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The validation of column-switching LC/MS as a high-throughput approach for direct analysis of di(2-ethylhexyl) phthalate released from PVC medical devices in intravenous solution

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Abstract

Health Canada reported recently that medical devices containing di(2-ethylhexyl) phthalate (DEHP) should not be used in the clinical treatment of infants, young boys, pregnant women, and nursing mothers. The risk assessment of DEHP released from PVC medical devices is an important issue for hospitalized patients. In this study, a simple, accurate, low-contamination and high-throughput analytical technique for the determination of DEHP in intravenous (IV) solution was developed using column-switching liquid chromatography/mass spectrometry (LC/MS) with an extraction mini-column. The sample preparation for on-line extraction involved simply mixing IV solution with internal standard as DEHP-d_4 in LC glass vials. The IV fat emulsion drug sample cannot be analyzed directly, hence this sample spiked with DEHP-d_4 solution was extracted by hexane and measured by column-switching LC/MS yielding an average recovery of 92.2% (C.V. = 7.8%, $n = 5$). A linear response was found for a variety of drugs tested within the validated range of 0.1 or 0.5–10 μg/ml with correlation coefficients (r) greater than 0.99. These results suggest that this method can assay background exposure to DEHP released from PVC medical devices in the patients. The method was applied to various IV solution samples to establish the first screening method for DEHP released from medical devices with respect to their safety.

Keywords: Di(2-ethylhexyl)phthalate; DEHP; PVC medical devices; Intravenous (IV) solution; Column-switching; LC/MS

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1. Introduction

The FDA Center for Devices and Radiological Health has published a review of the potential health risks faced by patients associated with di(2-ethylhexyl) phthalate (DEHP) leaching from polyvinyl chloride (PVC) medical devices [1]. Furthermore, Health Canada has reported that medical devices containing DEHP should not be used in the treatment of infants, young boys, pregnant women, and nursing mothers [2]. The risk assessment of DEHP released from PVC medical devices is an important issue for medical patients.

It has been reported that some species of phthalate esters including DEHP have reproductive and development toxicity [3,4]. Other research has shown that the clinical effects of DEHP are developmental toxicity, carcinogenicity and testicular toxicity [5–7]. However, the No Observable Adverse Effects Level (NOAEL) for clinical effects has not yet been firmly established.

PVC medical devices typically contain 10–40% DEHP by weight. DEHP is not chemically bound to the PVC polymer and may leach when medical PVC comes into contact with blood, drugs, or IV fluids. Many studies have been undertaken into human exposure to DEHP following treatment with PVC medical devices and these have been reviewed [1,2]. The studies have demonstrated that DEHP can leach from medical devices into patients in varying degrees. Most of this research has focused on quantitative measurements of human exposure to DEHP from medical devices, however, limited research has been conducted on the analytical technique and a high-throughput approach.

There are many analytical methods for the determination of DEHP in environmental water, air and plastics including gas chromatography and high performance liquid chromatography (HPLC) equipped with various detectors [8–12]. In addition, it was reported for the risk assessment in the clinical environment that many HPLC methods were developed for determination of DEHP in clinical solutions [13–15]. However, there are not found that highly sensitive and accurate methods for the determination of DEHP for clinical assessment.

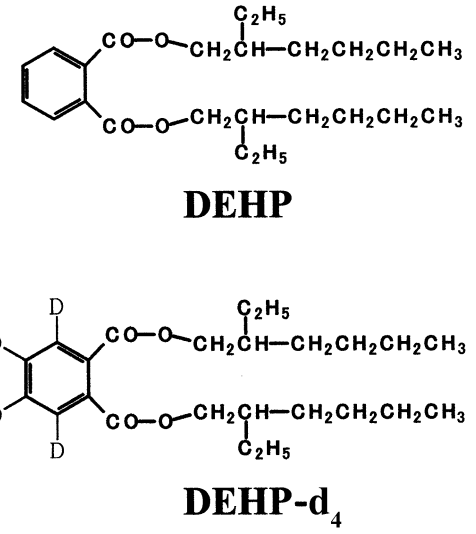

Fig. 1. Chemical structures of the DEHP and DEHP-d_4 surrogate compounds used for evaluation of column-switching assay.

