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Determination of 4-alkylphenols by novel derivatization and gas chromatography–mass spectrometry

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Abstract

A simple and sensitive method for the determination of alkylphenols in water samples has been developed using gas chromatography–mass spectrometry. Alkylphenols were determined after the extractive derivatization with pentafluoropyridine. The derivatization of alkylphenols efficiently proceeded to give the corresponding 4-tetrafluoropyridyl derivatives under the biphasic reaction system. The derivatization conditions including the phase-transfer catalyst, the amount of pentafluoropyridine, the reaction time, the concentration of NaOH and organic solvent were optimized. On the mass spectra of these derivatives, intense specific ion peaks were observed: *m*/*z* 256 for 4-*n*-alkylphenols and *m*/*z* 284 for 4-*tert*.-alkylphenols. Calibration curves were linear in the range of 20–1000 ng/l (200–10 000 ng/l for nonylphenol), and the detection limits varied between 6.93 and 15.7 ng/l (85.2 ng/l for nonylphenol). The average recoveries of the alkylphenols in a fortified river water sample (100 ng/l except for nonylphenol: 1000 ng/l) ranged from 91.1 to 112%. The relative standard deviations were found to be between 5.6 and 16%. This method was successfully applied to the determination of alkylphenols in river water.

Keywords: Water analysis; Derivatization, GC; 4-Alkylphenols; Pentafluoropyridine

1. Introduction

Scientific and public concern about endocrine disruptors has attracted much attention since the 1990s. In Japan, chemical substances suspected to be endocrine disruptors have been listed by the Ministry of the Environment, Government of Japan. They include alkylphenols which are frequently detected in the environment. Alkylphenols can be analyzed by gas chromatography–mass spectrometry (GC–MS) [1–11], liquid chromatography (LC) [12–14] and liquid chromatography–mass spectrometry (LC–MS) [15–17]. Among them, GC–MS is generally used for the determination of alkylphenols in environmental samples due to its high separation ability, low detection limit and wide use. Since alkylphenols are polar compounds, their GC–MS analysis is performed after extraction followed by derivatization such as acylation [1,2,11], alkylation [7,8], silylation [6,9,10] and so on. However, these methods require complicated procedures and a large amount of water sample, and are time-consuming.

It is known that perfluoroaromatic compounds

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react with phenols under basic conditions [18–20]. In 1967, De Pasquale and Tamborski reported that fluorophenol reacts with hexafluorobenzene [18]. In 1984, Jarman and McCague showed that octafluorotoluene and pentafluoropyridine can be used to protect the hydroxyl group of a phenol [20].

The objective of this study is to develop a simple and sensitive method for the analysis of alkylphenols using a perfluoroaromatic compound as a derivatizing reagent. The derivatization reactions of alkylphenols with perfluoroaromatic compounds were conducted under the biphasic reaction system using a phase transfer catalyst (PTC). The advantages of the biphasic reaction include decreasing the total number of preparative steps and obtaining extracts that can be directly analyzed by GC.

2. Experimental

2.1. Materials

Standard reagents: 4-*tert*.-butylphenol (C4), 4-*n*-pentylphenol (C5), 4-*n*-hexylphenol (C6), 4-*n*-heptylphenol (C7) and 4-*tert*.-octylphenol (C8) were obtained from TCI (Tokyo, Japan), and technical grade 4-nonylphenol (C9) was from Kishida Chemical (Osaka, Japan). Stock solutions of the alkylphenols (250 mg/l) were prepared by dissolving the alkylphenols in acetone. Pentafluoropyridine, pentafluoronitrobenzene, octafluorotoluene, pentafluorobenzonitrile and tetra-*n*-butylammonium hydrogensulfate (TBA) were purchased from TCI. The PTC solution (5000 mg/l) was prepared by dissolving TBA in methylene chloride. All solvents of pesticide grade and other chemicals were purchased from Wako (Osaka, Japan). Sodium hydroxide aqueous solution (5 *M*) was prepared before use. Silica gel [BW-127ZH (100–270 mesh)] was from Fuji Silysia (Aichi, Japan). Phenanthrene-d_{10} (internal standard for C4, C5, C6 and C8) and pyrene-d_{10} (internal standard for C7 and C9) were obtained from Kanto Kagaku (Tokyo, Japan) and Wako, respectively. The internal standard solution (5 mg/l) was prepared by dissolving both phenanthrene-d_{10} and pyrene-d_{10} in methylene chloride. All stock solutions were stored in the dark at 4 °C and properly diluted before use. Water was purified by using a Milli-Q system (Millipore, Bedford, MA, USA).

