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DERIVATIZATION OF THE MUTAGEN MX (3-CHLORO-4(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE) WITH BUTYL ALCOHOLS PRIOR TO GC-MS ANALYSIS

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Abstract—An extremely potent mutagen, 3-chloro-4(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) is commonly present in chlorinated drinking water. Due to its high mutagenic activity and according to WHO guidelines its concentration should be controlled in drinking waters. Determination of MX is difficult due to the low (ppt) levels at which the compound usually exists in drinking waters. Results obtained with butanols as MX derivatization agents are shown and derivatization with sec-butanol is presented as a method which significantly lowers GC/MS detection levels of MX. © 2001 Elsevier Science Ltd. All rights reserved

Key words—drinking water, derivatization methods, mutagenicity, MX

INTRODUCTION

Due to both technological and economical aspects, chlorination is the most frequently used method for water disinfection. For over 20 years chlorination has been known to generate numerous by-products of which some are proven dangerous to human health.

In 1986 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX)—a compound of extremely high mutagenic activity, comparable to that of aflatoxins, was identified in potable water (Kronberg *et al*., 1988; Meier *et al*., 1987) in many countries including Poland (Kronberg *et al*., 1988; Backlund, 1989; Suzuki and Nakanishi, 1990; Horth, 1990; Smeds *et al*., 1997; Nawrocki *et al*., 1995). On the basis of the available data it look like MX plays a significant role in the total mutagenic activity of water extracts.

In a standard analytical procedure for MX determination in potable water, sample derivatization with methanol is used, followed by GC–MS. It has some distinct advantages: simplicity, low-reaction temperature and ease of derivative separation from the substrate (mainly sulfuric acid excess). However, the main drawback of this method is the utilization of the [M-CH_3O] fragment for quantitative analysis. This fragment, although forming a characteristic triplet, has a low abundance compared to the base peak of $m/z = 147$ (Fig. 1). The base fragment ion forms a doublet together with $m/z = 149$, which reflects the cleavage of $CHCl_2$ group (Nawrocki *et al*., 1997). Despite its high intensity this doublet is not characteristic enough to be used in the MX identification by low resolution mass spectrometry. Electron capture detector (ECD) is usually only suitable to detect MX present in clean matrices while average chlorinated tap water cannot be analyzed by ECD due to the presence of hundreds of compounds at similar concentration levels.

Different derivatization methods can improve the detection of MX. Results achieved using propyl alcohols have been described by the authors in previous papers (Nawrocki *et al*., 1997, 1998). The present work is aimed at the application of butyl alcohols for the analysis of MX. The application of sec-butanol is emphasized as the most useful derivatization agent which improves MX detection and lowers the MX detection limit. Furthermore, the use of sec-butanol enantiomers allows a separation of MX optical isomers. This can be important for investigating the mechanism of mutagenesis of MX as well as carcinogenic activity.

MATERIALS AND METHODS

MX has been synthesized using the method of Padmapriya *et al*. (1985) at the Department of Organic Chemistry, ABO Akademii in Turku (Finland). A standard solution of

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75 ng μl^{-1} of MX was used in the experiments. The reaction of MX with butyl alcohols was the subject of interest and the following alcohols were used: *n*-butanol, isobutanol (2-methyl-1-propanol), sec-butanol (butanol-2), tert-butanol (2-methylpropanol-2). All were purchased from Sigma-Aldrich-Fluka. MX after methylation and isopropylation was used for comparison in mass spectrometry evaluation.

MX standard solutions were derivatized separately in 3% alcoholic solutions of sulfuric acid. Samples were allowed to react for 1 h at a temperature close to the alcohol's boiling point. These parameters were optimized if needed. The optimized derivatization parameters for the alcohols tested were as follows: methanol, 60°C for 1 h (Kronberg *et al.*, 1988); isopropanol, 85°C for 1 h (Nawrocki *et al.*, 1997); *n*-butanol, isobutanol, sec-butanol and tert-butanol, 90°C for 1 h. The derivatized samples were extracted three times with 0.3 ml of hexane. The extracts were analyzed using GC–ECD and peak intensities were compared. In case of a positive reaction result, the fragmentation intensities of $m/z = 199$, 201, 203 [M–OR] were compared by GC/MS.