Analysis of DEHP in various samples may present a difficult and serious problem because higher background is often encountered. This is due to DEHP contamination in many laboratory products, plastic tubing, room air, etc. In reality, the reported concentration of DEHP in air was 30–70 ng/m^3 [18]. Previous reported analytical methods may have the risk of contamination hence an over-estimation of DEHP concentration. Moreover, there is a requirement for the development of an easy, reliable and high-throughput analytical method for the determination of DEHP released from PVC medical materials.

The present study describes the development of a simple, accurate, low-contamination and high-throughput analytical technique for the determination of DEHP using column-switching liquid chromatography–mass spectrometry (LC/MS) with an extraction mini-column. The use of column-switching allows the entire extraction including load, wash and re-equilibration to be performed in a short time scale while the separation is in progress. By conducting extraction and separation in parallel, many labor intensive and time consuming off-line processes can be eliminated. This has the advantage of decreasing the risk of DEHP contamination. This novel method

has been successfully applied to investigate DEHP presence in intravenous solution and that released from PVC tubing.

2. Experimental

2.1. Reagents

DEHP and surrogate DEHP-d_4 standards were purchased from Kanto Chemical Industries Ltd (Tokyo, Japan). The structure is shown Fig. 1. HPLC-grade acetonitrile for the mobile phase was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Purified water was obtained using a Milli-Q gradient A10 Elix with an EDS polisher system (Millipore, Bedford, MA, USA). Acetic acid was purchased from Wako Pure Chemical Industries Ltd. Analysis-grade hexane for phthalic acid esters was obtained from Kanto Chemical Industries Ltd.

2.2. Intravenous (IV) solution samples

Sample A: Ciclosporin (the composition of inactives were dehydrated ethanol and polyoxyethene hydrogenated castor oil 60), B: Miconazole (the composition of inactive was poryoxyethene hydrogenated castor oil 60), C: 20% Soybean oil (the composition of inactives were concentrated glycerin and purified yolk lecithin), D: Tacrolimus hydrate (the composition of inactives were dehydrated ethanol and polyoxyethene hydrogenated castor oil 60); and E: Etoposide (the composition of inactives were polysorbate 80, dehydrated citric acid, macrogol 400 and dehydrated ethanol) were used in this study. Each of these drugs were kindly supplied from each corporation. The 5% glucose injection solution was purchased from Otsuka Pharmaceuticals Co. (Tokyo, Japan).

2.3. Equipment

The LC/MS used was an Agilent LC/MSD Superior Line (Agilent Technologies, Palo Alto, USA) equipped with an electrospray ionization (ESI) source. The Agilent pump was used to deliver mobile phase to elute the sample from the extraction column and to perform the separation on the analytical column. A Shimadzu LC-10AS pump (Shimadzu, Kyoto, Japan) was used to deliver mobile phase through the extraction column to load and wash the sample and to equilibrate the extraction column. A Mightysil RP-18 GP analytical column (100×2.0 mm, 5 μm) and a Mightysil RP-18 GP guard column (5×2.0 mm, 5 μm) from Kanto Chemical Industries Ltd were used for separation. The Waters Oasis HLB extraction column (20×2.1 mm, 25 μm) was used for extraction.

2.4. Standard solution and quantitative procedure

The DEHP and DEHP-d_4 stock solutions (1.0 mg/ml) were prepared in acetonitrile. Standard solutions of DEHP were prepared in acetonitrile or IV solution covering the calibration range. Quantitative analysis was performed using selected ion monitoring in order to maximize sensitivity. The DEHP concentration in each sample was calculated relative to DEHP-d_4 standards added to the samples prior to direct analysis giving a final detecting concentration of 10 μg/ml. Nine-point calibrations (0.1 or 0.5–10 μg/ml) were performed daily for all samples with internal standards.

