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ARTICLES

One-Step in-Syringe Dispersive Liquid–Liquid Microextraction and GC-FID Determination of Trace Amounts of di(2-Ethylhexyl) Phthalate and Its Metabolite in Human Urine Samples[1]

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Abstract—Di(2-ethylhexyl) phthalate (**DEHP**) is used as plasticizer in polyvinylchloride **(PVC)** plastics. Its metabolites and the parent phthalates are considered toxic. As the DEHP plasticizers are not chemically bound to PVC, they can migrate, evaporate or be leached into indoor air and atmosphere, foodstuff, and other materials. We have reported a novel, easy and available analytical method for the determination of DEHP and its metabolite, mono(2-ethylhexyl) phthalate (**MEHP**) in human urine samples by the in-syringe dispersive liquid–liquid microextraction method coupled with gas chromatography with flame ionization detector. The limits of detection and precision (RSD) were 2.5 μg/L and 1.4% for DEHP and 1.1 μg/L and 3.0% for MEHP, respectively. This method could be utilized for routine monitoring of the trace DEHP and MEHP in urine of human exposure to plasticizers.

Keywords: di(2-ethylhexyl) phthalate, urine analysis, GC-FID

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With an annual worldwide production of 1–4 million metric tons [1], di(2-ethylhexyl) phthalate is the most widely used commercial plasticizer. It is commonly used as a softener to improve material flexibility in plastic pipes (up to 30% in PVC by mass) [2], tubing (including those used in medical procedures), packing materials (including those used in food packaging) [3], and as a thickener in cosmetics, personal care products [4], and printing inks (including those used in food-wrap labels) [5]. DEHP is a viscous liquid at room temperature and is not covalently bonded to polymeric matrices. Therefore it readily diffuses from plastics and various other products into blood used in transfusions, food, and drinks [6]. Approximately 2% of the global phthalate production is released into the environment each year and part of this release is incorporated into the food chain [7]. DEHP has low acute toxicity and can be metabolized quickly in humans. In fact, 47% of the DEHP ingested is excreted in urine after metabolic hydroxylation and hydrolysis to produce mono(2-ethyl-5-hydroxyhexyl) phthalate, mono(2-ethyl-5-oxohexyl) phthalate, and MEHP within two days after ingestion [8]. According to the U.S. Department of Health and Human Services (DHHS), prior to 2002, inhalation of contaminated indoor air, ingestion of contaminated water and food, and exposure to DEHP from plastic medical products were the dominant pathways leading to human exposure of DEHP [9].

Until now, the methods for determination of DEHP and MEHP are only limited to liquid–liquid extraction coupled with gas chromatography–mass spectrometry (**GC–MS**) [10], solid-phase extraction coupled with HPLC–MS [11], liquid chromatography–MS [12] and GC–MS [13, 14]. All these techniques generate large amounts of toxic solvents and wastes, therefore there should be analytical methods to reduce or avoid side effects of the current methods. Recently, temperature controlled ionic liquid dispersive liquid–liquid microextraction (**IL DLLME**) method combined with HPLC was developed for the simultaneous determination of trace DEHP and MEHP in human urine [15]. In this method ionic liquids were used as the extraction solvents to replace the

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conventional organic solvents. There are some benefits that can be considered, including comfortable mixing with water, low volatility, low toxicity and good extraction capacity. Despite the advantages of temperature controlled IL DLLME, the lack of sustainable techniques for the removal of products from the room-temperature ionic liquids has limited their application [16].

The goal of this study is to evaluate the suitability of the in-syringe DLLME coupled with gas chromatography with flame ionization detector (**GC-FID**) method for the determination of these compounds in urine. We have tried green solvents used for process analysis. Green solvents are solvents that minimize the environmental effects resulting from our analytical activities [17]. Capello et al. [18] proposed a comprehensive framework for the environmental assessment of a solvent that covers major aspects of the environmental performance of solvents in chemical production, as well as important health and safety issues. In this article, the application of two environmental assessment methods with different scopes was estimated. The first method, the environmental health and safety (EHS) assessment method [19], is a screening method for exploration potential hazards of chemicals with a dangerous sum, which is from 0 to 9, while the second method, the life-cycle assessment (LCA) method [20], can be used for a detailed assessment of emissions to the environment, as well as resource use over the full life cycle of a solvent, containing the production, use, potential recycling, and disposal evaluated in terms of MJ_{eq} per kg of energy (net energy analysis) in solvent. The use of tetrahydrofuran, butylacetate, cyclohexanone, and 1-propanol is not recommended from a life-cycle perspective because these solvents cause heavy environmental effects during petrochemical production. In addition, formic acid, ethyl acetate, acetonitrile, dioxane, 1-butanol, and dimethylformamide are solvents of significantly heavy environmental effects. At the other end, hexane, heptane, and diethyl ether are environmentally favorable solvents. Since the metabolites and the parent phthalates are considered the toxic species, and since articles associated with this method have not been used for the determination of DEHP and its metabolite in biological samples, we determined the analytes by the in-syringe DLLME coupled with GC-FID. At the end, this recommended method was employed to investigate the levels of the target species in several real urine samples.

