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Ion-pair solid-phase extractive derivatization of 4-alkylphenols with pentafluoropyridine for gas chromatography–mass spectrometry

Miki Kojima, Norie Matsui, Shinji Tsunoi *, Minoru Tanaka

Research Center for Environmental Preservation, Osaka University, 2-4 Yamada-oka, Suita, Osaka 565-0871, Japan

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Abstract

The ion-pair solid-phase extraction (SPE) of 4-alkylphenols followed by derivatization with pentafluoropyridine is demonstrated. Under alkaline conditions, the 4-alkylphenols could be efficiently adsorbed on a C_{18} SPE cartridge conditioned with an ion-pair reagent, tetra-*n*-hexylammonium bromide. The ion pairs, ammonium phenolates, formed on the C_{18} solid phase, were eluted with a solvent containing the derivatizing reagent, pentafluoropyridine, and completely derivatized during the elution. After optimization of the adsorption and derivatization, we established a method for the determination of the 4-alkylphenols in water samples. The method showed good linearity between 20 and 1000 ng (200–10,000 ng for nonylphenol). By processing 20-ml samples, the method detection limits (MDL) were in the range of 5.2–8.9 ng/l for the 4-alkylphenols (76 ng/l for nonylphenol). To evaluate its applicability to a real aqueous matrix, several river water samples were analyzed.

Keywords: Derivatization with pentafluoropyridine; GC–MS; Sample preparation; Water analysis; Environmental analysis; 4-Alkylphenols; Ion-pair solid-phase extraction

1. Introduction

Solid-phase extraction (SPE) is widely used for the preconcentration of water samples [1]. For GC analysis of polar analytes, derivatization is generally carried out for good reproducibility at a trace level. The coupling of SPE with derivatization is classified mainly into three groups: (i) derivatization after elution from the solid phase, (ii) SPE after derivatization in water, and (iii) derivatization on the solid phase.

Derivatization after elution from a solid phase (i) is a conventional approach; however, it is accompanied by complicated procedures such as purification, extraction and concentration. SPE after derivatization in a water sample (ii) is called 'in situ derivatization'. Although the method is convenient, such derivatization is limited to the alkylation of organotins with sodium tetraalkylborate [2,3], arylation of organotins with sodium tetraarylborate [4], acetylation of phenols with acetic anhydride [5], and esterification of amines and phenols with chloroformates [6], probably due to the slow derivatization under the diluted conditions and to the lack of the stabilities of the reagent and derivative. Derivatization on a solid phase (iii) can also provide a convenient method [7]. Acids, phenols, amines, aldehydes, and ketones can be derivatized on solid phases with a variety of derivatizing reagent [5,7–15]. Except for the case of aldehydes and ketones [14–15], however, solid-phase derivatizations generally require high temperature and a long time for the derivatization.

Recently, Kuklenyik et al. reported the automated solid-phase derivatization of bisphenol A and alkylphenols in urine using pentafluorobenzyl bromide (PFBBr) and styrene–divinylbenzene copolymer (PS–DVB) [16]. In this work, the urine, conditioned by adding alkaline and an ion-pair reagent, is loaded onto the SPE cartridge containing PFBBr. Phenolic analytes adsorbed as ion pairs were then derivatized on the solid phase to give their PFB ethers. Very recently, we reported the solid-phase derivatization

* Corresponding author. Tel.: +81 668798977; fax: +81 668798978.
E-mail address: tsunoi@epc.osaka-u.ac.jp (S. Tsunoi).

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of phenols [17] with pentafluoropyridine [18]. We use a divinylbenzene-*N*-vinylpyrrolidone copolymer bearing a trimethylaminomethyl group (Oasis MAX). The phenols, adsorbed as phenolate ions, are derivatized and eluted with pentafluoropyridine in hexane. Although the use of Oasis MAX has a significant clean-up effect, it takes 10 min for the complete derivatization.

