Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1074 (2005) 155–161
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Development of a high-sensitivity quantitative analytical method for determining polycarbamate by gas chromatography-mass spectrometry incorporating temperature-programmable inlet on-column injection

Tatsuhiko Kawamoto *, Miho Yano, Nobuko Makihata

Hyogo Prefectural Institute of Public Health and Environmental Sciences, 2-1-29, Arata-cho, Hyogo-ku, Kobe 652-0032, Japan

Received 18 November 2004; received in revised form 14 March 2005; accepted 29 March 2005
Available online 11 April 2005

Abstract

A highly sensitive analytical method was developed using GC/MS with temperature-programmable inlet on-column injection (TPI on-column GC/MS) for determining methyl dimethyldithiocarbamate (DMDC-methyl) and dimethyl ethylenebisdithiocarbamate (EBDC-dimethyl), which are methyl derivatives of alkali decomposed polycarbamate. This method makes it possible to quantify 0.3 μg/l of polycarbamate in tap water, which is a 1/100 of the residual target value of 30 μg/l in Japan. Moreover, it now becomes possible to distinguish polycarbamate from other dithiocarbamate pesticides (DTCs) that have similar structures, including ziram and thiram, which only incorporate a DMDC side chain, or manzeb, maneb and zineb, which only incorporate an EBDC side chain, by simultaneously analyzing for DMDC-methyl and EBDC-dimethyl.

Keywords: Polycarbamate in tap water; Methyl dimethyldithiocarbamate; Dimethyl ethylenebisdithiocarbamate; Temperature-programmable inlet on-column GC/MS

1. Introduction

Polycarbamate (IUPAC: dizinc bis(dimethyldithiocarbamate) ethylenebis(dithiocarbamate), Fig. 1) is classified as a dithiocarbamate pesticide (DTC) and is used as a fungicide for fruit trees, vegetables and lawns. Ethylenethiourea (ETU), which is a decomposition product of polycarbamate [1], is suspected of causing teratogenic, carcinogenic, immunotoxic and mutagenic effects [2].

In order to control the quality of tap water, a target value for polycarbamate residues in water has been newly set at 30 μg/l, calculated from the value of 0.01 mg/kg/day that constitutes the acceptable daily intake (ADI) in Japan. Accordingly, the law requires that 1/100 of the target value must be detectable [3]. Therefore, it is necessary to develop an analytical method that offers high-sensitivity and selectivity.

The predominant methods for determining DTCs are based on their decomposition to carbon disulfide (CS_2) in an acid medium, usually followed by spectrometry [4–8] or head-space gas chromatography [9,10]. However, since these methods measure the decomposition products, they do not distinguish between different DTCs. Various methods have been developed in order to discriminate and determine different DTCs. These include a method involving capillary electrophoresis (CE) with UV detection [11], a method that combines HPLC with UV and an atomic absorption method [12] and an ion-pair HPLC technique [13]. However, none of these methods can be applied to the analysis of DTCs in tap water, due to their low sensitivity and poor selectivity. Blasco et al. [14] reported a sensitive and selective analysis method for DTCs using LC/MS. However, it was difficult to apply this method to the analysis of DTCs in tap water because polycarbamate is an insoluble metallic salt. Hanada et al. [15] developed an analytical method for determining the insoluble metallic salts of ethylenebisdithiocarbamates (EBDCs) by using LC/MS after the derivatization of the EBDC to dimethyl

* Corresponding author. Tel.: +81 78 511 6872; fax: +81 78 531 7080.
E-mail address: Tatsuhiko_Kawamoto@pref.hyogo.jp (T. Kawamoto).

0021-9673/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.chroma.2005.03.076

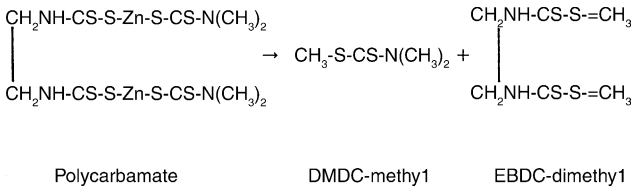

Fig. 1. Chemical structures of polycarbamate, DMDC-methyl and EBDC-dimethyl. Polycarbamate: dizinc bis(dimethyldithiocarbamate) ethylenebis (dithiocarbamate) DMDC-methyl: methyl dimethyldithiocarbamate EBDC-dimethyl: dimethyl ethylenebisdithiocarbamate.

ethylenebisdithiocarbamate (EBDC-dimethyl) in accordance with the method reported by Gustafsson and Fahlgren [16].

