Simultaneous Analysis of Residual 4-Alkylphenols in Synthetic Resin Products for Drug and Food Use Using Head-Space Gas Chromatography-Mass Spectrometry (HS-GC/MS)

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Investigation of the amounts of chemical substances excreted into environments is important in the consideration of their influence on humans and other biologics. We performed this study to investigate the excretion levels of 4-alkylphenols, represented by the endocrine disruptors 4-octylphenol and 4-nonylphenol, from chemical products and established a simultaneous analytical method in which 4-alkylphenols were trimethylsilylated and analyzed using head-space gas chromatography-mass spectrometry (HS-GC/MS). This is a simple and innovative method that proceeds to derivatization with a one-step procedure and dissolves the whole sample as well as eliminates impurities interfering with analysis. When this method was applied to synthetic resin products, 4-nonylphenol (detection limit $0.5 \mu g/ml$) was rapidly and simply analyzed with high sensitivity.

Key words — alkylphenol, head-space, gas chromatography-mass spectrometry, chemical endocrine disruptor, synthetic resin product

INTRODUCTION

Various environmental pollutants have a major influence on human health. These toxic compounds have been linked to adverse health effects including carcinogenesis due to exposure, and countermeasures have been taken against discharges into the air and water environments. However, many reports on the effects of chemicals that disrupt endocrine function on human health and wild biologics have recently been published, and 67 substances have been identified as chemicals suspected to have an endocrine-disrupting action.¹⁾ The future policy of the Ministry of the Environment on the problem of endocrine disruption by chemicals has been presented.²⁾ Among 4-alkylphenols, 4-octylphenol and 4-nonylphenol with C8 and C9 alkyl chains, respectively, have been reported to show various endocrine-disrupting actions in tests in fish and mammals³⁻⁶⁾ and designated as endocrine disruptors by the Ministry of the Environment. Histologic analysis has recently revealed that these two substances cause developmental retardation of the brain by impairing dopaminergic nerves. Moreover, they have been suggested to show toxicity toward not only dopaminergic nerves but also various other nerves, such as noradrenergic and serotonergic nerves.⁷⁾

4-Alkylphenols are used as additives in common synthetic resin products. Among 4-alkylphenols, nonylphenol is added to synthetic resin products or remains as an impurity or degradation product of tris(nonylphenol)phosphate used as an antioxidant.⁸⁾

Clarification of the movement, distribution, and alteration of chemical substances in environments is important for consideration of their influence on humans and other biologics. Products containing chemical substances are disposed of by burial in landfills and combustion, which ex-

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cretes chemical substances into the environment. Excreted 4-alkylphenols are measured using gas chromatography-mass spectrometry (GC/MS) after solvent or solid-phase extraction and derivatization.⁹⁾ In the analysis of 4-alkylphenols contained in chemical products, which may release substances into the environment, material and dissolution tests of containers and packages of food products and toys have been reported.^{10,11} However, these methods dissolve only chemical substances on the product surfaces and are unable to measure the total contents. To clarify the effects of chemical substances on the environment, accurate measurement of the total contents of chemical products as starting materials is necessary. We therefore attempted to measure the total contents of synthetic resin products reported to contain 4-alkylphenols.

We investigated an analytical method of 4alkylphenols contained in synthetic resin products using HS GC/MS that eliminates nonvolatile contaminants without pretreatment and established a rapid and simple simultaneous analytical method that extracts 4-alkylphenols and simultaneously derivatizes compounds using trimethylsilyl (TMS) reagent in a head-space vial. Furthermore, a more accurate analytical method was established using a surrogate substance for 4-nonylphenol. We initially performed simultaneous analysis of 4-alkylphenols using HS-GC/MS.

MATERIALS AND METHODS

Samples — Twenty-eight samples of synthetic resin products (drug containers, wrapping films, and household utensils) sold in department stores and specialty stores for household goods in July 2006 were used.