2.5. Sample preparation procedures of IV solution

The sample preparation procedure for the on-line extraction consisted of mixing IV solution with DEHP-d_4 internal standard in LC glass vials. The IV fat emulsion drug (Sample C) sample cannot be analyzed directly, hence 1 ml of this sample spiked with DEHP-d_4 solution was extracted using 1 ml of hexane. The hexane layer (0.5 ml) was transferred into a glass tube and evaporated to dryness in a water bath at 40 °C under nitrogen. The residue was dissolved in 0.5 ml of acetonitrile, and subsequently analyzed by column-switching LC/MS. Commonly, the off-line extraction techniques were solid-phase, liquid–liquid, soxhlet and membrane. However, those techniques may present a difficult and serious problem because higher background is often encountered. These analytical techniques may also

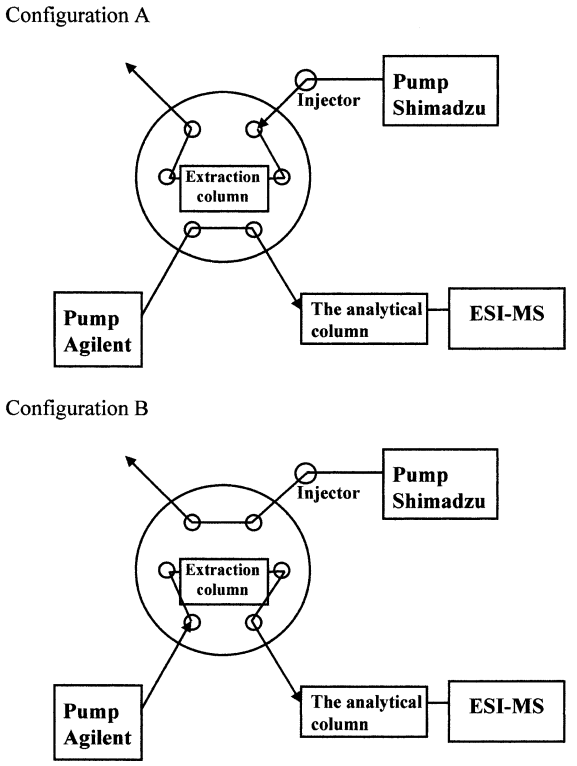

Fig. 2. Schematic representation of the column-switching LC/MS system.

face the risk of contamination and hence an overestimation of DEHP concentration. Therefore, this simple only hexane extraction can be bypassed.

2.6. Chromatographic and extraction conditions

The column-switching LC/MS system, as depicted in Fig. 2, was used for the direct injection of IV solution sample mixtures. After the IV solution sample (100 μl) was injected by the auto-sampler, the sample was loaded onto the extraction column in pure water at a flow rate of 0.2 ml/min controlled by the LC-10AS pump over 5.0 min. While the extraction column was directed to waste during the 5.0 min, the sample was extracted on the on-line extraction column. The matrices of compounds in the sample were removed while DEHP was retained on the extraction column. The extraction process was performed after closure of the on-line solid phase extraction. After 5.0 min, the switching valve was changed to position B (see Fig. 2). This configuration connected the extraction column to the analytical column and 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 using a mobile phase of water/0.1% acetic acid in acetonitrile (10/90, V/V) with a flow-rate of 0.2 ml/min. The effluent from the analytical column was directed to the electrospray MS without splitting. After an elution step of 10 min, the switching valve was changed back to the original position (configuration A in Fig. 2). The run time for the assay of the sample mixture was 20 min. The conditions used are summarized in Table 1.

Table 1
The condition of column-switching LC/MS system for determination of DEHP

Instument	Agilent 1100 MSD-SL Series & Shimadzu 10AS Pump
Analytical Column	Mightysil RP-18 GP (2.0 × 100 mm)
Mobile phase	Solvent A; H_20 Solvent B; H_20/CH_3CN (0.1 w/v% AcOH) = 1/9 (v/v)
Solvent Program	A (0-5 min), B (5-20 min)
Flow rate	0.2ml/min
Oven temp.	40 °C
Injection vol.	100 μl
Extraction Column	OASIS HLB (2.1 × 20 mm)
Ionization	Electrospray
Nebulizer	N_2 (35 psi)
Drying gas	N_2 (12 l/min, 350 °C
Fragmentor	110 V
Mode	Positive
SIM (*m/z*)	DEHP (*m/z* 391), DEHP-d_4 (*m/z* 395)

