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Headspace solid-phase microextraction of phthalic acid esters from vegetable oil employing solvent based matrix modification

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Abstract

A new solvent-free analytical procedure based on headspace solid-phase microextraction (SPME) coupled to gas chromatography employing an electron capture detector (GC/ECD) or alternatively a mass spectrometric detector (GC/MSD) has been developed for the determination of phthalic acid esters (dimethyl-[DMP], diethyl-[DEP], di-*n*-butyl-[DnBP], butylbenzyl-[BBP], di-2-ethylhexyl-[DEHP] and di-*n*-octyl [DnOP] phthalate) in vegetable oils. Four different fiber coatings were evaluated, among them polydimethylsiloxane with a thickness of 100 μm appeared to be the best choice for allowing extraction of the whole group of analytes. Various solvents were tested as sample matrix modification agents with the aim to facilitate the transfer of esters with low vapour pressure (DEHP and DnOP) from oil matrix into the headspace. The addition of methanol resulted in optimal set-up applicable for all phthalate esters. Temperature control and the way of sample stirring were recognized as critical points of the whole procedure. Primarily, because shaking rather than stirring of the sample is carried out using a CombiPal multipurpose sampler, the automation of the SPME method employing this instrument was found to be not fully suitable for efficient stripping of phthalates from the oil matrix into the sample headspace. Nevertheless, the optimized manual SPME method, encompassing GC/ECD or GC/MSD for the separation and detection of target analytes, offers a unique solution and showed acceptable performance characteristics: linear response in the range of 0.5–2 mg kg^{-1} and repeatability expressed as R.S.D. between 14 and 23% at the spiking level of 2 mg kg^{-1}.
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Keywords: Solid-phase microextraction; Phthalates; Di-2-ethylhexyl phthalate; Headspace analysis; Vegetable oil; Matrix modification

1. Introduction

Phthalic acid diesters (phthalates, PAEs) are used primarily as plasticizers in polymeric materials, such as polyvinylchloride or polyvinylidenchloride copolymers, increasing their flexibility only through weak secondary molecular interactions with polymer chains. Being not covalently bound to the vinyl polymer matrix, their migration from plasticized products to contact materials may occur through extraction (leaching) or evaporation processes. World wide production of PAEs and their frequent application in different products of everyday use has resulted in their widespread presence in all parts of the environment and, consequently, in food. The most frequently used ester, di-2-ethylhexyl phthalate (DEHP), became practically ubiquitous in the environment as a result of its massive use and persistent character.

The quest for a reasonable stand concerning the possible adverse impact of phthalates on human health is still an unresolved issue. The presence of phthalates in the environment has been discussed for decades, but a greater interest in phthalates during the eighties was provoked by the suspicion of the carcinogenic effect of di-2-ethylhexyl phthalate. DEHP has been shown to cause liver cancer in rats and mice [1,2]. The finding that some phthalates (benzylbutyl-[BBP], di-*n*-butyl-[DnBP], di-2-ethylhexyl-[DEHP] and diethyl [DEP] phthalate) show the ability to interfere with development of young mammals and to affect their reproductive system [3] has again prompted a growing interest in these substances in recent years [4]. Dimethyl phthalate together with benzylbutyl phthalate have been found to be the esters with the highest estrogenic potency among phthalates [5]. According to research carried out in this field, it is now clear that some phthalate metabolites, mainly monoesters, play an important role in the toxic action of PAEs [6].

Phthalates analysis may generally pose a serious problem due to the high risk of secondary contamination from chemicals, materials and laboratory equipment. Also the difficulty

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Table 1
Analyte characteristics: basic physico-chemical properties [41]

Analyte	Abbreviation	Molecular weight	H_2O solubility (mg L^{-1})	log K_{OW}[a]	Vapour pressure (mPa) [25 °C]
Dimethyl phthalate	DMP	194.19	4200	1.61	267
Diethyl phthalate	DEP	222.24	1100	2.38	133
Di-*n*-butyl phthalate	DnBP	278.35	11.2	4.45	3.60
Butylbenzyl phthalate	BBP	312.39	2.7	4.59	0.67
Di-2-ethylhexyl phthalate	DEHP	390.56	0.003	7.50	0.013
Di-*n*-octyl phthalate	DnOP	390.56	0.0005	8.06	0.013

[a] Partition coefficient octanol–water.

of separating co-extracts (lipids, pigments) makes the determination of low levels of PAEs in some matrices (food matrices above all) almost impossible, namely when capillary gas chromatography is used for the final separation and detection of analytes. Therefore, many procedures use high performance liquid chromatography (HPLC) to determine phthalates in complex matrices, such as plasma [7] or milk [8], regardless of the fact that lower sensitivity of determination has to be accepted in this case.

Several methods determining PAEs in fatty matrices have been published. Some procedures use adsorption column chromatography on Florisil or alumina and the elution of target analytes using various mixtures of diethyl ether in petroleum ether [9,10]. Page and Lacroix [11] employed a sweep co-distillation technique for phthalate separation in matrices, such as animal tissues, fats or cheeses with a high fat content. It should be noted that in most of these methods the separation of PAEs from matrix components was either incomplete or very laborious. With regard to these difficulties indirect methods consisting of the saponification of the parent phthalic acid esters and fats, and the subsequent determination of the resulting alcohol moiety or phthalic acid, were introduced [12,13]. In the early 1990s gel permeation chromatography (GPC) employing Bio-Beads S-X3 became the most often applied clean-up method. Unfortunately, the complete separation of lipids from the common PAEs presented in sample extracts could not be achieved with any conceivable mobile phase. To solve this problem one of two approaches was applied: either conversion of PAEs into easily separable dimethyl phthalate [14,15] or the so called "Stable Isotope Dilution" approach in which the deuterated analogue of analyte is added to the sample and used for recovery calculation after the intentional narrowing of GPC fraction containing phthalates [15–17].

Solid-phase microextraction in sample preparation became very popular in the late 1990s. The SPME procedure (sampling, extraction, concentration and sample introduction in one step) significantly reduces the risk of secondary contamination and substantially simplifies the overall analytical process. Consequently, SPME seems to be an 'ideal' technique for phthalate determination. Several studies employing direct SPME for extraction of phthalates from water have been published [18–21]. These methods have used polyacrylate [18,21], Carbowax-divinylbenzene [20] or polydimethylsiloxane-divinylbenzene fibers [19].