However, since polycarbamate is formed by two molecules of dimethyldithiocarbamate (DMDC) and one molecule of EBDC combined with zinc, this method cannot distinguish it from pesticides (manzeb, maneb, and zineb) that incorporate EBDC side chains. Moreover, in order to distinguish polycarbamate from pesticides such as ziram and thiram that have DMDC side chains, it is necessary to measure EBDC-dimethyl simultaneously with DMDC-methyl. Although we developed a method for the analysis of DMDC-methyl using LC/atmospheric pressure chemical ionization (APCI)/MS [17], it was difficult to conduct simultaneous analysis with EBDC-dimethyl, which can be measured by LC/ESI/MS with good sensitivity.

Therefore, we used the temperature-programmable inlet (TPI) on-column injection GC/MS method, applied to thermally-unstable pesticides [18,19], for the measurement of polycarbamate, and attempted the simultaneous analysis of DMDC-methyl and EBDC-dimethyl.

2. Experimental

2.1. Chemical and materials

Polycarbamate (>95%) was obtained from Kanto (Tokyo, Japan). DMDC-methyl (99.9%) and EBDC-dimethyl (96.3%) were obtained from Hayashi (Osaka, Japan). Dichloromethane, hexane and acetone (pesticide-grade), L-cysteine, EDTA-2Na, sodium hydroxide, tetrabutylammonium hydrogen sulfate, hydrochloric acid and methyl iodide were supplied by Wako (Osaka, Japan). Silicone-treated filter paper 1PS (Whatman, Maidstone, UK) was used for dehydration. Stock solutions (10 mg/10 ml) of DMDC-methyl and EBDC-dimethyl were prepared by dissolving in acetone. Working standard solutions were prepared by diluting the stock solutions with hexane. The standard solution of polycarbamate was prepared as a turbid liquid, which contained 10 mg in 1 l of distilled water. The solution was prepared immediately prior to use.

2.2. Sample preparation

The samples to be used in the GC/MS analysis were prepared by a modified version of the "pre-treatment method for polycarbamate analysis by HPLC" as detailed under the notice from the Department of the Environment [20].

The procedures used in the preparation are as follows: 200 ml of a water sample were poured into a 500 ml beaker, and then 0.4 g of L-cysteine and 12 g of EDTA-2Na were added to the sample. The pH was adjusted to between 9.6 and 10 with 12 M sodium hydroxide while stirring. After 60 min, 5 ml of 0.4 M tetrabutylammonium hydrogen sulfate was added and the pH of the water sample was adjusted to between 7.5 and 7.8 using 2 M hydrochloric acid. The sample was transferred into a separating funnel, and extraction was carried out by shaking twice for 10 min with 70 ml of 0.05 M methyl iodide in dichloromethane and hexane (3:1). Each organic layer was separated and combined. The organic extract was filtered using silicone-treated filter paper 1PS, and allowed to stand for 30 min. The organic extract was concentrated to 1 ml in Turbo Vap 500 (Zymark, Massachusetts, USA) and 5 ml of hexane was added, and concentrated again to 1 ml at 30 °C. The 2 μl was analyzed by GC/MS.

2.3. Instrument and conditions

GC/MS was carried out using an ion-trap type apparatus with a TPI system GCQ (Thermo Quest, Manchester, UK). The operating conditions of the GC/MS are given in Table 1.

