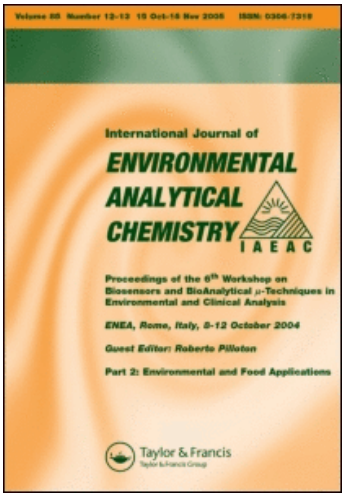

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A Comparison of Different Ways of Sample Preparation for the Determination of Phthalic Acid Esters in Water and Plant Matrices

K. Holadová[a]; J. Hajšlová[a]
[a] Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague, Czech Republic

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A COMPARISON OF DIFFERENT WAYS OF SAMPLE PREPARATION FOR THE DETERMINATION OF PHTHALIC ACID ESTERS IN WATER AND PLANT MATRICES

K. HOLADOVÁ and J. HAJŠLOVÁ

Institute of Chemical Technology, Department of Food Chemistry and Analysis, Technická 3, 16628 Prague, Czech Republic

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Different methods for the isolation of phthalates from water were tested and compared with the aim of evaluating the risk of secondary contamination of sample and to reach good values of recovery for six tested esters (i.e. dimethyl-, diethyl-, di-n-butyl-, benzylbutyl-, bis(2-ethylhexyl)- and di-n-octyl phthalates). Classic liquid-liquid extraction with hexane gave good recoveries for all six esters (70 – 100%, spiking level 20 µg/l), but the increased relative standard deviations document problems with cross contamination. Micro extraction with iso-octane is suitable for the determination of benzylbutyl-, bis(2-ethylhexyl)- and di-n-octyl phthalates even at low levels of contamination (1 µg/l) and also for di-n-butyl phthalate at higher levels (tens of µg/l). The detection limits for these esters ranged from 0.01 to 0.05 µg/l. The recovery of more polar phthalates (dimethyl-, diethyl phthalate) is very low. Solid phase extraction on octadecyl reverse phase with ethyl acetate as elution solvent was chosen from a variety of tested systems and can be successfully used for the determination of dimethyl-, diethyl, di-n-butyl- and benzylbutyl phthalates (recoveries 72 – 95%, spiking level 20 µg/l). However, recoveries of bis(2-ethylhexyl)- and di-n-octyl phthalate were not higher than 30%. Detection limits ranged from 0.05 to 0.10 µg/l.

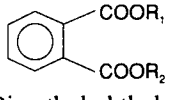
A method for the determination of phthalates in lettuce, using either alumina adsorption column chromatography or Florisil solid phase extraction as a clean-up step, was developed. Recoveries of all phthalates ranged from 60 to 110% (spiking level 1 mg/kg) and detection limits from 0.01 to 0.05 mg/kg.

GC-ECD or GC-MS were used for the identification and quantification of analytes.

KEY WORDS: Phthalate esters, water analysis, vegetable analysis, solid phase extraction, adsorption column chromatography, gas chromatography.

INTRODUCTION

Commercial phthalates (PAEs) encompasses a variety of compounds depending on the alkyl moiety of the ester. The following six esters are included in this study:



Dimethyl phthalate (DMP) ..... R_1, R_2 = $-CH_3$
Diethyl phthalate (DEP) ..... R_1, R_2 = $-C_2H_5$
Di-n-butyl phthalate (DnBP) ..... R_1, R_2 = $-C_4H_9$
Butylbenzyl phthalate (BBP) ..... R_1 = $-C_6H_5$, R_2 = $-C_4H_9$
Bis(2-ethylhexyl) phthalate (BEHP)... R_1, R_2 = $-CH_2(CH)(C_2H_5)C_4H_9$
Di-n-octyl phthalate (DnOP) ..... R_1, R_2 = $-C_8H_{17}$

The world production of PAEs shows a significant increase and it is estimated to be several million tons per year[1,2,3]. As a result of the wide use of materials containing phthalates, PAEs have become ubiquitous in the environment. The six esters, which were mentioned above, are included by several countries in their list of priority pollutants[4]. Tolerable Daily Intake for the most hazardous BEHP was defined as 25 μg/kg of body weight by the EEC Scientific Committee for Food (Commission of the European Community, 1991).

It is evident that reliable analytical procedures are necessary for both control and monitoring of various environmental samples as well as foodstuffs. For the clean-up of sample extracts prior to PAEs analysis in abiotic matrices several methods were described. However, there is a lack of information in the case of food analysis, especially vegetables.

In water analysis, the classic liquid-liquid extraction with dichloromethane[5-8], hexane[9,10] or petroleum ether[11] is still the most widely used method. Some authors tested procedures based on solid phase extraction with reverse phase (octyl or octadecyl silica) cartridges where analytes were eluted with either methanol[12] or acetonitrile[13]. Lessage[14] investigated dynamic thermal stripping (purge and trap method) and direct adsorption on Carbotrap followed by thermal desorption.