Reagents — For the mixture of seven alkylphenol compounds [4-*t*-butylphenol (0.1 mg/ml) (1), 4-*n*-pentylphenol (0.1 mg/ml) (2), 4-*n*-hexylphenol (0.1 mg/ml) (3), 4-*n*-heptylphenol (0.1 mg/ml) (4), 4-*t*-octylphenol[4-(1,1,3,3-tetramethylbutyl)phenol] (0.1 mg/ml) (5), 4-*n*-octylphenol (0.1 mg/ml) (6), and 4-nonylphenol mixture (1 mg/ml) (7)], reagents for environmental analytical use were purchased from Kanto Chemical Co. (Tokyo, Japan) and gradually diluted with dichloromethane to make standard solutions. ${}^{13}C_6$ -labeled 4-(1-methyl-1propylpentyl)phenol- ${}^{13}C_6$ (8) was synthesized, and the structure was confirmed based on the mass, nuclear magnetic resonance, and infrared absorption spectra. The labeled compound (8) was diluted with methanol to 1.1 mg/ml solution. Naphthalene- d_8 was purchased from Kanto Chemical Co. and then diluted to 0.1 mg/ml with methanol for use as an internal standard.

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) for GC, *N*-methyl-2-pyrrolidinone (NMP) used as organic solvent, methanol for the phthalate ester test, and dichloromethane for the residual pesticide test were purchased from Kanto Chemical Co. **Instruments** — The GC used was Model HP6890 from Agilent Co., (Santa Clara, CA, USA) the HS autosampler used was Model HS40XL from Perkin-Elmer Co., (Shelton, CT, USA) and the MS used was the Platform model from Micromass Co. The analytical conditions for HS-GC/MS are shown in Table 1.

The quantitative ions of the compounds measured using HS-GC/MS [selected ion monitoring (SIM)] are shown in Table $2.^{12}$ The peak of 4-nonylphenols is the sum of six components separated previously.

Preparation of Standard and Sample Solutions — Standard solution was prepared as follows: 2 ml of NMP was added to a HS vial, 20–200 μ l of standard solution, 2 μ l of 4-(1-methyl-1-propylpentyl)phenol-¹³C₆ solution as a surrogate substance, and 2 μ l of internal standard were syringe-spiked, and 250 μ l of BSTFA solution was added. The vial was immediately sealed with a polytetrafluoroethylene (PTFE)-coated silicon septum as shown in Fig. 1.

Table 1. Experimental Parameters for Measurements

HS
Sample temperature: 140°C
Needle temperature: 180°C
Transfer temperature: 200°C
Pressurize time: 1.5 min
Injection time: 0.16 min
GC
Column: EQUITY-5 Supelco (Bellefonte, PA, U.S.A.)
$0.25 \text{ mm i.d.} \times 30 \text{ m}, \text{ df: } 0.25 \mu\text{m}$
Temperature: 100° C (5 min) \rightarrow 10° C/min \rightarrow 280° C (5 min)
Injector: 220°C, split 5:1
Carrier gas: helium
Flow: 1 ml/min
MS
Ionization: Electron-impact ionization
Ionization energy: 70 eV
Ion source temperature: 180°C

Compound	MW	R T (min)	Base peak	Major
Compound	101. 00.	K.I. (IIIII)	fragment ion	fragment ion
4- <i>t</i> -butylphenol	222	11.26	207	222
4-n-pentylphenol	236	13.64	179	236
4- <i>n</i> -hexylphenol	250	14.90	179	250
4-n-heptylphenol	264	16.07	179	264
4- <i>t</i> -octylphenol	278	15.19	207	278
4-n-octylphenol	278	17.17	179	278
4-nonylphenol	292	16.31-16.81	207	292
4-nonylphenol- ¹³ C ₆	292	16.52	241	_
$nanhthalene_{d_{\alpha}}$ (Internal Standard)	136	8 4 5	136	

Table 2. Quantitative Ions of TMS 4-Alkylphenol and Internal Standard Using HS-GC/MS (SIM)



Fig. 1. In Situ TMS Derivatization in a Head-space Vial

For the sample solution, 200 mg of a sample cut into small pieces and 2 ml of NMP were added to a head-space vial and dissolved by mixing, and 2 μ l of 4-(1-methyl-1-propylpentyl)phenol-¹³C₆ solution as a surrogate substance and 2 μ l of internal standard were syringe-spiked, followed by the addition of 250 μ l of BSTFA solution, and the vial was sealed as described above.

The surrogate substance was added as a quantitative standard of 4-alkylphenol. The internal standard was added to confirm injection of the sample solution into the GC/MS system.

