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Determination of diethylhexyl phtalate in water by solid phase microextraction coupled to high performance liquid chromatography

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Abstract

Difficulties detected in the determination of the diethylhexylphthalate (DEHP) at trace levels by gas chromatography–mass spectrometry (GC–MS) using SPME, due to its ubiquitous distribution in the environment has been overcome and a new method for the determination of DEHP in drinking water has been proposed. The method is based on solid phase microextraction (SPME) coupled to high-performance liquid chromatography (HPLC). Detection was carried out spectrophotometrically. Calibration graph was linear in the range 10–110 μg/L with a regression coefficient of $r^2 = 0.998$ and a detection limit of 0.6 μg/L. The relative standard deviation was 5 and 2% ($n = 4$) for chromatographic areas and retention times, respectively. The usefulness of the SPME–HPLC technique was confirmed.

Keywords: Drinking water; DEHP; SPME/HPLC/UV–vis

1. Introduction

Diethylhexylphthalate (DEHP) is one of the main phthalates used as plasticizer in production of PVC and other polymers including rubber, cellulose and styrene. Because of this, DEHP is distributed ubiquitously in the environment and also in laboratory materials such as tubes, caps and gas chromatography septa. The US Environmental Protection Agency (EPA) has classified DEHP as a B2 substance, which is a possible human carcinogen substance. The most probable route of human exposure to DEHP is through food, with an average intake of 0.25 mg/d [1], being migration from plastics during processing and storage the main source [2]; exposure to DEHP can also occur due to the use of certain medical devices for blood transfusions, kidney dialysis and the use of respirators [3]. On the other, DEHP is present at high concentrations in PVC products used as basic materials in doll manufacturing; therefore children are exposed to DEHP due to the high migration ability of DEHP in PVC derivatives [4]. Several studies have shown that DEHP is embryotoxic and teratogenic in rodents [5]; in addition, adverse effects fertility, specifically on male and female reproductive systems including testicular atrophy, reduction in sperm mobility and concentration, increase in the number of abnormal sperm as well as histopathological damages have been described [6–8]. In addition, DEHP is often presented as interference in analytical trace determinations in different organic compound analysis.

There are some researches on DEHP determination in environmental samples such as marine sediments [9], source emissions [10], soils [11], surface waters [12,13], PVC materials [14] and toys [15]. DEHP analysis is carried out by GC or GC–MS. Liquid–liquid extraction (LLE), solid phase extraction (SPE), microwave-assisted solvent extraction (MASE) and supercritical-fluid extraction (SFE) have been mostly used for sample preparation. HPLC has been also used for DEHP determination; silica gel columns [16] and in-tube SPME [17] were used for clean-up purposes.

Solid phase microextraction (SPME) was first described by Belardi and Pawliszyn for headspace gas chromatography (HS-GC) [18]. As it is known SPME is based on the distribution of the sample analytes between the extracting phase immobilised on a fused-silica fibre and the headspace (liquid, solid or gaseous phase) or between the fiber and the liquid sample. When the equilibrium is reached, maximum amounts of the analytes are retained. SPME–GC involves thermal desorption of the ana-

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lytes in the GC injector port for further analysis and it has been widely used for the analysis of volatile compounds [19]. Problems still arise from reproducibility and conditioning the fiber in the gas chromatograph, which blocked the instrument during the conditioning step. On the other hand, in our experience, a not negligible fiber blank containing the DEHP chromatographic peak often appears when SPME–GC was applied; this makes difficult to determine DEHP compound at trace levels applying this technique.

For semi- and non-volatile organic compounds an interface has been developed for coupling SPME with HPLC [20]; this interface maintains the advantages of SPME such as solvent free for the preparation of the sample and time efficient.

In this paper, we confirm the mentioned difficulties to determine DEHP at trace levels by SPME–GC and we evaluate the possibilities of SPME–HPLC, proposing a new method for DEHP determination in drinking waters. Attention was also paid to conditioning of the fiber using a Nurka 390 device designed specifically for this purpose instead the GC injection port.

