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Determination of the potent mutagen 3-chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX) in water by gas chromatography with electron-capture detection

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ABSTRACT

A highly sensitive method for the determination of 3-chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX) in water by gas chromatography with electron-capture detection is described. MX was derivatized with 2,2,3,3,3-pentafluoropropanol and the product was easily decomposed by UV irradiation. The disappearance of the peak on the chromatogram after the irradiation was used for the identification of MX; the amount of MX was calculated from the decrease in peak height. The method was applied to the analysis of five samples of chlorinated domestic sewage. MX (141.6–2.2 ng/l) was detected in all samples with a detection limit of 0.8 ng/l. The recovery of MX was more than 97.5% at the level of 20.0 ng/l added.

INTRODUCTION

3-Chloro-4-dichloromethyl-5-hydroxy-2(5*H*)-franone (MX), an involatile, direct-acting and highly potent Ames mutagen, has been identified as a major mutagen in pulp chlorination liquors [1,2]. MX is formed by chlorination of naturally occurring humic substances [3–5]. Recently, MX was detected in tap water disinfected with chlorine in Finland [3,5], the USA [4], the UK [6], Netherlands [7] and Japan [8], and according to these reports the mutagenicity contribution of MX in the sample water was about 7–60%. Because of the high mutagenic potency of MX (20 000 revertants/μg MX), it is expected that requirements for checking the amount of MX in drinking water disinfected by chlorination will increase.

The determination of MX in water has conventionally been carried out by gas chromatography–mass spectrometry with selected ion monitoring (GC–MS–SIM) after the extraction of MX from water followed by high-performance liquid chromatographic (HPLC) purification and methylation with methanol and sulphuric acid [9]. Because of the extremely low concentration of MX in tap water, the GC–MS–SIM method required large volumes of sample water, high resolution and high sensitivity for analysis. Gas chromatography with electron-capture detection (GC–ECD) is highly sensitive to methylated MX (Me-MX). In this work, pentafluoropropylation instead of the conventional methylation was employed for the derivatization of MX in GC–ECD. It was expected that ECD would be more sensitive to pentafluoropropylated MX (PFP-MX) than Me-MX. PFP-MX was easily decomposed by UV irradiation, hence the disappearance of the PFP-MX peak on a gas chromatogram after UV

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irradiation could be used to identify MX. The method was applied to the determination of MX in a sample of chlorinated domestic sewage that had been treated with an activated sludge system. The results were compared with those obtained by GC–MS–SIM and were in good agreement.

EXPERIMENTAL

Materials

MX was synthesized and purified according to the procedure of Padmapriya et al. [10]. The synthesized MX was identified by GC–MS and its purity was confirmed by GC and HPLC to be more than 98%. MX was stored in ethyl acetate at 4°C, where MX is completely stable [11].

A standard solution of MX was prepared by dilution of the ethyl acetate stock solution with ethyl acetate and was stored in the same manner as the stock solution. 2,2,3,3,3-Pentafluoropropanol (PFP) was obtained from Nacalai Tesque (Kyoto, Japan) and was used as received.

Isolation of MX from chlorinated sewage

The isolation of mutagenic substances including MX from chlorinated sewage was performed as described previously [12]. Samples of 1 l of chlorinated sewage were dechlorinated with sodium thiosulphate, adjusted to pH 2.0 with hydrochloric acid and applied to two sequential columns, one packed with Amberlite XAD-2 and the other with Amberlite XAD-8 resin (Rohm and Hass, Philadelphia, PA, USA). The columns were washed with distilled water acidified with hydrochloric acid (pH 2), then the adsorbates on the resins were eluted with ethyl acetate. The eluates were dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure at 40°C. The residue containing non-volatile mutagens was resolved with a definite volume of ethyl acetate and this XAD extract was stored at −15°C in a freezer.

Fractionation of XAD extracts by HPLC

The XAD extracts were evaporated to dryness under reduced pressure at 40°C immediately before use. The residue was dissolved in 1.0 ml of methanol and the solution was subjected to preparative HPLC. The HPLC conditions were as follows: apparatus, LC-4A (Shimadzu, Kyoto, Japan); column, Capcell

Pak C_{18} (250 mm × 20 mm I.D.) (Shiseido, Tokyo, Japan); elution, methanol–0.05 M phosphate buffer (pH 3.0) (40:60) for 50 min followed by a linear gradient to 100% methanol during 30 min, then held at 100% methanol for 40 min; flow-rate, 5 ml/min; column temperature, ambient; detection, UV at 230 nm. Fractions of 5 ml up to 50 min and of 25 ml after 50 min were collected. Each fraction was evaporated to dryness under reduced pressure at 40°C, the residue was dissolved in ethyl acetate and the solution was stored in the same manner as the stock solution.

