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Trace determination of diethylphthalate in aqueous media by solid-phase microextraction–liquid chromatography

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Abstract

The development, optimisation and validation of a method for the determination of trace levels of diethylphthalate (DEP) in aqueous media is described. Extraction is by solid-phase microextraction followed by HPLC analysis. The compound is spiked into 10-ml aliquots of ultra-pure water to which 2.5 g of NaCl is added. DEP is separated from other co-extracted components on a Nucleosil C_{18} column 5 μm dp 250×3.2 mm I.D. The mobile phase is acetonitrile–water (52.5:47.5). delivered at a flow-rate of 0.5 ml/min. The column is thermostatted at 30°C and detection is by ultraviolet absorbance at 226 nm. Optimisation of the method is discussed, and the precision, accuracy and linearity using both a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre and a carbowax/templated resin (CW/TPR) fibre were determined. The PDMS/TPR fibre extracted the DEP more efficiently than the CW/TPR fibre with a limit of detection of 1 ng/ml for an immersion time of 15 min.

Keywords: Solid-phase microextraction; Diethylphthalate

1. Introduction

Solid-phase microextraction (SPME) is a convenient, solvent-free extraction technique, originally developed about 10 years ago by Pawliszyn and co-workers [1,2]. The most widely used system is that available commercially from Supelco whereby a fused-silica fibre coated with a thin film (7–100 μm) of sorbent material is introduced into the sample matrix or into the vapour above the condensed phase. After a pre-determined extraction period, typically 30–90 min, depending on the partition co-efficient of the analyte(s) between the coating and the matrix, desorption is effected by placing the fibre directly into the chromatograph. The coated fused-silica fibres currently available are polydimethylsiloxane (PDMS) PDMS/divinylbenzene (PDMS/DVB), polyacrylate, carbowax-divinylbenzene (CW/DVB) and carbowax/templated resin (CW/TPR) though fibres bonded with other sorbents are under development.

By the employment of a specially designed interface, SPME has been successfully combined with HPLC for the analysis of non-volatile and semi-volatile substances [3–6] including surfactants [7–9] and PAHs [10] in water; antioxidants in food [11], and pesticides in environmental samples [12].

Diethylphthalate (DEP) is widely used as a solvent and fixative in a wide variety of cosmetic products, and as a component of packaging for food

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and pharmaceutical products. It has been shown to leach into foods from packaging materials of which it is a component [13–15], and since phthalate esters have recently been shown to have weakly oestrogenic properties [16], public concern regarding the real safety of these substances has increased dramatically. As a result, this study was aimed at the development a method for the determination of trace levels of phthalates that may migrate into food and drinking water. DEP was chosen as the first test compound since it is more polar than the other widely used analogues (butyl- and diethyl hexyl phthalate) and would therefore present a greater challenge for the method development. SPME–LC was selected as a method of extraction in the analysis of trace levels of DEP, since as a solvent-free extraction technique it provides high concentration factors with the minimum of sample manipulation steps, thus minimising the potential for contamination of the sample.

This paper reports the development and optimisation of a SPME–HPLC method for the determination of DEP in aqueous samples. Four types of fibres were investigated, two of which warranted further investigation as they produced the greatest recoveries and the most acceptable peak shapes.

2. Experimental

2.1. Reagents and solutions

Lichrosolv gradient grade acetonitrile and HPLC grade methanol were obtained Merck (Darmstadt, Germany) and from Carlo Erba (Milan, Italy), respectively. Water for injections (B Braun Medical, Bologne, France) was used in the preparation of solutions and reagents, including the mobile phase. Sodium chloride was obtained from Prolabo (Paris, France); sodium carbonate from Carlo Erba, and diethylphthalate-DEP-(98%) was supplied by Fluka Chemika (Buchs, Switzerland). A 1 mg/ml stock solution of DEP was prepared in methanol. Working standards of 5 ppm and 500 ng/ml DEP were prepared by dilution of the stock solution in acetonitrile–water (10:90) with vigorous stirring. Test solutions (10 ml) were made adding the required volume of working standard solution to a 13-ml vial, adding the appropriate volume of water and, for extraction with the carbowax fibre, 2.5 g of either NaCl or Na_2CO_3. Prior to use, the salts were twice washed with acetonitrile to remove traces of DEP which may originate from the plastic containers in which they were stored.