Water samples were collected from the Ina, Neya and Hirano rivers. Ascorbic acid (1 g/l) was added to the samples as a preservative in order to avoid the degradation of the alkylphenols after sampling.

2.2. Synthesis of derivatives of alkylphenols with pentafluoropyridine

The following provides a representative procedure: to a NaOH solution (0.1 *M*, 200 ml) in a 500-ml separatory funnel was added TBA (20 mg in 150 ml of CH_2Cl_2), pentafluoropyridine (1 ml) and 4-*n*-heptylphenol (1 g) or 4-(1,1,3,3-tetramethylbutyl)phenol (1 g). The funnel was mechanically shaken at room temperature for 2 h. After neutralization of aqueous phase with conc. HCl, the organic phase was separated and collected in a flask. The aqueous phase was extracted with additional methylene chloride (50 ml). The combined organic phase was dehydrated over anhydrous sodium sulfate. The solvent was then removed by a rotary evaporator. Purification of the residue by column chromatography on silica gel (eluent: hexane–ethyl acetate, 10:1, v/v) gave 4-(4-*n*-heptylphenoxy)-2,3,5,6-tetrafluoropyridine in 91% yield or 4-[4-(1,1,3,3-tetramethylbutyl)phenoxy]-2,3,5,6-tetrafluoropyridine in 98% yield. The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra of the products were recorded on a JEOL JNM-GSX-400 system.

4 - (4 - *n* - Heptylphenoxy) - 2, 3, 5, 6 - tetrafluoropyridine: ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, J=6.96 Hz, 3H), 1.28–1.33 (m, 8H), 1.56–1.64 (m, 2H), 2.60 (t, J=7.70 Hz, 2H), 6.96 (dt, J=8.43 Hz, J=2.93 Hz, 2H), 7.17 (dt, J=9.29 Hz, J=2.93 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 14.09, 22.66, 29.14, 29.18, 31.47, 31.80, 35.20, 116.56, 129.78, 136.16 (ddt, J=261.98 Hz, J=28.96 Hz, J=7.82 Hz), 140.04, 144.20 (dtq, J=243.59 Hz, J=15.17 Hz, J=2.76 Hz), 153.92, 144.86 (complex m).

4-[4-(1, 1, 3, 3-Tetramethylbutyl) phenoxy]-2,3,5,6-tetrafluoropyridine: ^{1}H-NMR ($CDCl_3$) δ 0.72 (s, 9H), 1.37 (s, 6H), 1.73 (s, 2H), 6.97 (dt, J=8.80,

2H), 7.37 (dt, J=14.29 Hz, J=2.75 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 31.57, 31.75, 32.36, 38.37, 57.07, 116.14, 127.67, 136.09 (dt, J=262.44 Hz, J=23.75 Hz, J=6.67 Hz), 144.22 (ddq, J=244.51 Hz, J= 16.55 Hz), 145.23 (complex m).

2.3. GC–MS conditions

Analyses were performed on a Varian 3800 gas chromatograph directly connected to a Saturn 2000 ion-trap mass spectrometer (Varian, Walnut Creek, CA, USA). All injections were performed in the splitless mode with the split vent closed for 1 min. The injection port temperature was 280 °C. A DB-5MS column (30 m×0.25 mm I.D., 0.25 μm film thickness, J&W) was utilized. Helium (99.9999%) at a flow-rate 1.2 ml/min was used as the carrier gas. The GC oven temperature program was as follows: 60 °C for 1 min, followed by a 10 °C/min ramp to 280 °C and hold for 7 min (total analytical time: 30 min). The transfer line, manifold and ion trap temperatures were set at 280, 40 and 220 °C, respectively. Full scan electron ionization (EI) data were acquired under the following conditions: mass range 100–650 m/z, scan time 0.5 s, emission current 80 μA, automatic gain control (AGC) target 20 000.