To minimize injection inconsistency and facilitate the comparison of derivatization processes, equal volumes of the extracts of MX derivatives were mixed together. The mixture contained isopropyl–MX, *n*-butyl–MX, isobutyl–MX, sec-butyl–MX and methyl-MX (v/v 1:1:1:1:1). A similar procedure was described previously Nawrocki *et al.* (1997, 1998).

In a separate experiment enantiomers of sec-butyl alcohol were used.

Apparatus. The following gas chromatographs were used throughout the work:

(1) Hewlett-Packard HP 5890II with quadrupole mass spectrometer HP MSD 5971A. Separation was performed on a Supelco MDN-5 column (30 m × 0.25 mm × 0.25 μm).

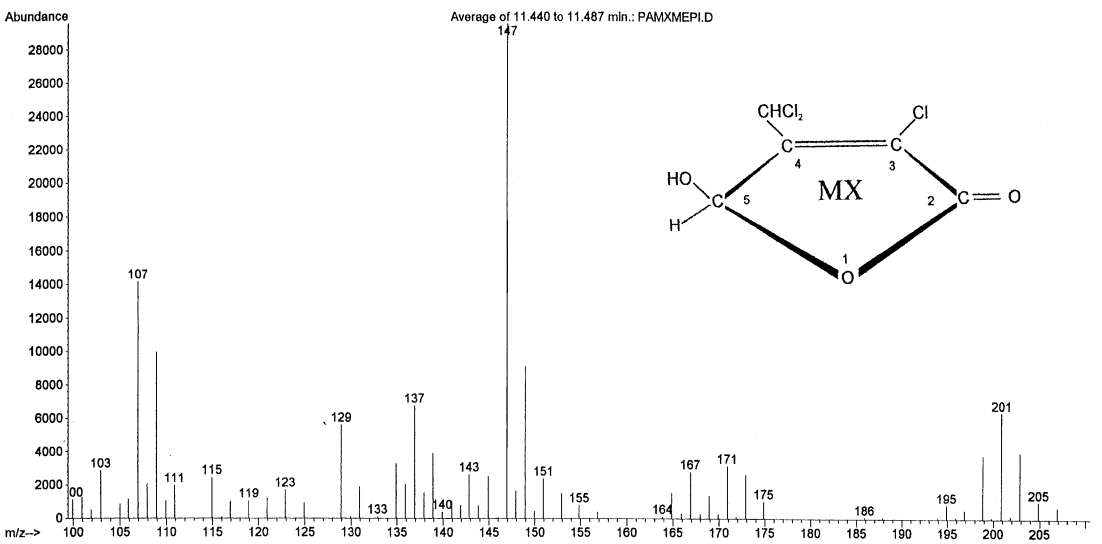

Fig. 1. The structure and full scan mass spectra of MX derivatized with methanol (MX + MeOH).

Table 1. (M–alkoxy group) fragment peak intensities and relative abundance ratios for MX derivatized with various alcohols

Sl. No.	Alcohol used	m/z fragments	Normalized intensity[a]	Relative abundances of fragment ions m/z 199, 201 and 203 (%)
1.	Methanol[b]	199	5	57 ± 2
		201	9	100
		203	5	61 ± 2
2.	Isopropanol[c]	199	100	100
		201	97	97 ± 2
		203	31	31 ± 2
3.	Isobutanol[d]	199	67	92 ± 2
		201	73	100
		203	28	38 ± 2
4.	Sec-butanol[e]	199	86	100
		201	82	95 ± 2
	Peak # 1	203	27	31 ± 1
		199	97	100
	Peak # 2	201	94	96 ± 2
		203	30	31 ± 1
5.	n-butanol	199	73	98 ± 2
		201	75	100
		203	26	34 ± 1

[a] The results are normalized to the intensity of m/z 199 ion resulting from fragmentation of pseudo-2-propyl ester of MX.
[b] $n = 3$.
[c] $n = 5$.
[d] $n = 3$.
[e] $n = 3$.

