ismatec, Glattbrugg-Zürich, Switzerland, Cat. No. ISM 942) using 2.29 mm i.d. solvent proof PharMed-tubes (Ismatec, Glattbrugg-Zürich, Switzerland, Cat. No. SC0334) through solid phase extraction cartridges. A 0.45 μm, 25 mm i.d. syringe filter was attached in front of SPE cartridges to remove larger sample particles. Some samples contained so much suspended solids that it was necessary to change the filter during pumping. After the filter, a Sep-Pak Plus tC_{18} cartridge (tC_{18}) containing 400 mg of sorbent (Waters, Wexford, Ireland, Cat. No. WAT036810) and an Oasis HLB Plus cartridge (HLB)containing 225 mg of sorbent (Waters, Wexford, Ireland, Cat. No. 186000132) were attached in train. A tC_{18} cartridge was used to remove large humic molecules and other interfering compounds and an Oasis HLB was used to adsorb the analytes. Cartridges in series were conditioned before sample pumping with 10 ml of acetone, methanol, and pH 2 MQ water (adjusted with HCl). After the sample pumping, 100 ml of pH 2 MQ water was further pumped through the cartridges to ensure complete transfer of the analytes from the sample vessels, tubing, and tC_{18} cartridge to the HLB cartridge, which was dried after pumping with air for 1 h to remove residual water. The HLB cartridge was eluted with 4 ml of acetone, the solvent was evaporated almost to dryness under a gentle stream of nitrogen, analytes were redissolved in 250 μl of 2% sulfuric acid in 2-propanol (v/v), and this solution was heated to 85 °C for 1 h for derivatization. After derivatization, 750 μl of MQ water was added and the solution was loaded to an Oasis HLB 1 cc/30 mg cartridge (Waters, Wexford, Ireland, Cat. No. WAT094225) conditioned with 1 ml of ethyl acetate, methanol, and 25% 2-propanol in MQ water (v/v). The cartridge was washed and neutralized with 1 ml of 40% methanol in water (v/v), dried under vacuum for 0.5 h, and analytes were finally eluted to an autosampler vial with 0.5 ml of ethyl acetate. Before elution, a solvent proof syringe filter (0.22 μm, 4 mm i.d.) was attached to the exit of the cartridge to ensure a particle-free extract. A PCB-30 syringe standard (5 ng) was added to the autosampler vial. Standards in organic solvent (prepared to compare system performance) were prepared exactly as water samples after the elution from the HLB cartridge.

2.3. Instrumental analysis

GC–MS analysis was performed with an HP 6890 gas chromatograph (Agilent, Little Falls, DE, USA) coupled to an Autospec Ultima high resolution mass spectrometer (Waters, Manchester, GB). The system was equipped with PTV injector and HP 6890 autosampler. Empty multi baffle liners (Agilent, Folsom, CA, USA, Cat. No. 5183-2037) were used in the PTV inlet. A DB-5MS (Agilent, Folsom, CA, USA, Cat. No. 122-5532) capillary column of 30 m length, 0.25 mm i.d., and 0.25 μm film thickness was used. In front of the column a 140 cm long, 0.32 μm i.d. deactivated retention gap was installed (Agilent, Folsom, CA, USA, Cat. No. 160-2325-10). MassLynx 4.0 software was used for instrument control and data analysis. The injection volume was 2 μl. The program for PTV splitless injection was: 70 °C for 0.01 min, 500 °C/min to 300 °C, hold 0.4 min, 700 °C/min to 350 °C, hold 2.5 min. Split valve was closed for 1 min, constant helium gas flow was 1 ml/min. Oven temperature program was: 50 °C for 2 min, 20 °C/min to 100 °C, 6 °C/min to 170 °C, 15 °C/min to 210 °C, and 40 °C/min to 260 °C, hold 3.0 min. The MS-parameters were: MS transfer line temperature 240 °C, temperature of ionization chamber 200 °C, energy of EI + ionization 35 eV, and trap current 600 μA. The two most intense ions of M–OR fragments (R = 2-propyl) were monitored for each analyte. Measured ions and their theoretical ratios (in parenthesis) were: 198.9120 and 200.9091 (0.96) for MX; 242.8615 and 244.8591 (1.62) for BMX-1; 288.8088 and 290.8066 (0.70) for BMX-2; 332.7585 and 334.7564 (0.98) for BMX-3; 238.8343 and 240.8323 (1.95) for MBA. Ions for PCB-30 were 255.9613 and 257.9584 (0.97). To ensure high acceleration voltage for every compound in the SIR-experiment, MBA, MX, and BMX-1 were run in the first time window, and PCB-30, BMX-2, and BMX-3 in the second. Sample peak identification was based on matching of the retention times and ion ratios with those of standards.

