ELSEVIER

Journal of Chromatography A, 972 (2002) 259–267

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Gas chromatographic–mass spectrometric method for quantification of 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone in chlorinated water samples

Andrea L. Rezemini[a], Jorge M. Vaz[b], Lilian R.F. Carvalho[a,*]

[a]*Instituto de Química, Universidade de São Paulo, C.P. 26077, 05599-970, São Paulo, SP, Brazil*

[b]*Instituto de Pesquisas Energéticas e Nucleares, São Paulo, SP, Brazil*

Received 4 March 2002; received in revised form 20 June 2002; accepted 19 July 2002

Abstract

A method for the determination of 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX), in drinking water by GC–MS with a limit of detection of 3.0 μg/l and a limit of quantification of 7.0 μg/l is presented. Clean-up by SPE and extraction of water samples with dichloromethane were carried out before the preconcentration of MX, which was derivatized directly in the injector of the GC, and the MX trimethylsilyl derivative was identified and quantitatively determined by MS.

Keywords: Water analysis; 3-Chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX)

1. Introduction

The main sources of toxic organic contaminants in drinking water are anthropogenic compounds from industrial and domestic discharges draining into the raw water supply and the conversion of nontoxic to toxic compounds by the disinfection practices used in drinking water treatment [1].

Among these drinking water treatment processes, chlorination is the most commonly used, and many chlorinated byproducts formed during this process can cause human health risks. Some of these compounds, such as volatile trihalomethanes and the nonvolatile organochlorine acids, including dichloroacetic acid and trichloroacetic acid, have been extensively studied [2].

As a result of recent analytical advances, new drinking water contamination problems have been observed. The use of short-term bioassays in combination with analytical measurements has constituted a powerful tool for identifying potentially genotoxic compounds in water samples. The chemical analysis techniques for identifying certain reaction byproducts of chlorination have shown the presence of several mutagenic compounds. For example, 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) is one of the most potent direct-acting mutagens ever tested in *S. typhimurium* TA 100 [3–5] with a mutagenic activity comparable to that of aflatoxin [11,12]. The MX precursors are not yet well known, however, it is known that humic substances present

*Corresponding author. Fax: +55-11-3815-5579.

E-mail address: lrfdcarv@iq.usp.br (L.R.F. Carvalho).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01093-2

in natural waters produce MX during the chlorination process [6–9]. Although MX has been found in drinking water only at low concentrations, ranging from a few nanograms per liter to 67×10^{-3} μg/l [10] it has been shown to account for approximately 50% of the total mutagenicity of chlorinated water.

Since the physical constants of MX are unknown, limited information is available for MX in water. The compound has been detected in chlorinated drinking water from Asia, Europe and North America [10,12,13], however no study of MX has been carried out in Brazil prior to this work. Gas chromatography with electron capture detection (GC–ECD) [12] and high- [11] and low- [15] resolution mass spectrometry detection techniques have been reported in the literature to determine MX in water. In such studies, liquid–liquid extraction (LLE) [11] and solid-phase extraction (SPE) [12] were employed prior to the chromatographic analyses. MX has been included in the list of chlorination byproducts in the most recent World Health Organization (WHO) guidelines as a potent mutagenic compound that should be controlled in drinking water. However, as yet, a limit has not been established, probably due to a lack of toxicity data and analytical difficulties [14].

Quantitative analyses of complex organic mixtures such as drinking water have been performed using GC techniques but precolumn derivatization has been required to obtain accurate analysis of these polar organic compounds [16]. Derivatization is typically performed by alkylation or reaction of the compound with trimethylsilyl reagents. The silylation procedure is often preferred due to its simplicity and speed of reaction [17]. Typical silylation derivatization procedures are performed off-line, thereby requiring additional sample processing and time for sample analysis. The methylation reaction of MX with alcoholic acid solution in vials at 70 °C has also been used to detect MX in water by GC–MS [10,11] however, it requires a longer time, between 2 and 12 h, and an additional step to transfer the derivatized MX to the organic solvent. On-line derivatization reduces these problems and has been shown to be quantitative [17,18].

