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DETERMINATION OF PHTHALATES IN BOTTLED MILK USING HEADSPACE SOLID-PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY

MIRELA MICLEAN[a,*], OANA CADAR[a], CECILIA ROMAN[a]

ABSTRACT. This study reports the level of contamination with phthalates of some commercial bottled milk collected in Cluj-Napoca, Romania. The investigated compounds were dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DOP). The method used for the determination of these phthalate esters in commercial bottled milk consists in headspace solid-phase microextraction (HS-SPME) technique (100 µm PDMS fibre) coupled with gas chromatography-mass spectrometry (GC-MS) in single ion monitoring (SIM) mode. The recoveries for spiked samples were over 75% and under 95% (RSD 8-13%). The obtained concentrations ranged between 2.12-3.93 and 36.8-77.1 ng/g for DBP and DEHP, respectively, DMP, DEP, BBP and DOP were not detected in any sample.

Keywords: *phthalates, bottled milk, HS-SPME-GC-MS*

INTRODUCTION

Phthalates, esters of the phthalic acid, are used as plasticizers that improve the extensibility, flexibility and workability of polymeric materials and they are worldwide produced in high amounts. The most important representative is bis(2-ethylhexyl) phthalate (DEHP) and represents a quarter of the total production of plasticizers [1].

Humans are exposed to phthalates through the food, air, water, cosmetics, pharmaceutical products, etc., but the main exposure occurs through food, due to the use of PVC in wrapping materials and food processing [2, 3].

Phthalates could easily migrate into foods, beverages and drinking water from the packaging or bottling material, being ingested into the body [4]. Thus, food and beverage packaging could contribute significantly to human xenobiotic exposure, in addition to the environmental contaminants [5]. Phthalates being lipophilic compounds tend to be distributed preferentially in fatty foods (milk, meat, fish, olive oil) [4, 6]. Plastic additives, such as bisphenol-A, phthalates, nonylphenol, are suspected to be endocrine disruptors exhibiting mutagenic and carcinogenic action and are considered as important organic

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[a] INCDO-INOE 2000, Research Institute for Analytical Instrumentation, Donath St., no 67, RO-400293 Cluj-Napoca, Romania, * mirela.miclean@icia.ro

pollutants in the environment [1]. Recent research has associated phthalates exposure with the abnormal sexual development and birth defects in humans [7], with cardiovascular, liver, urologic diseases [3]. The US Environmental Protection Agency and the European Union classified phthalates in their top priority lists for risk assessment [8, 9].

Due to the ubiquity of phthalates in the environment, bottled milk could be contaminated in many ways: contamination of water, air, soil, also during bottling process, or migration from the packaging material of the bottle to the milk, leaching from PVC tubing into raw milk during milking at dairy farms, etc. [10, 11].

Recently, many efforts have been made for the development of simple and sensitive analytical methods for determination of phthalates in different samples. Conventional extraction methods, such as liquid–liquid solvent extraction (LLE) and solid-phase extraction (SPE) consume high volumes of toxic organic solvents [1, 11].

Solid-phase microextraction (SPME), developed by Arthur and Pawliszyn (1990), is a simple, rapid and effective extraction technique due to the incorporation of sampling, extraction and concentration into a single solventless step, saving preparation time and the risk of secondary pollution of the sample by reagents and vessels is considerably reduced [8, 12, 13]. Also, the use of headspace extraction has the advantage of the elimination of the complex matrices (milk) [11].

Methods for determination of phthalates in milk samples were reported: SPME/GC-MS [11], ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) followed by gas chromatography–flame ionization detection (GC–FID) [14], selective molecularly imprinted solid-phase extraction (MISPE) technique coupled with spectrophotometry [15], LLE [16], automated solid phase extraction (SPE) coupled to isotope dilution–high-performance liquid chromatography (HPLC) [17].

The aim of this study was to investigate the level of contamination with phthalates of commercially available bottled milk samples (PET packaging) collected from the local markets in Cluj-Napoca, Romania, using HS-SPME/GC-MS method. According to our knowledge, there is a lack of information regarding the occurrence of these types of contaminants in bottled milk in the studied area and in Romania.