The main problem related to the PAEs analysis in foods - the separation of co-extracts - has not yet been resolved satisfactorily. Several methods for the determination of PAEs in fatty samples were published. These mainly consist in the saponification of parent esters and fats and determination of either the alcohol moiety or the phthalic acid[15,16]. Some procedures use adsorption column chromatography on Florisil or alumina eluted with different mixtures of diethyl ether in petroleum ether (usually 20% diethyl ether in petroleum ether)[17,18]. The solid phase extraction (SPE) for analysis of biological matrices has rarely been utilised. Lopez-Avila[19] who studied model mixtures of PAEs and other environmental pollutants indicates the possibility of separating PAEs from PCBs and organochlorine insecticides on SPE cartridges packed with Florisil or silica gel. Nevertheless, these SPE systems were not found to be suitable for the separation of PAEs and corn oil.

The only study concerning with the determination of PAEs in plant matrices was presented by Suzuki[20]. Extracts from vegetables were brought onto a $AgNO_3$-coated Florisil column and then phthalates were eluted by a mixture of 2% ethyl acetate in hexane.

The main objective of this study was to compare alternative ways of sample treatment prior to GC-ECD quantitation of PAEs. Liquid-liquid extraction, solid phase extraction and micro extraction were utilised for processing water samples. Adsorption column chromatography and solid phase extraction were tested for isolation and clean-up of extracts obtained from vegetable matrices.

MATERIALS

Reagents

Standards of phthalate esters: DMP, DEP, DnBP, BBP, BEHP, DnOP, purity min. 96% (Supelco, USA) – individual stock solutions (c=1 mg/ml) were prepared in methanol and stored at 4°C. Deuterated standards: BEHP (ring D_4), DnBP (ring D_4) purity min. 99% (Cambridge Isotope Laboratories, USA). Individual stock solutions (c=10 mg/ml) were prepared in methanol and stored at 4°C.

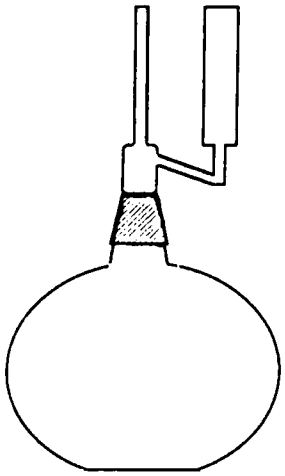


Figure 1 Micro extraction glass bottle with a special adapter.

Double distilled water: Distilled water was redistilled in an all glass still apparatus.
Solvents: Acetone, diethyl ether and ethyl acetate (Lachema Brno, CR) were rectified before use. Hexane, for residue analysis (Merck, Germany), isooctane, for UV-spectroscopy (Fluka, Switzerland) and acetonitrile CHROMASOLV (Riedel- de Haën, Germany) were used.

All solvents were tested prior to use by GC-ECD.

Sodium sulphate, anhydrous (Lachema Brno, CR): Heated for 5 hours at 500°C, cooled and stored in a dessicator.

Aluminium oxide: Basic, Brockmann Activity Grade I (J. T. Baker, USA) was activated at 400°C for 5 hours and deactivated with 3% (w/w) of water.

Solid phase extraction cartridges: Bakerbond SPE (J. T. Baker, USA):

reverse phase : – octadecyl (C18) bonded to silica gel, volume of cartridge 3 ml, weight of sorbent 0.5 g (cat. number 7020–03)
– octyl (C8) bonded to silica gel, volume of cartridge 3 ml, weight of sorbent 0.5 g (cat. number 7087–03)

alumina : volume of cartridge 6 ml, weight of sorbent 1 g (cat. number 7214–07)

Florisil : volume of cartridge 6 ml, weight of sorbent 1 g (cat. number 7213–07)

Apparatus

Glass columns for adsorption chromatography with fritted glass of porosity 1 μm (200 mm x 8 mm i.d.)

Glass bottles for micro extraction (0.5*l*) with a special adapter (see Figure 1)

Vacuum manifold Dorcus (Tessek Praha, CR)

Homogeniser Ika-Ultra-Thurrax T45 (Janke & Kunkel Ika-Werk Staufen, Germany)

Glassware: Detergent washed, rinsed with tap water, heated at 250°C, rinsed with acetone (4 hours) and stored covered with alumina foil.

Gas chromatographic systems

Gas chromatograph : Hewlett-Packard HP-5890 II
System 1) :

detector	: ^{63}Ni electron-capture
column	: HP – 5(30 m x 0.53 mm x 0.88 μm)
oven temperature	: 60°C held for 0.2 min, increase 20°C/min to 160°C, increase 7°C/min to 260°C, held for 10 min
carrier gas	: nitrogen (35 kPa)
make-up gas	: nitrogen, 30 ml/min
injector temperature	: 225°C, detector temperature : 300°C,
injection technique	: on-column, auto sampler HP 7673, 1 μl

System 2) :

Dual system	: 1 injection port with 2 (parallel) columns with different stationary
phases	and 2 ECD detectors.
detectors	: ^{63}Ni electron-capture
columns	: DB – 5(30 m x 0.25 mm x 0.1 μm), DB – 17(30 m x 0.25 mm x 0.11 μm)
oven temperature	: 60°C held for 2 min, increase 30°C/min to 160°C increase 5°C/min to 260°C, held for 5 min
carrier gas	: nitrogen at 200 kPa held for 2 min, decrease at 99 kPa/min to 80 kPa, next constant flow (0.5 ml/min)
make-up gas	: nitrogen, 60 ml/min.
injector temperature	: 225°C, detector temperature : 300°C
injection technique	: splitless, 1 – 3 μl

