J Pharm Anal Vol 1, No 2, 92 - 99 (2011) .

ORIGINAL ARTICLES

Determination of phthalate esters in physiological saline solution by monolithic silica spin column extraction method

Lu Lu[1,2], Yuki Hashi[3]*, Zhi-Hua Wang[1], Yuan Ma[2], Jin-Ming Lin[2]*

[1] *State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, China;* [2] *Department of Chemistry, Tsinghua University, Beijing 100084, China;* [3] *Shimadzu Global COE for Application & Technical Development, Shanghai 200052, China.*

Abstract: Monolithic silica spin column extraction (MonoSpin-SPE) was developed as a simple, sensitive, and eco-friendly pretreatment method which combined with ultra-fast liquid chromatography-mass spectrometry (UFLC-MS) to determine the levels of six phthalate esters, dimethyl-(DMP), diethyl-(DEP), dipropyl-[DPrP], butyl-benzyl-(BBP), dicyclohexyl-(DcHP), and di-*n*-octyl-(DOP) phthalate in physiological saline samples. Under optimized experimental conditions, the method was linear in the following ranges: 0.2 - 50 μg/L for DMP, DEP, DPrP, DcHP and DOP; 5 - 100 μg/L for BBP. The correlation coefficients (R^2) were in the range of 0.9951 - 0.9995 for all the analytes and the limits of detection (LODs) and limits of quantification (LOQs) were in the ranges of 0.02 - 0.9 μg/L and 0.08 - 2.7 μg/L, respectively. The pretreatment process showed good reproducibility with inter-day and intra-day relative standard deviations (RSDs) below 8.5% and 11.2%, respectively. This method was used to determine the levels of six phthalate esters in physiological saline samples and the recoveries ranged from 71.2% to 107.3%. DMP and DEP were found in actual physical saline samples (brand A and brand B).

Keywords: monolithic silica spin column; phthalate esters; physiological saline samples; ultra fast liquid chromatography-mass spectrometry (UFLC-MS)

1 Introduction

Phthalic acid esters (PAEs), commonly referred to as phthalates, are a group of chemical compounds that are widely used because they improve the softness and flexibility of plastics. These compounds have come to the attention of governments and the public in recent years because of their use as plasticizers in consumer products and medical devices, children's toys, and various kinds of packaging [1, 2]. Phthalates are not chemically bound to plastics, and therefore they can be released from the plastic and into the environment [3]. Humans are exposed to PAEs from food that has been contaminated during growth, processing, packaging or storage. Certain phthalates together with their metabolites and degradation products have been found in the liver, kidney, and testicles of humans [4]. Many studies have suggested that the presence of PAEs and their metabolites leads to reproductive and developmental problems in laboratory animals [5]. These compounds may also have adverse effects on human health [6], and current research indicates that measurable concentrations of phthalate metabolites can be found in almost all urine samples taken from most children [7]. The use of 0.9% saline is believed to have originated during the cholera pandemic that swept across Europe in 1831 [8]. Physiological saline can maintain the metabolism of water in human body and is widely used, particularly in hospitals. In order to facilitate transportation, plastic bags are frequently used to package of physiological saline, PAEs may be released from this plastic into the physiological saline solution.

Several regulatory bodies such as the US Environmental Protection Agency (US-EPA), European Union (EU), and the China National Environmental Monitoring Centre have classified PAEs such as diethyl phthalate (DEP), benzyl butyl phthalate (BBP), di-*n*-butyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) as priority environmental pollutants [9]. In particular, DBP, BBP, and DEHP are on the EU list of proposed substances that are suspected to lead to endocrine alteration [10]. Decision No. 2455/2001/EC of the EU parliament stipulates that dimethyl phthalate (DMP), DEP, BBP, DBP, DEHP, and dioctyl phthalate (DOP) be regarded as priority toxic pollutants [11]. DMP, DEP, BBP, and DBP have been detected in physiological saline samples [12]. However, there is no standard detection method to determine the levels of these compounds, and there are no regulations to limit the content of such substances in physiological saline samples.

Received 12 February 2011; Accepted 29 March 2011

* Corresponding authors. E-mail: jmlin@mail.tsinghua.edu.cn (J.-M. Lin), y-hashi@shimadzu.co.jp

