2.2. Standards

Concentrated solutions (1.0 mg ml^{-1}) of NP and OP were prepared as required by the addition of methanol with specific amounts of internal standard. The MS signals were monitored in the selected ion mode (SIM) at *m/z* 219 (NP), 205 (OP), 223 (NP-d_4), 209 (OP-d_4) and 224 (*m*-OP-d_5).

2.3. Instrumentation and analytical conditions

Liquid chromatography with electrospray mass spectrometry (LC–ESI–MS) was performed using an Agilent 1100 MSD-SL system (Agilent Technologies, Palo Alto, USA). The direct injection volume was 100 μl in the needle washing mode. The column used was Mightysil RP-18 (2.0 mm × 100 mm: 5 μm) with a Mightysil RP-18 GP pre-column (2.0 mm × 5 mm: 5 μm). The column oven was maintained at 40 °C.

The working conditions for ESI–MS were as follows: the drying nitrogen gas temperature was set at 350 °C and was introduced into the capillary region at a flow rate of 12 l min^{-1}; the capillary was held at a potential of 3500 V relative to the counter electrode in the negative-ion mode. The fragmentor voltage was 130 V during the chromatographic run. The chromatographic monitoring mode was gain 1.0. When working in the SIM mode, each *m/z* ion was designated as the $[M - H]^-$ of each compound.

The column-switching LC–MS coupled with an on-line extraction system consists of this LC–MS combined with an LC pump (Shimadzu LC-10AS pump: Shimadzu, Kyoto, Japan) and an extraction column (TSK-Precolumn BSA-ODS/S 4.6 mm × 10 mm: Tosoh, Tokyo, Japan). The column-switching LC–MS system, as depicted in Fig. 2, was used for the direct injection of urine sample. In addition, the system program was shown in Table 1. After a urine sample was injected by an auto-sampler, it was loaded onto the extraction column by flowing pure water at a rate of 0.5 ml min^{-1} using pump A (Shimadzu LC-10AS) for 5 min. While the extraction column was directed to drain during the 5 min run, the sample was extracted and purified on the on-line extraction column. After on-line extraction for 5 min, the position of the switching valve was changed (see Fig. 2: configuration B). This configuration connected the back-flashing extraction column to the analytical column and the MS detector in the flow path of pump B (Agilent 1100 system). Separation was carried out using a gradient mobile phase of 0.1 mM ammonium acetate in water-acetonitrile at a flow rate of 0.2 ml min^{-1}. The gradient mode was as follows: 0 to 10 min using 40% acetonitrile based solution, then 10 to 20 min using a linear increase from 40 to 80% (v/v) acetonitrile solution, and holding at 80%. After detection of these compounds, the switching valve was returned to its original position (see Fig. 2: configuration A). The run time for the assay of the sample mixture was 30 min.

Table 1
Time program of column-switching LC–MS coupled with an on-line extraction condition

Time (min)	Event	Column position (configrations are shown in Fig. 2)	Mobile phase (A–B, v/v)[a]
0.0	Sample injection	Configuration A	60:40
5.0	Valve changed	Configuration B	60:40
10.0		Configuration B	60:40
	Gradient		↓
20.0		Configuration B	20:80
30.0		Configuration A	20:80

[a] Pump B solvent: (A) 0.1 mM ammonium acetate in water; (B) acetonitrile.

In the quantitative procedure, standard solutions of OP and NP were prepared in aqueous solution to cover the calibration range. Quantitative analysis was performed in the SIM mode in order to maximize sensitivity. OP and NP concentrations in each sample were calculated relative to the stable isotopically labeled internal standards added to the sample prior to direct analysis, and gave a final detecting concentration of 50 ng ml^{-1}. Six-point calibrations (0.2–12.5 ng ml^{-1}) were performed daily for all samples with internal standards.

2.4. Human urine samples

Urine samples were obtained from 10 healthy volunteers aged 21–28. All samples were stored at −80 °C prior to use.

2.5. Enzymatic deconjugation of glucuronidated forms of OP and NP in urine

One milliliter of human urine sample was buffered with ammonium acetate (200 μl, 1.0 M, pH 6.8).

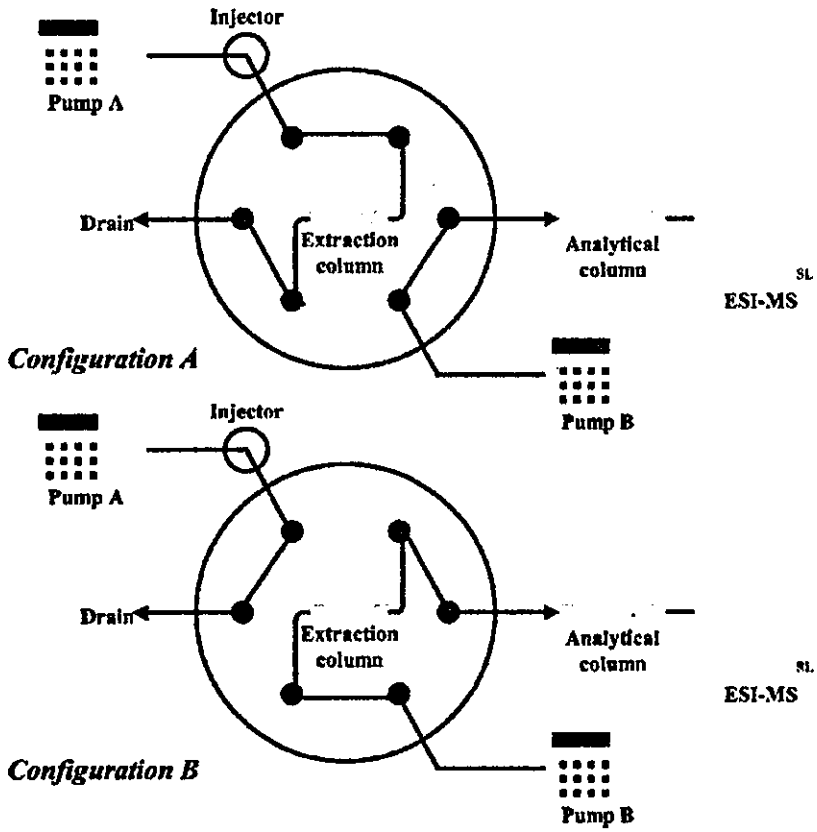

Fig. 2. Schematic representation of the column-switching LC–MS system. LC–ESI–MS was performed using an Agilent 1100 MSD-SL system (pump B + ESI–MS). Sample preparation system was performed using a Shimadzu LC-10AS pump (pump A).

After *E. coli* β-glucuronidase (15 μl, 89 U ml^{-1}; Fluka) was added, the sample was sealed in a glass tube and gently mixed. Quantitative glucuronidase hydrolysis to release the free OP and NP was accomplished by incubating at 37 °C for 3 h. After enzymatic deconjugation, the sample were filled with water to a total volume of 1.5 ml. This treatment is sufficient to deconjugate the glucuronidase of glucuronidated bisphenol A and phthalate monoesters [22–24].

3. Results and discussion

3.1. Optimization of column-switching LC–MS detection and conditions

In the mass spectral analysis using the ESI–MS detector with flow-through injection analysis of the standard solutions (1.0 μg ml^{-1}), the molecular ions that were designated as the $[M - H]^-$ ions were observed as the main peaks (Table 2). Then, we examined the optimal ionization for detecting these compounds. The parameters affecting the determination of compounds by LC–MS are the fragmentor voltage and the mobile phase. In order to determine the optimum fragmentor voltage for the detection of OP and NP, the *m/z*

Table 2
Ions monitored (*m/z*) for the determination of standards and stable isotopically labeled internal standards

Compound	Quantitation ion (*m/z*)
4-*tert*-Octylphenol (OP)	$[M - H]^-$ 205
4-Nonylphenol (NP)	$[M - H]^-$ 219
[2,3,5,6-$^2H_4$]4-*tert*-Octylphenol (OP-d_4)	$[M - H]^-$ 209
[2,3,5,6-$^2H_4$]4-*n*-Nonylphenol (NP-d_4)	$[M - H]^-$ 223
4-(1-Methyl)octylphenol-d_5 (*m*-OP-d_5)	$[M - H]^-$ 224