2.7. MS conditions

The working conditions for electrospray ionization MS were as follows; the drying nitrogen gas temperature was set at 350 °C and the gas was introduced into the capillary region at a flow rate of 12 l/min; the capillary was held at a potential of 3500 V relative to the counter electrode for the positive-ion mode. The fragmentor voltage was

Table 2
Experimental conditions used for determination of DEHP released from PVC

IV solution sample	The sample preparation	Quantitative range (μg/ml)	Correlation
Sample A	Sample A (250 mg/5 ml) 1A 5% TZ 250 ml 7.5 ml/h: 24 h medication	0.5–10.0	0.999
Sample B	Sample B (200 mg/20 ml) 1A 5% TZ 250 ml 270 ml/h: 1 h medication	0.1–10.0	0.996
Sample C	Sample C 100 ml 20 ml/h: 5 h medication	0.5–10.0	0.999
Sample D	Sample D (5 mg/ml) 5% TZ 500 ml 1.4 ml/h: 24 h medication	0.5–10.0	0.999
Sample E	Sample E (100 mg/5 ml) 5% TZ 250 ml 4.25 ml/min: 1 h medication	0.1–10.0	0.997

TZ, the 5% glucose injection solution; 1A, an ampoule.

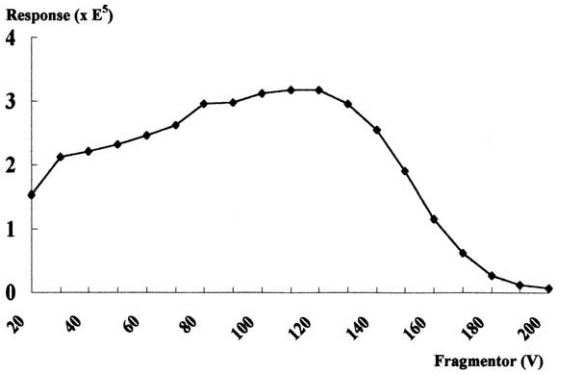

Fig. 3. Effect of fragmentor voltage on the peak response of DEHP ($[M+H]^+$). Concentration of DEHP; 5.0 μg/ml.

110 V during the chromatographic run. When working in the selected ion monitoring (SIM) mode the *m/z* 391 and 395 ions were assigned as the $[M+H]^+$ of DEHP and the $[M+H]^+$ of DEHP-d_4, respectively.

2.8. Sampling the IV solution using PVC tubing

The test solutions were prepared under the conditions described in Yokohama City University Hospital, as summarized in Table 2. In addition, PVC (X1-50) and non-PVC (N1-FL50) tubings (size: 50 cm) were used for investigation of the released DEHP in IV solutions.

3. Results and discussion

3.1. Analysis of DEHP by LC/MS and online system

In the mass spectral analysis using ESI-MS with flow through injection analysis of standard solutions, the *m/z* 391 and 395 ions were observed as the main peak for DEHP ($[M+H]^+$) and DEHP-d_4 ($[M+H]^+$), respectively. The most important parameters affecting LC/MS for determination of compounds are the fragmentor voltage and the mobile phase effect. In order to establish the optimum fragmentor voltage for the detection of DEHP, the signals of the *m/z* 391 versus fragmentor voltage were investigated (Fig. 3). The optimal fragmentor voltage was 110 V for DEHP. The ionization of samples at the LC/MS interface is affected by the mobile phase, hence a mobile phase containing a volatile-acid or salt is used frequently. In this case, a low level of response of DEHP was observed using a water/acetonitrile mobile phase. However, the response of DEHP was increased by addition of 0.1% acetic acid to the mobile phase. The main signals of *m/z* showed a maximum in 0.1% acetic acid at 110 V for DEHP.

