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Evaluation of a method for phthalate extraction from milk related to the milk dilution ratio

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Abstract: Liquid–liquid extraction techniques coupled with gas chromatography–mass spectrometry (GC–MS) were compared for the extraction and the determination of four phthalates: dimethyl phthalate (DMP), di-*n*-butyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-(2-ethylhexyl) phthalate (DEHP) in six different kinds of milk-based samples. Extraction factors: sample preparation, organic solvent type and volume, salt effect, agitation and the extraction time were optimized. The ions of the base peak (*m*/*z* 149 for DBP, BBP and DEHP and *m*/*z* 163 for DMP) for the investigated phthalates were selected for the screening studies. The acquisition was performed in the selected ion-monitoring mode. The response of the mass selective detector (MSD) for GC–MS phthalate calibration standards was linear between 0.25 and 2.50 μg mL^{-1} with calculated limit of detection (*LOD*) values between 0.01 to 0.04 μg mL^{-1} and limit of quantitation (*LOQ*) values of 0.05 to 0.12 μg mL^{-1}, while repeatability was between 1.7 to 4.9 % relative standard deviation (*RSD*). The study demonstrated an increase in the recovery of less polar phthalates in matrix milk standards on matrix dilution. Recovery for hydrophilic phthalates, such as DMP, was not changed by matrix dilution and it was continuously low for the investigated method. Two spiking levels, tested for the influence of matrix dilution on phthalate recovery, showed the same trend.

Keywords: extraction efficiency; phthalate esters; gas chromatography-mass spectrometry; milk samples.

INTRODUCTION

Phthalates present one of the ubiquitous chemicals in the environment. Since their usage is mainly as plasticizers for polymers, such as poly(vinyl chloride) (PVC), over one million tons of phthalates are produced in western Europe each year.[1] The most important congeners are: di-2-ethylhexyl phthalate (DEHP),

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which accounts for about 50 % of the world production of phthalates,[1] dimethyl phthalate (DMP), di-*n*-butyl phthalate (DBP) and benzyl butyl phthalate (BBP),which is prohibited by the EU in toys and childcare articles if they could be placed in the mouth by children.[2] Phthalates have long been considered to be potential endocrine disrupters, and several of them have shown reproductive effects in animals.[3–5]

The structures of the four studied phthalates as the most commonly used phthalate esters based on the 1,2-benzenedicarboxylic acid are given in Fig. S-1 of the Supplementary material to this paper.

The permanence of phthalates in polymer materials is low because phthalates are not chemically bound to the polymer. Their migration from food-packaging materials was reported as a route for food contamination with phthalates. Since food is one of the major sources of human exposure to phthalates, in order to assess human exposure to these substances, monitoring of phthalates levels in various foods should be performed. Fatty and oily foods are primarily contaminated with phthalates due to their lipophilic character.[6] Although human intake of phthalates may originate from many food sources and routes, there is a special interest in monitoring the contamination of milk and milk products because they constitute a primary food source, especially for children. Tolerable daily intakes (*TDI*) were specified by the European Food Safety Authority (EFSA) for several phthalates, and they are 0.01, 0.05 and 0.5 and mg kg^{-1} body weight day^{-1} for DBP, DEHP and BBP, respectively.[7–9] The physicochemical properties of phthalates (Table S-I of the Supplementary material) and their amount and frequency of usage in food-packaging materials could determine their migration and leaching levels and thus the possibility of food contamination and human exposure.

The majority of publications deal with phthalate determination in simple matrices, such as water[10–12] or biological fluids,[13] while publications about phthalates in food samples with fatty matrices[14–16] are less frequent. Due to usually low level of phthalates and generally high complexity of the matrices of food sample, extraction and clean up of the sample are usually considered as necessary and critical steps in phthalate determination.

However, as indicated in this paper, the crucial point in phthalate extraction from complex milk matrices is considered the choice of solvent for the extraction and sample dilution step. The influence and importance of these two aspects on the recovery of phthalates, as well as analytical methodology (agitation method, extraction time, *etc*.) are considered herein.