The SPME technique can be applied not only in water analysis but also, providing that headspace sampling mode is used, in examination of complex liquid samples, such as human body fluids [22–24], wine [25] or beer [26] and even solid matrices like fruit or vegetables [27–29]. Applications of SPME in food analysis were reviewed by Kataoka et al. [30].

Low volatility of target analytes may be a limiting factor in SPME headspace analysis. In this case, extraction at elevated temperature is recommended to increase the vapour pressure of analytes [15,31]. However, the negative temperature effect on the coating-headspace partition coefficient of analytes in the system, and the consequent reduction of the extracted amount of analytes, should be considered. Mass transfer from a liquid sample to the headspace can also be enhanced by stirring of the sample. Whenever the matrix retains analytes strongly, a modification of the matrix should be considered to facilitate their transport from the matrix to the headspace. In the case of liquid samples, 'salting-out' and pH adjustment are the most commonly used techniques [32–36]. The addition of organic solvent or water to solid [37–39] or liquid matrices [22,34,40] has also been reported.

The aim of our study was to investigate the applicability of HS-SPME for the simple and fast analysis of phthalates in vegetable oil samples. In addition to heating and stirring of the sample, solvent based matrix modification was used to enhance phthalate amounts in the headspace of an oil sample. A list of the target analytes together with selected physico-chemical properties relevant to our experiments is summarized in Table 1.

2. Experimental

2.1. Chemicals and materials

Dimethyl phthalate (98.8%), diethyl phthalate (99.9%), di-*n*-butyl phthalate (99.4%), butylbenzyl phthalate (97%), di-2-ethylhexyl phthalate (99%) and di-*n*-octyl phthalate (94%) were purchased as neat compounds (Dr. Ehrenstorfer GmbH, Germany). The individual stock solutions (1 g L^{-1}) were prepared in methanol (Merck, Germany) and stored at 4 °C. They were used for preparation of standard mixtures in methanol (50 mg L^{-1} each) and in *n*-hexane (Merck, Germany) (2 mg mL^{-1} each). The solvents used for matrix modification (acetonitrile, *N*,*N*-dimethylformamide [both Riedel-de Haën, USA], methanol and *n*-hexane [both Merck, Germany]) were of analytical grade. The purity of the nitrogen and helium used was 99.998 and 99.996%, respectively. The vegetable (rape) oil was selected from a set of oils purchased at a retail market and examined for the presence of phthalates. Gel-permeation chromatography was employed,

under severe measures preventing cross-contamination, for oil samples processing. The minimum contaminated oil (di-*n*-butyl phthalate and di-2-ethylhexyl phthalate at the LOQ level (0.1 mg kg^{-1})) was used for SPME method optimization.

To minimize the risk of secondary contamination the following precautions were taken: (i) all solvents were checked for the presence of phthalates and re-distilled if necessary. (ii) The water used as a modifier was distilled in all glass apparatus and stored in a glass bottle for no more than one week. (iii) After standard washing using a detergent, all laboratory glassware was rinsed with re-distilled acetone (checked for the presence of phthalates) and baked out in a laboratory oven at 250 °C for 2 h. Clean glassware was stored covered with an aluminium foil, and again rinsed with acetone prior to use.

2.2. *SPME fibers*

The SPME fibers used in this study were purchased from Supelco (USA). Silica fibers coated with polydimethylsiloxane (thickness 100 μm, PDMS 100), polyacrylate (thickness 85 μm, PA), Carboxen/polydimethylsiloxane (thickness 75 μm, CX/PDMS) and polydimethylsiloxane/divinylbenzene (thickness 65 μm, PDMS/DVB) were tested. Before being used for the first time, new fibers were conditioned in accordance with the recommendations of the producer. Each day, before the samples analyses started, the fibers were thermally cleaned in a GC injector held at 250 °C (30 min). Then a blank analysis was performed to verify that no extraneous compounds were desorbed from the fiber.

2.3. *Optimized SPME procedure*

SPME of phthalates was carried out manually, using a PDMS 100 fiber and 20 min extraction from the headspace above 1 g of magnetically stirred (stir position #4, heat position #2, Nuova Stir Plate, Thermolyne, USA) oil sample modified with 1 mL methanol. The temperature was held at 40 °C (measured in headspace) and the sample was incubated for 60 min at 40 °C prior to extraction.

2.4. *GC analysis*

2.4.1. *Manual SPME*

All desorptions in manual SPME experiments, unless otherwise stated, were performed using a HP 5890 Series II gas chromatograph equipped with a split-splitless injector (liner volume 250 μL), electronic pressure control (EPC) and an electron capture detector (^{63}Ni-ECD) (Hewlett-Packard, USA). A capillary column DB-35 (35% phenyl, 65% polymethylsiloxane) of 30 m length × 0.25 mm i.d. coated with 0.15 μm film (J&W Scientific, USA) was employed for separation of analytes. The data was processed using a GC ChemStation HP 3365 (Hewlett-Packard, USA).

The initial oven temperature was set at 45 °C for 5 min, increased to 130 °C at 20 °C min^{-1}, then increased to 240 °C at 3 °C min^{-1} and finally increased to 270 °C at 20 °C min^{-1} (held 2.5 min, total GC run time was 50 min). Nitrogen was used both as carrier and make-up gas at flow rates of 1 and 30 mL min^{-1}, respectively. The injector and detector temperatures were 250 and 300 °C, respectively.

In experiments focused on the dynamics and repeatability of PAEs extraction, the manual SPME was combined with gas chromatography coupled to a mass spectrometric detector, for conditions see Section 2.4.2.

2.4.2. *Automated SPME*

All automated SPME experiments were performed using the CombiPal multipurpose sampler (CTC analytics, USA) connected to a TRACE 2000 gas chromatograph (Thermo Quest, USA) equipped with a PTV injector (liner volume 160 μL), digital pressure flow control (DPFC) and a mass spectrometric—ion trap detector Polaris Q (Finnigan, USA). A capillary column RTX-5 (5% phenyl, 95% polymethylsiloxane) of 30 m length × 0.25 mm i.d. coated with 0.25 μm film (Restec, USA) was employed for the separation of analytes. The data was processed using an XCALIBUR software, Version 1.2.2 (Finnigan).

