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High-throughput determination of mono- and di(2-ethylhexyl)phthalate migration from PVC tubing to drugs using liquid chromatography–tandem mass spectrometry

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Abstract

The risk assessment of di(2-ethylhexyl) phthalate (DEHP) that migrated from polyvinyl chloride (PVC) medical devices is an important issue for hospitalized patients. Many studies have been conducted to determine the level of DEHP migration. A recent report has indicated that DEHP in blood bags was hydrolyzed by esterase to mono(2-ethylhexyl) phthalate (MEHP). Therefore, a method for the simultaneous determination of DEHP and MEHP was developed. The migration of DEHP and MEHP from PVC tubing to drugs was examined. Although we detected MEHP in the drugs, we found no enzymatic activity involved in the migration process. Some reports have indicated that hydrolysis may have occurred during sterilization by autoclaving. However, we did not perform any heat treatment. It is speculated that the MEHP migrated directly from the PVC tubing. The simultaneous determination of DEHP and MEHP is required for risk assessment, as MEHP may be even more toxic than the parent compound.
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Keywords: Liquid chromatography–tandem mass spectrometry; Di(2-ethylhexyl)phthalate; Mono(2-ethylhexyl)phthalate; Drugs

1. Introduction

Polyvinyl chloride (PVC) is one of the most widely used polymeric materials in medicine. Flexible PVC is used for the manufacture of blood and blood component storage bags, intravenous solution dispensing sets, blood tubing, and so on. As PVC per se is a rigid polymer, additives in the form of plasticizers are incorporated into it to increase its flexibility and low-temperature properties. The esters of phthalic acid, particularly di(2-ethylhexyl)phthalate (DEHP), are the most preferred plasticizers for medical grade PVC. However, because these additives are not bound to the base polymer by covalent bonds, their permanence is low. The migration of DEHP from PVC medical devices has been reported [1–5].

DEHP in PVC products easily migrates into foods, drugs and body fluids [6–8]. The general toxicity of DEHP has been evaluated [5,9–12], and a risk assessment study has suggested that it is relatively safe for humans. Recently, however, it has been considered that the level of DEHP exposure to humans, particularly high risk patients, must be monitored, based on the finding that DEHP exerts an adverse effect on young rodents. The US Food and Drug Administration's Center for Devices and Radiological Health and Health Canada have reported the risk assessment of DEHP that migrated from PVC medical devices in hospitalized patients [13,14].

It has been reported that DEHP is hydrolyzed enzymatically to mono(2-ethylhexyl)phthalate (MEHP) [15], and that MEHP may be even more toxic than the parent compound. In vitro studies have found that MEHP inhibits FSH-stimulated cAMP accumulation in cultured Sertoli cells [16–20], in addition to reducing 17β-estradiol production and aromatase mRNA expression [21,22]. These results suggest that MEHP

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is an active metabolite of DEHP, and that any toxic effects of orally ingested DEHP are more likely to be due to the properties of the corresponding monoester rather than the intact DEHP.

Therefore, a method for determining DEHP and MEHP with high sensitivity, precision, and selectivity is required. Most of the conventional simultaneous analyses of DEHP and MEHP involve liquid chromatography(LC)/ultraviolet(UV) detection [23,24], LC/mass spectrometry(MS) [2,7] and gas chromatography(GC)/MS [25–27]. However, those methods lack sensitivity, precision and selectivity. Inoue and co-workers [2,7] have reported the utility of the column-switching LC/MS method for the direct analysis of DEHP because of its high throughput and low contamination. In addition, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has high sensitivity and selectivity. Therefore, the column-switching LC–MS/MS method was developed.

2. Experimental (materials and methods)

2.1. Chemicals and materials

Environmental analytical grade DEHP and DEHP-d_4 were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). MEHP and MEHP-d_4 were purchased from Hayashi Pure Chemical Industries (Osaka, Japan). The structures of DEHP, MEHP and their surrogate standards are shown in Fig. 1. Phthalic acid esters, analytical grade acetonitrile and acetone were used in the experiments. The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

The test material was PVC tubing that is used for transfusion, infusion, and donation of blood. This was kindly supplied by two manufacturers and was not sterilized prior to use.

The drugs used for the DEHP and MEHP migration tests were Prograf® (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan), FLORID®-F (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan), and Lastet injection (Nippon Kayaku Co., Ltd., Tokyo, Japan). These were used after dilution with 5% glucose solution for injection (Otsuka Pharmaceuticals Co., Tokyo, Japan) to the desired concentration based on the package inserts.

