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INVESTIGATION OF AMMONIUM HYDROXIDE EFFECT ON DnBP EXTRACTION FROM MILK SAMPLES

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Abstract

In this study, influence of ammonium hydroxide addition on di-n-buthyl phthalate (DnBP) extraction from milk samples was investigated. Ammonium hydroxide addition was done before extraction step. Effects of ammonium hydroxide were studied using spiked milk samples with known amount of DnBP standard solution in concentration range 0.25 to 1.25 mg L^{-1}. DnBP determination was carried out by gas chromatography – mass spectrometry (GC-MS). Obtained results indicate that extraction method which included addition of ammonium hydroxide higher mean recovery for all spiked concentrations of DnBP (51.91%) than extraction without addition of ammonium hydroxide (29.90%). The recoveries were 25.54% to 35.27% at five spiked levels for samples treated by extraction procedure without addition ammonium hydroxide, and in range from 47.88% to 61.50% for samples treated by extraction procedure with addition of ammonium hydroxide.

Keywords: phthalate, milk, extraction, ammonium hydroxide

INTRODUCTION

Phthalates are used as plasticizers in producing of polymeric materials in order to increase their physical properties such as flexibility, transparency and softness. Millions of tons of phthalates are produced over the world annually and the most popular plasticizer is di-2-ethylhexyl phthalate (DEHP), followed by di-*n*-buthyl phthalate (D*n*BP), di-iso-decyl phthalate (D*i*DP) and di-iso-nonyl phthalate (D*i*NP) [1]. Phthalates are not physically bonded to plastics and can migrate into air, groundwater, soil, food and then come into food chain [2]. A lot of packaging materials are made of polymeric material that could contain phthalates. The most used phthalates in polymers of food packaging materials are DEHP and DBP [3-5]. These phthalates could metabolize to bioactive phthalate monoesters and they are listed as endocrine disruptors [6].

Due to fact that phthalates are not physically bonded to plastic material, in case of contact with fat-containing food they tend to migrate in food, due to their lipophilic structure [7]. Milk as a complex matrix, can contains proteins and different amounts of fat, and it causes that different phthalates can be bound to this matrix constituents with different affinity, depending of their structure and length of chain. Bearing in mind that milk is a major source

of nutrition for infants and children, determination of D*n*BP and DEHP presence in milk is of increasing interest [8].

Techniques such as solvent extraction and solid phase extraction are used to clean up and concentrate milk samples before analysis. It is very important to find appropriate solvent or combination of few solvents to obtain high recovery [9]. The main objective of this work was to investigate if addition of ammonium hydroxide can improve extraction of D*n*BP from milk samples based on fact that addition of ammonium hydroxide causes disruption of milk fat globule and forms free fatty acids which then remain in the aqueous phase and are not extracted.

MATERIALS AND METHODS

Reagents and equipment

D*n*BP standard, dibuthyl adipate (DBA) and hexane were purchased from Sigma Aldrich, USA. Ethanol was purchased from Baker, USA. All used solvents were HPLC grade and screened to determine the phthalate background.

All sample and standard manipulation was done avoiding any contact with plastic equipment. Amounts of DnBP and DBA standard were accurately weighted out by analytical balance with precision at ±0.0001 g (Kern, Germany) and stock solutions were prepared by hexane diluting. Stock and working standards were stored in fridge.

Sample preparation

Milk samples with 2.8% milk fat content were purchased from local supermarket (Niš, Serbia). Effects of solvent extraction were studied by spiking known amount of D*n*BP standard solution in concentration range from 0.25 to 1.25 mg L^{-1} in samples before extraction steps. A volume of 10 mL milk sample was put in a glass centrifuge tube and 5 mL of ethanol was added. Mixture was treated under ultrasonic conditions for 30 min and centrifuged for 10 min at 4000 rpm. After this step, 10 mL of supernatant was transferred into clean polypropylene tube, 5 mL of hexane was added, and mixture was shaken and centrifuged once more by the same conditions. Hexane layer was analysed by GC-MS. All spiked samples and blank sample were analysed in three replicates. This procedure was repeated for the same milk samples with additional step. Before addition of ethanol, 1 mL of ammonium hydroxide was added to each analysed sample.

GC-MS analysis

Analysis was carried out by gas chromatograph coupled to mass spectrometer (Hewlett Packard 6890 series GC System with autosampler connected with Agilent 5973 Mass Selective Detector (Electron Ionization MSD-EI, single quadrupole)). The separation was achieved with 30 m × 0.25 mm × 0.25 μm a non-polar AGILENT DB-5MS column coated with 5% phenyl, 95% dimethylpolysiloxane. The MSD was used in the single ion-monitoring (SIM) mode. The identification of target compounds was based on the relative retention time, the presence of target ions and their relative abundance. The most abundant ion *m/z* 149 was chosen for quantification of D*n*BP. Ion *m/z* 185 was chosen as representative ion of DBA internal standard. The dwell time was 100 ms.