The objective of this study was to develop an effective coupling of SPE with derivatization. On the other hand, Pocurull and Borrull et al. reported ion-pair SPE with PS–DVB was efficient for the preconcentration of phenols [19,20]. Li and Lee showed the usefulness of the ion-pair SPE using C_{18} and cetyltrimethylammonium bromide [21,22]. These results prompt us to develop an efficient way in combination of SPE with derivatization. In this study, we demonstrate the efficient coupling of SPE with derivatization of 4-alkylphenols using pentafluoropyridine and ion-pair SPE. The derivatization of the 4-alkylphenols can be simultaneously performed with the elution from the SPE cartridge by the use of the derivatizing reagent containing solvent as an eluent.

2. Experimental

2.1. Materials

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); technical grade nonylphenol (C9) was from Kishida (Osaka, Japan). Stock solutions of the 4-alkylphenols (10 mg/l) were prepared by dissolving them in acetone and properly diluted before use. Pentafluoropyridine, tetra-*n*-butylammonium hydrogensulfate (TBA), tetra-*n*-pentylammonium bromide (TPA), and tetra-*n*-hexylammonium bromide (THA) were also purchased from TCI. All solvents were of pesticide grade; other chemicals were purchased from Wako (Osaka, Japan) or Kishida. Sodium hydroxide solution and the aqueous solution of an ion-pair reagent (1 mM) were prepared before use. Deuterated *tert*-octylphenol (C8d) was synthesized in our laboratory and used as a surrogate compound which can provide an accurate analysis [23]. The surrogate solution (50 mg/l) was prepared by dissolving in acetone. Phenanthrene-d_{10} (internal standard) was obtained from Kanto Kagaku (Tokyo, Japan). The internal standard solution (5 mg/l) was prepared by dissolving phenanthrene-d_{10} in hexane. All solutions were stored in the dark at 4 °C. Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Solid-phase extraction cartridges, Bond Elut C18-HF (500 mg, 3 ml) and ENV (500 mg, 3 ml) were obtained from Varian (USA), and Oasis HLB (500 mg, 6 ml) was from Waters (USA). The SPE was performed on a GL-SPE vacuum manifold system (GL Sciences, Tokyo, Japan).

River water samples were collected from the Ina (Hyogo), Kanzaki, Yodo, and Neya rivers (Osaka), and filtered using a 0.45 μm membrane filter (Millipore) before use.

2.2. GC–MS conditions

Analyses were performed on a 3800 gas chromatograph directly connected to a Saturn 2000 ion-trap mass spectrometer (Varian, USA). All injections were performed in the splitless mode with the split vent closed for 1 min. The injection port temperature was 280 °C. An ID-BPX5 column (30 m × 0.25 mm I.D., 0.25 μm film thickness, SGE, Australia) was utilized. Helium (99.9999%) at a flow rate of 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 210 °C, respectively. Full scan 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.

2.3. Sample preparation

Ten microliters of the surrogate solution (50 mg/l) was added to a 20 ml water sample. An SPE cartridge, Bond Elut C18-HF, was successively conditioned with 5 ml of acetone, 10 ml of water, and 5 ml of 1 mM THA solution. To the cartridge, the 20 ml water sample, adjusted to 0.3 M NaOH, was loaded at a flow rate of 5–10 ml/min. Then, 20 ml of pure water was passed through the cartridge to remove excess NaOH. After centrifuging the SPE cartridge for 5 min, the solid phase was dried under vacuum for 30 min. Derivatization and elution was performed by passing pentafluoropyridine solution (0.1% pentafluoropyridine in 2 ml acetone) through the cartridge. The eluate was concentrated to 0.2 ml under a gentle stream of nitrogen (0.5 l/min). The concentrate was subjected to column chromatography (1.5 g of sodium sulfate and 0.5 g of silica gel) and then eluted with 5 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 to the concentrate. An aliquot (2 μl) of the solution was then injected into the GC–MS apparatus.

Quantification and qualification were performed at $m/z = 284$ and 256 for the branched 4-alkylphenols, respectively. With the linear 4-alkylphenols, $m/z = 256$ and M^+ (molecular ion) were used for quantification and qualification, respectively. Pyrene-d_{10} (internal standard) was monitored at $m/z = 212$. For example, in the case of the branched 4-alkylphenols, the peak area ratio of $m/z = 284$ to 212 was used for the quantification and optimization of conditions. The peak area ratio of nonylphenol was reduced to 1/10.