In our work, an alternative method for quantification of MX at trace level involving on-line based trimethylsilyl derivatization by GC–MS was developed and the optimum conditions were defined. Prior to chromatographic analysis, LLE followed by a concentration step is proposed for MX determination in chlorinated water samples.

2. Experimental

2.1. Chemicals

The MX standard (98%) was purchased from Sigma. MX (1.5 g/l) was stored in ethyl acetate at −20 °C. The derivatization reagent, bis-(trimethylsilyl)trifluoroacetamide (BSTFA), was obtained from Merck. Stock solutions (5×10^{3} μg/l) of the MX standard in acetonitrile, chloroform, ethyl acetate, dichloromethane (DCM) and tetrahydrofuran (THF) were prepared. Ethyl acetate, methanol (MeOH) and DCM were used for the extraction tests. All HPLC grade solvents were purchased from EM Science. Na_2SO_4 and NaCl (Merck) were used for the LLE tests. The water used for the experiments was purified, 18 MΩ resisitivity, by a Nanopure system (Branstand).

2.2. Derivatization procedure

In order to monitor the formation of the reaction product, aliquots of 0.5 μl of the stock solutions were injected into the GC–MS apparatus. The procedure was carried out at room temperature using different injection times (30, 60, 90, 120, 180 and 240 min of solution preparation) and different solvents. Stock solutions of MX compound in ethyl acetate were analyzed using on-line derivatization at 250 °C in the chromatograph injector port, and off-line derivatization at 25 and 75 °C in the oven at different reaction time periods; after 30, 90, 120, 180 and 240 min of the BSTFA addition, stock solutions were injected into the chromatograph.

Additional amounts of the derivatization reagent were added to the aliquots of MX in order to find the BSTFA:MX ratio that gave the best conditions for MX detection. The BSTFA:MX molar ratio ranged from 2.0×10^{2} to 16.5×10^{3}.

2.3. Gas chromatographic–mass spectrometric analysis

A Shimadzu GCMS-QP5000 with the mass selective detector operating in the scan mode and the selective ion monitoring (SIM) mode, and electron impact ionization (EI) at 70 eV, was used to analyze MX solutions (5.0 mg/l). Data analysis was performed using the CLASS-5000 version 2.10 software. A fused-silica capillary column, DB-5 (HP-5MS), 30 m×0.25 mm I.D., with 0.25 μm film thickness, was used.

The MS parameters were; ion source temperature: 240 °C; mass range: 45–350 *m/z* in the scan mode; fragment ions monitored by SIM mode: 93, 107, 135, 137 and 275 *m/z*; solvent cut time: 5 min; detector voltage: 1.50 kV (scan) and 2.5 kV (SIM).

The GC parameters were; carrier gas: helium at 32 kPa; column flow: 0.8 ml/min; total flow: 50.3 ml/min; linear velocity: 32 cm/s; injection volume: 0.5 μl; temperature program: from 40 to 310 °C at 20 °C/min; splitless injection at 150, 250 and 340 °C; split opening at 0.5, 1.0, 1.5, 2.0 and 2.5 min after injection.

A five-point multipoint calibration curve for the MX in ethyl acetate, ranging from 3 to 100 μg/l was obtained under the following conditions: injector temperature of 250 °C; splitless time of 2 min and on-line derivatization using a BSTFA:MX molar ratio of 8.0×10^2.

The fragment ions *m/z* 93, 107, 135, 137 and 275 were used for identification (similar ratios of the peak areas corresponding to the fragment ions selected were found). To optimize on -line derivatization, the ion *m/z* 135 was used for MX quantification. However, *m/z* 275 was chosen for MX quantification in water because EI-spectra of all water samples studied presented peaks at *m/z* 93, 107, 135 and 137 at the same retention time as D-MX, while no peak was present at *m/z* 275.