2.4. Derivatization procedure

To a TBA solution (500 mg/l in CH_2Cl_2, 10 ml) in a 100-ml separatory funnel were sequentially added an NaOH solution (5 *M*, 1 ml), pentafluoropyridine (100 μl) and a water sample (50 ml). The funnel was mechanically shaken at room temperature for 30 min. After neutralization of the aqueous phase with conc. HCl, the organic phase was collected through a short column of anhydrous sodium sulfate. The aqueous phase was extracted with additional methylene chloride (2 ml). The combined organic phase was concentrated to 0.2 ml under a gentle stream of nitrogen. The concentrate was subjected to column chromatography on silica gel (0.9 g) and then eluted with 6 ml of hexane–ethyl acetate (9:1, v/v). The eluate was concentrated to 0.4 ml under a gentle stream of nitrogen, and 100 μl of internal standard solution was added. An aliquot (2 μl) of the solution was injected into the GC–MS apparatus.

3. Results and discussion

3.1. Optimization of the procedure

The derivatization reactions of the alkylphenols with perfluoroaromatic compounds were carried out under the biphasic reaction system described above. The derivatization reaction can be supposedly affected by many parameters: derivatizing reagent, phase transfer catalyst, solvent, reaction time, concentration of NaOH and amount of the reagents. These parameters were investigated using a 100 ng/l mixed standard solution except for 4-nonylphenol (1000 ng/l).

3.1.1. Derivatizing reagent

The reactivities of four perfluoroaromatic compounds, pentafluoropyridine, pentafluoronitrobenzene, octafluorotoluene and pentafluorobenzonitrile, with the analytes were compared. Except for octafluorotoluene, the other three derivatizing reagents indicated similar reactivities (Fig. 1). The derivatives with pentafluoronitrobenzene and pentafluorobenzonitrile had longer retention times than those with pentafluoropyridine. When pentafluoronitrobenzene was used as a derivatizing reagent, two derivatives could not be analyzed due to the overlap of interfering peaks. In the case of pentafluoropyridine, no removal of excess reagent was required due to its low boiling point (83 °C). Therefore, pentafluoropyridine was the derivatizing reagent of choice.

We synthesized the derivatives with pentafluoropyridine and purified them by column chromatog-

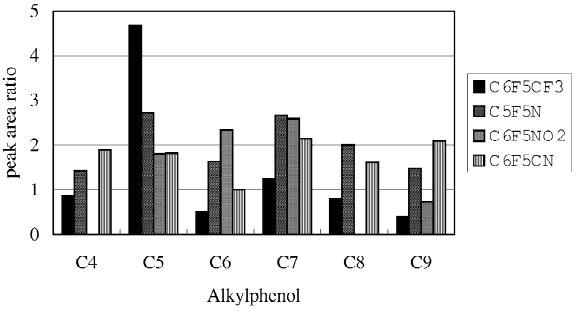

Fig. 1. Effect of derivatizing reagents on GC–MS sensitivity (100 ng/l).