3. Results and discussion

3.1. Optimization of ion-pair SPE

We examined three types of solid phase, C_{18} (Bond Elut C18-HF), SDB (Bond Elut ENV), and Oasis HLB (Waters).

Since the SDB and Oasis HLB are the polymer based solid phases among these three solid phases, it took a long time to obtain the complete dryness of the cartridges after sample loading due to their larger volumes. Therefore we selected C_{18} as the solid phase that retained an ion pair. Unless otherwise stated, the conditions for the ion-pair solid-phase extractive derivatization were examined using a 20 ml NaOH solution spiked with 4-alkylphenols (1 μg each, except for C9: 10 μg) according to the method described in Section 2.3. It is well known that C_{18} solid phase is decomposed under the alkaline conditions. However, the decomposition of C_{18} solid phase was not observed under the conditions used in this study. We consider that the reason why C_{18} was not decomposed is due to a small amount of sample (20–100 ml).

3.1.1. Comparison of efficiency of ion-pair formation: in the water phase versus on the solid phase

At first, we examined two methods for the formation of the ion pair by using the C_{18} cartridge. One was formation of the ion pair in the water phase. The other was formation of the ion pair on a solid phase. The same amount of TBA was used and the efficiencies were compared. For formation in the water phase, TBA solution (10 mM, 100 μl) and 4-alkylphenol were added to a water sample (20 ml) adjusted by alkaline. For formation on the solid phase, a water sample to which 4-alkylphenol and alkaline were added, were passed through the solid phase loaded with TBA solution (0.2 mM, 5 ml). The results are shown in Fig. 1. The formation of the ion pairs on the solid phase showed higher efficiency with all the 4-alkylphenols. The 4-alkylphenols, C4, C5, and C6, gave especially better results on the solid phase.

3.1.2. Ion-pair reagent and its concentration

By using the C_{18} cartridge, several ion-pair reagents were investigated by the following procedures: to the SPE cartridge successively conditioned with acetone (5 ml), water (10 ml), and ion-pair reagent solution (0.05 mM, 5 ml), a 20 ml water sample, adjusted to 0.3 M NaOH, was loaded. After centrifuging and air-drying the cartridge, 2% pentafluoropyridine solution (in 5 ml CH_2Cl_2) was added and eluted. The eluate was concentrated, and then injected into GC–MS. The results are shown in Fig. 2. When TBA was used, the peak area ratios of all the 4-alkylphenols were low. We consider that this may be due to a relatively low amount of TBA retained on the solid phase. The ion-pair reagents having longer alkyl groups gave better results.

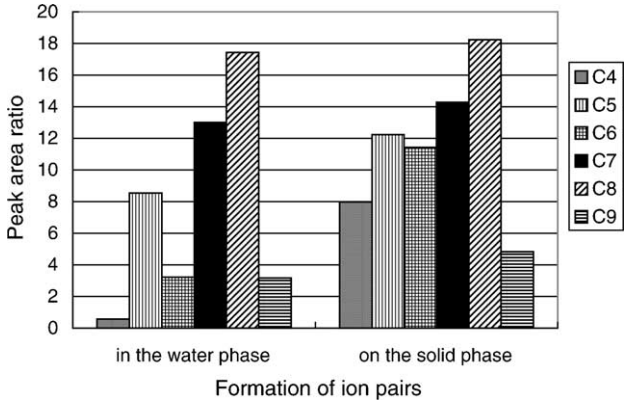

Fig. 1. Comparison of ion-pair formation. (Conditions: ion-pair reagent, TBA (10 mM, 100 μl); eluent, 2% pentafluoropyridine in CH_2Cl_2.)