RESULTS AND DISCUSSION

The recovery was determined on five replicates of milk with “zero” phthalates spiked with phthalate standard mix at 1.0 µg/L of each analyte, analyzed by HS-SPME/GC-MS. Mean recoveries are ranged from 75 to 99% and the coefficient of variation varied between 8 and 13%, as shown in Table 1.

Good linear correlation coefficients (R2) were found for the compounds, as shown in Table 1.

The limit of detection (LOD) was calculated from the measured value of the milk with "zero" phthalates sample (mean + 3 standard deviation) analyzed using HS-SPME/GC-MS procedure, and the limit of quantification (LOQ) was calculated as three times detection limit.

Table 1. Method performance for phthalates in milk

	DMP	DEP	DBP	BBP	DEHP	DOP
R^2	0.9975	0.9984	0.9991	0.9982	0.9760	0.9662
LOD (ng/g)	0.25	0.16	0.13	0.21	1.22	0.36
LOQ (ng/g)	0.75	0.48	0.39	0.63	3.66	1.08
Recovery (RSD%)	75 (9)	80 (11)	88 (10)	90 (9)	93 (8)	96 (13)

The investigated milk samples represent 4 different types of commercial milk and were bottled in polyethylene terephthalate (PET) packaging. The results of phthalates from the four investigated bottled milk samples are shown in Table 2. The results were calculated as the arithmetic means of concentrations obtained for the same analyzed milk samples.

Table 2. Mean concentration (ng/g) of phthalate esters in the bottled milk samples

Sample	DMP	DEP	DBP	BBP	DEHP	DOP	%Fat
1	< LOD	< LOD	2.81	< LOD	36.8	< LOD	2.5
2	< LOD	< LOD	3.93	< LOD	77.1	< LOD	3.0
3	< LOD	< LOD	2.12	< LOD	68.2	< LOD	2.0
4	< LOD	< LOD	3.88	< LOD	42.6	< LOD	3.0

ND=not detected, concentrations below the detection limit

DMP, DEP, BBP and DOP were not detected in any sample. DBP and DEHP were detected in all milk samples, in the range of 2.12-3.93 ng/g and 36.8-77.1 ng/g, respectively. The results obtained for DBP are comparable with those obtained by Feng et al. (2005) and the obtained concentrations of DEHP are lower than those obtained by the same authors for processed cow milk samples [11]. The obtained concentrations of DEHP fall within the range reported by Sharman et al. (1994) in commercial milk (50-130 ng/g) [19], except for the samples 1 and 4, which are lower (36.8 and 42.6 ng/g, respectively).

CONCLUSIONS

Due to the high consumption of bottled milk and due to the potential health risk of phthalates, the control of these consuming products is of special concern.

In this study, solid phase microextraction using PDMS-100 µm fiber followed by capillary gas chromatography coupled to mass spectrometry in the SIM mode acquisition was used for analysis of six phthalates from different bottled milk samples collected from markets in Cluj-Napoca, Romania. The only two phthalates were found in the investigated samples, namely DBP and DEHP with the concentrations in the range of 2.85-6.28 ng/g and 36.84-112.3 ng/g, respectively. Therefore, commercial milk could be considered as a source of human exposure to phthalates.

EXPERIMENTAL SECTION

Reagents, materials and apparatus

A standard stock solution containing six phthalate esters, dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DOP), in iso-octane at a level of 1000 µg/mL per compound was purchased from LGC Standards (Wesel, Germany). The sodium chloride (99.5%) was supplied from Merck (Darmstadt, Germany). Iso-octane (Suprasolv) was purchased from Merck (Darmstadt, Germany) and was used for the preparation of the calibration standard solutions by serial dilutions. The working standard solutions of phthalates at 0.1, 0.5, 2.5, 5, 10, and 20 µg/mL concentrations were prepared by diluting the individual stock solutions in iso-octane. All solutions were stored at 4ºC. The SPME device for manual sampling consisting of a holder assembly and 100 µm polydimethylsiloxane (PDMS) fiber was obtained from Supelco (Bellefonte, PA, USA). The fiber was conditioned prior to use according to the manufacturer’s requirements by heating in the injection port of the chromatographic system.