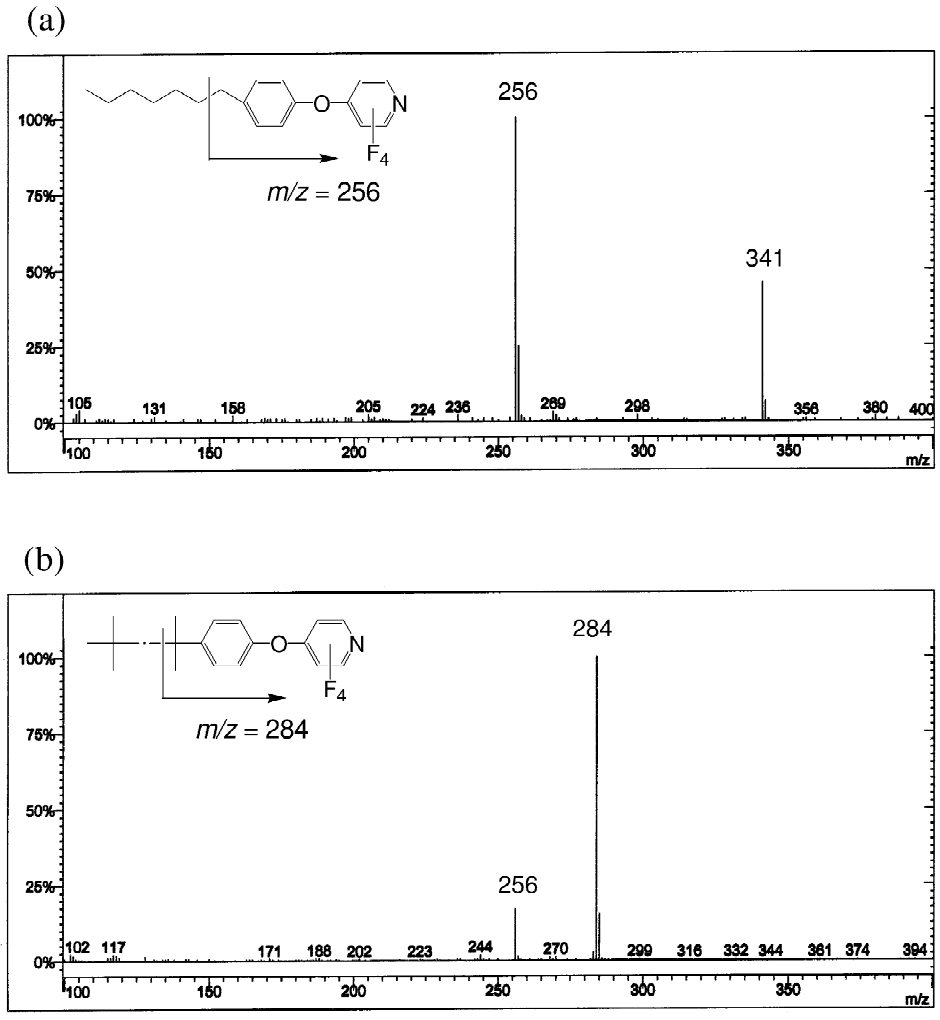

Fig. 2. Mass spectra of 4-(4-heptylphenoxy)-2,3,5,6-tetrafluoropyridine (a) and 4-[4-(1,1,3,3-tetramethylbutyl)phenoxy]-2,3,5,6-tetrafluoropyridine (b).

raphy on silica gel. The structures of the derivatives were confirmed by ^{1}H- and ^{13}C-NMR and GC–MS. In the ^{13}C-NMR, the three pyridine carbon signals indicate that the nucleophilic aromatic substitution selectively occurs at the 4-position of the pyridine ring.

Mass spectra of the derivatives with pentafluoropyridine are shown in Fig. 2. The spectrum of the derivatized 4-*n*-heptylphenol (Fig. 2a) contains a molecular ion at *m*/*z* 341 and a fragment ion at *m*/*z* 256. The spectrum of the derivatized 4-*tert*.-octylphenol (Fig. 2b) contains two fragment ions at *m*/*z* 284 and 256. The peaks at *m*/*z* 256 for the derivatized 4-*n*-heptylphenol and *m*/*z* 284 for the derivatized 4-*tert*.-octylphenol result from benzyl cleavage of the molecular ions. The determination was performed using the base peaks of *m*/*z* 256 for the linear alkylphenols and *m*/*z* 284 for the branched alkylphenols. These derivatives are six times more sensitive in GC–MS than the corresponding underivatized alkylphenols.

3.1.2. Phase transfer catalyst

The reaction was investigated using the following three phase transfer catalysts: tetramethylammonium, tetra-*n*-butylammonium and cetyltrimethylammo-

nium hydrogensulfate. With TBA, the derivatization yield of each analyte was almost quantitative. However, with the other two catalysts, complete reactions were not attained within 30 min. Therefore, TBA was selected as the phase transfer catalyst.

