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Quantitative Analysis of Phthalates Plasticizers in Traditional Egyptian Foods (Koushary and Foul Medams), Black Tea, Instant Coffee and Bottled Waters by Solid Phase Extraction-Capillary Gas Chromatography-Mass Spectroscopy

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Abstract: In the present study, method of solid-phase extraction followed by capillary gas chromatography coupled to mass spectrometry (SPE-GC-MS) was used for quantitative analysis of trace levels of phthalates in the most tow Egyptians traditional food (foul medams and koushary) and drinks (black tea and instant black coffee) and bottled water samples. Method performance was evaluated in terms of accuracy, linearity, limits of detection and recovery. Also the practical application of extraction and analysis method was explained.

Key words: Phthalate, plasticizers, GC-MS, solid, phase, Egyptian, foods

INTRODUCTION

Foodstuffs packed in plastics is the potential migration of plastic additives such as plasticizers, stabilizers, antioxidants and residual monomers from the plastic packaging material into the contained foodstuffs (Goulas *et al*., 1998). Phthalate esters are the major class of plasticizers permitted by FDA for use in food-contact plastics. Phthalate esters are synthetic compounds widely used as polymer additives in plastics, rubber, cellulose and styrene industry, to improve their softness and flexibility. They are present in many consumer products including children toys, cosmetics, personal care products, blood bags, organic solvents, packaging, paper coatings and insecticides, etc. (Agency for Toxic Substances and Disease Registry, 2000; David *et al*., 2001).

Due to widespread use, phthalates are considered as ubiquitous environmental pollutants. Since their physical rather than chemical incorporation in the polymeric matrix, phthalates easily migrate into foods, beverages and drinking water from the packaging or bottling material or manufacturing processes, being ingested and absorbed into the body, as well as during blood transfusions from PVC blood bags (Balafas *et al*., 1999; Wahl *et al*., 1999). Also highest levels tend to be found in fatty foods, such as milk and dairy products, fish, meat and vegetable oils (Schettler, 2006).

Phthalates are suspected to be endocrine disrupting chemicals exhibiting carcinogenic action (McKee *et al*., 2004). Due to their potential risks for human health and environment, several phthalates has been listed as priority substances by many national and international regulatory organizations. The European Union (EU) published a candidate list of substances with evidence or potential endocrine disrupting action, which includes di-n-butyl phthalate, butyl benzyl phthalate and Di-2-Ethylhexyl Phthalate (DEHP). Since DEHP is the most widespread phthalate produced and used, it was incorporated in the list of priority substances in the field of water policy established by EU and the World Health Organization (WHO), has established a guideline value of 8.0 μg L^{-1} in fresh and drinking waters (European Union Council, 2001; WHO, 2003).

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Capillary gas chromatography with flame ionization, electron capture or mass spectrometry detection and high performance liquid chromatography with diode-array and tandem mass spectrometry detection prior to sample preparation methodologies, i.e. liquid–liquid extraction and solid-phase extraction, have been purposed for the determination of phthalates in several types of matrices such as water (US Environmental Protection Agency, 2001; Cortazar *et al*., 2002), milk (Calafat *et al*., 2004), urine (Silva *et al*., 2004), serum and plastics (Li *et al*., 2004) and packing materials (Balafas *et al*., 1999). All above methods are highly performance, but very expensive and need more time for many steps of each method.

Dietary intake from contaminated food is likely to be the largest single source of phthalate exposure in the general population. Phthalate levels in food, however, are widely variable and data may not reflect current exposure levels. Maximal daily intake estimates were 0.48 μg/kg/day for di-n-butyl phthalate, 4.9-18 μg/kg/day for bis (2-ethylhexyl) phthalate and 0.11-0.29 μg/kg/day for butyl benzyl phthalate (Schettler, 2006).

Koshary and Foul medams are commonly consumed in Egypt as traditional foods and served hot and mostly in plastics packages.

In the present study, solid phase extraction followed by capillary gas chromatography coupled to mass spectrometry (SPE-GC-MS) was used as simple, accurate and economic method for quantitative analysis of trace levels of phthalates in the most tow Egyptians traditional food, foul medams and koushary and traditional drinks, black tea and instant black coffee and bottled water which has increasing in consumption in recent years. The performance of the method was evaluated in terms of accuracy, linearity, limits of detection and recovery percentage.

MATERIALS AND METHODS

Chemicals

Phthalate Esters standard individual and Mix (500 μg mL^{-1} each in methanol) including Dimethyl Phthalate (DMP), Diethyl Phthalate (DEP), di-n- butyl phthalate (DBP), Butyl Benzyl Phthalate (BBP), bis(2-ethylhexyl) adipate (DEHA), bis(2-ethylhexyl) phthalate (DEHP), were purchased from Supelco (Bellefonte, PA, USA). HPLC-grade methanol (MeOH, 99.9%), were obtained from Fluka Chemie AG (Buchs, Switzerland). Ultra-pure water was prepared from Milli-Q water purification systems (Millipore, Bedford, MA, USA). An Oasis HLB glass (6 mL; 0.5 g) cartritges were purchased from Waters Inc., Milford, USA.