The initial oven temperature was set at 50 °C for 5 min, then increased to 180 °C at 20 °C min^{-1}, then increased to 250 °C at 5 °C min^{-1} and finally increased to 270 °C at 10 °C min^{-1} (held 13 min, total GC run time was 40 min). Helium was used as carrier gas at the flow rate of 1 mL min^{-1}. The injector and the transfer line temperatures were set at 250 °C and 270 °C, respectively. The detector was operated simultaneously in a SegmentScan (SGSC, analysed *m*/*z* 50–100, 101–150, 151–200, 201–250, 251–300, 301–350, 351–400) and a Selected Ion Monitoring mode (SIM, monitored *m*/*z* DMP 163; DEP, DnBP, BBP, DEHP and DnOP 149).

3. Results and discussion

The decision to initiate the study presented was based on previous long-term experience with the determination of PAEs in fatty matrices, or, strictly speaking, on continuous unsuccessful attempts to find an analytical approach enabling their reliable determination in such matrices. Among various analytical approaches (liquid–liquid extraction, adsorption column chromatography, solid-phase extraction) tested in our laboratory, gel permeation chromatography using gel Bio-Beads S-X3 and the mixture of cyclohexane:dichloromethane as a mobile phase, seemed to be the best choice for the separation of PAEs from fats. This technique finally enabled gas chromatographic determination of phthalates in cleaned samples using an electron capture detector, although extreme care must be taken to avoid secondary contamination of the sample (even though in the case of vegetable oil the only sample handling step prior to GPC clean-up is its dilution in a mobile phase). In any case, the solvents had to be freshly distilled and checked for phthalate presence, all glassware had to be specially cleaned, stored and rinsed with 'phthalate-free' solvent prior to their use. Obviously, these unavoidable steps make the analytical procedure laborious, as well as costly. According to our experience, the best approach to PAEs analysis is therefore to omit or at least minimize sample handling and the

use of chemicals whenever possible. In this context, the idea to test the potential of SPME for PAEs analysis was entirely natural.

With regards to the matrix character, headspace sampling of phthalates occurring in vegetable oil was the only conceivable option. However, as shown in Table 1, the physico-chemical properties of phthalates vary in a wide range, with low vapour pressure (hence low transfer into a vapour phase) and the lipophilic character of PAEs with a long alkyl chain, making optimization of headspace SPME sampling complicated. Having relatively high vapour pressures and less lipophilic character, the most polar analytes in the selected PAEs group, DMP and DEP do not represent such a problem. Feng et al. [36] noticed in their study dealing with the application of SPME for monitoring of PAEs in cow milk, that the extraction efficiency was strongly dependent on the content of fat in milk samples. This inverse correlation was documented by Page and Lacroix in their paper concerned with the SPME sampling of volatile compounds in vegetable oil as well [31]. The transport of analytes from the oil to the headspace was facilitated by stirring of a heated sample. To our knowledge, this and 'the salting-out effect' are the only approaches applied in phthalate analysis so far. As with the 'salting-out effect', addition of a polar solvent immiscible with oil may push out relatively lipophilic analytes into the headspace. Based on this assumption, the optimization steps of the whole procedure are described in detail below.

3.1. Optimization of SPME procedure—GC/ECD determination

3.1.1. Selection of fiber coating

Some of the knowledge obtained from our previous study employing SPME in PAEs analysis in water [21] also applies to the current optimization process. According to our experience, a 5 min desorption period at 250 °C was shown to be sufficiently effective to remove phthalates from the fiber. However, to keep it clean and ready for subsequent analysis and to avoid any undesirable sorption of compounds from the laboratory environment, the fiber was left in the injector port until the end of the GC run.

An oil spiked at the level of 5 mg kg^{-1} (each analyte) served as a sample in fiber testing experiments. The spiking procedure consisted of the following steps: 5 μL of the stock standard solution in hexane (concentration 2 mg mL^{-1}) were transferred into a 4 mL screw cap vial, the hexane then being evaporated with a gentle stream of nitrogen; two grams of rape oil were weighed into the vial; finally, to ensure sample homogeneity, 10 min sonication (Sonorex Super RK 510 H, Bondelin Electronic, Germany) was followed by 30 min shaking (1000 min^{-1}, MS1 Minishaker, IKA, USA).

A piece of stainless wire, used as a stirring bar, was put into the vial, a cap with an aluminium lined septum was fixed and the samples were then heated for 60 min at 40 °C (measured in headspace) to equilibrate. In the course of the following 20 min sorption at 40 °C (measured in headspace), the fiber was placed in position to prevent contact of its tip with the surface of the magnetically stirred sample (stir position #4, heat position #2, Nuova Stir Plate, Thermolyne, USA).

Three types of stationary phases were tested at this stage of our study: (1) polyacrylate 85 μm (PA), (2) Carboxen/polydimethylsiloxane 75 μm (CX/PDMS) and (3) polydimethylsiloxane 100 μm (PDMS 100). In accordance with theoretical assumptions, extraction efficiency was strongly influenced by the volatility and polarity of the particular ester (see Fig. 1).

The first tested PA fiber enabled the most efficient extraction of the most polar PAEs, DMP, DEP and DnBP. The volatility of these esters is relatively high in comparison with these with a long alkyl chain (BBP, DEHP and DnOP) (see Table 1). Contrary to our expectation based on the results obtained during direct SPME of phthalates from water samples [18,21], the heating of the sample (laboratory temperature versus 40 ± 1 °C, measured in the headspace) did not result in improved extraction potential of the PA fiber for the non-polar phthalates. However, the extracted amounts of DMP, DEP and DnBP increased in spite of the fact that the retention ability of the fiber decreased with increased temperature [42].

Using a stationary phase CX/PDMS, suitable mainly for analyses of volatile compounds [31], only small peaks of the most volatile esters DMP and DEP from the whole group were recorded in the chromatogram.

According to literature data, the third tested PDMS 100 fiber seems to be, the most efficient in headspace analyses of various analytes in fat-containing samples [31,36]. Likewise in our experiments, the PDMS 100 fiber provided the best result too. Five of the six esters were extracted using this fiber as can be seen in Fig. 1, however, the extracted amounts of polar phthalates (DMP and DEP), compared to the PA fiber, decreased. The extension of the extraction time from 20 to 60 min (results not displayed) resulted in increased responses of DMP and DEP, while responses of the later-eluted phthalates were not so enhanced. Similar observations were described by Page and Lacroix [31], in their paper dealing with the determination of volatile compounds in vegetable oil. Using the PDMS 100 fiber, the equilibrium for phthalates with a long chain was reached in our system within 20 min; however, due to the relatively low vapour pressures of these analytes, the sensitivity of determination was very low. The small peaks of DnBP, BBP and DEHP in Fig. 1 illustrate this fact. Still the PDMS 100 fiber provided the best result among the fibers tested, being the only one able to extract esters other than DMP and DEP. The following experiments focused on matrix modification were carried out employing this fiber.