We next examined the concentration of TBA. The derivatization yield increased with the increasing concentration of TBA in the region from 10 to 500 mg/l and then decreased for each analyte. The results of C4, C6 and C9 out of six alkylphenols are plainly shown in Fig. 3. The optimization was mainly investigated in the range of 5–500 mg/l, because some noise appeared on the chromatograms and decreased the sensitivities over 500 mg/l. The quantitative yield was obtained at 500 mg/l of TBA.

3.1.3. Amount of pentafluoropyridine

The amount of pentafluoropyridine was examined in the range of 5–200 μl. A 5-μl volume of pentafluoropyridine produced poor reproducibility of the derivatization. The addition of more than 10 μl of pentafluoropyridine resulted in complete derivatization. The addition of more than 200 μl of pentafluoropyridine showed a high background level. Consequently, the amount of pentafluoropyridine was fixed at 100 μl.

3.1.4. NaOH concentration

The NaOH concentration was examined in the range of 0.01–0.2 *M*. It was found that increasing the NaOH concentration led to an increase in the amount of the derivatives, and the efficiency of the reaction was almost constant at the NaOH concentration of more than 0.1 *M* (Fig. 4). However, the background level in the mass chromatogram became higher at

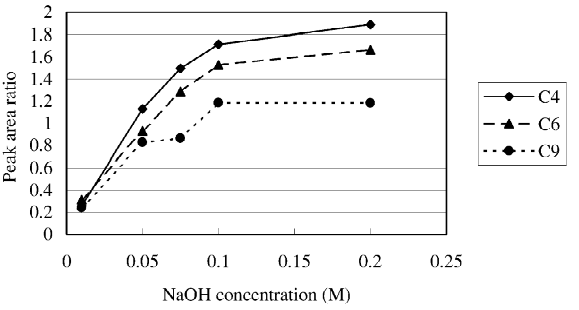

Fig. 4. Effect of NaOH concentration on GC–MS sensitivity.

0.2 *M* NaOH. We chose 0.1 *M* NaOH for this method.

3.1.5. Derivatization time

The derivatization yields were evaluated by varying the reaction time at room temperature (Fig. 5). The derivatization was completed within 15 min, and the reaction time was tentatively fixed at 30 min.

3.2. Quantitative calibration and reproducibilities

The peak area ratios of analytes to the internal standard were used for their quantification. Calibration curves were constructed of five concentration levels. As a result, good linear relationships were obtained in a range from 20 to 1000 ng/l (Table 1). The detection limits obtained from the standard deviation at the 50 ng/l level were 6.93–15.7 ng/l. The reproducibilities were evaluated on five replicates using pure water spiked at the 100 ng/l level. The relative standard deviations (RSDs) were between 6.5 and 12%.

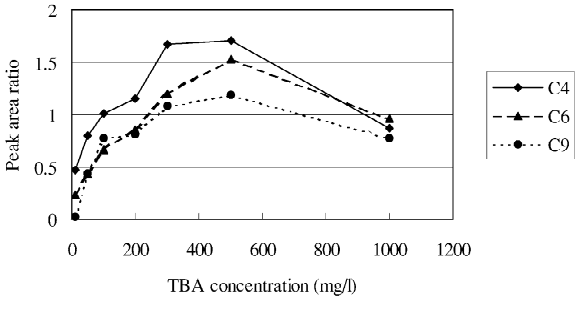

Fig. 3. Effect of TBA concentration on GC–MS sensitivity.

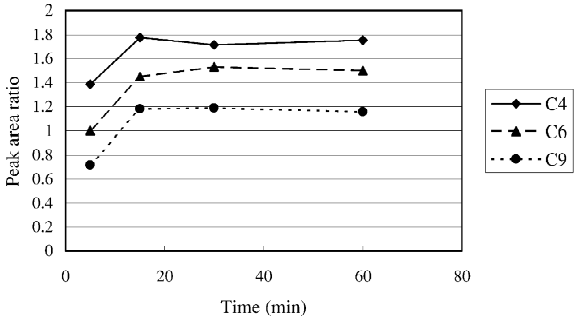

Fig. 5. Effect of reaction time on GC–MS sensitivity.