2.4. Extraction–concentration procedure

Different solid sorbents (150 mg), such as grafitized carbon, alumina, Florisil, silica gel, XAD-4 and -8 (1:1) and C_{18} were tested for the SPE. An aliquot of 50 μl of MX stock solution (5.0 mg/l in DCM) was added to the top of each solid sorbent cartridge and 0.5 ml (5 times) of organic solvent was used for elution of MX. Ethyl acetate, MeOH and DCM were tested as sorbent. In order to evaluate MX recovery, 1 μl of each eluted solution with 1% (v/v) BSTFA was injected into the chromatograph (on-line derivatization). The efficiency of MX recovery was high using grafitized carbon, Florisil and C_{18}, whereas practically no MX was found in the eluted solutions using the other solid sorbents.

The recovery of MX was determined using grafitized carbon, Florisil and C_{18}. An aliquot of 10 μl of MX (5.0 mg/l in DCM) was added to the deionized water (100 ml) and the prepared solution was passed through each solid sorbent followed by 0.5 ml of the organic solvent (5 times). Ethyl acetate was the most efficient solvent for both grafitized carbon and C_{18}, and MeOH the most efficient for Florisil. Different pH values (2.0 and 5.8) and extraction with and without salt (Na_2SO_4) were investigated. For these investigations 1 μl of each eluted solution with 1% (v/v) BSTFA was injected into the chromatograph (on-line derivatization).

MX in DCM at 0.01, 0.05, 0.1, 0.5 and 1 μg/l concentrations was added to deionized water (100 ml) to evaluate MX recovery using LLE. MX concentration procedures in ethyl acetate and DCM at both ambient temperature and 40 °C were studied previously.

After DCM extraction, the organic extract was concentrated, using a waterbath at 40 °C and nitrogen gas to near dryness (~5 μl). The residue was dissolved in 45 μl ethyl acetate, and an aliquot of 0.5 μl BSTFA was added to the solution. The prepared solution was injected into the chromatograph (on-line derivatization).

The efficiency of MX recovery in water (100 ml) by LLE under different extraction conditions, such as: presence/absence of salt (NaCl and Na_2SO_4 saturated solutions), pH equal to 2.0 and 4.0, with/without clean-up (C_{18}), diethyl ether, hexane, chloroform, ethyl acetate and DCM as extractor solvent, solvent volume of 10 ml and 5 ml, three times, and extraction time of 5 and 30 min, was studied.

The optimized conditions for analysis were: 10 ml of DCM, three times, at pH 2.0 without addition of salt in 5 min of agitation and using clean-up with C_{18}. MX aliquots were added to the real samples and extractions/concentrations at optimized conditions

were performed to evaluate MX recoveries in complex matrices. Finally, the optimized method was applied to chlorinated water samples.

3. Results and discussion

3.1. MX determination by GC–MS

Previous efforts to identify MX by GC–MS without derivatization were unsuccessful in our laboratory. Since the MX peak was not exhibited in the total ion chromatogram, an alternative analytical procedure, the derivatization technique, was necessary for detection.

Among derivatization agents, BSTFA was chosen because it is a strong electron donor and no extraction with organic solvent is necessary [19–21]. The electron impact mass spectrum of the product formed, 3-chloro-4-(dichloromethyl)-5-(trimethylsilyl)oxi-2[5H]-furanone (D-MX), obtained from GC–MS is presented in Fig. 1a. Some fragment ions for D-MX in the mass spectrum are suggested (Fig. 1a). A typical total ion chromatogram of D-MX (retention time 8.2 min) is illustrated in Fig. 1b.

In Fig. 2, a calibration curve for D-MX in ethyl acetate is shown. MS detector performance could be observed through linear response ($r^2 = 0.99$) to D-MX in the concentration range of 3–100 μg/l.