Traditional extraction methods used for PAEs detection, such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) [13-16], are time-consuming and require large amounts of organic solvents. Control samples may show high levels of PAEs due to contamination from phthalates in the laboratory environment [17]. Therefore, a simple, rapid, inexpensive and eco-friendly analytical method is required to detect trace levels of phthalates in complex samples. In our study, an improved sample pretreatment method named MonoSpin-SPE was developed to enrich trace levels of phthalates from physiological saline solutions. The MonoSpin C18 (disc type) column consists of octadecyl groups that are chemically bonded to a monolithic silica spin column and this column was used for solid-phase extraction. In recent years, monolithic silica has been developed for high-performance liquid chromatography separation [18]. This material is different from classical silica in that it consists of silica rods instead of particles. The surface area per unit volume of the monolithic silica is larger than that of the normal particle-type silica. Pretreatment of sample with this column has many advantages: it is easy to perform, and requires less eluent volume and less time to purify analytes from the sample compared with normal SPE procedures. The eluent does not need to be evaporated, reducing loss of the target compounds. The advantages of monolithic silica prompted us to use it as a new SPE tool for the extraction of analytes in samples. This kind of column can be used for various matrices. Shintani *et al*. [19], for example, reported the extraction of phenol in water by using an in-tube solid-phase microextraction monolithic silica column. Saito *et al*. [20] reported a specific and sensitive LC-MS method to characterize amitraz and its metabolite in serum by monolithic silica spin column extraction. In order to achieve the simultaneous extraction of multiple biological samples that contain drugs, Nakamoto *et al*. [21] developed a spin column that was packed with a monolithic silica disk in a microtube holder. In this experiment, all the steps of sample preparation could be achieved by centrifugation. The volumes of sample and organic solvent needed were only 5 mL and 200 μL, respectively, in our sample processing procedure and the time we required was about 15 min.

Phthalates are generally analyzed by gas chromatography (GC) [22, 23]. High performance liquid chromatography (HPLC) can be used as an alternative analytical technique and is particularly useful for the analysis of isomeric mixtures and metabolites of phthalates because this method does not require prior derivatization of the sample [24, 25]. Phthalates can be detected by UV detection, flame ionization detection (FID), or mass spectrometry (MS). The most important detection technique for phthalate analysis is MS, which has high sensitivity and specificity, and excludes interference from impurities.

The aim of this study was to develop a simple, rapid, sensitive and UFLC-MS method in combination with MonoSpin-SPE extraction to detect PAEs in physiological saline samples. In this study, the pretreatment and detection of a single sample could be completed within 30 min. To the best of our knowledge, there are no published papers in which such a method has been used to detect phthalates in physiological saline samples.

2 Experimental

2.1 Reagents and chemicals

All chemicals were of analytical grade. Six phthalates, DMP (99.6%), DEP (99.7%), dipropyl phthalate (DPrP, 99.8%), BBP (97.6%), dicyclohexyl phthalate (DcHP, 99.7%) and DOP (99.7%), were purchased from Sigma-Aldrich (Beijing, China). HPLC-grade methanol, acetone, acetonitrile, and dichloromethane were obtained from Merck (Darmstadt, Germany). The water used in the experiments was obtained from Watson's (Quchenshi, Shanghai, China) and had been treated by passing through a Millipore system (USA). MonoSpin C18 consists of a spin column, waste fluid tube, and recovery tube, all of which were supplied by GL Sciences Inc. (Tokyo, Japan). Physiological saline samples were purchased from the Jiangsui Yabang Pharmaceutical Co., Ltd. (brand A, plastic bag), Anhui Shuanghe Pharmaceutical Co., Ltd. (brand B, plastic bag), Shandong Kangning Pharmaceutical Co., Ltd. (brand C, glass bottle) and Shandong Hualu Pharmaceutical Co., Ltd. (brand D, glass bottle).

2.2 Preparation of standard solutions

Individual stock standard solutions were prepared in methanol at a concentration of 1000.0 mg/L. From these solutions, a working mixture was prepared in methanol on a monthly basis. It contained all the standards at a concentration of 100 mg/L each. These solutions were protected from light and stored at −30 ℃ in volumetric flasks. Standard working solutions of different concentrations (in the range of 0.2 − 100 μg/L) that were used on a daily basis were prepared weekly by diluting the stock solutions with methanol. All solutions were stored at 4 ℃.

2.3 Instruments

Chromatographic analyses were performed on a Shimadzu LCMS 2020 system (Shimadzu Corporation, Kyoto, Japan) with two LC-20AD pumps, an SIL-20AC autosampler, CTO-20AC column oven, and LCMS 2020 mass spectrometer. A personal computer equipped with a Shimadzu Labsolution LCMS 5.1 system was used to process the MS data. The analytes were separated on a Shim-pack XR-ODS Ⅱ column (75 mm × 2.0 mm i.d., 2.2 μm).

2.4 Conditions for chromatography and MS

The mobile phase consisted of a mixture of methanol and water (v/v). The flow rate and injection volume were 0.4 mL/min and 2 μL, respectively, and the column tempera-

ture was 40 ℃. All of the compounds were eluted within 8 min. A rest time was used to re-establish the column equilibrium.

