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A Research for Reduction the Pollution in the Pretreatment Process of PAEs in Human Serum

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***Abstract*—Phthalate acid esters (PAEs) are wide spreading the environment. In order to reduce pollution in the process of human serum PAEs pretreatment, some experimental conditions had been investigated. Shortening the exposure time of the eluent and nitrogen blowing time, purifying silica gel and using glass chromatography column with glass piston could reduce PAEs pollution. The method had a good linearity with correlation coefficient r≥0.9993, good repeatability with RSD between 2.0 % and 15.3 % , sensitive with LOD varying**

from 0.5 to 1.5 ng/mL and was satisfied to determine real serum samples.

Keywords- PAEs; Pollution; Human serum.

I. INTRODUCTION

PAEs are widely used in rubber and plastic to enhance the flexibility [1] and can be found in building materials, medical devices, manufacture of plastics, cosmetics and children's toys [2]. PAEs can be absorbed through the skin and harmful to human health as endocrine disrupter [3-4]. The human serum samples are difficult to get, and how to detect accurately trace level PAEs in human serum sample with complicated matrix is particularly important. Our study was based on the study of Shao et al [5] , tried to reduce PAEs pollution source in determination of PAEs in human serum samples and got the satisfied results.

II. EXPERIMENTAL

A. Instrumentation and Reagents

Trace DSQ gas chromatograph-mass spectrometer (ThermoFisher Corp., USA), nitrogen blowing instrument EFAA-DC12, Laborota 400 efficient rotary evaporator (Heidolph Corp., Germany).

Hexane, dichloride methane, and methanol of HPLC grade were purchased from Bizcomr, which were distilled prior to use. Acetone and ether of analytical grade were distilled prior to use. Silica gel and cotton were extracted by Soxhlet extraction with dichloride methane for 72 h and then put into a desiccator. Anhydrous sodium sulfate and sodium chloride were baked at 450 ^{0}C for 5 h in muffle and then put into a desiccator.

The 2 mg/mL of PAEs mixed standard solution including dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), dioctyl phthalate (DNOP), benzyl butyl phthalate (BBP), Di(2-ethylhexyl) phthalate (DEHP)and 0.1mg/mL of DBP-D4 standard solution were purchased from Accustandards (New Haven, CT). Benzyl benzoate (BB) standard solutions were purchased from Dr. Ehrenstorfer GmbH (Augsburg).

B. Collection and Preservation of Serum Sample

The blood samples were provided by a hospital and the serum samples were collected by centrifugation, then they were stored in a refrigerator under -80^{0}Ccondition.

C. Sample Pretreatment

In a 50mL glass centrifuge tube, 1.0 mL serum was added, then recovery indicators DBP-D4, and 1 mL of 6 mol/L HCl were also put into it. This solution was shaken. Then 10 mL ether was added and violently shaken. The organic layer and aqueous layer were separated by centrifugation. Then the aqueous layer was extracted twice by 5 mL ether. All organic layers were merged together. This organic layer was dehydrated by anhydrous Na2SO4. Then the solvent was removed by rotary evaporation, the residues were purified by glass chromatograph column filled with silica gel to get PAEs (10 mL eluent: acetone/hexane, 2:8). After most of the eluent was removed by rotary evaporation, the residues were transferred to the vessels by small amount of solvent, then dried by nitrogen blowing. Prior to instrumental analysis, the internal standard BB was added, and the volume was settled to 300 μL with hexane. An aliquot of 1 μL was withdrawn and analyzed by GC-MS. All the experiments were performed in triplicate.

TABLE I. THE RETENTION TIME,CHARACTERISTIC IONS, LINEAR RANGE AND RSD OF PAES

Analytes	Rt(min)	Quantitative ions (m/z)	Qualitative ions (m/z)	Linear range (ng/mL)	r	RSD
DMP	5.72	163, 194	163	5-300	0.9997	2.0%
DEP	7.05	149, 177	149	5-300	0.9996	4.6%
DBP	12.00	149, 167	149	5-300	0.9994	5.1%
BBP	18.21	91, 149	149	5-300	0.9993	3.7%
DEHP	21.13	149, 167, 279	149	5-300	0.9996	15.3%
DNOP	23.99	149, 279	149	5-300	0.9994	4.7%
DBP-d4	11.98	153	153	3-180	0.9996	3.1%
BB	9.39	91, 105	105	—	—	—

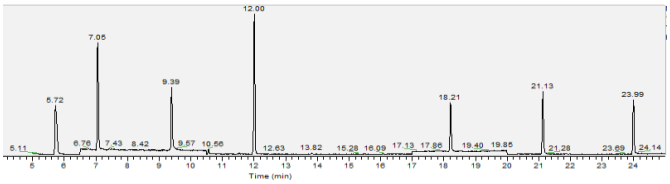

Figure 1. The chromatogram of PAEs

III. RESULTS AND DISCUSSION

A. Effect of the Exposure Time of the Eluent

A 10 mL mixture of distilled acetone/distilled hexane (2:8, V:V) was exposed to air for some time, then concentrated by rotary evaporation and nitrogen blowing, and then diluted to 300 μL for GC-MS analysis. As seen in Table 2, the results showed that the longer the reagent was exposed to air, the higher contamination for DBP. The other five PAEs were not affected by the exposure time. Therefore, in order to reduce the contamination of DBP, the eluent should be used as soon as possible if they were distilled.

