Chromatography, Vol.25 No.1 (2004) **Original**

Original

Simultaneous Detection of Endocrine Disruptors of 4−Nonyl Phenols and 4−Octyl Phenols in Commodity Products Using Positive Chemical Ionization−Gas Chromatography/Mass Spectrometry

Tomoo Nakagawa*, Naeko Sugaya*, Katsumi Sakurai*, Junichi Nakagawa**,
Koichi Usukura***, Nobuhiko Onda*** and Koichi Oguma****

**Yokohama City Institute of Health, 1−2−17 Takigashira, Isogo−ku, Yokohama, 234−0011, Japan*

***Tokyo Metropolitan Research Laboratory of Public Health, 3−24−1 Hyakunincho, Shinjuku−ku, Tokyo, 169−0073, Japan*

****PerkinElmer Japan, 2−8−4 Kitasaiwai, Nishi−ku, Yokohama, 220−0004, Japan*

*****Department of Materials Technology, Faculty of Engineering, Chiba University, 1−33 Yayoi−cho, Inage−ku, Chiba, 263−8522, Japan*

Received for review December 20, 2003. Revised manuscript received February 2, 2004. Accepted February 3, 2004

Abstract

Alkyl phenol is the general name for phenol compounds with different length or branched alkyl groups. Among the alkyl phenol group, a series of chemicals called nonyl phenols and octyl phenols that have C 9 or C 8 alkyl chains at the para position relative to the phenolic hydroxyl group are used for additives of synthetic resin products and raw materials for non−ionic surfactants, are classified as the endocrine disruptors. In this study a rapid, highly selective and sensitive analytical method using positive chemical ionization (PCI)−GC/MS has been established to determine trimethylsilyl derivatives of nonyl phenols and octyl phenols. Using this method, 18 isomers of 4−nonyl phenol and 8 isomers of 4−octyl phenol were separated and confirmed for the commercial resin products.

Keywords: endocrine disruptor; nonyl phenols; octyl phenols; positive chemical ionization; GC/MS; commodity products

Introduction

Nonyl phenols and an octyl phenol (4−(1,1,3,3−tetramethylbutyl)−phenol) are compounds identified as endocrine disrupting chemicals by the Japanese Ministry of the Environment [1]. Those alkyl phenols are the compounds that have various alkyl groups with different length or branched alkyl chains at the para position relative to the phenolic hydroxyl group [2−3]. Among them, those that have C 9 and C 8 alkyl chains are used as additives in synthetic resin products. Nonyl phenols are deliberately added as an antioxidant or corrosion inhibitor, or present as an impurity of stabilising agents, tris(nonyl phenyl) phosphite (TNP), or heat decomposition products formed during the production process [4−5]. Since the synthetic resin products contain numerous numbers of additives designed to improve the function of the material, the chemical analysis becomes complicated and time−consuming. Therefore, a rapid and high selective analytical method has been required.

Of the analytical methods that have been used to detect alkyl phenol compounds, some have utilised gas chromatography separation with hydrogen flame ionization detector (FID) [6] or electron capture detector (ECD) [7], high performance liquid chromatography (HPLC) with ultra violet / visible absorption (UV) or fluorescence (FL) detector [8]. However, gas chromatography/mass spectrometry (GC/MS) is a superior method, with high sensitivity and selectivity [9]. In our previous publication [10], the chromatograms with better shaped peaks were obtained by derivatisation of the al-

---

To whom correspondence should be addressed.
Tel: +81−45−314−8381, Fax: +81−45−314−5142
E−mail: nobuhiko.onda@perkinelmer.com

kyl phenol hydroxy group into trimethylsilyl (TMS) derivatives, and at the same time the molecular ions and unique fragments were found by "soft ionization" using positive chemical ionization (PCI).

In this publication, we report the separation and confirmation of 4−nonyl phenol isomers from synthetic resin products, and also 4−octyl phenols that have not previously been reported, were found in the leaching followed by use of TMS derivatisation and PCI−GC/MS method.

Experimental

Samples and Reagents

Thirty one (31) home and office use products made of synthetic resin, such as small plastic cases, stickers, pen and card cases were purchased from a market in February, 2003. The resin major materials on the label of these samples were confirmed by Fourier transform infrared analysis (FT−IR).