Commonly, previous off-line methods have attempted to directly measure the phthalate diesters (included DEHP) in sample but were fraught and happen with DEHP contamination problems [16,17]. This contamination is from a variety

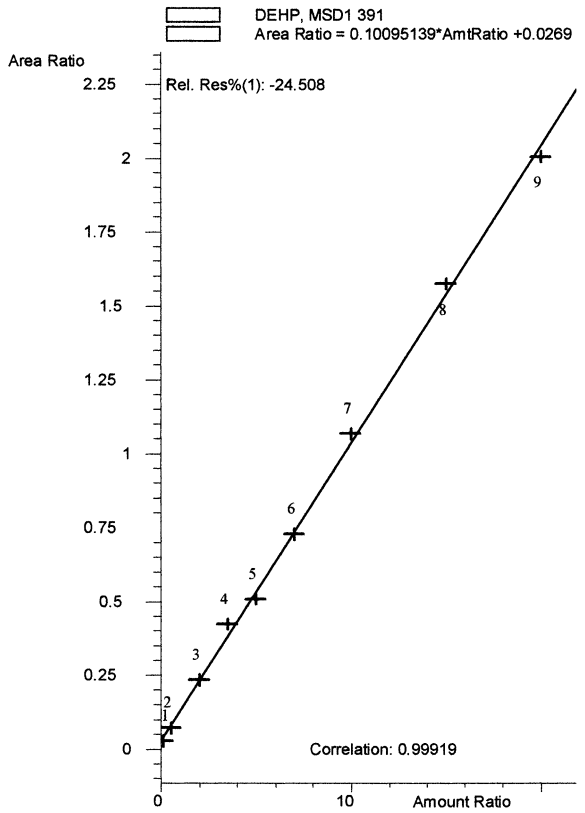

Fig. 4. Calibration curve obtained for peak ratio (DEHP/DEHP-d_4).

laboratory products, plastic tubing, and preparation instruments. In any event, it is need that non-contamination and accurate analytical method and preparation were developed. Therefore, we developed that the determination of DEHP by this on-line system was contamination free, highly sensitive and accurate analysis.

3.2. Data analysis and quantification of DEHP

Analysis of preliminary IV solution samples was used to set target ranges of quality control at blank levels. The quality control was prepared from raw IV solution that had not passed through PVC tubing. These DEHP background peaks in IV solutions were trace levels (peak area < 500, S_0). In addition, the precision (SD) was under 10.0 (SD_0). The limit of quantification (LOQ) was calculated using these factors as follows:

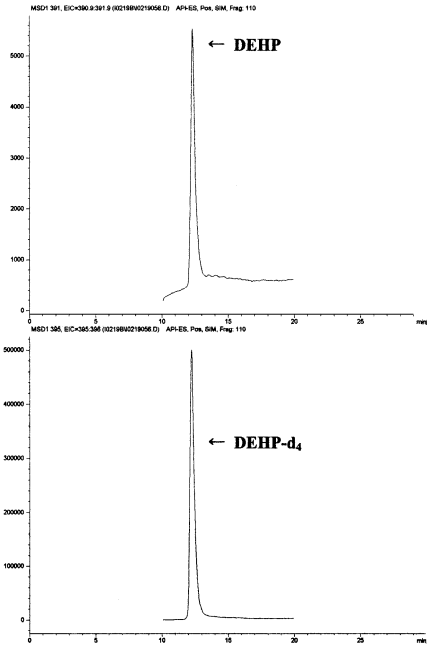

Fig. 5. Chromatograms of DEHP and DEHP-d_4 in Sample B. Migration time range from 50 to 60 min. Concentration level of DEHP; 0.13 μg/ml.

$$\text{LOQ} > \text{Concentration level } (10 \times [S_0 + \text{SD}_0]). \quad (1)$$

The LOQ concentration of DEHP in this system was set at 0.1 or 0.5 μg/ml from previous reports of leaching DEHP from PVC medical devices [1,2,13–15]. In addition, the limit of detection (LOD) was calculated by concentration level ($3 \times [S_0 + \text{SD}_0]$). The result of LOD of DEHP was 0.01 μg/ml. These LOQ and LOD were achieved by on-line Column-switching LC with electrospray MS coupled with on-line of extraction.