3.1.2. Matrix modification

Matrix modification is commonly used to facilitate the transport of strongly retained analytes from the sample matrix to the headspace. An increase of ionic strength ('the salting-out' effect) and/or pH-adjustment are the typical approaches applied in the case of analysis of water or high moisture samples [22,32,35,36,40]. The addition of solvent to solid samples (soil and sediments) is used to decrease the strong sorption of analytes [37–39].

For the experiments, encompassing matrix modification rape oil spiked at the level of 5 mg kg^{-1} (each analyte) was prepared

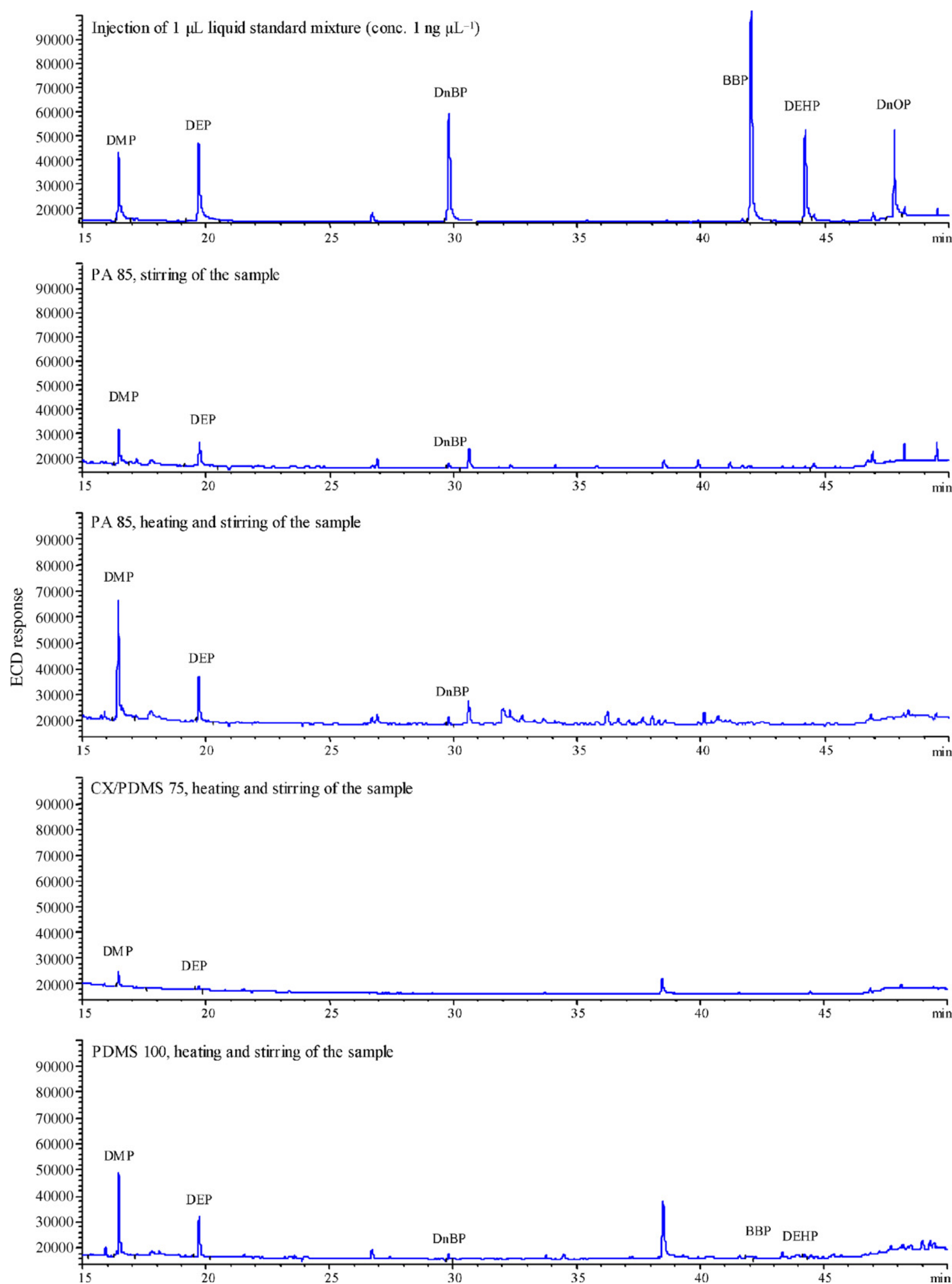

Fig. 1. Comparison of effectiveness of SPME fibers for phthalate extraction; rape seed oil without modification, spiking level 5 mg kg^{-1} (each analyte), laboratory temperature (25 °C) or 40 °C in headspace.

according to the following procedure: a volume of 100 µL was taken from the individual PAE stock solutions and placed into a round-bottom flask; the solvent (600 µL in total) was blown down under a gentle stream of nitrogen; 20 g of oil were then weighed into the flask; finally, to ensure sample homogeneity, 10 min ultrasonic treatment (Sonorex Super RK 510 H, Bondelin Electronic, Germany) was followed by 30 min shaking (220 min^{-1}, HS 250 Basic shaker, IKA, USA).

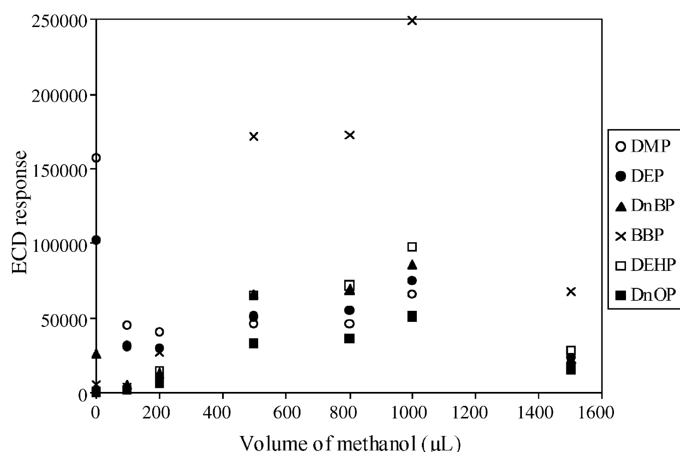

Fig. 2. Dependence of PAEs extraction efficiency on the volume of methanol added to 1 g of vegetable oil: spiking level 5 mg kg^{-1} (each analyte), temperature 40 °C in headspace, PDMS 100, manual SPME–GC/ECD (results averaged over two analyses).