2. Experimental

2.1. Instrumentation

The SPME/HPLC system consisted on: (1) a SPME NURKA390 fiber cleaner (Nurka, Madrid, Spain). A heating block device with a cleaning port to fit the fiber into a small chamber with a temperature range from 35 to 320 °C through which an N_2 (CARBUROS METÁLICOS, Madrid, Spain) flow goes through at 0.2 bar; (2) a SPME NURKA390 fiber Rotatory Device (Nurka, Madrid, Spain); (3) an HPLC system consisted of the following components: HPLC pump Waters Model 590 (Waters, Milford, USA); a Luna Phenomenex C18 (Phenomenex, CA, USA), 4.6 mm × 30 mm, 5 μm column; an UV detector Waters Lambda-Max Model 481 (Waters, Milford, USA); and a Waters Data Module integrator (Waters, Milford, USA); (4) a SPME/HPLC interface with a Rheodyne valve (SUPELCO, CA, USA). This interface consists of a standard six port HPLC valve with a fiber desorption chamber (total volume 60 μL) installed in place of the sample loop of the HPLC injector valve, and a bracket for benchtop or ring stand mounting. A sealing needle is present in the needle guide. Static and dynamic modes are possible.

In the static mode, the valve is switched to the load position, so that the desorption chamber is off-line. The desorption chamber is filled with the mobile phase (or other typical solvent having lower eluting strength than the mobile phase) and the analyte is desorbed from the fiber. Then the valve is switched to the inject position so the mobile phase flows through the desorption chamber to the HPLC column.

In the dynamic mode, analytes are desorbed on-line from the fiber by a stream of HPLC mobile phase. A schematic diagram of the set up is shown if Fig. 1.

2.2. DEHP standard

A solid DEHP standard, from Sigma–Aldrich (St. Louis, MO), with a purity of 99% was used. Analytical solutions were prepared in acetonitrile and further diluted as required from a 1000 mg/L stock solution. Purified water was obtained by passing tap water through a C_{18} cartridge.

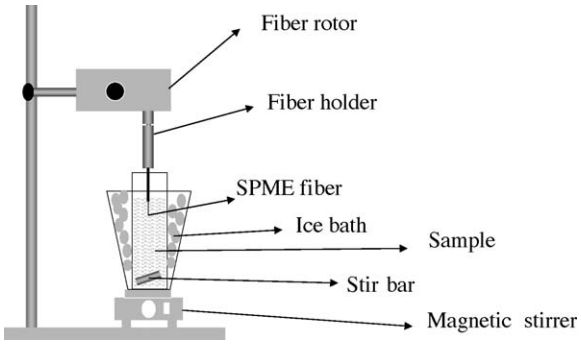

Fig. 1. Schematic diagram of the set up for the determination of DEHP.

2.3. Solvents

HPLC grade acetonitrile and methanol from Scharlau (Barcelona, Spain) were used. Water was obtained by means of a Milli-Q apparatus from Millipore (Milford, MA).

2.4. SPME fiber

A poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) 60 μm was used for DEHP determinations. The following fibres were also tested: poly(dimethylsiloxane) (PDMS) 7 and 100 μm, poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) 65 μm, polyacrilate (PA) 85 μm, Carbowax/divinylbenzene (CW/DVB) 65 μm and Carboxen/poly(dimethylsiloxane) (Carboxen/PDMS) 75 μm (SUPELCO, CA).

2.5. Water samples

Four different drinking mineral waters, all of them available at the supermarket, were analysed, as well as the Milli-Q water and water packed in plastic bag.