Alkyl phenol standards: 4−*t*−butyl phenol, 4−*n*−pentyl phenol, 4−*n*−hexyl phenol, 4−*n*−heptyl phenol, 4−*t*−octyl phenol (4−(1,1,3,3−tetramethylbutyl)−phenol), 4−*n*−octyl phenol, nonyl phenol mixture (environmental analysis grade) and TMS derivatisation reagent of Bis (trimethylsilyl) trifluoroacetamide (BSTFA) were obtained from Kanto−Kagaku (Tokyo, Japan). As a leaching solvent, *n*−heptane (phthalic acid analysis grade) and dichloromethane (pesticide residue analysis grade) from the same reagent supplier were used without any purification. Highly pure nitrogen gas (purity 99.999%) and pure isobutane gas for the chemical ionization were from Takachiho Chemicals (Tokyo, Japan).

Instruments

For the measurement of PCI−GC/MS and EI (Electron Ionization)−GC/MS, a model Clarus 500 Gas Chromatography/Mass Spectrometer of PerkinElmer (Shelton, CT, USA) was used. The capillary column used was a PTE−5 of Supelco (Bellefonte, PA, USA). The analytical condition of GC/MS is shown in Table 1. For the FT−IR analysis a model Spectrum One FT−IR combined with a Universal ATR (PerkinElmer, Shelton, CT, USA) was used.

Table 1. Experimental parameters for GC / MS measurements

GC
Column: PTE−5 (30 m × 0.25 mm I. D., df: 0.25 μm)
Temperature: 60℃ (1 min) −10℃/min−280℃ (5 min)
Carrier gas: helium
Injector: 280℃, Splitless

PCI−MS
Sample: 2 μl
Reagent gas: isobutane (99.999%)
Ionization Energy: 70 eV
Ion source temperature: 150℃
Mass range: 60−400 *m/e*

Leaching of alkyl phenols from resin sample

Leaching was performed by the leaching method of Pharmaceutical Society of Japan for commodity tests (Methods of Analysis in Health Science) [11] : a sample (2 cm x 5 cm) were washed with purified water and then air−dried. As shown in Fig.1, the sample specimen was placed in 20 mL of n−heptane at 25℃ for 1 hour. After removing the specimen, the leaching solution was transferred to a round bottom flask, and concentrated by rotary evaporation, dried under blowing nitrogen gas and then was dissolved with 0.5 mL of dichloromethane. Thereafter, 0.2 mL of TMS derivatisation reagent (BSTFA) was added to the solution and then made up to exactly 1 mL with dichlomethane. After a vigorous shake the solution was left at room temperature for 1 hour for the complete derivatisation.

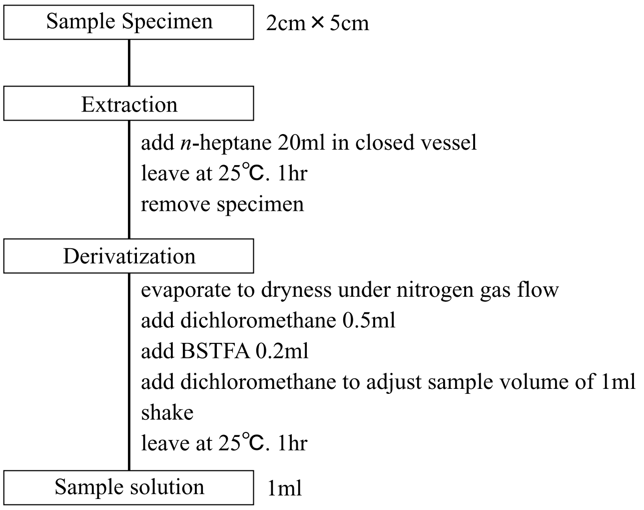

Figure 1. Sample preparation for the determination of 4−alkyl phenols BSTFA: Bis (trimethylsilyl) trifluoroacetamide)

Preparation of blank and standard solutions

A sample blank solution was prepared as described above without sample material. The standard solution was prepared as follows: 100 mg each of alkyl phenol standards was added with dichloromethane to make a 1 mg/mL standard stock solution which was further diluted with dichloromethane to create several different concentrations of standard solutions.