System 3):

detector	: HP – 5972 mass selective
column	: HP – 5(30 m x 0.25 mm x 0.25 μm)
oven temperature	: 60°C held for 2 min, increase 20°C/min to 160°C increase 7°C/min to 260°C, held for 5 min
carrier gas	: helium at 200 kPa held for 2 min, decrease at 99 kPa/min to 60 kPa, next constant flow (0.5 ml/min)
injector temperature	: 225°C, detector temperature : 280°C
injection technique	: splitless, 1 μl
ionization voltage	: 70 eV

Ions selected for monitoring (dwell time 100 ms) were as follows :

DMP, m/z 163, 194	DEP, m/z 149, 177
DnBP (ring – D_4), m/z 153, 227	BBP, m/z 149, 206
BEHP (ring – D_4), m/z 153, 171	DnOP, m/z 149, 167

Blank samples

With respect to the fact that almost all laboratory materials (e.g. solvents, glassware, chemicals, filtration papers) contain PAEs, it is necessary to run blank samples during sample analysis. We used all materials and procedures without a sample matrix for testing of secondary contamination.

Real samples

Water samples : tap water and samples of drinking water available in Czech supermarkets in glass bottles with soft PVC seal caps containing up to 35% of dialkyl phthalates:
water "OASA" (Pražské cukrárny a sodovkárny, CR) – two different samples mineral water "KORUNNÍ KYSELKA" (Karlovarská korunní kyselka s.r.o., CR) – two different samples
Lettuce sample : sample was bought on the market-square in Prague

METHODS

Determination of PAEs in drinking water

Micro Extraction (ME)

A 300 ml sample together with 1 ml of iso-octane was agitated for 2 hours in 0,5 ℓ bottle. Then a special adapter (see Figure 1) was attached and the bottle was filled via the side arm funnel with distilled water so that the iso-octane layer reached the narrow part of adapter. The iso-octane layer was directly injected into the gas chromatograph (system 1). Recovery of this method was tested on redistilled water spiked with PAEs at different levels of contamination (1–10 μg/l).

Liquid-Liquid Extraction (LLE)

A 500 ml sample was extracted three times in a 1ℓ separatory funnel with 40 ml of hexane. The organic layer was filtered through anhydrous sodium sulphate and the combined extracts were evaporated to dryness. The residue was dissolved in 1 ml of iso-octane and analysed by GC-ECD (system 1). Recovery of this method was tested on redistilled water spiked with PAEs at different levels of contamination.

Solid Phase Extraction (SPE)

a) *Development*

Two types of reverse phase cartridges (C8, C18) with various solvents or solvent mixtures for eluting of PAEs were tested using a Dorcus vacuum manifold (see Table 1).
The procedure commonly used included the following steps:

- cartridge conditioning : 2.5 ml of elution solvent, 2.5 ml of redistilled water
- sample application : 200 ml of redistilled water spiked with PAEs at different levels of contamination - flow rate max. 4 ml/min
- drying of sorbent : 15 min under a stream of N_2
- elution of PAEs : see Table 1
- evaporation of elution solvent to dryness
- addition of 0.5 ml of iso-octane
- injection to GC-ECD (system1)

Table 1 Characterisation of experiments carried out during the developement of PAEs analysis in water by means of SPE.

Experiment	*Sorbent*	*Elution solvent*	*Volume of elution solvent /ml/*		
No.			*F 1**	*F 2**	*F 3**
I	a) octyl b) octadecyl	acetone	2	2	–
II	a) octyl b) octadecyl	ethyl acetate	2	2	2
III	a) octyl b) octadecyl	hex : dee (3:1)**	2	2	2
IV	octyl	hexane	2	2	2
V	octyl	diethyl ether	2	2	2
VI	octyl	hex : dee (1:1)**	2	2	2
VII	octyl	acetonitrile	2	2	2

* F1, F2, F3 – fraction 1,2,3
** hex – hexane
dee – diethyl ether

b) *Determination of PAEs in real water samples*

The SPE method described as experiment No. II b) in Table 1 was used (that means sorbent C18 and elution solvent ethyl acetate).

Procedural steps:

– cartridge conditioning : 2.5 ml of ethyl acetate, 2.5 ml of redistilled water
– sample application : 200 ml at a max. flow rate of 4 ml/min
– drying of sorbent : 15 min under a stream of N_2
– elution of PAEs : 4 ml of ethyl acetate
– evaporation of solvent to dryness
– addition of 0.5 ml of iso-octane
– injection into GC-ECD (system 1)

Determination of PAEs in lettuce

Extraction of samples

Approx. 30 g of lettuce was homogenised for 5 min together with 60 g of anhydrous sodium sulphate and 150 ml of a solvent mixture of hexane and acetone (2:1, v/v). The extract was filtered through a Büchner funnel and after the evaporation of solvents, the residue was dissolved in an appropriate volume of hexane to obtain a concentration of 1g of lettuce/ml hexane.