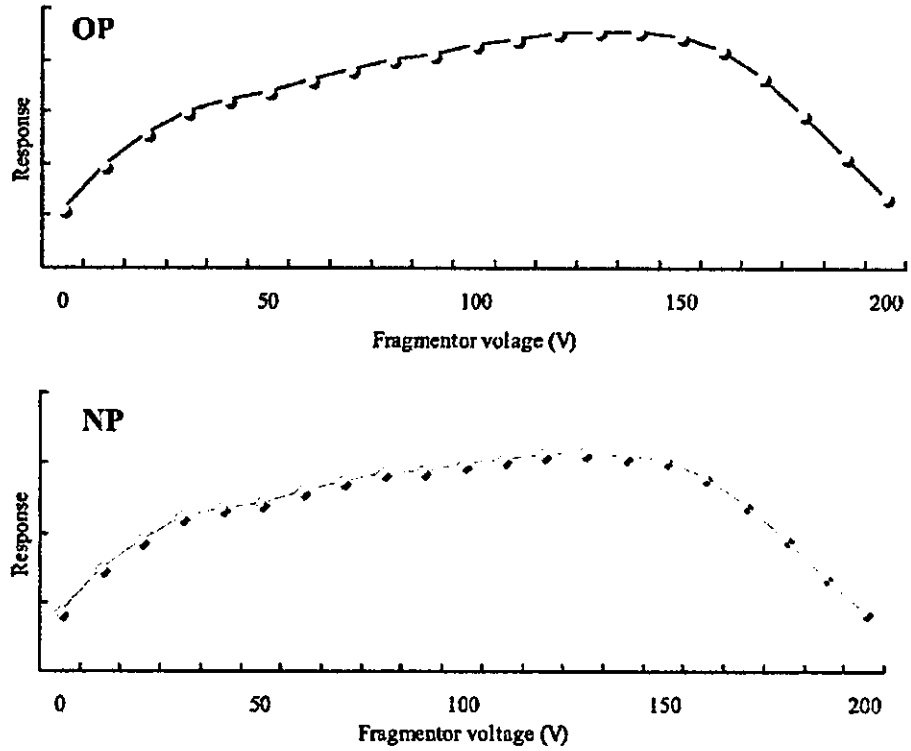

Fig. 3. Effect of fragmentor voltage on the peak responses of OP (*m/z* 205) and NP (*m/z* 219). Analytical conditions: electrospray MS (drying nitrogen gas temperature set at 350 °C; capillary region flow rate, 12 l min^{-1}; capillary potential 3500 V relative to the counter electrode; negative-ion mode; fragmentor voltage range 0–200 V; and SIM mode). Carrier solution: 0.1 mM ammonium acetate in water-acetonitrile (40:60, v/v) at a flow rate of 0.2 ml min^{-1}.

signals for OP and NP were plotted against the fragmentor voltage (Fig. 3). The effect of the mobile phase was also investigated (Fig. 4). The main *m/z* signals showed a maximum in 0.1 mM ammonium acetate as the mobile phase at 130 V for OP and NP.

The calculated detection limits of OP and NP were 0.05 and 0.1 ng ml^{-1}, respectively, for column-switching LC–MS detection with the ratio of the compound's signal to the background signal (S/N) = 3 (Table 3). In addition, the quantitation limit calculated when S/N = 5 was 0.3 ng ml^{-1} in urine (Table 3). Peak area ratios with respect to each internal standard were plotted, and the response was found to be linear over the validated range with correlation coefficients (r) higher of 0.999 (Table 3). Therefore, the method enables the precise determination of standards and may be applied to the detection of trace amounts of OP and NP in human urine samples.

3.2. Recovery from the coupled on-line solid-phase extraction with internal standards

A previously reported method for the determination of alkylphenol ethoxylate metabolites in aquatic environmental samples was used for the correction by the stable isotopically labeled internal standards [25]. Recovery was calculated relative to the stable isotopically labeled *n*-nonylphenol internal standard that was added to the sample prior to extraction. By LC on a reversed-phase column, 4-nonylphenol (mixed type) and 4-*n*-nonylphenol compounds were well separated on the basis of the branched alkyl chain [25]. Therefore, we examined whether 4-*n*-nonylphenol internal standard may be used for correcting the recovery of OP and NP because these compounds are different in terms of chemical and physical behaviors. The absolute recovery using this method is

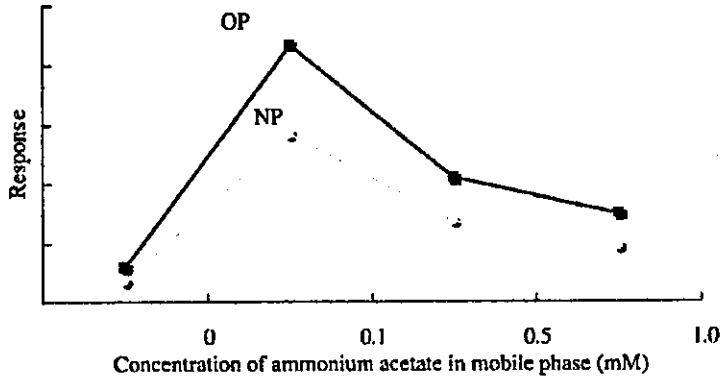

Fig. 4. Effect of mobile phase on the peak responses of OP (*m/z* 205) and NP (*m/z* 219). Analytical conditions: electrospray MS (drying nitrogen gas temperature set at 350 °C; capillary region flow rate, 12 l min^{-1}; capillary potential 3500 V relative to the counter electrode; negative-ion mode, fragmentor voltage 130 V; and SIM mode). Carrier solution: 0–1.0 mM ammonium acetate in water-acetonitrile (40:60, v/v) at a flow rate of 0.2 ml min^{-1}.

shown in Table 4. Average recoveries of NP were 51.7 and 51.9% (relative standard deviation (R.S.D.) 3.9 and 3.4%, respectively) by using NP-d_4 of the straight alkyl chain type. On the other hand, the average recoveries of NP from urine sample spiked with *m*-OP-d_5, which is a stable isotopically labeled nonylphenol of the branched alkyl chain type, were 97.0 and 85.7% (R.S.D. 4.1 and 1.7%, respectively) (Table 4). In addition, *m*-OP-d_5 internal standard was useful for correcting the recovery of OP in urine samples (Table 4). In other words, OP-d_4 and *m*-OP-d_5 are similar in terms of correction of the recovery of OP from urine sample. For this reason, the accuracy of the method was confirmed for both recoveries of OP and NP relative to only the stable isotopically labeled *m*-OP-d_5 internal standard. The method was successfully applied to pretreated urine samples. Thus, we decided to use this method for the simple and selective on-line pretreatment and quantitative determination of OP and NP in urine samples.

3.3. Background OP and NP levels in urine sample by experiments

The analysis of trace levels of OP and NP in biological samples is complicated by contamination, particularly by leaching from plastics. Thus, care must be taken to control contamination during experiments and where possible, to eliminate the source of contamination. For example, for the collection of human urine use of a plastic cup may contaminate the sample. We therefore investigated this potential source of contamination using control urine for the determination of leaching additives in plastic, and found that OP and NP were not detected (<0.1 ng ml^{-1}); therefore, plastic cup was not a source of contamination in these experiments. Investigations of OP and NP contamination of the Milli-Q water and LC system also gave negative results. However, trace levels of NP (15.6 ng ml^{-1}) in blank sample were detected after injecting pure water into a glass vial. This proves that the screw cap septum is a potential source of contamination. Based on

Table 3
Validation data of column-switching LC–MS system

Compound	Retention time (min)	Detection limit (S/N = 3) (ng ml^{-1})	Quantitation limit in urine (ng ml^{-1})	Calibration curve[a] (*r*) (0.2–100 ng ml^{-1})
4-*tert*-Octylphenol (OP)	23.3	0.05	0.3	0.999
4-Nonylphenol (NP)	25.0	0.1	0.3	0.999

[a] Peak area ratios with respect to standard and *m*-OP-d_5 internal standard (retention time: 26.5 min) were plotted.

Table 4
Recovery levels of OP and NP levels in human urine sample spike the stable isotopically labeled internal standard (IS)

Spike amount (ng ml^{-1})	NP: average[a] (R.S.D.)		OP: average[a] (R.S.D.)	
	IS NP-d_4	*m*-OP-d_5	OP-d_4	*m*-OP-d_5
1.0	51.7 (3.9)	97.0 (4.1)	101.4 (2.4)	96.2 (3.3)
5.0	51.9 (3.4)	85.5 (1.7)	99.1 (3.3)	99.6 (1.1)

[a] Background levels in the unspiked urine can be neglected (S/N < 3), $N = 6$.

this finding, we used a non-contaminated septum for our study.

In addition, OP and NP contamination in reagents used for measuring these compounds in urine was determined by this method. Trace amounts of OP (<0.1 ng ml^{-1}) in the *E. coli* β-glucuronidase solution were detected. If we take into consideration the quantitation limit calculated for S/N > 5 (0.3 ng ml^{-1}), this OP contamination may be disregarded in monitoring OP in human urine samples.

3.4. Measurement of NP and OP in human urine samples

Healthy volunteers were enrolled in this study. As expected, the average urinary creatinine level was 11.4 mg dl^{-1} (Table 5). All urine sample analyzed by this column-switching LC–MS system demonstrated no detectable OP and NP before deglucuronidation. After deglucuronidation, trace amounts of urinary NP in healthy volunteer G were detected. The urinary NP levels in the healthy volunteers varied from n.d. < 0.3 to 0.96 ng ml^{-1} (Table 5). On the other hand, urinary OP even was not detected after deglucuronidation (n.d. < 0.3 ng ml^{-1}).

We used this method to assess OP and NP levels in human urine samples to provide a reference range. In addition, variations in human metabolism were also be examined by monitoring both free and glucuronidated OP and NP in urine.