Measurement of Samples — In previous analysis of 4-alkylphenols generally performed, removal of contaminants was necessary because various materials are contained in samples. Our method is capable of eliminating nonvolatile contaminants by HS sampling, but TMS derivatization may be affected by contaminants. Thus measured values were corrected using the isotope dilution method, in which a substance with a different molecular weight but the same physicochemical properties showing the same sample treatment pattern as the substance of interest was added and measured as a surrogate substance. For the surrogate substance, 4-(1-methyl-1-propylpentyl)phenol- $^{13}C_6$ synthesized by us was used. The ^{13}C label with a more stable molecular structure of the backbone than deuterium was used because deuterium may be dissociated from the deuterium label by heating and pressure during HS sampling.

Various measurement conditions are shown in Tables 1, 2, and Fig. 1.

RESULTS AND DISCUSSION

HS Solvent

HS-GC samples a portion of HS and analyzes volatile components in the HS evaporated from the sample at a specified temperature in a sealed This method is used mainly for the meavial. surement of volatile organic compounds in water. However, a less volatile solvent under high temperature and pressure is necessary for the measurement of 4-alkylphenols because 4-alkylphenols have a very high boiling point (237–297°C). Thus solvents with relatively high boiling and ignition points, NMP, triethyleneglycoldimethylether, and dimethylsulfoxide, were investigated, and the sensitivity of HS-GC/MS was high when NMP was used as the solvent. NMP was selected for the solvent because it is known to be a good solvent of many organic and inorganic compounds, polar gas, and natural and synthetic macromolecules excluding lower aliphatic hydrocarbon and thus is appropriate for the dissolution of synthetic resin products.¹³⁾

HS Sampling Conditions

The sample temperature and heating time were

investigated. When measurement was performed at sample temperatures of 60, 100, 120, 130, and 140°C, the sensitivity was the highest at 140°C. For the heating time, measurement was performed after heating for 5, 10, 20, and 30 min, and good results were obtained when heating for 20 and 30 min at 140°C, with no difference in the sensitivity.

The pressure time was changed within a range of 0.2-2.0 min. The sensitivity was high when the pressure time was 1.5 min or less, but double-injection occurred with pressure for 2.0 min.

When 4-alkylphenols were not derivatized to TMS, measurement using HS-GC/MS was not possible under any condition. This may have been due to the much lower volatility of 4-alkylphenols than TMS derivatives.

Based on these findings, the optimal sample temperature, sample heating time, and pressure time were 140° C, 20 min, and 1.5 min, respectively.

Internal Standard

Under the above HS sampling conditions, 4chlorotoluene- d_4 , 1,4-dichlorobenzene- d_4 , naphthalene- d_8 , phenanthrene- d_{10} , acenaphthene- d_{10} , chrysene- d_{12} , and perylene- d_{12} were investigated. 4-Chlorotoluene- d_4 , 1,4-dichlorobenzene- d_4 , phenanthrene- d_{10} , chrysene- d_{12} , and perylene- d_{12} were not detected. Naphthalene- d_8 and acenaphthene d_{10} were detected at retention times of 8.45 and 13.30 min, respectively, and thus naphthalene- d_8 was selected as the internal standard.

TMS Derivatization Conditions

TMS derivatization of the hydroxyl group of 4-alkylphenols by BSTFA was investigated. The reaction temperature was set to room temperature $(25^{\circ}C)$, and the reaction time was changed from 60 to 300 min. As shown in Fig. 2, no marked changes with the reaction time were noted in the detection sensitivity, because the reaction may have been completed by heating at 140°C for 20 min under the HS sampling conditions. Based on these findings, the reaction time was set at 60 min.

The mass fragmentgrams of the eight 4-



Fig. 2. Effects of Reaction Time on TMS 4-Alkylphenols Concentration: 4-nonylphenol mixture 10 µg/ml, other 4-alkylphenols 1 µg/ml.



Concentration: 4-nonylphenol 10 µg/ml, other 4-alkylphenols 1 µg/ml, internal standard 0.1 µg/ml.



Fig. 4. Mass Spectrum of 4-o-Trimethylsilyl-nonylphenol- $^{13}C_6$ Used as a Surrogate Substance (1 µg/ml)



Vertical line: peak ratio (Standard, Internal Standard); transverse: concentration ($\mu g/ml$) (n = 3)

alkylphenols and the internal standard measured with HS-GC/MS(SIM) under the conditions investigated above are shown in Fig. 3. The mass spectrum of the TMS derivative of 4-(1-methyl-1-propylpentyl)phenol- $^{13}C_6$ is shown in Fig. 4.