2.2. Instrumentation and operating conditions

The mobile phase (acetonitrile–H_2O 52.5:47.5) was isocratically delivered at a flow-rate of 0.5 ml/min by a SpectraSystem P4000 dual piston pump (ThermoSeparations products, France). Separation was carried out on a 250×3.0 mm I.D. ChromCart column (Macherey-Nagel, Hoerdt, France) packed with Nucelosil C18-50 dp 5 μm, which was thermostatted at 30°C. Under these operating conditions, the retention time of DEP was 7.2±0.2 min. with direct injection and 7.3±0.3 min after extraction. Detection was by UV absorbance at 226 nm using a ThermoSeparations Spectrofocus Scanning UV–Vis detector. The chromatograph was controlled, and data was collected using TSP PC1000 software installed on a Dell Pentium PC. For direct injection, 20 μl aliquots were introduced into the chromatograph using a Rheodyne injector which was used to initiate data collection by switching of the valve.

For desorption from the fibres, the loop in the Rheodyne injection valve (V_1, Fig. 1), was replaced by a Supelco (Bellefonte, PA, USA) SPME–HPLC interface with Valco valve, thus permitting four different flow path configurations depending on the orientation of the two valves (V_1 and V_2): In Fig. 1A-Load Position, the interface is isolated from the chromatographic system and this enables insertion and removal of the fibre assembly. When the fibre assembly has been placed in the interface and sealed, an aliquot of a solvent in which the analytes are highly soluble may be introduced into V_2 to immerse the fibre in the desorption solvent for a pre-determined period of time (typically 10–30 s). Rotation of V_1 to the Injection Position causes the mobile phase to be diverted into the interface whereupon it sweeps the desorbed compounds onto the HPLC column.

For the purposes of calibration, for example, the fibre may be isolated from the rest of the interface by rotation of V_2 (Fig. 1B) and the interface may now