3. Results and discussion

3.1. Initial MX recovery tests with HPLC

The currently very popular Oasis HLB sorbent is a hydrophilic-lipophilic balance polymeric material, which

consists of hydrophilic *N*-vinylpyrrolidone and lipophilic divinylbenzene monomers. According to the manufacturer, this material retains polar molecules better than traditional silica-based sorbents like C_{18}. *N*-Vinylpyrrolidone, which contains a hetero-atom and a carbonyl group in the 5-ring structure is relatively similar to the ring-form of MX, therefore one might anticipate that MX would exhibit good retention to this sorbent. Oasis HLB Plus cartridges containing 225 mg of sorbent were tested as a replacement for the traditional, large, XAD resins, since the Plus-format enables easy automation of sample preparation with a tubing pump. The test was conducted by pumping through the cartridges 1000 ml of acidified (pH 2) MQ water and City A tap water to which 12.4 μg of MX was added. tC_{18} cartridges were not used in these experiments. MX was eluted with ethyl acetate, solvent evaporated, MX redissolved in 1 ml of MQ-water (pH 4), and the amount of MX measured with HPLC. The original amount of MX in City A tap water was not detectable by HPLC. The same amount of MX was dissolved directly in 1 ml of MQ water (pH 4) and peak areas compared. Recoveries were very close to 100% for both MQ water and City A tap water. Subsequently, ethyl acetate eluent was changed to acetone, because less acetone was required for complete elution.

3.2. Selection of derivatization method and back extraction after derivatization

According to Nawrocki et al., derivatization of MX with 2-propanol offers some important advantages in GC–MS analysis in comparison to traditional derivatization with methanol [18]. The most abundant fragment ions with 2-propanol (199 and 201) were at higher masses and have larger intensity than the most abundant fragments with methanol (147 and 149), which means that there will be less interference from low molecular mass compounds and better sensitivity with 2-propanol derivatives. Furthermore, in the study by Lu et al. an unknown compound which possesses the same characteristic ions and the retention time as methylated MX was formed during the chlorination of aromatic acids and other phenolic compounds making quantification of MX less reliable [21]. For this reason, derivatization with both methanol and 2-propanol was evaluated. The most abundant fragment ions for methyl- and 2-propyl derivatives of MX and BMXs were selected from a scan experiment. When MX and BMXs were run in the SIM-mode, the same concentration of propylated analytes gave significantly larger peaks and these peaks were at higher masses than the corresponding methylated analytes. Derivatization of dried sample extract with 250 μl 2-propanol containing 2% sulfuric acid at 85 °C for 1 h was thus selected.

After derivatization, the analytes have to be transferred to organic solvent from acidic alcohol. In the previous studies this has been performed by liquid–liquid extraction with hexane [9,14,18]. To ensure essentially 100% transfer of analytes to organic solvent and to further clean up the sample, we incorporated a second solid phase extraction step into the method. After derivatization, 750 μl of MQ water was added to 250 μl of acidic 2-propanol containing the analytes. The solution was loaded onto an Oasis HLB 1 cc/30 mg cartridge, which was subsequently washed and neutralized with 1 ml 40% methanol in MQ water, which gave complete recovery for every compound (including the internal standard). A volume of 0.5 ml ethyl acetate was sufficient for 100% elution. A sample enrichment factor of 1000 was thus achieved. We found that this solid phase extraction yielded about twice as high peak areas than liquid–liquid extraction with hexane.