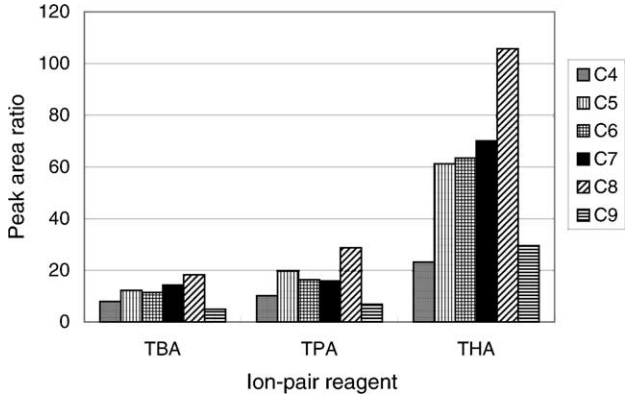

Fig. 2. Effect of ion-pair reagents on adsorption of 4-alkylphenols. (Conditions: ion-pair reagent, 0.2 mM (5 ml); eluent, 2% pentafluoropyridine in CH_2Cl_2.)

Next, we examined the concentration of THA. Five milliliters of the THA aqueous solution was used in the concentration range of 0.2–1.1 mM. The THA concentration of 1 mM gave the highest responses.

3.1.3. NaOH concentration in the water sample

The NaOH concentration was examined in the range of 0.1–0.4 M because pK_a value of alkylphenols is ca 10 [17]. In the range, the responses of the analytes were almost constant. The NaOH concentration was set to 0.3 M in consideration of analysis of environmental water. Under the conditions, no decomposition of the C_{18} solid phase was observed.

As a result of the above observations, the following optimum conditions were chosen for the ion-pair SPE of the 4-alkylphenols: (a) after the usual conditioning, 1 mM THA aqueous solution (5 ml) was loaded onto a C_{18} SPE cartridge, (b) 20 ml water sample (adjusted to 0.3 M NaOH) was passed through the cartridge.

3.2. Optimization of derivatization and elution

3.2.1. Organic solvent

Three organic solvents, methanol, dichloromethane and acetone, were investigated for derivatization and elution in the presence of the derivatizing reagent, pentafluoropyridine. The 4-alkylphenols adsorbed on the C_{18} solid phase were derivatized and eluted with 5 ml of an organic solvent containing 2% pentafluoropyridine (Fig. 3). When methanol was used, the response was apparently low. Acetone gave the highest recovery through the derivatization and elution of all the 4-alkylphenols. Dichloromethane showed slightly low responses probably due to its water immiscible character.

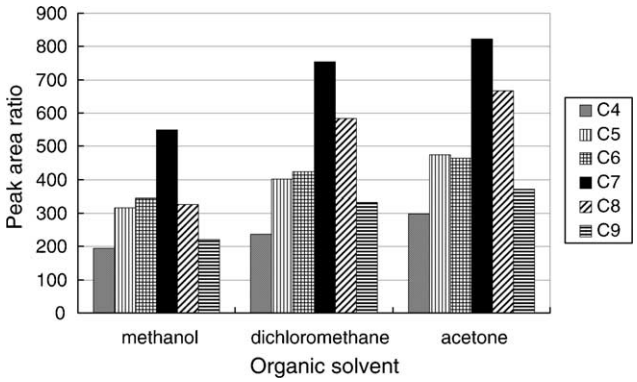

Fig. 3. Effect of organic solvent on desorption of derivatized 4-alkylphenols. (Conditions: eluent, 2% pentafluoropyridine in the organic solvent.)

3.2.2. Pentafluoropyridine concentration

The concentration of pentafluoropyridine was examined in the range of 0.002–1% using acetone as an eluent. The responses of the derivatives were constant under the conditions more than 0.1% pentafluoropyridine. For the derivatization of the 4-alkylphenols, 0.1% pentafluoropyridine was sufficient.