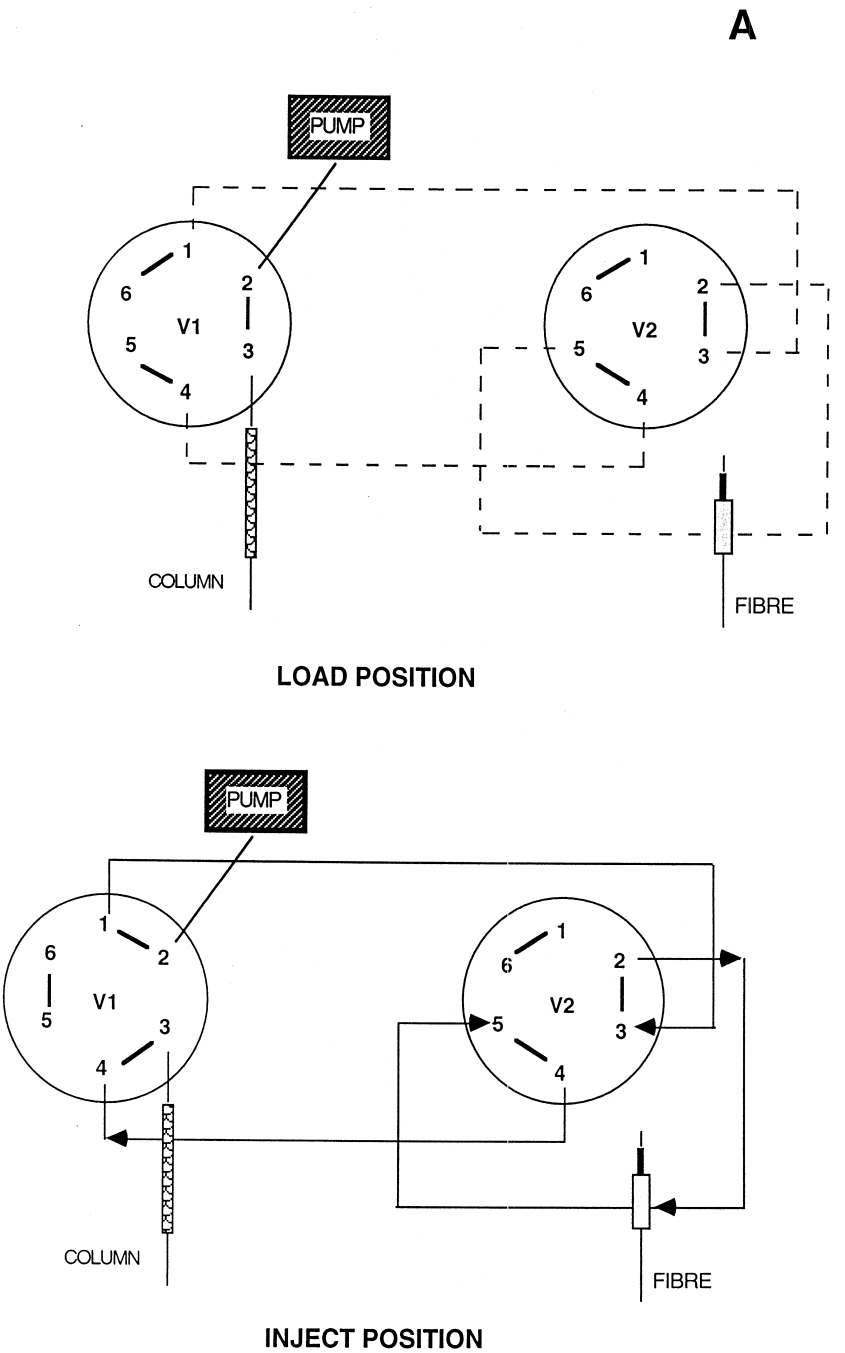

Fig. 1. Schematic representation of flow paths through Rheodyne valve and Valco valve in Interface. (A) Fibre In-line with interface. Load Fibre position, the column is isolated from the interface, permitting the fibre to be positioned. Inject position, the fibre is flushed with the mobile phase. (B) Fibre isolated from interface. Load position, the column is isolated from the interface permitting loading of the interface with sample. Inject position, the contents of the interface are switched onto the column.