3.5. Risk estimations based on the daily intake and the main source of human exposure

In a previous study where the pharmacokinetic behavior OP and NP was investigated, the elimination

Table 5
Concentrations of OP and NP in urine samples from volunteers

Volunteer	Case	Old	Creatinine (mg dl^{-1})	OP[a] (ng ml^{-1})		NP[b] (ng ml^{-1})	
				None	β-Glucuronidase	None	β-Glucuronidase
A		20	9.14	n.d.	n.d.	n.d.	n.d.
B		23	11.7	n.d.	n.d.	n.d.	n.d.
C		23	10.3	n.d.	n.d.	n.d.	n.d.
D		21	30.4	n.d.	n.d.	n.d.	n.d.
F		24	10.1	n.d.	n.d.	nd.	n.d.
F		22	2.69	n.d.	n.d.	n.d.	n.d.
G		22	12.7	n.d.	n.d.	n.d.	0.96
H	1	23	5.48	n.d.	n.d.	n.d.	n.d.
	2[c]		13.9	n.d.	n.d.	n.d.	109.7
I	1	28	13.5	n.d.	n.d.	n.d.	n.d.
	2[c]		9.02	n.d.	n.d.	n.d.	15.9
J	1	26	10.4	n.d.	n.d.	n.d.	n.d.
	2[c]		8.99	n.d.	n.d.	n.d.	110.9

[a] n.d. indicates OP concentrations lower than 0.3 ng ml^{-1}.
[b] n.d. indicates NP concentrations lower than 0.3 ng ml^{-1}.
[c] These volunteers had taken a meal of rice or meat wrapped in PVC film.

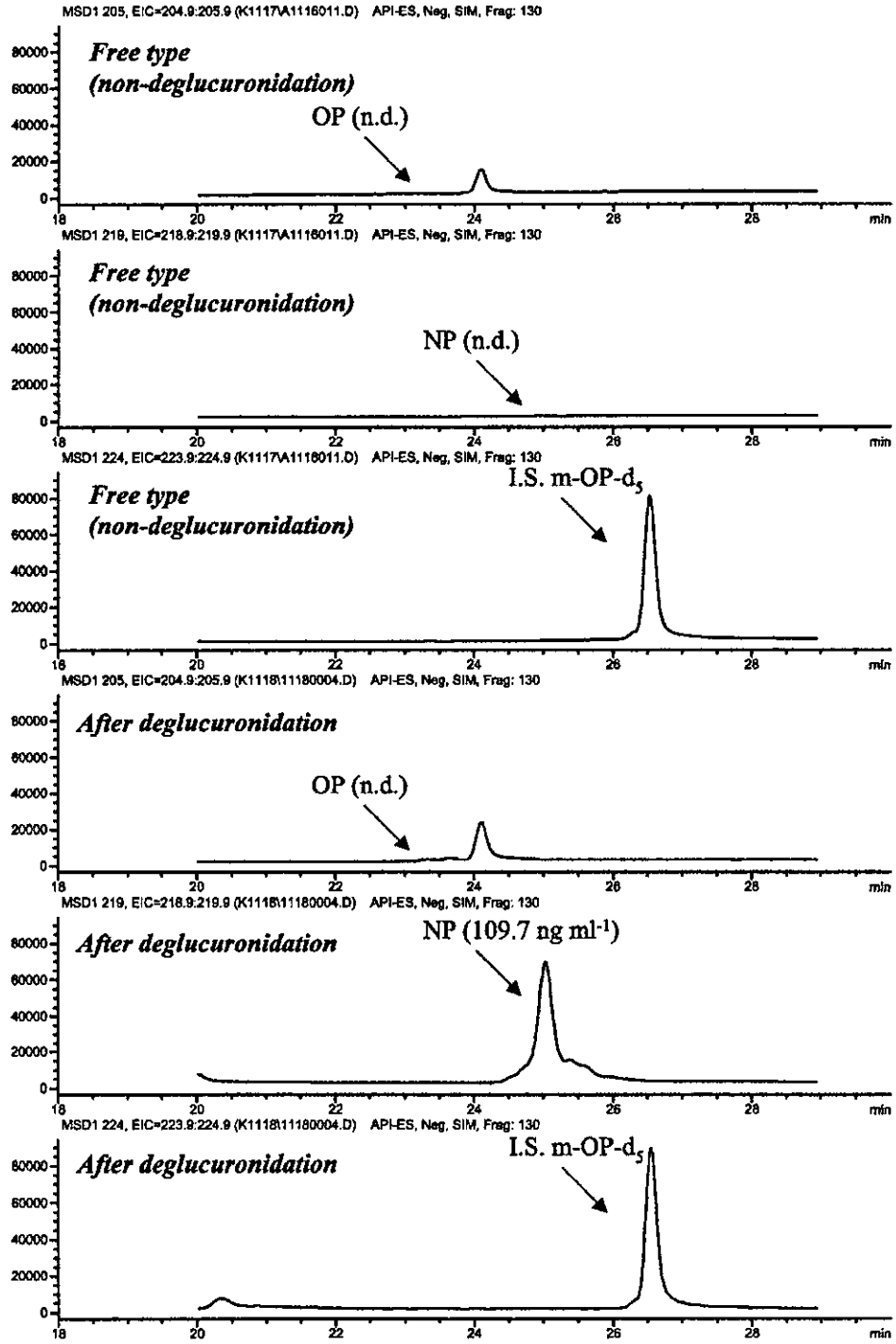

Fig. 5. Chromatograms of OP, NP and internal standard [4-(1-methyl)octylphenol-d_5: *m*-OP-d_5] in human urine sample (volunteer H, case 2).

half-life from blood and the bioavailability (determined from oral and intravenous AUCs) were found to be 2–3 h and 20%, respectively [26]. The low bioavailability was due to extensive metabolism during the first passage in the liver and excretion via the urine. To evaluate the potential estrogenic effects of these compounds on human, the levels of human exposure must be investigated. In an estimation of the daily intake of NP, the oral intake of NP by non-occupationally exposed human was found to be less than 0.16 mg per day [27]. In an other study, NP in fish products available commercially was detected at the level of 9–800 ng g^{-1} [28]. That study suggested that most of the NP detected in the fish products was due to migration from food wrapping film.

We investigated the main source of human exposure by the levels of NP in human urine samples. The volunteers had taken a meal of rice or meat wrapped in polyvinyl chloride (PVC) film. The experiment plan is following: PVC film was most contamination of NP than other in our study [16], these foods wrapped using PVC film were re-heated in a microwave oven for 1 min, volunteer H and J had taken rice and meat wrapped in PVC film, volunteer I had taken rice wrapped in PVC film, and rough estimations of intake amount of NP in this meal were above 35 μg (volunteer H and J) and below 35 μg (volunteer I) using our report [16]. Than, three urine samples on first time after meal were used that we examined the NP exposure using the present method.

The urinary glucuronidated NP was detected at the levels of 109.7 (volunteer H), 15.9 (volunteer I), and 110.9 ng ml^{-1} (volunteer J) (Table 5, case 2). Fig. 5 shows the chromatograms obtained from volunteer H's urine sample. It is therefore reasonable to surmise that the main source of human exposure is the ingestion of food contaminated by NP that leached from the PVC film. Recently, there have been on the contamination and leaching of NP from PVC films [17–19]. In addition, the potential exposure to NP due to the use of tris(nonylphenyl) phosphate in food-contacting materials was calculated [29]. In our previous study, we demonstrated that NP may be leached from food wrapping film into foods in varying degrees [17]. Moreover, daily exposure to NP in healthy human may take place by taking a meal of NP-contaminated foods from the food wrapping film. Certain populations may be exposed to NP; in addition, infants and the developing fetus may be exposed at critical points of their development. However, little research has been conducted on the effects of NP on the human health including epidemiological, analytical, and toxicological studies. We confirmed that there is a need for developing non-NP-containing or contamination-free plastic food containers for use in our daily lives.

Acknowledgements

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Notes

Determination of Trace Amounts of Bisphenol A in Urine by Negative-Ion Chemical-Ionization-Gas Chromatography/Mass Spectrometry

Tadashi TSUKIOKA,*1 John BROCK,*2 Sam GRAISER,*2 Johnny NGUYEN,*2 Hiroyuki NAKAZAWA,*3 and Tsunehisa MAKINO*4

*1 *Nagano Research Institute for Health and Pollution, 1978 Komemura-Amori, Nagano 380-0944, Japan*
*2 *Centers for Disease Control and Prevention, 4770 Buford Highway NE Atlanta, GA, USA*
*3 *Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan*
*4 *Tokai University, Bouseidai, Isehara, Kanagawa 259-1193, Japan*

We improved an analytical method for determining trace amounts of bisphenol A (BPA) in urine. BPA was subjected to enzymolysis and then to solid phase extraction with a C_{18} cartridge. The extract was eluted with methanol, and the eluate was concentrated under a nitrogen stream, and then pentafluorobenzylized in an alkali solution. The obtained pentafluorobenzylized compound was purifed using a florisil cartridge, followed by a determination using NCI-GC/MS. This method exhibited an excellent selectivity and reproducibility with a determination limit of 0.1 ng/ml.

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Introduction

BPA, a suspected endocrine disruptor, has been widely used as a plastic material for polycarbonate and epoxy resins. There has been great concern about the release of bisphenol A (BPA) into the environment, migration into food stuffs, exposure to humans, and potential endocrine disrupting effects on humans. There has been no report on the daily intake of BPA by humans. We improved an analytical method for determining trace amounts of BPA in urine so that the intake of BPA by humans could be estimated.