3.3. Comparison of split/splitless and PTV-injectors

In the initial experiments where MX and BMXs were spiked to MQ water and analyzed with hot splitless injection and a deactivated glasswool plug in the liner, no peaks for BMXs at 20 ng/l level were detected. On the other hand, a good peak for MX was observed at the same concentration, indicating a tendency of BMXs to be adsorbed onto the deactivated glasswool. The use of a clean and empty, deactivated single tapered liner yielded large and sharp peaks for BMXs. This is a very important observation not mentioned in the previous methods where BMXs have been analyzed at low concentrations [9,16]. Fig. 2 shows chromatograms for the same sample injected with and without a glasswool plug in the injection liner of the split/splitless injector. After the removal of the glasswool plug from the liner, a retention gap was always used in front of the analytical column to prevent the accumulation of sample dirt in front of the column. Subsequently, it was observed that the same concentration of BMXs in a real sample matrix gave significantly larger peaks than the respective amount in organic solvent, i.e., an enhancing matrix effect. No sample clean-up with tC_{18} cartridges was done in these early experiments. This observation prompted us to test whether softer sample introduction with PTV injector would increase peak sizes in solvent standards more than in real water samples. The PTV splitless injection was selected, because sufficient sensitivity was achieved already with a 2 μl injection volume. Larger injection volumes with the solvent split mode were tested briefly, but they resulted in more rapid deterioration of the results due to faster system contamination. The effective use of solvent split would have required very careful optimization of conditions, but we did not consider that the slight gain in sensitivity was worth such considerable effort. Table 1 compares the peak areas of MX and BMXs in real samples and organic solvents with PTV splitless and hot splitless injection. Analyte peak areas were normalized with respect to the syringe standard PCB-30. As can be seen from Table 1, the peak areas of MX are practically the same with both methods of sample introduction. There is a slight increase in the normalized peak areas of the internal standard MBA, and a substantial increase in the peak areas of BMXs with the PTV injector. Peak areas of BMXs increase more for solvent standards than for matrix

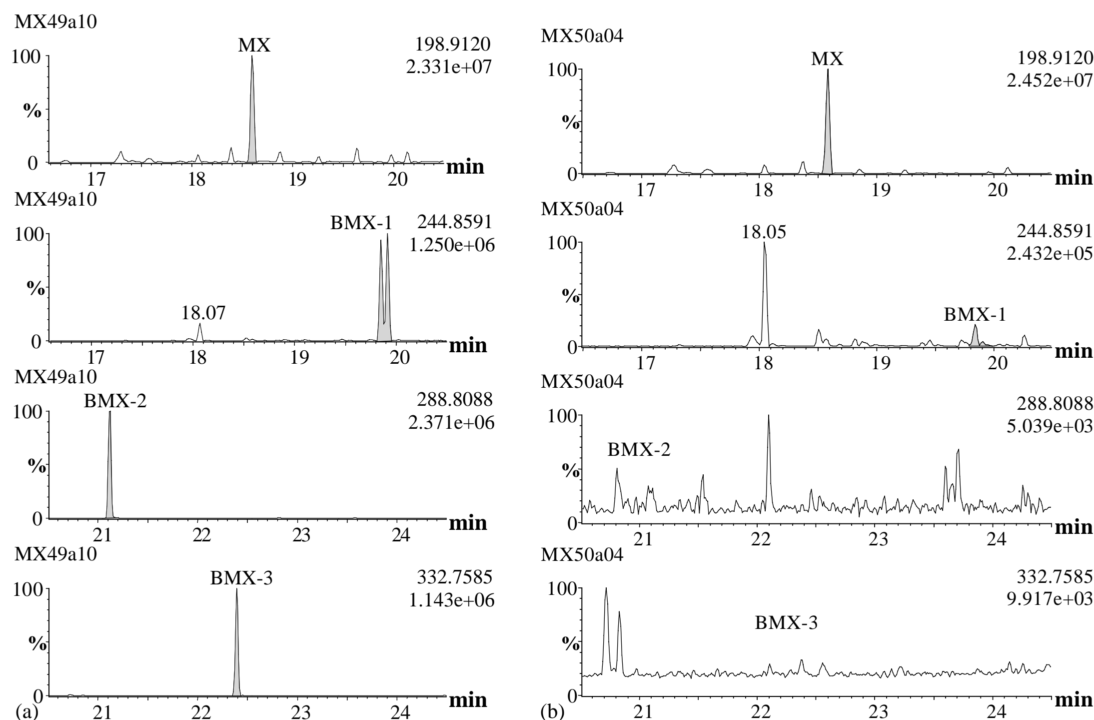

Fig. 2. Chromatograms of City A tap water (1 l, tC_{18} not used) spiked with 20 ng/l of each hydroxy furanone, (a) without glasswool plug in the injection liner, (b) with glasswool plug. Oven temperature program was as in Section 2, injection temperature in the hot splitless injection 160 °C, injection volume 2 μl. For BMX-1, two diastereomers were separated.