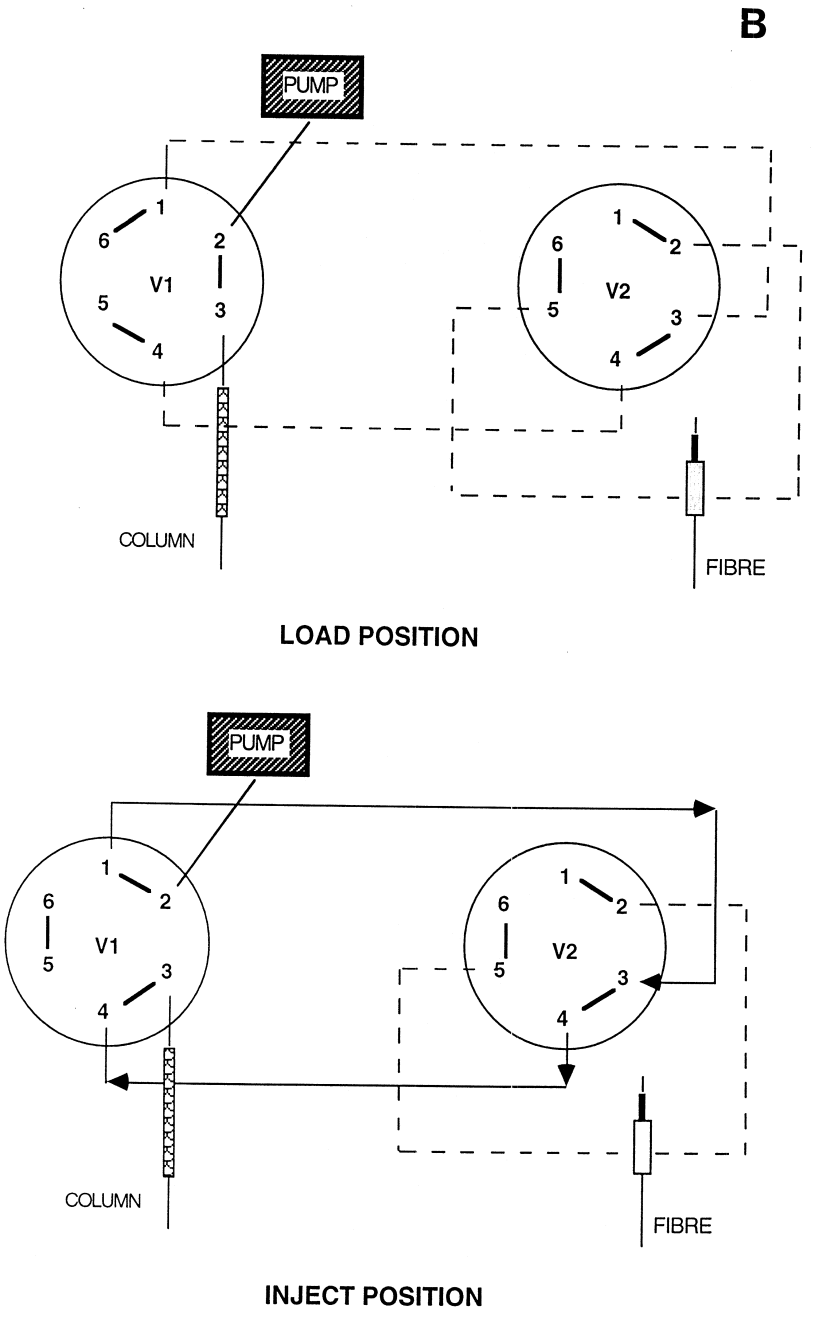

Fig. 1. (*continued*)

serve as an injection loop, the volume of which has been estimated to be 80 μl [6]. The sample may be introduced via the injection port of V_1 while in the Load position, and by switching to the Injection Position, the contents of the interface are swept onto the HPLC column.

The fibres used were obtained from Supelco and were polydimethylsiloxane (PDMS) 100 μm,

PDMS/divinylbenzene (PDMS/DVB) 60 μm without epoxy resin, polyacrylate, 85 μm, and carbowax/templated resin (CW/TPR) 50 μm without epoxy resin. Fibres were supplied as 'assemblies' which are placed in an SPME holder (5-7331) specifically for SPME-HPLC.

2.3. Conditioning of fibre

Before first usage, the fibre was immersed for 5 min in acetonitrile (without stirring) after which it was withdrawn into the holder by raising the plunger. The holder containing the fibre was then transferred to the SPME/HPLC interface where it was conditioned by flushing with the mobile phase for 30 min, as recommended by the manufacturer.

2.4. Extraction procedure

After conditioning, the fibre was air dried for 1 min. The sample solution was covered with a stopper which was punctured to provide an aperture to permit passage of the fibre. The depth of immersion was kept constant and the solution was vigorously stirred with a magnetic stirrer at a constant stirring rate (75% of maximum) for all extractions, which were carried out at ambient temperature (22°C±2°C). After extraction, the fibre was withdrawn and the SPME fibre holder and was placed in the interface with both valves in the load position (Fig. 1). By turning first the interface valve, and then the Rheodyne valve to the inject position, the mobile phase was diverted through the interface whereby the components were swept from the fibre onto the column. To investigate the static desorption mode, 500 μl of desorption solution (mobile phase or acetonitrile) was injected into the injection port (position 6) of the Valco valve with both it and the Rheodyne valve in the load position. The fibre was allowed to soak in the desorption solution for 30 s and by switching the valve, the solution surrounding the fibre was swept onto the column by the mobile phase. After each desorption, the valve was switched twice to ensure that no DEP remained on the fibre after the first desorption step. The fibre was air-dried for 1 min before immersion into the next solution.

2.5. Calculations

All measurements were based on peak areas and the amount recovered was calculated on the assumption that all the compound sorbed was released into the mobile phase during desorption.

3. Results and discussion

3.1. Choice of fibre

Four different fibres were selected for preliminary investigation for the analysis: polydimethylsiloxane/divinylbenzene film thickness, 60 μm (PDMS/DVB); carbowax/templated resin 50 μm (CW/TPR); PDMS 100 μm and polyacrylate 85 μm (PA). The fibres were conditioned as described in the experimental section and aligned with mobile phase flow stream to demonstrate that there were no interfering compounds originating in the fibre.