There have been reports on the determination of trace amounts of BPA using HPLC,[1,2] LC/MS,[3-7] and GC/MS.[8-13] Analyses using HPLC and LC/MS have an advantage in that they do not require derivatization, but analyses using HPLC exhibit poor selectivity, separability and sensitivity, and analyses using LC/MS exhibit poor sensitivity. Analyses using GC/MS enable a direct anaylsis, but require derivatization for determination at low concentrations. Thus, the Japanese Ministry of the Environment proposed a method[13] in which BPA is trimethylsilylized (TMS), and then subjected to GC/MS-SIM using electron ionization (EI). Analyses using negative-ion chemical ionization (NCI), which makes use of an electron-capture reaction, can achieve a highly sensitive and selective detection of chemicals having a high electron affinity. Nakamura *et al.*[8] determined BPA in river water at pg/ml levels using NCI-GC/MS after pentafluorobenzylization of the BPA. Brock *et al.*[9] determined BPA in urine and Yoshimura *et al.*[10] determined it in serum using NCI-GC/MS after pentafluorobenzylization of the BPA. A highly sensitive analysis is required for biological samples, since only small amounts of biological samples can be obtained because of the existence of a large number of coexisting substances. We investigated in detail the methods of Brock *et al.*[9] and Yoshimura *et al.*,[10] while improving the method of derivatizing into a pentafluorobenzylized compound, followed by determining by means of NCI-GC/MS with a determination limit of 0.1 ng/ml in a 2 ml urine sample. This method has excellent selectivity, determination limit, and precision, and is applicable to real samples.

Experimental

Reagents

Sodium hydroxide was purchased from Mallinckrodt and tetrabutylammoniumhydrogensulfate was obtained from Kodak. Dichloromethane, ethyl acetate, 2,2,2-trimethylpentane, methanol, and purified water were purchased from Caledon. Pentafluorobenzylbromide was a product of Superuco and bisphenol A and formic acid were products of Aldrich. ^{13}C-BPA was purchased from Cambridge Isotope. C_{18} cartridges and florisil cartridges were obtained from J. T. Baker, and C-cartridges were purchased from Superuco. Ammonium acetate was a product of Sigma, and β-glucuronidase solution (200 U/ml) was purchased from Roche Biochemical.

BPA free-water was obtained by using a C-cartridge. Ammonium acetate solution containing 1% β-glucuronidase was prepared by dissolving β-glucuronidase in a 1 M ammonium acetate solution that had been adjusted to have a pH of 6.5 by glacial acetic acid so that the concentration of β-glucuronidase would be 1%.

Urine samples were collected in glass bottles in an amount of about 200 ml after informed consent.

Method

Two milliliters of a sample were put in a 15 ml test tube, to which 20 µl of a 0.076 µg/ml ^{13}C-BPA solution was added as a surrogate compound. To the mixture, 200 µl of ammonium

acetate solution containing 1% β-glucuronidase was added, followed by enzymolysis at 37°C for 90 min. To the resultant mixture, 1 ml of 32% formic acid was added, followed by irradiation with ultrasonic waves for 5 min. The thus-obtained mixture was loaded into a C_{18} column that had been prewashed with 10 ml of methanol and 5 ml of purified water. After washing with 5 ml of a 10% methanol solution, the mixture was eluted into a 15 ml centrifuging tube with 3 ml of methanol; to the resultant eluate, 0.5 ml of a 0.2 M sodium hydroxide solution was added, followed by blowing nitrogen gas, to thereby concentrate the eluate to approximately 0.2 ml. To the concentrated eluate, 2 ml of dichloromethane, 0.5 ml of a 0.2 M sodium hydroxide solution, 0.5 ml of a 0.1 M tetrabutylammoniumhydrogensulfate solution, and 20 µl of pentafluorobenzylbromide were added; the tube was sealed with an aluminium closure, followed by irradiation with ultrasonic waves for 20 min using an ultrasonic washer. Then, the resultant eluate was subjected to centrifugation at 3000 rpm for 5 min, and the lower layer was collected with a pipet into a 10 ml centrifuging tube, followed by blowing nitrogen gas, to thereby allow dichloromethane to evaporate. After the residue was dissolved in 0.5 ml of isooctane, the mixture was loaded into a florisil cartridge that had been prewashed with 10 ml of isooctane, followed by washing with 10 ml of isooctane containing 1% ethyl acetate. Subsequently, BPA was eluted with 5 ml of isooctane containing 10% ethyl acetate, and the eluted BPA was concentrated to dryness with nitrogen gas, and then dissolved in 0.5 ml of isooctane. The resultant mixture was subjected to GC/MS.

GC/MS conditions

GC/MS conditions were as follows: GC, Agilent 6800; column, HP-5MS, 30 m × 0.25 mm × 0.25 mm; column temperatures, 60°C - 15°C/min - 215°C (7 min) - 20°C/min - 300°C (5 min); inlet temperature, 245°C; carrier gas, He, flow velocity 1 ml/min; MS, Agilent 5973; reaction gas, methane, flow velocity, 2.5 ml/min; ion-source temperature, 230°C; monitor ions (*m/z*): 407 (BPA), 419 (^{13}C-BPA).

Results and Discussion

Hydrolysis of a glucuronide conjugate

BPA is considered to be excreted in urine as a glucuronide conjugate and a sulfate conjugate.[14] Because Brock *et al.*[9] did not investigate in detail the hydrolysis of the glucuronide conjugate of BPA in urine, we investigated the amount of β-glucuronidase and the time required for the hydrolysis of a glucuronide conjugate of BPA. Figure 1(A) shows the BPA concentrations when 1 - 5 µl of β-glucuronidase was added to 2 ml of urine, followed by enzymolysis at 37°C for 90 min. A glucuronide conjugate of BPA was found to be hydrolyzed by almost 100% by the addition of 1 µl of the enzyme to 2 ml of urine. We chose to add 2 µl of the enzyme. Figure 1(B) shows the relationship between the BPA concentration and the hydrolysis time when 2 µl of β-glucuronidase was added to 2 ml of urine, followed by enzymolysis at 37°C. The result shows that hydrolysis was almost completed in 60 min. In this method, we chose to perform enzymolysis for 90 min.

Extraction, washing, and elution using a C_{18} cartridge

The extraction of phenols using a C_{18} cartridge is generally performed with a pH of 3, or lower. In this method, 1 ml of 32% formic acid was added to denature protein in urine, resulting in a pH of 3 or lower. Since a variety of substances

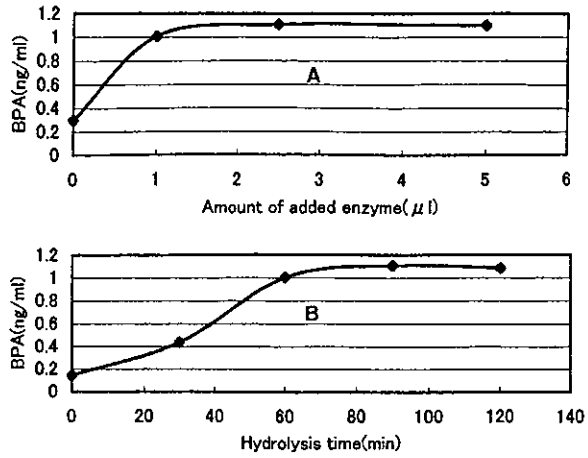

Fig. 1 Hydrolysis of glucuronide conjugate of BPA. (A) amount of added enzyme and BPA concentration; (B) hydrolysis time and BPA concentration.

exist in urine, the removal of as many substances as possible, except for the target substance, with a washing solvent and derivatization is required. In this method, the use of 10 ml of a 10% methanol solution as a washing solvent was found to cause the no elution of the target substance, and the use of 3 ml of methanol was found to cause the elution of the target substance by almost 100%.

Reaction with pentafluorobenzylbromide

Brock *et al.*[9] and Yoshimura *et al.*[10] used a circulator for pentafluorobenzylization. However, this method has a drawback in that data may not be obtained due to leakage from the stopper. We studied the use of ultrasonic waves for pentafluorobenzylization, and found that the production ratio obtained after irradiation with ultrasonic waves for 20 min was comparable to the production ratio obtained by using a circulator. In the NCI mode, the predominant ion (*m/z* = 407) is formed by the loss of one of the pentafluorobenzyl moieties. Based on the obtained NCI mass spectrum, we chose *m/z* = 407 (BPA) and *m/z* = 419 (^{13}C-BPA) as fragment ions for the determination.

Clean-up using a florisil cartridge

NCI is generally believed to have relatively few interfering peaks. In reality, however, without any clean-up, interfering substances and peaks would overlap, producing errors. We compared the clean-up using a florisil and a silicagel cartridge, and found that a florisil cartridge achieved a greater clean-up effect. Accordingly, we chose a florisil cartridge for this method. Pentafluorobenzylized compounds of BPA were found not to elute unless the polarity of the elution solvent was increased. Thus, in this method, after washing with 10 ml of isooctane containing 1% ethyl acetate, BPA was eluted with 5 ml of isooctane containing 10% ethyl acetate. Washing with isooctane containing 1% ethyl acetate caused pentafluorobenzylized compounds, such as nonylphenol and octylphenol, to elute. When the concentration of ethyl acetate was 20% or higher, interfering substances were found to elute and overlap with the BPA peaks, causing positive interference. Accordingly, we chose the concentration to be 10%.

Preparation of a calibration curve and a recovery test

A calibration curve was prepared in the following manner.