(2) Fisons 8000 with ECD detector in the GC–ECD evaluations. The following columns were used; RTX-5 (30 m × 0.32 mm × 0.25 μm) and CP-Sil CP-8 (60 m × 0.25 mm × 0.11 μm).

(3) Hewlett-Packard HP 5890II coupled to AMD (AMD, Germany) double focusing high resolution mass spectrometer. Compounds were separated on a DB-1 column (15 m × 0.2 mm × 0.25 μm).

RESULTS AND DISCUSSION

In the usual way of MX analysis in tap water extracts, the hydroxyl group of the compound is methylated to yield a methoxy group. Identification of the methyl pseudoester is done on the basis of the mass spectrum. Unfortunately, the two isotopic ions characterized by the highest intensities ($m/z = 147$, 149) cannot be used due to their low specificity in complex water extracts. For qualitative and quantitative purposes, the triplet of isotopic fragments [M– OCH_3] ($m/z = 199$, 201, 203) resulting from the presence of three chlorine atoms (Bruner, 1993) in the MX molecule is used. The drawback of this cluster is its low relative intensity, which causes difficulties when MX is present in water at trace concentration. In the present investigation butanols were selected for derivatization to give an isotopic cluster $m/z = 199$, 201 and 203 of higher abundance than that resulting from methylation. The following criteria are considered: formation of butyl–MX, intensity of the [M–alkoxyl group] fragment and application possibilities of butyl derivatization.

Generation of MX butyl derivatives

Positive derivatization results were observed for all alcohols except tert-butanol. Therefore, for further experiments the following alcohols have been chosen: methanol, isopropanol, *n*-butanol, isobutanol and sec-butanol (as racemate and separate enantiomers R(−) and S(+)). Extracted ion chromatograms (EIC) for the ions of $m/z = 199$, 201 and 203 confirmed the results obtained using GC/ECD, indicating both the formation of MX butyl derivatives and their separation by GC.

For sec-butyl alcohol two well-resolved peaks were obtained. The presence of two peaks follows from the fact that the C5 atom in the MX molecule is asymmetric. Sec-butyl alcohol is also a chiral molecule. After derivatization, four diastereoisomers are formed (RR, RS, SR, SS) of which two pairs have been separated.

The retention times of butylated MX derivatives are distinctively different with the exception of the isobutanol derivative and the first of the two peaks of the sec-butyl derivatives. Measurement of the abundance of the second peak of MX–sec-butanol solved the problem of the quantitation of the overlapping peaks, because ratios of abundance of adequate isotopic ion ($m/z = 199$, 201 and 203) from first and second peaks of MX derivatized with sec-butanol are constant. With these data it is easy to calculate the abundance of isotopic ions which originated from MX–isobutyl derivative and first peak of MX–sec-butyl derivative.

The main obstacles of butyl alcohols application for MX derivatization (comparing to the application of methanol) are caused by better solubility of butyl alcohols in hexane (hexane is routinely used for extraction of MX derivative from post-reaction mixture) and the higher boiling points. Thus, this procedure is slightly more complicated than derivatization with methanol since

- separation of products from the substrates is more difficult (sulfuric acid excess),
- higher temperature of the process.

Using high-temperature reaction vials solved the problem of the higher process temperature.

MS characterization of butylated MX

Intensities of [M–alkoxy group] fragments. As mentioned before, to facilitate a comparison of the results obtained for particular alcohols, equal volumes of alkoxy–MX hexane extracts were mixed together. Thus, both the derivatization efficiency and the alkoxy–MX recovery were assessed together for all alcohols. Table 1 and Fig. 2 give the relative intensities of the isotope peaks of MX derivatized with butyl alcohols [M–alkoxy group].