Mass analysis was performed using an ESI source in positive ion mode. The following conditions were used: nebulizing gas, 1.5 L/min; dry gas, 10 L/min; detection voltage, 1.1 kV; DL temperature, 250 ℃; and heat block temperature, 450 ℃. The LC-ESI-MS analysis for the six phthalates was performed in scan mode. Phthalates were identified on the basis of the retention times and mass spectra of the phthalate standards. To improve the sensitivity for phthalates, the selected ion monitoring mode was used. The characteristic ions selected for quantitative studies are listed in Table 1 and corresponded to the $[M+Na]^+$ ions.

2.5 Typical procedure

A schematic diagram of the novel MonoSpin-SPE apparatus is shown in Figure 1. The method used followed the literature [20] with little modification.

Step 1 (Conditioning): The spin column was attached to the waste fluid tube, and 100 μL of methanol was added to the spin column. This was centrifuged at 5000 rpm for 2 min. After centrifugation, 100 μL of water was placed in the spin column and the procedure was repeated. The waste fluid (200 μL) was removed from the waste fluid tube.

Step 2 (Adsorption): The sample solution (5 mL) was placed in the spin column and centrifuged at 5000 rpm for 15 min. In this step, analytes were adsorbed by the C18 sorbent. In order to obtain adsorption equilibrium, adsorption conditions should be optimized.

Step 3 (Rinsing): 200 μL of water was added, and then centrifuged at 5000 rpm for 2 min.

Step 4 (Elution): The spin column was placed in the recovery tube, and then 50 μL of acetone was added to the spin column, which was then centrifuged at 5000 rpm for 2 min. This step was then repeated with a second aliquot (50 μL) of acetone. 100 μL of eluent was obtained and detected by LC/MS.

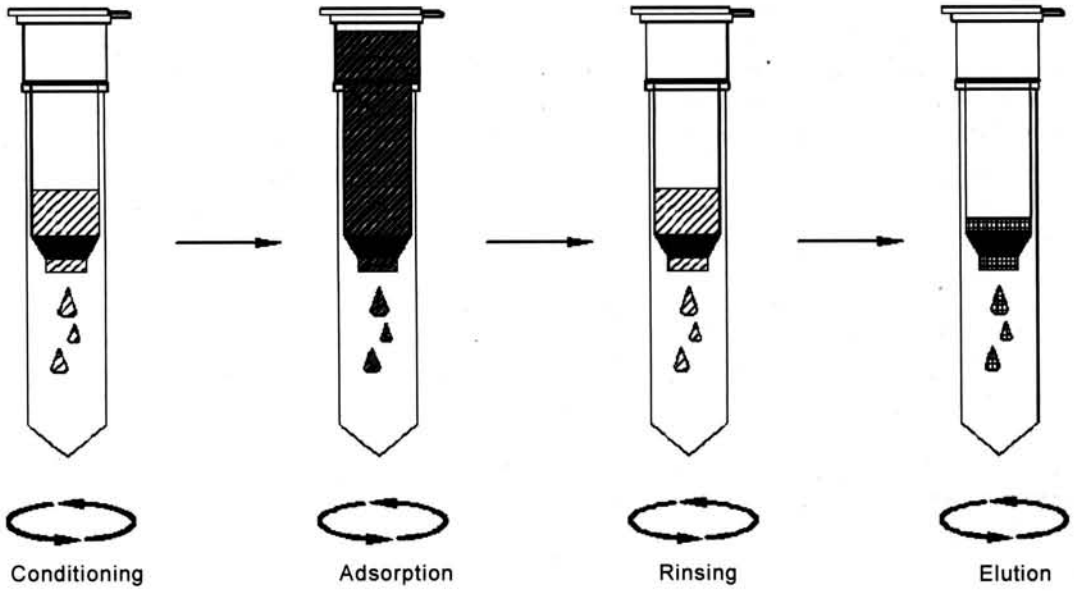

Figure 1 Schematic diagram of the MonoSpin-SPE (monolithic silica spin column extraction) apparatus

3 Results and discussion

3.1 Optimization of the MS parameters

The mass spectrograms and product ions of DMP, DEP, DPrP, BBP, DcHP, and DOP were clearly observed in full-scan mode by infusing individual standard solutions (50 μg/L) dissolved in methanol. The molecular ion $[M+Na]^+$ was selected as the quantitative ion for all compounds, as shown in Table 1.

Table 1 Retention time and ions selected for the analysis of the target phthalates

Compounds	Retention time (min)	Quantification ions (m/z)
DMP	1.613	217.2
DEP	2.766	245.2
DPrP	3.873	273.2
BBP	4.661	335.1
DcHP	5.412	353.2
DOP	8.016	413.2

To achieve maximum sensitivity for each analyte, 2 μL of a mixed standard solution of PAEs (50 μg/L) was injected into the mass spectrometer using an initial chromatographic mobile phase flow rate of 0.4 mL/min. The following

parameters were optimized: nebulizing gas, dry gas, detection voltage, DL temperature, and heat block temperature, and to the optimized conditions were 1.5 L/min, 10 L/min, 1.1 kV, 250 ℃, and 450 ℃, respectively.