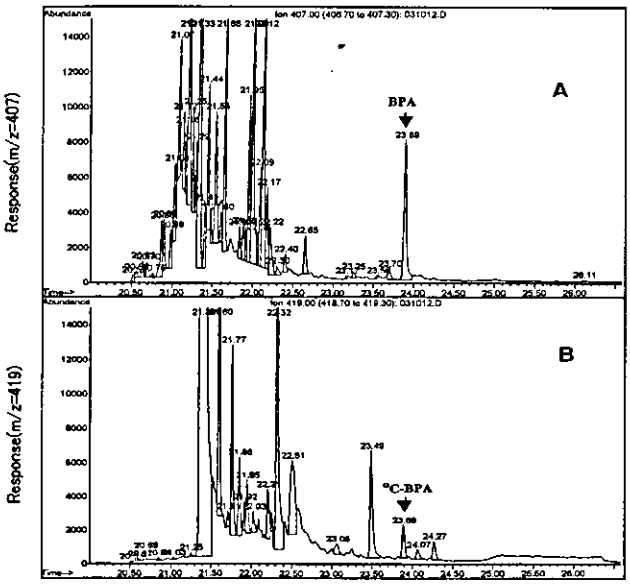

Fig. 2 SIM chromatograms of urine sample. (A) BPA; (B) ^{13}C-BPA.

Standard solutions having concentrations in the range of 0.05 μg/ml to 1.0 μg/ml were prepared, and 20 μl of the solution was put in a test tube, to which a surrogate compound was added. Then, the reaction and its succeeding procedures described in *Method* were performed and a calibration curve was prepared based on the area ratio in relation to the surrogate compound. The calibration curve remained linear up to concentrations of 2 μg/ml for the GC/MS inlet. One nanogram of BPA was added to 2 ml of urine to perform a recovery test, and the obtained recovery was found to be 83% with a standard deviation of 7.4% ($n = 5$).

Application to real samples

The proposed method was applied to urine samples ($n = 6$). BPA was detected in all of the urine samples in the range of 0.2 to 3.8 ng/ml with the average being 1.6 ng/ml. Figure 2 shows an SIM chromatogram of the urine samples.

Conclusion

We improved an analytical method for determining trace amounts of BPA in urine so that the exposure of humans to BPA could be estimated. BPA in urine was subjected to enzymolysis, and then to solid phase extraction with a C_{18} cartridge. The extract was eluted with methanol, and the eluate was concentrated by blowing nitrogen gas, and then pentafluorobenzylized in an alkali solution. The obtained pentafluorobenzylized compound was cleaned up using a florisil cartridge, followed by determination by NCI-GC/MS. This method exhibited an excellent selectivity and reproducibility with a determination limit of 0.1 ng/ml. When this method was applied to real samples, BPA was detected in all of them.

Acknowledgements

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Development of Analytical Method for Determining Trace Amounts of BPA in Urine Samples and Estimation of Exposure to BPA

Tadashi TSUKIOKA[1], Jun-ichi TERASAWA[1], Shouichiro SATO[1], Yoshiyuki HATAYAMA[1], Tsunehisa MAKINO[2] and Hiroyuki NAKAZAWA[3]

[1]Nagano research Institute for Health and Pollution
(1978 Komemura, Amori, Nagano 380-0944)
[2]Tokai University
(Bouseidai, Isehara, Kanagawa 259-1193)
[3]Hoshi University
(2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501)

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Abstract

We have developed a reliable analytical method for determining trace amounts of bisphenol A (BPA), a suspected endocrine disruptor, in urine samples by use of GC/MS so that daily exposure of human bodies to BPA can be estimated. We administered BPA-d_{16} (100 μg) to volunteers in order to conduct an excretion experiment and found that the BPA was absorbed quickly through the digestive tract and excreted mainly as a glucuronide conjugate into urine, in an amount of almost 100 % in 24 hours. The results suggest that determining the BPA content of urine samples enables estimation of the exposure to BPA.

The results of our analysis of urine from adults show that the average total BPA concentration was 0.82 ng/mℓ (0.14~5.47 ng/mℓ, n=91) and that the average free BPA concentration was 0.08 ng/mℓ (0.01~0.27 ng/mℓ, n=11). Also, from a determination of whole-day urine samples, the exposure to BPA was estimated to be 1.68 μg/day (0.48~4.5 μg/day, n=22) on the average.

Key words: BPA, endocrine disruption, GC/MS, exposure

INTRODUCTION

Bisphenol A [2,2-bis(4-hydroxyphenyl)propane; BPA], a suspected endocrine disruptor, has been widely used in large amounts as a raw material for polycarbonate, epoxy resin, phenol resin, polyester, antioxidant, and a stabilizer for vinyl chloride. This has led to a great concern about human exposure to BPA and potential effects on the human body. BPA does not exhibit high acute toxicity; LD_{50} in rats is 3250 mg/kg (oral)[1]. Some reports have stated that BPA is an endocrine disruptor: BPA shows a weak estrogenic activity in an E-SCREEN test on the cellular level[2], and promotes the multiplication of human MSF-7 breast cancer cells[3]. In animal tests, upon exposure to BPA female mice show a decrease in the number of litters delivered and the number of offspring per litter, as well as a decrease in birth weight, and male mice show a decrease in the number of offspring, an increase in the weight of seminal vesicle, and a deterioration in the motility of sperm cells[4].

Conventionally, exposures to a substance via differ-

ent pathways are estimated from the concentrations of the substance in food, air, water, and the like, as well as the amounts of intake, and the estimated amounts are added together in order to estimate the amount of exposure to a chemical substance. However, the conventional method has a drawback in that a large amount of work is required. If the exposure to BPA can be estimated from the amount of excreted BPA in urine, the amount of work and cost will be substantially reduced.

Reported methods for determining BPA include GC/MS[5-8], LC-MS[9-12], HPLC[13,14], and ELISA[15]. However, most of the instruments used for collection, storage, and clean-up of samples are composed of high polymer materials. Thus they may contaminate samples, resulting in an overestimation when trace amounts of BPA in biological samples are to be determined. Accordingly, urgent demand exists for a highly reliable and sensitive analytical method for determining BPA that is free from the risk of contamination.

In our method, the BPA in urine is subjected to an enzymolysis and then to a solid phase extraction by use of a C_{18} cartridge. The extract is trimethylsilylated (TMS), and the TMS derivative obtained is purified with a florisil cartridge, and then determined using GC/MS-SIM. We aim to estimate the human daily exposure to BPA by determining the concentration and amount of excreted BPA in urine samples by employing our method.

EXPERIMENTAL SECTION

Reagents

BPA was a product of Wako Pure Chemicals. ^{13}C-BPA was purchased from Cambridge Isotope. Purified water was obtained by filtering super pure water through an activated charcoal cartridge. (β-Glucuronidase was a product of Wako Pure Chemicals intended for biochemical use. C_{18} cartridge used was a Supelclean ENVI-18 (0.5 g) from Supelco, and the florisil cartridge was a Supelclean ENVI-florisil (0.5 g) from Supelco. BSTFA (*N,O*-bis[trimethylsilyl]trifluoroacetamide) was purchased from GL Sciences, Inc. Methanol, ethyl acetate, *n*-hexane, and acetone were products of Kanto Kagaku intended for analysis of agricultural chemical residues. Phosphoric acid was a product of Wako Pure Chemicals of special grade.

Apparatus

The apparatus used were an ultrasonic washer (Shibata SU-3TH), a vacuum pump (Yamato WP-25), a solid phase extraction equipment (Spelco VISIPREP DL), a super pure water production equipment (Millipore Milli-Q-ST), and a GC/MS (JEOL GC-mate).

Collection of urine samples

All the urine samples were collected after the procedure of informed consent.

Exposure experiment: A subject orally consumed 100 mℓ of a drink containing 100 μg of BPA-d_{16}, and urine samples were collected at predetermined intervals for 26.5 hours after intake (n=1). Twelve male and thirteen female volunteers each orally consumed 100 mℓ of a drink containing 50 μg of BPA-d_{16}, and urine samples were collected for 5 hours after intake.

Urine samples were collected from 46 male and 23 female volunteers at least twice, and samples from the same subject were combined.

Whole-day urine samples were collected from 11 male and 11 female volunteers.

Method

Total BPA: 100 mℓ of a urine sample was placed in a conical flask equipped with a ground stopper, and 100 $\mu\ell$ of β-glucuronidase solution and 0.1 μg of ^{13}C-BPA were added to the flask. The mixture was subjected to an enzymolysis at 37 ℃ for 90 minutes. To the resultant mixture, 1 mℓ of 7.5 M phosphoric acid was added in order to adjust pH to 3 or lower. The mixture thus obtained was loaded onto a C_{18} cartridge that had been activated with 5 mℓ of methanol and 10 mℓ of purified water, to extract BPA. After the cartridge was washed with 10 mℓ of 10 % methanol, 3 mℓ of methanol was added to elute BPA. The eluate was collected in a 100 mℓ eggplant-shaped flask. To the eluate, 20 mℓ of ethyl acetate was added, and concentrated to dryness by use of a rotary evaporator. To the flask, 200 $\mu\ell$ of BSTFA and 2 mℓ of acetone were added, and the mixture was allowed to stand overnight to be trimethylsilylated, and the stripped of acetone by use of a rotary evaporator. To the resultant mixture, 2 mℓ of *n*-hexane was added and dissolved by use of an ultrasonic washer. The resultant mixture was loaded onto a florisil cartridge that had been prewashed with 5 mℓ of *n*-hexane, and the eluate was collected in a test tube. Subsequently, the flask was

washed twice with 2 mℓ of *n*-hexane each time, and the mixtures obtained were loaded onto the cartridge. The eluate was added to the previously obtained eluate in a test tube. The resultant eluate was concentrated to 1 mℓ by blowing nitrogen gas, and then subjected to an analysis by GC/MS-SIM.