In order to obtain all data from water samples, the instrument detection limit of 3:1 signal-to-noise was 3 μg/l and quantification limit of 10:1 signal-to-

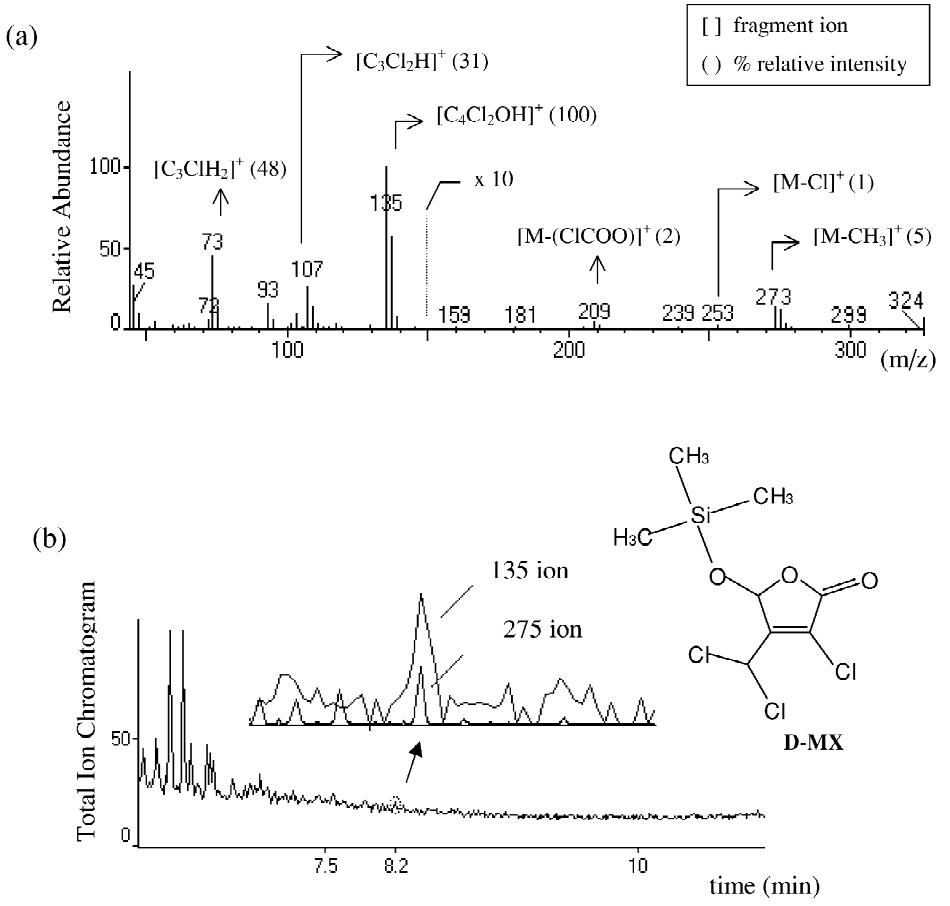

Fig. 1. (a) Electron impact mass spectrum and some fragment ions proposed for D-MX. (b) Total ion chromatogram of D-MX at 3 μg/l (LOD).

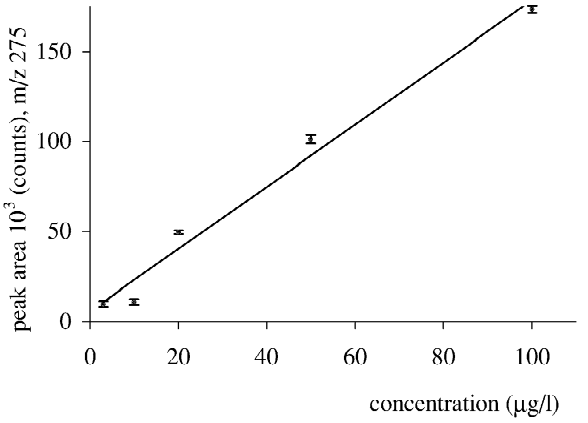

Fig. 2. Calibration curve of D-MX using on-line derivatization (replicate analysis, $n=3$).

noise was 7 μg/l. Repeatability ≤4.0% (RSD) and reproducibility ≤8.0% (RSD) were obtained at MX concentration of 3 μg/l. All these parameters were calculated based on m/z 275.