3.2 Optimization of the HPLC separation

The mobile phases acetonitrile [0.1% (v/v) acetic acid]: water [0.1% (v/v) acetic acid], and methanol: water were compared. A better resolution was obtained using methanol: water as the mobile phase. BBP and DEHP were still present in the flow tubing; DEHP is an isomer of DOP. In order to separate the target compounds and decrease analysis time, different binary gradient programs were employed. The initial volume ratio of A (water)/B (methanol) was 50/50: the volume ratio of B was increased from 50 to 90 in 4 min and maintained for 1.5 min at a volume ratio of 90 B, then the B volume ratio was increased to 100 in 2 min and maintained for 1 min. The B volume ratio was decreased to 50 in 1 min and this ratio was maintained for 5.5 min to reequilibrate the column. Under these conditions, the six compounds were well separated within 8 min. Figure 2 shows the chromatogram for the separation of a mixture of these compounds under optimized conditions.

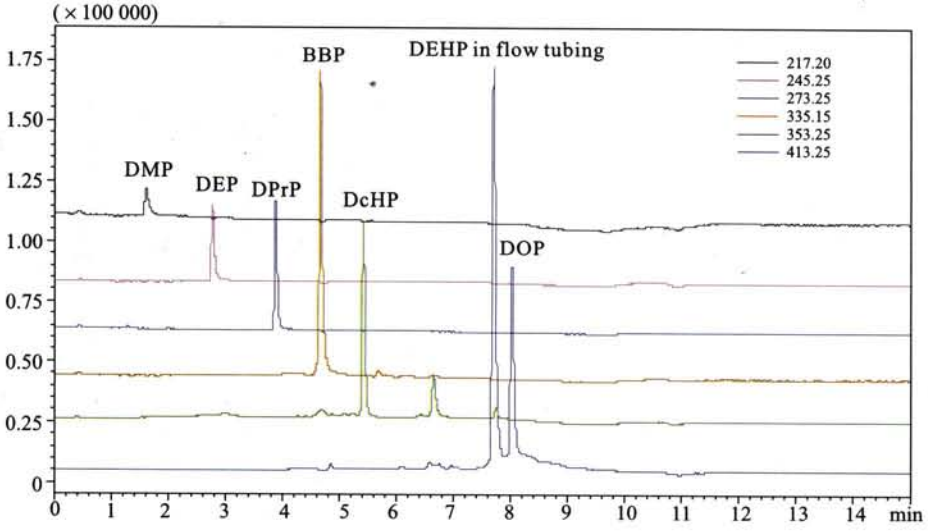

Figure 2 The selected ion monitoring chromatogram for standard species (concentration 50 μg/L) of six phthalates.

3.3 Method development

Various parameters including the type and volume of eluent, eluting temperature, centrifugal speed, and sample volume affect the MonoSpin-SPE performance and efficiency, and these factors were therefore optimized.

3.3.1 Selection of the eluent and elution temperature

Selection of a suitable eluent that can enhance both the elution performance and capacity of target compounds is essential in the MonoSpin-SPE method. In this study, HPLC/MS was used, which necessitated the use of a water-miscible eluent. Four organic solvents: methanol, acetonitrile, acetone and dichloromethane were tested as eluent. As shown in Figure 3, good extraction efficiency of DMP, DEP, DPrP, BBP, DcHP, and DOP was achieved when acetone was used as the eluent solvent, and the repeatability was also good. These compounds are low to medium polarity (log P 1.6 − 8.3), making acetone a suitable to extract these compounds. Dichloromethane is not suitable for LC-MS analysis. Therefore, acetone was chosen as the eluent in the following experiments. This solvent has the added advantages of low toxicity and cost. Prior to use, these organic solvents and water were injected into the LC/MS to detect whether any phthalates were present. Chromatographic-grade organic solvents were used together with ultrapure water. No contamination was detected in the solvent.

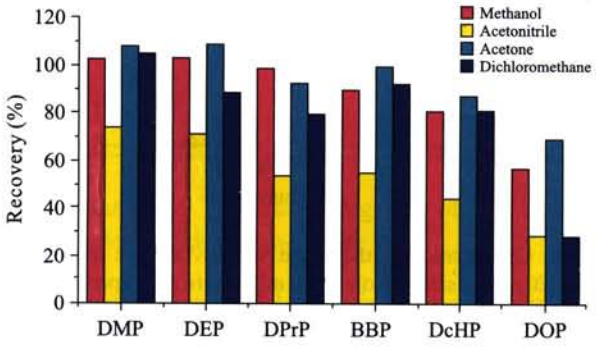

Figure 3 Optimization of the eluent. Sample spiked concentration, 20 μg/L; sample volume, 2 mL; centrifugal speed, 5000 rpm; adsorption time, 15 min; elution temperature, 20 ℃; eluent volume, 50 μL; elution time, 2 min; and centrifugal speed, 5000 rpm.

