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Rapid screening of edible oils for phthalates using phase-transfer catalyst-assisted hydrolysis and liquid phase microextraction coupled to high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

Edible oil is easily contaminated with phthalic acid esters (PAEs). Conventional procedures to analyze individual PAEs require very rigorous experimental conditions that are extremely labor-intensive due to significant procedural contaminations generated by the ubiquitous presence of PAEs in the laboratory environment. In this study, a rapid screening method for PAEs in edible oil was successfully developed. Using a phase-transfer catalyst (terabutylammonium bromide) during oil/water biphasic base hydrolysis of PAEs, the hydrolysis time was decreased from a previously reported time of 20 h to 10 min (80 °C). The resulting phthalic acid in the acidified hydrolysate was extracted with 600 μL of tributyl phosphate and then analyzed by high performance liquid chromatography–tandem mass spectrometry in 6 min. Parameters affecting the hydrolysis of PAEs and the extraction of phthalic acid were optimized, and the analytical method was validated. No obvious matrix effect existed in the edible oils whether an external or internal standard method was used. The detection limit was 1.0 μmol kg^{-1}, and the quantification limit was 1.3 μmol kg^{-1}. The recovery rates varied from 86 to 107% with relative standard deviations equal to or lower than 9.9% in all of the tested conditions. Twenty-six samples were analyzed, and the background corrected total PAE content was found to be in the range of <LOD–52.1 μmol kg^{-1}. This fast and reliable method was not only a practical way to screen oil samples but can also be used as an indicator of false positive or overestimated results in conventional analysis of PAEs. In addition, it presents a new and promising methodology to deal with oil matrices.

1. Introduction

Phthalates are ubiquitous environmental chemicals, and some of them were found to be carcinogenic, mutagenic and antiandrogenic in rodents in the early 1970s [1]. Since then, potential adverse effects of PAEs on human health have been an academic and public focus. Several reviews have summarized that human exposure to PAEs may be associated with abnormal reproductive outcomes [2,3], development of asthma and allergies [4], obesogens [5], children's cognitive development and behavioral problems [6].

Studies have shown that diet is a significant source of human exposure to PAEs in addition to inhalation, dermal and endovenous routes [7–9]. PAEs characteristically have high log K_{ow} values >4 and therefore tend to be distributed mostly in oily foods. In Italy, Nanni et al. studied 172 vegetable oils and found that the highest levels of PAEs were from bis-(2-ethylhexyl) phthalate (DEHP, 7 mg kg^{-1}) and di-iso-nonyl phthalate (DINP, 5.5 mg kg^{-1}) [10]. Cavaliere et al. indicated that the most abundant PAE in olive oil was DEHP, which had a mean value of 1.67 mg kg^{-1} (n = 16) [11]. In the Czech Republic, Jarošovaí et al. reported 110.96 mg kg^{-1} di-n-butyl phthalate (DBP) and 20.46 mg kg^{-1} DEHP (n = 26) in soybean oil [12]. In China, Wu et al. [13] reported that the DBP content of 16.7% (5:30) of edible oil samples exceeded the specific migration limit (SML) of 0.3 mg kg^{-1} that was established in Directive 2007/19/EC of the European Union, while the DEHP content of 10% (3:30) of

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samples was above the regular migration limit of 1.5 mg kg^{-1}. We obtained a very similar result when detecting 28 edible samples using QuEChERS coupled with ionic liquid-based microextraction [14]. These assay findings showed that contamination of PAEs in edible oils is much more ubiquitous than in other foods, and therefore PAE detection represents a very important goal for consumer health and confidence.

The analysis of PAEs in oils is complicated because lipidic components are co-extracted with PAEs. Conventional methods of sample pre-treatment involve liquid–liquid extraction followed by solid phase extraction (SPE) or gel permeation chromatography (GPC) cleanup [11–13]. These methods have serious issues providing accurate quantification due to potential contamination of the laboratory environment, including the air, glassware, materials, instruments, consumables, reagents and solvents; the contamination often varies irregularly, resulting in false positive or overestimated results. Furthermore, in some cases blank values can be so high that trace amounts of PAEs are difficult to detect. Standard methods have strict requirements: highly purified organic solvents, salts baked at 500–700 °C for 8 h, specially cleaned glassware, conducting experiments in dust-proof rooms to avoid contamination of PAEs from the air, and repeated blank procedure analyses. These additional steps make conventional methods extremely labor-intensive and time-consuming. Despite the strict conditions, the procedural blank cannot be completely diminished because some of the secondary contaminations are random and cannot be simulated by blank tests. Guo et al. summarized potential PAE contamination sources and suggested that liquid–liquid extraction methods can substantially decrease background levels compared with GPC or SPE [15]. Marega et al. discussed blank problems related to the syringe needle [16]. Although some simple and miniaturized techniques, such as direct injection of diluted oil into GC–MS [10], headspace solid-phase microextraction (HS–SPME) [17] and multiple hollow fiber liquid-phase microextraction (MHF–LPME) [18] have been introduced, these methods are not widely accepted outside the academic field. By far, accurate analysis of PAEs in edible oil remains a challenging task.