Calibration Curves

The calibration curves prepared based on HS-GC/MS(SIM) measurement of the TMS derivatives of 4-*t*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*n*-heptylphenol, 4-*t*-octylphenol, 4*n*-octylpehnol, and 4-nonylphenol are shown in Fig. 5. Calibration curves were calculated from peak values using the internal standard. Good correlation coefficients of $R^2 = 0.9962-0.9998$ were obtained for 4-nonylphenol and the other compounds within the ranges of 1–100 and 0.1–10µg/ml, respectively. The lower detection limits were obtained from the General Test Methods section in the 14th revision of the *Japanese Pharmacopoeia*,¹⁴⁾ and the value was 0.5µg/ml for 4-nonylphenol and 0.05µg/ml for the other compounds.

Repeatability

For investigation of the repeatability of each compound, the analysis was repeated six times using 1 µg/ml solutions of 4-*t*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*n*-heptylphenol, 4-*t*-octylphenol, 4-*n*-octylphenol, and 4-(1-methyl-1-propylpentyl)phenol- ${}^{13}C_6$ and 10 µg/ml solutions of 4-nonylphenol, and the relative standard deviation was 4.7–8.9%.

Addition and Recovery of Surrogate Substance

The surrogate substance was added to each sample at a final concentration of 1 μ g/ml, following the preparation procedure used for the sample solution, and the yield was investigated. The yield from No. 21 sponge was 54%, showing a low yield. This may have been due to the insolubility of the sample with NMP and absorption of the surrogate substance by the sponge. In the other samples, the yield was 79–124%, with a mean of 104%.

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Solubility of Various Materials of Synthetic Resin Products in NMP

The materials of samples measured are listed in Table 3. When the samples cut into small pieces were weighed (200 mg), combined with 2 ml of NMP, and heated at 140°C for 10 min, No. 19 silicone rubber and No. 21 polyurethane were not dissolved, and residues of Nos. 13 and 14 polyethylene remained. However, NMP was suitable for dissolving other materials: polyvinyl chloride, polyvinylidene chloride, acrylonitrile-butadienestyrene (ABS), polycarbonate, and polystyrene, for which residual 4-nonylphenols after dissolution have been reported.

Measurement of 4-Alkylphenols in Synthetic Resin Products for Drug and Food Use

For the samples, chemical products, mainly synthetic resin products, from which 4-alkylphenols were previously detected on dissolution and mate-

	Sample	Material	4-t-Butylphenol	4-n-Pentylphenol	4-n-Hexylphenol
1	Tablet sheet	—	N.D.	N.D.	N.D.
2	Tablet sheet	Polyvinyl chloride	N.D.	N.D.	N.D.
3	Tablet sheet		N.D.	N.D.	N.D.
4	Eyedrop container	—	N.D.	N.D.	N.D.
5	Eyedrop container	—	N.D.	N.D.	N.D.
6	Eyedrop container	—	N.D.	N.D.	N.D.
7	Wrapping film	Polyvinyl chloride	N.D.	N.D.	N.D.
8	Wrapping film	Polyvinyl chloride	N.D.	N.D.	N.D.
9	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.
10	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.
11	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.
12	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.
13	Wrapping film	Polyethylene	N.D.	N.D.	N.D.
14	Wrapping film	polyethylene	N.D.	N.D.	N.D.
15	Plate	ABS	N.D.	N.D.	N.D.
16	Plate	Polycarbonate	67	N.D.	N.D.
17	Plate	Polystyrene	N.D.	N.D.	N.D.
18	Disposable cup	Polystyrene	N.D.	N.D.	N.D.
19	Bond	Silicon rubber*	N.D.	N.D.	N.D.
20	Bond	Polyvinyl acetate	N.D.	N.D.	N.D.
21	Sponge	Polyurethane*	N.D.	N.D.	N.D.
22	Drug case	Vinyl	N.D.	N.D.	N.D.
23	Drug case	ABS	N.D.	N.D.	N.D.
24	Card case	Polyvinyl chloride	N.D.	N.D.	N.D.
25	Vinyl bag	Polyvinyl chloride	N.D.	N.D.	N.D.
26	Тоу	Polyvinyl chloride	N.D.	N.D.	N.D.
27	Тоу	Polyvinyl chloride	N.D.	N.D.	N.D.
28	Air frame	Polyvinyl chloride	N.D.	N.D.	N.D.