3.1.1. Off-line derivatization versus on-line derivatization

In this work, off-line (in the oven) and on-line derivatization (in the GC injector port) techniques by silylation were investigated (Table 1). When the derivatization reaction occurs in the oven, D-MX peak area values are similar when the time is increased from 90 to 120 min at both the temperatures studied (25 and 75 °C). The derivatization in the GC injector port resulted in D-MX peak area values very close to those of the oven derivatization reaction in 90 min. Such results led us to consider the application of the on-line silylation derivatization procedure using the inlet of GC to form D-MX.

Table 1
Derivatization conditions of D-MX in ethyl acetate and detector responses

Time (min)	Normalized value of the D-MX peak area (135 ion), average[a] ± SD		
	250 °C[b], on-line	25 °C[c], at oven	75 °C[c], at oven
0	0.90±0.02	–	–
30	–	0.32±0.01	0.66±0.02
90	–	0.89±0.04	0.97±0.05
120	–	1.00±0.03	0.98±0.03
180	–	0.91±0.07	0.84±0.06
240	–	0.62±0.07	0.55±0.03

[a] Replicate analysis, $n=3$.
[b] Injector temperature.
[c] Oven temperature.

3.1.2. Optimization of derivatization parameters

The on-line derivatization procedure utilizes the GC inlet as a gas-phase reactor for silylation derivatization. In our study, the derivatization efficiency of the technique was optimized with respect to four parameters: the effect of the solvent on the derivatization, the inlet temperature, the inlet splitless time, and the amount of BSTFA injected with the sample.

3.1.3. Solvent effect on the derivatization

The chromatographic behavior of D-MX was evaluated using different organic solvents (acetonitrile, chloroform, ethyl acetate and THF) and different reaction times at ambient temperature. The results of the MX analyses, in triplicate, are presented in Fig. 3.

The largest peak areas were observed using THF, followed by ethyl acetate. Peak areas obtained with THF increased significantly, except at 120 min, when its area was slightly smaller than that obtained for ethyl acetate. Using acetonitrile and chloroform, the peak areas of D-MX decreased at the time periods studied compared to those of THF.

When using THF, many peaks were observed in the chromatograms. THF impurities probably take part in chemical reactions occurring during the on-line derivatization procedure. Thus, ethyl acetate was chosen as solvent for the chromatographic analysis of MX.

3.1.4. Influence of the injector temperature

In order to evaluate the performance of the technique in achieving complete derivatization of MX, excess BSTFA was injected over a range of injector temperatures (injector port) of 150–340° (Table 2). The splitless time was fixed at 2 min. The optimal inlet temperature was determined to be 250 °C, with a slight decrease in efficiency at lower and higher temperatures. The smallest D-MX peak

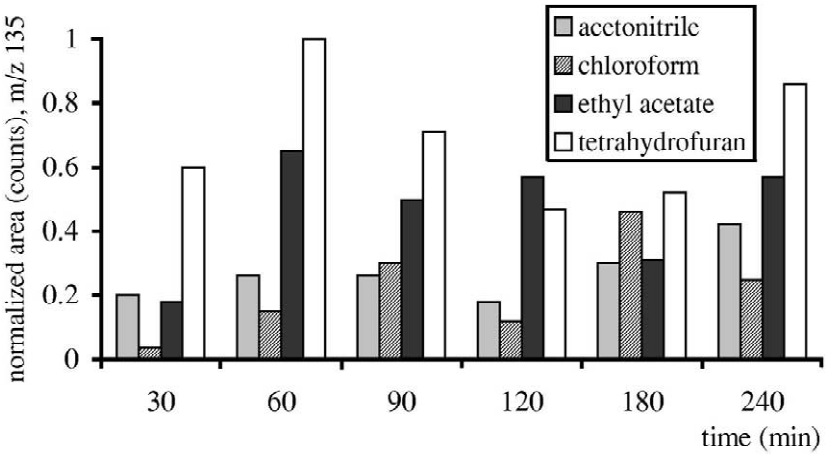

Fig. 3. Solvent effect on the D-MX detectability: measurements using acetonitrile, chloroform, ethyl acetate and THF at different reaction times and ambient temperature.

area value was obtained with the highest temperature (340 °C), probably due to the thermal degradation of MX leading to a decarboxylation of the compound [11].