The length of time for which the fibre should be immersed in the sample solution is difficult to anticipate since the time to reach equilibrium is highly dependent on the distribution coefficient of a particular analyte between the coating and the matrix, which in turn depends on nature of the analyte, the type and thickness of fibre coating, in addition to experimental parameters such as the temperature, and whether, by what means and by how much the sample is agitated. Based on a study of the literature, an immersion time of 60 min was chosen for preliminary experiments to determine the optimum fibre for the extraction of DEP from water. Fresh solutions of 90 ng/ml DEP were used for each immersion with stirring at 75% of the maximum. This was the fastest stirring rate that could be used for the size of sample container before rotation of the stirring bar became erratic.

In all cases, a peak was found that had the same retention time as DEP by direct injection, however, the peak obtained with the PDMS fibre was very broad by comparison with the directly injected sample, an observation which may be attributed to the fact that PDMS is highly viscous; the film (100 μm) is relatively thick and diffusion from the fibre into the mobile phase may have been retarded by a slow rate of mass transfer in the film. Therefore, this

fibre was excluded from further study. The polyacrylate fibre yielded a very low recovery by comparison with the carbowax fibre (see below) which may be explained by the fact that it is less polar than either the CW/TPR or PDMS/DVB fibres and may therefore not have as high an affinity for DEP as the more polar phases, and so it too was excluded from subsequent experiments. Both the CW/TPR and PDMS/DVB fibres were promising in initial experiments, showing a peak for DEP that corresponded with that obtained by direct injection, both in terms of retention time and peak width indicating a fast desorption of DEP with little or no contribution from the interface configuration to the total band broadening in the system.

3.2. Carbowax/templated resin (CW/TPR) fibre

Using an aqueous test solution of 20 ng/ml DEP and an immersion time of 45 min, the percent extracted was determined to be less than 0.25%. The experiment was then repeated in the static mode. In this case, the interface was filled with either acetonitrile or the mobile phase by injection through the Valco valve and then the fibre was soaked in the surrounding liquid for 30 s before the mobile phase was diverted through the interface. This measure produced a larger peak than with dynamic desorption (recovery was estimated to be 1.94%) for both acetonitrile and the mobile phase, but the peak was significantly broader and many interfering peaks were also seen.

The effect of adding sodium chloride to the sample solution was investigated, since this approach has been widely used to promote transfer of analytes onto the fibre [3,6,17]. However when a blank solution containing NaCl only was subjected to analysis, an interfering peak appeared at the retention time of DEP. Assuming that this peak may originate from the plastic containers in which the salt was stored, the interfering peak was successfully removed by twice extracting the salt with acetonitrile in which diethylphthalate is highly soluble. Adding increasing quantities of NaCl to a 20 ng/ml solution of DEP resulted in a corresponding increase in the amount recovered, until at saline concentration of 25% NaCl the amount recovered after 45 min immersion was 3.84%. Both Na_2CO_3 and LiCl were also investigated as salting-out agents, and Na_2SO_4 was found to give a similar extraction recovery to NaCl. Again, however, it proved necessary to carry out a double-extraction with acetonitrile and it was used at concentration of 20%, rather than 25% due to its lower solubility than NaCl.

3.3. Polydimethylsiloxane/divinylbenzene (PDMS/DVP) fibre

The PDMS/DVB fibre gave higher recovery than the carbowax fibre based on 60 min immersion in a 20 ng/ml aqueous (unsalted) solution of DEP, indicating that it has a greater affinity for this compound. Furthermore, it was found that with an immersion time of just 15 min, the PDMS/DVB fibre yielded similar recoveries to 45 min immersion with the carbowax fibre: recovery was approximately 3.7% following 15 min immersion in a 5 ng/ml aqueous (unsalted) solution of DEP. When either NaCl or Na_2SO_4 were added to the solution at a concentration of 25%, recovery was not found to increase, and so subsequent investigations were carried out on unsalted solutions. The effect of extraction time (Fig. 2) shows that the amount extracted continued to increase even after 5 h immersion, indicating that it would not be feasible to carry out extractions under equilibrium conditions. However, it has been demonstrated in a detailed