GC/MS measurements

Monitored ions of the target compounds measured by PCI method are shown in Table 2. The calibration curve was constructed by injecting 1 μL of standard solutions into PCI−GC/MS

Table 2. Monitor ions of TMS 4−alkyl phenols

Compound	M.W.	Base peak fragment ion	Major fragment ion
4−*t*−butyl phenol	222	223	207
4−*n*−pentyl phenol	236	237	179 (279)
4−*n*−hexyl phenol	250	251	179 (293)
4−*n*−heptyl phenol	264	265	179 (307)
4−*t*−octyl phenol	278	279	207
4−*n*−octyl phenol	278	279	179 (321)
nonyl phenols	292	293	127

( ) is the adduct ion (m/z) with CI gas of isobutane.

system and from the measured peak areas. An 1 μL of the leaching sample solutions and the blank solution were injected into PCI−GC/MS using the splitless mode and analysed.

Quantification of nonyl phenols

The chromatographic peaks detected for m/z of 293 were summed into a peak area, and this peak was used for the calibration curve.

Results and Discussion

There are some reports that 4−alkyl phenol compounds can leach from wrapping films for food products, and synthetic resin food plates [12−13]. The concerned compounds leached from synthetic resin products can be easily transferred into food. Kawamura et al. studied the leaching tests of nonyl phenol residues in synthetic resin products for food such as PVC wrapping film and gloves, and plastic toys using *n*−heptane as a pseudo food oil [14−15]. The leaching method doesn't report the total amount of nonyl

Table 3. Nonyl phenols released from household utensils

(#)	Sample name	Sample weight (g)	Nonyl phenols		Phthalic acid ester	Plastics	C_8 /C_9*
			μg/cm^2	μg/g			
1	Mat	1.48	0.20	3	＋＋	PVC	0
2	Mat	1.37	2.89	42	＋＋	PVC	0.20
3	Sheet	0.05	0	0	＋	PE	0
4	Sheet	0.03	0	0	±	PE	0
5	Sheet	2.46	0.31	3	＋＋	PVC	0
6	Sheet	3.00	0	0	＋＋	PVC	0
7	Bag	0.40	5.60	280	＋＋	PVC	0.05
8	Bag	0.52	0	3	＋＋	PVC	0
9	Bag	0.24	0	9	＋	PVC	0.05
10	Letter bag	0.24	2.80	233	＋＋	PVC	0
11	Carry bag	0.19	0	0	＋	PP	0
12	Pen case	0.49	0	0	＋＋	PVC	0.03
13	Pen case	0.36	0	0	＋＋	PVC	0
14	Soft case	0.35	10.2	581	＋＋	PVC	0
15	Soft card case	0.48	0	0	＋＋	PVC	0
16	Soft card case	0.48	0	0	＋＋	PVC	0
17	Soft card case	0.38	0	0	＋＋	PVC	0
18	Card case	0.31	0	0	−	PP	0
19	Pass case	0.83	0.35	8	＋＋	PVC	0
20	Card holder	0.06	0	0	±	PP	0
21	Card holder	0.18	0	0	−	PP	0
22	Card holder	0.20	0	0	−	PP	0
23	Card holder	0.04	0	0	−	PP	0
24	Seal	0.11	0	0	−	PET	0
25	Seal	0.39	0	0	＋＋	PVC	0
26	Seal	0.05	0	0	＋＋	PET	0
27	Header label	0.08	0	0	＋	−	0
28	Frame	0.24	1.95	162	＋	PVC	0
29	Rain coat	0.06	0	0	−	EVA	0
30	Rain wear (trousers)	0.09	0	7	＋＋	PVC	0
31	Frost cover	0.06	0	0	−	PE	0

PVC: Polyvinyl chloride　PE : Polyethylene　PP : Polypropylene
PET : Polyethylene terephthalate　EVA: Polyvinyl acetate
* Peak height ratio of octyl phenols to nonyl phenols (not calibrated)

phenol in the specimen, but it is easy and simple to apply as a rapid quantitative method. The samples shown in Table 3 were applied to this test in this study.