For quantitation of DEHP in IV solutions, the peak ratio of DEHP to DEHP-d_4, the stable isotope-labeled internal standard, was calculated. The calibration curve was obtained for peak ratio (DEHP/DEHP-d_4) versus DEHP concentration levels using HP ChemStation soft from Agilent Technology. The solutions for the calibration curves were blank IV solution containing each concentration level of DEHP. A linear calibration curve was obtained over the cover range, as summarized in Table 2. The responses were found to be linear in the validated range with correlation coefficients (r) greater than 0.99. Fig. 4 shows the

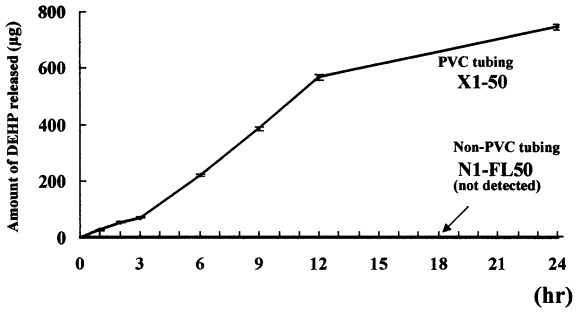

Fig. 6. The release of DEHP from PVC tubing in Sample A solution ($n = 3$). PVC tubing: total amount of leaching DEHP 745.7 μg. Non-PVC tubing: not detected.

calibration curve obtained from ratio of DEHP standard/internal standard. In addition, we determined that the recovery of DEHP (5.0 μg/ml in IV fat emulsion drug; Sample C) by using the extraction and column-switching LC/MS method yielded an average recovery of 92.2% (C.V. = 7.8%, $n = 5$). These results show the method gives a highly precise determination of standards with potential application to the detection of leaching amounts of DEHP in IV solution from PVC medical devices.

3.3. Investigation of DEHP released from PVC tubing for intravenous solution

The method presented in this study was successfully implemented for the high-throughput analysis of DEHP in IV solution. The chromatograms for IV solution (Sample B) passed through PVC are shown in Fig. 5. All preparations were performed by Chemical Hazard Laboratory Room in Hoshi University. The total amounts of DEHP leached from PVC tubing under the conditions listed Table 2 were found to be; 745.7 (Sample A), 30.5 (Sample B), 361.7 (Sample C), 625.2 (Sample D), and 69.5 μg (Sample E), respectively. The relationship between the cumulative amount of leached DEHP in Sample A IV solution and the time exposed to PVC tubing was investigated, as shown in Fig. 6. We propose other IV solutions should also be investigated or to be investigated in terms of the release behavior of DEHP from PVC tubings and devices and the health risk assessment posed by the use of DEHP in PVC medical devices. However, it is difficult that the relationship between IV solution's composition and release behavior of DEHP was simplify illustrated [15,19].

4. Conclusions

These data suggest that this method can assay background exposure to DEHP released from PVC medical devices in the patients. The method was applied to a selection of IV solution samples to establish the first screening of DEHP released from medical devices with respect to their safety. We demonstrate that column-switching LC/MS with internal standard is the method of choice for the accurate analysis of DEHP levels and that this may be extended to include other IV solution samples. Detectable levels of DEHP were found using PVC tubing. This rapid, selective and accurate method will help to elucidate the practicality of using PVC medical devices in relation to the risk potential.

Based on this results, the Ministry of Health, Labour and Welfare of Japan has published its information (Pharmaceuticals and Medical Devices Safety Information, No. 182) of the potential health risks patients face from DEHP leaching from PVC medical devices. In 2000, the Japanese Government established a tolerable daily intake (TDI) for DEHP based on data for testicular and reproductive toxicity [4]. In safety assessment of DEHP released from PVC medical devices, the rationale for establishing this TDI for DEHP and the exposure problem are discussed. We examined medical procedures using PVC medical tubing and generally conclude that patient exposures to DEHP were below the TDI (40–140 μg/kg weight/day) levels. However, we have fears for patient's safety using PVC medical devices because children, especially infants, undergoing certain medical procedures, may represent a population at increased risk from the effects of DEHP.

Acknowledgements

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