standards indicating that especially with solvent standards PTV splitless injection permits a more complete transfer of the analytes to the column than hot splitless injection. Switching from hot splitless injection to PTV injection thus decreased the enhancing matrix effect, but did not eliminate it. When matrix matched standards and City A tap water in Table 1 were quantified with solvent standards of Table 1, the recoveries for BMXs varied from 150 to 200% with PTV injection (data not shown). Anyway, subsequent experiments were done with PTV injector in the splitless mode.

Table 1
Comparison of peak areas with PTV splitless and hot splitless injection. Analyte responses were normalized with respect to the syringe standard PCB-30

Sample	PTV[a]/hot splitless injection[b] (%)					
	MX	BMX-1	BMX-2	BMX-3	MBA	PCB-30[c]
UV-disinfected water[d]	98	154	155	179	112	122
City A tap water	106	160	159	189	111	135
Solvent standard[d]	97	206	186	214	114	150

The amount of MX, BMXs, and MBA added was 50 ng/compound/500 ml to each sample type. Water samples were extracted with HLB, propylated, and analyzed with GC–MS using PTV or hot splitless injection.

[a] PTV injector and oven temperature programs as in Section 2.

[b] Hot splitless injector temperature was 160 °C, oven temperature program as in Section 2.

[c] Peak areas compared directly.

[d] Results are average of two parallel samples.

3.4. Use of calibration standards prepared in real sample matrix

After these experiments it was concluded that the use of matrix matched calibration standards would be necessary to achieve accurate quantification of BMXs. We prepared blank matrix from City A tap water, which is chlorinated drinking water containing a few nanogram per liter of MX and traces of BMX-1. City A tap water is processed from humus rich lake water originating from swamp areas in Eastern Finland. Raw water of City A contains less than 5 μg/l of bromide indicating low formation potential of brominated disinfection by-products. Since MX is not stable under basic conditions [15], removal of background analytes was accomplished by adjusting the water to pH 12 with NaOH, and leaving it at laboratory temperature and under irradiation for 3 days. This resulted in complete removal of MX and BMXs. Calibration standards were prepared by spiking analytes to this blank matrix, which was used to quantify spiked City A and City B tap waters. These experiments had two aims. Firstly, to find out whether the properties of City A tap water matrix remain the same during the removal process of the background analytes to enable the quantification of freshly spiked City A tap water. Secondly, to find out whether the prepared blank matrix is universal enough to enable quantification of the very different spiked City B tap water, the raw water of which originates from sulfate rich old sea bed area in west coast of Finland. City B raw water

Table 2
Average spike recoveries and recovery corrected original concentrations of MX and BMXs in City A and City B tap waters

Sample	Spiked concentration	Measured concentration (ng/l)			
		MX	BMX-1	BMX-2	BMX-3
City A tap water	0 ng/analyte/l (1)	9.6	1.8	0.0	0.0
	0 ng/analyte/l (2)	10.3	2.2	0.0	0.0
	15 ng/analyte/l (1)	24.0	18.9[a]	23.0[b]	25.7
	15 ng/analyte/l (2)	23.5	15.8[a]	16.5[b]	15.8
	Average recovery (%)	92	111	122	138
	Recovery corrected concentration (ng/l)	10.8	1.8	0.0	0.0
City B tap water	0 ng/analyte/l (1)	3.7	38.5	27.6	67.9[c]
	0 ng/analyte/l (2)	6.5	75.2	44.8	–[c,d]
	15 ng/analyte/l (1)	18.3	82.1[a]	71.8[b]	185.0[c]
	15 ng/analyte/l (2)	19.7	133.0[a]	100.3[b]	–[c,d]
	Average recovery (%)	93	367	308	781
	Recovery corrected concentration (ng/l)	5.5	15.5	11.8	8.7