2.6. Procedure

2.6.1. DEHP solid phase microextraction

After thermal conditioning of the fibre for 12 h at 200 °C and 0.2 bars under a nitrogen stream in the SPME NURKA390 fiber cleaner, the poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) 60 μm fiber was introduced into a 1000 mL vessel containing 500 mL of the water sample or purified tap water for external standard calibration, which was refrigerated at 2–4 °C in an ice bath. The sample was stirred magnetically while rotating the fibre in opposite side without vibrations using the rotatory device. After extraction, solvent desorption was carried out inside the SPME/HPLC interface. The fiber was inserted into the desorption chamber which contained 60 μL of 100% acetonitrile. During 5 min, the analytes were desorbed in the static mode from the SPME fiber in the load position. Finally, they were eluted in the dynamic mode on the HPLC

column for analysis by switching the valve to the inject position during 2 min.

2.6.2. DEHP HPLC method

The system was operated at 0.6 mL/min of 100% acetonitrile as mobile phase. The wavelength used for detection was 224 nm. The external standard calibration graph at five concentration levels containing DEHP in the range of 10–110 μg/L was prepared from the stock standard solution. DEHP quantitation was attained from peak area data.

3. Results and discussion

3.1. Source of DEHP contamination on SPME–GC–MS

DEHP determination at low levels was firstly tried by SPME–GC–MS but without success. The blanks presented variable significant intensities of the DEHP peak. This was attributed to DEHP contamination from the rubber septa, potentially coming from the GC injection port or from the fitting of the SPME fiber.

In order to determine the origin of this DEHP contamination, a series of blanks was run. Firstly, blanks were obtained operating the SPME fiber in the usual way. After 10 running experiments the DEHP peak always appeared which areas were due to amounts of DEHP in the range 7–454 ng with a R.S.D. of 90%. Moreover, operating in the same way, even though the fiber was cut off, similar DEHP peaks appeared when the device of the fiber was lowered but not if the plunger was not depressed to low the fiber. From this experiments, we decided that the DEHP source was not the GC septum port nor the own SPME fiber but the septum of the SPME fiber. It seems that the carrier gas contacts the SPME fiber septum after lowering the fiber (Fig. 2a).

At this point, DEHP determination was tried by HPLC-UV. Blanks were running by following the DEHP–SPME procedure specified in experimental using purified tap water obtained as indicated above. The DEHP peak did not appeared in any case. This was attributed to the absence of contact of the mobile phase with the SPME fiber septum, as indicated in Fig. 2b.

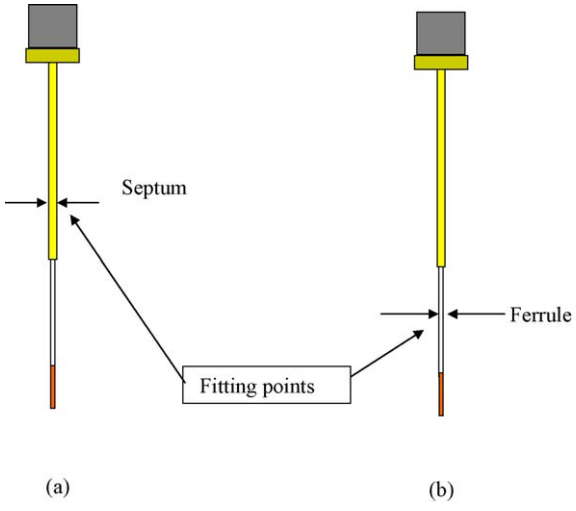

Fig. 2. Fitting of SPME fiber: (a) GC port and (b) LC interface.

3.2. Analytical characteristics of the SPME–HPLC method for standards

The HPLC system was operated at a flow rate of 0.6 mL/min with pure acetonitrile as a mobile phase otherwise retention factors were higher than 20 and a wide tailing peak was observed. Detection was at 224 nm and the Luna Phenomenex column specified in the experimental section was used. The apparent wideness of the peak was not a problem in this case because of this simple sample.