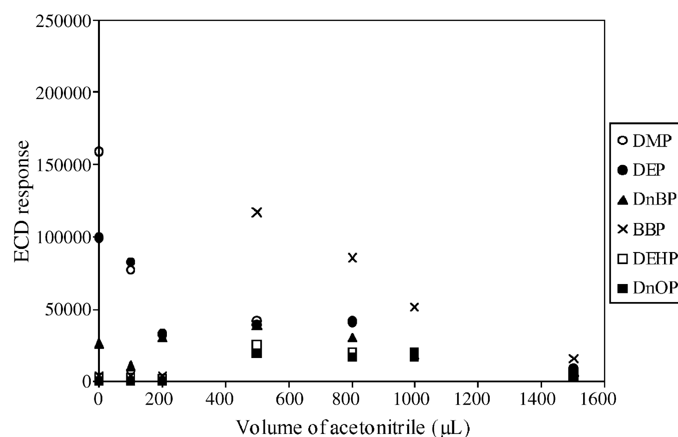

Fig. 3. Dependence of PAEs extraction efficiency on the volume of acetonitrile added to 1 g of vegetable oil: spiking level 5 mg kg^{-1} (each analyte), temperature 40 °C in headspace, PDMS 100, manual SPME–GC/ECD (results averaged over two analyses).

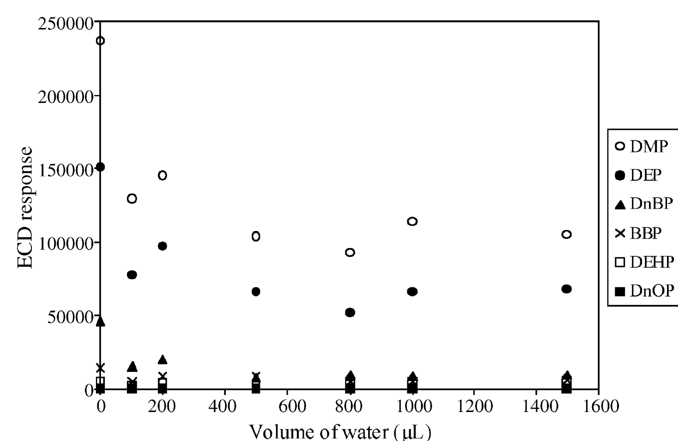

Fig. 4. Dependence of PAEs extraction efficiency on the volume of water added to 1 g of vegetable oil: spiking level 5 mg kg^{-1} (each analyte), temperature 40 °C in headspace, PDMS 100, manual SPME–GC/ECD (results averaged over two analyses).

One gram of the spiked oil was transferred into a 4 mL screw cap vial, together with a piece of stainless wire, and a respective volume of modifying solvent was then added. The capped vial was then shaken for 10 min at 1000 min^{-1} (MS1 Minishaker, IKA, USA) to homogenize the sample, and after that incubated at 40 °C for 60 min to equilibrate. During the 20 min sorption period, when the temperature of the headspace was maintained at 40 ± 1 °C, the tip of the fiber was placed in close proximity to the sample surface, in a position that prevented its contact with the sample during magnetic stirring (stir position #4, heat position #2, Nuova Stir Plate, Thermolyne, USA).

The addition of five solvents of different physico-chemical properties (hexane, methanol, acetonitrile, dimethylformamide and water) was tested during this step with the aim of enhancing the transfer of PAEs from vegetable oil to the sample headspace. The main criterion for the assessment of the effectiveness of individual modifiers was the yield of non-polar esters with a long alkyl chain. The volumes and selected characteristics of used solvents are summarized in Table 2.

In the first set of experiments, hexane was employed as a matrix modifier. As soon as the added amount exceeded 500 μL, degradation of fiber coating was observed. With respect to this problem, no further experiments were conducted using this solvent.

The results obtained for the other solvents are summarized in Figs. 2–5. The general trend observed was reduced yield of DMP and DEP in the presence of solvents. This phenomenon was probably caused by the good solubility of these ‘polar’ esters in polar solvents added to non-polar oil matrix. Despite this, the sensitivity of the method for DMP and DEP remained sufficient.

The extraction of the other four esters was also affected, but to differing extents depending on the volume and type of solvent added. Promising results were achieved using methanol and acetonitrile (see Figs. 2 and 3). Heating of the sample (40 °C, measured in the headspace) induced repeated boiling and condensation of these relatively low boiling modifiers (boiling point 64.7 °C for methanol, 81.6 °C for acetonitrile). Under these con-

Table 2
Characteristics of modifiers tested [43]

Modifier	Polarity index (P')	Boiling point (°C)	Volume added into 1 g of oil sample (μL)
Hexane	0	68.7	0, 100, 500
Methanol	5.1	64.7	0, 100, 200, 500, 800, 1000, 1500
Acetonitrile	5.8	81.6	0, 100, 200, 500, 800, 1000, 1500
Dimethylformamide	6.4	153.0	0, 100, 200, 500, 800, 1000, 1500
Water	10.2	100.0	0, 100, 200, 500, 800, 1000, 1500