Parallel water samples (1) and (2) (500 ml) were extracted with HLB, propylated, analyzed with GC–MS using PTV-injection, and quantified with matrix matched calibration standards. tC_{18} clean up was not used. Sample sequence was: Standards-Samples (1)–Standards-Samples (2)–Standards.
[a] Exact spiked concentration was 13.8 ng/l.
[b] Exact spiked concentration was 16.2 ng/l.
[c] Ion ratio differs from theoretical ratio.
[d] Calculated concentration was too large to permit the use of the negative second order calibration curve.

contains several hundred micrograms per liter of bromide, which can result in the formation of BMXs during chlorination. No sample clean-up with tC_{18}-cartridges was done in these experiments. Recoveries from these experiments are given in Table 2, from which some important conclusions can be drawn. The average recoveries for MX are very similar for both tap waters indicating good accuracy irrespective of the differences in the sample matrixes. The average recoveries for BMXs in tap water of City A are relatively close to 100% if compared to those for City B, which vary from 300 to almost 800%. There are some possible explanations for these very large enhancing matrix effects (spike recoveries). Firstly, the condition of the GC column had degraded during the previous experiments and peaks obtained for BMXs were quite small. This makes their accurate quantification less reliable. According to Suzuki, BMXs are very sensitive to active sites in the column and even different lots of the same column can show different performance [22]. Our experience confirms this observation. In the chromatogram of City B tap water there were great many interfering peaks especially around BMX-3. Thus, likely due to coelution, the ion ratios of BMX-3 were not correct. It is possible that these coeluting peaks are not so sensitive to the active sites in the column, and become more pronounced than they would with less a active column leading to erratic ion ratios for BMX-3. Secondly, it is quite likely that there are some actual differences in the matrix effect evoked by the tap water of City B as compared to that of City A. We believe that more than 300% recoveries for BMX-1 and BMX-2 are not only a result of coelution or smaller than usual peaks, because the ion ratios were correct in these cases.

The results in Table 2 highlighted the need for more exhaustive sample clean up procedure. This can be concluded from the fact that measured concentrations of spiked City B tap water were higher in set (2) than in set (1). Even though peak sizes significantly deteriorated for every sample in set (2), the deterioration was more pronounced for matrix matched standards resulting in higher measured concentrations for spiked/unspiked City B tap water. In the following experiments special attention was paid to the GC system cleanliness: a new column was purchased, clean liner and retentions gap were installed for every sample set, and the sample clean up procedure was improved.

3.5. Impact of further sample clean up

Rezemini et al. used clean up with C_{18} sorbent, since it retained impurities whereas MX was eluted by sample water [19]. We chose a trifunctional silane tC_{18} sorbent for further sample clean up, since it tolerates better the low pH of acidified samples. We found that the previously yellow sample extracts turned colorless after treatment with tC_{18} cartridges, which we attached in series before the HLB cartridges. The suitability of tC_{18} cartridges for clean up was first tested by spiking water samples before and after SPE. It was found that MX and BMXs were not retained on tC_{18}. The performance of this clean up procedure was then tested by spiking 15 ng/analyte/l to tap water samples from City A, City B, and City C (catchment area of City C raw water contains marshland and old seabed area). The measured concentrations and spike recoveries are presented in Table 3, and a chromatogram of unspiked City B tap water is shown in Fig. 3. In this experiment, the average recoveries ranged "only" from 70 to 126%, and concentrations in parallel sets (1) and (2) showed much better reproducibility, especially for tap water of City B. Surprisingly, the highest deviation was found in the tap water of City A from which the calibration standards had been prepared. Peak sizes decreased less

Table 3
Average spike recoveries and recovery corrected original concentrations of MX and BMXs in City A, City B, and City C tap waters

