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Fast analysis of phthalates in freeze-dried baby foods by ultrasound-vortex-assisted liquid-liquid microextraction coupled with GC-IT/MS☆

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ABSTRACT

This paper is focused on the determination of phthalates (PAEs), compounds "plausibly" endocrine disruptors, in baby food products by means of a method based on ultrasound-vortex-assisted liquid-liquid microextraction coupled with GC-IT/MS (UVALLME-GC-IT/MS). Particularly, the whole procedure allows the determination of six phthalates such as DMP, DEP, DBP, iBcEP, BBP and DEHP. After dissolution of 0.1 g product sample and addition of anthracene as Internal Standard, 250 μL of *n*-heptane are used as extraction solvent. The solution, held for 5 min on the vortex mixer and for 6 min in an ultrasonic bath at 100 W for favoring the solvent dispersion and consequently the analyte extraction, is centrifuged at 4000 rpm for 30 min. About 100 μL of heptane are recovered and 1 μL is injected into the GC-IT/MS. All the analytical parameters investigated are deeply discussed: under the best conditions, the percentage recoveries range between 96.2 and 109.2% with an RSD ≤10.5% whereas the Limit of Detections (LODs) and the Limit of Quantifications (LOQs) are below 11 and 20 ng g^{-1}, respectively, for all the PAEs except for iBcEP (23 and 43 ng g^{-1}, respectively). The linear dynamic range of this procedure is between 10 and 5000 ng g^{-1} with R^2 ≥0.92. The method has been applied to real commercial freeze-dried samples (chicken and turkey meats) available on the Italian pharmaceutical market: three PAEs were preliminary identified, i.e. DEP (14 ng g^{-1}), DBP (11 ng g^{-1}) and DEHP (64 ng g^{-1}).

1. Introduction

Baby food is a soft, liquid paste or an easily chewed easily consumed food, made specifically for babies, roughly between the ages of four/six months and two years because since ever infant nutrition has been considered a very important issue: a good diet helps the baby to grow up healthy and, simultaneously, protects them against diseases. In 1996 Bradbury [1] launched the alarm about the possible phthalate intake risk by babies from babymilk formulae.

Phthalate esters (PAEs) are compounds worldwide employed as additives in plastics and consumer products [2] from which they can leach and migrate into the food or other materials [3]. During these last two decades PAEs have received a great attention [4] because they are thought to be endocrine disruptors [5,6]. Adult humans are exposed via food, medicine, cosmetics and environment (i.e. ingestion, inhalation and dermal contact) [7–10]; on the other hand, babies exhibit highest intake and consequently they are exposed to the main risks [11–14].

The Scopus data-base reports more than 5000 papers using "Phthalate" and "analysis" as keywords, meaning the large importance of such issue in analytical chemistry. This number decreases drastically (less than 20) if the "baby food" words are added; most of them addressed to PAEs determination in both breast milk [13]; infant formula [1,10,15,16]; liquid milk [13,14,17,18] or migrated from metallic package or plastic baby bottles [19–22]; whereas only the paper by Petersen and Breindahl [15] deals the PAE determination in 11 different baby foods (fruit; cereals; rice mixed with fruit or meat mixed with vegetables). After sample dissolution with 100 mL of pentane; extraction and clean-up of samples were performed by means of 5 mL ethylacetate/cyclohexane (1 + 1) and the relative supernatant was undergone to Gel Permeation Chromatography (GPC) and dryness for obtaining a final solution to be injected into the GC Mass Spectrometry (GC–MS). By this procedure the authors determined 4 PAEs; i.e. di-*n*-butylphthalate (DBP); butyl-

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benzylphthalate (BBP); di-2-(ethylhexyl)phthalate (DEHP) and di-2-(ethylhexyl)adipate (DEHA) with interesting Limits of Detection (0.10; 0.004; 0.07 and 0.03 mg kg^{-1}; respectively) and Limits of Quantification (0.35; 0.015; 0.25 and 0.10 mg kg^{-1}; respectively) but with recoveries widely spread (87–128%; 93–101%; 76–116% and 86–102%; respectively; with spiking level of 0.2 mg kg^{-1} of each PAE). These last data are important because they confirm that the main analytical difficulties comes from the sample treatment due to the matrix complexity. In fact; baby food comes in multiple varieties and tastes (i.e.; cereals; fruits; meat; fish; vegetables); it may be table food that the rest of the family is eating that has been mashed or otherwise broken down; or it can be purchased ready-made from producers. Along with them; commercial freeze-dried products are also very common baby foodstuff in Italy.

Although the literature on the PAE determination is increasing [4,23,24], this determination is still difficult: it is necessary to put great attention to avoid the sample contamination due to glassware, solvent and standards, and simultaneously to arrange a dedicated laboratory [25]. In fact, over glassware and reagents, PAEs are present in environment, including indoor air and this can produce false positive outputs: the final solution could be the building of laboratories free of materials containing PAEs, such as PVC flooring and electric cables, and minimizing the indoor air contamination [4]. In our study, the PAE clean-up protocol is performed by liquid-liquid extraction (LLE) [26], solid-phase extraction (SPE) [27–29], solid-phase microextraction (SPME) [30–34] or liquid-phase microextraction (LPME) [35,36]; recently Russo et al. applied the Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-Extraction (USVADLLME) for determining PAEs (or other organic compounds) in soft drinks, light alcoholic beverages, wine and spirits [25,37–39].