When sample extraction is performed with solvent mixtures of low polarity, fats are co-extracted together with the phthalates and thus, the fats must be removed and the membranes of milk fat globules should be disrupted before the chromatographic analysis. Otherwise, phthalates may not be effectively extracted, yielding low recoveries. The optimization of extraction efficiency of phthalates

from milk could alternatively be achieved by precipitation of milk proteins by addition of NaCl for salting-out followed by the addition of acetonitrile[17] or through primary addition of acetone or an alcohol.[18] Gel permeation chromatography (GPC) is often used for this purpose.[6,19] When the sample is extracted with polar solvents, such as methanol or acetonitrile, the extract contains other interfering organic impurities in addition to fat. These may require solid-phase extraction (SPE) on different sorbents.[20–22] In each of these sample preparation steps, the possibility of contamination of the sample is high because even in pure solvents, solid phase extractants, laboratory water, laboratory air and laboratory glassware, phthalates could be detected.[23] This can be negligible source of contamination that could be reduced by establishing whether the reduction of the accuracy of phthalate determination is higher due to phthalate contamination of the sample by using clean-up procedures or due to the complexity of co-eluting substances, which could influence the sensitivity of phthalate determination by MS analysis.

Since the extraction procedure could be the source of a matrix effect and is an essential step in the evaluation process of phthalate determination, different liquid solvents and solvent mixtures were investigated in this study regarding the extraction efficiency of phthalates, the possibility of phase separation, visibility of phase separation, clearness of the extracts, formation of emulsions, availability of reagents and duration of the extraction. In addition to the selection of the solvent type, different methods/procedures for extraction were also investigated in order to obtain the most optimal method for the extraction phthalates from milk and dairy products.

Bearing in mind that in milk with high fat content, phthalates cannot be easily extracted due to phospholipid–protein membranes that encapsulate the fat droplets containing lipophilic phthalate, dilution of milk samples prior to the analysis could enable a better extraction efficiency of phthalates and relatively clean extracts to be obtained. Moreover, sample dilution is an easy and effective method to reduce interfering compounds and to diminish the matrix effect. The obtained extracts, even without a clean-up step, could be used for GC–MS analysis, as shown herein.

EXPERIMENTAL

Reagents and materials

All solvents (HPLC grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA), except *n*-hexane that was purchased from Fisher Scientific (Pittsburgh, PA, USA). Dimethyl phthalate (DMP), di-*n*-butyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-(2--ethylhexyl) phthalate (DEHP) were purchased, in the highest available purity, from Sigma––Aldrich (St. Louis, MO, USA). Dibutyl adipate (DBA) was purchased from Fluka (Buchs, Switzerland) and used as an internal standard. Water from a Milli-Q system (Millipore, Bedford, MA, USA) was used.

All reagents and water used for the analyses were checked for contamination with phthalates. To avoid phthalate contamination, all the employed laboratory dishes were made of glass, previously washed with water and soap, tap and ultrapure water, rinsed with acetone and *n*-hexane and dried at 200 °C in a clean oven for 4 h.[23]

All stock, intermediate and working solutions were prepared in *n*-hexane. Individual stock solutions of each phthalate were prepared at a concentration of 1000 μg mL^{-1} and stored at 4 °C. The stock solutions were stable for up to one month. A mixed stock solution of phthalates was prepared at a concentration of 100 μg mL^{-1} for each phthalate. With stepwise dilutions, individual working solutions of 1 μg mL^{-1} were obtained for each phthalate to identify their retention times. The working solutions were stable for 10 days. Furthermore, the mixed working solutions of all phthalates at 0.25, 0.50, 1.00, 1.50 and 2.50 μg mL^{-1} were also prepared as calibration standards with DBA as the internal standard at a concentration of 1 μg mL^{-1}. Between some other commonly used internal standards, such as benzyl benzoate[20] or isotope-labeled standards, DBA was adopted as an internal standard being approved for this purpose according to our findings and literature data.[24]

The calibration curves were linear in the range from 0.25 to 2.5 μg mL^{-1} with correlation coefficients higher than 0.990. The linear dynamic range was broader and covered the range from 2.50 to 50 μg mL^{-1}. Samples of nine systems of commercial milk-based samples diluted with water, from 0 to 50vol. %, were all spiked phthalates at two concentration levels, 3.0 and 6.0 μg mL^{-1}.