2.2. Instrumentation

A Series 1100 liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000™ (Applied Biosystems Japan, Tokyo, Japan) equipped with a Turbo Ionspray™ ionization source. Mass spectrometry data were processed with Analyst 1.3.2 software. A Shimadzu (Kyoto, Japan) LC-10 AS pump was used for providing flow through the extraction column to load and wash the sample and to equilibrate the extraction column. A Mightysil® RP-18 GP column (5 mm × 2.0 mm, 5 μm particle size) from Kanto Chemical was used for the separation. An Oasis® HLB extraction column (20 mm × 2.1 mm, 25 μm particle size) from Waters was used for the extraction and clean-up.

2.3. Standard solution and quantitative procedure

DEHP, DEHP-d_4, MEHP and MEHP-d_4 stock solutions were prepared in acetonitrile. They were mixed to make the desired ratio and serially diluted with 50% acetonitrile for the preparation of calibration curves.

2.4. Chromatographic and extraction conditions

The column switching system was used for sample injection. After 20 μl of the sample was injected with an

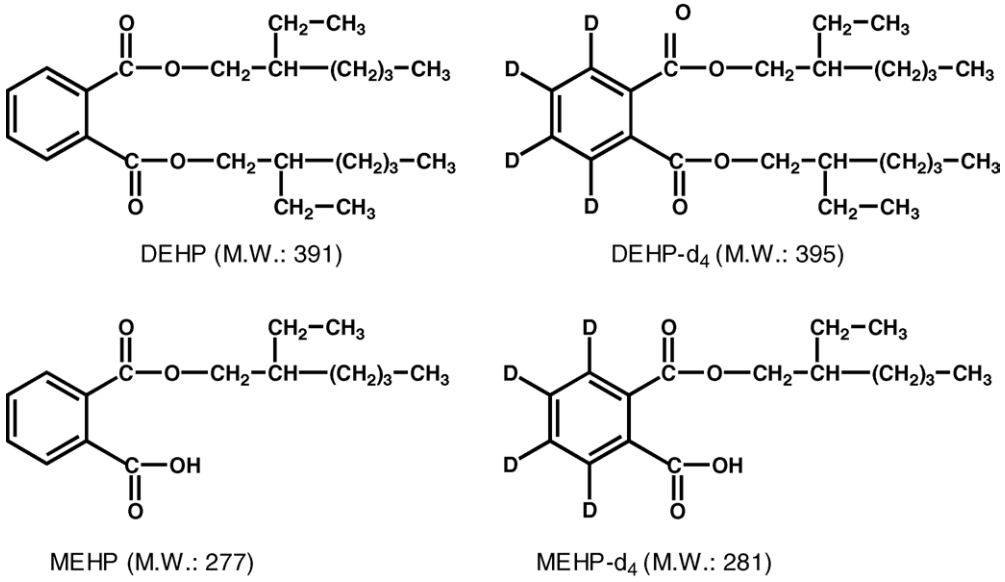

Fig. 1. Chemical structures of DEHP, MEHP and their surrogate compounds.

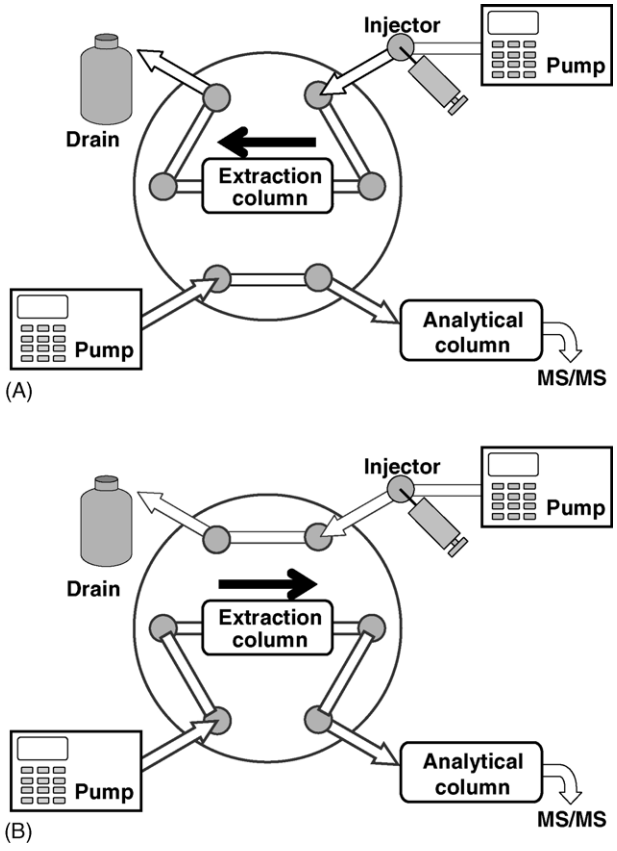

Fig. 2. Schematic representation of the column-switching LC–MS/MS system. (A) Configuration for sample loading and washing; (B) Configuration for sample elution.