Separation of 4−alkyl phenols and the mass spectrum

The soft synthetic resin products contain a lot of plasticizer and antioxidants. The leaching method for those requires a clean−up procedure before the measurements. After leaching, Isobe et al. used 5% H_2O (w/w) in activated column chromatography to separate fractions and purify samples, and then analysed 4−alkyl phenols by EI−GC/MS (SIM mode) [13]. In the present study a lot of phthalate esters were detected, but direct separation and determination without clean−up procedure was possible because of selective detection of the TMS derivatised and of high specificity of PCI method: isobutane is “softer” reagent gas, and analysis was done by the lesser fragmentation as previously reported [10]. For all of mass spectra of the separated 4−nonyl phenol TMS derivatives, a strong molecular ion of m/z 293 of M+1 was detected as the base peak. Compared with EI−GC/MS method, this is a superior method with less interference of sample contaminants. The fragment with m/z 279 of M+1 was used for the qualitative analysis and determination of 4−octyl phenols.

For the 31 tested samples, the results are shown in Table 3. The compound of 4−nonyl phenols (0.20−10.0 μg/cm^2) were detected in 8 samples of stationary such as letter bags, desk mat, and pen case. Also 4−octyl phenols were detected in 4 samples at about 2−3 % of 4−nonylphenols as calculated from the peak area ratio.

Detection of 4−octyl phenols by PCI−GC/MS

Among the octyl phenols, both of 4−*n*−octyl phenol and 4−*t*−octyl phenol (4−(1,1,3,3−tetramethylbutyl)−phenol), were not detected from the samples. However, for the samples of 2, 7, 10, 14 in Table 3, 8 isomers of 4−octyl phenols were detected: in Fig.2 the chromatogram of the sample #10 (synthetic resin letter bag) and in Fig.4 the mass spectrum for each peak are shown. Octyl phenols have isomers as well as nonyl phenols, but those have not been reported to date, with the above exception of 4−*n*−octyl phenol and 4−*t*−octyl phenol (4−(1,1,3,3−tetramethylbutyl)phenol). The PCI−GC/MS spectra of these 8 isomers have little fragmentation, and an M+1 (m/z 279) ion is detected as the strong fragment of 4−octyl phenol derivatives. This mass number agrees with the sum (m/z 279) of molecular weight (206) of 4−octyl phenol increased by a mass of 72 by trimethylsilyl derivatisation and increased by a mass of 1 by proton transfer. On the other hand, a peak of m/z 207 is an unique fragment for the tertiary alkylated TMS derivative of phenols [10] and m/z 113 fragment is interpreted as the mass of octyl group of $C_8H_{17}^+$. These results suggest all of the 8 detected compounds are the structural isomers of 4−octyl phenols. Since some spectra such as (d), (e), and (h) in Fig.3, are contaminated with high concentration of 4−nonyl phenol, therefore M+1 ion of 4−octyl phenols doesn’t become the base peak. However, quantitation using the mass chromatograms doesn’t have problem because of the difference in mass number. All of the detected 4−octyl phenol compounds do not exhibit M+43 fragment that is typical for 4−*n*−alkyl phenol, and also m/z 179 fragment ion that is specific to primary alkyl phenols [10] are not detected. One can conclude from those results that 4−*n*−octyl phenol doesn’t exist in those 8 com-