The effect of temperature on the elution efficiency was studied in the range of 5 − 25 ℃. The recovery of PAEs relative to the temperature of the organic solvent is shown in

Figure 4. The recovery increased with an increasing elution temperature; probably due to increased molecular movement, allowing rapid equilibration of the two phases. Considering the volatility of the eluent, an elution temperature of 25 ℃ was selected to ensure good recovery in subsequent experiments.

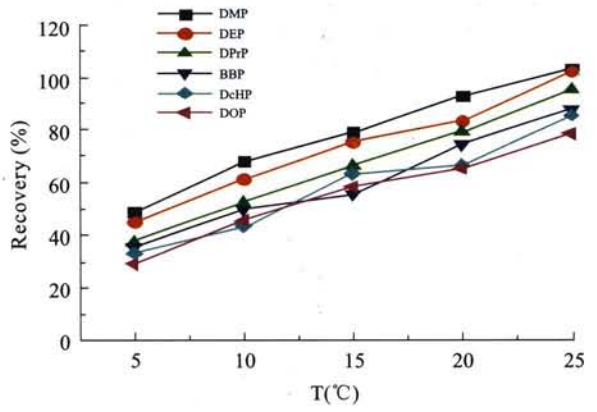

Figure 4 Optimization of the elution temperature. Sample spiked concentration, 20 μg/L; sample volume, 2 mL; centrifugal speed, 5000 rpm; adsorption time, 15 min; eluent, acetone; eluent volume, 50 μL; elution time, 2 min; and centrifugal speed, 5000 rpm.

3.3.2 Optimization of eluent volume

To ensure that the target compounds were completely eluated from the C18 sorbent, the effect of the eluent volume on the elution efficiency was studied in the range of 20 – 200 μL. PAE recovery relative to the volume of organic solvent used is shown in Figure 5.

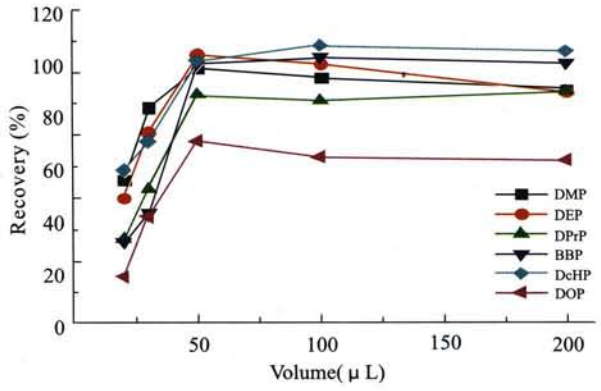

Figure 5 Optimization of the eluent volume. Sample spiked concentration, 20 μg/L; sample volume, 2 mL; centrifugal speed, 5000 rpm; adsorption time, 15 min; eluent, acetone; elution temperature, 25 ℃; elution time, 2 min; and centrifugal speed, 5000 rpm.

The results showed that the recovery of phthalates increased with increasing volumes of acetone from 20 to 50 μL. When the volume of acetone was more than 50 μL, the recoveries of DcHP, BBP and DPrP became constant and those of DMP, DEP and DOP decreased. In order to ensure complete recovery, 50 μL of acetone was used to elute the loaded spin column twice, to give a total of 100 μL of eluent which was then analyzed by LC/MS.

3.3.3 Optimization of the centrifugal speed and adsorption time

Equilibrium between the sorbent and sample solution is determined by the time taken with the solvent to pass through the column. To ensure good equilibrium and to reduce the time required for analysis, the rotary speed of the centrifuge was optimized in the range of 3000 – 7000 rpm. The effect of rotary speed on the recovery of the six phthalates is shown in Figure 6.

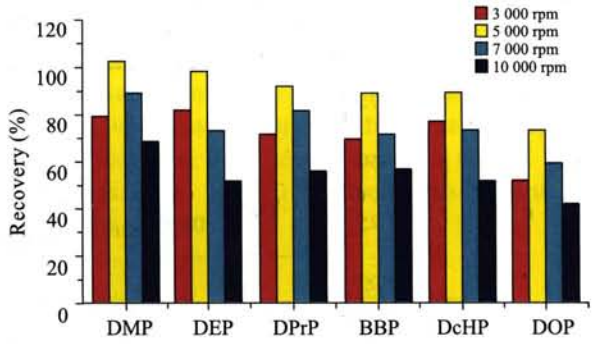

Figure 6 Optimization of the centrifugal speed. Sample spiked concentration, 20 μg/L; sample volume, 2 mL; adsorption time, 15 min; eluent, acetone; eluent volume, 100 μL; elution temperature, 25 ℃; and eluton time, 2 min.

