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Simultaneous determination of phthalate di- and monoesters in poly(vinylchloride) products and human saliva by gas chromatography–mass spectrometry

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Abstract

A gas chromatographic–mass spectrometric (GC–MS) method using selected ion monitoring (SIM) is described for the simultaneous determination of phthalate di- and monoesters in poly(vinylchloride) (PVC) products. The method consists of the following four procedures; (1) liquid–liquid extraction with ethyl acetate from the acidified aqueous homogenates of the PVC products, (2) esterification with trimethylsilyldiazomethane (TMSD) and methanol, (3) clean-up using Florisil column chromatography and (4) quantitative determination of methylated phthalate monoesters by GC–MS using SIM. The methylated monoesters show a characteristic mass fragment pattern at m/z 163, 149 and 91. The calibration curves for the monoesters were linear from 0.05 to 10 ng (injection volume 1 μl). Overall recoveries ranged from 86.6 to 94.3%. The limits of detections for these methylated derivatives were in the range of 2.0–5.0 ng/g ($S/N=3$). This method was applied to phthalate monoesters in PVC toy products. Mono-*n*-butyl phthalate and mono-2-ethylhexyl phthalate were found at levels of 6.42–11.62 μg/g and 30.50–41.81 μg/g, respectively. No monoethyl phthalate, mono-*n*-hexyl phthalate and monobenzyl phthalate were found in the toy products. The method was also applied to these compounds in human saliva.

Keywords: Phthalate esters

1. Introduction

Dialkyl phthalates are widely used as plasticizers to impart softness and flexibility to normally rigid plastics such as poly(vinylchloride) (PVC). Medical devices and toys are often made of PVC, containing the predominant plasticizers, di-2-ethylhexyl phthalate (DEHP), di-*n*-butyl phthalate (DBP) or diisononyl phthalate (DINP) [1,2]. Some dialkyl phthalates have induced testicular toxicity and other effects on the male and female reproductive tract at high dosages in rats and other animals [3,4]. Jobling et al. showed that DBP binds to the estrogen

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receptor, displaces the natural ligand from the receptor, then acts as an estrogen antagonist [5]. Recently, it has been clarified that mono-*n*-butyl phthalate (MBuP) can cause adverse effects for the development of the reproductive system in male rats [6]. DEHP has been also known as a peroxisome proliferator that induces tumors in rodent livers [7]. This effect is probably not exerted by DEHP itself but by one or more of its metabolites [8]. Lake et al. demonstrated that hepatic changes produced by DEHP could be reproduced by administration of mono-2-ethylhexyl phthalate (MEHP) [9].

Dialkyl phthalates have been found to be metabolized to the monoesters by enzymes present in many tissues. It is generally accepted that orally-ingested dialkyl phthalates are quantitatively hydrolyzed by esterases in the wall of the small intestine and pancreatic lipases and not by gut flora [10,11].

In recent years much work has been carried out to develop methods for the determination of phthalate dialkyl esters in biological samples [12], plastic toy products [13] and foods [14], and the monoalkyl esters in biological specimen [15,16]. The methods differ in detail, but almost all include extraction, derivatization, clean-up and analysis. The techniques used for concentrating plasticizers from solid and liquid samples include extraction with organic solvents [12–15] or solid-phase extraction [16]. The derivatization of phthalate monoalkyl esters is often carried out by esterification with pentafluoropropanol–pentafluoropropionic anhydride (PFP–OH-PFPA) [15], diazomethane [17] and other materials [18]. The clean-up is normally carried out by column chromatography [14,19]. For the determination of phthalate esters, gas chromatography [20–22], gas chromatography–mass spectrometry (GC–MS) [12–15,19] and high-performance liquid chromatography (HPLC) [16,23] have been used. Although routine analysis of priority important phthalate esters in solid and liquid samples have been reported by earlier workers, only a few papers have been published on the determination of phthalate monoesters in plastic products and biological samples.

In this paper, we report a rapid and sensitive procedure for simultaneous determination of phthalate di- and monoesters in PVC products and human saliva by GC–MS using selected ion monitoring (SIM).

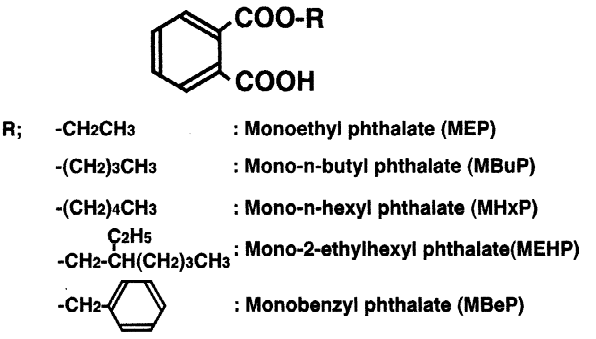

Fig. 1. Structures of phthalate monoesters.

