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Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry

Wei Jia[a,b], Xiaogang Chu[a,b,*], Yun Ling[b], Junrong Huang[a], James Chang[c]

[a] College of Chemistry & Chemical Engineering, Shaanxi University of Science & Technology, Xi'an 710021, China
[b] Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing 100123, China
[c] ThermoFisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, United States

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ABSTRACT

A new analytical method was developed and validated for simultaneous analysis of 27 phthalates in milk and milk products. Response surface methodology was employed to optimize a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation method. Ultrahigh-performance liquid chromatography and electrospray ionization quadrupole Orbitrap high-resolution mass spectrometry (UHPLC/ESI Q-Orbitrap) was used for the separation and detection of all the analytes. The method was validated by taking into consideration the guidelines specified in Commission Decision 2002/657/EC and 2007/19/EC. The extraction recoveries were in a range of 90.7% to 104.6%, with coefficient of variation <5.6%. The 27 compounds behave dynamic range in the 0.1–1000 μg kg^{-1} concentration, with correlation coefficient >0.99. The limits of detection for the analytes are in the range 0.32–2.6 μg kg^{-1}. This method has been successfully applied on screening of phthalates in 96 commercial milk and milk product samples.

1. Introduction

1,2-Benzenedicarboxylic-acid esters, also known as phthalate-acid esters (PAEs), have long been used as industrial plasticizer in a wide range of consumer products. The worldwide annual production of phthalates is approximately 6.0 million metric tons per year [1–3]. Because of their non-covalent bonding properties and extensive use, phthalates are released ubiquitous in the environment. The food packaging and food processing can also introduce these compounds in the food change. These may result in direct contamination of feed and food products, bioaccumulation in tissues, and transfer through the food chain [4–6].

A recent food safety concern has arisen from the unapproved use of certain phthalates as direct food additives in a broad range of food manufactured in China [7]. These phthalates were illegally substituted for food grad emulsifiers in formulating clouding agents meant to provide turbidity to selected food products, mainly distilled spirits and beverages. Some of these products apparently might have been exported to various parts of the world [8].

Phthalates are considered to be potential mutagenic, carcinogenic activity and endocrine disrupters, with fetal animals being particularly sensitive [9,10]. Although the intake of phthalates may originate from many sources, there is special interest in monitoring the contamination of milk and milk products because they constitute a primary food source, especially for children [11–13].

For monitoring purposes, broad range analytical methods are needed to reduce analytical costs and allow for a more frequent monitoring of phthalates in milk and milk products. For the detection and quantification of phthalates, chromatographic techniques like high performance liquid chromatography (HPLC) with diode array detector (DAD) [14], gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS) have been used [15–17]. Under GC–MS (electron ionization, EI) conditions, the fragment at m/z 149 is the common ion for most phthalates. This is a major limitation in using GC–MS for the determination of phthalates isomeric mixtures, primarily because of the occurrence of coeluting isomers with varying composition of alkyl substitution [18,19]. LC–MS is a suitable technique for the analysis of phthalates because no derivatization step is required as in GC–MS. Tandem quadrupole MS has been widely accepted

* Corresponding author at: Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing 100123, China. Tel.: +86 010 85778904; fax: +86 010 857707750.
E-mail address: foodjiawei@yahoo.com.cn (X. Chu).

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as the main tool in the structural characterization, identification, and quantitative analysis of phthalates owing to its efficiency, superior sensitivity and specificity [20–22]. However, LC–tandem quadrupole MS is not suitable for simultaneous screening of a large number of phthalates. Besides, false positives caused by complex food matrices are frequently encountered [23]; no studies were reported to simultaneously detect over 25 different phthalates in food samples. From last year the role of UHPLC-Q-Orbitrap is increasingly built up as enabling tool in food safety analysis for it can provide detailed structural information. In spite of the potential value of the application, to the best of our knowledge, so far no people has reported the application of Q-Orbitrap mass spectrometry combined with high performance liquid chromatography for simultaneous determination for a group of phthalates in foods [24].

The analysis of phthalates in milk and milk products is a difficult task because of the lipophilic properties of most phthalates. When sample extraction is performed by solvent mixtures of low polarity, fats are co-extracted together with phthalates. The chromatographic analysis requires the application of previous extraction and clean-up steps in order to remove lipids and proteins. A wide variety of sample preparation has been reported in literature for phthalates, such as liquid–liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME) and dilute-and-shoot (DAS) [25–28]. However, some of these methods still have some limitation, such as high variability in results, high requirement for clean sample, time consuming as well as expensive, which make them inadequate for routine analyses. Obviously, elimination or simplification of the sample preparation would reduce the risk of contamination. Hence, new straightforward approaches involving simpler and fewer steps would be welcome for a more effective clean-up of complex matrices such as milk and milk products samples. In this way, QuEChERS has been checked elsewhere for the extraction of mycotoxins, plant toxins, pesticide and veterinary drug residues in food and feed, but to date, no work focused on the determination of phthalates in milk and milk products using QuEChERS has been published [29–31].