The results indicated that 5000 rpm was the optimal speed. When the speed was less than 5000 rpm, the physiological saline samples did not pass through the column completely, and the analytes were only adsorbed partially by the sorbents, leading to reduced recoveries. At speed greated than 5000 rpm, the time taken by the sample solution to pass through the column was too short to ensure complete adsorption of the analytes. The equilibrium between sorbent and sample solutions was not established. Therefore, 5000 rpm was selected for subsequent experiments.

Under the optimum centrifugal speed, 15 min was sufficient to allow the compounds to reach equilibrium between the sorbent and solution.

3.3.4 Sample volume and pH effect

An increase in the sample volume leads to an increase in the amounts of target compounds adsorbed to the C18 sorbent, which in turn improves the sensitivity of the method. However, sorbents have a breakthrough volume, therefore the sample volume was optimized. To examine the effect of sample volume, five different sample volumes (1, 2, 3, 4, 5 and 8 mL) were tested. The results showed that the largest analytical response was obtained when 5 mL of sample solution was used. When 8 mL was used, the recovery of PAEs decreased. The matrix sample capacity of the sorbent was limited, so 5 mL may be not suitable for highly concentrated samples.

Under the following optimized conditions, including

sample volume, 5 mL; centrifugal speed, 5000 rpm; adsorption time, 15 min; adsorption temperature, 25 ℃; eluent, acetone; elution temperature, 25 ℃; eluent volume, 100 μL; and elution time, 2 min, the effect of sample pH was studied. Because phthalates are neutral, a pH value of 7 was appropriate. In this experiment, BBP was detected as a contaminant from the flow tubing at a stable concentration. The concentration of BBP due to the flow tubing should therefore be taken into account when calculating the content of BBP in physiological saline solutions.

3.3.5 Validation of the method

Calibration curves were obtained by analyzing standard solutions of the six PAEs added to the ultrapure water using the above method in the following linear ranges: 0.2 – 50 μg/L for DMP, DEP, DPrP, DcHP and DOP; 5 – 100 μg/L for BBP. The correlation coefficients (R^2) were in the range of 0.9951 – 0.9995 for all the analytes (Table 2).

Table 2 Linearity, limit of detection (LOD), limit of quantitative (LOQ) and repeatability of the proposed method

Compounds	Linear equation	Linear range (μg/L)	R^2	RSD (%, n = 5)	LOD (μg/L)	LOQ (μg/L)
DMP	$y = 79776x + 100248$	0.2 – 50	0.9961	4.3	0.05	0.14
DEP	$y = 103664x + 360964$	0.2 – 50	0.9953	5.1	0.02	0.08
DPrP	$y = 107073x + 137015$	0.2 – 50	0.9956	3.3	0.03	0.10
BBP	$y = 99507x + 2E + 06$	5.0 – 100	0.9983	5.4	0.90	2.70
DcHP	$y = 84380x + 75123$	0.2 – 50	0.9951	3.7	0.04	0.11
DOP	$y = 137923x + 48973$	0.2 – 50	0.9995	8.9	0.04	0.11

x = the concentrations of target compounds. y = the peak area of target compounds. RSD with 10 μg/L of each PAE. LOD was defined at S/N = 3. LOQ was defined at S/N = 10.

The limits of detection (LODs) and limits of quantification (LOQs) are regarded as the minimum concentrations of analytes that can be confidently identified and quantified, respectively, by this method. The LODs and LOQs were estimated as the analyte concentration that produced a signal/noise ratio of 3 : 1 and 10 : 1, and these were in the ranges of 0.02 – 0.9 μg/L and 0.08 – 2.7 μg/L, respectively, for the PAEs. These values were lower than those reported in other studies. Pérez Feás *et al*. obtained LODs of 0.99 μg/L DMP, 22.13 μg/L DEP, and 5.32 μg/L BBP in physiological saline samples [12].

3.4 Analysis of a real sample

The proposed MonoSpin-SPE technique was applied to the determination of the levels of PAEs in physiological saline samples. Precision was evaluated by measuring the intra-day and inter-day relative standard deviations (RSDs). The intra-day precision was determined by analyzing spiked saline samples five times a day at three different fortified concentrations of 5, 10, and 50 μg/L (Table 3).

Table 3 Recovery and reproducibility of the method in analysis of the real samples

Analytes	5 μg/L				10 μg/L				50 μg/L			
	Intra-day (n = 5)		Inter-day (n = 3)		Intra-day (n = 5)		Inter-day (n = 3)		Intra-day (n = 5)		Inter-day (n = 3)	
	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)
DMP	6.1	105.2	7.6	102.6	5.6	101.8	6.1	97.2	4.1	96.3	4.8	97.2
DEP	5.9	94.3	6.8	90.3	5.3	95.1	5.6	98.4	5.2	91.2	6.1	92.3
DPrP	5.2	93.6	8.2	97.7	3.7	107.3	7.4	103.6	3.4	92.6	4.6	94.2
BBP	6.9	89.7	7.3	93.5	4.2	94.8	5.9	92.1	3.5	87.2	4.1	85.3
DcHP	6.5	87.4	7.9	83.4	4.9	97.6	6.2	94.8	4.8	82.3	5.8	88.4
DOP	8.7	79.3	11.2	71.5	7.6	71.2	9.9	76.4	8.5	71.5	9.7	74.2