Clean-up of extracts

a) *Adsorption column chromatography*

Glass columns were filled with 5 g of basic alumina (deactivated with 3% of water) in hexane. 1 ml of sample extract in hexane (corresponding to 1 g of lettuce) was pipetted onto the top of this column and PAEs were then eluted with a solvent mixture of hexane : diethyl ether (4:1, v/v). The first 10 ml of elution mixture were discarded, the second 100 ml fraction was collected, the solvent was evaporated to dryness and the residue was dissolved in 0.5 ml of iso-octane and analysed by GC-ECD (system 2,1).

Recovery of this method was tested on lettuce extract spiked with standard mixture of PAEs (DMP, DEP, BBP, DnOP) and deuterated PAEs (BEHP-ring D_4 and DnBP-ring D_4) analysed by GC-MS (system 3).

b) *Solid phase extraction*

The SPE cartridge (packed with 1 g of Florisil or alumina) was conditioned with 3 ml of hexane before use. Then, 0.5 ml of sample extract in hexane (corresponding to 0.5 g of lettuce) was transferred through the cartridge at a max. flow rate 4 ml/min. Phthalates were eluted with a solvent mixture hexane : diethyl ether (4:1, v/v) as follows :

Florisil : 1 ml discarded, 7 ml collected

alumina : 6 ml collected

The eluate was evaporated to dryness and the residue was dissolved in 0.5 ml of iso-octane and analysed by GC-ECD (system 2,1).

Recovery of this method was tested on a lettuce extract spiked with a standard mixture of PAEs (DMP, DEP, DnBP, BBP, BEHP, DnOP) and analysed by GC-ECD (system 2).

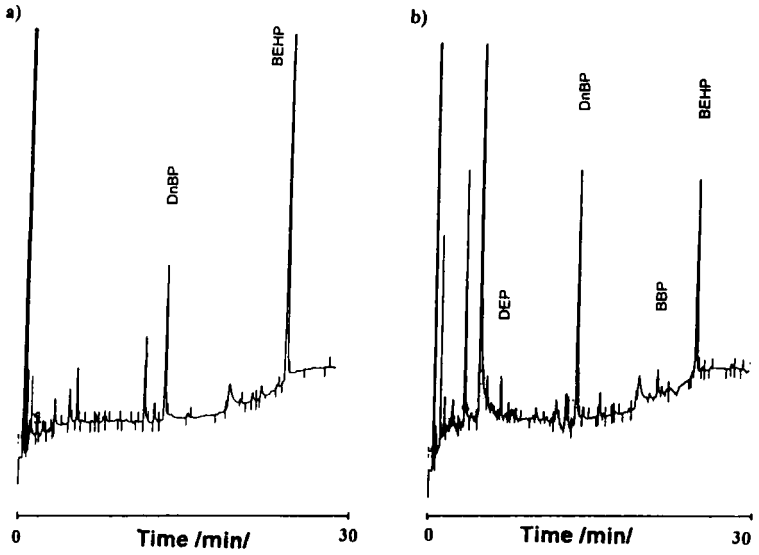


Figure 2 Chromatograms of extracts of mineral water Korunni kyselka processed by both micro extraction and solid phase extraction analysed by GC-ECD (system 1) a) micro extraction, injection represents 0.3 ml of matrix b) solid phase extraction, injection represents 0.4 ml of matrix.

RESULTS AND DISCUSSION

Gas chromatographic analysis

Three systems of gas chromatographic analysis were used in the present study. *System 1*, with a single wide-bore column and electron capture detector, was employed for the routine analyses of PAEs. The chromatogram obtained under these conditions is demonstrated in Figure 2. The *second system*, with two capillary columns with different stationary phases and two electron capture detectors, was used to correctly identify the analytes in cases where interferences from the matrices occurred in the chromatogram recorded by system 1. Chromatograms obtained in this system 2 are presented in Figures 3 and 4. The *third system* with mass selective detector served only for the investigation of methods recoveries. In order to avoid incorrect results caused by secondary contamination, labelled (deuterated) standards were utilised for this purpose. The chromatogram obtained in this system 3 is shown in Figure 5.

Determination of phthalates in water

We compared three different types of water sample preparations with the aim of finding the best one from the point of view of good recoveries for all six esters as well as from the point of simplicity and possibility of miniaturisation.

Micro extraction

Micro extraction is a very simple and non-laborious method often used in routine laboratories for the isolation of non-polar organic compounds such as PCBs. The disadvantage of this procedure is that only relatively clean, particle-free samples can be processed in this way (i.e. drinking water, ground water in some cases), because of the absence of a clean-up step and due to the occurrence of emulsions.

We attempted to utilise this method for the determination of phthalates exhibiting wide range of polarities : from non-polar (BEHP, DnOP) to moderately polar (DMP, DEP). We were able to determine, with the extraction solvent iso-octane, BBP, BEHP and DnOP, those even at low levels of contamination. The determination of DnBP at higher levels (tens of µg/l) was also possible. The recovery of more polar phthalates (DMP, DEP), in accordance with our expectations, was very low. The recovery values of the method (calculated in %) and detection limits (in µg/l) are summarised in Table 2.

Table 2 Recoveries ± RSD (in %) of phthalates from water using micro extraction (extraction solvent iso-octane) and limits of detection.