Table 3. 4-Alkylphenols in Synthetic Resin Products (Drug Containers, Wrapping Film, and Household Utensils) (µg/g)

ABS: acrylonitrile-butadiene-styrene, -: not indicated, N.D.: not detected, *: not dissolved.

			Tuble 61 Continue	ieu		
	Sample	Material	4-t-Octylphenol	4-n-Heptylphenol	4-n-Octylphenol	4-Nonylphenol
1	Tablet sheet		N.D.	N.D.	N.D.	N.D.
2	Tablet sheet	Polyvinyl chloride	N.D.	N.D.	N.D.	N.D.
3	Tablet sheet		N.D.	N.D.	N.D.	N.D.
4	Eyedrop container		N.D.	N.D.	N.D.	N.D.
5	Eyedrop container		N.D.	N.D.	N.D.	N.D.
6	Eyedrop container	_	N.D.	N.D.	N.D.	N.D.
7	Wrapping film	Polyvinyl chloride	N.D.	N.D.	N.D.	N.D.
8	Wrapping film	Polyvinyl chloride	N.D.	N.D.	N.D.	N.D.
9	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.	N.D.
10	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.	N.D.
11	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.	N.D.
12	Wrapping film	Polyvinylidene chloride	N.D.	N.D.	N.D.	N.D.
13	Wrapping film	Polyethylene	N.D.	N.D.	N.D.	N.D.
14	Wrapping film	Polyethylene	N.D.	N.D.	N.D.	N.D.
15	Plate	ABS	N.D.	N.D.	N.D.	N.D.
16	Plate	Polycabonate	N.D.	N.D.	N.D.	N.D.
17	Plate	Polystyrene	N.D.	N.D.	N.D.	N.D.
18	Disposable cup	Polystyrene	N.D.	N.D.	N.D.	N.D.
19	Bond	Silicon rubber*	N.D.	N.D.	N.D.	N.D.
20	Bond	Polyvinyl acetate	N.D.	N.D.	N.D.	N.D.
21	Sponge	Polyurethane*	N.D.	N.D.	N.D.	N.D.
22	Drug case	Vinyl	N.D.	N.D.	N.D.	N.D.
23	Drug case	ABS	N.D.	N.D.	N.D.	N.D.
24	Card case	Polyvinyl chloride	N.D.	N.D.	N.D.	405
25	Vinyl bag	Polyvinyl chloride	N.D.	N.D.	N.D.	2430
26	Тоу	Polyvinyl chloride	N.D.	N.D.	N.D.	1320
27	Тоу	Polyvinyl chloride	N.D.	N.D.	N.D.	249
28	Air frame	Polyvinyl chloride	N.D.	N.D.	N.D.	116





Fig. 6. Mass Fragmentgram of Sample No. 25

rial tests were selected. The 4-alkylphenol measurement results of 28 samples are shown in Table 3. None of the seven compounds was detected in six samples of drug containers (Nos. 1–6) or eight samples of wrapping film (Nos. 7–14). Among samples of items not for drug and food use, 4-*t*-butylphenol was detected in No. 16 polycarbonate plate, and 116–2430 μ g/g of 4-nonylphenol was detected in Nos. 24–28, which are household utensils. The mass fragmentgram of No. 25 is shown in Fig. 6. No 4-alkylphenol was detected in film wrap for foods previously analyzed,⁸⁾ but 4-nonylphenol was detected in household utensils at 5-35-fold higher levels than those we previously reported.¹²⁾ The pre-

vious method eluted only substances on synthetic resin product surfaces using solvent and were unable to measure the total contents of the products. In addition to solvent extraction of the surface, evaporation, redissolution with other solvents, and derivatization are necessary. This is a simple method that proceeds to derivatization in a one-step procedure and dissolves the whole sample as well as eliminates impurities interfering with analysis.

Summary

We established a simultaneous analysis method of 4-alkylphenols contained in chemical products, which may release substances into the environment. When this method was applied for the analysis of 4-alkylphenols in synthetic resin products, 4alkylphenols could be simultaneously detected with high sensitivity. Since NMP used as the solvent has good ability to dissolve various substances, this method may be widely applicable in the analysis not only of synthetic resin products but also of environmental samples and industrial materials.

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