Table 1
Quantitative calibration and reproducibility

4-Alkylphenol	Ions (m/z) for determination and identification	Correlation coefficient (R)	Regression equation	Detection limit[b] (ng/l)	Reproducibility[c] (RSD, $n=5$) (%)
C4	284/256	0.9997	$y=0.01853x+0.09617$	10.9	11
C5	256/313	0.9998	$y=0.01475x+0.2050$	15.7	12
C6	256/327	0.9998	$y=0.01603x+0.03971$	8.90	8.4
C7	256/341	0.9967	$y=0.02532x-0.1163$	6.93	6.5
C8	284/256	0.9997	$y=0.02360x-0.01606$	12.5	12
C9[a]	284/256	0.9993	$y=0.009080x+0.7638$	85.2	8.0

Concentration range; 20–1000 ng/l (C4–C8), 200–10 000 ng/l (C9). y=Peak area ratio, x=concentration of analyte (ng/l).
[a] Isomeric mixture of branched nonyl groups.
[b] Calculated as standard deviation$\times t$, where $t=1.895$ from one-sided t-distribution at 95% confidence level ($n=8$, at 50 ng/l).
[c] Spiked level: 100 ng/l (C4–C8), 1000 ng/l (C9).

3.3. Recovery from river water

We conducted the recovery test from river water under the optimum conditions (Table 2). The spiked level of alkylphenols is 100 ng/l except for 4-nonylphenol (1000 ng/l). The recoveries of the spiked analytes ranged from 91.1 to 112%, and the reproducibilities of this method were found to be RSD 5.6–16% for five replicates. The derivatization time of 30 min was sufficient to allow the complete reaction even with the river water sample. We examined the stability of the derivatives and found them stable for more than 30 days at room temperature. These results indicate the applicability of the method to environmental water samples.

3.4. Application to the river water

This method was used for the analysis of the alkylphenols in polluted river water (Table 3). Water samples were collected from two rivers running through Osaka city. The analytical results are shown in Table 3. Three branched alkylphenols, 4-*tert*.-butylphenol, 4-*tert*.-octylphenol and 4-nonylphenol were detected in all the samples. The RSD values were in the range of 5.7–18%.

As an example, the mass chromatogram for sample B is shown in Fig. 6. In this chromatogram few interfering peaks are observed, thus indicating good performance of this method.

Table 2
Recovery test from river water[a]

Alkylphenol	Spiked level (ng/l)	Recovery (%)	Reproducibility (RSD, $n=5$) (%)
C4	100	112	5.6
C5	100	105	16
C6	100	91.8	14
C7	100	112	15
C8	100	108	5.7
C9[b]	1000	91.1	16

[a] Taken from Ina river.
[b] Isomeric mixture of branched nonyl groups.

4. Conclusions

The novel derivatization of alkylphenols with

Table 3
Concentration of alkylphenols in river water

Sample	Concentration (ng/l)[a]					
	C4	C5	C6	C7	C8	C9
A	47.4 (16)	nd	nd	nd	48.1 (13)	472 (15)
B	80.1 (9.1)	nd	nd	nd	313 (5.7)	2820 (10)
C	72.0 (8.0)	nd	nd	nd	401 (18)	2170 (13)

A: Kemabashi, B: Kyobashi, C: Kamishiromibashi, nd: not detected.
[a] Mean value. RSDs are in parentheses ($n=5$).