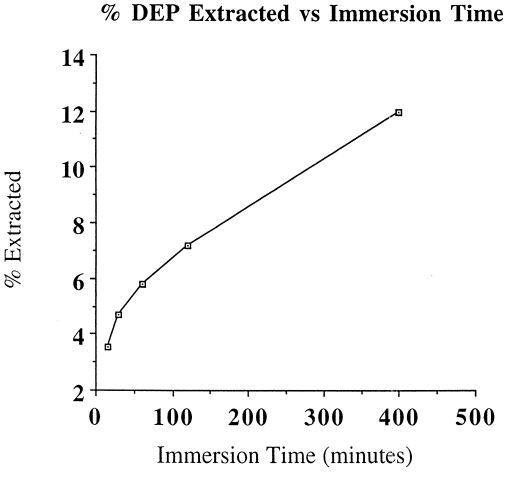

Fig. 2. Effect of immersion time on % recovery-Polydimethylsiloxane/divinylbenzene fibre.

Table 1
PDMS/DVB Fibre. Repeatability and linearity (n=5)[a]

Concentration (ng/ml)	Mean peak area	C.V. %	Recovery %
5	8481.8	3.14	3.69
10	16670	0.11	3.63
25	40638	4.57	3.54
50	67057.8	5.22	2.98
75	89429	2.25	2.60
r (5–75 ng/ml)=0.994			
r (5–50 ng/ml)=1			

[a] Recovery was calculated from the following formula ($2.A_{ext}.100/9183.5.x$) where 9183.5 is the mean peak area ($n=5$) obtained by direct injection of 2 ng (20 μl of a 100 ng ml^{-1} solution) and x corresponds to the total amount of DEP (ng) in the sample.

study in SPME in non-equilibrium situations [18], that quantitation is possible before equilibrium has been reached, provided that the extraction conditions (time, sample volume, stirring rate etc.) are kept constant.

The linearity of the procedure, expressed in terms of the correlation coefficient, was 0.995 between 5 and 75 ng/ml, a figure which increased to 1.0 over the concentration range 5–50 ng/ml (Table 1). These findings would suggest that as the concentration increases, the fibre becomes saturated and recovery is no longer a linear function of concentration, an observation that concurs with the fact that the yield is greater at lower concentrations. The fact that the method is linear over just one order of