EXPERIMENTAL

Reagents. Mono(2-ethylhexyl) phthalate of high purity was purchased from Sigma-Aldrich (Louis, USA). Di(2-ethylhexyl) phthalate esters, benzyl benzoate (**BBZ**) and solvents: methanol, *n*-hexane, acetone, ethanol and acetic acid were supplied from Merck (Darmstadt, Germany). The stock standard solutions of 1000 mg/L of each compound were prepared in methanol. The working standard solution of 200 mg/L was prepared weekly in methanol.

Instruments. The analysis was performed by an Agilent 7890A gas chromatograph (Palo Alto, CA, USA) equipped with a split–splitless injector and a flame ionization detector. An HP-5 Agilent fused-silica capillary column (30 m ± 0.32 mm i.d., 0.25 μm film thickness) was applied for separation of analytes. Nitrogen (99.999% purity) was used as the carrier gas at the constant flow rate of 1 mL/min. The temperatures of injector and detector were set at 398 and 410°C, respectively. The injection port was operated in splitless mode. Oven temperature was held at 90°C for 1 min, increased to 150°C at the rate 20 grad/min, then increased to 250°C at the rate of 40 grad/min; after that increased to 300°C at 20 grad/min and then held at 300°C for 3 min.

Experimental procedure. Human urine samples were provided by healthy volunteer in our lab. These samples were kept frozen at −20°C before extraction. The frozen urine samples were thawed at room temperature and centrifuged for 15 min at 4000 rpm. Then, supernatants were decanted into clean glass tube and filtered through a 0.45 μm filter; 1 mL of filtrate was diluted to 5 mL and applied for the extraction process. The in-syringe DLLME was used in this study. Several solvents (methanol, *n*-hexane, acetone, ethanol and acetic acid) were tested for the extraction. Finally, the *n*-hexane–acetic acid (1 : 1, v/v) proved to be the most efficient in extracting DEHP and MEHP from human urine. At the beginning, 5 mL urine sample is aspirated in the 10 mL glass syringe by means of glass tubing adapted to the tip of the syringe. Then, 600 μL of the extraction mixture, containing 300 μL of acetic acid (disperser solvent) and 300 μL of *n*-hexane (extractant) are sprayed by using the 1000-μL glass syringe, a cloudy solution being immediately formed. Later on, the plunger of the 10 mL-syringe is slowly moved to the initial point allowing the recovery of extractant from the wall and the lower part of the syringe while the urine sample is removed from the unit. Finally, the extractant phase containing the target analytes can be easily recovered from the syringe tip and injected into the gas chromatograph with FID.

RESULTS AND DISCUSSION

Enrichment factor, linear range, precision, repeatability and accuracy of the method were determined to evaluate the method performance. All the evaluating experiments were carried out with blank urine sample spiked with 200 μg/L of BBZ as an internal standard

Table 1. Some quantitative data obtained after in-syringe DLLME GC-FID determination of the DEHP and MEHP

Compound	LOD[a], μg/L	r^2	Linear range, μg/L	Enrichment factor	RSD[b], %	LOQ[c], μg/L
DEHP	2.5	0.9977	20–3000	71	1.4	8
MEHP	1.1	0.9972	20–3000	64	3.0	3

[a] Limit of detection for $S/N = 3$, [b] relative standard deviation at concentration of 100 μg/L of each analyte ($n = 5$), [c] limit of quantification for $S/N = 10$.

Table 2. Comparison of in-syringe DLLME GC-FID with other methods for the determination of MEHP

Method	LOD, μg/L	Linear range, μg/L	LOQ, μg/L	Reference
LLE[a]–GC–MS	25	0–1000	–	[10]
SPE[b]–HPLC–MS	0.33	1–500	–	[11]
TCIL-DLLME[c] HPLC–MS	0.96	20–1920	3.1	[15]
In-syringe DLLME GC-FID	1.1	20–3000	3.6	Present work