2. Experimental

2.1. Chemicals and glassware

The phthalate monoesters are shown in Fig. 1. Monoethyl phthalate (MEP), diethyl phthalate (DEP) and DEHP were purchased from Wako (Osaka, Japan) and MBuP, mono-*n*-hexyl phthalate (MHxP), MEHP, monobenzyl phthalate (MBeP) and di-*n*-hexyl phthalate (DHxP) from Tokyo Kasei Kogyo (Tokyo, Japan). DBP and butyl-benzyl phthalate (BBP) were obtained from Kanto (Tokyo, Japan). The purity of MEP, MBuP, MHxP and MEHP were each over 90%. MBeP was 98% purity. DEP, DBP, DHXP, DEHP and BBP were more than 99.0% purity. Trimethylsilyldiazomethane (TMSD) was purchased from Aldrich (Milwaukee, USA). All other solvents and reagents were analytical grade, and confirmed as being dialkyl phthalate free. Sodium chloride and Florisil® (60–100 mesh) were purchased from Wako, and heated at 500 °C for 5 h. The Florisil® 100 g was impregnated with 6 ml water by shaking for 30 min and standing for 2 h in a 500-ml sample bottle before use. Glassware was heated at temperatures over 230 °C for at least 5 h before use.

2.2. Preparation of Florisil and Oasis® HLB columns

Florisil columns were prepared as follows: a glass column (300×15 mm ∅) packed with glass wool at the bottom was filled with 1 g of Florisil using *n*-hexane, along with 1 g of sodium sulfate

(anhydrate). Monoesters in human saliva were cleaned up by solid-phase extraction using Oasis hydrophilic lipophilic balance (HLB) cartridges (3 ml) from Waters (Milford, MA, USA). Prior to use, the cartridges were washed with 5 ml of ethyl acetate, followed by 5 ml of methanol and 10 ml of an 0.5% acetic acid aqueous solution.

2.3. Instruments

The GC operating conditions were as follows: apparatus, GCMS-QP5050 (Shimadzu, Kyoto, Japan); column, DB1 (30 m×0.25 mm I.D., film thickness 0.25 μm); carrier gas, helium at 2.0 ml/min; column temperature, 100 °C (1 min)→10 °C/min→200 °C (0 min)→2 °C/min→230 °C (0 min) →20 °C/min→270 °C (1 min); injection port and interface temperature, 260 °C. The MS conditions for electron impact (EI) ionization of monophthalates were as follows: ion energy, 70 eV; ion source temperature, 260 °C; selected ions, *m*/*z* 163, 149 and 91.

2.4. Analysis of monoalkyl phthalates in PVC products

Three PVC ball toys containing DBP and DEHP as plasticizers, and two PVC soft doll toys containing DINP as plasticizers were used for determination of phthalate monoesters. The amount of monoesters in these PVC products was determined after cutting off small pieces at an angle was measured as follows. The 1.0-g sample was dissolved in 10 ml of tetrahydrofuran, followed by addition of acetonitrile (10 ml), 5% sodium chloride solution (30 ml) and 0.01 *M* sodium hydroxide (5 ml). These solutions were filtered using glass wool. Dialkyl phthalates in the filtrate were extracted with *n*-hexane (20 ml×2). The monoesters in the aqueous acetonitrile layer acidified to pH 3 with 0.01 *M* hydrochloric acid were extracted with ethyl acetate (20 ml×2). The extracts were evaporated to dryness. The residues were added to 0.5 ml ethyl acetate and 100 μl methanol, and then 30 μl TMSD for derivatization at room temperature for 30 min [24] (Fig. 2). The derivatized solutions were evaporated to dryness under nitrogen, and the residues were dissolved in 1 ml *n*-hexane. These solutions were loaded on the Florisil® column and washed with 10 ml of *n*-hexane, and then eluted with 20 ml of 0.5% acetonitrile–*n*-hexane. Methylated monophthalates in the elutes were quantified by GC–MS.

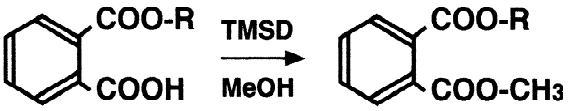

Fig. 2. Methylation of phthalate monoesters with TMSD.