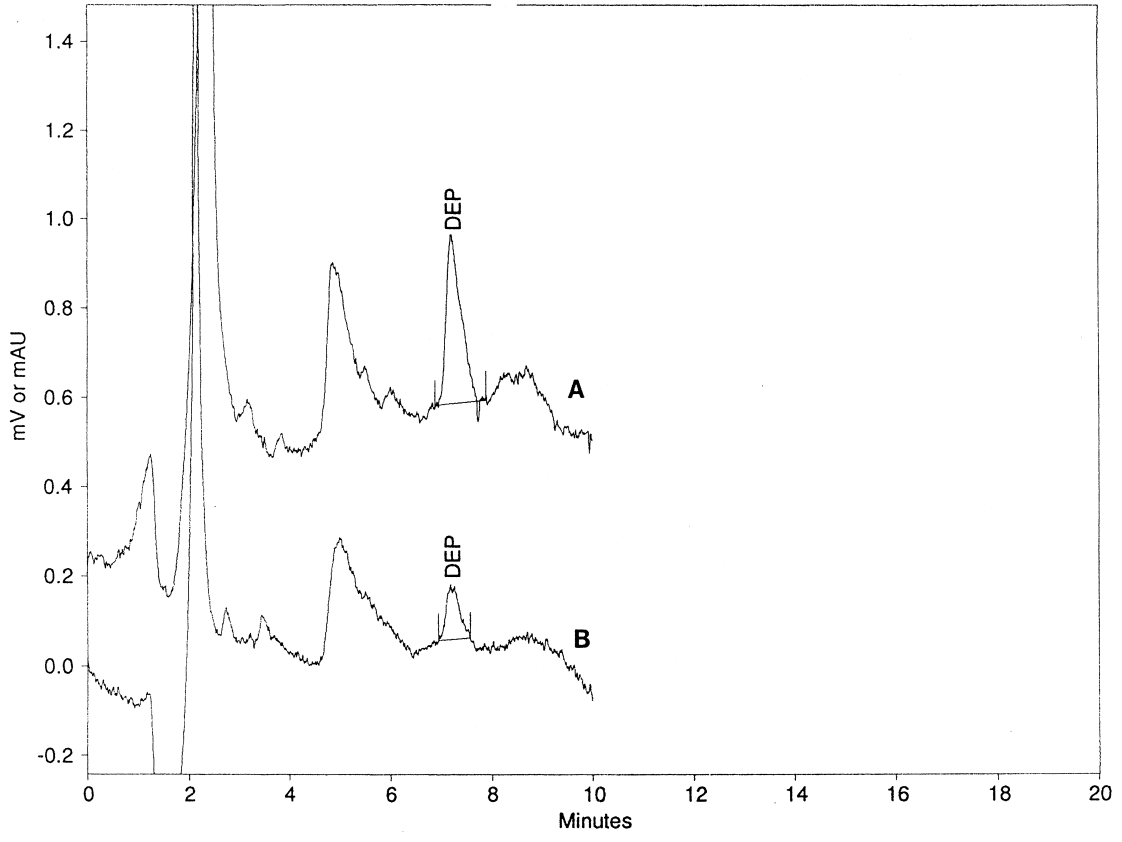

Fig. 3. Effect of immersion time on recovery of 1 ng/ml DEP-(PDMS/DVB fibre). (A) Immersion time 90 min. (B) Immersion time 15 min. Chromatographic conditions given in text.

magnitude is a potential drawback of the system, however, due to the unique nature of the SPME technique, saturation of the fibre may be avoided by simply reducing the immersion time. Likewise, lower concentrations of analyte may be more easily detected if the analysis time is increased appropriately. Another possibility for more concentrated solutions is to use a fibre with a greater film thickness, though this can result in significantly increased sorption and desorption times, due to the slow rate of mass transfer within the film itself. The PDMS fibre also showed good repeatability, with a mean relative standard deviation of 3.06% for five replicate extractions over the concentration range 5–75 ng/ml.

The limit of quantitation was in this study was 5 ng/ml but as may be seen from Fig. 3, it is possible to detect as little as 1 ng/ml after 15 min immersion. In order to detect lower concentrations, it is necessary to increase the immersion time significantly; in this case, the peak area of the 1 ng/ml solution was increased four-fold by increasing the immersion time to 90 min, This would indicate that to realise the analysis of DEP at real trace levels (sub ng/ml), it would be necessary to allow for immersion times of 2 h or greater, which renders the method considerably more time consuming than some of the currently used methods for phthalate analysis such as GC-MS [19] or SPE-GC [19,20]. On the other hand, however, with the SPME technique reasonable enrichment factors are possible, as demonstrated in Fig. 4 which shows a chromatogram following SPME extraction of a 10 ng/ml solution as compared to direct injection (20 μl) of a 100 ng/ml solution.

The PDMS/DVB fibre was used to test various water sources for the presence of DEP. The sources used were mineral waters stored in a plastic bottle