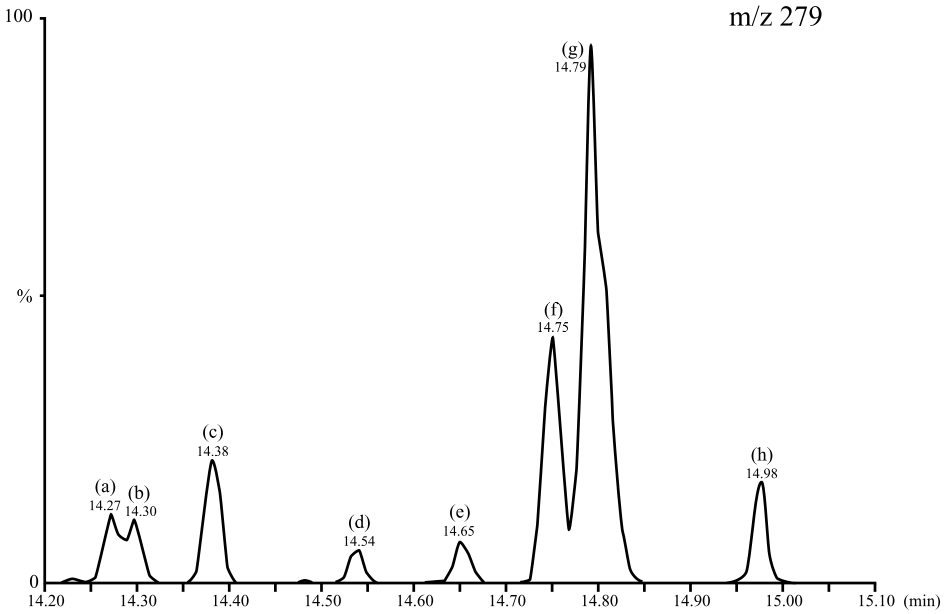

Figure 2. Separation of TMS derivatives (a−h) of “octyl phenols” in a sample #10 (PVC)