Table 1 reveals that the intensity of the triplet of $m/z = 199$, 201 and 203 of the isopropyl MX derivative is the highest of all the alcohols used. The intensities of the triplet from isobutanol derivatized MX are 13, 8 and 5 times higher than those from methylated MX. Similar intensities are observed for the *n*-butylated derivative. For sec-butyl alcohol, the

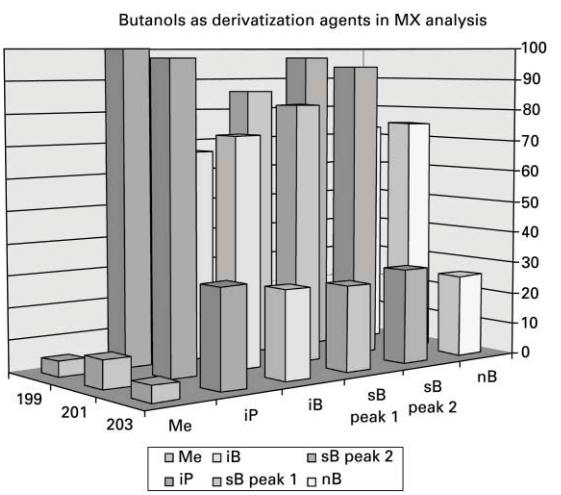

Fig. 2. Relative abundance of fragments [M.–OR] ($m/z = 199$, 201 and 203) for MX derivatized with butyl alcohols.

intensities of the ion triplets are comparable to those of isopropyl derivatives. Thus, the derivatization of MX with isopropyl alcohol as well as with butyl alcohols is much more favorable for the detection of MX in complex water extracts than methylation.

Full range mass spectra of butyl–MX are shown in Figs 3–5 (the mass spectra for the second peak of MX–sec-butanol derivative, with higher retention time, are practically identical to that with lower retention time—as in Fig. 5). The intensity ratios of isotopic ions $m/z = 199$, 201 and 203 generated from sec-butyl pseudoester and that of isopropyl one are similar to the theoretical values (for fragment ions containing three chlorine atoms, Bruner (1993)). In the case of *n*-butyl and isobutyl derivatives, the intensity ratios are different. The dominating ion is of $m/z = 201$, and instead of the triplet $m/z = 199$, 201 and 203 we observe a sextet cluster ($m/z = 199$, 200, 201, 202, 203 and 204). The even mass ions are also present for ethyl, propyl and sec-butyl derivatives, but their intensities are much smaller.

According to the data of elemental analysis of the isotopic ions (shown in Table 2) it can be concluded that the presence of the even mass ions at $m/z = 200$, 202 and 204 is related to the protonated fragment [M + H – OR].

Separation of MX enantiomers. The formation of diastereoisomers in the reaction of MX with sec-butyl alcohol can be utilized for the separation of MX enantiomers. When racemic sec-butanol is used, two pairs of diastereoisomers are separated. Derivatization with R(−) or S(+) sec-butanol allows a separation of the MX enantiomers. The mass spectra of the resolved diastereoisomers do not show any differences in the fragments nor in their

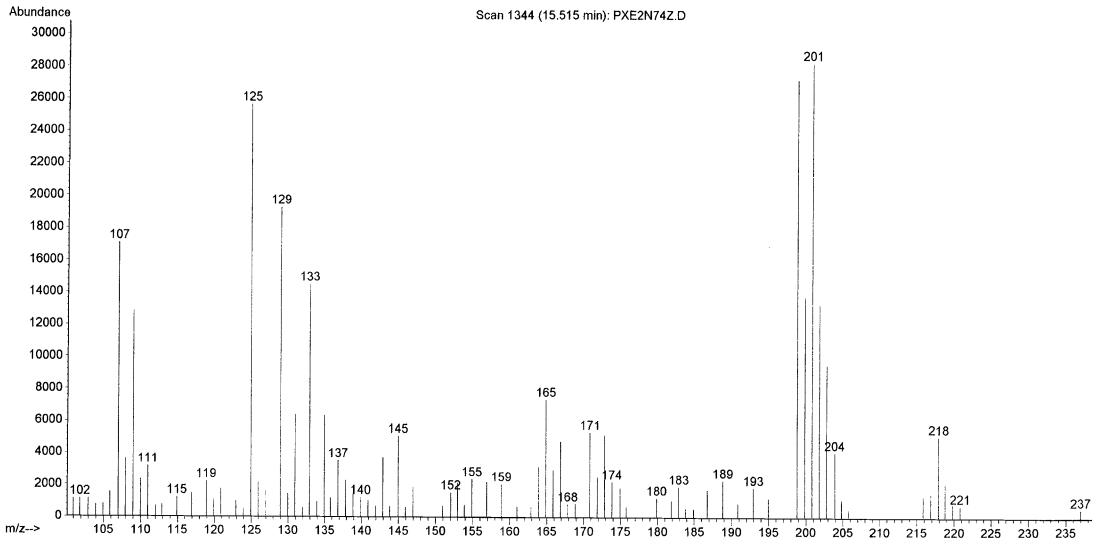

Fig. 3. The full scan mass spectra of MX derivatized with *n*-butanol.