Sample	Spiked concentration	Measured concentration (ng/l)			
		MX	BMX-1	BMX-2	BMX-3
City A tap water	0 ng/analyte/l (1)	16.4	1.7	0.0	0.0
	0 ng/analyte/l (2)	16.0	1.7	0.0	0.0
	15 ng/analyte/l (1)	24.2	18.5[a]	19.8[b]	20.6
	15 ng/analyte/l (2)	29.2	16.4[a]	16.1[b]	16.3
	Average recovery (%)	70	114	111	123
	Recovery corrected concentration (ng/l)	23.1	1.5	0.0	0.0
City B tap water	0 ng/analyte/l (1)	4.3	19.8	13.0	14.7
	0 ng/analyte/l (2)	3.8	18.4	16.5	18.9
	15 ng/analyte/l (1)	17.2	29.1[a]	29.3[b]	31.0
	15 ng/analyte/l (2)	17.6	29.1[a]	31.0[b]	33.3
	Average recovery	89	72	95	102
	Recovery corrected concentration (ng/l)	4.6	26.4	15.5	16.4
City C tap water	0 ng/analyte/l (1)	1.8	0.0	0.0	0.0
	0 ng/analyte/l (2)	2.0	0.0	0.0	0.0
	15 ng/analyte/l (1)	16.4	17.9[a]	18.9[b]	18.0
	15 ng/analyte/l (2)	16.8	16.8[a]	17.0[b]	18.3
	Average recovery (%)	98	126	111	121
	Recovery corrected concentration (ng/l)	1.9	0.0	0.0	0.0

Parallel water samples (1) and (2) (500 ml) were cleaned up with tC_{18}, extracted with HLB, propylated, analyzed with GC–MS using PTV-injection, and quantified with matrix matched calibration standards. Sample sequence was: Standards-Samples (1)–Standards-Samples (2)–Standards.

[a] Exact spiked concentration was 13.8 ng/l.

[b] Exact spiked concentration was 16.2 ng/l.

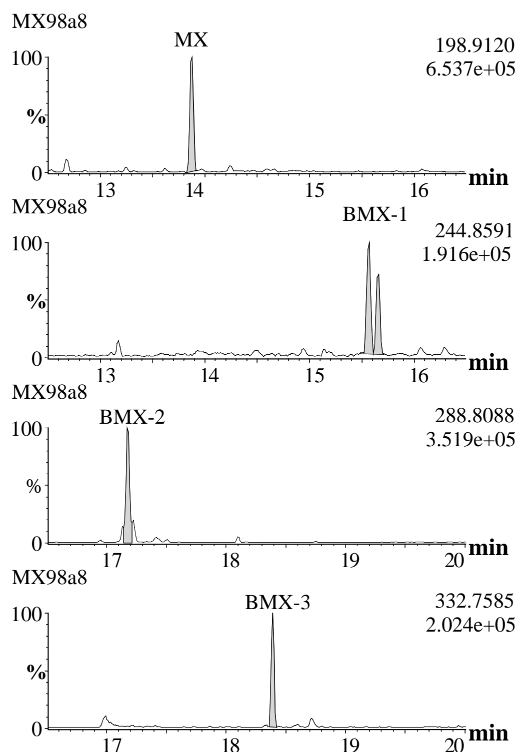

Fig. 3. Chromatograms of unspiked City B tap water, conditions as in Section 2. Recovery corrected concentrations were 4.6 ng/l for MX, 26.4 ng/l for BMX-1, 15.5 ng/l for BMX-2, and 16.4 ng/l for BMX-3. For BMX-1, two diastereomers were separated.

and peak shapes remained good during the course of the sequence as compared to the samples shown in Table 2. Thus, at least in the case of these three sample matrixes, which represent relatively different kinds of raw waters, clean up with tC_{18} cartridges proved effective in minimizing the differences in matrix effects. This enabled reasonably accurate quantification of MX and BMXs using matrix matched calibration. However, more spiked sample data needs to be gathered to evaluate the universality of the calibration method. The limits of quantitation (LOQs) were calculated with MassLynx software as eight times the average baseline noise, and it can be calculated separately for every sample. For the samples shown in Table 3 LOQs were 0.08–0.2 ng/l for MX, 0.6–1.0 ng/l for BMX-1, 0.2–0.4 ng/l for BMX-1, and 0.3–0.6 ng/l for BMX-3, depending on the activity of the GC system.