Pentafluoropropylation of MX

The solvent was removed from the standard solution of MX and the fractions obtained by preparative HPLC under a stream of dry nitrogen immediately before use. To the residue, 100 μl of PFP and 10 μl of concentrated sulphuric acid were added and the mixture was heated for 30 min at 70°C. After cooling, 1 ml of 8% sodium hydrogen carbonate solution was added, then PFP-MX was extracted twice with 1-ml volumes of n-hexane. The extract was concentrated to 0.5 ml.

Detection and determination of PFP-MX by GC–ECD

Part of the n-hexane solution of PFP-MX was injected into a GC–ECD system. The GC–ECD conditions were as follows: apparatus, Shimadzu GC-4BM equipped with an electron-capture detector; column, SPB-5 fused-silica capillary (15 m × 0.53 mm I.D.; film thickness 1.5 μm) (Supelco, Bellefonte, PA, USA); carrier gas, nitrogen at 6 ml/min; injection port and detector temperatures, 200°C; column temperature, 110°C. The remaining 0.25 ml of n-hexane solution was transferred into a small quartz vessel and irradiated with a UV lamp (Toshiba GL-15 bactericidal lamp, 18 W, 15 cm long) placed parallel to the surface of the solution and about 30 cm from it for 90 min. After irradiation, the solution was diluted to the original volume with n-hexane and analysed by GC–ECD. PFP-MX was decomposed by the UV irradiation and the peak corresponding to it disappeared. This peak showed a retention time identical with that of authentic PFP-MX. The amount of MX was calculated from the height of this peak.

TABLE I

RELATIONSHIP BETWEEN VOLUME OF PFP AND PEAK HEIGHT OF PFP DERIVATIVE OF MX

The reaction mixture containing 1 μg of MX, 10 μl of sulphuric acid and PFP was allowed to react for 30 min at 70°C and extracted three times with 1-ml volumes of n-hexane. The PEP derivative of MX was detected by GC–ECD.

PFP volume (μl)	Peak height (mm)
0	0.0
10	142.2
50	267.8
100	293.6
200	150.0
300	108.5

TABLE II

RELATIONSHIPS AMONG REACTION TIME, REACTION TEMPERATURE AND PEAK HEIGHT OF PFP DERIVATIVE OF MX

A reaction mixture containing 100 μl of PFP was used; the other conditions were as in Table I.

Reaction temperature (°C)	Reaction time (min)	Peak height (mm)
60	30	102.8
70	0	0.0
	15	249.2
	30	330.0
	60	335.7
	90	327.0
80	30	136.1
90	30	59.6

Determination of PFP-MX by GC–MS–SIM

Authentic PFP-MX and pentafluoropropylated samples obtained from chlorinated sewage were analysed by GC–MS–SIM. The GC and MS conditions were as follows: apparatus, Shimadzu GC-9A gas chromatograph and MS QP-1000 mass spectrometer; column, 3% silicone OV-101/Shimalite W (AW-DMCS) (1 m × 2.6 mm I.D.) (Shimadzu); column temperature, increased from 80 to 200°C at 6°C/min; carrier gas, helium at 30 ml/min; injection temperature, 250°C; electron energy, 70 eV; ion source temperature, 250°C. Fragment ions of m/z 265, 267, 199 and 201 were chosen for SIM.

RESULTS AND DISCUSSION

MX could be pentafluoropropylated in the same manner as the conventional methylation of MX.
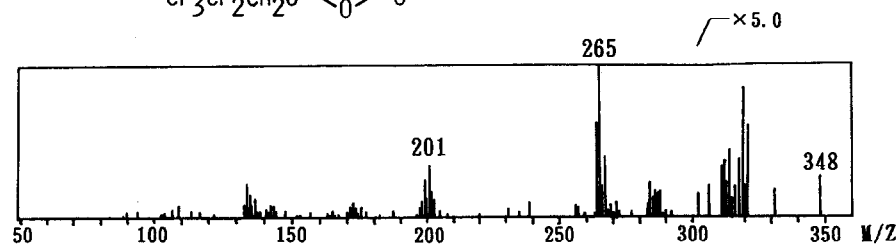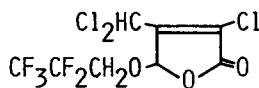
PFP-MX gave one peak in GC–ECD. A mass spectrum of the peak is shown in Fig. 1. A number of specific ion peaks, m/z 348 (M^+, weak), 265 and 267 ($M^+ - CHCl_2$, prominent), 199, 201 and 203 ($M^+ - OCH_2CF_2CF_3$), were observed. The results showed the hydroxyl group at the 5-position in MX was pentafluoropropylated. The set of specific ions of m/z 265, 267, 199 and 201 was used for GC–MS–SIM.