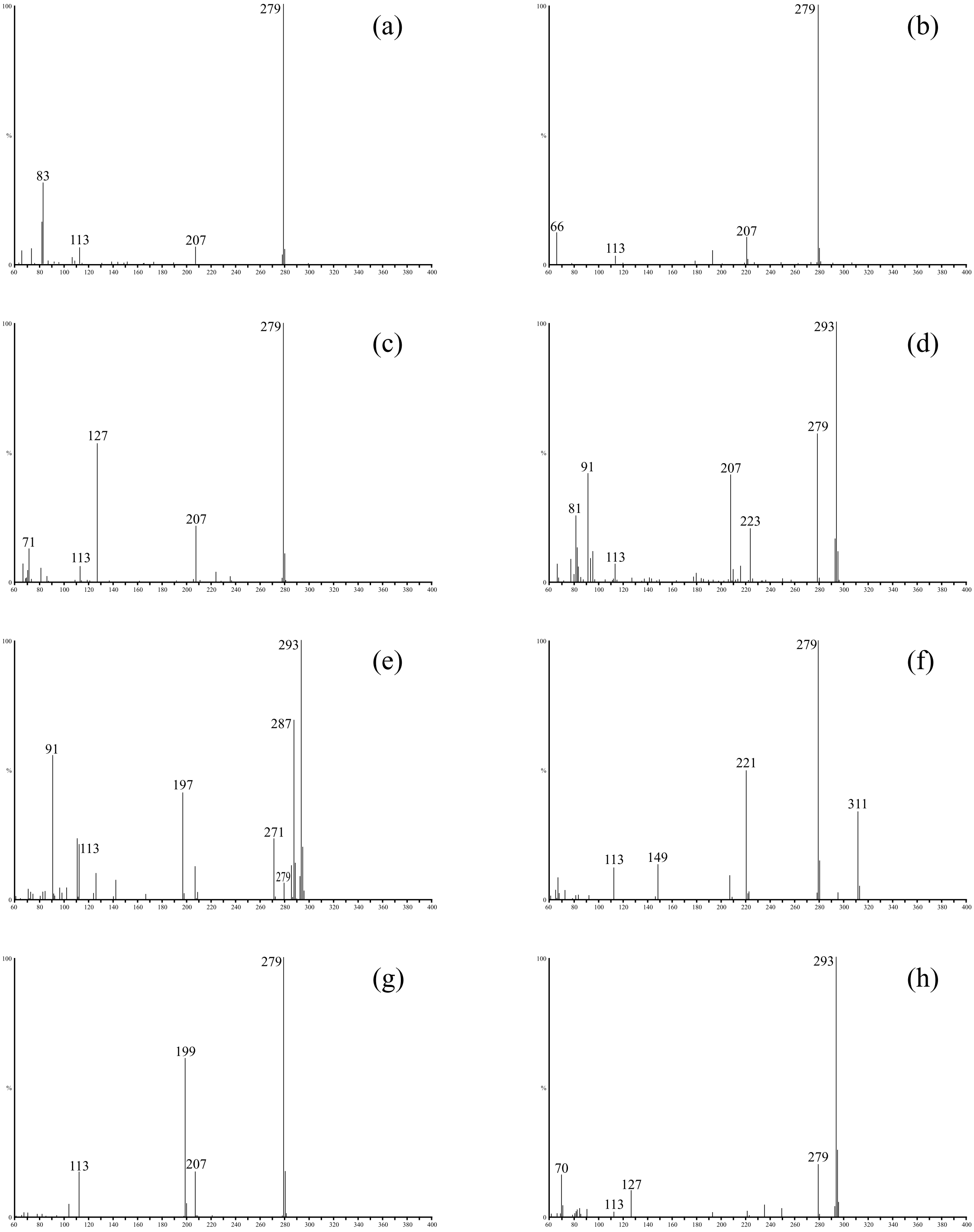

Figure 3. PCI spectrum of the peaks (a−h) in Fig.2.

pounds.

Separation and determination of 18 compounds of 4−nonyl phenol isomers by PCI−GC/MS

The quantitation was performed based on the calibration curve shown in Fig. 4, where the peak area of 4−nonyl phenols is the sum of 9 components separated previously [10]. As shown in Fig.5, the amount of 4−nonyl phenols was detected in high concentration (2.80 μg/cm^2) for sample #10 (synthetic resin letter bag), and 9 of new isomers of (1)−(9) in addition to 9 isomers of (a)−(i) that previously reported [10] were separated. The spectra obtained from PCI−GC/MS had less fragmentation and M+1 ion of 4−nonyl phenol derivatives as base peak was detected from all of the spectra. Each peak agreed as well with the sum (m/z 293) of molecular

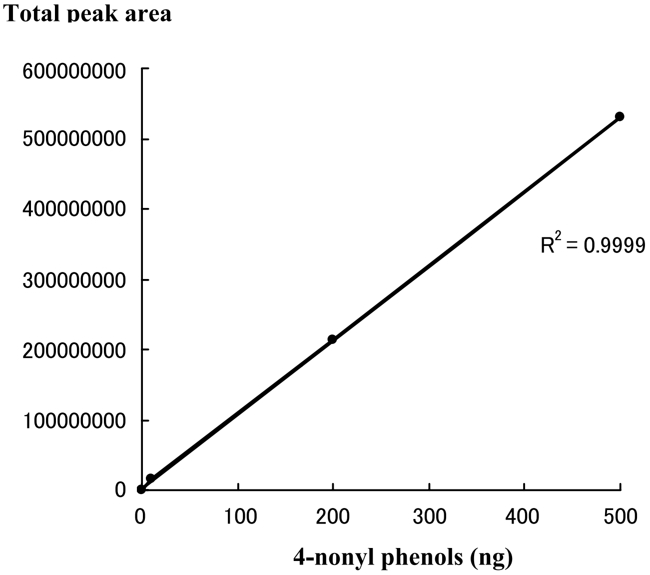

Figure 4. Calibration curve for TMS derivative of 4−nonyl phenols
Total peak area: the sum of peak area of 9 isomers of (a)−(i) found in 4−nonyl phenols standard [10]