3. Results and discussion

3.1. Generation of decomposition products in the GC injection port

Fig. 2 shows the comparative chromatogram of DMDC-methyl and EBDC-dimethyl by temperature difference at the GC/MS injection port. The peaks of the thermal decomposition product appeared at GC injection temperatures of 250 °C

Table 1
GC/MS operating conditions

GC
Injection method: TPI on-column injection, injection volume: 2 μl
Injection temperature: 35 °C (1 min) → 80 °C/min → 240 °C (42 min) → 80 °C/min → 300 °C (2.5 min)
Injection method: conventional splitless injection, injection volume: 2 μl
Injection temperature: 250 °C, 300 °C
Coolant: liquid CO_2
Oven temperature: 35 °C (4 min) → 20 °C/min → 180 °C → 2 °C/min → 200 °C (5 min) → 2 °C/min → 240 °C → 20 °C/min → 300 °C (4 min)
Carrier gas: helium, 40 cm/s (EPC)
Column: DB-5MS (film thickness: 0.1 μm, 30 m × 0.25 mm i.d.)
MS
Ion source temperature: 200 °C (EI), transferline temperature: 275 °C
Emission current: 285 μA, ionization volt: 70 eV
Mass range: 60–250 *m/z*
Mode: full scan
Quantitative ion: DMDC-methyl; *m/z* 135, EBDC-dimethyl; *m/z* 144