This paper would like to assess the application of the entire procedure based (UVALLME-GC-IT/MS) to these two complex matrices, i.e. baby foods and freeze-dried products. The analytical method has been modified according to the matrices investigated: the role of the disperser solvent was evaluated as well as the effect of the different nature between soft (baby food) and freeze-dried matrix was investigated in terms of analytical parameters.

2. Experimental

2.1. Materials and chemicals

Standards of the phthalates investigated in this study such as dimethyl phthalate (abbreviation DMP; $C_{10}H_{10}O_4$; molecular weight (MW) 194; mass-to-charge ratio (*m*/*z*) 163), diethyl phthalate (DEP; $C_{12}H_{14}O_4$; MW 222; *m*/*z* 149–177), dibutyl phthalate (DBP; $C_{16}H_{22}O_4$; MW 278; *m*/*z* 149–205), butyl cyclohexyl phthalate (iBcEP; $C_{18}H_{24}O_4$; MW 304; *m*/*z* 149–223), benzyl butyl phthalate (BBP; $C_{19}H_{20}O_4$; MW 312; *m*/*z* 149–206) and diethylhexyl phthalate (DEHP; $C_{24}H_{38}O_4$; MW 390; *m*/*z* 149–167), were obtained from Sigma-Aldrich (Milan, Italy); ethanol, *n*-heptane, cyclohexane, isooctane and benzene were of pesticide grade (Carlo Erba, Milan, Italy) whereas sodium chloride (Carlo Erba) was of analytical reagent grade.

Anthracene (10 μg mL^{-1}) ($C_{14}H_{10}$; MW 178; *m*/*z* 178), purchased from LabService Analytica (Anzola Emilia, Bologna, Italy), was used as Internal Standard (I.S.): 2 μL were added to each sample before performing the analytical process.

For avoiding the cross-contamination due to reagents (expecially for minimizing the background contamination due to NaCl), materials and laboratory (e.g., glassware) equipment, which is still fundamental issue in PAE analysis, all the chemicals and instruments were undergone to severe cleaning procedure. The details are reported in previous papers [40,41]. Briefly, the glassware was soaked and washed in acetone, dried at 140 °C for at least 4 h; NaCl was heated for 4 h at 140 °C and, after cooling, kept in a tightly sealed glass vial. For PAE standard solutions (0.1 mg mL^{-1} of each PAE), absolute ethanol was used: each solution was further diluted by ethanol to obtain solutions at different PAE concentrations for spiking the samples.

2.2. Extraction and clean-up process

Aliquot of each sample (0.1 g of baby food or freeze-dried sample) was dissolved in 10 mL of warm distilled water. Before starting the clean-up, the sample was vortexed for 2 min for favoring the hydration of the lyophilized sample. After addition of I.S. (2 μL of anthracene at 10 μg mL^{-1} concentration), NaCl (concentration 10 g L^{-1}) and 250 μL of *n*-heptane (as extraction solvent), the solution was undergone to vortex for 5 min followed by 6 min ultrasound bath. These operations were repeated three times to obtain a stable emulsion: the solution was centrifugated at 4000 rpm for 30 min to form the micro-drop. 1 μL of the surnatant were directly injected into the GC-IT/MS for determining PAEs.

Baby foods were undergone to freeze-drying by means of a benchtop manifold freeze-drier (mod. LIO5P, Cinquepascal, Milan, Italy) at −52 °C and 0.080 mbar for 4 h.

2.3. GC-IT/MS measurement

The GC-IT/MS instrument used for analysis was a TraceGC coupled with a PolarisQ mass spectrometer (Thermo Finnigan, Bremen, Germany) equipped with a data acquisition software (Xcalibur 1.4.1). The GC analysis was performed by laboratory-made fused-silica capillary column with chemically bonded phase [42–44]: SE-54, 5% phenyl-95% dimethylpolysiloxane column, 30 m × 250 μm i.d.; theoretical plate number, *N*, 120,000 for *n*-dodecane at 90 °C; capacity factor, K*í*, 7.3; film thickness, d_f, 0.25 μm; optimum linear velocity of carrier gas, hydrogen, u_{opt}, 34.5 cm s^{-1}; utilization of theoretical efficiency, 95%). These characteristics were verified and monitored so to be the column analogous to commercial one, with the advantage to be economic. After 50 runs the column undergoes a cleaning procedure.

A 1 μL sample was injected into the programmable temperature vaporization (PTV) injector in splitless mode. Five seconds after injection the vaporizer was heated from 110 to 280 °C at 800 °C min^{-1} and kept for 5 min whereas the splitter valve was opened after 2 min. The GC-IT/MS transfer line temperature was 270 °C, the temperature of the ion source was 250 °C. Helium (IP 5.5) was used as carrier gas (flow rate 0.3 mL min^{-1}). The oven temperature program was: 100 °C for 1 min, after up to 300 °C at 10 °C min^{-1}, held for 5 min. The IT/MS operated in Electron Ionization mode (70 eV): the analytes were qualitatively identified in full-scan mode (*m*/*z* 75–450) and quantified in SIM mode.

The PAE levels were determined by means of calibration graphs of the $Area_{(PAE)}/Area_{(IS)}$ ratio plotted against each PAE concentration. All the samples were determined in triplicate.

3. Results & discussion

3.1. Evaluation of the analytical methodology

The analytical method set up in this paper shows substantial improvements related to previous papers [25,37,39]: the emulsification occurs without disperser solvent and the emulsion breaking is performed by means of NaCl. Each step of the entire protocol has been investigated and the analytical parameters studied: all the procedure has been optimized using a freeze-dried product, i.e. turkey with different levels of PAEs.

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