3.1.5. Influence of splitless time

The splitless time was varied from 0.5 to 2.5 min while the injector was maintained at 250 °C. As Table 3 shows, there is an increase in efficiency when the splitless time is ≥1.5 min. Increasing the splitless time from 2.0 to 2.5 min did not improve the on-line derivatization efficiency, therefore an inlet splitless time of 2.0 min was employed in our final procedure.

Table 2
Effect of the injector temperature on detector response using splitless time of 2 min

Injector temperature (°C)	Normalized value of the D-MX peak area (135 ion), average[a] ± SD
150	0.93 ± 0.02
250	1.00 ± 0.02
340	0.79 ± 0.08

[a] Replicate analysis, $n = 3$.

Table 3
Effect of the splitless time on detector response using injector temperature of 250 °C

Splitless time (min)	Normalized value of the D-MX peak area (135 ion), average[a] ± SD
0.5	0.47 ± 0.04
1.0	0.75 ± 0.03
1.5	0.98 ± 0.03
2.0	1.00 ± 0.02
2.5	0.94 ± 0.02

[a] Average, $n = 3$.

Table 4
Effect of the BSTFA:MX ratio on detector response

BSTFA:MX ratio (mol:mol)	Normalized value of the D-MX peak area (135 ion), average[a] ± SD
2.0×10^2	0.44 ± 0.05
8.0×10^2	0.51 ± 0.02
1.7×10^3	0.51 ± 0.02
3.3×10^3	0.41 ± 0.08
6.6×10^3	0.60 ± 0.01
8.3×10^3	1.00 ± 0.02
13.2×10^3	0.85 ± 0.07
16.5×10^3	0.87 ± 0.08

[a] Replicate analysis, $n = 3$.

3.1.6. Amount of derivatized reagent injected with MX

The effect of the derivatization reagent:MX ratio on the detector response was evaluated. Different BSTFA:MX molar ratios, ranging from 2.0×10^{2} to 16.5×10^{3}, were used. Table 4 shows that the maximum derivatization efficiency is reached at a ratio of 8.3×10^{3}. An excess of BSTFA is required due to the fact that BSTFA reacts with the OH group of MX and other OH groups present in the glass insert and glass vial used. Organic extracts can also react with BSTFA as they contain some moisture, and BSTFA can be also consumed by contaminants containing hydroxyls in the water such as carboxylic acids, that have been detected in deionized water and real samples studied.

Therefore, the best conditions for MX quantitative analysis were: injector temperature of 250 °C, splitless time of 2 min, and an 8.3×10^{3} BSTFA:MX ratio.

3.2. Extraction–concentration procedure for MX analysis in water

First, SPE as an extraction–concentration method for MX analysis was investigated by adding 5.0 mg/l of MX to water. For this study, some solid sorbents and organic solvents were selected (Section 2.4). The results, presented in Fig. 4a, show that percentages of MX recovery in water were very low with grafitized carbon (5%) and Florisil (9%), and there was no improvement using C_{18}. Since SPE proved to be inadequate for the purpose, the LLE method was evaluated even though much more organic solvent is required.

The LLE optimization study showed that among the solvents studied, only DCM is useful, since MX remained in the residue after evaporation. Clean-up by SPE and extraction of water samples with DCM were applied before preconcentration of MX. C_{18} was chosen as purification sorbent since impurities were retained on it whereas MX was eluted (Fig. 4a).