Free BPA : To 200 mℓ of a urine sample, which had not been subjected to enzymolysis, 2 mℓ of 7.5 M phosphoric acid and 0.05 μg of ^{13}C-BPA were added. The subsequent procedure was the same as that described above for total BPA.

Calibration curve: BPA was placed in test tubes stepwise in an amount of 10~200 ng, and 100 ng of ^{13}C-BPA was added as a surrogate, and then 200 μℓ of BSTFA was added. The volume of the resultant mixture was increased to 1 mℓ by adding acetone. The mixture obtained was allowed to stand overnight and then subjected to a GC/MS-SIM. A calibration curve was constructed on the basis of area ratios relative to ^{13}C-BPA.

GC/MS conditions

GC separation was carried out with an HP-5890 series Ⅱ. GC conditions were as follows: column DB-5MS, inner diameter 0.32 mm, length 30 m, film thickness 0.25 μm; column temperatures 70 ℃ (2 min) —20 ℃ /min—150 ℃—10 ℃ /min—300 ℃ (5 min), inlet port temperature 250 ℃; carrier gas He, flow velocity 1 mℓ/min; injection method splitless, purge off 1 min.

MS analysis was carried out with an JEOL GC-mate. The SIM conditions were as follows: ion source temperature 230 ℃; ionization voltage 70V; monitor ions (m/z), BPA (357, 372), BPA-d_{16} (368), and ^{13}C-BPA (369).

RESULTS AND DISCUSSION

Enzymolysis of conjugates

Insoluble compounds taken into the body are converted into highly water-soluble glucuronide conjugates during the metabolic process before excretion. Phenol compounds are believed to be excreted mainly as a glucuronide conjugate. Thus, we studied the amount of β-glucuronidase and the incubation time required for decomposition of the glucuronide conjugate of BPA. The results show that the decomposition was almost completed by addition of 50 μℓ of β-glucuronidase to 100 mℓ of urine followed by incubation at 37 ℃ for 60 minutes. In our analysis using real samples, we chose to add 100 μℓ of β-glucuronidase and perform enzymolysis at 37 ℃ for 90 minutes, in order to provide a margin of safety.

Extraction, washing, and elution using the C_{18} cartridge

Phenol compounds are generally subjected to solid phase extraction at a pH of 3 or lower. The C_{18} cartridge used in our analysis allows extraction under this condition. In our analysis, after the cartridge was washed with 10 % methanol, an addition of 3 mℓ of methanol allowed elution of BPA. No elution of BPA from the cartridge was observed.

Trimethylsilylation of BPA

Since BPA is absorbed in a GC column when subjected to direct analysis at low concentrations, determination of BPA at extremely low concentrations is difficult and requires derivatization. Commonly used forms of derivatization include pentafluorobenzylization (PFB) and trimethylsilylation (TMS). For our analysis, since we use urine samples which can be collected in a large amount, we chose TMS. Since urine samples are considered to contain a variety of substances that act on BSTFA, 200 μℓ of BSTFA was added to each sample, and the resultant mixture was allowed to stand overnight. Fig. 1 shows the mass spectrum of BPA-TMS, and Fig. 2 shows the mass spectrum of ^{13}C-BPA-TMS.

Clean-up using the florisil cartridge

Since urine samples contain a variety of metabolites, trimethylsilylation of extracts was found to produce a tar-like substance and require a clean-up treatment for determination. We studied a clean-up using a florisil cartridge and found that *n*-hexane caused trimethylsilylated compounds of BPA to elute easily and that clean-up was achieved by merely allowing a reagent dissolved in *n*-hexane to pass through the cartridge.

Excretion of BPA

In order to study the kinetics of excretion of BPA, 100 mℓ of a drink containing 100 μg of BPA-d_{16} was administered, and urine samples were collected at predetermined intervals for 26.5 hours after the intake. The results are shown in Fig. 3. The concentration of BPA in urine reached 90 ng/mℓ after 30 minutes, then

decreased to 26 ng/mℓ 60 minutes after intake, and 5 hours after intake, the concentration further decreased to the proximity of the concentration before the intake. BPA was found to be absorbed quickly through the digestive tract and excreted mainly as a glucuronide conjugate into urine in almost 100 % in 24 hours.

BPA concentrations in urine

The average BPA concentration in urine samples was 0.81 ng/mℓ (0.14~5.47 ng/mℓ), and three subjects had a concentration higher than 2 ng/mℓ. The subject who had the highest concentration, 5.47 ng/mℓ, had an extremely small amount of urine. Fig. 4 shows an SIM chromatogram of urine sample and Fig. 5 shows a histogram which has a bell shape with the mean almost at the center. The analysis of whole-day urine samples (Table 1) shows that the average concentration of BPA was 0.81 ng/mℓ (0.24~2.03 ng/mℓ),

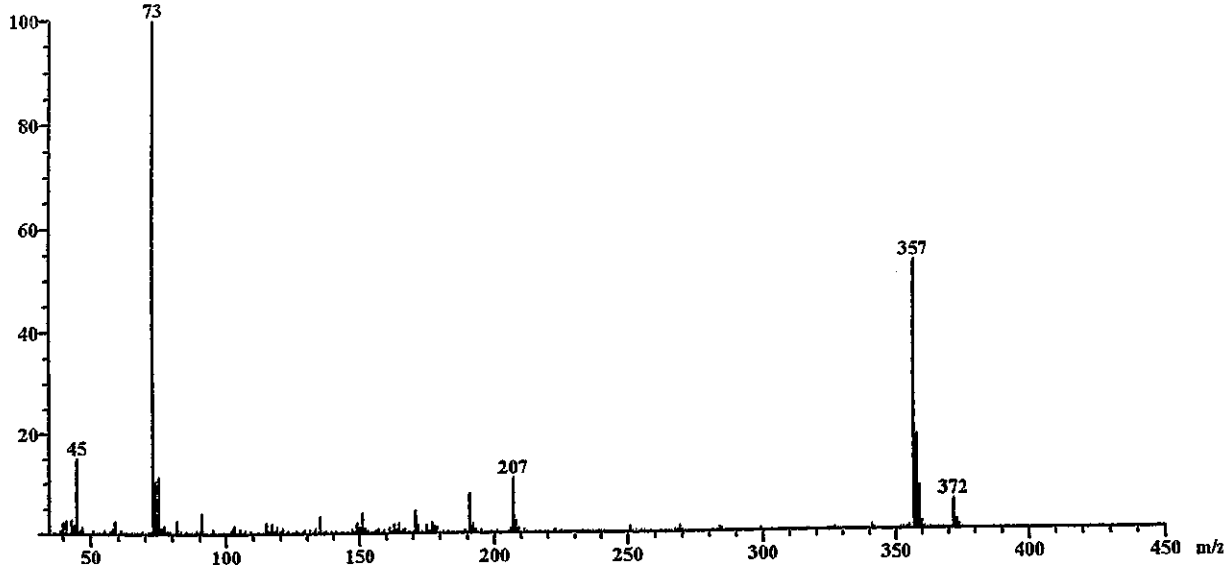

Fig. 1 EI mass spectrum of the BPA derivative. Measurement conditions: column DB-5MS, inner diameter 0.32 ㎛, length 30 m, film thickness 0.25 ㎛; column temperature 70℃ (2min)-20℃/min-150℃-10℃/min-300℃(5min); carrier gas He at a flow rate of 1 mℓ/min; ion source temperature 230℃: ionization voltage 70V

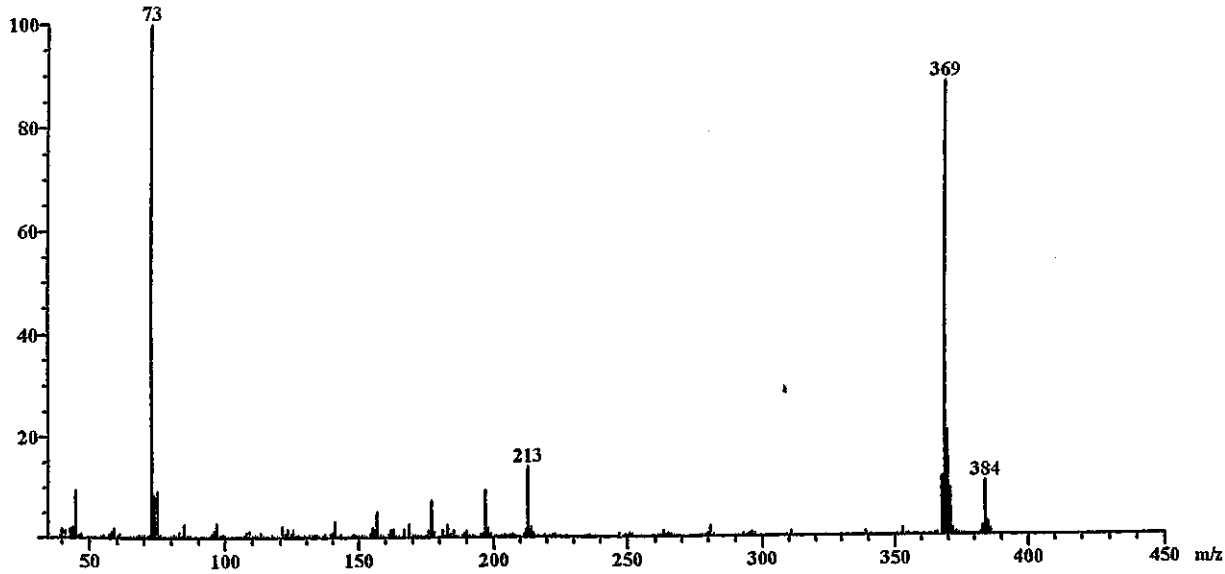

Fig. 2 EI mass spectrum of the ^{13}C-BPA derivative. Measurement conditions were the same as Fig. 1

the average amount of urine was 2055 mℓ (1030～3900 mℓ), and that the average amount of excreted BPA was 1.68 μg (0.48～4.5 μg/day). The amount of excreted BPA was found to be significantly less than the amount we had expected. This may be due to a drop in exposure to BPA achieved by an improvement in inner coatings of cans and the like. The excreted BPA is considered to be derived from food, but the type of food it is derived from remains unknown.