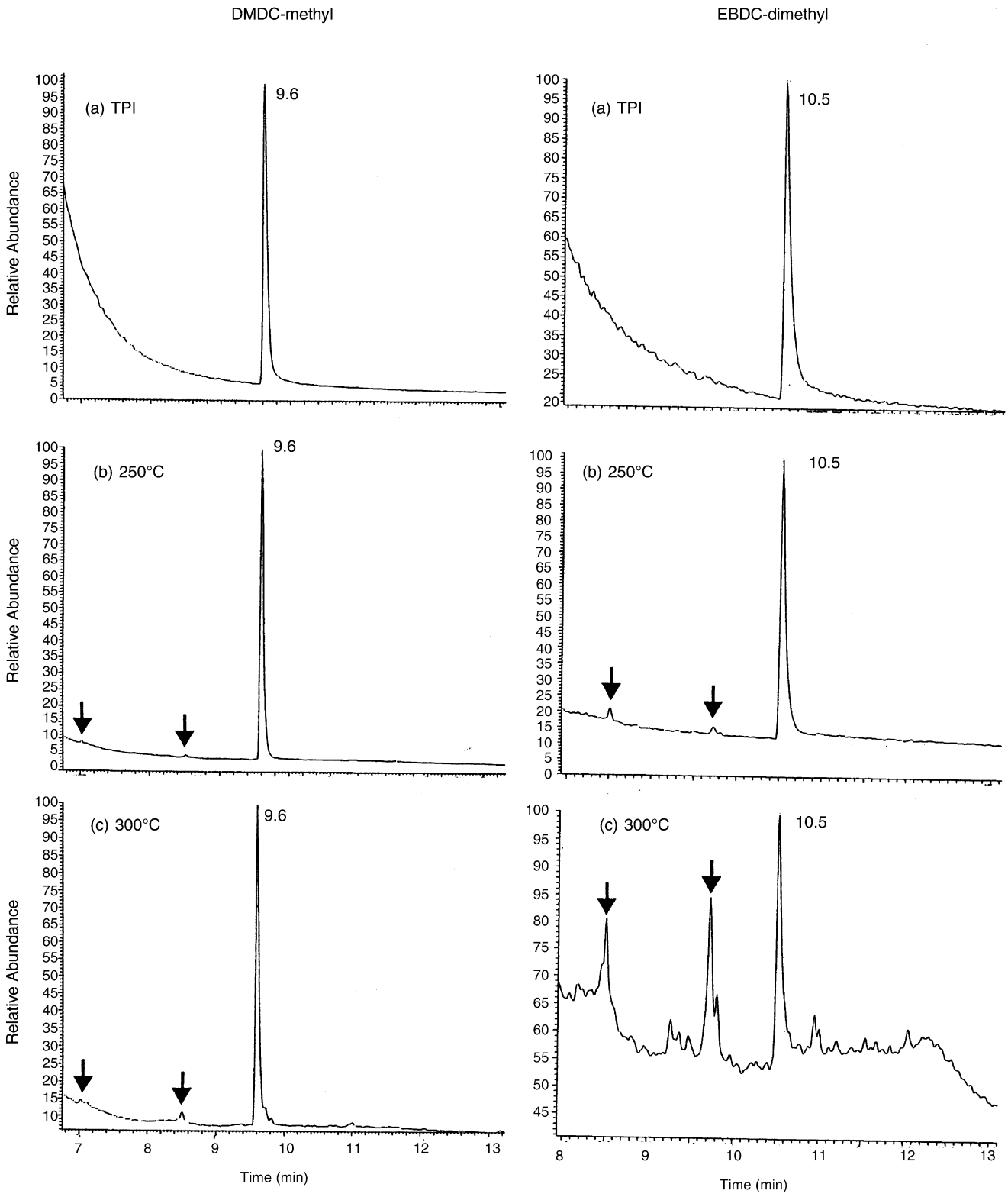

Fig. 2. Generation of decomposition products in the GC injection port. The left-hand side column shows the DMDC-methyl and the right-hand side column shows the EBDC-dimethyl. Each concentration of DMDC-methyl and EBDC-dimethyl was 1 mg/l (→: decomposition product).

and 300 °C. The more intense peak was at the higher temperature (300 °C). This tendency was especially remarkable in EBDC-dimethyl. Therefore, we investigated the simultaneous analytical method using TPI on-column injection GC/MS.

3.2. Total ion chromatogram and mass spectra

Fig. 3 shows total ion chromatogram and mass spectra of DMDC-methyl and EBDC-dimethyl obtained by TPI on-column injection GC/MS. Sharp peaks for DMDC-methyl and EBDC-dimethyl were detected at retention time (Rt) 9.6 min and 10.5 min, respectively.

In the case of DMDC-methyl, *m/z* 135 and *m/z* 88 were observed as strong peaks. The peak for *m/z* 135 was a molecular ion of DMDC-methyl, and the *m/z* 88 peak was thought to be the fragment ion corresponding to $[(CH_3)_2NC{=}S]^+$. In EBDC-dimethyl, *m/z* 144 was observed as having the most intense fragment ion, and *m/z* 72 was observed as the second most intense fragment ion. The peaks for *m/z* 144 and *m/z* 72 were thought to be due to fragment ions corresponding to $[S{=}CH{-}NH{=}CH{-}CH{=}N{-}C^{\bullet}{=}S]^+$ and $[CH_2{=}NC{=}S]^+$, respectively. For DMDC-methyl, *m/z* 135 (the molecular ion) was chosen as the quantitative ion, while *m/z* 88 was chosen as the qualitative ion. In the case of EBDC-dimethyl, *m/z* 144, which was the most intense fragment ion, was chosen as quantitative ion and *m/z* 72 was chosen as the qualitative ion.

3.3. Calibration curve, instrument detection limit and quantitation limit

Standard solutions of DMDC-methyl and EBDC-dimethyl with concentrations from 10 μg/l to 1000 μg/l were analyzed. Table 2 shows the regression equation, the correlation coefficient of the calibration curve, the instrument detection limit (IDL, $S/N = 3$) and the instrument quantitation limit (IQL, $S/N = 10$) for the compounds. Their correlation coefficients of the calibration curves were 0.997 and 0.995, respectively, and the degree of linearity was satisfactory. The

