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A simple and selective method for determination of phthalate biomarkers in vegetable samples by high pressure liquid chromatography–electrospray ionization-tandem mass spectrometry

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ABSTRACT

In the present study, solid-phase extraction cartridges including silica reversed-phase Isolute C_{18}, polymeric reversed-phase Oasis HLB and mixed-mode anion-exchange Oasis MAX, and liquid–liquid extractions with ethyl acetate, *n*-hexane, dichloromethane and its mixtures were compared for clean-up of phthalate monoesters from vegetable samples. Best recoveries and minimised matrix effects were achieved using ethyl acetate/*n*-hexane liquid–liquid extraction for these target compounds. A simple and selective method, based on sample preparation by ultrasonic extraction and liquid–liquid extraction clean-up, for the determination of phthalate monoesters in vegetable samples by liquid chromatography/electrospray ionisation-tandem mass spectrometry was developed. The method detection limits for phthalate monoesters ranged from 0.013 to 0.120 ng g^{-1}. Good linearity ($r^2 > 0.991$) between MQLs and 1000× MQLs was achieved. The intra- and inter-day relative standard deviation values were less than 11.8%. The method was successfully used to determine phthalate monoester metabolites in the vegetable samples.

1. Introduction

Phthalates are a class of synthetic compounds mainly used as non-reactive plasticisers in polyvinylchloride (PVC), polyvinyl acetates, cellulosics and polyurethanes, and as non-plasticisers in the manufacturing of cosmetics, paints, glues, photographic films, and insect repellents (Net, Sempéré, Delmont, Paluselli, & Ouddane, 2015; Staples, Peterson, Parkerton, & Adams, 1997). Due to widespread use of phthalate-containing products, phthalates have been detected in various environmental matrices (Adeogun et al., 2015; Hassanzadeh, Sari, Khodabandeh, & Bahramifar, 2014; Kim et al., 2015; Ma, Christie, Luo, & Teng, 2013; Meng et al., 2014; Morrison, Li, Mishra, & Buechlein, 2015; Wu et al., 2015; Zhang et al., 2015). Elevated levels of phthalates are ubiquitous in agricultural soils irrigated with treated wastewater, commonly called reclaimed or recycled water, and amended with biosolids (Kong et al., 2012; Niu, Xu, Xu, Yun, & Liu, 2014; Wang et al., 2015). Similar to other hydrophobic organic pollutants, phthalates may tend to accumulate in growing crops, potentially causing damage to the crops, and/or entering the food chain (Liao, Yen, & Wang, 2009; Ma et al., 2013; Meng et al., 2014; Wang, Fan, & Wang, 2015; Wu et al., 2013; Yin, Lin, Wang, & Zhang, 2003). Currently, the occurrence, fate, and potential exposure risks of phthalates in vegetable/soil systems are causing increasing concern (Net et al., 2015; Wang et al., 2015).

Once in the vegetables, phthalates are rapidly metabolised by hydrolysis to their corresponding monoester metabolites. These phthalate monoester metabolites are non-persistent with short half-lives, which make them ideal biomarkers of the vegetables' exposure to phthalates (Högberg et al., 2008; Marie, Vendittelli, & Sauvant-Rochat, 2015; Net et al., 2015). In assessing vegetable exposure to phthalates, it is essential to have a sensitive and selective analytical method that can quantify phthalate monoester metabolites in vegetable samples. Recently, high pressure liquid chromatography–electrospray ionisation (ESI)-tandem mass spectrometry (LC–ESI-MS/MS) using multiple reaction monitoring (MRM) has emerged as an effective method for the analysis of phthalate monoester metabolites (Net et al., 2015). To date, LC–ESI-MS/MS has been applied to the determination of phthalate monoesters in urine, hair and milk samples (Chang, Lin, & Chang, 2013; Högberg et al., 2008; Kim et al., 2015; Servaes et al., 2013). However, to the best of our knowledge, there is no reported

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analytical method utilising LC–MS/MS for the determination of phthalate monoester metabolites in vegetable samples.

For the determination of phthalate monoester metabolites in vegetable samples by LC–ESI-MS/MS, sample preparation, including extraction and clean-up procedures, is required, in order to isolate phthalate monoester metabolites from the vegetable samples, remove interfering compounds and achieve a sufficient sensitivity. Nowadays, several extraction techniques have been tested for plant samples, including Soxhlet extraction (Rodríguez-Solana, Salgado, Domínguez, & Cortés-Diéguez, 2014), ultrasound-assisted extraction (UAE) (Rezaie, Farhoosh, Iranshahi, Sharif, & Golmohamadzadeh, 2015; Zhou et al., 2014), pressurized liquid extraction (PLE) (Shang, Kim, & Um, 2014), accelerated solvent extraction (ASE) (Rodríguez-Solana et al., 2014; Wang, Fan, et al., 2015) and microwave-assisted extraction (MAE) (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015; Zhang et al., 2014). UAE is one of the upcoming extraction techniques that can offer high reproducibility in short times, simplified manipulation, reduced solvent consumption and temperature, and lower energy input (Rezaie et al., 2015). Ultrasonic cavitations create shear forces that disrupt cell walls mechanically and increase mass transfer processes. Moreover, there is no chemical reaction in UAE, which can prevent chemical degradation of target compounds (Zhou et al., 2014). However, the extraction efficiency of phthalate monoester metabolites in complex plant matrices depends on the extent to which they are associated with the pigments, lipids and other lipophilic matrix substances; the co-extraction of lipids and other lipophilic matrix substances can alter the ionisation efficiency of LC–ESI-MS/MS, which may lead to serious matrix effects (González-Curbelo, Herrera-Herrera, Ravelo-Pérez, & Hernández-Borges, 2012). Therefore, the clean-up of UAE extracts is a key procedure in the determination of phthalate monoester metabolites by LC–ESI-MS/MS. Solid-phase extraction (SPE) and liquid–liquid extraction (LLE) are the most common clean-up methods for phthalate monoester metabolites from biological samples (Chang et al., 2013; Itoh et al., 2009; Kim, Song, Choi, Lee, & Pyo, 2014; Monfort, Ventura, Balcells, & Segura, 2012). The objectives of this study were to (1) optimise, in terms of efficiency, the sample pre-treatment involved in UAE and SPE/LLE clean-up procedure, and (2) develop a simple and selective analytical method for the simultaneous determination of phthalate monoester metabolites in vegetable samples.