2.5. Analysis of monoalkyl phthalates in human saliva

The amount of phthalate monoesters in the saliva of a volunteer was measured as follows. Human saliva (1 ml) produced by chewing a polypropylene disk without dialkyl phthalates for 15 min was acidified to pH 4 with 0.01 *M* hydrochloride, mixed with acetonitrile (1 ml), and then centrifuged at 3000 rpm (1350 x *g*) for 10 min. The supernatants were mixed with 8 ml of 0.1% acetic acid and loaded onto an Oasis HLB cartridges, and then rinsed with 5 ml of distilled water–methanol (9:1, v/v). Organic compounds remaining in the column were eluted with 10 ml of methanol–ethyl acetate (1:9, v/v). These eluates were evaporated to dryness under nitrogen. The residues were subjected to derivatization by the procedure described above. Methylated monoesters were quantified by GC–MS.

3. Results and discussion

3.1. Gas chromatographic–mass spectrometric characterization of methylated phthalate monoesters

In order to reveal the detailed GC behavior of the compounds of interest, capillary columns with different polarities were tested for the chromatography of methylated phthalate monoesters (Fig. 3). Retention time data for these compounds on the three capillary columns are summarized in Table 1, and presented graphically in Fig. 4 as a plot of the retention times against the chain length (carbon numbers) of monoalkyls in the molecule. Sharp peaks without the

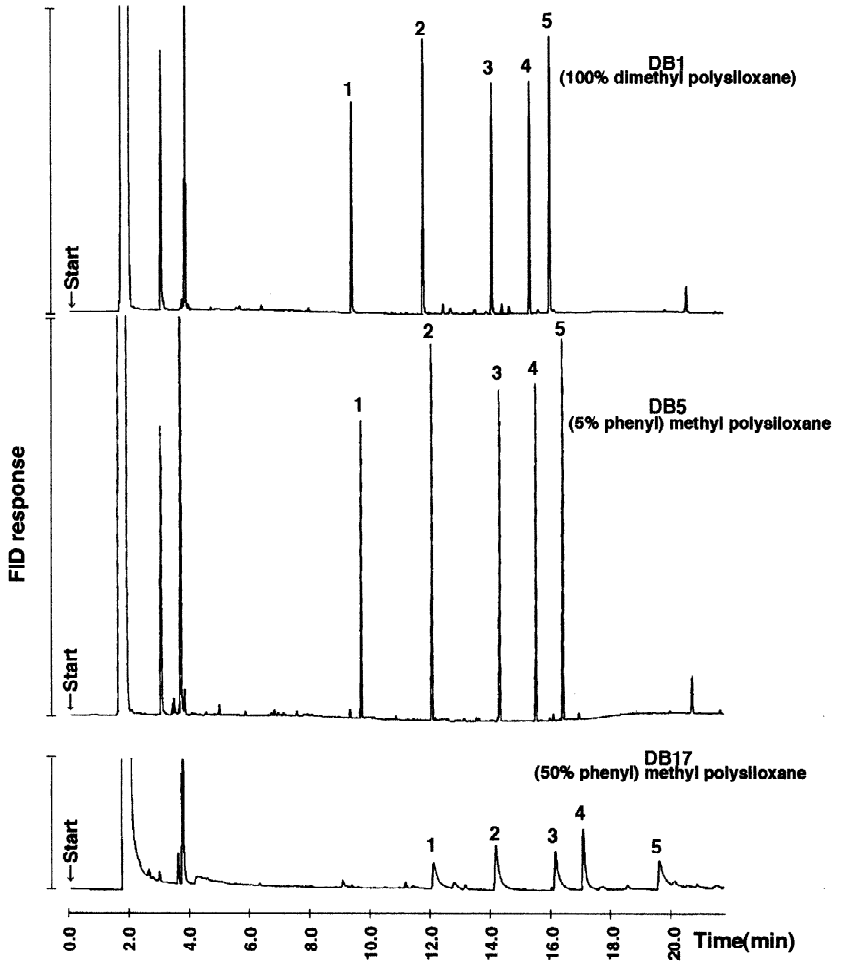

Fig. 3. Gas chromatograms (FID) of methylated phthalate monoesters on the three kinds of columns with different polarities. 1, Methylethyl phthalate; 2, methyl-*n*-butyl phthalate; 3, methyl-*n*-hexyl phthalate; 4, methyl-2-ethylhexyl phthalate; 5, methyl-benzyl phthalate. GC conditions: column temperature, 100 °C (1 min)→10 °C/min →270 °C; injection and detection port temperatures, 270 °C; carrier gas, helium (1.8 ml/min); detector, FID.

