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Determination of phthalate esters in vegetable oils using direct immersion solid-phase microextraction and fast gas chromatography coupled with triple quadrupole mass spectrometry

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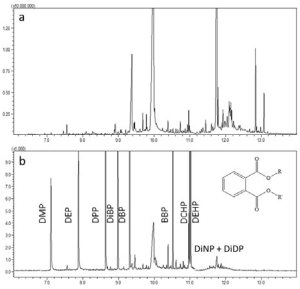
HIGHLIGHTS

- A comparison of different fibers, namely PDMS and Carbopack Z/PDMS, was performed.
- A comparison of head-space and direct immersion extraction mode was performed.
- A fast GC-QqQ method was optimized for phthalates determination in vegetable oils.
- The final DI-SPME-GC-QqQ MS method was fully validated.

GRAPHICAL ABSTRACT



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ABSTRACT

Phthalates are a group of synthetic compounds mainly used as plasticizers, which have been classified as endocrine-disrupting chemicals and potential human-cancer causing agents. They can be found in high amounts in foods, deriving mainly from plastic packaging. The analytical determination of these compounds is very challenging since they are ubiquitous. Therefore, minimization of sample manipulation is highly desirable.

The present work exploited the application of a solid-phase microextraction method for the analysis of phthalates in vegetable oil. A preliminary comparison between a polydimethylsiloxane (PDMS) and a Carbopack Z/PDMS fiber was carried out both in the headspace and direct immersion extraction modes. Before immersing the fiber, a rapid liquid–liquid extraction was performed using acetonitrile to remove the bulk of triglycerides. PDMS in the direct immersion mode showed the best performance. The method was fully validated obtaining a good linearity with a coefficient of correlation of over 0.9960 for all compounds, repeatability and accuracy values generally better than 10%, and very good limit of quantification values.

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1. Introduction

Phthalic acid esters (PAEs), commonly known as phthalates, are a group of organic and synthetic chemical compounds applied in a wide range of industrial areas according to their physico-chemical characteristics, which are mainly affected by the structure of the side chains. PAEs are widely used as film-forming agents, solvents and denaturants, and especially as plasticizers to make plastic stronger and more flexible. PAEs are not chemically bound to the polymeric matrix, but they are present as a freely mobile and leachable phase; therefore, they can easily migrate into food or water, or evaporate into the environment. Bis(2-ethylhexyl) phthalate (DEHP) is the most frequently employed PAE and it is highly ubiquitous, also due to its persistent character. DEHP, along with diisobutyl phthalate (DiBP) and dibutyl phthalate (DBP) are the most commonly detected in the air [1–4] and foods [5–8], even though, in recent years, two higher-molecular-weight isomer mixtures, namely diisononyl (DiNP) and diisodecyl (DiDP) phthalates, have been used more intensively to replace the above-mentioned PAEs, in particular DEHP [9].

PAEs and their metabolites are classified as endocrine-disrupting chemicals and are suspected human-cancer causing agents; furthermore, they have been linked to adverse health effects particularly in relation to early life exposures [10–14]. Due to their toxicity, PAEs have been included in the priority list of pollutants in several countries [15]. Furthermore, the European Food Safety Authority (EFSA) defined a tolerable daily intake (TDI) for certain PAEs between 0.03 and 0.20 mg kg^{-1} of body weight [16].

Among the different sources of human exposure, foods play an important role [17]. In particular, direct migration from packaging films is one of the main sources, especially into fatty foods due to the lipophilic nature of PAEs [5,18]. However, the content of PAEs in food has not been regulated yet; the only regulations in force today refer to the migration of PAEs from food contact materials (Directive 2007/19/EC; China GB 9685-2008 Hygienic Standard), and establish a list of substances allowed in such materials.