The observation that removal of the colored fraction reduced the matrix effects between different sample matrixes prompted us to consider whether measurement of molecular size fractions of humus according to Vartiainen et al. [23] could shed more light on which specific fractions were responsible for the matrix effects. This size exclusion method produces seven discrete peaks in the order of decreasing molecular weight. We presumed that the size of humus peaks would positively correlate with the enhancing matrix effect. The measurements were conducted from acidified City A and City B tap water samples before and after tC_{18} clean up. In the untreated tap water of City A, the large molecular fraction peaks 1–4 were larger than in City B, but peaks 5–7 were of equal size. Thus, this measurement could not

Table 4
Average spike recoveries and recovery corrected original concentrations of silylated MX and BMXs in City B, City A, and City C tap waters

Sample	Spiked concentration	Measured concentration (ng/l)			
		MX	BMX-1	BMX-2	BMX-3
City A tap water	0 ng/analyte/l (1)	11.8	0.0	0.0	0.0
	0 ng/analyte/l (2)	7.3	0.0	0.0	0.0
	15 ng/analyte/l (1)	21.3	21.0[a]	22.1[b]	27.1
	15 ng/analyte/l (2)	22.2	18.0[a]	19.7[b]	22.9
	Average recovery (%)	81	141	129	167
	Recovery corrected concentration (ng/l)	11.7	0.0	0.0	0.0
City B tap water	0 ng/analyte/l (1)	1.6	11.2	9.7	30.2
	0 ng/analyte/l (2)	2.1	15.6	12.2	33.2
	15 ng/analyte/l (1)	8.9	20.8[a]	28.1[b]	58.1
	15 ng/analyte/l (2)	13.0	29.7[a]	36.1[b]	59.5
	Average recovery (%)	61	86	131	181
	Recovery corrected concentration (ng/l)	3.0	15.6	8.4	17.5
City C tap water	0 ng/analyte/l (1)	0.9	0.0	0.0	0.0
	0 ng/analyte/l (2)	1.0	0.0	0.0	0.0
	15 ng/analyte/l (1)	12.7	14.6[a]	16.6[b]	16.5
	15 ng/analyte/l (2)	16.9	20.5[a]	22.4[b]	24.5
	Average recovery (%)	92	127	120	137
	Recovery corrected concentration (ng/l)	1.0	0.0	0.0	0.0

Parallel water samples (1) and (2) (500 ml) were cleaned up with tC_{18} cartridges, extracted with HLB, analyzed with GC–MS using hot splitless injection with on-line silylation, and quantified with matrix matched calibration standards. Sample sequence was: Standards-Samples (1)–Standards-Samples (2)–Standards.

[a] Exact spiked concentration was 13.8 ng/l.

[b] Exact spiked concentration was 16.2 ng/l.

explain the larger enhancing matrix effect with the untreated City B tap water. Treatment with tC_{18} caused a 70–80% reduction in the peak sizes of City A tap water, and 60–75% in City B tap water, after which the peak sizes were approximately equal in both samples.

3.6. Derivatization of sample extracts inside the liner

Some attempts were made to simplify the analysis protocol by silylating the analytes with bis(trimethylsilyl)trifluoroacetamide inside the injection liner with a slight modification of the silylation method of Rezemini and Vaz[19]. Briefly, the primary extract in acetone was evaporated to almost dryness and redissolved in ethyl acetate, transferred into an autosampler tube and adjusted to 100 μl before 10 μl of BSTFA was added. The injection volume was 1 μl, the injection temperature of hot splitless injection 160 °C, and the most abundant $M–CH_3$ ions were monitored. Matrix matched calibration standards and water samples as in Table 3 were prepared and analyzed. The results from the silylation experiments are presented in Table 4. Recoveries varied more for silylated samples, from 61 to 181%. When compared to propylation, larger recoveries for silylation were observed especially for BMX-2 and BMX-3. Also, in most cases, parallel silylated samples varied more from each other. The peak sizes of silylated analytes were larger in most cases, partly due to the more concentrated sample extracts, but part of the difference may have been attributable to the nature of the silylation process itself, e.g. perhaps its derivatization efficiency was larger. On the other hand, in the chromatograms of silylated analytes there were more interfering peaks especially for MX and BMX-1, which to some extent nullified the benefits of the increased peak sizes. For BMX-2 and BMX-3 the situation was rather equal. On-line silylation would save a considerable amount of time, which makes it an attractive alternative to propylation, but this method still requires some more fine tuning to make it sufficiently accurate reliable. LOQs for silylation were 0.05–0.1 ng/l for MX; 0.3–0.7 ng/l for BMX-1; 0.1–0.2 ng/l for BMX-2; and 0.2–0.3 for BMX-3 depending on the activity of the GC system.