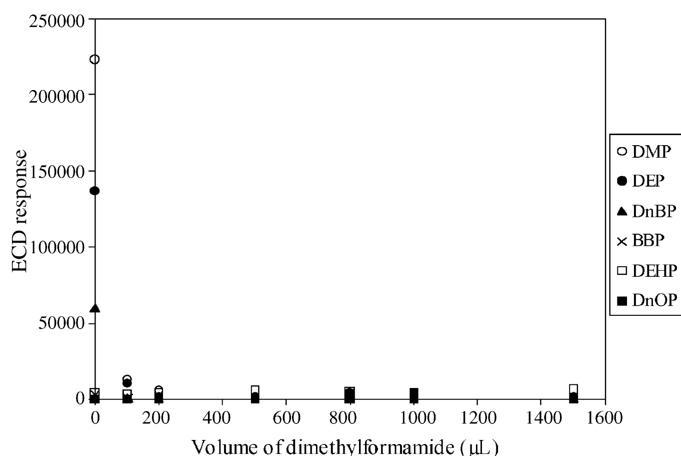

Fig. 5. Dependence of PAEs extraction efficiency on the volume of dimethylformamide added to 1 g of vegetable oil: spiking level 5 mg kg^{-1} (each analyte), temperature 40 °C in headspace, PDMS 100, manual SPME–GC/ECD (results averaged over two analyses).

ditions, stripping of phthalates from the oil matrix occurred. The possibility should be considered that the positive influence of these two solvents might be associated with their lower boiling point rather than with their polarity. Magnetic stirring played a very important role in this process and its proper execution (regular motion and speed) appeared to be the critical factor in achieving not only good repeatability, but also a high PAEs yield.

The results of experiments with water addition showed decreased amounts of all target analytes (see Fig. 4). However, the addition of this modifier did not affect the extraction of polar phthalates (DMP and DEP) to such an extent as methanol or acetonitrile (compare Figs. 2–4).

The addition of the last-tested dimethylformamide dramatically decreased the extracted amounts of all phthalates (see Fig. 5).

The best results, i.e. the highest yields of extracted phthalates, were achieved when 1 mL methanol was added to 1 g of oil sample. The use of a solvent with a lower boiling point, such as diethyl ether (boiling point 36.5 °C), could probably result in more effective phthalate stripping, but, on the other hand damage of the stationary phase (as in the case of hexane addition), together with undesirable pressurizing of the vial, could be expected.

3.2. Optimization of SPME procedure—GC/MSD determination

3.2.1. Dynamics of PAEs extraction

Once the positive result of matrix modification trials had been obtained, experiments, focusing on the dynamics of PAEs extraction from the modified oil sample, were conducted using gas chromatography and a mass spectrometric detector (GC/MSD) with 1, 2, 5, 10, 20 and 30 min sorption times. Rape oil spiked at the level of 1 mg kg^{-1} (each analyte) was prepared in accordance with the procedure described in Section 3.1.2 (a volume of 20 μL was taken from the individual PAE stock solutions). One gram of spiked sample was modified with 1 mL

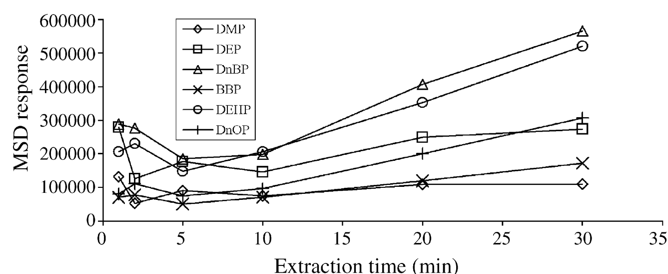

Fig. 6. Extraction time profiles of PAEs: rape seed oil, 1 mL methanol added, spiking level 1 mg kg^{-1} (each analyte), temperature 40 °C in headspace, PDMS 100, manual SPME–GC/MSD (results averaged over two analyses).

methanol, and homogenization, incubation and extraction was conducted in accordance with the procedure also described in Section 3.1.2. After the extraction a 5 min desorption of analytes at 250 °C was performed. The fiber was kept in the injector port until the end of the GC run (40 min).

Analyte extraction time profiles are shown in Fig. 6. In the presence of methanol all PAEs demonstrated quite similar behaviour, showing a more or less stable extracted yield up to 10 min sorption, and a gradual increase behind this point. The only exception was DMP, in the case of which equilibrium was reached so quickly that its extracted yield remained unchanged within the whole time period tested. Although equilibrium cannot be achieved for all tested phthalates within this period, the sorption time of 20 min was chosen for further analyses as a compromise between laboratory sample throughput and method sensitivity.

3.2.2. Comparison of PDMS and PDMS/DVB fibers

Based on the results of studies [19] indicating that a good sensitivity of phthalate determination can be reached using a PDMS/DVB fiber, in addition we decided to compare this fiber with the PDMS 100 fiber employed in our experiments thus far. The comparison was carried out using 1 g of oil sample, spiked at the level of 1 mg kg^{-1} and modified with 1 mL methanol, which was extracted using 20 min sorption at temperature 40 °C. Manual SPME was again combined with GC/MSD determination; the results obtained are shown in Fig. 7. The use of a PDMS 100 fiber still yielded a slightly higher sensitivity of determination.

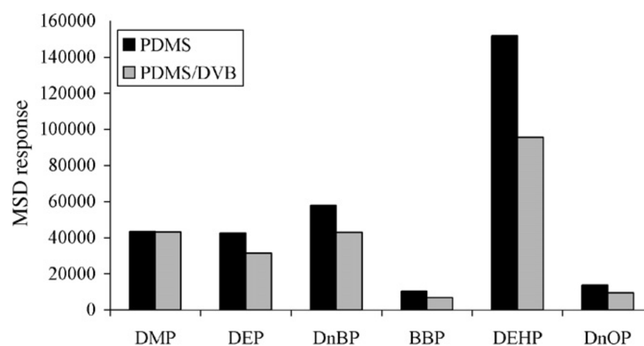

Fig. 7. Comparison of PDMS100 and PDMS/DVB fibers: rape seed oil, 1 mL methanol added, spiking level 1 mg kg^{-1} (each analyte), temperature 40 °C in headspace, manual SPME–GC/MSD (results averaged over two analyses).

3.2.3. Automation of SPME procedure

When the possibility of automating the whole procedure using a CombiPal multipurpose sampler was considered, facilitation of the demanding manual SPME (in the sense of precise execution) was a major factor. However, the main benefit anticipated was the potential improvement in method robustness as a consequence of the improved stability of sample heating inside a closed insulated unit.