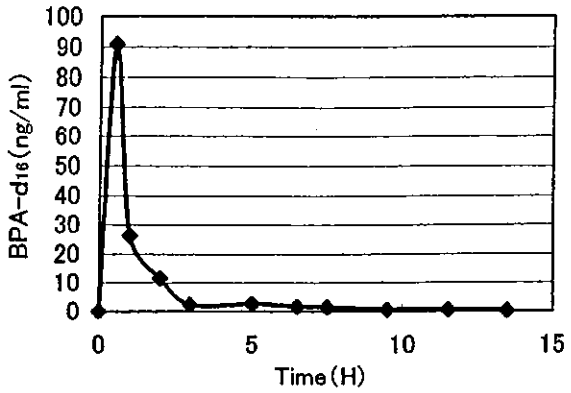

Fig. 3 Relationship between the concentration of the total BPA-d_{16} in urine and the elapsed time after 100 mℓ of a drink containing 100 μg of BPA-d_{16} was consumed

The conjugates of BPA and free BPA are considered to have different endocrine disrupting effects. Thus, determination of free BPA in urine was required. A drink containing 50 μg of BPA-d_{16} was given to volunteers, and urine samples were collected for 5 hours after intake. The results are shown in Fig. 6. The average total BPA concentration was 57.2 ng/mℓ (26.5～80 ng/mℓ), and the average free BPA concentration was 1.13 ng/mℓ (0.13～5.8 ng/mℓ). The average amount of excreted BPA was 38 μg (17.6～48.6 (μg), which means that 76 % of the given BPA was excreted after 5 hours. The average ratio of free BPA was 2.0 % (0.34～8.1 %). The subjects who showed higher ratios of free BPA may have been affected by their body conditions; in particular, liver function on the day of the experiment. Normal urine samples were also analyzed. The average total BPA concentration was 0.56 ng/mℓ (0.19～1.38 ng/mℓ), and the average free BPA concentration was 0.08 ng/mℓ (0.01～0.27 ng/mℓ). The average ratio of free BPA was 12 % (2.6

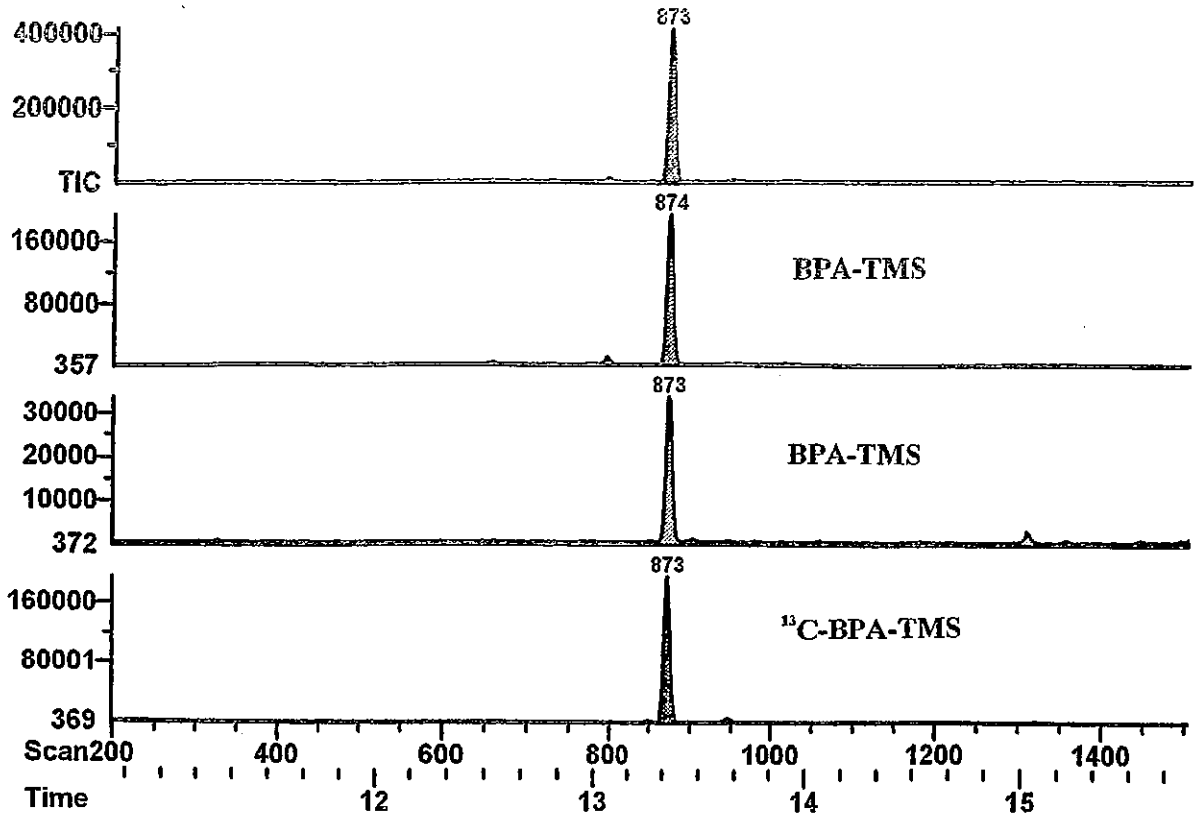

Fig. 4 SIM chromatogram of a real urine sample. Measurement conditions were the same as given in 2.5 GC/MS conditions

−29 %).

CONCLUSION

1. We developed a reliable analytical method for determining trace amounts of BPA in urine.
2. We conducted an excretion experiment by administering 100 μg of BPA-d_{16} to volunteers and found that BPA was absorbed quickly through the digestive tract and excreted mainly as a glucuronide conjugate into urine, in almost 100 % in 24 hours. Thus, the determination of the BPA in whole-day urine samples is considered to enable the estimation of the exposure to

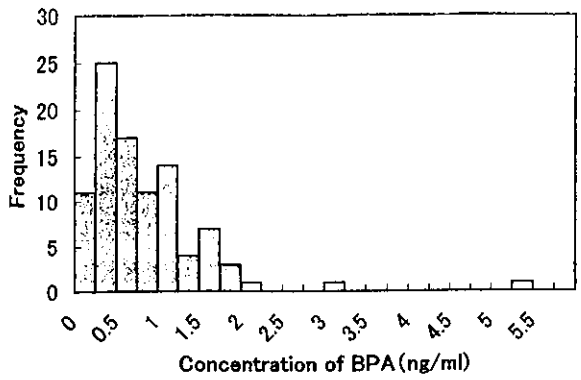

Fig. 5 Histogram of the BPA in urine samples (n =91)

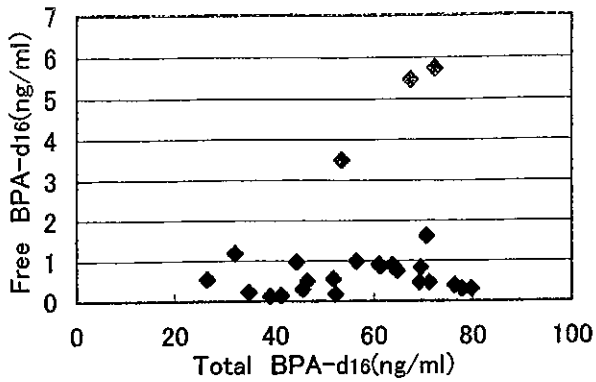

Fig. 6 Relationship between the concentrations of the total BPA-d_{16} and the free BPA-d_{16} in urine after 100 mℓ of a drink containing 50 μg of BPA-d_{16} was consumed

Table 1 Results of whole day urine samples

No.	Volume(ml)	BPA(ng/ml)	BPA(μg/day)
1	1567	0.36	0.56
2	1832	1.20	2.20
3	1846	0.57	1.05
4	1030	1.17	1.21
5	1910	0.64	1.22
6	2271	0.27	0.61
7	2125	0.58	1.23
8	2586	1.28	3.31
9	1296	0.37	0.48
10	2293	0.24	0.55
11	2137	0.82	1.75
12	1146	0.80	0.92
13	2664	0.43	1.15
14	1903	2.03	3.86
15	2800	1.57	4.40
16	2120	1.20	2.54
17	1950	0.93	1.81
18	1843	0.45	0.83
19	1460	0.62	0.91
20	1490	0.40	0.60
21	3060	1.48	4.53
22	3900	0.34	1.33
Ave.	2055±665	0.81±0.49	1.68±1.26

BPA.

3. The average total BPA concentration in urine samples was 0.82 ng/mℓ (0.14~5.47 ng/mℓ, n=91), and the average free BPA concentration was 0.08 ng/mℓ (0.01~0.27 ng/mℓ, n=11).