Finally, the volume of acetone containing 0.1% pentafluoropyridine was examined under the optimum conditions. No significant changes were observed in the range of 1–5 ml. We selected 2 ml of acetone in which 2 μl of pentafluoropyridine was contained. Compared with our previous work using Oasis MAX [18], the amount of pentafluoropyridine used in the present study is one hundredth, indicating the high efficiency of the present ion-pair solid-phase derivatization. Moreover, a special derivatization time is not required. Under the optimum elution and derivatization conditions (0.1% pentafluoropyridine in 2 ml acetone), the 4-alkylphenols could be desorbed from the solid phase and derivatized with pentafluoropyridine. When analyzing the eluate immediately, a similar response was obtained with all the 4-alkylphenols. This result indicates that the derivatization with pentafluoropyridine is accomplished during the elution.

3.3. Amount of the water sample

To obtain a lower MDL, we also examined the effect of water sample size. By applying 100 ml of the water sample, the peak area ratios of C4 and C5 clearly decreased, meanwhile those of C8 and C9 did not change. We consider that this decrease is due to the leakage of the ion-pair reagent from the solid phase. The peak area ratio apparently decreased by loading 100 ml pure water after THA conditioning (data not shown). Li and Lee showed that hydrophobic interaction is important for the adsorption of phenols even with ion-pair SPE [21]. Our results indicate that hydrophobic interaction assists the ionic interaction and that the more hydrophobic 4-alkylphenols, C8 and C9, are adsorbed more easily than C4 and C5.

To achieve better adsorption of the shorter 4-alkylphenols, C4 and C5, the solid phase was conditioned with a higher THA concentration. The ion-pair reagent of higher concentration improved the adsorption of the shorter 4-alkylphenols. These results indicate that the C_{18} solid phase is not saturated with THA when loading 5 ml of 1 mM THA. To analyze 100 ml of water sample, the conditioning with 10 mM THA (5 ml) is indispensable.

3.4. Quantitative calibration and reproducibilities

Calibration was done for the whole analytical procedure, including enrichment with SPE, derivatization and elution of the analytes, and measurement with GC–MS (Table 1). All calibration curves were linear in the range of 20–1000 ng/l for the alkylphenols (200–10,000 ng/l for C9). When applying a 20-ml water sample, the method detection limits (MDL) were between 5.2 ng/l for C4 and 76 ng/l for C9.

3.5. Application to river water samples

The recovery test from river water was carried out using two river waters, the Ina (a low polluted river) and Kanzaki (a highly polluted river). Table 2 summarizes the average recovery of all the 4-alkylphenols in the fortified river waters. The average recoveries from the low polluted river at a concentration of 200 ng/l are in the range of 83–109% with good reproducibilities (RSD = 6.4–15%). Similar recoveries were obtained from the highly polluted river with similar reproducibilities.

This method was applied to the analysis of several river water samples. The water samples were collected from

Table 1
Quantitative calibration and method detection limit

4-Alkylphenol	Regression equation[a]	Correlation coefficient (R)	Reproducibility (RSD, %)[b]	Method detection limit (ng/l)[c]
C4	$y = 9.039x + 0.03699$	0.9994	10	5.2
C5	$y = 13.16x + 0.2365$	0.9990	7.0	8.9
C6	$y = 12.65x + 0.09361$	0.9998	5.5	7.3
C7	$y = 15.65x + 0.07506$	0.9999	4.1	6.0
C8	$y = 19.15x - 0.03518$	0.9989	3.0	7.9
C9	$y = 6.478x + 2.497$	0.9998	12	76

Linear range: 20–1000 ng/l (C4–C8), 200–10000 ng/l (C9). Water sample: 20 ml.

[a] y = Peak area ratio, x = amount of analyte (μg).

[b] At 100 ng/l (C9: 1000 ng/l).

[c] Calculated as standard deviation × t, where $t = 1.895$ from one-sided t-distribution at 95% confidence level ($n = 8$, at 50 ng/l for C4–C8 and 500 ng/l for C9).