Samples

Egyptians traditional food (koushary and foal medams) were obtained in one day from different local restaurants located in Giza city, one sample set was packed in plastic bags and other sample set was packed in glass dishes. Bottled water samples were obtained from different markets and the expiration date was within 6 months. Black tea and instant black coffee were prepared in laboratory, one sample set in plastic cups and other set in glass cups.

Samples Preparation

Food samples (1000 g) were homogenized with 500 mL of ultra-pure water for 15 min, then the mixture was centrifuged at 10000 g during 20 min, then supernatant was recovered and extracted with solid phase cartridge. Water samples (1 L) directly extracted with solid phase cartridge. One liter (5 cups) of black tea or coffee were directly extracted after 5 min of preparation.

Samples Extraction

Five hundred milliliter sample (supernatant or water) was filtrated through the SPE cartridge at flow rate 1.0 mL min^{-1} by vacuum using a vacuum manifold. After this, cartridges were flushed with

2×20 mL aliquots of ultra-pure water followed by reapplication of the vacuum for 15 min to remove residual water. Each cartridge was eluted with 2 mL methanol and the eluent was concentrated to <1 mL by evaporation under a stream of N_2 gas and then adjusted to 1.25 mL (1 g) with methanol and stored until analysis. A laboratory blank prepared by SPE of 1 L of ultra-pure water was included with each sample set. Also spiked sample at level 10 μg L^{-1} was included to evaluate method recovery.

GC-MS Instrument Operating Conditions

GC-MS analysis were performed on a HP 6890 Series gas chromatograph coupled to a HP 5973 mass selective detector (HP Technologies, USA). The GC analysis was performed on a HP-5MS (80 m × 0.25 mm I.D., 0.25 μm film thickness) capillary column (5% diphenyl, 95% dimethylpolysiloxane; HP, USA). Injection system having a septumless sampling head was used and helium as carrier gas was maintained in the constant pressure mode and the inlet pressure was 35 psi. The oven temperature was programmed from 60°C (3 min) to 120°C at a rate of 5°C/min and from 120 to 230 at rate of 8°C/min and final temperature held for 10 min The MS was operated in Selected-Ion Monitoring (SIM) mode and several groups having target ions were monitored at different time windows defined by the corresponding retention times. Two ions of each phthalate were chosen, according to the mass spectra characteristic features obtained in the full-scan mode (from 35 to 550 m/z and by comparison with the NIST library reference spectral bank and data recording and instrument control were performed by the HP ChemStation software (HP Technologies, USA).

Statistical Analysis

Each of the measurements described was carried out in at least three replicate. Unpaired t-tests were performed using the dada analysis in the spreadsheet program (Microsoft, 2003). The probability level used in evaluating test statistics was p = 0.05, 0.01.

RESULTS AND DISCUSSION

Six phthalate esters were selected for the present study, since they prove to be the most common widespread phthalate contaminants (Scientific Committee on Food, 1999). A standard mixture (10 mg L^{-1}) was analyzed by capillary GC-MS in the full-scan mode to record the mass spectral fragmentation pattern of each compound which were chosen to attain the best response in the selective ion detector mode acquisition and good sensitivity and selectivity. The instrumental calibration was performed with standard mixtures ranging from 15 to 100.0 μg L^{-1}, (r^2>0.85) for the six phthalates, using the corresponding target ion abundances. To evaluate the instrumental precision, sensitivity was checked through the limit of detection (LOD) between 40 to 100 ng L^{-1} was measured daily (Table 1). The result of spiked water matrices (n = 3) at the 10 μg L^{-1} level, producing excellent recoveries (>85%) and good selectivity and sensitivity was observed when comparing with blank assays for almost all compounds under study (Fig. 1). The laboratory blank is very important since the contamination is a major problem in the analysis of phthalates, especially from unclean plastic-

Table 1: Retention time (Rt), selected ions (m/z), linear range (μg L^{-1}), limit of detection (LOD (ng L^{-1}), correlation coefficient (r^2) and average of recovery percentage (%R) of selected compounds

Phthalates	Rt (min)	Selected ions	Linear range	LOD (ng L^{-1})	Accuracy
Dimethyl phthalate (DMP)	8.78	163.770	15-100	60	0.90
Diethyl phthalate (DEP)	10.76	149.177	20-100	40	0.95
Di-n-butyl phthalate (DBP)	11.92	149.150	15-100	70	0.85
Butyl benzyl phthalate (BBP)	14.72	149.910	20-100	50	0.92
Bis(2-ethylhexyl) adipate (DEHA)	16.08	149.167	25-100	100	0.80
Bis(2-ethylhexyl) phthalate (DEHP)	19.11	129.112	15-100	60	0.90

Linear range based on 6 levels of concentration, Recovery percentage based on spiked sample at level 100 μg L^{-1}