Dairy samples

Optimization of the phthalate liquid–liquid extraction procedure was realized using six samples of dairy products: raw bovine milk, commercial (pasteurized) milk, thawed milk, whey, human milk and yogurt. Samples of raw bovine milk were collected in glass bottles from a dairy farm in south Serbia avoiding any contact with plastic materials. This milk had not been pasteurized before analysis and was used as collected. Samples of commercial milk from a Serbian dairy were purchased at Serbian market and used as received. The commercial milk was bottled in a plastic (polyethylene terephthalate, PET) bottle.[6] The shelf life of milk is 10 days after packaging and the milk was used within this period. Thawed commercial milk was obtained after thawing overnight the milk that had been frozen for 24 h in the original packing. Whey produced in a cheese manufacturing process was purchased from the same dairy farm as the raw bovine milk. Samples of human milk were collected in three successive days by a postpartum 35-year old woman (5^{th} week postpartum). Milking was carried out manually, with previously cleaned breasts and hands, directly into a glass container that was intended for this purpose. The yogurt used in this study was made from cow's milk, commercially available from a Serbian market and stored in a plastic (PET) bottle.

All nine systems of commercial milk-based samples diluted with water were used for the selection of optimum solvent type as extractant considering the possibility of phase separation, visibility of phase separation, clearness of the extracts, formation of emulsion, availability of the reagents and duration of extraction. In addition to selection of the solvent type, different methods/procedures for extraction were also investigated in order to obtain the most optimal method for the extraction of phthalates from milk and dairy products.

For the determination of extraction efficiency of phthalates in milk, only the commercial milk was used. The milk was diluted with water from 0 to 50 vol. %.

Before the analysis, samples of raw bovine milk, thawed milk and human milk were stored in a glass bottle in a refrigerator at 4 °C.

GC–MS analysis

Gas chromatographic analysis was performed on a gas chromatograph 6890 (Hewlett--Packard) equipped with a mass selective detector (MSD) 5973 (Agilent) and a DB-5 MS capillary column (30 m×250 mm×0.25 mm). The mass spectra were recorded under an electron impact ionization voltage of 70 eV. The gas chromatograph was operated in the split less injection mode. The oven temperatue was programmed from 60 °C (1 min) to 220 °C (1 min) at a rate of 20 °C min^{-1} and then to 280 °C (4 min) at a rate of 5 °C min^{-1}. The MSD was used in the single ion-monitoring mode (SIM) at *m*/*z* 149 and 163. The identification and quantification of target compounds was based on the relative retention time, the presence of target ions and their relative abundance. The target ion was *m/z* 149 for DBP, BBP and DEHP and *m/z* 163 for DMP. Linearity was investigated in the range 0.25–2.5 μg mL^{-1}. The linear dynamic range for the investigated phthalates by GC–MS was 0.25–50 μg mL^{-1}. The limit of detection (*LOD*) and limit of quantitation (*LOQ*) for each phthalate were calculated from six replicated measurements of a low concentration spiked standard solution according to the Analytical Detection Limit Guidance from the Wisconsin Department of Natural Resources.[25]

The laboratory contamination was monitored with blank samples obtained from Milli-Q water treated in the same manner as the milk samples.

Extraction procedures

Fourteen extraction procedures were examined. Each entailed different conditions (type of extractant, extraction time, agitation and settling). Two solvent mixtures were studied for extraction, acetone/*n*-hexane at a 1:1 volume ratio and methanol/*n*-hexane at a 1:3 volume ratio. The salting out effect was examined using acetonitrile as the extraction agent with the addition of NaCl to saturation. The liquid–liquid extractions for these three systems were performed by adding a volume of the extraction agents to a volume of sample in a 2:1 ratio, followed by vigorously hand shaking for 1 h and left standing for 24 h. In extraction methods using ethyl acetate, *n*-hexane, acetonitrile, acetone, dichloromethane, dichloroethane, trichloroethylene and trichloroethane, the volume ratio of the extraction agent to a milk sample was 2:1, agitation was performed in an ultrasonic bath for 1 h and hand-shaking for 1 h. The extractions with extractants soluble in the feed solution, *i.e.*, acetone and alcohols (ethanol, methanol and 2-propanol), the volume ratio of extractant to sample was also 1:2, agitation in an ultrasonic bath for 1 h and standing overnight at room temperature were applied.