The inter-day precision was determined by analyzing spiked samples at three fortified concentrations for three consecutive days. The results are shown in Table 4. The intra-day and inter-day RSDs ranged from 3.5% to 8.5% and from 4.1% to 11.2%, respectively. At all three fortified concentrations, the recoveries of the six PAEs were in the range of 71.2% – 107.3%. The recovery of DOP (71.2% – 79.3%) was not as good as others due to its high hydrophobicity (log P = 8.3), leading to inefficient recovery by organic solvents from the C18 sorbent. These results indicated that the MonoSpin-SPE method can be successfully used to analyze trace levels of PAEs present in physiological saline samples. The chromatograms of the blank and spiked samples are shown in Figure 7.

Table 4 Concentrations (μg/L) of PAEs found in different physiological saline samples

Physiological saline solutions	Concentration (μg/L)					
	DMP	DEP	DPrP	BBP	DcHP	DOP
Brand A	10.75	2.37	ND	ND	<LOD	ND
Brand B	11.40	2.62	ND	ND	<LOD	ND
Brand C	ND	ND	ND	ND	<LOD	ND
Brand D	ND	ND	ND	ND	<LOD	ND

ND, not deteted.

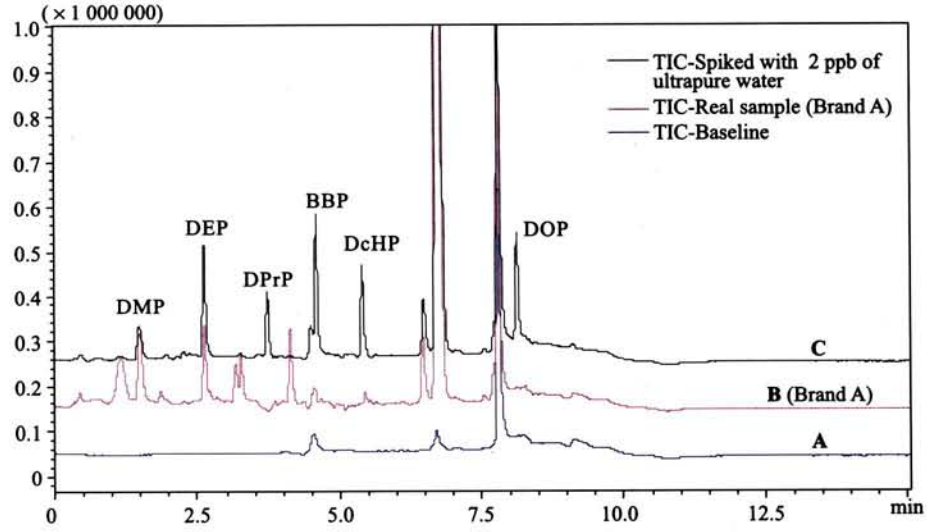

Figure 7 Chromatograms of DMP, DEP, DPrP, BBP, DcHP and DOP in real samples. **A**, baseline; **B**, real sample (brand A) using MonoSpin C18; **C**, spiked with 2 μg/L of ultrapure water using MonoSpin C18.

Because the brand A and B physiological saline solutions are stored in the plastic bags, the phthalates may be released from the package or during production. DMP and DEP were detected, as shown in Table 4. The concentrations of DMP were higher than 8.0 μg/L, which is the EU limit set for these compounds in drinking water samples [11]. Brand C and D physiological saline solutions were preserved in the glass bottles, and no PAEs were detected in these samples. For the purpose of reducing PAEs pollution, glass bottles should therefore be adopted as far as possible.

4 Conclusion

A MonoSpin-SPE-LC-MS method has been developed, and has been shown to be efficient and sensitive for the determination of phthalates in saline samples. A centrifuge was used to accelerate the sample pretreatment process. The detection limit of this method was lower than or equal to the values reported in other studies. However, sample preparation was faster in this method and less toxic organic solvent was required. A pretreatment time of 25 min and organic solvent volume of 200 μL were used in this assay. Therefore, this method is a promising alternative to the traditional techniques used for phthalate detection.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 90813015, 20935002).