For these experiments, rape oil, spiked at the level of $1\,mg\,kg^{-1}$ (each analyte), was prepared in accordance with the procedures described in Sections 3.2.1 and 3.1.2. Two or three grams of the spiked oil were transferred into a 10 mL vial together with 2 mL (2-g sample) or 1 mL (3-g sample) methanol. The vial neck was then covered with aluminium foil and, after being capped with a magnetic cap, the vial was shaken for 10 min at $1000\,min^{-1}$ to homogenize the sample. The agitated (agitator speed 500 rpm, on time 5 s, off time 2 s) samples were incubated at 40 or 60 °C (according to the extraction temperature used) for 60 min to equilibrate, before automated headspace SPME (20 min sorption) employing a PDMS 100 fiber was carried out. After the extraction, 5 min thermal desorption followed in a GC injection port kept at 250 °C. The fiber was left in the GC injector port until the end of analysis (40 min).

Unfortunately, the expectation of improvement proved to be false. None of the arrangements tested provided acceptable determination sensitivity; no phthalate peaks were identified at the level of $1\,mg\,kg^{-1}$ (see Fig. 8). Taking into account that the sensitivity of the final GC/MSD determination was proved in combination with manual SPME (see Fig. 9), several other possible explanations exist for this phenomenon. Even if the sample amount was double (2 g) or triple (3 g) the amount in the manual arrangement (1 g) and the volume of vials used by CombiPal (10 mL) was just 2.5 times higher than the volume of vials used for manual SPME (4 mL), the enlarged headspace could still play a negative role. However, based on the findings gained during our manual SPME, which showed that the way of moving the magnetic stirrer inside the oil sample dramatically affects phthalate transfer to the headspace, it is, in our opinion, more likely that the shaking performed by the CombiPal simply does not sufficiently support this transfer.

3.3. Performance characteristics of the optimized method

Based on the results described above, the final determination of SPME method performance was carried out using a PDMS 100 fiber and 20 min phthalate extraction from the headspace above 1 g of magnetically stirred oil sample modified with 1 mL methanol. The temperature was maintained at 40 °C (measured in headspace); the sample was incubated for 60 min at the same temperature prior to extraction. The repeatability of the SPME method was determined by performing five replicate analyses of vegetable oil spiked at levels of 5, 2 and $1\,mg\,kg^{-1}$, and expressed as a relative standard deviation (R.S.D.). Linearity testing was carried out using vegetable oils spiked at levels of

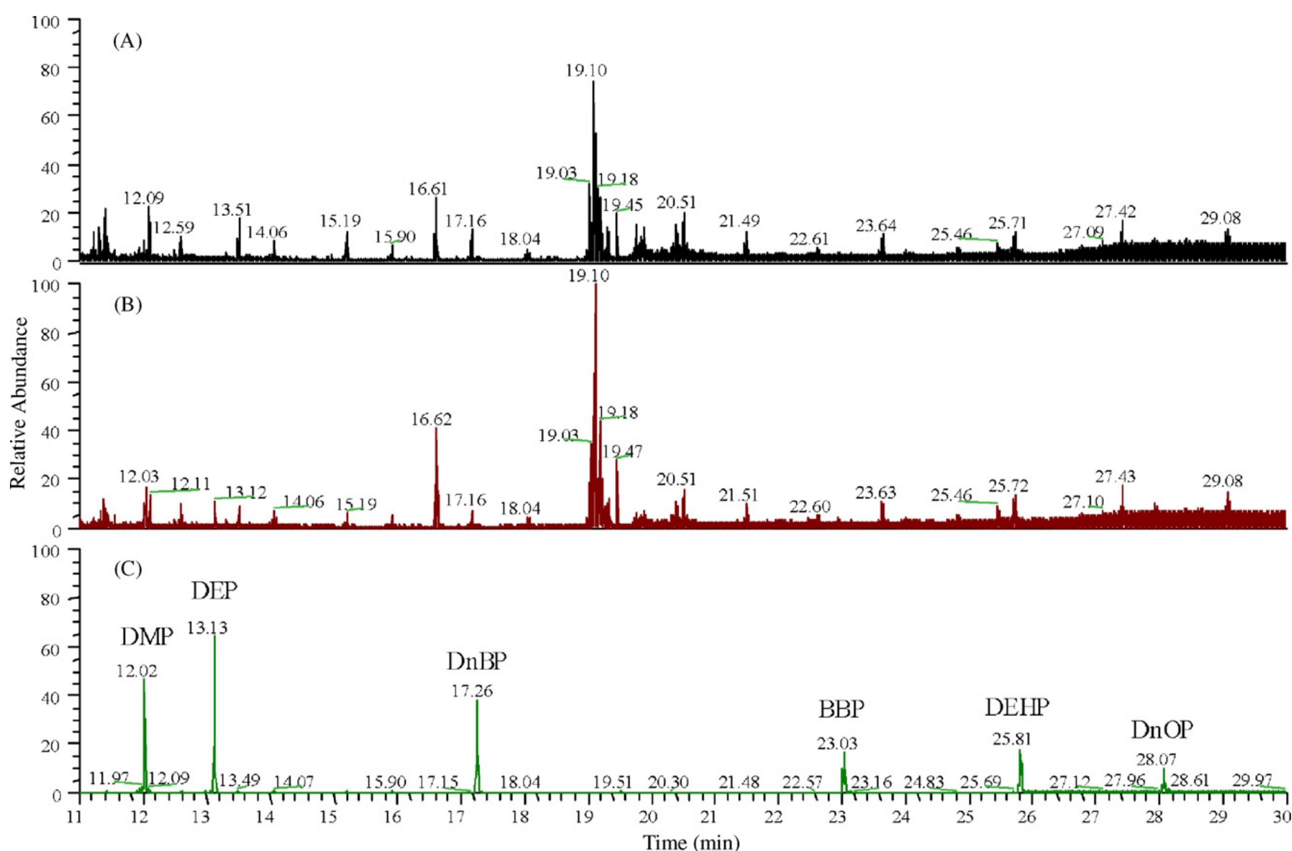

Fig. 8. Chromatogram of vegetable oil sample analysed by automated SPME–GC/MSD method: 3 g of oil, 1 mL methanol, sorption 20 min, temperature 60 °C. (A) Blank oil sample; (B) oil sample spiked at the level of $1\,mg\,kg^{-1}$; (C) PAEs standard mixture-liquid injection (1 μL, i.e. 10 ng).