A magnetic stirrer/temperature-controlled water bath IKA RET with IKA ETS-D5 digital thermometer was from IKA Werke Gmbh (Staufen, Germany). The carrier gas used for GC was helium (>99.9999%) supplied by Linde Gas (Cluj-Napoca, Romania).

SPME procedure

The SPME was performed according to the methods described by Feng [11] and Cao [18]. 5 g NaCl were weighed into a 20 mL vial, then 10g of milk sample and a magnetic stirring bar were added and the vial was tightly closed with the vial cap. The vial was placed on a preheated water bath (90ºC) on the hot plate and the sample was continuously stirred at a constant speed (700 rotations/min). The SPME syringe was introduced by the septum and the fiber was exposed to the headspace for 60 min. After the extraction, the fiber was immediately inserted into the GC injection port and allowed 10 min for desorption. All samples were analyzed in duplicate. Also, a blank analysis was performed using 10 g of milk with “zero” phthalates in the vial and the fiber was exposed under the same conditions as the standards and the samples. The concentration of phthalates in samples was calculated after subtraction of the blank value.

The SPME fiber was checked for its fiber blank after each sample run to avoid any carryover effect from the previous sample run.

In this study, PDMS-100 μm fiber was used, although this fiber had lower extraction efficiency for DMP and DEP, but 100% efficiency for DBP, BBP, DEHP, DOP, since DEHP and DBP are the two major phthalates detected in milk, in former studies [11].

GC-MS analysis

The analysis was performed on Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) HP 6890 series GC, equipped with a split/splitless injector and a HP 5975 mass selective detector system. The MS was operated at the electron impact (EI) mode (70 eV). Desorption of the fiber into the injection port was carried out in the splitless mode at 280ºC for 5 min and then maintained in injection port 30 min with purge gas turned on, before the next extraction. A HP-5MS, 5% diphenyl 95% dimethyl polysiloxane capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) from Agilent Technologies was used. Oven temperature was set at 100ºC, increased at 8ºC/min up to 260ºC, increased at 35ºC/min up to 310ºC and held for 10 min and the running time being 31.43 min. The MSD transfer line heater, ion source and quadrupole analyzer temperatures were set at 320, 230 and 150ºC, respectively.

The qualitative and quantitative analyses were performed by comparison with the external standards. The target and the qualifier ions were determined by injection of standards under the same chromatographic conditions using full-scan with the mass/charge ratio ranging from 100 to 550 *m/z*. A quantitative analysis was made using selected ion-monitoring (SIM) by acquiring the signals of the target ions (as quantifier).

The compounds of interest were identified by comparing the retention time with that of the standard compounds. The retention times, target and qualifying ions of the investigated phthalates are shown in Table 3.

Table 3. Retention time and selected ions for the analysis of the phthalates

Compound	Retention time (min)	Quantification ions	Identification ions
DMP	9.463	163	77, 194
DEP	11.434	149	177, 104
DBP	16.189	149	223, 104
BBP	20.503	149	91, 206
DEHP	21.723	149, 167	279
DOP	22.769	149	279, 104

The milk with “zero” phthalates, used as blank, was obtained from a private cow (in Coruşu village, Cluj county, away from any pollution source); the milk was carefully manual milked and transported to the laboratory in a clean glass bottle.

The blank values of the analytical procedure were determined by extracting the milk with “zero” phthalates in which the phthalates standard was not added. Only a small chromatographic peak for DEHP was recorded in the chromatogram of the blank procedure. The concentrations of phthalates in real samples were calculated after subtraction of the blank value.

The calibration was performed using multilevel spiked samples: 10g of milk with “zero” phthalates samples were spiked with 10 µL of the following working standard solutions of phthalate: 0.1, 0.5, 2.5, 5.0, 10 and 20 µg/mL (in iso-octane) in a 20 mL clear glass vials with Teflon-lined silicone rubber septum (Agilent Technologies), then analyzed using HS-SPME/GC-MS procedure. The obtained theoretical concentrations were: 0.1, 0.5, 2.5, 5.0, 10 and 20 ng/g milk.

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