In industry, various isomers of PAEs are produced by reacting phthalic acid (PA) with mixtures of alcohols, typically from methanol up to tridecanol, and are used in different products. Despite that 16 PAEs have been specified to be analyzed in edible oil [19], some other PAEs may exist but are as yet unknown due to the lack of corresponding standard compounds. There is no feasible way to detect all of the PAEs in edible oil.

In view of the issues mentioned above, the analysis of total phthalate is a good way to preliminarily assess total PAE contamination, to screen oil samples before conducting a more laborious and expensive standard method, and to ascertain the likelihood of false positive or overestimated results.

Methods that are based on alkaline hydrolysis of PAEs to PA followed by PA esterification to DMP prior to GC analysis [20] or acid hydrolysis to PA prior to SPE–HPLC–MS/MS analysis have been developed to detect total phthalate in urine [21]. Both of these procedures are not applicable to fatty samples. Currently, only one method has been reported that is compatible with fatty foods, and this method requires 20 h for base hydrolysis of PAEs to free PA [22]. Clearly, this method is not a practical solution to handle a large number of real samples.

In this context, the aim of this work was to develop a rapid and reliable procedure to screen edible oils for PAEs by measuring total PAEs. To achieve this goal, the most difficult task was decreasing the hydrolysis time of PAEs contained in oil matrices. However, due to their highly hydrophobic characteristics, PAEs tend to be partitioned in the oil phase rather than in the alkaline water phase, thus severely suppressing hydrolysis. In organic chemistry, a phase-transfer catalyst (PTC) is a powerful tool to accelerate chemical reactions of mutually insoluble species (hydrophobe and hydrophile) in the presence of an oil–water interface, in conjunction with high selectivity to the desired product, mild conditions and decreased energy consumption [23]. However, there is scarcely any literature that describes sample preparation methods [24].

Herein, PAEs of edible oil were hydrolyzed directly with the assistance of a PTC, which rapidly converted the oil matrix into an aqueous matrix and converted PAEs into PA simultaneously. In addition, molecular complex-based liquid-phase microextraction (LPME), a simple and environmentally friendly technique for extracting highly polar compounds [25], was used to directly recover PA in the acidized hydrolysate before HPLC–MS/MS analysis. Parameters affecting the hydrolysis of PAEs and the efficiency of LPME were systematically examined to achieve the best analytical performance. Once optimized and evaluated, the method was applied to detect PAEs in twenty-six edible oils collected locally.

2. Experimental

2.1. Chemicals and reagents

Phthalic acid (PA), PA-d_4 (IS), dimethyl phthalate (DMP), di-n-butyl phthalate (DBP), bis(2-ethylhexyl) phthalate (DEHP), di-n-decyl phthalate (DDP), and di-iso-decyl phthalate (DIDP) were of high purity grade (>99%). DMP, DEHP, DDP and DIDP were purchased from Aladdin Chemistry (Shanghai, China). DBP, PA and PA-d_4 were purchased from Sigma Aldrich Chemical (St. Louis, MO, USA).

Individual stock solutions of the standards and the IS were prepared by dissolving the correct amount of each analyte to 0.5 mg mL^{-1} in hexane except for PA and PA-d_4, which were prepared in methanol. Working standard solutions were prepared from the individual solutions after appropriate dilution with the same solvent. All of the solutions were stored at −20 °C.

Tetrabutylammonium bromide (TBAB) (>99%), 1-octanol (99.5%), tetrabutylammonium chloride (TBAC) (>97%), hexadecyltrimethyl ammonium bromide (CTAB) (99%), formic acid (99%) and acetic acid (99.5%) were purchased from Aladdin Chemistry (Shanghai, China). 1-Hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$) was purchased from Cheng Jie Chemical (Shanghai, China). Tributyl phosphate (TBP) (≥98.5) and diethyl carbonate (DEC) (>99%) were purchased from Sinopharm (Beijing, China). Analytical-grade hydrogen chloride (36–38%) and potassium hydroxide (≥99.99%) were provided from Tianjin BODI Chemical Reagent (Tianjing, China). HPLC–grade methanol, ethanol, acetonitrile, acetone and hexane were purchased from Kemiou Chemical Reagent (Tianjin, China).

2.2. Chromatographic instruments and apparatus

Chromatographic analysis to optimize the hydrolysis parameters was performed on a Shimadzu Prominence LC-20A (Kyoto, Japan) equipped with two LC-20AT pumps, a 7725i manual sample injector and a SPD-M20A photodiode array detector (DAD). The HPLC system was operated by LC-Solution software (version 1.25). Separations were conducted on a GL Sciences Inertsil ODS-3 column (250 mm × 4.6 mm i.d., 5 μm). A mixture of aqueous acetic acid (1.0%) and methanol (30/70, v/v) at a flow rate of 1 mL min^{-1} was used as the mobile phase in isocratic elution mode. The injection volume was 20 μL. The DAD detector (190–800 nm) was used to identify target compounds in real samples by comparison of the spectrum, with a wavelength of 240 nm for quantitative analysis.

Chromatographic analyses for optimizing the LPME parameters, validating the method and detecting real samples were