MX was allowed to react with various amount of PFP in the presence of concentrated sulphuric acid and the reaction products were extracted with n-hexane. As shown in Table I, the maximum peak height was obtained in the presence of 100 μl of PFP. In the presence of more than 200 μl of PFP, the

Fig. 1. Mass spectrum of PFP derivative of MX.

mixture was separated into three phases (water, PFP and n-hexane phases) in the extraction process and the peak height of PFP-MX decreased. It was thought that PFP-MX was distributed between the n-hexane and PFP phases, and the recovery of PFP-MX in the n-hexane phase was lowered. Table II shows the relationship among the peak height, the reaction temperature and the reaction time. The maximum peak height was obtained by heating at 70°C for 30 min. From these results, it was decided that pentafluoropropylation of MX was carried out in the presence of 100 μl of PFP and 10 μl of concentrated sulphuric acid with heating at 70°C for 30 min. The electron-capture detector was about 3.5 times more sensitive to PFP-MX than Me-MX.

When XAD extracts obtained from an actual sample were pentafluoropropylated and analysed by GC–ECD without further purification, there were large peaks of impurities in the chromatogram and the peak of PFP-MX was completely obscured (Fig. 2). Hence purification of the XAD extracts by preparative HPLC was necessary in the GC–ECD method. The gas chromatograms of authentic PFP-MX and purified XAD extracts are shown in

Fig. 3. Typical gas chromatograms with ECD of PFP derivatives of authentic MX and chlorinated sewage purified by preparative HPLC before and after UV irradiation. n-Hexane solutions (0.25 ml) containing PFP derivatives of authentic MX (0.2 μg, A) or chlorinated sewage purified by preparative HPLC (B) were analysed by GC–ECD before (A-1 and B-1) and after (A-2 and B-2) UV irradiation for 90 min.
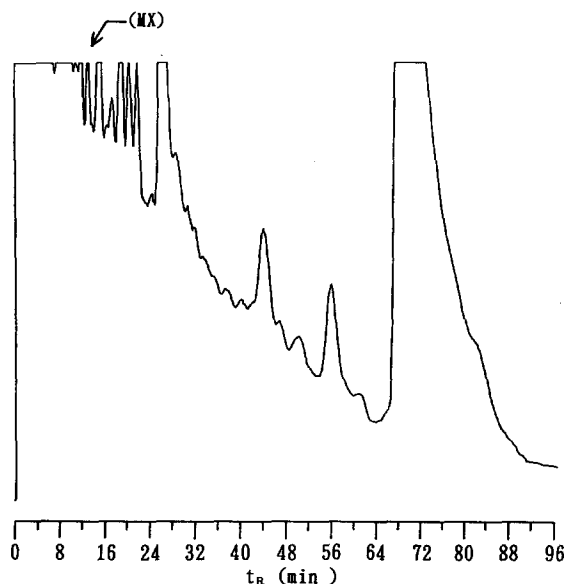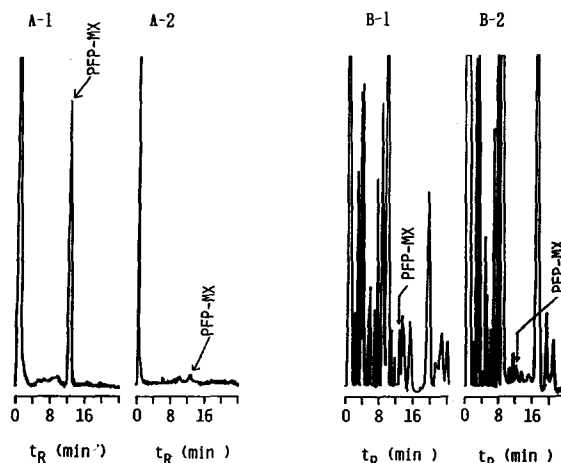

Fig. 2. Typical gas chromatogram with ECD of chlorinated sewage derivatized with PFP before preparative HPLC purification. XAD extracts corresponding to 1 l of original chlorinated sewage were derivatized with PFP without further purification and analysed by GC–ECD.