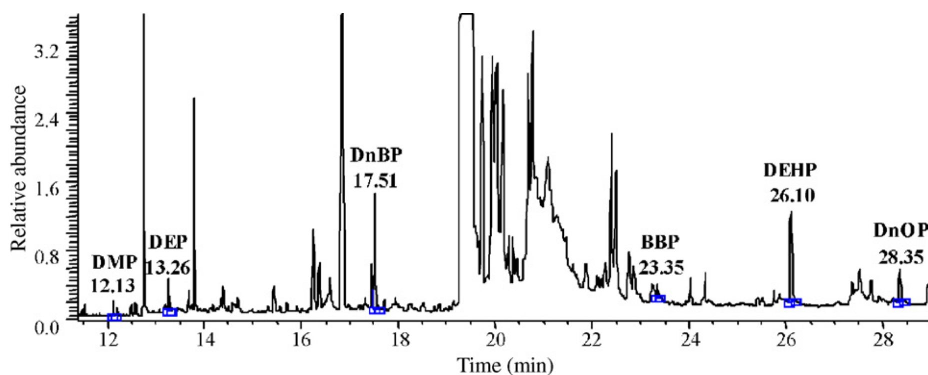

Fig. 9. Chromatogram of vegetable oil sample spiked at the level of 1 mg kg^{-1} analysed by manual SPME–GC/MSD method: 1 g of oil, 1 mL methanol, sorption 20 min, temperature 40 °C.

Table 3
Performance characteristics of the final SPME method

	Repeatability R.S.D. (%), $n = 5$			Linearity r^{2a} (0.5–2 mg kg^{-1})	LOD[a] (mg kg^{-1})	LOQ[a] (mg kg^{-1})
	5 mg kg^{-1} [a]	2 mg kg^{-1} [b]	1 mg kg^{-1} [b]			
DMP	9	14	11	0.970	0.2	0.5
DEP	10	16	22	0.980	0.2	0.5
DnBP	9	23	24	0.994	0.2	0.5
BBP	6	17	13	0.987	0.06	0.2
DEHP	8	17	18	0.999	0.2	0.5
DnOP	9	17	17	0.940	0.3	0.5

[a] Manual SPME—GC/ECD.
[b] Manual SPME—GC/MSD.

0.5, 1, 2, 5 and 10 mg kg^{-1}. The resulting performance characteristics are summarized in Table 3.

R.S.D. values at the level of 5, 2 and 1 mg kg^{-1}, ranging between 6 and 10%, 14 and 23%, and 11 and 24%, respectively, were achieved. The method showed acceptable correlation coefficients (r^2) for all target analytes within the range 0.5–2 mg kg^{-1}. The level 5 mg kg^{-1}, for which measurement of precision was carried out, was still within the dynamic range of the method (although outside the linearity range). The limits of quantification, calculated using signal to noise ratio S/N = 9/1,

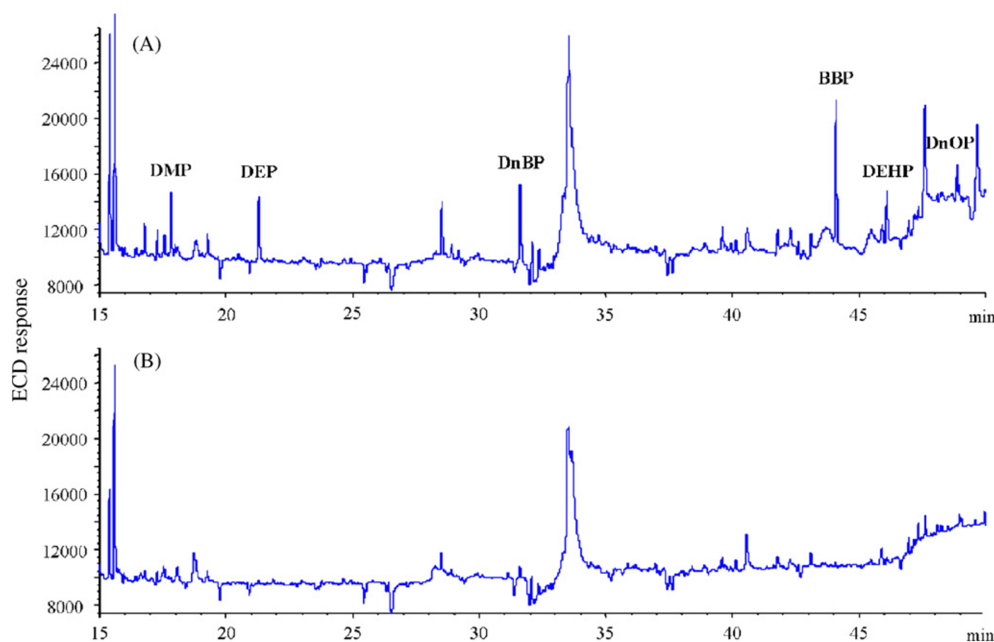

Fig. 10. Chromatograms of sample spiked at the level of 2 mg kg^{-1} (A) and blank vegetable oil sample (B), analysed by optimized manual SPME–GC/ECD method.

ranged between 0.2 and 0.5 mg kg^{-1}. It should be noted that neither certified reference material nor a relevant proficiency test is available for an accuracy check.

To achieve the performance characteristics presented, rigorous keeping of all extraction conditions was necessary. Extraction temperature and stirring technique were identified as the most critical parameters. Chromatograms of a blank vegetable oil sample and the sample spiked at the level of 2 mg kg^{-1} are shown in Fig. 10.

4. Conclusion

Gas chromatographic analysis of low levels of phthalates in fatty matrices is a difficult issue, mainly due to difficulties with their separation from bulk lipids and the risk of secondary contamination, the latter leading to overestimation of results. The minimization of sample handling is therefore important to obtain precise data. In this context, headspace SPME represents a real challenge, although semivolatile and relatively lipophilic analytes, such as PAEs, in combination with fatty matrices do not represent an optimal set-up.

The promising results of the study presented, in spite of the negative preconditions, showed that alternative ways could be found of reaching a solution. The newly developed SPME method, employing a PDMS 100 fiber together with methanol as the matrix modifier, enables headspace phthalate determination in vegetable oil samples. The method is not only very simple, fast and inexpensive, but, in comparison with conventional procedures, significantly minimizes the risk of secondary contamination. However, reliable results can only be obtained by maintaining careful temperature control and employing intensive stirring by a magnetic stirrer. Despite this, the SPME method employing methanol for the stripping of analytes into sample headspace, offers a unique solution for the analysis of phthalic acid esters in vegetable oil samples.

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