RESULTS AND DISCUSSION

The chromatogram in Figure 1 shows that the separation of D*n*BP and DBA, as internal standard, occurred within a running time of 15 min. Retention times for DBA and DEHP were 9.940 and 11.557 min, respectively.

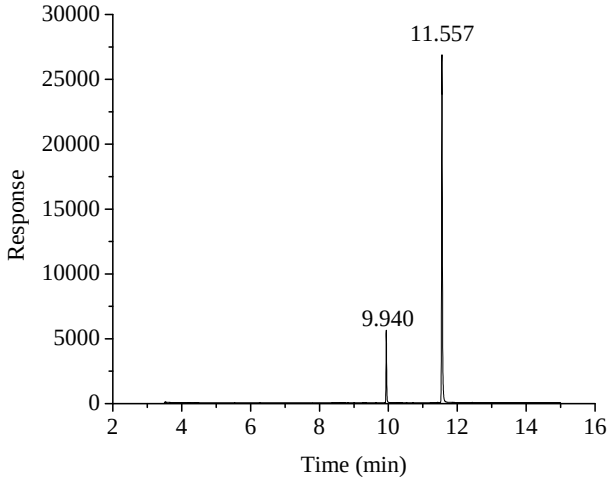

Figure 1 *Chromatogram of a standard solution containing DnBP in concentration 0.25 µg mL^{-1} and DBA in concentration 1 µg mL^{-1}*

All quantifications were performed by an external calibration method based on response ratios between internal standard and D*n*BP. The analytical curve obtained for D*n*BP in concentration range 0.25–10.0 µg mL^{-1} was linear for the given range with coefficient of determination, R^2, 0.99922 (Figure 2).

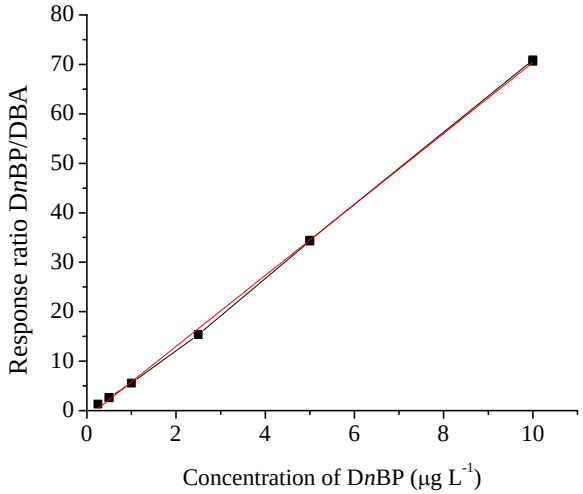

Figure 2 *Analytical curve for DnBP for concentration range 0.25–10.0 µg cm^{-3}*

Recovery values were calculated for all spiked milk samples. Mean values of recovery obtained by using two different extraction procedures for all investigated spiked samples are given in Table 1.

Table 1 *Recovery values obtained by extraction without and with addition of NH_4OH*

Spiked concentration of D*n*BP (mg L^{-1})	Recovery (%)	
	Without NH_4OH	With NH_4OH
0.25	29.73	61.50
0.50	25.54	52.25
0.75	27.00	49.83
1.00	31.96	47.88
1.25	35.27	48.10
Mean value of recovery (%)	29.90	51.91

D*n*BP concentration correlation between hexane extract from both procedures and milk matrix are showed in Figure 3.

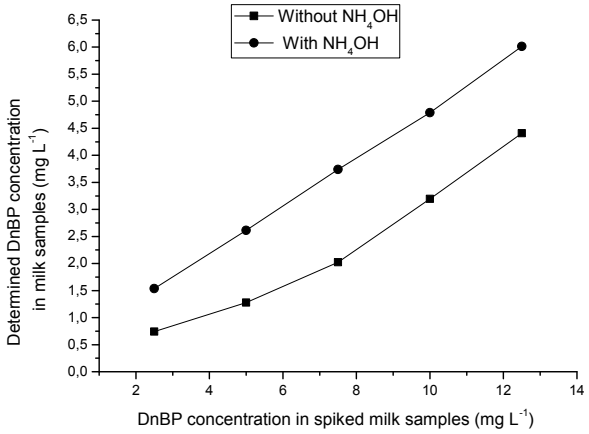

Figure 3 *Correlation between spiked and determined DnBP concentration in milk samples (mg L^{-1})*

Obtained results indicate that extraction method which included addition of ammonium hydroxide showed higher recovery for all spiked concentrations of DnBP. These results could be explained by fact that ammonium hydroxide causes disruption of milk fat globule. This process allows better extraction of DnBP from samples that contains free fatty acids in the aqueous phase.

CONCLUSION

The effectiveness of ammonium hydroxide addition on D*n*BP extraction from milk samples was investigated. Results showed that addition of ammonium hydroxide causes more efficient extraction with mean recovery value of 51.91% compare to mean recovery value

obtained by extraction without addition of ammonium hydroxide (29.90%). The highest value of recovery was obtained from milk samples with the lowest concentration of D*n*BP (61.50%) by procedure with addition of ammonium hydroxide.

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