From an analytical viewpoint, PAEs pose several problems, mainly related to the cumbersome operations needed to minimize secondary contamination that may occur in any step of the analytical procedure, including sampling, sample preparation and chromatographic analysis, possibly leading to over-estimated contamination levels. Only a few groups have discussed such a problem in detail, highlighting that PAEs are commonly present in the laboratory environment, especially in the air, organic solvents, chemicals, adsorbed on glassware and other devices (*e.g.* syringes, in particular with Teflon-capped plungers) used for analysis, as well as in the final analytical instrument (*e.g.* carrier gas, ferrules, liners, etc.) [19,20]. A frequent assessment of blanks is readily suggested and the limit of quantification (LOQ) has to account for such an unavoidable level. Moreover, sample preparation should be as simple as possible to avoid further contamination. Many methods have been proposed to analyze PAEs in foods and vegetable oils, mainly followed by a gas chromatography-mass spectrometry (GC-MS) determination. Dugo and co-workers [21] presented a simple method based on a liquid–liquid extraction (LLE) of vegetable oil samples with 1 + 1 mL of acetonitrile (ACN), where the extract was directly injected in a GC-MS system, obtaining LOQ values slightly lower than 0.5 mg kg^{-1}. Shortly after, Mariani and co-workers [22], starting from the same extraction procedure (oil extracted with 2 mL of ACN), added further purification steps, namely a purification on alumina to remove the free fatty acids interference and solid-phase extraction with silica gel to remove the triacylglycerols (TAGs). The final determination was carried out in a GC-MS system. Cavalieri et al. developed a gel permeation chromatography clean-up method, followed by GC-tandem MS (MS/MS), to investigate the potential of MS/MS for the unequivocal confirmation and accurate quantification of PAEs at low LOQ levels in fatty matrices [18]. Fankhauser-Noti and co-workers [23] proposed a very straightforward method for the analysis of oils and fatty extracts by direct injection of a diluted oily solution, without preliminary extraction and clean-up steps, into a programmed-temperature vaporizer injector. The injector was heated to 260 °C to transfer PAEs into the separation column, but leaving the high boiling components in the inlet and avoiding their entrance into the analytical column. After the transfer, a backflush system allowed the automatic cleaning of the pre-column and the inlet. Although the method was very effective, with very satisfactory LOQ values, it required a modification of the traditional GC injector and a thorough washing of the inlet liner after about 50 mg of injected oil.

Solid-phase microextraction (SPME) has been investigated to minimize sample manipulation in the preparation step for PAEs analysis in vegetable oils. In particular, Rios and co-workers [24] exposed different SPME fibers to the headspace (HS) of 1 g of olive oil heated at 250 °C. Polydimethylsiloxane (PDMS) fiber proved to be the most selective fiber, even though the high operation temperature negatively affected its durability. Holadová and co-workers [25] used a PDMS fiber to extract PAEs from the HS of 1 g of vegetable oil (modified with methanol) at 40 °C, obtaining LOQ values of about 0.5 mg kg^{-1} for 6 PAEs.

The aim of the present work was to optimize a rapid and simple SPME method, to minimize sample manipulation, followed by a fast GC-triple quadrupole (QqQ) MS determination. The starting point was the method proposed by Halodová and co-workers [25] performing SPME in the HS mode. A comparison between the performance obtained using a Carbopack Z/PDMS (used with satisfactory results in previous works for polycyclic aromatic hydrocarbon extraction from vegetable oils [26–29]) and a PDMS fiber both in HS and direct immersion (DI) extraction modes was carried out. The latter extraction mode was chosen and optimized, introducing a simple LLE step to reduce TAG interferences.

2. Materials and methods

2.1. Chemicals and materials

HPLC-grade ACN was provided by Baker (USA), acetone and *n*-hexane (*n*-Hex) were from AppliChem (Germany). Dimethyl phthalate (99%, DMP), diethyl phthalate (99.5%, DEP), dipropyl phthalate (pestanal®, DPP), diisobutyl phthalate (TraceCERT®, DiBP), dibutyl phthalate (99%, DBP), benzylbutyl phthalate (>98%, BBP), dicyclohexyl phthalate (99%, DCHP), dietylhexyl phthalate (pestanal®, DEHP), diisononyl phthalate (99%, DiNP), diisodecyl phthalate (>98%, DiDP) were purchased from Sigma–Aldrich/Supelco (Bellefonte, USA). A stock solution (10,000 mg L^{-1}) was prepared mixing all the previously mentioned PAEs in *n*-Hex and stored at 4 °C. Since edible oil or fat standards, certified for plasticizers, were not commercially available, a sample of extra virgin olive oil (EVO) with minimal PAEs content was spiked with 2 mg kg^{-1} of the PAEs standard solution, and it was used for SPME method optimization. Solvents were checked for the presence of PAEs every day, all the glassware was rinsed with acetone and ACN, and kept at 100 °C before use. Special care was taken with vials, caps and septa before use. The chromatographic system was initially and regularly checked for the absence of PAEs by running blank injections.

2.2. Samples

Eight vegetable oils (2 EVOs, 1 olive oil, 1 peanut oil, 2 sunflower oils, 1 soybean oil, 1 mixed seed oil) were purchased from a local