Spiking level (µg/l of water)	*DMP*	*DEP*	*DnBP*	*BBP*	*BEHP*	*DnOP*
10	4	42	88 ± 14	105 ± 3	94 ± 6	89 ± 5
1	0	31	25 ± 6	112 ± 9	66 ± 10	94 ± 2
Det. limit (µg/l)	n.d.	n.d.	n.d.	0.01	0.01	0.05

n.d. ... not determined

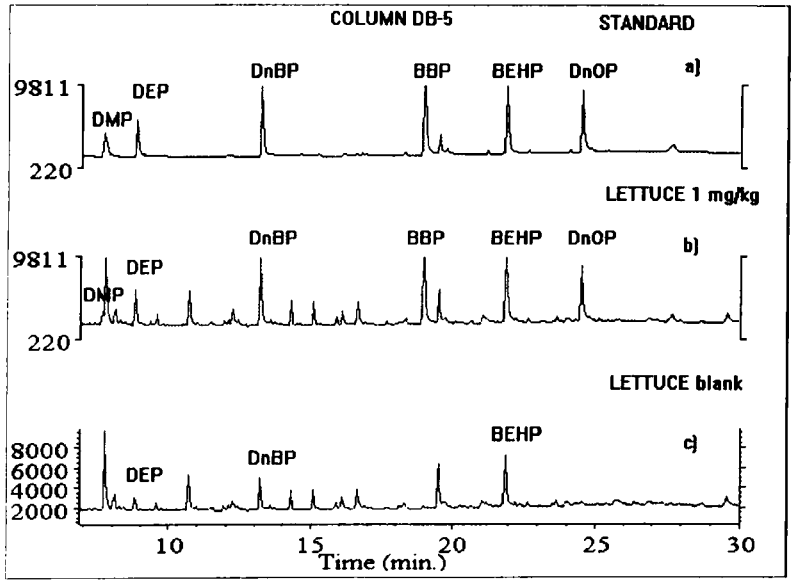


Figure 3 Chromatograms of the standard mixture of PAEs and lettuce extracts cleaned by SPE (sorbent Florisil). Dual GC-ECD system (system 2 – column DB–5).
a) standard mixture of PAEs (c = 1 μg/ml)
b) lettuce extract spiked by PAEs at the level 1 mg/kg (injection represents 1 mg of original matrix)
c) blank lettuce extract (injection represents 1 mg of original matrix)

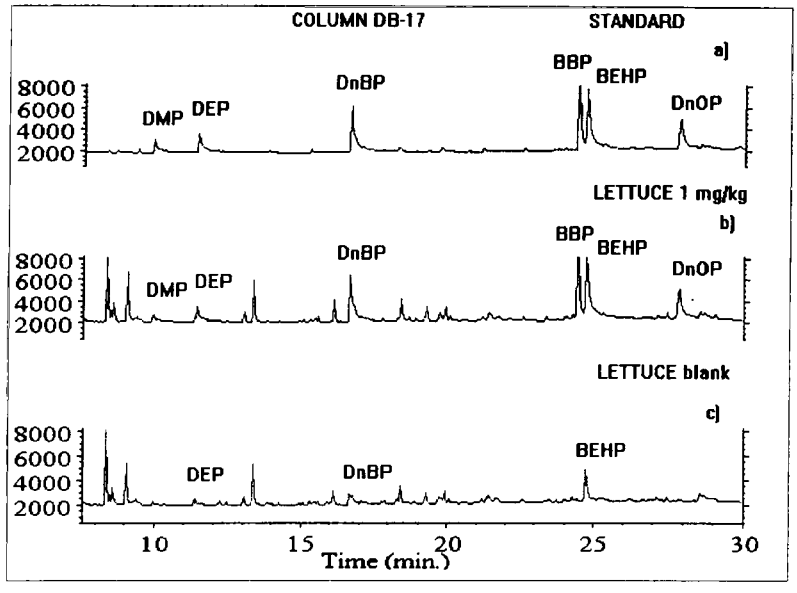


Figure 4 Chromatograms of the standard mixture of PAEs and lettuce extracts cleaned by SPE (sorbent Florisil). Dual GC-ECD system (system 2 – column DB–17).
a) standard mixture of PAEs (c = 1 μg/ml)
b) lettuce extract spiked by PAEs at the level 1 mg/kg (injection represents 1 mg of original matrix)
c) blank lettuce extract (injection represents 1 mg of original matrix)

Table 3 Recoveries ± RSD (in %) of phthalates from water using liquid-liquid extraction (extraction solvent : hexane).

Spiking level (µg/l of water)	*DMP*	*DEP*	*DnBP*	*BBP*	*BEHP*	*DnOP*
20	80 ± 18	100 ± 29	80 ± 15	100 ± 15	70 ± 9	83 ± 10
5	70 ± 20	94 ± 16	97 ± 11	92 ± 9	80 ± 16	86 ± 20

Liquid-liquid extraction

The liquid-liquid extraction is a very common simple method. However, its drawback is high time consumption and, above all, handling of large volumes of solvents and several pieces of glassware and other materials may pose (especially in PAEs analysis) a risk of secondary contamination and thus bad repeatability. On the other hand, good recovery values were obtained for all six phthalate esters using hexane as extraction solvent (see Table 3). The higher values of relative standard deviation are related to the above mentioned occurrence of cross contamination. The recovery values of the method (calculated in %) are summarised in Table 3.