TABLE II. THEEFFECT OF EXPOSURE TIME OF THE ELUENT

Exposure time	10 minutes	3.5 hours	10 days
C_{DBP}/ (ng/mL)	<50	≈100	>300

B. Effect of the Concentration Methods

Two methods were chosen to concentrate the eluent. One was by rotary evaporation and nitrogen blowing, the other was just by nitrogen blowing. The results (shown in Table 3) indicated that the concentration method of just by nitrogen blowing could bring more serious DBP contamination for the longer time to concentration. Hence, the eluent was concentrated by rotary evaporation and nitrogen blowing.

TABLE III. EFFECT OF THE CONCENTRATION METHODS

Concentration method	Rotary evaporation and nitrogen blowing	Nitrogen blowing
C_{DBP}/ (ng/mL)	>50ng/mL	>300ng/mL

C. *Effect of the Purity of Silica Gel*

There were three kinds of silica gel, the first was with no treatment, the second was purified by dichloromethane for 72 hours, and the last was purified by dichloromethane for 72 hours, but hadbeen put invacuum dryer for one month. The three kinds of silica gel were put into10 ml of distilled hexane / distilled acetone (8:2, V:V) for 30 minutes, then concentrated by rotary evaporation and nitrogen blowing, and finally diluted to 300 μLfor GC-MS analysis.The results showed that the purity of silica gel had obvious effect on the contamination of PAEs (Table 4). Consequently, silica gel should be purified by dichloromethane for 72 hours and used quickly.

TABLE IV. EFFECT OF THE PURITY OF SILICA GEL

Silica gel	With no treatment	Purified for 72 hours	Purified for 72 hours, then put in vacuum dryer for one month
PAEs	DMP, DEP, DBP, DEHP detected	Not detected	DBP 500ng/mL
contamination	DBP>1000ng/mL DEHP>100ng/mL		

D. *Effect of the Material of Piston of Glass Chromatography Column*

Glass chromatography columns, one with polytetrafluoroethylene (PTFE) piston, the other with glass piston, were eluted with10 ml of distilled hexane / distilled acetone (8:2, V:V),then concentrated by rotary evaporation and nitrogen blowing, and finally diluted to 300 μL for GC-MS analysis. The results were shown in Table 5, and it showed that PTFE piston had some pollution to DBP, so glass chromatography column with glass piston was used.

TABLE V. EFFECT OF THE MATERIAL OF PISTON OF GLASS CHROMATOGRAPHY COLUMN

Glass chromatography column	With glass piston	With PTFE piston
DBP contamination	Not detected	20ng/mL

E. *Evaluation Method*

1) *Repeatability and Limit of Detection*

Under the optimal experimental conditions, the linearity, precision and limit of detection of the proposed method were studied. To obtain the method linearity, the standard solutions from 5 to 300ng/mL were analyzed by GC–MS. Each analyte exhibited good linearity with correlation coefficient r≥0.9993 (shown in Table 1). The relative standard deviation (RSD) values varied from 2.0 % to15.3 %. The limit of detection (LOD) values were from 0.5~1.5 ng/mL (S/N=3).A typical chromatogram was shown in Fig.1.

2) *Accuracy*

In order to guarantee the reliability and accuracy of the data, a procedural blank, a spiked procedural blank, a spiked sample, an internal standard (BB) quantification and recovery indicator (DBP-D4) were used to process quality control. For each batch of 10 serum samples, a procedural blank and a spiked procedural blank were processed. Some of PAEs were detected in the procedural blank (shown in Table 6). The recoveries of PAEs were 38.6%-80.0% in the procedural blank samples, and 39.0%-70.1% in the serum samples (Table 7), which met trace analysis of organic matters in samples with complicated matrix.

TABLE VI. PAES IN THE PROCEDURAL BLANK

Components	DMP	DEP	DBP	DEHP
Content (ng/mL)	1-11.91	11.48-19.05	23.35-34.26	17.7-19.69
average value(ng/mL)	4.59	13.89	28.74	18.99

F. *Analysis of Real Samples*

Under the optimal experimental conditions, the method was applied to analyze 10 human serum samples. Every serum had two copies and they were performed simultaneously by two labors. The system error of the two investigators was 0-3.21%. After subtracting the procedural blank, the results were shown in Table 8. DNOP was below the detection limit and the other five targets were detected which indicated that the body had been polluted by PAEs.

TABLE VII. THERECOVERIES OF PAES IN PROCEDURAL BLANK SAMPLES AND SERUM SAMPLES (%)

Analytes	DMP, DEP	DBP	DEHP	BBP	DONP	DBP-D4
Solvent addition	38.6-47.4	60.9-68.1	51.0-75.3	63.2-80.0	53.7-73.6	63.0-77.2
Serum addition	39.0-47.2	47.2-51.1	41.0-55.4	59.8-64.3	50.6-55.5	66.7-70.1

TABLE VIII. PAES CONTENT OF 10 HUMAN SERUM SAMPLES

Analytes	DMP	DEP	DBP	BBP	DEHP	DNOP
The detection rate (%)	100	50	100	100	100	0
Min-value (ng/mL)	8.3	ND	64.1	2.6	109.8	ND
Max-value(ng/mL)	16.3	1.3	152.3	9.1	301.5	ND
Mid-value (ng/mL)	10.4	0.5	106	4.2	251	ND

ND: means no detection.

IV. CONCLUSIONS

In this work, we tried the best to avoid the new PAEs pollution in the pretreatment, and got the ideal results. Shortening the exposure time of the eluent and nitrogen blowing time could effectively reduce DBP pollution. Silica gel purity and glass chromatography column with glass piston also contributed much to reducing the PAEs pollution.

The method has good repeatability, low limit of detection and high accuracy. It could be used to determine PAEs in human serum.

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