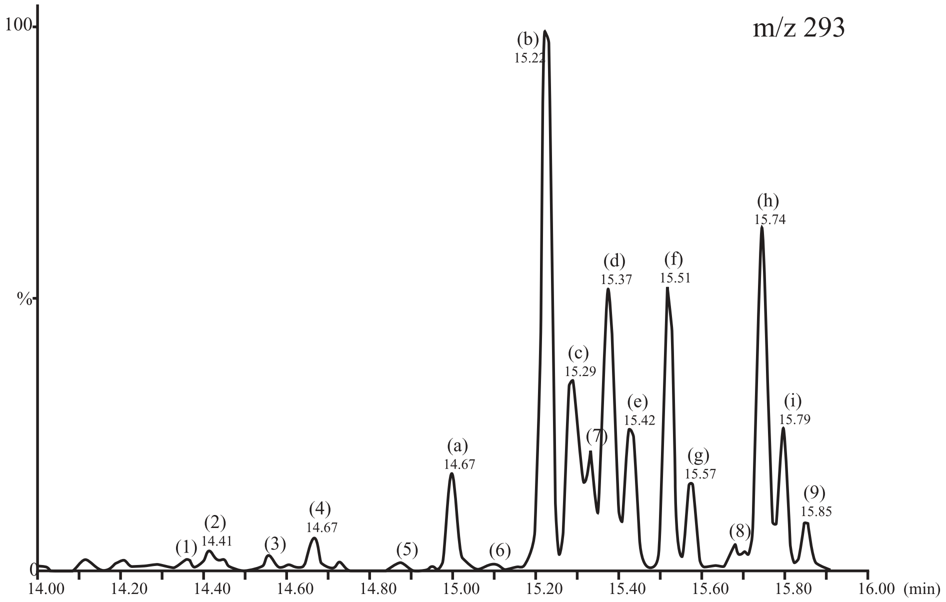

Figure 5. Separation of TMS derivatives (1−9 and a−i) of "nonyl phenols" in a sample #10

weight (220) of 4−nonyl phenol, increase mass 72 by trimethylsilyl derivatisation, and the increase of mass 1 of proton transfer. Therefore all of 18 compounds separated from sample #10 were concluded as structural isomers.

The accurate quantitation of 4−nonyl phenols is limited for the mixture of the structural isomers, and further there is none of the single compound standard available except 4−*n*−nonyl phenol. It elutes very late and does not fall in the so−called class of the nonyl phenols [10]. Total peak area of 9 isomers of (a)−(i) in Fig.5 accounts for more than 90% of the peak area found for m/z 293 mass chromatogram.

Synthetic resin products containing 4−alkyl phenol

The detection of 4−alkyl phenols and plasticizers is shown in Table 3. The base materials of synthetic resin products were confirmed by Fourier transform infrared analysis. The major materials in the 31 samples are polyvinyl chloride (PVC) (18 samples), polypropylene (PP) (6 samples), polyethylene (PE) (3 samples), polyethylene terephthalate (PET) (2 samples), and polyvinyl acetate (EVA) (1 sample). Many additives such as plasticizer and antioxidants etc. have been used in the soft synthetic resin products. In 22 samples the plasticizers (phthalate esters) were detected. Also 4−nonyl phenols were detected for 8 samples of synthetic resin

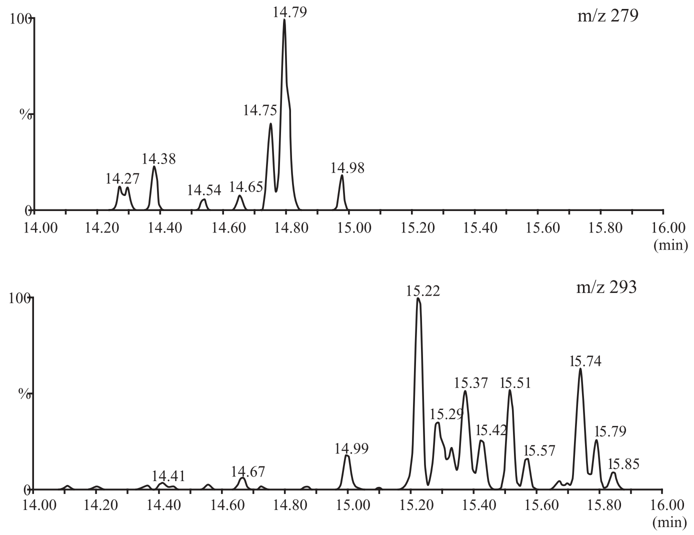

Figure 6. Separation of TMS derivatives of ”octyl phenols ” (upper) and “nonyl phenols” (lower)

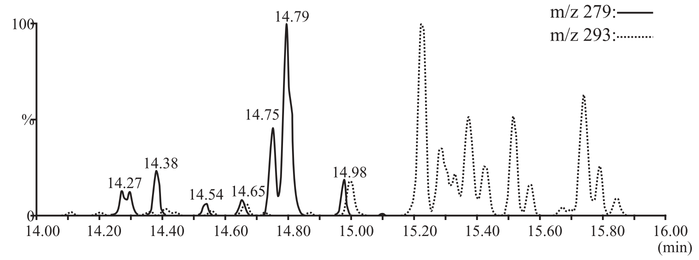

Figure 7. Overlapping of TMS derivatives of “octyl phenols” (m/z 279) and “nonyl phenols” (m/z 293). The scale of octyl and nonyl phenols are normalized with each largest peak.