Solid phase extraction (SPE)

The small volumes of solvents and sorbents used in this method make it especially attractive for trace analysis.

We tested the SPE cartridges packed with two types of reverse phases (octyl and octadecyl) with different elution solvents. The values of recoveries obtained in all examined systems are summarised in Table 4 and 5. As can be seen from these results, almost all tested SPE systems, except No. IV (i.e. octylsilica eluted with hexane) are applicable for the determination of DMP, DEP, DnBP and BBP. The problem is the low recovery of BEHP and DnOP. The first used elution solvent, acetone, was from this point of view unsuccessful. Therefore, other elution solvents such as ethyl acetate, acetonitrile, hexane, diethyl ether and their mixtures were tested. Considering the results obtained (see Table 1 and 4) we concluded, that for the analysis of the six phthalates in one run on octyl (C8) SPE cartridges, the most efficient elution mixture is hexane : diethyl ether (3 : 1) (experiment No. III a). In this system, values of recoveries for DMP, DEP, DnBP and BBP range between 71–100% and are about 40% for BEHP and DnOP. The results obtained with ethyl acetate are almost comparable (experiment No. II a). On the contrary, for eluting of all six PAEs from octadecyl (C18) SPE columns (see Table 1 and 5) ethyl acetate (experiment No. II b) seems to be better.

The method No. II b (C18 – ethyl acetate) works with lower elution volume (2 – 4 ml) than the method No. III a (C8 – hexane : diethyl ether (3:1), 6 ml) and recovery of PAEs by both these methods is comparable - this is the reason why the real samples were analysed on C18 cartridges with ethyl acetate as elution solvent.

From the comparison of PAEs elution volumes on both types of sorbents, we can draw the conclusion that more polar phthalates (DMP, DEP, DnBP, BBP) are adsorbed more strongly on the octylsilica than on the octadecylsilica and thus they require elution with higher volumes of solvents.

In any of the evaluated systems, recoveries of BEHP and DnOP were not higher than 40%. This result is unsatisfactory and further investigations should be carried out. We

Table 4 Recoveries (calculated in %) of PAEs from water sample analysed by means of SPE, sorbent : octyl (C8) silica; spiking level 20 µg/l (See also Table 1).

	Exp. I a) acetone			*Exp. II a) ethyl acetate*				*Exp. III a) hex:dee (3:1)*				*Exp. IV hexane (hex)*				*Exp. V diethyl ether (dee)*				*Exp. VI hex:dee (1:1)*				*Exp. VII acetonitrile*			
PAE	F1	F2	ΣF	F1	F2	F3	ΣF	F1	F2	F3	ΣF	F1	F2	F3	ΣF	F1	F2	F3	ΣF	F1	F2	F3	ΣF	F1	F2	F3	ΣF
DMP	70	20	90	64	27	10	100	38	23	10	71 ± 8	0	0	0	0	51	18	9	78	39	21	12	72	52	25	24	91
DEP	110	10	120	61	20	8	89	69	20	11	100 ± 10	0	0	4	4	78	14	13	105	67	20	11	98	59	18	8	85
DnBP	85	15	100	61	12	0	73	75	19	1	95 ± 15	47	27	10	84	57	2	0	59	47	5	0	52	39	3	0	42
BBP	80	10	90	67	10	5	82	77	15	1	93 ± 9	23	21	12	56	59	5	1	65	63	8	2	73	54	7	6	67
BEHP	8	2	10	35	1	0	36	38	4	1	43 ± 5	7	0	0	7	28	4	0	32	13	2	0	15	25	4	0	29
DnOP	6	0	6	33	0	0	33	40	6	0	46 ± 9	6	0	0	6	35	0	0	35	21	0	0	21	23	5	0	28

Table 5 Recoveries (calculated in %) of PAEs from water sample analysed by means of SPE, sorbent : octadecyl (C8) silica; spiking level a) 20 µg/l, b) 5 µg/l (see also Table 1).

a)	*Exp. I b) acetone*			*Exp. II b) ethyl acetate*				*Exp. III b) hex.:dee (3:1)*			
PAE	F1	F2	ΣF	F1	F2	F3	ΣF	F1	F2	F3	ΣF
DMP	72	0	72	95	0	0	95 ± 14	70	5	0	76
DEP	108	0	108	80	0	0	80 ± 8	102	2	0	104
DnBP	83	0	83	72	4	0	76 ± 1	90	7	0	97
BBP	86	3	89	72	13	0	85 ± 2	89	4	0	93
BEHP	10	5	15	30	0	0	30 ± 2	21	2	0	23
DnOP	0	0	0	30	0	0	30 ± 1	21	0	0	21

b)	*Exp. II b) ethyl acetate*	*Det. limit*
PAE	F1+F2	µg/l
DMP	74 ± 14	0.10
DEP	108 ± 8	0.10
DnBP	70 ± 1	0.05
BBP	99 ± 2	0.05
BEHP	32 ± 2	0.10
DnOP	23 ± 1	0.10

Table 6 Levels of PAEs (in μg/l) in real water samples analysed by mirco extraction (ME) and solid phase extraction (SPE, exp. No. II b) - results are corrected for recoveries and blank sample.