Fig. 4b shows that the recovery of MX in water by LLE under optimized conditions was efficient using concentrations of 0.01, 0.05, 0.1, 0.5 and 1 μg/l. Compared to the SPE results, higher recovery values

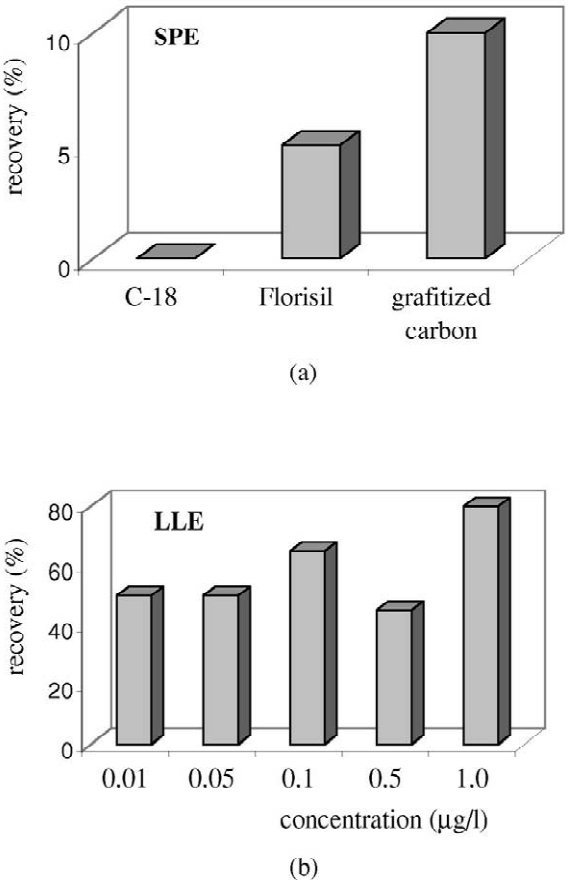

Fig. 4. MX recovery from deionized water by (a) SPE using different solid sorbents, $[MX]=5\times10^{3}$ μg/l; (b) LLE using different MX concentrations, 0.01, 0.05, 0.1, 0.5 and 1 μg/l.

(45–80%) with a good precision (RSD≤4%; $n=3$) were obtained using the LLE method. MX concentrations ranging from 0.01 to 1.0 μg/l were studied. Notwithstanding the low MX concentrations, LLE presented a reasonable sensitivity as signal-to-noise responses and their RSD values were 30±4, 35±3, 104±9, 28±4 and 209±6 for MX concentrations, in deionized water, of 0.01, 0.05, 0.1, 0.5 and 1 μg/l, respectively.

In this work, all extraction experiments were done at a pH of 2 since the closed MX tautomeric form, which presents higher a mutagenicity than the open form, dominates at pH<3 [22].

Table 5
MX in chlorinated water samples from São Paulo City, Brazil (concentration in 10^{-3} μg/l)

Water sample	Site 1		Site 2	
	Concentration (10^{-3} μg/l), $n=6$	Recovery[b] (%), $n=2$	Concentration (10^{-3} μg/l), $n=3$	Recovery[b] (%), $n=2$
Reservoir[a]	N.d.	45±3	N.d.	48±3
Chlorinated (initial)	6.3±0.8	49±4	N.d.	47±2
Chlorinated (final)	21.6±3.4	52±2	3.3[c]±1.1	50±3
Domestic tap water	14.7±2.1	49±2	N.d.	49±4

N.d. = not detected.
[a] Untreated water sample.
[b] [MX] = 50×10^{-3} μg/l.
[c] <LOQ.

3.3. Applications

The proposed method was applied to water samples collected before and after chlorination (initial and final) from two water suppliers located in different regions of São Paulo City, Brazil, during the dry season (October, 2001). At one site (site 1), located in an urban area, there is a domestic source affecting the water reservoir, while at the other site (site 2), located in a suburban area with dense vegetation, there is no major source.

The results of MX concentrations found in water samples are shown in Table 5. The MX recovery tests in real samples, shown in Table 5, presented similar results to those found for MX recovery in deionized water (Fig. 4b).