Five DEHP concentration levels were used to prepare calibration graph in the range of 10–110 μg/L. Linearity was found with regression coefficient of 0.998. The relative standard deviation percentages (R.S.D.%) were determined from four other analytical solutions, each one of them prepared separately, obtaining values of 5% for chromatographic areas and 2% using retention times. Detection limit (DL) was 0.6 μg/L using the 3 (s/n) criterion.

3.3. Cleaning of the fiber

In the above experiments, we observed memory effect of the fiber due to DEHP. So conditioning of the fiber was carried out until DEHP disappeared.

By treating the fiber with usual solvents such as acetonitrile and methanol was not successful. Consequently, thermal clean up was used. Optimum conditions are specified in the experimental section. The passing through of an inert gas stream such as nitrogen helped to clean the fiber and decrease the conditioning time, increasing the work life of the fiber. For this purpose, we employed the Nurka390 fiber cleaner device instead of the injection port of the chromatograph, allowing the use of the GC chromatograph.

3.4. Optimization of solid-phase microextraction

The studied variables for standards and the obtained results are summarised in Table 1. A chromatogram obtained with the optimised conditions is shown in Fig. 3.

The variables studied to optimise included conditioning of the fibre, extraction time, extraction temperature, agitation method, sample volume, desorption time and the nature of the desorption solvent. Fibers investigated are specified in the experimental section. The data of DEHP extraction efficiency obtained with other fiber coatings are shown in Table 2. Among these, better results were found by using the poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) 60 μm fiber.

Fibers were thoroughly conditioned by inserting them into the NURKA 390 injection port conditioning device for 12 h at 200 °C before its use as they were received from Supelco. After each analysis the fiber was cleaned up again at 200 °C for 30 min using the NURKA 390 fiber cleaner. In this way the fibre was used for at least 25 analyses without significantly change of the

Table 1
Optimization of variables in SPME

Retention process													
Variable Studied	Extraction time[1], min			Extraction temperature[1], ºC		Agitation mode[2]				Sample volume[4], mL		
Studied range	15	30	45	Ice bath	Room temperature	No	Sample	Fiber	Sample & fiber	250	500	1000
DEHP Peak area	4	19	7.5	19	5	3.8	5.6	4	12	3.3	5	2.5
RSD%, n=3	9	7	11	9	6	8	9	10	5	7	4	8

Desorption process									
Variable Studied	Static time[2], min			Dynamic time[3], min			Solvent[4]		
Studied range	1	2	5	1	2	5	MeOH	ACN	ACN:H_2O; 80:20; v:v
DEHP Peak area	8.1	12.5	11	2.1	7	6.7	2.8	5.1	5
RSD%, n=3	7	6	12	5	8	9	7	7	11

Spiked with DEPH in ng/ml at: [1] 260; [2] 170; [3] 100 and [4] 70.

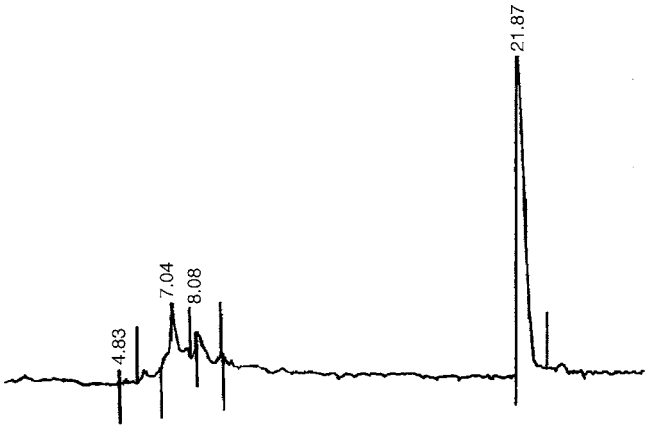

Fig. 3. Chromatogram of spiked tap water with DEHP standard. *Conditions:* Luna Phenomenex (250 mm × 4.6 mm) column; mobile phase, 100% acetonitrile; flow rate, 0.6 mL/min; UV detection 224 nm.

signal. Clean-up temperature could be higher but the life of the fiber was shorter.