RESULTS AND DISCUSSION

GC–MS acquisition

A chromatogram of the investigated phthalates is given in Fig. S-2 of the Supplementary material. The four phthalates were separated using the selected chromatographic conditions. The chromatogram shows that the separation of the phthalates (using the optimized conditions) occurred within a running time of 20 min and that GC–MS method is well suited for the simultaneous determination of the 4 phthalates. For the considered range of phthalate concentrations, 0.25–2.50 μg ml^{-1}, the response of the mass-selective detector was linear. The correlation coefficients (R^2) ranged from 0.990 to 0.999. The limits of detection (*LOD*) values, calculated according to the Winefordner and Long criterion,[26] were 0.01––0.04 μg ml^{-1}. The precision of the GC–MS method, expressed as the relative standard deviation (*RSD*, $n = 3$), was found to be in the range 1.7–4.9 %.

The retention times, selected masses and the scan start times for each phthalate studied by GC–MS are listed in Table I.

TABLE I. Target ions, retention times and scan start times for the investigated phthalates determined by GC-MS; target ions observed in SIM are shown in bold

Phthalate	*m*/*z*	Retention time, min	Scan start time, min
DMP	**163**, 194	8.03	7.80
DBP	**149**, 150, 223, 205	11.57	11.20
BBP	**149**, 91, 206, 238	16.02	15.50
DEHP	**149**, 167, 279, 150	18.39	17.90

Optimization of extraction procedure

The choice of the optimum extraction solvent for separation was determined from a consideration of several criteria: high boiling point and a low vapor pressure in order to reduce the risk of evaporation, high selectivity that enables fewer stages to be used, insolubility of solvent for prevention of solvent losses, good chromatographic behavior, and high partitioning coefficient of the analyte. Based on these considerations, several extraction solvents and mixture of solvents were investigated. As milk forms stable emulsions with the majority of solvents, all the investigated extractants were characterized by the appearance of extract and raffinate, the possibility to define the phase boundary, the possibility to perform phase separation, clearness of the extracts and formation of emulsions. Among the investigated solvents and solvent systems, *n*-hexane presented the best extractant because it gave a homogenous and clear extract with precipitated raffinate phase, thus a well-defined interfacial boundary and the possibility to separate the phases (Table II).

Agitation of samples with extractants enhanced the extraction efficiency and reduced the extraction time for reaching the equilibrium. In this study, stirring, shaking and ultrasonic treatment were investigated for the extraction of the phthalates from nine milk-based samples. Too vigorous agitation was avoided since it produced stable emulsions without visible phase boundary and the possibility of phase separation. In order to achieve effective phase separation, shaking of the samples and extractants was chosen as the best agitation method.

The extraction time to obtain higher peak areas of phthalates relative to internal standard peak area was investigated in the range of 10 to 30 min. An extraction time of 15 min was selected for extraction since the systems reached the steady state during this period.

The addition of an inorganic salt into a mixture of milk and a water-miscible organic solvent, such as acetonitrile, caused separation of the solvent from the mixture and the formation of a two-phase system. The results revealed that salt addition, although providing for a well-defined interfacial boundary, was not a satisfac-

TABLE II. Extraction systems studied for phthalate extraction from milk-based samples