At site 1, MX was found in all chlorinated water samples. Higher MX concentration (21.6×10^{-3} μg/l) was observed in water sample collected in the final chlorination process. However, MX was not detected in samples from site 2, except that collected at the final chlorination process, in which it was found at very low concentration (3.3×10^{-3} μg/l).

4. Conclusions

A GC–MS method based on the on-line derivatization of MX by BSTFA is proposed and a detailed description of the method optimization to improve the quantitative analysis is presented. The method offers useful analytical parameters, such as short time of analysis, and satisfactory detection (3.0 μg/l) and quantification limits (7.0 μg/l).

The optimized method for quantification of MX at trace level was applied to chlorinated water samples and relatively high MX recoveries were obtained for those samples.

Acknowledgements

A.L.R. wishes to acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the doctoral fellowship.

References

[1] G. Baldauf, H.J. Brauch, B. Haist-Gulde, A. Bruchet, J. Mallevialle, B.E. Rittmann, D.V.D. Kooij, A.M.V. Dijk-Looijaard, in: Drinking Water and Drinking Water Treatment, Springer, 1995, p. 1.
[2] H.J. Kool, in: Health Risk in Relation to Drinking Water Treatment, Wiley, New York, 1994, p. 3.
[3] L. Kronberg, Wat. Sci. Tech. 40 (1999) 31.
[4] A. Smeds, T. Vartiainen, J. Maki-Paakkanen, L. Kronberg, Environ. Sci. Technol. 31 (1997) 1033.
[5] J.R. Meier, S. Monarca, K.S. Patterson, M. Villarini, F.B. Daniel, M. Moretti, R. Pasquini, Toxicology 110 (1996) 59.
[6] Z. Huixian, L. Junhe, C. Zhou, Y. Chengyong, Z. Jinqi, Z. Wen, Water Res. 34 (2000) 225.
[7] X. Xu, L. Lin, Z. Huixian, L. Yongbin, W. Liansheng, Z. Jinqi, Chemosphere 35 (1997) 1709.
[8] V. Langvik, O. Hormi, L. Tikkanen, B. Holmbom, Chemosphere 22 (1991) 547.
[9] V. Langvik, O. Hormi, Chemosphere 28 (1994) 1111.
[10] L. Kronberg, B. Holmbom, M. Reunanen, L. Tikkanen, Environ. Sci. Technol. 22 (1988) 1097.
[11] M.J. Charles, G. Chen, R. Kanniganti, G.D. Marbury, Environ. Sci. Technol. 26 (1992) 1030.

[12] S. Ogawa, H. Kita, Y. Hanasaki, S. Fukui, H. Kami, J. Chromatogr. 643 (1993) 221.
[13] A.A. Padmapriya, G. Just, N.G. Lewis, Can. J. Chem. 63 (1985) 828.
[14] J. Nawrocki, P. Andrzejewski, L. Kronberg, H. Jelén, J. Chromatogr. A 790 (1997) 242.
[15] J. Nawrocki, P. Andrzejewski, H. Jelén, E. Wasowicz, Water Res. 35 (2001) 1891.
[16] K.S. Docherty, P.J. Ziemann, J. Chromatogr. A 921 (2001) 265.
[17] R. Evershed, in: K. Blau, J. Halket (Eds.), Handbook of Derivatives for Chromatography, Wiley, New York, 1993, p. 53.
[18] B. Holmbom, R.H. Voss, R.D. Mortimer, A. Wong, Environ. Sci. Technol. 18 (1984) 333.
[19] J.L. Little, J. Chromatogr. A 844 (1999) 1.
[20] T.-Y. Chu, C.-H. Chang, Y.-C. Liao, Y.-C. Chen, Talanta 54 (2001) 1163.
[21] J. Drozd, in: Chemical Derivatization in Gas Chromatography, Plenum, New York, 1975, p. 304.
[22] H. Horth, M. Fielding, H.A. James, M.J. Thomas, T. Gibson, P. Wilcox, in: Production of Organic Chemicals and Mutagens During Chlorination of Amino Acids in Water, Wiley, New York, 1998, p. 107.