Table 2
Recoveries from river water samples (20 ml) and their concentrations

4-Alkyl phenol	Recovery (%)[a]		Concentration (ng/l)[b]			
	Ina river	Kanzaki river	Ina river	Kanzaki river	Yodo river	Neya river
C4	83 (10)	84 (10)	12[c] (19)	29 (13)	25 (14)	23 (17)
C5	100 (11)	103 (7.0)	–	–	–	–
C6	109 (6.4)	111 (6.2)	–	–	–	–
C7	108 (14)	118 (9.0)	–	–	–	–
C8	92 (15)	103 (13)	113 (17)	67 (13)	48 (12)	44 (5.0)
C9	108 (8.7)	103 (7.1)	188[c] (10)	288 (11)	118[c] (10)	282 (3.4)
C8d[d]	113 (8.1)	108 (10)	98 (13)	106 (7.6)	97 (12)	95 (5.2)

The relative standard deviation (RSD) is given in parentheses ($n = 5$).
[a] Average recovery at 200 ng/l (C9: 2000 ng/l).
[b] Average concentration ($n = 5$).
[c] Estimated values by extrapolating the calibration curves.
[d] Average recovery at 25 μg/l ($n = 5$).

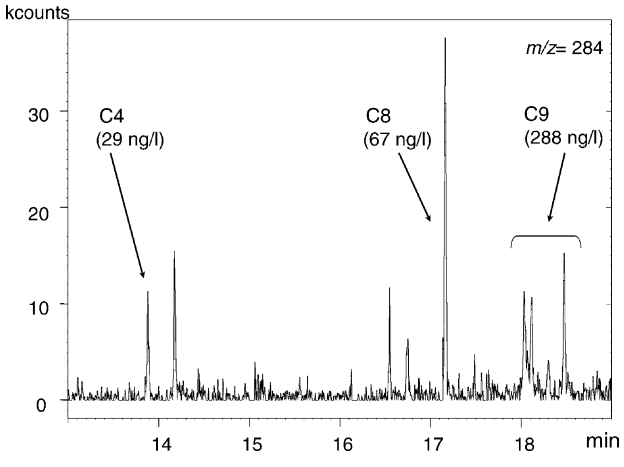

Fig. 4. Mass chromatogram for analytes in the Kanzaki river.

two rivers (the Yodo and Neya rivers) running through Osaka City in addition to the Ina and Kanzaki rivers. The analytical results are also shown in Table 2. Three branched 4-alkylphenols, 4-*tert*-butylphenol, 4-*tert*-octylphenol and 4-nonylphenol were detected in all the samples. The RSD values were in the range of 3.4–19%. For all cases, the recoveries of surrogate were more than 95% with good reproducibilities. A typical mass chromatogram is shown in Fig. 4.

4. Comparison with previously reported method using pentafluoropyridine

We introduced three methods for the determination of 4-alkylphenols using pentafluoropyridine as a derivatizing reagent. One is a liquid–liquid extractive derivatization under phase transfer conditions (LLE) [18] and other two are solid-phase extractive derivatization systems, anion-exchange SPE [17] and ion-pair SPE (this work). All the methods can provide good recoveries with good reproducibilities.

The solid-phase systems brought some advantages, such as a simple operation, short analysis time, and low detection limits (for C4–C8 LLE: 6.9–16 ng/l (50 ml water), anion-exchange SPE: 0.45–2.3 ng/l (100 ml water), ion-pair SPE: 5.2–8.9 ng/l (20 ml water)). On the other hand, the LLE system can provide a low cost analysis.

Compared to the anion-exchange SPE, the procedure using the ion-pair SPE is simple although requiring the use of an ion-pair reagent. Moreover, the ion-pair SPE could use a conventional solid phase and the derivatization could be efficiently performed with a small amount of pentafluoropyridine during elution from a C_{18} SPE cartridge.

5. Conclusion

The efficient combination of solid-phase extraction of the 4-alkylphenols with derivatization has been demonstrated by using pentafluoropyridine as a derivatizing reagent. The derivatization of the 4-alkylphenols with pentafluoropyridine is suitable for solid-phase derivatization due to the fast derivatization and low boiling point of pentafluoropyridine. The derivatization could be efficiently performed during elution from a C_{18} SPE cartridge. The total analytical procedure for the present method is very simple, as if it did not include the derivatization, indicating the strong possibility of on-line SPE coupled with derivatization.

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