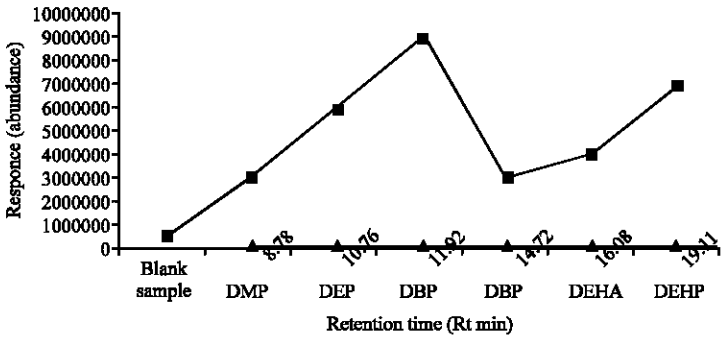

Fig. 1: The comparison between the responses (abundance) of six phthalates in spiked sample with blank sample

Table 2: Levels (Mean±SD, n = 5) of phthalate (μg L^{-1} for water, tea and coffee and μg kg^{-1} for koushary and foul medams) detected in real samples

Bottled water	DMP	DEP	DBP	BBP	DEHA	DEHP
20 days	nd	nd	nd	nd	nd	nd
26 days	nd	nd	nd	nd	nd	nd
35 days	nd	nd	0.44±0.05	nd	nd	nd
42 days	nd	nd	0.99±0.21	0.28±0.08	nd	nd
50 days	nd	0.87±0.19	1.04±0.23	0.23±0.02	nd	nd
70 days	0.76±0.11	0.98±0.17	0.78±0.11	0.32±0.07	nd	nd
80 days	0.74±0.15	0.95±0.11	0.98±0.13	0.30±0.05	nd	nd
100 days	0.75±0.20	0.65±0.15	0.76±0.09	0.25±0.08	nd	nd
125 days	0.81±0.23	0.76±0.09	0.88±0.12	0.22±0.03	nd	nd
150 days	0.76±0.09	0.90±0.19	0.95±0.19	0.31±0.06	nd	nd
Foul medams[a]	1.02±0.24	1.22±0.21	nd	0.94±0.04	0.33±0.08	nd
Foul medams[b]	nd	nd	nd	nd	nd	nd
Kosharya[a]	1.83±0.11	nd	1.43±0.24	2.54±0.19	nd	0.43±0.07
Koshary[b]	nd	nd	nd	nd	nd	nd
Black tea[a]	0.11±0.05	nd	0.17±0.04	nd	0.23±0.04	nd
Black tea[b]	nd	nd	nd	nd	nd	nd
Black coffee[a]	0.76±0.14	nd	0.54±0.07	0.94±0.19	nd	1.22±0.23
Black coffee[b]	nd	nd	nd	nd	nd	nd

a: In plastic bag or cup, b: In glass dish or cup, nd: Not detected

containing glassware, organic solvents and many items in laboratory settings and even from septum in injection system (Polo *et al*., 2005). In present study, any contact with plastic material was avoided and before use, all laboratory glassware was properly washed several times with acetone and ultra-pure water. Furthermore, phthalate contamination from the septum bleeding was avoided during the present study, extraction cartridges were glass. The mass spectra of blank sample showed in Fig. 1.

The LODs determined in present study were low enough to detect phthalate contamination, comparable with previous reports in literature in water samples (Polo *et al*., 2005) and other food samples (Calafat *et al*., 2004). Data of real samples recorded in Table 2, from the data, six phthalates were detected in all sample but not in the same sample. The DBP and BBP were the most abundant phthalate in bottled water samples, All levels were below the restricting limits set by the international regulatory organisms (European Union Council, 2000).

Data in Table 2 showed that foul medams served in plastic bag had higher DEHA content than black tea (significantly at p = 0.05), also served in plastic cup (0.33 and 0.23 μg L^{-1}, respectively). Foul medams had high temperature, long contact time with plastic bag and high fat (oil) content, where these parameters increase the migration of DEHA and oil act as un-polar media for migration of DEHA (un-polar). Schettler (2006) found that the migration of the compound did occur, that it increased with

length of contact time and temperature and the direct contact between the film and foods with a high fat content at the surface. All plasticizers are poorly water soluble substances. This is especially true for DEHP, DEHA which show true water solubility in the lower μg L^{-1}-range. The results in Table 2 showed that DEHP and DEHA didn't detected in bottled water because they are very poorly water soluble substances. All DEHP and DEHA detected levels were significantly ($p = 0.01$) lower than the tolerance daily intake of 50 μg kg^{-1} body weight (Scientific Committee on Food, 1999).

CONCLUSION

Based on the data of method performance, solid phase extraction followed by capillary gas chromatography coupled to mass spectrometry could be used for analysis of trace levels of phthalates with high accuracy and recovery addition to ease of use and. Egyptian food, tea and coffee which selected in present study usually served hot in plastic materials either cups or dishes contained phthalates. Generally, the diet has been considered the prime source of phthalates exposure in the population. According to the above mentioned results, foods should be prepared, served and packaged in a glass or polyolefin containers.

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