auto-sampler, it was loaded onto the extraction column by flowing pure water at the rate of 1 ml/min using the LC-10 AS pump for 3 min. While the eluate from the extraction column was directed to waste during the 3 min, the sample was extracted on the on-line extraction column. The matrices in the sample were eluted whereas DEHP and MEHP were retained on the extraction column. Then, the extraction process was performed after the on-line solid phase extraction was accomplished. After 3 min, the switching valve was changed to configuration B (Fig. 2). This configuration connected the extraction column to the analytical column and the MS detector in the flow path of the Agilent LC pump. The column oven was maintained at 40 °C for LC. The separation was carried out with a mobile phase of acetonitrile/water (90/10, v/v) at a flow-rate of 0.2 ml/min. The eluate from the analytical column was directed to the electrospray MS. After elution for 8 min, the switching valve was returned to the original position (configuration A in Fig. 2). The time program for the column-switching LC–MS/MS system is summarized in Table 1.

2.5. MS/MS conditions

The working parameters for turbo ionspray ionization MS/MS were as follows: declustering potentials, 81 V (DEHP and DEHP-d_4) and −60 V (MEHP and MEHP-d_4); curtain gas flow-rates, 20 psi (DEHP and DEHP-d_4) and 30 psi (MEHP and MEHP-d_4); nebulizer gas (N_2) pressure, 30 psi; and turbo ionspray gas (N_2) pressure, 0 psi. The ion source temperature was maintained at 650 °C and the turbo ionspray voltages for DEHP (DEHP-d_4) and MEHP (MEHP-d_4) were 5500 and −4500 V, respectively. DEHP and DEHP-d_4 were detected in the positive mode, whereas MEHP and MEHP-d_4 were detected in the negative mode. The product ion mass spectra of DEHP, DEHP-d_4, MEHP and MEHP-d_4 obtained by the LC–MS/MS system are shown in Fig. 3. The combinations of precursor ion and product ions were as follows: DEHP (precursor ion → product ion, m/z 391 → 149), DEHP-d_4 (m/z 395 → 153), MEHP (m/z 277 → 134), and MEHP-d_4 (m/z 281 → 138). The collision gas (N_2) pressures were set at 2 units (DEHP and DEHP-d_4) and 1 unit (MEHP and MEHP-d_4).

Table 1
Time program for the proposed column switching-LC/MS/MS method

Time (min)	Solvent A (%)	Solvent B (%)	Configuration
0.0	100	0	Loading and washing
3.0	100	0	
3.1	0	100	Elution and separation
8.0	0	100	
8.1	100	0	Conditioning

Solvent A: water; solvent B: acetonitrile/water = 90/10 (v/v).

2.6. Migration test

The two kinds of PVC tubing were cut to 10 cm length and filled with the drugs (tube length, 8 cm). The tubing was subjected to extraction with shaking at room temperature for 1 h. The extracts were pipetted into another test tube, and put in vials containing 50 ng of DEHP-d_4 or MEHP-d_4. Then, the samples were appropriate dilute, consequently subjected to LC–MS/MS.

3. Results and discussion

3.1. Analysis of DEHP and MEHP by on-line SPE-LC–MS/MS

Our previous report [7] which performed simultaneous determination of DEHP and MEHP in serum using column switching-LC/MS, required almost 30 min for analysis. In addition, other report [8] using LC–MS/MS method required 30 min to perform the DEHP and MEHP simultaneous analysis in serum. On the other hand, the column switching system combined with LC–MS/MS method performed the high-throughput and high-precision analysis that needs almost 10 min.

In this method, the limits of quantificaion (signal-to-noise ratio > 10) of DEHP and MEHP were 2.5 and 0.75 ng/ml with