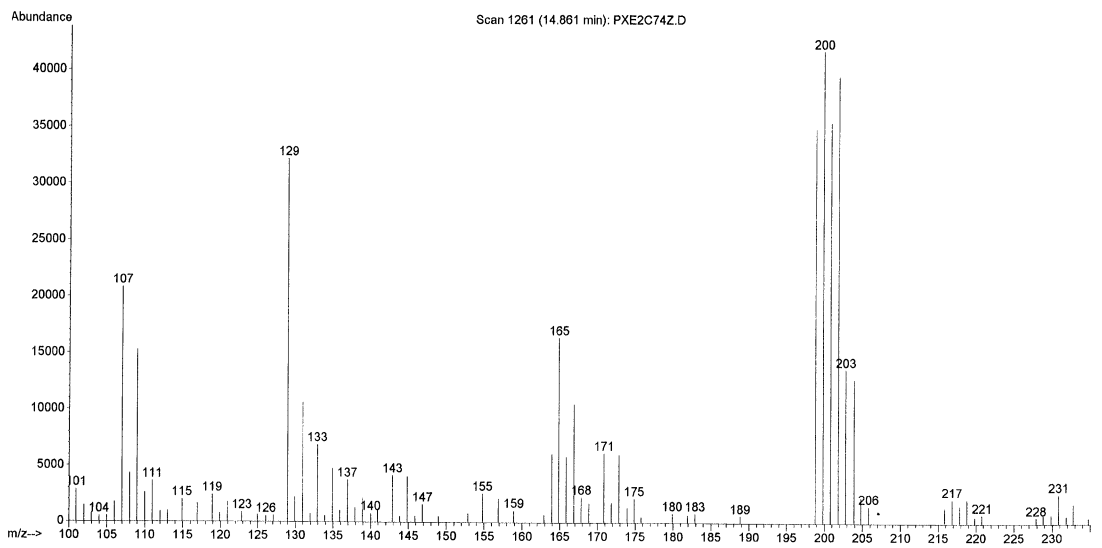

Fig. 4. The full scan mass spectra of MX derivatized with iso-butanol.