Table 1
Retention times of the individual methylated monophthalates on the three capillary columns of different polarities

Compounds	Amounts (ng)	Retention time (min)		
		DB1	DB5	DB17
Me-EP	10	9.41	9.73	12.00
Me-BuP	10	11.79	12.08	14.06
Me-HxP	10	14.07	14.33	16.06
Me-EHP	10	15.32	15.53	17.02
Me-Bep	10	16.00	16.44	19.47

Me-EP, methylethyl phthalate; Me-Bup, methyl-*n*-butyl phthalate; Me-HxP, methyl-*n*-hexyl phthalate, Me-EHP, methyl-2-ethylhexyl phthalate; Me-Bep, methylbenzyl phthalate; GC operating conditions as in Fig. 3.

tailing were observed for the methylated monoesters when these compounds were chromatographed on the nonpolar (DB1) and weak polar (DB5) columns, as compared with those obtained for their parent materials.

Since it is known that plots of log t_R (relative retention times) for many organic compounds on nonpolar column obtained under an isothermal condition against their boiling points give approximately straight lines, the retention times of compounds with shorter length in the monoalkyl moieties and having low boiling points are expected to be smaller than those of compounds with longer chain length and

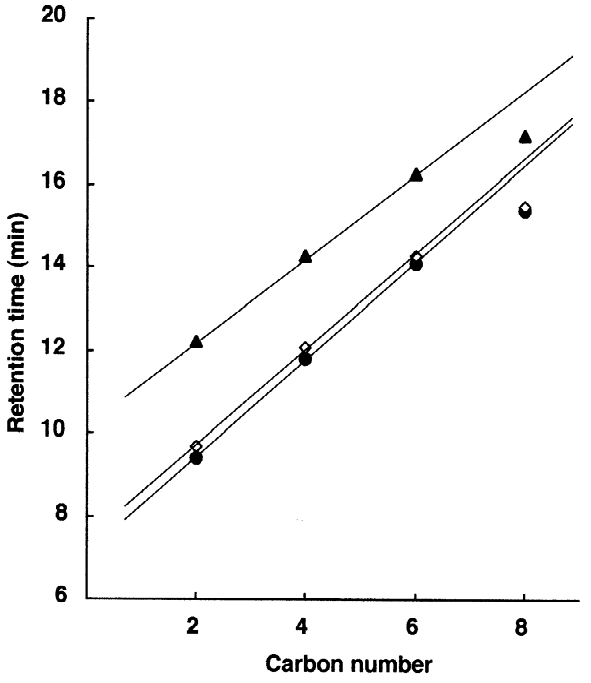

Fig. 4. Effect of the carbon number of ester in monoalkyl phthalates on the retention times on three capillary columns with different polarities. ●, DB1, 100% dimethyl polysiloxane; ◇, DB5, (5% phenyl) methyl polysiloxane; ▲, DB17, (50% phenyl) methyl polysiloxane; GC conditions as in Fig. 3.

high boiling points, when these compounds are chromatographed on a nonpolar column. Figs. 3 and 4 show that the methylated monoalkyl phthalates on the DB1, DB5 and DB17 capillary columns are eluted in the order of increasing carbon number of monoalkyl moieties. The plots of retention times against number of carbons in the monoesters are found to be linear for phthalic acid esters, with few exceptions, even though these compounds were chromatographed with temperature programming. However, the MEHP (C_8) deviated from the linear lines irrespective of the column investigated.

Fig. 5 shows the electron impact-mass spectra (EI-MS) of methylated MEP, MBuP, MHxP, MEHP and MBeP corresponding to the peaks appearing in the chromatogram in Fig. 3. The EI-MS of these compounds were very similar, with the base peak at m/z 163 ($[C6H4(CO)2OCH3]^+$) and a large peak at m/z 149 ($[C6H4(CO)2OH]^+$). The characteristic peak at m/z 181 resulted from a 'double hydrogen rearrangement involving the long side chain' on methylated MBuP, MHxP and MEHP [25]. The mass spectrum of methylated MBeP was shown as the base peak at m/z 91 arising from the benzyl group ($[C6\ H5CH2]^+$) and a comparatively large peak at m/z 163 and 149. Similar EI spectra of these methylated monoalkyl phthalates as shown in Fig. 5, with the exception of MEP, have been observed to those of DEHP metabolites in rat urine after methylation with diazomethane [17].