4. From the determination of whole-day urine samples, the exposure to BPA was estimated to be 1.68 μg/day (0.48~4.5 μg/day, n=22) on the average.

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GC及びGC/MSによる文具、化粧品、家庭用品等に含まれる可塑剤フタル酸及びアジピン酸エステル類の分析

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岡　尚男[a)]、伊藤裕子[a)]、後藤智美[a)]、猪飼誉友[a)]、近藤文雄[a)]、松本　浩[a)]、牧野恒久[b)]、中澤裕之[c)]

a)愛知県衛生研究所
b)東海大学医学部
c)星薬科大学薬学部

Analysis of Plasticizer Phthalate and Adipate Esters in Stationeries, Cosmetics, and Household Commodities by GC and GC/MS

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Hisao Oka[a)], Yuko Ito[a)], Tomomi Goto[a)], Yoshitomo Ikai[a)], Fumio Kondo[a)], Hiroshi Matsumoto[a)], Tsunehisa Makino[b)], and Hiroyuki Nakazawa[c)]

a) Aichi Prefectural Institute of Public Health
b) School of Medicine, Tokai University
c) Faculty of Pharmaceutical Sciences, Hoshi University

Abstract

Fourteen plasticizers (12 phthalates and 2 adipates) were analysed in commercially available stationeries, cosmetics, and household commodities by GC and GC/MS. Among 66 samples, dibutyl phthalate (DBP), di (2-ethylhexyl) phthalate (DEHP), diheptyl phthalate (DHpP), diisononyl phthalate (DINP), and di (2-ethylhexyl) adipate (DEHA) were detected in 32 samples (eraser: 20 samples, plastic bags for fiber-tipped pen: 4 samples, soft floor carpet: 5 samples, plastic tile: 1 sample, and plastic glove: 2 samples). Among them, 15 samples contained two different kinds of the plasticizers and 4 samples have three different kinds of the plasticizers. Erasers contained the plasticizers with high frequency of 95 % (20/21) and high concentrations ranging from 2.4 % to 35.2 %, and plastic bags showed frequency of 80 % (4/5) and the concentrations around 4.4-18.0 %.

Keywords：フタル酸エステル、アジピン酸エステル、文房具、化粧品
GC/MS; phthalate; adipate; stationery; cosmetic

I　緒言

フタル酸エステル及びアジピン酸エステル類はプラスチック製消しゴムの可塑剤として、あるいは化粧品、インク、接着剤、塗料等の成分保留剤として使用され[1-3]、我々の生活環境中に氾濫している[4,5]。これらの可塑剤の安全性に関する最近の研究ではげっ歯目類等の動物実験において、肝細胞内ペロオキシソーム増殖作用[6-8]、催奇形性[9-11]、アンドロゲン依存性の組織[12,13]への影響が報告されている。一方、これらの可塑剤は消化管、肺及び皮膚から速やかに吸収され[14]、特に側鎖の短いものは皮膚吸収性が高いとされていることから、我々はこれら可塑剤の暴露を日常的に受けているものと考えられる。したがって、これら文具、化粧品、家庭用品等からの可塑剤の暴露量を早急に把握する必要がある。しかし、現在までのところ、文具等をはじめとしたこれらのものに含まれる可塑剤の質的及び量的な調査は実施されていないので、早急な実施が必要と考えられる。そこで、文具、化粧品、家庭用品等中に含まれるフタル酸エステル及びアジピン酸エステル類を分析し、これら可塑剤の含有実態を調査することとした。

連絡先：〒462-8576　名古屋市北区辻町流7-6　愛知県衛生研究所　岡　尚男
Corresponding author: Hisao Oka, Aichi Prefectural Institute of Public Health
Tsuji-machi, Kita-ku, Nagoya 462-8576, Japan

II 実験

1. 試薬および標準溶液

n-ヘキサンは残留農薬測定用2000(和光純薬製)を、アセトンはフタル酸エステル測定用(関東化学製)を用いた。フタル酸ジエチル(DEP)、フタル酸ジプロピル(DPrP)、フタル酸ジイソブチル(DIBP)、フタル酸ジブチル(DBP)、フタル酸ジペンチル(DPeP)、フタル酸ブチルベンジル(BBP)、フタル酸ジヘキシル(DHxP)、フタル酸2-エチルヘキシル(DEHP)、フタル酸ジシクロヘキシル(DcHP)、フタル酸ジヘプチル(DHpP)、フタル酸ジオクチル(DOcP)、フタル酸ジイソノニル(DINP)、アジピン酸2-エチルヘキシル(DEHA)、アジピン酸ジイソノニル(DINA)については、すべて環境分析用(関東化学製)を用いた。フタル酸エステル類及びアジピン酸エステル類の標準溶液は、それぞれの濃度が1 μg/mLになるようにn-ヘキサンで調製し、それを適宜n-ヘキサンで希釈して用いた。

2. 試薬

名古屋市内の小売店で購入した文具49検体、化粧品9検体、家庭用品8検体を用いた。

3. 試験溶液の調製

クッションフロアはその表面をはぎ取ったのち5 mm角に細切し、消しゴム等その他の固形試料はそのまま5 mm角に細切し、いずれもその2.5 gをあらかじめアセトン及びn-ヘキサンで洗浄した50 mLメスフラスコに秤量した。n-ヘキサン40 mLを加え、一昼夜放置し、メスアップした。

インク、マニキュア等の液状試料は、あらかじめアセトン及びn-ヘキサンで洗浄した直径125 mmのガラス製シャーレに直径55 mmのNo.5Aのろ紙を置き、その上に0.5 gの試料を薄くのばした。その後、シャーレに20 mLのn-ヘキサンを加え、一昼夜放置し、あらかじめアセトン及びn-ヘキサンで洗浄した20 mLメスフラスコにn-ヘキサン溶液を移し、メスアップした。

4. 分析条件

4.1 GC条件

装置:GC-14B(FID検出器付、島津製作所製)
カラム:DB-5(0.53 mm×30 m、1.5 μm、J&W製)
カラム温度:200℃から280℃まで毎分5℃で昇温後、280℃で4分間保持した。
注入口及び検出器温度:280℃
キャリアガス:窒素、40 mL/min

4.2 GC/MS条件

装置:AUTO MASS SYSTEM II(日本電子製)カラム:HP-5MS(0.25 mm×30 m、0.25 μm、ヒューレットパッカード製)
カラム温度:150℃で1分間保持後、280℃まで毎分10℃で昇温し、280℃で5分間保持した。
イオン源温度:210℃ イオン化:EI イオン化電圧:70 eV検出方法:スキャン法(m/z 50-500) 試料注入方式:スプリットレス(1分間)

III 結果と考察

1. GCによる定量及びGC/MSによる同定

Table 1に示す14種の可塑剤(フタル酸エステル:12種類、アジピン酸エステル:2種類)の標準品を、実験の部に示すGC条件下で分析した。それらの保持時間はTable 1に、また検出頻度の高いフタル酸エステルとアジピン酸エステルのクロマトグラムをFig. 1Aに示した。検量線は50 μg/mLから2000 μg/mLの間で良好な直線性を示し、文具等の分析を行うために十分な定量性が得られたので、以下の研究では実験の部に示すGC条件を用いて可塑剤を定量することとした。

また、検出された可塑剤を同定するために実験の部に示すGC/MS条件下で14種の可塑剤の標準品を分析したところ、すべてのマススペクトルからモノエステル体に由来するイオン(DHpP:m/z 265、DBP:m/z 223、DEHP:m/z 279、DINP:m/z 293、DEHA:m/z 241)と、無水フタル酸に由来するイオン(DHpP、DBP、DEHP、DINP:m/z 149、DEHA:m/z 129)が観察され、良好に同定できることが判明した。したがって、以下の研究ではこのGC/MS条件を用いて可塑剤を同定することとした。

Table 1 Retention times of phthalates and adipates

Plasticizers	Retention time (min)
Diethyl phthalate (DEP)	1.27
Dipropyl phthalate (DPrP)	2.24
Diisobutyl phthalate (DIBP)	2.83
Dibutyl phthalate (DBP)	3.41
Dipentyl phthalate (DPeP)	5.27
Butyl benzyl phthalate (BBP)	7.41
Dihexyl phthalate (DHxP)	7.41
Diheptyl phthalate (DHpP, 4 peaks)	7.90-9.19
Di (2-ethylhexyl) adipate (DEHA)	8.00
Di (2-ethylhexyl) phthalate (DEHP)	9.88
Dicyclohexyl phthalate (DcHP)	9.68
Diisononyl adipate(DINA)	10.96
Diisononyl phthalate (DINP, 8 peaks)	12.00-14.42
Dioctyl phthalate (DOcP)	12.15

2. 文具、化粧品、家庭用品等の分析

市販の消しゴム等文具49検体、マニキュア等化粧品9検体、クッションフロア等家庭用品8検体について、実験の部に示す方法で、14種類の可塑剤(フタル酸エステル:12種類、アジピン酸エステル:2種類)を分析した。代表的な検体のクロマトグラムをFig. 1B～Eに、それらのマススペクトルをFig. 2に示した。GCクロマトグラム上のピークは、対応する可塑剤の保持時間に完全一致し、マススペクトルはそれぞれの可塑剤に由来するモノエステル体及び無水体のイオンが出現していたことから、それぞれのピークはFig. 1のクロ