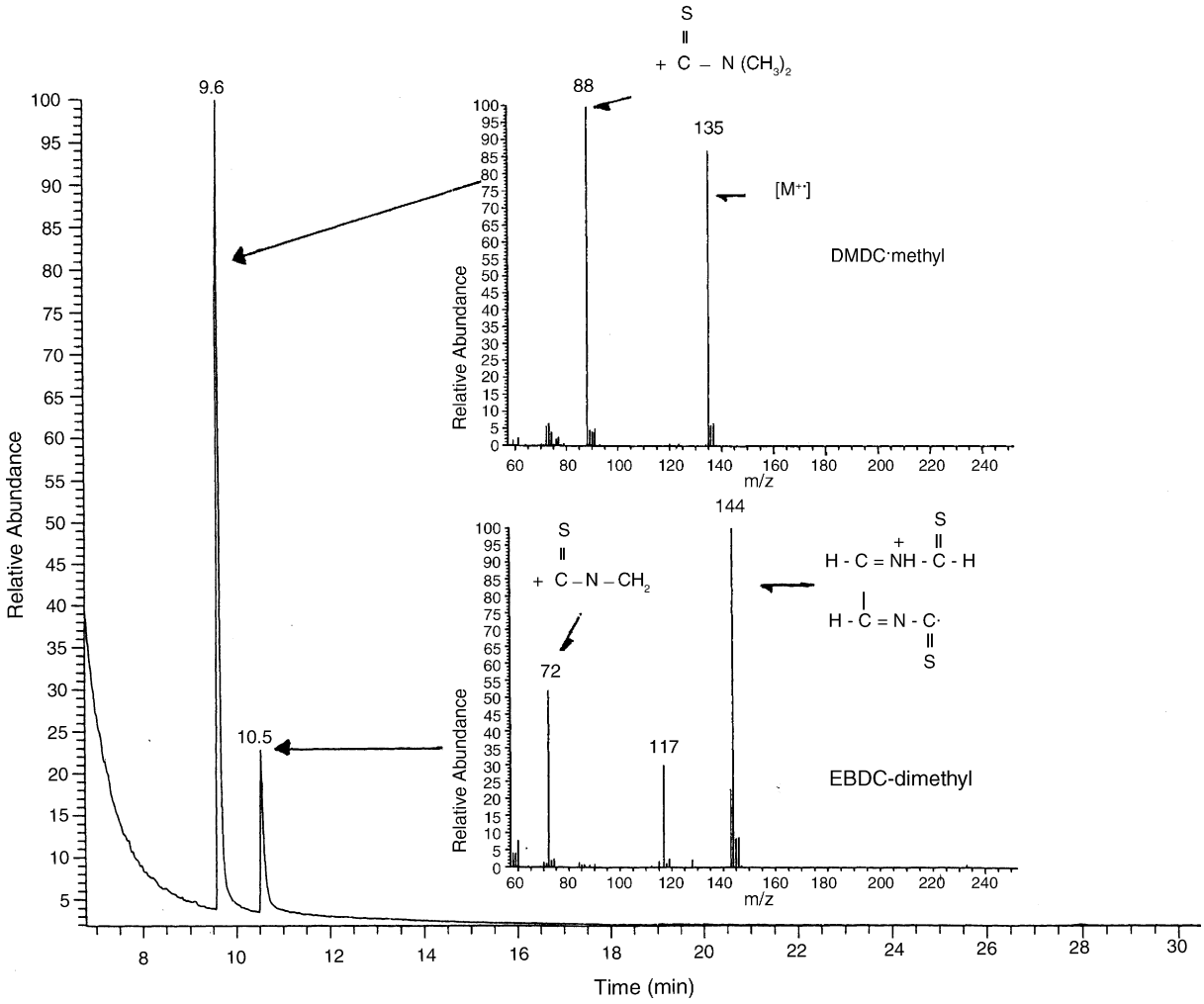

Fig. 3. Total ion chromatogram and mass spectra of DMDC-methyl and EBDC-dimethyl. Each concentration of DMDC-methyl and EBDC-dimethyl was 1 mg/l.

Table 2
Instrument detection limits, instrument quantitation limits and linearity of calibration curve for DMDC-methyl and EDBC-dimethyl

Compounds	Rt.	IDL (μg/l)	IQL (μg/l)	LDR (μg/l)	Regression equation	*r*
DMDC-methyl	9.6	3.3	12	10–100	$y = 193x + 568$	0.997
EBDC-dimethyl	10.6	11	36	25–250	$y = 29x + 838$	0.995

LDR: linear dynamic range; *r*: correlation coefficient.