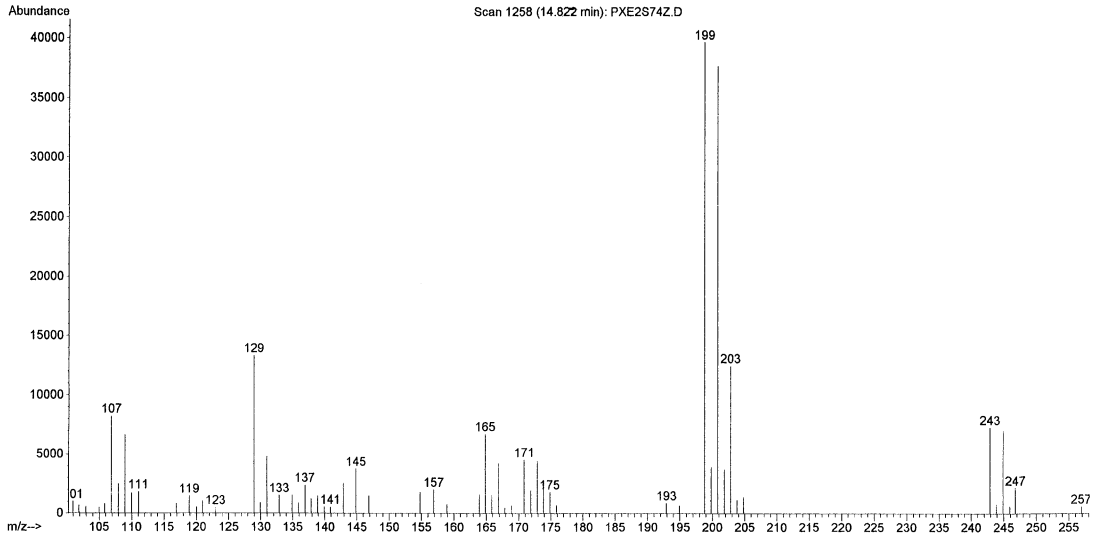

Fig. 5. The full scan mass spectra of MX derivatized with sec-butanol, peak #1 (lower RT).

Table 2. Identification of fragments (M. –OR) of the MX derivatized with butyl alcohols by HR mass spectrometry[a]

No	Alcohol used	m/z received	m/z calculated	Mass deviation (ppm)[b]	Sum formula	Identified fragment
1.	Sec-butanol Peak # 1	198.91350	198.91203	−7.4	$C_5H_2O_2Cl_3^{35}$	[M – OR]
2.	Sec-butanol Peak # 1	199.91981	199.91986	0.3	$C_5H_3O_2Cl_3^{35}$	[M + H –OR]
3.	Sec-butanol Peak # 1	200.90842	200.90909	3.3	$C_5H_2O_2Cl_3^{35}Cl^{37}$	[M – OR]
4.	Sec-butanol Peak # 1	201.91648	201.91692	2.2	$C_5H_2O_2Cl_3^{35}Cl^{37}$	[M + H – OR
5.	Sec-butanol Peak # 1	202.90671	202.90614	−2.8	$C_5H_2O_2Cl^{35}Cl_2^{37}$	[M –OR]
6.	Sec-butanol Peak # 2	198.9189	198.91203	0.7	$C_5H_2O_2Cl_3^{35}$	[M –OR]
7.	Sec-butanol Peak # 2	199.91815	199.91986	8.6	$C_5H_3O_2Cl_3^{35}$	[M. + H –OR]
8.	Sec-butanol Peak # 2	200.90956	200.90909	−2.3	$C_5H_2O_2Cl_2^{35}Cl^{37}$	[M –OR]
9.	Sec-butanol Peak # 2	202.90677	202.90614	−3.1	$C_5H_2O_2Cl^{35}Cl_2^{37}$	[M –OR]
10.	Iso-butanol	198.91197	198.91203	0.3	$C_5H_2O_2Cl_3^{35}$	[M.–OR]
11.	Iso-butanol	199.92162	199.91986	−8.8	$C_5H_3O_2Cl_3^{35}$	[M. + H –OR]
12.	Iso-butanol	200.91084	200.90909	−8.7	$C_5H_2O_2Cl_2^{35}Cl^{37}$	[M.–OR]
13.	Iso-butanol	201.91620	201.91692	3.5	$C_5H_3O_2Cl_2^{35}Cl^{37}$	[M + H –OR]
14.	Iso-butanol	202.90767	202.90614	−7.5	$C_5H_2O_2Cl^{35}Cl_2^{37}$	[M –OR]
15.	Iso-butanol	203.91379	203.91396	0.8	$C_5H_3O_2Cl^{35}Cl_2^{37}$	[M + H –OR]
16.	n-butanol	198.91206	198.91203	−0.1	$C_5H_2O_2Cl_3^{35}$	[M –OR]
17.	n-butanol	199.91816	199.91986	8.5	$C_5H_3O_2Cl_3^{35}$	[M. + H –OR]
18.	n-butanol	200.90749	200.90909	8.0	$C_5H_2O_2Cl_2^{35}Cl^{37}$	[M –OR]
19.	n-butanol	201.91550	201.91692	7.0	$C_5H_3O_2Cl_2^{35}Cl^{37}$	[M. + H –OR]
20.	n-butanol	202.90577	202.90614	1.8	$C_5H_2O_2Cl^{35}Cl_2^{37}$	[M –OR]

[a] ATT: Accepted mass error ± 10.0 ppm; Isotopic ions of fragmentation [M.–alkoxy group] which appear on mass spectra but did not fulfill the acceptable mass error criteria (i.e. ± 10.0 ppm) are not included in the table.
[b] Mass difference between columns 3 and 4.

intensities (see Fig. 5). The practical importance of the separation of MX enantiomers has not been fully established yet.