Extractant	Appearance of:		Interfacial boundary	Phase separation
	Extract	Raffinate		
Acetone:*n*-hexane 1000 mL diluted milk sample was mixed with 10 mL acetone and 10 mL *n*-hexane, shaken for 30 min and repeated for extract enrichment. After settling overnight, the *n*-hexane/acetone phase was taken. The enriched extract was evaporated to dryness and re-dissolved in *n*-hexane.	Heterogeneous, creaming	Yellow, opaque	Not defined	–
Acetonitrile:NaCl 1000 mL diluted milk sample was mixed with 20 mL acetonitrile, 20 g sodium chloride, shaken for 30 min and repeated for extract enrichment. After settling overnight, the *n*-hexane/acetone phase was taken. The enriched extract was evaporated to dryness and redissolved in *n*-hexane.	Homogenous, clear	Precipitate	Well defined	–
Methanol:*n*-hexane 1000 mL diluted milk sample was mixed with 5 mL methanol and 15 mL *n*-hexane, shaken for 30 min and repeated for extract enrichment. After settling overnight, the *n*-hexane/acetone phase was taken. The enriched extract was evaporated to dryness and redissolved in *n*-hexane.	Heterogeneous, opaque	Voluminous precipitate	Not defined	–
Acetonitrile 1000 mL diluted milk sample was mixed with 20 mL acetonitrile. Agitation: ultrasonic bath for 1 h and hand shaking for 1 h. Extract phase submitted to new sample volume for enrichment with a final extract to solvent volume ratio of 1:50. Settling overnight. The combined extract was evaporated to dryness and redissolved in *n*-hexane.	Heterogeneous, yellow	Voluminous precipitate	Not defined	–
Ethyl acetate The same procedure as for acetonitrile	Homogenous, clear	Coalescence with creaming	Well defined	+
Cyclohexane The same procedure as for acetonitrile	Homogenous, clear	Foaming, precipitate	Well defined	+
n-Hexane The same procedure as for acetonitrile	Homogenous, clear	Precipitate	Well defined	+
Dichloromethane The same procedure as for acetonitrile	Heterogeneous, opaque	Precipitate	Well defined	+

TABLE II. Continued

Extractant	Appearance of		Interfacial boundary	Phase separation
	Extract	Raffinate		
Trichloroethane	Heterogeneous, opaque	Precipitate	Well defined	+
The same procedure as for acetonitrile				
Dichloroethane	Heterogeneous, opaque	Precipitate	Well defined	+
The same procedure as for acetonitrile				
Extractants soluble in feed solution	Appearance of extract and raffinate		Interfacial boundary	Phase separation
Methanol	Heterogeneous, voluminous precipitate, flocculation		Not defined	–
Ethanol				–
2-Propanol				–
Acetone				–

tory method due to low phase separation and adhesion of milk globules on the wall of the separation funnel.

Based on literature data, the effect of the sample to solvent ratio on the total extracted phthalates from milk products was found to be the best at the 1:20 level. Various amounts of a mixture of solvents were used in order to determine the optimum quantity of the extracting solvent, based on the appearance of extract, phase boundary and possibility of phase separation.[27]

This work showed that due to dilution of milk samples, the satisfactory ratio of solvent to sample volume was found to be at the 1:50 level, when the extract phase is several times submitted to a new sample volume, leading to better concentration of analyte and reduction in solvent consumption. In this way, enrichment of the extract phase with phthalates could be achieved by repeating the extraction procedure.

The study showed that the influence of milk type was not a critical factor in the determination of an adequate extraction procedure. All the observed effects for optimization of the extraction procedure were more or less the same for all six investigated samples of dairy products. Based on all the obtained results and observations during optimization of the extraction procedure, the parameters that provided the most efficient phthalate extraction were: ratio of sample volume to solvent volume, 1:2; the procedure of enrichment of the extract phase by submitting the extract to a new sample volume, to obtain a final extract to sample ratio 1:50, leading also to a reduction in solvent consumption; shaking as agitation method in order to avoid the formation of stable emulsions and an extraction time in the range 15 to 30 min.