3.7. Some remarks on the analytical problems encountered

Trace analysis of highly active compounds such as BMXs creates problems which sometimes are difficult to overcome even with the greatest care. As mentioned earlier, the selection of a column with little activity is very important. When we analyzed the same sample with 60 m DB-5MS column instead of 30 m, peaks were clearly smaller. This may be in part due to the individual column, but in general we would recommend the use of a shorter column where the analytes are likely to encounter fewer active sites. When a suitable column is found, it should be reserved for BMXs exclusively. Careful sample clean up, clean liners, and the use of a deactivated retention gap are important to extend column

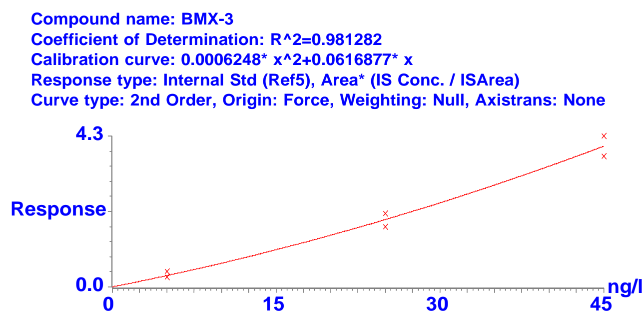

Fig. 4. Typical calibration curve of BMX-3. Six real samples were run between matrix matched calibration standards.

lifetime and to maintain column performance. Also, samples should be analyzed as soon as possible after preparation, because propylated derivatives of BMXs are not very stable.

Even when careful sample clean up is achieved, sequences should be kept relatively short (less than 30 injections) to maintain reasonable peak sizes of BMXs, which tend to decrease more than the peak size of the internal standard during the course of the sequence. For this reason it is important to perform frequent calibration to obtain the average runtime response factors. This is illustrated in Fig. 4, which shows a typical calibration curve for BMX-2. Correlation co efficients of second order curves are not very good, because the responses of calibration standards ran before and after samples follow a different curve. They varied from 0.9 to 0.99 depending on the activity of the GC system. Perhaps the use of suitable analyte protectants as demonstrated for pesticides by Anastassiades et al. [2] would represent a solution to the system contamination. The best protectants found by these workers require that there has to be some water in the final extract, which in our case would necessitate the use of a solvent other than ethyl acetate. Also, little is known of the stability of MX and BMXs derivatives in a water containing solution. No experiments were made in this study to answer these questions.

The heart of the quantification problem is the lack of suitable (labeled) internal standards. MBA works reasonable well for MX, but despite its structural similarity to BMXs, it is much less sensitive to the active sites in the GC system. We also observed another factor with MBA, which complicated the use of calibration standards prepared in organic solvents. In pure organic solvent, the efficiency of propyl derivative formation of MBA was dependent on the concentration of the other hydroxyfuranones present. At higher analyte concentrations, less MBA derivative was formed in the solvent standard. When used for sample quantification, this results in the underestimation of analyte concentrations in real samples at higher concentrations, because proportionally more MBA derivative is formed in the real samples. In some unknown way, the sample matrix seems to balance MBA derivative formation.

4. Conclusions

A GC–MS method using calibration standards prepared in real sample matrix was developed for the determination of MX and BMXs. In the three tested tap waters recoveries between 70 and 126% were obtained. Although the accuracy was not excellent, reproducibility was greatly improved for different drinking water matrixes by the use of PTV splitless injection, proper sample clean up, and frequent maintenance of the GC system. High sensitivity of BMXs towards the active sites in the GC system is a problem, which requires extreme vigilance on the part of the analyst. The raw waters used for the tap waters tested originated from humus rich surface waters and sulfate rich, acidic, old sea bed. When different kinds of water matrixes have to be analyzed, it is advisable to test the suitability of the matrix matched calibration standards by using spiked samples to ensure reasonable accuracy of the results.

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