	Tap Water		*Dist. water*		*Oasa 1*		*Oasa 2*		*Korunní kys. 1*		*Korunní kys. 2*	
PAE	*ME*	*SPE*	*ME*	*SPE*	*ME*	*SPE*	*ME*	*SPE*	*ME*	*SPE*	*ME*	*SPE*
DMP	*	*	*	*	*	*	*	*	*	*	*	*
DEP	0.72	0.74	1.23	0.62	0.23	0.24	*	0.29	*	0.39	*	0.17
DnBP	4.23	2.00	11.2	8.00	2.93	2.02	2.73	1.59	3.87	2.40	1.83	1.65
BBP	*	0.10	*	0.04	*	0.06	*	0.06	*	0.04	*	0.06
BEHP	0.66	2.00	0.94	*	3.51	3.61	2.90	3.29	5.29	7.00	3.57	1.77
DnOP	*	*	*	*	*	*	*	*	*	*	*	

* ... values below detection limit.

assumed that with respect to a lipophilic character of esters with long alkyl chains, these are strongly adsorbed on the both non polar sorbents and therefore used solvents are not sufficiently strong for their elution. This assumption was confirmed by the analysis (micro extraction) of a water sample (spiked with PAEs) that passed through the octyl SPE cartridge. No phthalates were detected, which means that all of them were sorbed on the cartridge.

Determination of PAEs in real water samples

From the comparison of Table 2 and 5 it can be seen that the micro and the solid phase extractions are in our arrangement of experiments complementary (the microextraction is convenient for the determination of less-polar esters and the SPE is better for the analysis of those more-polar). Accordingly, two methods were used for the determination of PAEs in real water samples. Obtained results calculated in μg/l are summarised in Table 6.

The results obtained by micro extraction and solid phase extraction are well comparable. The most often present phthalates in the analysed samples were DEP, DnBP and BEHP. The distilled water was more contaminated than the tap water. This fact is probably due to the storage of the distilled water in plastic bottles. The levels of PAEs in water Oasa and mineral water Korunní kyselka are comparable with the levels in tap water and generally were low. With respect to the concentration limit of BEHP in drinking water proposed by EPA (4 μg/l), only one sample (Korunní kyselka 1) exceeded the tolerance level. Figure 2 shows representative chromatograms of extracts of mineral water Korunní kyselka processed both by micro and solid phase extraction and analysed by GC-ECD (system 1).

Determination of PAEs in lettuce

Since in the Czech Proposal for the Directive on Food Contaminants Maximum Residue Limits for the amount (the sum) of DnBP and BEHP in vegetable and fruits (about 1 mg/kg) are declared, there exists a need for the control of their residues in these matrices. As it was stated earlier, methods for this purpose are not available and, therefore, in our next experiments we tried to meet this demand and to develop a functional analytical procedure.

Table 7 PAEs recovery values (calculated in %) from lettuce using alumina columns or SPE on Florisil or alumina cartridges as a clean-up step.

	Alumina column	*SPE alumina*	*SPE Florisil*	
PAE	*1 mg/kg*	*1mg/kg*	*0.5 mg/kg*	*1 mg/kg*
DMP	76 ± 5	50 ± 10	77 ± 3	60 ± 1
DEP	76 ± 4	120 ± 10	70 ± 13	110 ± 13
DnBP	74 ± 5	100 ± 15	70 ± 13	81 ± 2
BBP	71 ± 2	110 ± 6	83 ± 2	105 ± 1
BEHP	81 ± 4	100 ± 9	60 ± 15	80 ± 10
DnOP	100 ± 9	109 ± 8	58 ± 6	103 ± 15

For the extraction of PAEs from vegetable matrices (lettuce was tested) the common procedure (involving extraction with a mixture of acetone and hexane), convenient for moderately polar substances, was used. As a clean-up step both classic adsorption column chromatography and solid phase extraction on Florisil or alumina cartridges were tested. The recovery values (in %) of these methods at different contamination levels are summarised in Table 7. They do not differ too much and range from 50 to 120% on the spiking level 1 mg/kg.

The clean-up effect of adsorption column chromatography on alumina is quite satisfactory. In fraction 1 (= 10 ml of elution mixture) yellow pigments are eluted and in the second (= 100 ml of elution mixture) are PAEs. The green pigments remain on the column. In the case of adsorption column chromatography it is possible to increase the sample loading by up to 3 g per column.

In the case of SPE on alumina cartridges the yellow pigments are unfortunately partially eluted in the PAEs fraction. The clean-up effect of SPE Florisil cartridges seems to be similar to adsorption column chromatography on alumina, the difference is that the yellows pigments remain in the cartridge.

It is possible to use successfully both alumina column and Florisil SPE cartridges for the clean-up of lettuce extracts. The detection limits for these methods, for all six PAEs, range from 0.05 to 0.01 mg/kg. Further investigations are planned to extend the application of these procedures to other kinds of materials.

Alumina column chromatography and SPE on Florisil cartridges were used for the determination of PAEs levels in the real sample of lettuce. All results obtained (in

Table 8 Levels of PAEs in a lettuce sample cleaned by adsorption column chromatography on alumina and SPE on Florisil cartridges. The results are calculated in mg/kg and are corrected for blank sample.