References

[1] Castillo M, Barceló D. Analysis of industrial effluents to determine endocrine-disrupting chemicals. *Trends Anal Chem*, 1997, 16(10):574-583.
[2] Lovekamp-Swan T, Davis BJ. Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ Health Perspect*, 2003, 111(2): 139-145.
[3] Heudorf U, Sundermann VM. Phthalates: toxicology and exposure. *Int J Environ Health*, 2007, 210(5):623-634.
[4] Arcadi FA, Costa C, Imperatore C, *et al*. Oral toxicity of bis (2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. *Food Chem Toxicol*, 1998, 36(11):963-970.
[5] Saillenfait AM, Gallissot F, Sabaté JP. Evaluation of the development toxicity of diallyl phthalate administered orally to rats. *Food Chem Toxicol*, 2008, 46(6):2150-2156.
[6] Matsumoto M, Koizumi MH, Ema M. Protentional adverse effects of phthalic acid esters on human health: a review of recent studies on reproduction. *Regul Toxicol Pharm*, 2008, 50(1):37-49.
[7] Sathyanarayana S. Phthalates and children's health. *Curr Probi Prediatr Adolesc Health Care*, 2008, 38(2):34-49.
[8] Awad S, Allison SP, Lobo DN. The history of 0.9% saline. *Clin Nutr*, 2008, 27(2):179-188.
[9] US-EPA. *Code of Federal Regulation*. 40 CFR Part 136, 1992.
[10] Communication from the Commission to the Council and the European Parliament on the implementation of the community strategy for endocrine disrupters—a range of substances suspected of interfering with the hormone systems of humans and wildlife, COM(1999) 706; COM(2001) 262 final, Brussels, 2001.
[11] Decision No 2455/2001/EC of the European Parliament and council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC.
[12] Férez Feás P, Barciela Alonso MC, Peña-Vázquez E, *et al*. Phthalates determination in physiological saline solutions by HPLC-ES-MS. *Talanta*, 2008, 75(5):1184-1189.
[13] Carlo MD, Pepe A, Sacchetti G, *et al*. Determination of phthalate esters in wine using solid-phase extraction and gas chromatography-mass spectrometry. *Food Chem*, 2008, 111(3):771-777.
[14] Jiménez FJL, Rubio S, Bendito DP. Determination of phthalate esters in sewage by hemimicelles-based solid-phase extraction and liquid chromatography-mass spectrometry. *Anal Chim Acta*, 2005, 551(1-2):142-149.
[15] Niu HY, Cai YQ, Wei FS, *et al*. Cetyltrimethylammonium bromide-coated titanate nanotubes for solid-phase extraction of phthalate esters from natural waters prior to high-performance liquid chromatography analysis. *J Chromatogr A*, 2007, 1172(2):113-120.
[16] Zhao RS, Wang S, Yuan JP, *et al*. Investigation of feasibility of bamboo charcoal as solid-phase extraction adsorbent for the enrichment and determination of four phthalate esters in environmental water samples. *J Chromatogr A*, 2008, 1183(1-2):15-20.
[17] Prokpková G, Holadová K, Poustka J, *et al*. Development of a solid-phase microextraction method for the determination of phthalic acid esters

in water. *Anal Chim Acta*, 2002, 457(2):211-223.
[18] Kato M, Sakai-Kato K, Toyo'oka T, *et al*. Effect of preparatory conditions on the performance of photopolymetized sol-gel monoliths for capillary electrochromatography. *J Chromatogr A*, 2002, 961(1):45-51.
[19] Shintani Y, Zhou X, Furuno M, *et al*. Monolithic silica column for in-tube solid-phase microextraction coupled to high performance liquid chromatography. *J Chromatogr A*, 2003, 985(1-2):351-357.
[20] Saito T, Yamamoto R, Inoue S, *et al*. Simutaneous determination of amitrz and its metabolite in human serum by monolithic silica spin column extraction and liquid chromatography-mass spectrometry. *J Chromatogr B*, 2008, 867(1):99-104.
[21] Nakamoto A, Nishida M, Saito T, *et al*. Monolithic silica spin column extraction and simultaneous derivatisation of amphetamines and 3,4-methylenedioxyamphetamines in human urine for gas chromatographic-mass spectrometric detection. *Anal Chim Acta*, 2010, 661(1):42-46.
[22] Prieto A, Telleria O, Etxebarria N, *et al*. Simultaneous preconcentration of a wide variety of organic pollutants in water samples. Comparison of stir bar sorptive extraction and membrane-assisted solvent extraction. *J Chromatogr A*, 2008, 1214(1-2):1-10.
[23] Carrillo JD, Martínez MP, Tena MT. Determination of phthalates in wine by headspace solid-phase microextraction followed by gas chromatography-mass spectrometry use of deuterated phthalates as internal standards. *J Chromatogr A*, 2008, 1181(1-2):125-130.
[24] Yao JR, Xu H, Lü LL, *et al*. A novel liquid-phase microextraction method combined with high performance liquid chromatography for analysis of phthalate esters in landfill leachates. *Anal Chim Acta*, 2008, 616(1):42-48.
[25] Orsi DD, Gagliardi L, Porràa R, *et al*. A enviromentally friendly reversed-phase liquid chromatography method for phthalates determination in nail cosmetics. *Anal Chim Acta*, 2006, 555(2):238-241.