A GC–MS method has been reported by Sjoberg and Bendesson [15] for the determination of some metabolites of DEHP in blood plasma, after treatment with PFP–OH-PFPA. This esterification requires heating. The reaction of fatty carboxylic acids with TMSD–methanol proceeds quickly at room temperature with an excellent yield, and is safe compared to the use of diazomethane [24]. In the present work, it was confirmed that the reaction of phthalate monoesters with TMSD–methanol proceeds quickly at room temperature and results in excellent yields. In addition, the regression analysis for these methyl derivatives showed that the responses of all analytes were linear within the range 0.05–10 ng (injection volume 1μl), and the correlation coefficients were 0.9995–0.9999, as shown in Table 2. The relative standard deviation (RSD) values of the peak areas injected with 1 ng of these methyl derivatives into the GC–MS were $<0.9\%$ ($n=7$).The entire analytical procedure gives limits of detection (LOD) (peak-to noise ratio, 3:1) in a very low range. Furthermore, it was found that simultaneous determination of methylated mono- and dialkyl phthalates is possible by GC–MS-SIM by simply changing the oven temperature program (Fig. 6).

3.2. Analysis of phthalate monoesters in the PVC products

The phthalate monoesters were extracted from PVC products according to the method reported by Tsumura et al. [14], with minor modifications. We observed that the monoesters were not readily extracted by the *n*-hexane extraction from acidified sample solutions which removed dialkyl phthalates. Therefore, ethyl acetate was used as the extraction medium for monoesters. The recoveries of five monophthalates by the extraction method, at a spiking level of 100 ng/g, were in the range of 86.6–

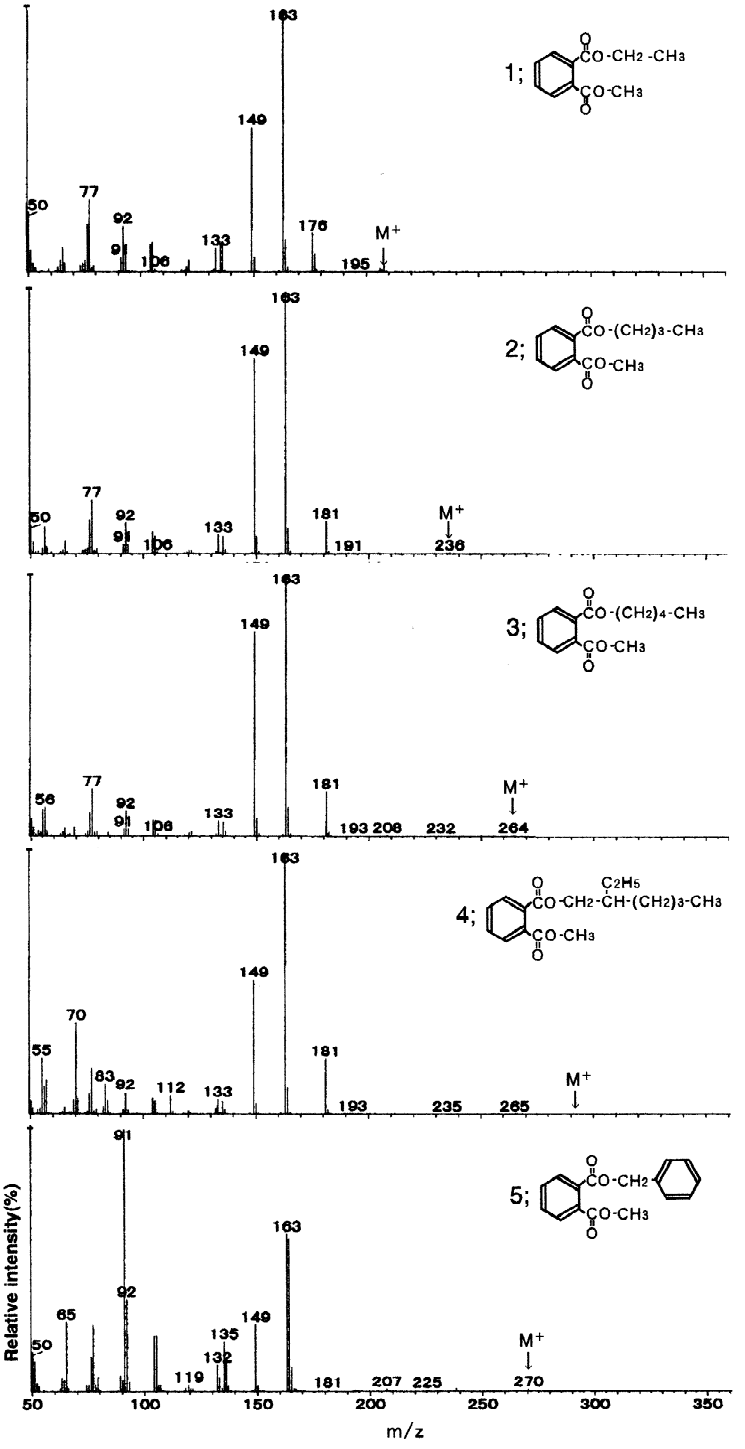

Fig. 5. Electron impact mass spectra of methylated phthalate monoesters obtained peaks 1–5, respectively (cf. Fig. 3.). MS operating conditions: ion energy, 70 eV (EI); ion source temperature, 260 °C.