In this paper, we describe the development of an easy-to-use sample preparation based on QuEChERS for the simultaneous extraction of the 27 most important phthalates from milk (cow, fat content > 2%), milk beverages (cow, protein content > 0.7%) and yogurt (cow, fat content > 3%). Coupled with an optimized UHPLC/ESI Q-Orbitrap, this method was successfully applied on screening of phthalates in milk and milk products samples from local market.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade. Acetic acid, formic acid (FAc), ammonium formate, sodium acetate, sodium chloride and anhydrous magnesium sulfate ($MgSO_4$) were purchased from Sigma–Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (MeCN) and methanol (MeOH) were sourced from J.T. Baker (Deventer, Holland). BAKERBOND® octadecyl (C_{18}), bondesil primary secondary amine (PSA), and ceramic homogenizers obtained from Agilent Technologies (Harbor City, USA). Ultrafree-MC centrifugal filter devices (0.22 μm) of Millipore (Millipore, Brussels, Belgium) were used. Trifluoroacetic acid was obtained from Fluka (Buch, Switzerland). Ultrapure Water (resistivity, 18.2 MΩ) was purified on a Milli-Q Plus apparatus (Millipore, Brussels, Belgium).

Standards of dimethyl adipate (DMeP), dimethyl phthalate (DMP), bis(2-methoxyethyl) phthalate (DMEP), bis(2-ethoxyethyl) phthalate (DEEP), diethyl phthalate (DEP), diallyl phthalate (DAP), diisopropyl phthalate (DIPrP), dipropyl phthalate (DPrP), diphenyl phthalate (DPhP), dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), bis(2-butoxyethyl) phthalate (DBEP), dibenzyl phthalate (DBeP), benzyl butyl phthalate (BBP), dibutyl adipate (DBuP), bis-iso-pentyl ester (DIPP), dipentyl phthalate (DPP), dicyclohexyl phthalate (DCHP), bis(4-methylpentyl) phthalate (BMPP), dihexyl phthalate (DHXP), diheptyl phthalate (DHP), bis(2-ethylhexyl) adipate (DEeP), diisononyl-phthalate (DINP), dinonyl phthalate (DNP), diisodecyl-o-phthalate (DIDP), dioctyl phthalate (DNOP) and bis(2-ethylhexyl) phthalate (DEHP) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The purity of all references compounds were >98%.

Stock solutions of individual compounds were prepared in MeOH (1000 mg mL^{-1}) and stored at −20 °C in the dark. Then, a multicompound working standard solution at a concentration of 100 mg L^{-1} of each compound was prepared by combining suitable aliquots of each individual standard stock solution and diluting them with appropriate amounts of MeOH and stored in screw-capped glass tubes at −20 °C in the dark.

Special care was taken to avoid the contact of solvents and reagents with plastic materials. To minimize the risk of secondary contamination, glass materials were used in place of plastic materials. All glassware was cleaned prior to the analysis according to the recommendations specified in U.S. EPA Method 506. All solvents were checked for the presence of phthalates before use.

2.2. Instrumentation

The UHPLC/ESI Q-Orbitrap system consisted of an Accela 1250 LC pump and a CTC Analytics PAL open autosampler coupled with a Q Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The system was controlled by Exactive Tune 1.1 and Xcalibur 2.2 software (Thermo Fisher Scientific, San Jose, USA).

2.3. Analytical procedure

2.3.1. Sample preparation

After homogenization on a Polytron PT-2000 (Kinematica, Switzerland) for 30 s, 15.0 g of each sample (milk, milk beverages or yogurt sample) was weighed in glass centrifuge tube (50 mL), fortified with the 27 different phthalates and let to stand for 15 min. 10 mL volume of MeCN with 1% acetic acid was added as an extraction solvent and the tube was tightly capped and vigorously mixed for 1 min using a vortex (Scientific Industries, New York, USA) mixer at maximum speed. $MgSO_4$ (6 g), anhydrous sodium acetate (1.45 g) and ceramic homogenizers were added to the tube, to induce phase separation. After that, the tube was immediately shaken for 1 min, and then centrifuged for 5 min at 4000 rcf (relative centrifugation force) at 4 °C (Beckman Couler, Brea, USA). Then the upper layer (8 mL) was submitted to a dispersive SPE clean up with a mixture of 1.2 g of $MgSO_4$, 405 mg of PSA and 95 mg of C_{18}. The glass tube was vortexed for 1 min and centrifuged for 5 min at 4000 rcf at 4 °C. An aliquot of the final upper layer (200 μL) was transferred into a Mini-UniPrep vial, 300 μL MeOH and 500 μL 8 mM ammonium formate buffer were added. After the vial was capped, vortexed for 30 s. Finally the extract was taken for UHPLC/ESI Q-Orbitrap analysis.

Methods blanks were prepared in the same way using prescreened water instead of milk sample.

2.3.2. Experimental design for response surface methodology (RSM)

Response surface methodology (RSM) was employed to investigate the variations in recovery rates with respect to the preparation of conditions including extraction solvent volume, the amounts of sodium acetate, PSA, and C_{18}. The optimal composition of the four variables was determined by using a central composite design