Phthalate analysis in standard n-*hexane solutions*

Phthalates were identified by GC–MS in the full scan mode and quantified in the SIM mode. Linear calibration curves for the phthalates dissolved in *n*-hexane were obtained in the concentration range 0.25–2.50 μg mL^{-1}. The linearity range

and respective correlation coefficients (R^2) calculated in the range 0.25–2.50 μg mL^{-1}, the *LOD* and the *LOQ* values for each phthalate investigated in this study and determined by GC–MS (in SIM mode) are reported in Table III. The R^2 values for all the phthalates in the linear range were above 0.990. The *LOD* and *LOQ* values of each phthalate analyzed in this study were adequate for estimating such compounds in milk samples: the *LOD* values ranged between 0.01 and 0.04 μg mL^{-1} whereas *LOQ* values ranged between 0.05 and 0.12 μg mL^{-1} with *RSD* values between 1.7–4.9 %. These values were determined according to the Knoll definition,[28] *i.e.*, an analyte concentration that produced a chromatographic peak equal to three times (*LOD*) and seven times (*LOQ*) the standard deviation of the baseline noise. In comparison with other extraction methods, this method provided comparable *LOD* and *LOQ* values.[11,19]

TABLE III. Linearity range and the respective values of correlation coefficients (R^2), *LOD* and *LOQ* values, and repeatability (*RSD*) of each phthalate; linearity range: 0.25–2.50 μg mL^{-1}

Phthalate	R^2	*LOD* / μg mL^{-1}	*LOQ* / μg mL^{-1}	*RSD* / %
DMP	0.999	0.04	0.12	4.9
DBP	0.999	0.01	0.05	1.7
BBP	0.992	0.02	0.08	2.4
DEHP	0.990	0.04	0.12	3.6

Phthalate analysis in matrix extracts

Validation of the phthalate quantification method in milk samples, considering the possible matrix effect, was performed with successive milk dilution. The high percentage of fat in milk may affect on the one hand the extraction efficiency of the phthalates and the analytical sensitivity, precision and stability of the response of the chromatographic system on the other. Therefore, for an estimation of the correlation of the milk fat content with the recovery for each phthalate, milk samples with different level of dilution were spiked and examined. For an estimation of the analytical sensitivity, the slopes of the curves obtained for diluted milk samples spiked with phthalates were examined and compared to the slopes of the calibration curves of phthalate standards in *n*-hexane.

As expected, higher phthalate recoveries were obtained by diluting the milk samples in water, whereby the recovery efficiency was improved by 60–80 %, compared to a dilution of 50 vol. % (Table IV). The low recovery rate (only 10 %) for the sample with 50 % milk content was expected due to the hydrophobic nature of phthalates, which are more soluble in milk fat globules giving lower distribution ratio between the extract and the milk sample. The recovery of phthalates was the highest for water samples (without any milk), which is in accordance with previous investigations performed with commercial bottled water samples.[29,30]

TABLE IV. Phthalates recoveries (%) from spiked diluted milk samples with 3 ppb of each phthalate

Milk content, vol. %	DMP	DBP	BBP	DEHP
0	14.00	75.00	125.00	135.00
0.5	10.82	62.30	89.51	73.61
2	7.10	60.86	92.34	69.22
3	10.12	61.78	90.18	68.00
6	13.10	54.14	72.41	65.00
10	13.10	41.72	57.24	71.03
15	13.83	34.04	46.81	60.00
30	13.33	25.45	37.27	45.00
50	11.76	4.41	8.66	10.95

The lowest recovery values were for DMP which is the phthalate with the lowest molar mass, only one carbon atom in hydrocarbon side chain and with even 10^3 times higher water solubility than the rest of investigated phthalates. Generally, higher recovery is observed for the high-molecular weight phthalates (log K_{ow} = 7.6 for DEHP), while low-molecular weight phthalates are more water-soluble relatively hydrophilic and thus the recovery decreases (log K_{ow} = = 1.5 for DMP). The same trend is observed for lower spike phthalate concentration of 3 ppb (Table IV) and for higher spike phthalate concentration of 6 ppb, as shown in Fig. 1.