Table 2
Linearity of calibration curves for determination of methylated phthalate monoesters and their recovery data and the limit of detections in PVC product

Compounds	Monitored ions[a] (*m*/*z*)		Calibration curve[b] (correlation coefficient)	Precision of the assay		
	A	B		Recovery[c] (%)	RSD (%)	LOD (ng/g)
Me-EP	163	149, 176	0.9995	86.6	3.1	5.0
Me-BuP	163	149, 181	0.9999	92.4	2.6	2.0
Me-HxP	163	149, 181	0.9999	94.3	2.2	2.0
Me-EHP	163	149, 181	0.9999	88.4	3.1	2.0
Me-BeP	91	163, 149	0.9997	87.9	3.5	4.0

[a] The A ions were for quantification; B ions were for confirmation.
[b] Each calibration curve represents five data points, in the range 0.05–10 μg/ml, with six replicates at each data point.
[c] Recovery values are means ($n=4$); spiking level in PVC product was 100 ng/g.

94.3% (mean value was 90.0%), as shown in Table 2. The within-day precisions of this method were $<3.5\%$ ($n=4$), and the between-days precisions (repetitive analysis on 3 different days) were $<4.3\%$ ($n=4$).

Table 3 shows the occurrence of phthalate monoesters in the PVC products. MBuP and MEHP detected in the three PVC toy products which contained DBP and DEHP were in the ranges 6.42–11.82 and 30.50–41.81 μg/g, respectively. We found that the residual MBuP and MEHP were 0.005–0.009 and 0.019–0.020% for the DBP and DEHP content, respectively. It seemed that these phthalate monoesters remained as an impurity in the plasticizer. MEHP was detected in the two products that contained DINP in the range of n.d. to 0.04%. No MEP, MHxP and MBeP were detected in the PVC products.

3.3. Analysis of phthalate monoesters in the human saliva

The phthalate monoesters were extracted from acidified human saliva with acetonitrile according to the method described by Sjoberg and Bendesson [15], with minor modifications. The recoveries of five monophthalates by this extraction method, at a spiking level of 50 nmol/ml, were in the range of 94.0–97.8% (mean value 95.9%), and the coefficient of variations were $<2.6\%$ ($n=6$).

A 50-nmol amount of dialkyl phthalates was incubated in human saliva at 37 °C for 30 min in accordance with Ref. [26]. It was determined that 6.7, 33.1, 5.0 and 0.9 nmol of MEP, MBuP, MHxP and MEHP were formed by hydrolysis of DEP, DBP, DHxP and DEHP in human saliva, respectively. The formation of monoalkyl phthalates in human saliva differs according to the length of the alkyl group. Fig. 7 show the chromatogram of monoalkyl phthalates formed from 50 nmol of the diesters (12.5 nmol of DEP, DBP, DHxP and DEHP) in human saliva. The peaks of phthalate mono- and diesters with small interferences appeared on TIC and SIM chromatograms by monitoring of *m*/*z* 163 and 149, respectively. Sjoberg and Bendesson suggested that GC–MS analysis of MEHP in rat plasma, which was ex-

Table 3
Occurrence of monophthalates in polyvinyl chloride (PVC) products

PVC products	Contents ($\times 10^4$ μg/g)			Monophthalates (μg/g)				
	DBP	DEHP	DINP	MEP	MBuP	MHxP	MEHP	MBeP
Ball A	12.2	15.9	–	N.d.	6.42	N.d	30.50	N.d
Ball B	10.0	18.5	–	N.d.	9.30	N.d.	36.44	N.d.
Ball C	15.9	21.4	–	N.d.	11.82	N.d.	41.81	N.d.
Soft doll A	–	–	24.3	N.d.	N.d.	N.d.	N.d.	N.d.
Soft doll B	–	–	33.9	N.d.	N.d.	N.d.	0.04	N.d.

N.d., <0.005 μg/g (MEP), <0.002 μg/g (MBuP, MHxP and MEHP) and <0.004 μg/g (MBeP).