Some points could be emphasised. It was essential to cool the water sample because solubility of the analyte decreases. Regarding to agitation and as reported in the literature [21], agitation reduces equilibration time during the extraction process because mass transfer from the solution to the fiber is improved. Four different agitation modes for extraction were tested: static, fiber rotation, sample stirring, and both sample stirring and fiber rotation. Best results were obtained when both, strong stirring with glass-coated mini-stirrers and rotation of the fiber, using the rotatory extraction device NURKA390 were applied.

Table 2
SPME fibers selection

Fiber coating	Thickness (μm)	Extraction efficiency (%)
PDMS	100	55
PDMS	7	14
PDMS/DVB	65	97
PDMS/DVB	60	97
PA	85	16
CW/DVB	65	44

As it has been mentioned in the experimental section, analytes can be desorbed using either static or dynamic mode. Desorption times investigated were 1, 2 and 5 min for both modes; best results were found allowing the fiber for 5 min to static desorption inside the chamber. However, the best desorption time for the dynamic mode was 2 min.

3.5. Determination of DEHP in drinking mineral water

The proposed method was applied to determine DEHP in four different drinking mineral waters available at the supermarket, a Milli-Q water sample and a sample of water packed in plastic bag. The results obtained are summarised in Table 3. DEHP was not detected in any of drinking mineral waters. However DEHP was found in Milli-Q water and in the water sample packed in plastic bags; concentration was 22 and 9 μg/L with a relative standard deviation of 9 and 3% ($n = 4$), respectively. Figs. 4 and 5 show the chromatograms obtained for the mineral water sample number 1 and for the Milli-Q water sample, respectively.

Table 3
Determination of DEHP in water samples

Sample	DEHP (μg/L)	R.S.D.% ($n = 4$)
1	n.d.	–
2	n.d.	–
3	n.d.	–
4	n.d.	–
Milli-Q	22	9
Water packed	9	3

n.d., not detected.

Fig. 4. Chromatogram of mineral water sample 1. *Conditions:* Luna Phenomenex (250 mm × 4.6 mm) column; mobile phase, 100% acetonitrile; flow rate, 0.6 mL/min; UV detection 224 nm.

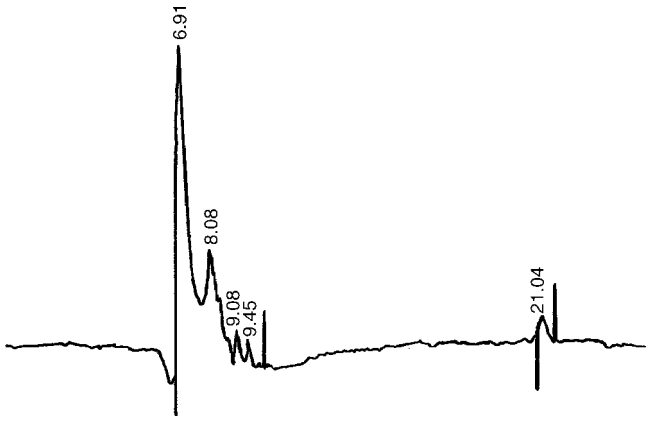

Fig. 5. Chromatogram of the Milli-Q water. *Conditions:* Luna Phenomenex (250 mm × 4.6 mm) column; mobile phase, 100% acetonitrile; flow rate, 0.6 mL/min; UV detection 224 nm.

4. Conclusions

The fiber blank for DEHP was negligible when SPME–HPLC was applied. This allowed the use of this technique for trace DEHP determinations in drinking water, thus increasing the applicability of SPME/HPLC.

The thermal treatment of the fiber before the analysis was essential and the temperature chosen was the appropriate to make the analysis without damaging the fiber and obtaining a good reproducibility. The suitable agitation of both fibre and the sample was also critical to get good results.

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