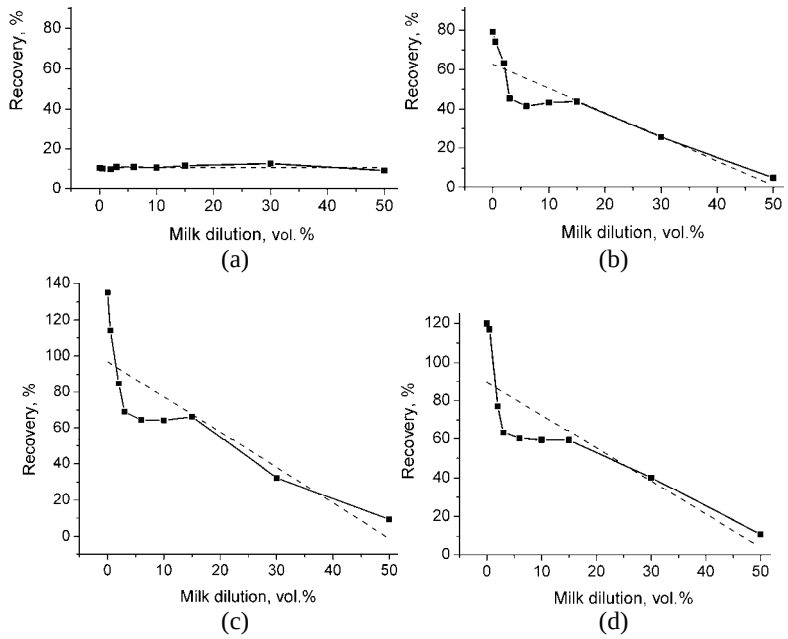

Fig. 1. Recoveries (average values) for the determination of phthalates in water dissolved milk samples spiked with 6 μg ml^{-1} of each phthalate: a) DMP, b) DBP, c) BBP and d) DEHP.

The analytical sensitivity of diluted milk samples spiked with phthalates were examined and compared to the calibration phthalate standards in *n*-hexane regarding the slopes of the obtained standard curves (Fig. 2).

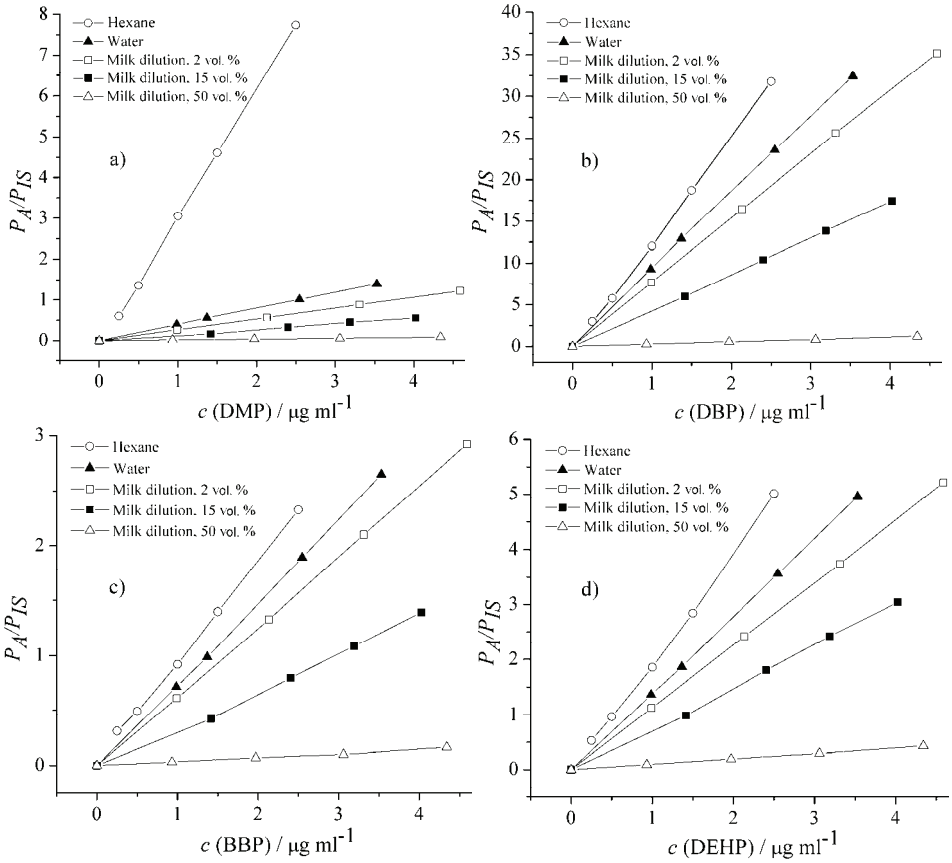

Fig. 2. The slopes of the curves for each phthalate prepared in *n*-hexane and milk matrix: a) DMP, b) DBP, c) BBP and d) DEHP. P_A and P_{IS} presents the values for chromatogram peak are of analyte and internal standard.