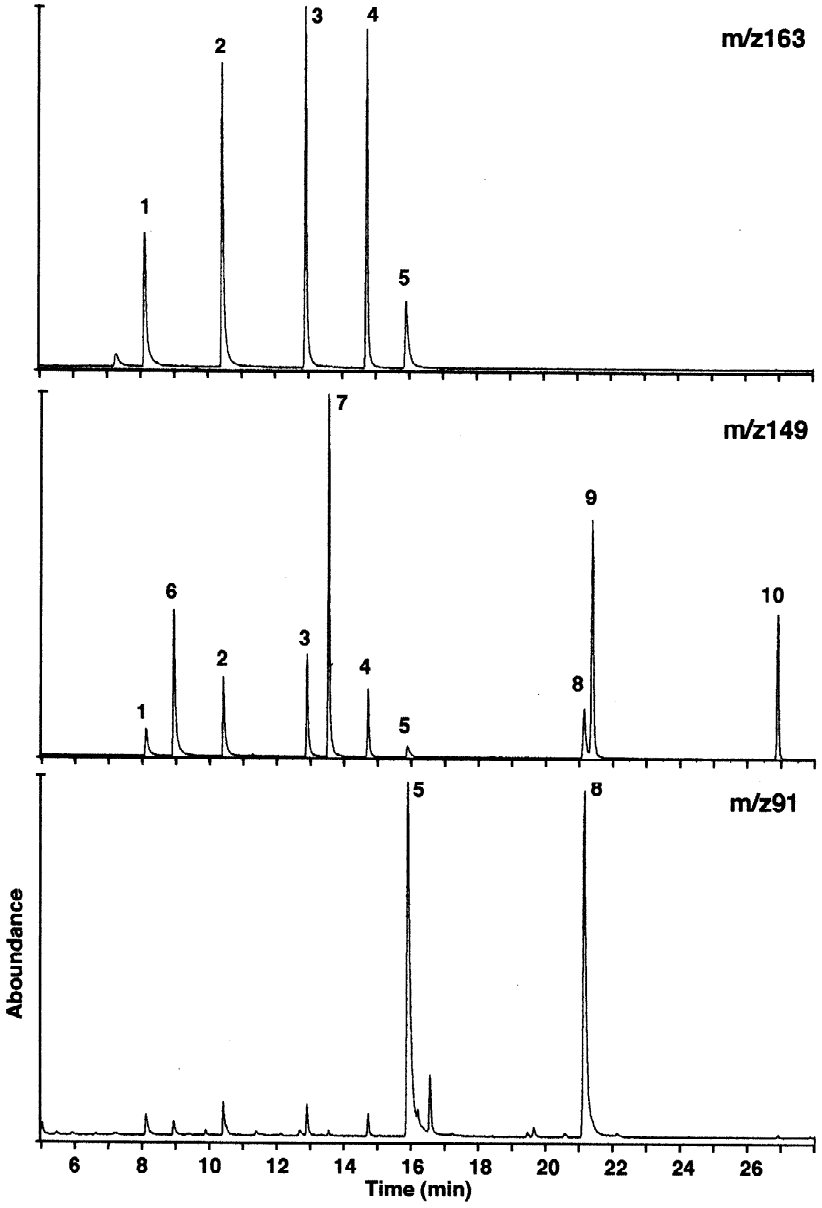

Fig. 6. Typical selected ion chromatograms of methylated phthalate mono-esters and diesters. Peaks 1–5 as in Fig. 3; 6 = diethyl phthalate; 7 = di-*n*-phthalate; 8 = benzyl-*n*-butyl phthalate; 9 = di-*n*-hexyl phthalate; 10 = di-2-ethylhexyl phthalate; GC operating conditions: column, DB1; column temperature, 100 °C (1 min)→10 °C/min→200 °C→2 °C/min→230 °C→20 °C/min→270 °C, other details as in Figs. 3 and 5. MS operating conditions: ions energy, 70 eV (EI); ions source temperature, 260 °C.

tracted with diethyl ether and derivatized with PFPA–PFP-OH, resulted in less interference from endogenous compounds [15]. We found that the monitoring of *m*/*z*163, 149 and 91 can be more sensitive for the determination of phthalate monoesters in human saliva after solid-phase extraction with the Oasis HLB cartridge and methylation with TMSD–methanol.