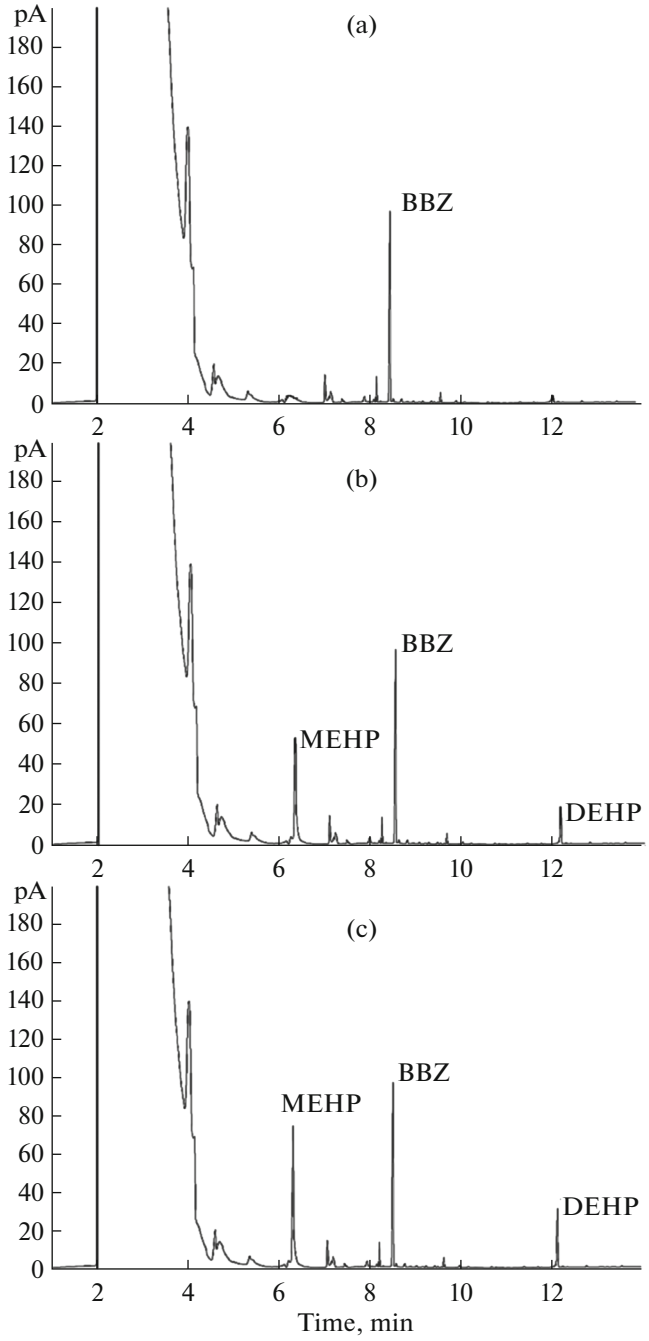
[a] Liquid–liquid extraction, [b] solid-phase extraction, [c] temperature controlled ionic liquid dispersive liquid–liquid microextraction.

under the optimum conditions. Enrichment factor (**EF**) is calculated according to the following equation: $EF = c_{dil}/c_0$, where c_{dil} is the concentration of analyte in the diluents for GC-FID analysis and c_0 is the concentration of analyte originally present in the sample solution, the results were summarized in Table 1. Until now, the method of analysis used to determine MEHP is limited to conventional methods in Table 2. This method compared to others has good repeatability, acceptable LODs and LOQs, and broad linear ranges. The validated analytical methodology was applied to the determination of DEHP and MEHP in three urine samples. The analytes were detected in GC-FID chromatograms of samples. The results are summarized in Table 3 and the typical chromatograms of blank urine sample, PVC worker urine sample and PVC worker urine sample spiked with 100 μg/L of MEHP and DEHP are shown in Figs. 1a–1c, respectively. The accuracy of the method was confirmed by a spike recovery test: 0.1, 0.3 and 0.6 μg/mL of MEHP and DEHP were separately spiked to the human urine sample originally containing 0.1 μg/mL MEHP and DEHP. Then the three sets of spiked urine samples were extracted with the same method and analyzed by using the GC-FID procedure. The recoveries of the analytes are illustrated in Table 4. Relative recoveries higher than 79% for all analytes in all samples demon-

Table 3. DEHP and MEHP contents (μg/L) of three samples of urine

Analyte	Normal person	PVC worker	Diabetic patient
MEHP	1.4 ± 1[a]	12 ± 1	96 ± 1
DEHP	ND[b]	3.8 ± 1	21 ± 2

[a] Mean concentration ± standard deviation ($n = 3$), [b] not detected.



Chromatograms of human blank urine (a), PVC worker urine sample containing MEHP and DEHP (b), and PVC worker urine sample spiked with 100 μg/L MEHP and DEHP (c).

strate that the method is suitable for the determination of DEHP and MEHP at trace concentrations in urine samples.

CONCLUSIONS

A quick, simple, and affordable method for trace plasticizer has been developed. The method reduces the amount of solvent necessary for the whole procedure and thus it can be considered as a green sample preparation method. The method has good repeatability, low LODs and LOQs, and broad linear range. The obtained results show that this method could be utilized for routine monitoring of the trace DEHP and its metabolite in urine of human exposure to plasticizers.

Table 4. Recoveries of the analytes in the spiked human urine sample (n =3)

Sample	DEHP		MEHP	
	added, μg/mL	recovered ± SD[a]	added, μg/mL	recovered ± SD
Normal person	0.1	86 ± 7	0.1	99 ± 7
	0.3	97 ± 7	0.3	96 ± 5
	0.6	98 ± 3	0.6	92 ± 4
PVC worker	0.1	99 ± 5	0.1	94 ± 3
	0.3	81 ± 4	0.3	99 ± 4
	0.6	95 ± 8	0.6	92 ± 3
Diabetic patient	0.1	98 ± 4	0.1	93 ± 5
	0.3	79 ± 3	0.3	88 ± 4
	0.6	97 ± 6	0.6	82 ± 6

[a] Mean extraction recovery ± standard deviation (n = 3).

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