Fig. 3A-1 and B-1, respectively. As shown in Fig. 3B-1, a peak showing a retention time (t_R) identical with that of PFP-MX was found in the actual sample. A method other than t_R for the identification of PFP-MX in the chromatogram was further investigated. As shown in Figs. 3A-2 and 4, the peak of authentic PFP-MX completely disappeared after UV irradiation for 90 min. The peak height identified as PFP-MX in the actual sample decreased after irradiation for 90 min and was constant after 90 min (Fig. 4). When authentic PFP-MX was added to the actual sample and irradiated for 90 min, the peak height also decreased to the same constant level as that in the actual sample (Fig. 4). These results suggested that PFP-MX was not separated from some component(s) of an actual sample and overlapped them. However, they also suggested that PFP-MX contained in the peak was completely decomposed by UV irradiation for 90 min. Thus, in addition to identification via t_R, the disappearance of the peak or the decrease in the peak height after UV irradiation for 90 min was used for the routine identification of MX. The decreased peak height was used for the calculation of the amount of MX.
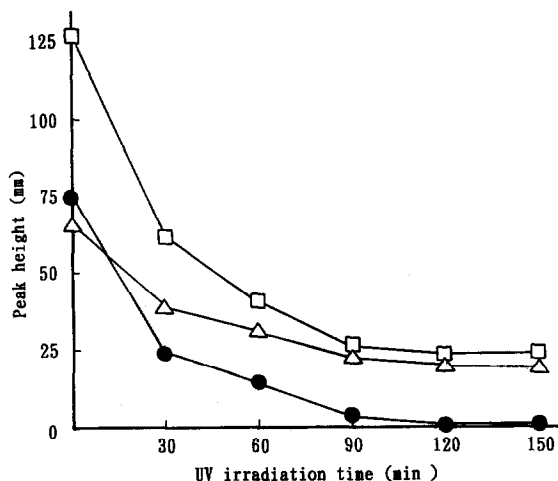
The recovery of MX through the all analytical

Fig. 4. Decrease in peak height of PFP-MX in gas chromatograms with ECD of authentic sample, chlorinated sewage and chlorinated sewage spiked with authentic MX on UV irradiation. n-Hexane solution (0.25 ml) containing PFP derivative of authentic MX (0.2 μg) (●), chlorinated sewage (△) or chlorinated sewage spiked with 0.2 μg of authentic MX (□) was subjected to UV irradiation for various times and was analysed by GC–ECD.

procedures was examined by use of the GC–ECD method with UV irradiation. Authentic MX was added to an actual sample immediately after the dechlorination process. As shown in Table III, the recoveries of 20 and 40 ng/l of MX added to an actual sample were more than 97.5%. Table IV shows the results of the determination of MX in five chlorinated domestic sewage samples by GC–ECD.

TABLE III

RECOVERY OF MX FROM CHLORINATED DOMESTIC SEWAGE SPIKED WITH MX

The amount of MX was calculated from decrease in peak height on UV irradiation.

Spike level (ng/l)	MX	
	Found (ng/l)[a]	Recovery (%)
0.0	30.8 ± 2.02	–
20.0	50.3 ± 0.59	97.5 ± 2.94
40.0	70.9 ± 5.23	95.3 ± 6.01

[a] Each value is the mean ± S.D. of three determinations.

TABLE IV

CONTENT OF MX IN CHLORINATED DOMESTIC SEWAGE

Sample No.	TOC[a] (mg/l)	Content of MX (ng/l)[b]	
		GC–ECD method[c]	GC–MS–SIM method
1	40.5	141.6 ± 8.2	134.0 ± 9.1
2	45.5	37.2 ± 3.5	34.6 ± 2.8
3	17.5	30.8 ± 2.0	28.5 ± 3.3
4	42.2	10.9 ± 0.7	N.D.[e]
5	11.0	2.2 ± 0.4	N.D.[e]
6[d]	353.2	N.D.[e]	N.D.[f]