2. Experimental

2.1. Reagents and materials

Monomethyl phthalate (MMP), monoethyl phthalate (MEP), monoisobutyl phthalate (MiBP), mono-*n*-butyl phthalate (MnBP), monobenzyl phthalate(MBzP), mono-n-hexyl phthalate (MHP), mono-(2-ethylhexyl) phthalate (MEHP), mono-*n*-octyl phthalate (MnOP), d_4-mono-*n*-butyl phthalate (d_4-MnBP) and d_4-mono-2-ethylhexyl phthalate (d_4-MEHP) were purchased from CDN Isotopes, Inc. (Quebec, Canada). Isolute C_{18} (6 cm^3, 200 mg) SPE cartridges were obtained from Biotage (Uppsala, Sweden). Oasis HLB (6 cm^3, 200 mg), and Oasis MAX (6 cm^3, 200 mg) SPE cartridges were purchased from Waters (Milford, MA). HPLC-grade reagents, including acetone (Ac), *n*-hexane (Hex), acetonitrile (ACN), ethyl acetate (EtOAc), dichloromethane (DCM), methanol (MeOH), and formic acid (FA) were purchased from Sigma–Aldrich (St. Louis, MO). Milli-Q water, produced using a Milli-Q purification system (Millipore Corporation, Billerica, MA), extracted with DCM and Hex, respectively, and then redistilled. Laboratory glassware was soaked overnight in K_2CrO_7/H_2SO_4 solution, washed with tap water and redistilled water, baked at 300 °C for 12 h, and then rinsed with acetone, DCM and Hex, respectively.

2.2. Standard solution preparation

Individual stock standard solutions of the phthalate monoesters and d_4-MnBP were prepared at 0.1 mg mL^{-1} in MeOH or ACN. Mixed working solutions of the phthalate monoesters were prepared by dilution of the individual stock standard solutions in MeOH or ACN. All solutions were stored in darkness at −20 °C.

2.3. Biological material

Fresh cucumber (*Cucumis sativus* L.) was collected from suburban plastic film greenhouses in Guangzhou, China. The vegetable samples were weighed, cut into small pieces, homogenised, and then stored at −20 °C until analysis.

2.4. Sample extraction

The cucumber samples (10.00 g fresh weight (FW)) were weighed into 25-mL polytetrafluoroethylene centrifuge tubes, spiked with phthalate monoesters and d_4-MnBP, stored in dark at 4 °C overnight. Afterwards, the extraction solvents were added and samples placed in a 10 °C ultrasonic water bath (KH300SP, 25 kHz, 300 W; Kunshan Ultrasonic Instrument Co. Jiangsu, China) for 10 min. The organic phase was separated by centrifuging at 12,000*g* under 10 °C for 10 min. Three replicates were carried out. The combined extracts were evaporated to dryness. The following experiments were tested as clean-up protocols.

2.5. Sample purification

2.5.1. Liquid–liquid extraction

The extracted supernatants or the standard solutions were redissolved in 2.0 mL 0.50% (v/v) FA in water, extracted with 5.0 mL EtOAc, Hex, DCM and its solvent mixtures, respectively. The organic phases were collected. Three replicates were carried out. The combined extracts were evaporated to dryness, and reconstituted in 50 μL of mobile phase for LC–ESI-MS/MS analysis (Kim et al., 2014).

2.5.2. Oasis HLB cartridge

The extracted supernatants or the standard solutions were redissolved in 2.0 mL 0.5% (v/v) FA in water, loaded on Oasis HLB cartridges (6 cm^3, 200 mg; Waters Corp., Milford, MA) preconditioned with 2.0 mL MeOH and 2.0 mL water. The cartridge was washed with 1.0 mL of water and 1.0 mL 40% MeOH in water, and phthalate monoesters were eluted with 2.0 mL of ACN. The eluates were dried and reconstituted in 50 μL of LC mobile phase for LC–ESI-MS/MS analysis (Chang et al., 2013).

2.5.3. Oasis MAX cartridge

The extracted supernatants or the standard solutions were redissolved in 2.0 mL 0.50% (v/v) FA in water, loaded on Oasis MAX cartridges (6 cm^3, 200 mg; Waters Corp., Milford, MA) preconditioned with 2.0 mL ACN and 2.0 mL water. The cartridge was washed with 1.0 mL water and 2.0 mL ACN and phthalate monoesters were eluted using 2.0 mL 1.00% FA in ACN. The eluates were dried and reconstituted in 50 μL of LC mobile phase for LC–ESI-MS/MS analysis (Itoh et al., 2009).

2.5.4. Isolute C_{18} cartridge

The extracted supernatants or the standard solutions were redissolved in 2.0 mL 1.0% FA in ACN, loaded on Isolute C_{18} cartridges (6 cm^3, 200 mg; Biotage, Sweden) preconditioned with 2.0 mL MeOH and 2.0 mL water. The cartridge was washed with 2.0 mL water, and phthalate monoesters were eluted using 2.0 mL 1.0% FA in ACN. The eluates were dried and reconstituted