Table 3
Recoveries of polycarbamate

	Added (μg/l)	DMDC-methyl		EBDC-dimethyl	
		Recovery (%)	RSD (%, $n = 3$)	Recovery (%)	RSD (%, $n = 3$)
Distilled water	3	102	3.4	78	3.6
Distilled water	0.3	79	5.8	56	11
Tap water	3	58	4.1	47	4.9
Tap water + VC	3	90	3.7	70	3.5
River water	3	99	3.0	71	5.0

0.6 μg or 0.06 μg of polycarbamate was added to 200 ml of distilled water, tap water and river water. VC: ascorbic acid sodium salt.

IQL of DMDC-methyl was 12 μg/l, and IDL was 3.3 μg/l (these are 26 μg/l and 7.2 μg/l, respectively, when it converts into the polycarbamate). Theoretically, with this sensitivity, 0.13 μg/l of polycarbamate could be determined quantitatively, and 0.036 μg/l could be detected in a 200-fold condensed pretreated water sample. On the other hand, the IQL of EBDC-dimethyl was 36 μg/l, and IDL was 11 μg/l (these are 87 μg/l and 27 μg/l, respectively, when it converts into the polycarbamate). Again, with this sensitivity, 0.44 μg/l of polycarbamate could theoretically be determined quantitatively, and 0.14 μg/l could be detected in a 200-fold condensed pretreated water sample.

The sensitivity of DMDC-methyl by the present method was 10 times as high as the sensitivity by our previously-reported LC/ESI/MS method [17]. Moreover, in the case of EBDC-dimethyl, the sensitivity of our present method was