Potential applications of butylated MX

Butyl alcohols react easily with MX forming alkoxy derivatives. This opens up the possibility of using these derivatives for the analysis of MX by GC–MS.

For the determination of MX in potable water, high-resolution mass spectrometry is routinely used. Improved detectability of the triplet ions would allow us to use cheaper analytical method with low-resolution mass spectrometry. GC–LRMS is used mainly in the selected ion monitoring (SIM) mode. The unique group of ions used for identification and quantitation of MX is an isotope cluster of m/z 199, 201 and 203. Identification of MX (using LR/MS) is performed by a comparison of two parameters:

- retention time of a standard and the MX peak in a sample and,
- the relative intensities of the cluster peaks m/z 199, 201 and 203 (56 : 100 : 63 for methanol and 100 : 97 : 32 for MX derivatized with isopropanol or other alcohols).

The most abundant ion in a cluster ($m/z = 201$ for methylated MX and $m/z = 199$ for other derivatives) is selected as a target ion, whereas the others serve as qualifiers.

Abundance of isotopic ion depends, at least, on two parameters:

- first is derivatization efficiency,
- second is relative intensity of chosen isotopic ions compared to the total intensity of all ions resulting from the fragmentation of the compound.

Thus, the limit of detection (LOD) for MX derivatized with alcohols depends on intensity of the cluster (m/z 199, 201 and 203 resulting from a cleavage of the alcoxy group). For derivatization with isopropyl alcohol the $m/z = 199$, 201 and 203 cluster is the most abundant, the LOD of derivatized MX is estimated at 220 pg inj^{-1}. The limit of detection for methylated MX is tenfold higher at 2000 pg inj^{-1}. Thus, the real samples, due to low MX concentrations in tap water (ppt level), have to be preconcentrated 15,000–100,000 times prior to GC/MS analysis.

For all tested alcohols except sec-butanol, one chromatographic peak is obtained as a result of derivatization. The ion of the lowest abundance in the cluster determines the limit of detection (qualifiers $m/z = 199$ for methanol and $m/z = 203$ for other alcohols). In the case of sec-butyl alcohols used for MX derivatization, two well-resolved peaks of two diastereoisomers are obtained. Although the intensities of ion fragments are slightly lower than the corresponding ones for isopropyl derivative (see Table 1), there are two additional parameters for identification of MX. The first one is the difference in retention times of the enantiomers. In this case it is possible to identify MX without monitoring the lowest intensity ion. The other one is the presence of two clusters of isotopic ions with $m/z = 199$, 201 and 203. Then the intensity ratio of adequate isotopic ions can be used for identification of MX (i.e. the abundance of fragment $m/z = 199$ of the peak #1 to the abundance of fragment $m/z = 199$ of the peak #2 ratio, should be the same as in sec-butylated MX standard).

As a result, the detection limit is lowered as it is determined by the abundance of the qualifier ion $m/z = 201$ which is relatively more abundant compared to $m/z = 203$. Therefore, the detection limit is approximately three times lower (ratio of intensities of $m/z = 201$ and 203, see Fig. 2.) and equals 75 pg inj^{-1}.

The resolution of MX enantiomers can contribute to the evaluation of the mutagenic activity of optical isomers of hydroxyfuranones.

CONCLUSIONS

1. Butyl alcohols (except tert-butanol) react easily with MX. From the point of view of GC/MS analysis, each of the butyl alcohols is better than methanol for MX derivatization.
2. Butyl alcohols yield a high intensity triplet at $m/z = 199$, 201 and 203, which can be used for the quantitative and qualitative analysis of MX.
3. Derivatization of MX with sec-alcohol can facilitate the analysis of MX by low-resolution mass spectrometry in the cases of crude, impure matrices. In this case, the detection limit is lower than that obtained using isopropanol as derivatization agent.
4. The separation of MX enantiomers can shed new light on the mutagenic activity of MX enantiomers.

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