products as above mentioned. Four samples (sample 2, 7, 10, and 14) that exhibit the high concentration of 4−octyl phenol contain also 4−nonyl phenols. Mass chromatogram of sample #10 is shown in Fig.6 for each of those 4−alkyl phenols. Fig.7 shows the overlapping of the elution of isomers of 4−octyl phenol and 4−nonyl phenol (the scale was normalized (100%) using the largest peak of each). The amount of 4−octyl phenol detected was about 0.03−0.20 μg/cm^2 of 4−nonyl phenol. As an interesting result, those 4−alkyl phenols detected samples are all made of PVC.

Conclusion

This study established a highly sensitive analytical method using positive chemical ionization PCI−GC/MS following TMS derivatisation of nonyl phenol and octyl phenol in synthetic resin products. Leaching results for the resin products show that 18 isomers of 4−nonyl phenol and 8 isomers of 4−octyl phenol were separated and confirmed. All of 4−octyl phenols were detected from PVC resin products that contain the high concentration of 4−nonyl phenols. From the soft synthetic resin products, phthalate esters were commonly found.

References

[1] Japan Environmental Agency: *Environmental Agency’s Initiatives to Issues of Exogenous Endocrine Disruptors−Environmental Hormone Strategic Project SPEED ’98,* **1998**, Tokyo, Japan (in Japanese).

[2] B. D. Bhatt, J. V. Prasad, G. Kalpana, and S. All, *J. Chromatogr. Sci.*, **1992**, *30*, 203.

[3] T. F. Wheeler, J. R. Heim, M. R. LaTorre, and A. B. Janes, *J. Chromatogr. Sci.*, **1997**, *35*, 19 .

[4] Y. Kawamura, C. Tagai, T. Maehara, and T. Yamada, *Shokuhin Eiseigaku Zasshi*, **1999**, *40*, 274 (in Japanese).

[5] S. Yamada, ed., “*Review of PVC Stabilizers*”, **1982**, Polymer Industry Laboratory Co. Ltd., Tokyo, Japan (in Japanese).

[6] E. Stephanou and W. Giger, *Environ. Sci. Technol.*, **1982**, *16*, 800.

[7] C. Wahlberg, L. Renberg, and U. Wideqvist, *Chemosphere*, **1990**, *20*, 179.

[8] K. K . Chee, M. K. Wong, and H. K. Lee, *J. Liq. Chrom. Technol.*, **1996**, *19*, 259.

[9] Japan Environmental Agency, Bureau of Water Quality Pro-

tection, Water Quality Control Office, “*Temporary Manual for Investigation of Chemicals of Exogenous Endocrine Disruptors in Water, Sediment and Biology*”, **1998**, Tokyo, Japan (in Japanese).

[10] T. Nakagawa, N. Sugaya, K. Sakurai, J. Nakagawa, K. Usukura, and N. Onda, *Analytical Sciences,* **2001**, *17*, i 1597.

[11] Pharmacuetical Society of Japan, ed., “*Hygiene Test Methodologies−Notes 2000*”, **2000**, Kimbara Publisher, Tokyo, Japan (in Japanese).

[12] Y. Kawanaka, M. Torigai, S. Yun, T. Hasiba, and K. Iwashima, *J. Environ. Chem.,* **2000**, *10*, 73.

[13] T. Isobe, N. Nakada, Y. Mato, H. Nishiyama, H. Kumata, and H. Takada, *J. Environ. Chem.,* **2002**, *41*, 621.

[14] Y. Kawamura, T. Maehara, H. Iijima, and T. Yamada, *Shokuhin Eiseigaku Zasshi,* **2000**, *41*, 212 (in Japanese).

[15] Y. Kawamura, T. Maehara, C. Wakui, and T. Yamada, *Shokuhin Eiseigaku Zasshi,* **2000**, *41*, 331 (in Japanese).