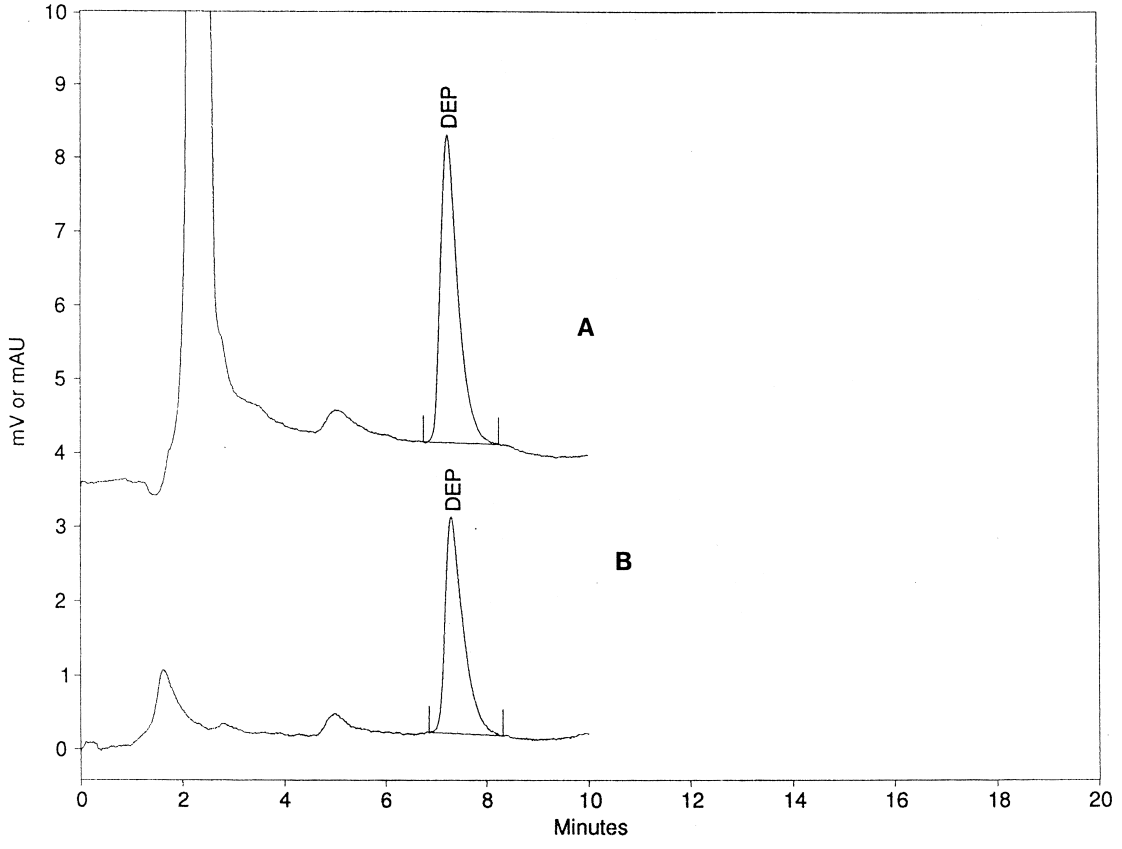

Fig. 4. SPME Extraction vs. direct injection-(PDMS/DVB fibre). (A) 10 ng/ml DEP (10 ml) following SPME extraction. (B) Direct injection (20 μl) of 100 ng/ml DEP. Chromatographic conditions given in text.

and a glass bottle at ambient temperature for 1 month, and tap water stored at 37°C for 1 month. The fibre was placed in 10 ml aliquots of each sample, and extraction was carried out following the standard protocol of stirring at 75% maximum and immersion for 15 min. When no peaks were observed after this immersion time, the fibre was exposed to the samples for 60 min. This resulted in chromatographic profiles which relative to that of the aqueous blank suggested that other compounds may be leaching from the bottles, investigation of which will form part of a later study.

4. Conclusion

The SPME technique has been shown to suitable for the extraction of low concentrations of DEP from aqueous solution; limits of detection in the UV in the low ng/ml regions are attainable after 15 min immersion in the case of the PDMS/DVB fibre and after 45 min immersion with the CW/TPR fibre. Addition of salt at a concentration of 25% was found to increase the amount recovered with the CW/TPR fibre, but had little impact on the recovery of the PDMS/DVB fibre. The method appeared to be linear and reproducible for both fibres but it proved critical that the operating conditions (stirring rate, depth of immersion, time lapse between extraction and injection) were thoroughly consistent. The principal advantage of the SPME method would appear to be the fact that it permits a simple one-step extraction and that it does not involve the use of organic solvents, therefore eliminating some of the expense in terms of purchase and disposal of materials, in addition to minimising the potential for sample contamination. The principal drawback which emerged in this study was that the linear range of the fibre was rather limited and the fact that in order to achieve real trace analysis it was necessary to immerse the fibre in the sample for a long period of time. It is possible that the PDMS/DVB phase was not highly adapted to the particular analyte, although it was superior to the other three tested, and it would be interesting to study the absorption profiles using fibres coated with traditional solid-phase extraction materials, such as C_{18}, to effect a real comparison between SPME and SPE.

Acknowledgements

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