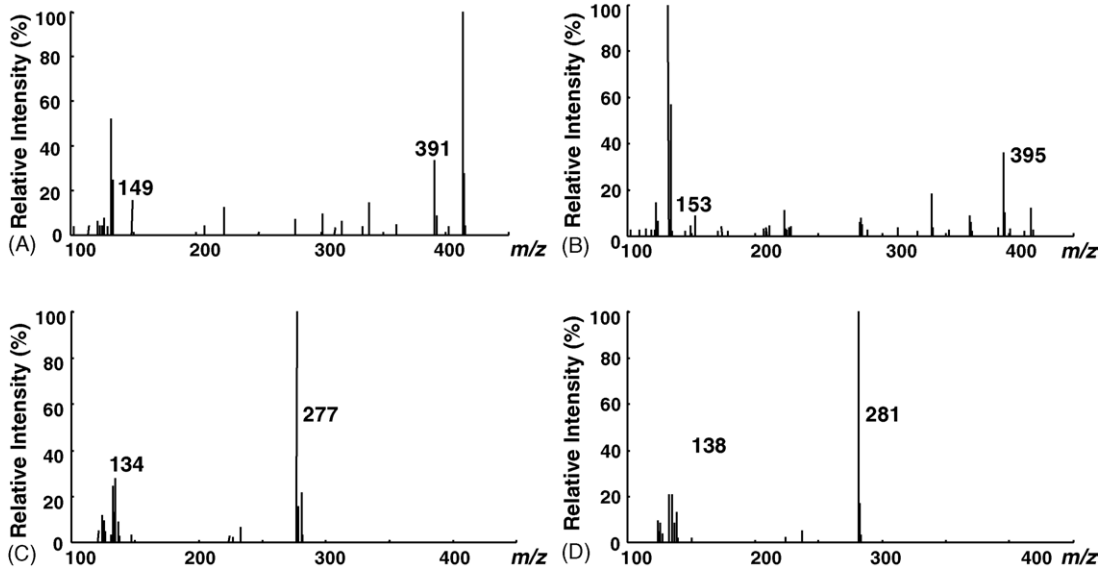

Fig. 3. Product ion spectra of DEHP, MEHP and their surrogate compounds. (A) DEHP (B) DEHP-d_4 (surrogate compound for DEHP). (C) MEHP (D) MEHP-d_4 (surrogate compound for MEHP).

Table 2
Validation data for determination of DEHP and MEHP migration from PVC tubing to drugs

	DEHP		MEHP	
	Quantitative range (ng/ml)	Correlation (r)	Quantitative range (ng/ml)	Correlation (r)
Glucose	2.5–100	0.999	0.75–100	0.999
Prograf®	2.5–50	0.999	0.5–50	0.999
FLORID®-F	2.5–50	0.999	0.25–50	0.996
Lastet inj.	5–50	0.999	0.5–50	0.999

the standard solutions, respectively. For DEHP measurement, the calibration curve was obtained by plotting the peak-area ratio (DEHP/DEHP-d_4) versus DEHP concentration, and was linear over the range of 2.5–500 ng/ml (r = 0.998). For MEHP measurement, the calibration curve was obtained by plotting the peak-area ratio (MEHP/MEHP-d_4) versus MEHP concentration, and was linear over the range of 0.75–500 ng/ml (r = 0.997). DEHP and MEHP concentrations in the drugs were measured; however, as the the matrices of the drugs were different from each other, a known concentration of the standard solution was added to the drugs, and a calibration curve was obtained for each drug (Table 2)

We examined the recovery using 5% glucose solution. The average recoveries of DEHP and MEHP were 99.2% (R.S.D. = 3.2%, n = 6) and 109.0% (R.S.D. = 3.4%, n = 6), respectively (Table 3). The chromatograms obtained by the recovery test are shown in Fig. 4.

Table 3
Recoveries of DEHP and MEHP from glucose solution

50 ng/ml spiked	Recovery ± S.D. (%)
DEHP	99.2 ± 3.2
MEHP	109.0 ± 3.4

5% glucose solution, n = 6.

3.2. Determination of DEHP and MEHP migration from PVC tubing

The proposed method was applied to the determination of DEHP and MEHP migration from the PVC tubing (Table 4). The level of DEHP migration was almost the same for the two tubing, that is, it was speculated that the DEHP content

Table 4
Levels of DEHP and MEHP migration to various drugs from PVC tubing

Sample	DEHP concentration (mean ± S.D., μg/ml)	
	Company A	Company B
Glucose	0.12 ± 0.03	0.13 ± 0.06
Prograf®	4.60 ± 0.17	4.40 ± 0.10
FLORID®-F	53.99 ± 3.63	54.64 ± 2.90
Lastet inj.	27.04 ± 0.62	28.88 ± 1.53
	MEHP concentration (mean ± S.D., μg/ml)	
	Company A	Company B
Glucose	0.56 ± 0.05	0.20 ± 0.00
Prograf®	0.39 ± 0.04	0.12 ± 0.01
FLORID®-F	ND*	ND*
Lastet inj.	ND*	ND*

n = 3. The samples were appropriate dilute, consequently subjected to LC–MS/MS.
* One thousandth dilution were performed.