PAE	*Alumina column*	*SPE Florisil*
DMP	*	*
DEP	0.02	0.19
DnBP	0.15	0.20
BBP	*	*
BEHP	0.12	0.08
DnOP	*	*

* ... values below detection limit

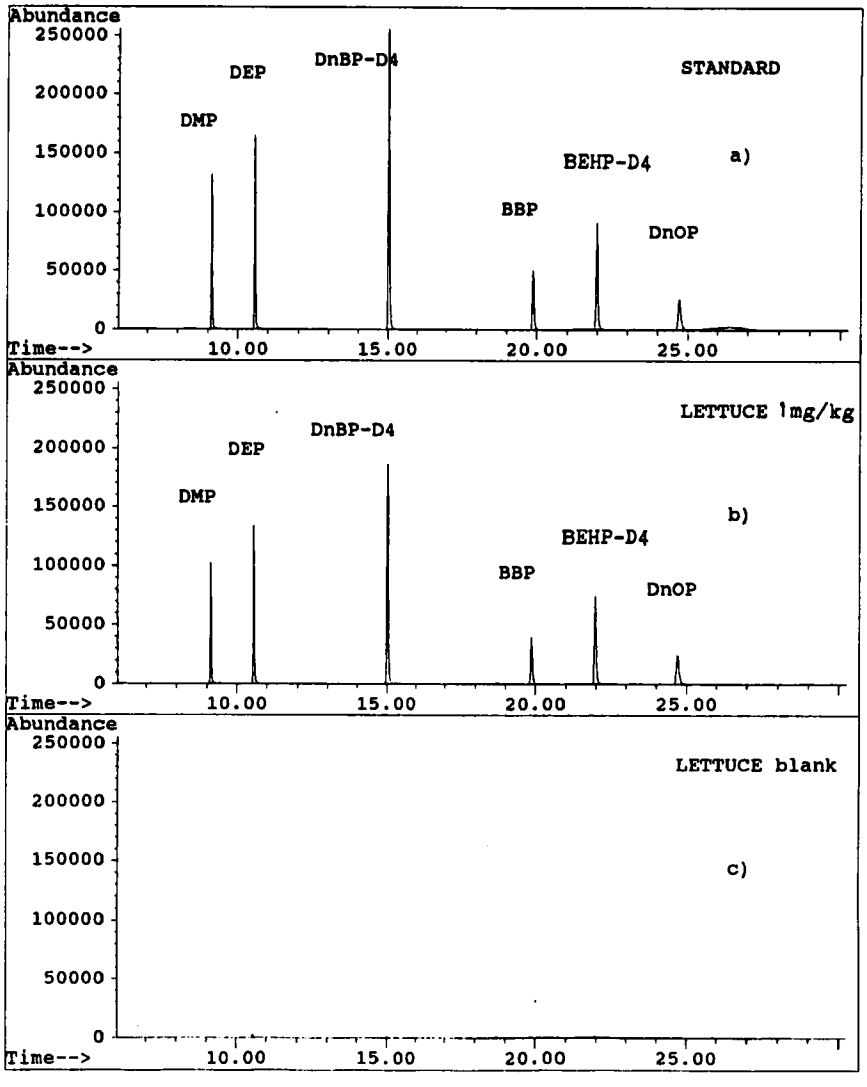


Figure 5 Chromatograms of the standard mixture of PAEs and lettuce extracts cleaned by adsorption column chromatography (sorbent = alumina), analysed by GC–MS (system 3).
a) standard mixture of PAEs (c = 2 μg/ml)
b) lettuce extract spiked by PAEs at the level 1 mg/kg (injection represents 1 mg of original matrix)
c) blank lettuce extract (injection represents 1 mg of original matrix)

mg/kg) are summarised in Table 8. These show a good agreement for both methods and do not indicate a high contamination of the examined material. Figures 3 and 4 show chromatograms of the lettuce extract cleaned by solid phase extraction (sorbent = Florisil) and analysed by dual GC-ECD (system 2). Figure 5 presents the chromatogram of the lettuce extract cleaned by adsorption column chromatography (sorbent = alumina) and analysed by GC-MS (system 3).

CONCLUSIONS

Two miniaturised methods (micro extraction and solid phase extraction) for the water sample processing prior to gas chromatographic analysis of PAEs were compared with classic liquid-liquid extraction. The micro extraction with iso-octane and solid phase extraction on octadecyl cartridges, with ethyl acetate as an elution solvent, seem to be complementary. While with micro extraction relatively high recoveries for more hydrophobic PAEs can be achieved, SPE provides better results for more polar PAEs with shorter alkyl chains. In any of the evaluated SPE systems, recoveries of BEHP and DnOP did not exceed 40%, further investigations have to be done.

The analysis of PAEs in real water samples did not indicate serious contamination problem.

With respect to the demand for analyses of plant matrices, the method for the determination of PAEs in such type of samples utilising either alumina adsorption column chromatography or Florisil solid phase extraction as a clean-up step prior to GC-ECD was developed. Both of these procedures give good recovery values. Further investigations are aimed to document the applicability of this procedure to other kinds of plant materials.

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