[a] Content of total organic carbon.
[b] Each value is the mean ± S.D. of three determinations.
[c] The amount of MX was calculated from the decrease in peak height on UV irradiation.
[d] Domestic sewage not treated with activated sludge system.
[e] Not detected (below 0.8 ng/l).
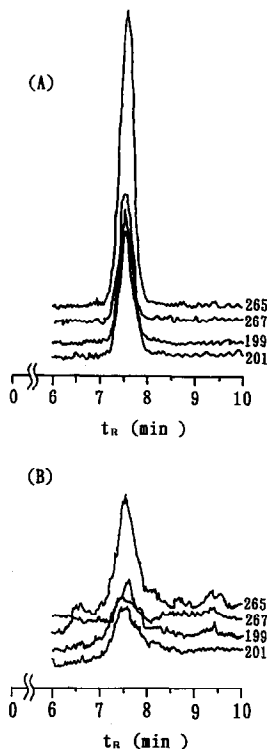[f] Not detected (below 23 ng/l).

Fig. 5. Typical GC–MS–SIM traces of PFP derivatives of (A) authentic MX and (B) chlorinated sewage.

MX was found in all samples in the range 141.6–2.2 ng/l. The detection limit was lower than 0.8 ng/l of MX in water (signal-to-noise ratio = 2). Detection and determination by the GC–MS–SIM method (Fig. 5) was carried out simultaneously. In three out of five samples the results obtained by both the two were in good agreement. It was confirmed from these results that the decrease in the height of the peak that showed a t_R identical with that of authentic PFP-MX in the gas chromatogram obtained with ECD is attributable only to the decomposition of PFP-MX by UV irradiation, and not to the other substances coeluted with MX. Hence measurement of the peak height of PFP-MX after UV irradiation was reasonable for the determination of MX in the GC–ECD method. In the two samples, MX could be determined by the GC–ECD method, but could not be detected by the GC–MS–SIM method because the amount of MX was below the detection limit (23 ng/l of MX in water under the conditions used). MX was not detected by either method in a sample of chlorinated domestic sewage that had not been treated with an activated sludge system (sample No. 6). The mutagenicity contribution of MX detected has been reported elsewhere [13].

CONCLUSION

An advantage of the proposed GC–ECD method is that pentafluoropropylation of MX is used instead of conventional methylation. The electron-capture detector was about 3.5 times more sensitive to PFP-MX than Me-MX and the detection limit was lowered. When a GC separation was carried out, insufficient separation among PFP-MX and impurities derived from an actual sample was observed. This problem was solved by the purification of MX by preparative HPLC and by identification

based on the disappearance of the peak of PFP-MX on UV irradiation. The method was successfully applied to chlorinated domestic sewage for the determination of MX. The method proposed here is useful and convenient for the routine determination of MX in water.

ACKNOWLEDGEMENTS

REFERENCES

1 B. Holmbom, R. H. Voss, R. D. Mortimer and A. Wang, *Tappi*, 64 (1981) 172.
2 B. Holmbom, R. H. Voss, R. D. Mortimer and A. Wang, *Environ. Sci. Technol.*, 18 (1984) 333.
3 J. Hemming, B. Holmbom, M. Reunannen and L. Kronberg, *Chemosphere*, 15 (1986) 549.
4 J. R. Meier, R. B. Knohl, W. E. Coleman, H. P. Ringhand, J. W. Munch, W. H. Kaylor, R. P. Streicher and F. C. Kopfler, *Mutat. Res.*, 189 (1987) 363.
5 L. Kronberg and T. Vartiainen, *Mutat. Res.*, 206 (1988) 177.
6 H. Horth, M. Fielding, H. A. James, M. J. Thomas, T. Gibson and P. Wilcox, presented at *6th Conference on Water Chlorination, Oak Ridge, TN, May 3–8, 1987*.
7 P. Backlund, E. Wondergem, K. Voogd and A. De Jong, *Sci. Total Environ.*, 84 (1989) 273.
8 N. Suzuki and J. Nakanishi, *Chemosphere*, 21 (1990) 387.
9 L. Kronberg, B. Holmbom, M. Reunannen and L. Tikkanen, *Environ. Sci. Technol.*, 22 (1988) 1097.
10 A. A. Padmapriya, G. Just and N. G. Lewis, *Can. J. Chem.*, 63 (1985) 828.
11 B. Holmbom, L. Kronberg and A. Smeds, *Chemosphere*, 18 (1989) 2237.
12 S. Fukui, Y. Yoshimura, S. Ogawa and Y. Hanazaki, *Chemosphere*, 21 (1990) 705.
13 S. Fukui, S. Ogawa, H. Kita, Y. Hanazaki and H. Kami, *Chemosphere*, 24 (1992) 927.