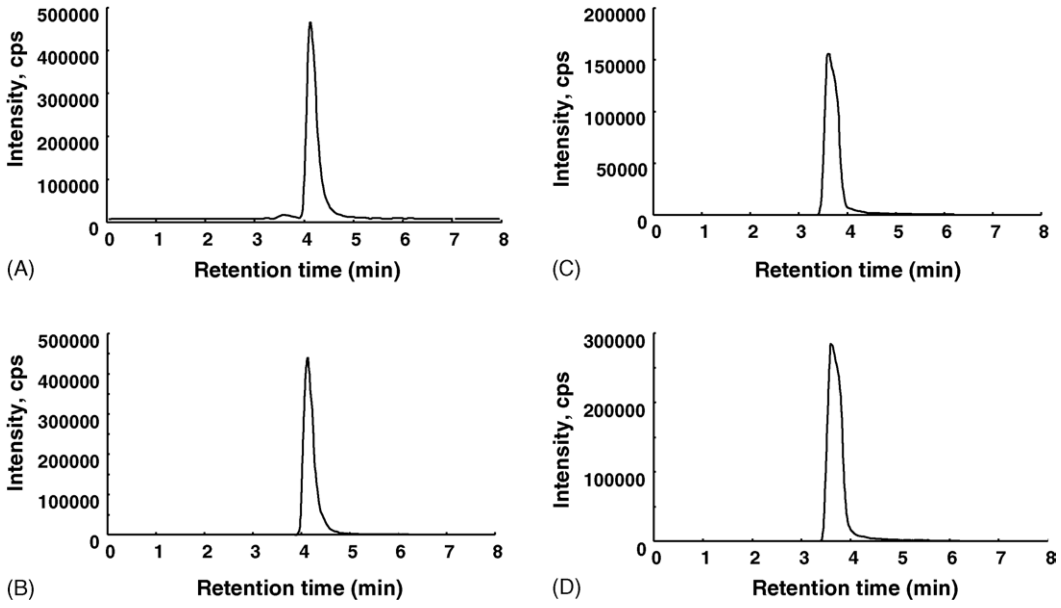

Fig. 4. MRM chromatograms of DEHP, MEHP and their surrogate compounds in glucose solution spiked with 50 ng of DEHP or MEHP. (A) DEHP (m/z 391 → 149); (B) DEHP-d_4 (m/z 395 → 153); (C) MEHP (m/z 277 → 134); (D) MEHP-d_4 (m/z 281 → 138).

was almost the same for the two tubings. On the other hand, MEHP was detected in 5% glucose solution and Prograf. The level of DEHP migration to the same drug was almost the same for the two tubings. By contrast, the level of MEHP migration differed by almost threefold between the two tubings even when the same drug was used.

The 5% glucose solution has been used for dilution of all drug, however, drug additives including surfactants and their concentration were different (Table 5). Owing to the other report [28], DEHP migration was dependent on the concentration of drug additives such as HCO-60. High concentration of drug additives such as HCO-60 as shown FLORID®-F and Lastet inj. in the Table 5 might be contributed to migrate the DEHP. In comparison, MEHP was more hydrophilic than DEHP (DEHP: log P = 7.19, MEHP: log P = 3.35 calculated by log P predictor from ChemSilico) so that MEHP have less migration than DEHP.

It was thought that 5% glucose solution had very little effect on DEHP migration. When we measured the levels of DEHP and MEHP migration with 5% glucose solution, the level of DEHP migration was found to be lower than that of MEHP migration. It has been reported that DEHP was hydrolyzed by such enzymes as lipases to MEHP in blood bags. However, that the drugs used in this study have enzymatic activity is not plausible. Some reports have indicated that hydrolysis may have occurred on sterilization by autoclaving [29,30]. However, we did not perform any heat treatment in this study. In addition, the level of MEHP migration was different between the two tubings. Moreover, we comfirmed that MEHP was also migrated from PVC sheet with just water. Taken together, we hypothesized that MEHP already existed in the PVC tubing and migrated directly from it.

To date, the mechanism underlying the migration of DEHP from PVC medical devices remains unknown. Further research of MEHP and DEHP migration from PVC medical devices is required.

Table 5
Additives in diluted drugs

Drugs	Additives	Concentration
Glucose	Nothing	–
Prograf®	Polyoxyethylated hydrogenated castor oil (HCO-60)	80 ppq
	Dehydrated ethanol	Unknown
FLORID®-F	Polyoxyethylated hydrogenated castor oil (HCO-60)	1000 ppm
Lastet inj.	Polyethylene glycol 400 (PEG-400)	240 ppm
	Polysorbate 80 (Tween 80)	32 ppm
	Ethanol	Unknown
	Citric acid	Unknown

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