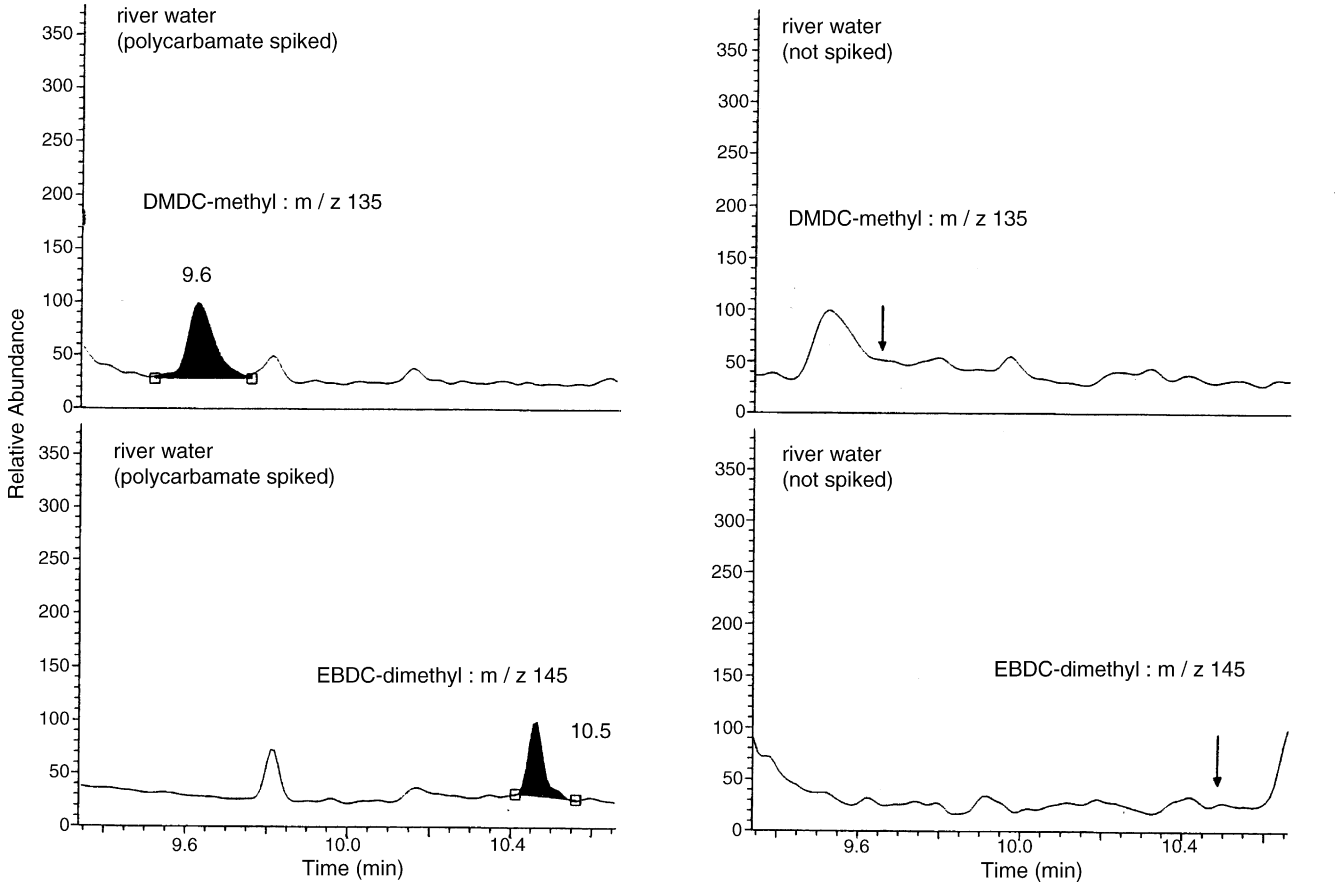

Fig. 4. GC/MS SIM chromatograms of river water samples. The concentration of polycarbamate in river water was 0.5 μg/l.

about 200-times higher than the previous results obtained by using HPLC-UV [16,21,22], and was higher than Hanada's method using LC/ESI/MS [15]. Therefore, we used DMDC-methyl for the quantitative analysis of polycarbamate and used EBDC-dimethyl for qualitative analysis in order to distinguish it from other pesticides.

3.4. Recovery tests and application to actual samples

To evaluate the accuracy and the precision of the present method, three replicates of the recovery tests were carried out (Table 3). Polycarbamate was added to 200 ml of distilled water to yield solutions with concentrations of 3 μg/l and 0.3 μg/l. Quantitative analysis using DMDC-methyl showed recovery rates of 102% and 79%, respectively. Although the recovery rate was slightly lower at a concentration of 0.3 μg/l, because the coefficient of variation was 5.8%, 1/100 of the target value could be measured with sufficient reproducibility. The recovery rates of EBDC-dimethyl in the 3 μg/l and 0.3 μg/l polycarbamate solutions were 78% and 56%, which were relatively low compared with DMDC-methyl. However, because this side chain does not exist in ziram and thiram, the results were considered to be satisfactory for the qualitative measurement of polycarbamate.

In order to confirm the applicability of the present method to actual samples, we conducted additional experiments using river water (collected from the River Ishii in Kobe), tap water to which ascorbic acid sodium salt (VC) was added in order to remove any residual chlorine, and untreated tap water (residual chlorine: 1 mg/l). It was confirmed that the samples do not contain any polycarbamate. The recovery rates of DMDC-methyl in the 3 μg/l polycarbamate solution were 99% in the river water and 90% in the tap water with VC, which were considered to be good results. On the other hand, the recovery rate in tap water was just 58%. It has been reported that DTCs such as sulfallate and thiram are decomposed with residual chlorine in tap water [23–25]. It was assumed that polycarbamate was also decomposed by the residual chlorine in tap water. Therefore, it is necessary to add VC to remove residual chlorine during the analysis of polycarbamate in tap water, similar to the addition of VC as an anti-oxidant during the official method [26] for the analysis of pesticides in tap water.

From these results, it was considered that the analytical method can be applied to raw water and to tap water samples. Chromatograms for river water containing 0.5 μg/l of polycarbamate and for the sample without polycarbamate are shown in Fig. 4. No interference peaks were observed in the river water without the polycarbamate. Quantitation by DMDC-methyl and confirmation by EBDC-dimethyl were possible at this concentration.

After this, raw water and tap water (river water 6, river-bed water 1, lake water 1, shallow well 5, deep well 2) collected from 15 monitoring points in Hyogo Prefecture on October 19, 2004 were analyzed. As a result, a trace of polycarbamate was detected in one raw water sample (river water sample).