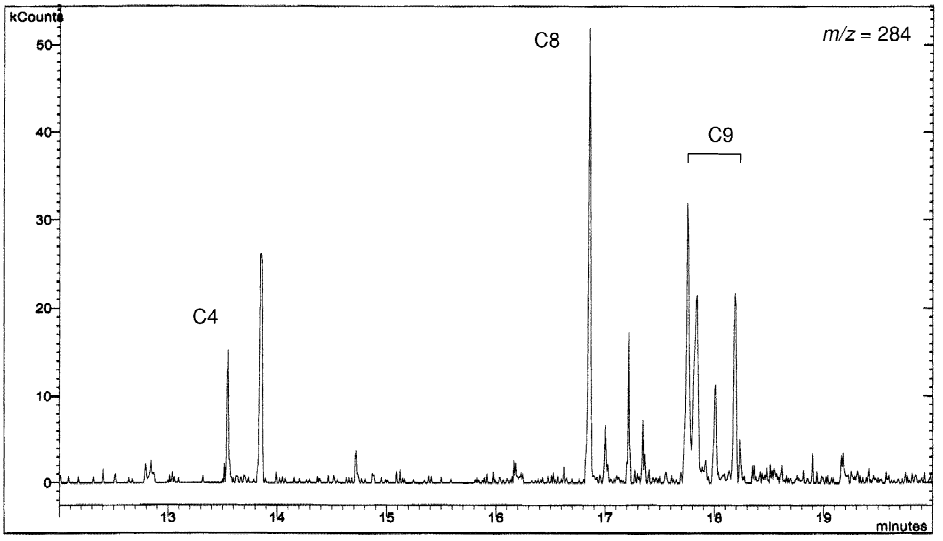

Fig. 6. Mass chromatogram of the derivatization products for sample B.

pentafluoropyridine has been demonstrated using GC–MS. This method has some advantages over the conventional derivatizations. The procedure of this derivatization is simple due to extractive derivatization and requires no specialized equipment. Pentafluoropyridine is easy to handle and has a low boiling point. The derivatization proceeds quantitatively at room temperature and the resulting derivatives are stable for more than 1 month. The detection limits of ca. 10 ng/l of the analytes (except for nonylphenol) can be achieved using 50 ml of water sample. The application of this method to other analytes is promising and quite interesting.

References

[1] H.-B. Lee, T.E. Peart, Anal. Chem. 67 (1995) 1976.
[2] T.R. Croley, B.C. Lynn Jr., Rapid Commun. Mass Spectrom. 12 (1998) 171.
[3] J.-G. Lin, R. Arunkumar, C.-H. Liu, J. Chromatogr. A 840 (1999) 71.
[4] T. Tsuda, A. Takino, M. Kojima, H. Harada, K. Muraki, J. Chromatogr. B 723 (1999) 273.
[5] W.-H. Ding, J.C.H. Fann, J. Chromatogr. A 866 (2000) 79.
[6] H.G.J. Mol, S. Sunarto, O.M. Steijger, J. Chromatogr. A 879 (2000) 97.
[7] U. Bolz, W. Köner, H. Hagenmaier, Chemosphere 40 (2000) 929.
[8] S. Nakamura, M. Takino, S. Daishima, Bunseki Kagaku 49 (2000) 329.
[9] M.I.H. Helaleh, S. Fujii, T. Korenaga, Anal. Chim. Acta 428 (2001) 227.
[10] M.I.H. Helaleh, Y. Takabayashi, S. Fujii, T. Korenaga, Talanta 54 (2001) 1039.
[11] R.J.W. Meesters, H.Fr. Söhroder, Anal. Chem. 74 (2002) 3566.
[12] T. Tsuda, K. Suga, E. Kaneda, M. Ohsuga, J. Chromatogr. B 746 (2000) 305.
[13] K. Inoue, Y. Yoshie, S. Kondo, Y. Toshimura, H. Nakazawa, J. Chromatogr. A 946 (2002) 291.
[14] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marcé, J. Chromatogr. A 964 (2002) 153.
[15] P.L. Ferguson, C.R. Iden, B.J. Brownawell, Anal. Chem. 72 (2000) 4322.
[16] J. Yonekubo, S. Sasaki, M. Ichiki, M. Kanai, H. Sasaki, Bunseki Kagaku 48 (1999) 571.
[17] M. Petrović, D. Barceló, Anal. Chem. 72 (2000) 4560.
[18] R.J. De Pasquale, C. Tamborski, J. Org. Chem. 32 (1967) 3163.
[19] A.H. Haines, K.C. Symes, J. Chem. Soc., Perkin I (1973) 53.
[20] M. Jarman, R. McCague, J. Chem. Soc., Chem. Commun. (1984) 125.