The good separation of phthalate diesters and the monoesters produced by hydrolysis of the com-

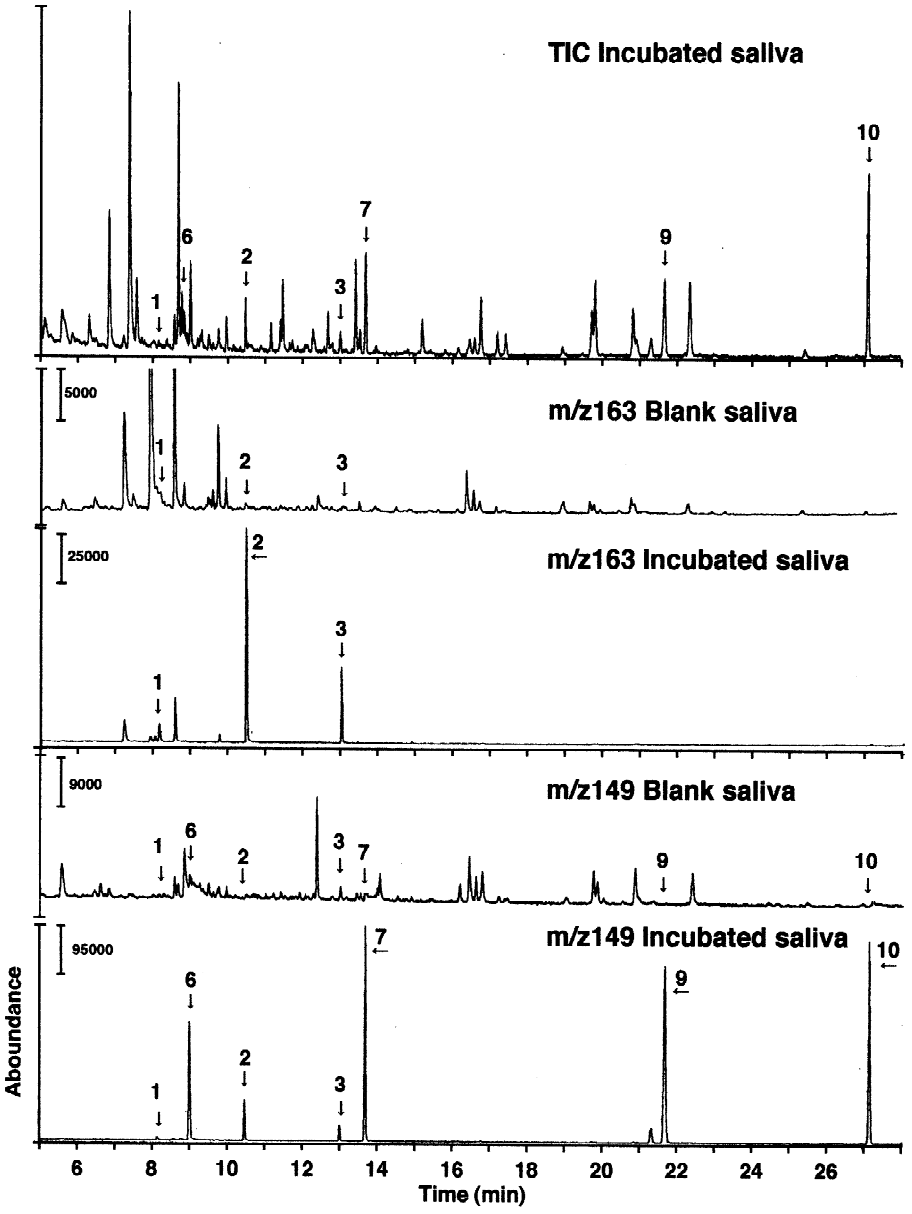

Fig. 7. Typical total ion current and selected ion chromatograms of methylated monoalkyl phthalates in human saliva. Saliva obtained from volunteer who had chewed PP disks was mixed with 12.5 nmol DEP, EBP, DEHP, respectively, and incubated at 37 °C for 30 min. Peaks 1–10 as in Fig. 6. GC and MS operating conditions as in Fig. 6.

pounds simultaneously obtained with these methods indicates that the methods will be valuable for application to the study of the behavior of these compounds in human saliva and in various environments.

4. Conclusion

The present results show that simultaneous GC coupled with electron-impact MS, with monitoring of the ions *m*/*z*163, 149 and 91, is a useful method

for the routine determination of phthalate di- and monoesters in PVC products and in human saliva by methylation with TMSD–methanol. The calibration curves for the monoesters were linear in the range 0.05–5.00 μg/ml. This method has been applied to phthalate monoesters in PVC toy products where MBuP and MEHP was detected in the range of 6.42–11.82 μg/g and 0.04–41.81 μg/g, respectively. This method could been also applied to the determination of phthalate di- and monoesters in human saliva.

Acknowledgements

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