4. Conclusions

A highly sensitive simultaneous analytical method for DMDC-methyl and EBDC-dimethyl, which are alkali decomposition methyl derivatives of polycarbamate, was developed using the TPI on-column GC/MS. Using this method, it is now possible to quantify 0.3 μg/l of polycarbamate, which is 1/100 of the residual target value of 30 μg/l in tap water in Japan. Furthermore, the ability to distinguish between polycarbamate and other pesticides having similar chemical structures has also become possible.

Acknowledgment

We are thankful to Mr. Kazuo Fujimori (Hyogo Prefectural Institute of Public Health and Environmental Sciences) for giving useful information about sample preparation.

References

[1] H. Kumagai, K. Nakamoto, M. Nakamura, S. Hirose, Y. Ichikawa, J. Endo, H. Ikari, Biosci. Biotech. Biochem. 56 (1992) 828.
[2] World Health Organization, Environmental Health Criteria 78, Dithiocarbamate Pesticides, ETU and PTU: A General Introduction, WHO, Geneva, Switzerland, 1998.
[3] Notification No. 1010004 of Health Service Bureau, Ministry of Health, Labour and Welfare, Octorber 10, 2003.
[4] W. Schwack, S. Nyanzi, Z. Lebensm. Unters. Forsch. 198 (1994) 8.
[5] J.K. Dubey, H.J. Stan, J. Food Sci. Technol. 35 (1998) 482.
[6] S. Heise, H. Weber, L. Aldder, Fresenius J. Anal. Chem. 366 (2000) 851.
[7] E.D. Caldas, M.H. Conceicao, M.C.C. Miranda, L.C.K.R. de Souza, J.F. Lima, J. Agric. Food Chem. 49 (2001) 4521.
[8] Assocition of Official Analytical Chemists, Official Methods of Analysis, 16th ed., AOAC, Arlington, 2000.
[9] N. Ahmad, L. Guo, P. Mandarkas, S. Appleby, J. AOAC Int. 78 (1995) 1238.
[10] R.C. Perz, H. Van Lishaut, W. Schwack, J. Agric. Food Chem. 48 (2000) 792.
[11] A.K. Malik, W. Faubel, Talanta 52 (2000) 341.
[12] C.C. Lo, M.H. Ho, M.D. Hung, J. Agric. Food Chem. 44 (1996) 2720.
[13] K.W. Weissmahr, C.L. Houghton, D.L. Sedlak, Anal. Chem. 70 (1998) 4800.
[14] C. Blasco, G. Font, Y. Pico, J. Chromatogr. A 1028 (2004) 267.
[15] Y. Hanada, T. Tanizaki, M. Koga, H. Shiraishi, M. Soma, Anal. Sci. 18 (2002) 441.
[16] K.H. Gustafsson, C.H. Fahlgren, J. Agric. Food Chem. 31 (1983) 1137.
[17] N. Makihata, M. Yano, T. Kawamoto, Abstr. Jap. Soc. Anal. Chem. 53 (2004) 286.
[18] T. Kawamoto, K. Teranishi, Bull. Hyogo Prefect. Inst. Public Health 33 (1998) 124.
[19] T. Kawamoto, N. Makihata, Anal. Sci. 19 (2003) 1605.
[20] Notification No. 234 of Water Environment Department, Environmental Management Bureau, Ministry of the Environment, December 28, 2001.
[21] T. Yoshizawa, Y. Sugama, J. Inomata, Ann. Rep. Tochigi Prefect. Inst. Public Health 14 (1984) 73.
[22] K.H. Gustafsson, R.A. Thomson, J. Agric. Food Chem. 29 (1981) 729.

[23] T. Okumura, J. JSWE 15 (1992) 62.
[24] M. Ohto, A. Yamamoto, A. Matsunaga, N. Takayanagi, T. Kemmei, Y. Saito, E. Mizukami, J. Environ. Chem. 3 (1993) 59.
[25] S. Kodama, A. Yamamoto, M. Ohto, A. Matsunaga, J. Agric. Food Chem. 47 (1999) 2914.
[26] Water Supply and Environmental Sanitation Department, Environmental Health Bureau, Ministry of Health and Welfare, Standard Method for the Examination of Water, Japan Water Works Association, Tokyo, 2001.