The concentrations of phthalates analyzed in the matrix extracts were in general lower than in *n*-hexane, showing a negative matrix effect that made for a less stable response of the chromatographic system and lower analytical sensitivity and precision.

The values of the slopes of the curve, obtained by linear regression and tabulated in Table V, show that the response and precision were the lowest for the milk sample with the highest fat content and for DMP as the most water soluble phthalate. This suggested a strategy for the elimination of matrix effect, *i.e.*,

dilution of the milk extract, especially for more hydrophobic phthalates such as the majority of commonly used phthalates.

TABLE V. Slopes of the curve obtained from solvent (*n*-hexane) and matrix (water and diluted milk samples)

Phthalate	*n*-Hexane	Water	Milk content, vol. %		
			2	15	50
DBP	12.8829	9.17614	7.6756	4.3494	0.28192
DEHP	1.9924	1.4067	1.135	0.7638	0.099
BBP	0.905	0.75284	0.6380	0.3490	0.0371
DMP	3.1800	0.39551	0.2669	0.14242	0.02068

CONCLUSIONS

The method of extraction of phthalates from milk samples by *n*-hexane was shown to be a simple and effective procedure. The validation results of the method were satisfactory, since the recovery data and relative standard deviation values indicated good method accuracy and precision (R^2 >0.990) for phthalates when evaluated in a milk matrix. An increase in the recovery of less polar phthalates in matrix milk standards by matrix dilution was observed. Recovery for hydrophilic phthalates, such as DMP, was not changed by matrix dilution and was continuously low for the investigated method. The same trend for influence of matrix dilution on phthalate recovery was observed at two phthalate spiking levels. Elimination of the matrix effect by dilution of the milk extract, especially for more hydrophobic phthalates such as the majority of commonly used phthalates, was shown to be a satisfactory method.

SUPPLEMENTARY MATERIAL

The chemical structures of the four studied phthalates, GC–MS chromatogram of a standard solution containing phthalates and physicochemical properties of the four studied phthalate esters are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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И З В О Д

ЕВАЛУАЦИЈА МЕТОДЕ ЕКСТРАКЦИЈЕ ФТАЛАТА ИЗ МЛЕКА У ЗАВИСНОСТИ ОД САДРЖАЈА МАСТИ У МЛЕКУ

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У циљу одређивања диметил-фталата (DMP), ди-*n*-бутил-фталата (DBP), бензил бутил-фталата (BBP) и ди-(2-етилхексил)-фталата (DEHP) у шест различитих узорака млека упоређени су резултати добијени течно–течном екстракцијом куплованом са

гасном хроматографијом–масеном спектрометријом. Извршена је оптимизација екстракционих фактора: припрема узорака, врста и запремина органског растварача, ефекат исољавања и мешања и време екстракције. Одабрани су базни максимуми испитиваних фталата (*m*/*z* 149 за DBP, BBP и DEHP и *m*/*z* 163 за DMP) за даље истраживање. Снимање је извршено у моду мониторинга изабраног јона. Калибрациона права је линеарна у опсегу 0,25 до 2,50 μg mL^{-1} са израчунатим *LOD* вредностима између 0,01 и 0,04 μg mL^{-1} и *LOQ* вредностима између 0,05 μg и 0,12 μg mL^{-1}, са *RSD* између 1,7 и 4,9 %. Истраживање је показало раст измереног аналита ("recovery") за мање поларне фталате разблаживањем матрикса млека. "Recovery" за хидрофилне фталате, као што је ДМП, се не мења разблаживањем матрикса млека и константантно је низак за испитивану методу. Две концентрације унутрашњег стандарда ("spike"), тестиране ради испитивања утицаја разблажења млека на "recovery" фталата, показују исти тренд.

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