Journal of Chromatography A, 1456 (2016) 196–204

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Solution for blank and matrix difficulties encountered during phthalate analysis of edible oils by high performance liquid chromatography coupled with tandem mass spectrometry

Adam Vavrouš[a,b,*], Jana Pavloušková[a], Václav Ševčík[a,b], Karel Vrbík[a], Radomír Čabala[b,c]

[a] National Institute of Public Health, Unit for Chemical Safety of Products, Srobarova 48, 100 00 Prague 10, Czech Republic
[b] Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Albertov 6, 128 43 Prague 2, Czech Republic
[c] Institute of Forensic Medicine and Toxicology, First Faculty of Medicine, Charles University in Prague and General University Hospital, 121 08 Prague 2, Czech Republic

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 3 June 2016
Accepted 3 June 2016
Available online 4 June 2016

Keywords:
Phthalates
Edible oils
Solid phase extraction (SPE)
High performance liquid chromatography (HPLC)
Tandem mass spectrometry (MS/MS)
Blank problems

ABSTRACT

Worldwide production of phthalates has led to their undesirable presence in the food chain. Particularly edible oils have become an area of growing concern owing to numerous reported occurrences of phthalates. The analytical methods used in this field face difficulties associated mainly with matrix complexity or phthalate contamination which this study has aimed to describe and resolve. The proposed procedure consisting of liquid-liquid extraction, solid phase extraction and high performance liquid chromatography coupled with tandem mass spectrometry allowed us to analyze simultaneously 6 individual phthalates and 2 phthalate isomeric mixtures. DSC−18 SPE phase was selected for cleanup owing to the most efficient co-extract removal (assessed using high resolution mass spectrometry). Several sources of phthalate contamination were identified, however, the mobile phase was the most serious. The key improvement was achieved by equipping a contamination trap, a 50-mm reverse phase HPLC column, generating a delay between target and mobile phase peaks of the same compounds. RSDs ranging between 2.4 and 16 % confirm good precision and LOQs between 5.5 and 110 $\mu g\,kg^{-1}$ reflect satisfactory blank management. With up to 19 occurrences in 25 analyzed edible oil samples and levels up to 33 $mg\,kg^{-1}$, bis(2-ethylhexyl), diisononyl and diisodecyl phthalates were the most important contaminants.

1. Introduction

Diesters of phthalic acid (phthalates) are high production volume chemicals finding use as plasticizers and solvents in a vast range of applications. The main drawback of their worldwide use is that they have been associated with a number of serious health problems [1]. Due to their presence in the environment, food and products of everyday use, there are many human exposure paths, however, diet is considered the major one [2]. Phthalate contamination of food and beverages has been frequently attributed to food contact materials (FCMs), e.g. PVC tubing and gaskets, packaging films, paper and board, PET bottles [3], which are capable of releasing phthalates into contacted foodstuffs. Specific migration limits (SMLs) have been set in Europe for selected phthalates migrating from plastic FCMs [4]. Owing to the lipophilic character of phthalates, fatty commodities are especially susceptible to phthalate migration and have become an area of great concern, which inspired us to survey edible oils on the Czech market.

This analytical task has always been challenging. Raw extracts in organic solvents typically undergo a treatment by lowered temperature [5], liquid-liquid extraction (LLE) by *n*-hexane [6], solid phase extraction (SPE) [7–9], dispersive solid phase extraction (dSPE) [10,11], dispersive liquid-liquid microextraction (DLLME) [10], gel permeation chromatography (GPC) [12], or online SPE [13] in order to remove present fat. Some studies attempted to straightforward sample handling by developing a headspace-solid phase microextraction (SPME) technique [14–16] or thermal desorption of diluted oil sample in a GC injection port [17,18].

Phthalate contamination and the corresponding raised procedural blanks also frequently cause problems. There are numerous sources of phthalates contributing to sample contamination during analysis, e.g. disposable plastic items, organic solvents or sorbents [2]. An injector or a gas supply were also reported to introduce considerable amounts of phthalates during a GC analysis [19]. Even if

* Corresponding author at: National Institute of Public Health, Unit for Chemical Safety of Products, Srobarova 48, 100 00 Prague 10, Czech Republic.
E-mail address: adam.vavrous@szu.cz (A. Vavrouš).

http://dx.doi.org/10.1016/j.chroma.2016.06.014
0021-9673/

phthalate-free materials are used and analytical system contamination is eliminated, phthalate vapors present in the laboratory air are still capable of contaminating all surfaces including glassware, plastic items or a GC autosampler syringe needle [20], so these must typically undergo routine cleaning procedures. Unfortunately, it remains unclear, which role phthalate contamination plays in HPLC and what are the measures to avoid it. Admittedly, GC represents the dominant determination tool nowadays since it provides better chromatographic resolution of individual phthalates than HPLC. However, when it comes to analysis of isomeric mixtures of phthalates, broadened and overlapped elution zones constituted of partially separated isomers are typically observed in GC. By contrast, chromatographically separated peaks of isomeric mixtures can be achieved by using reverse phase HPLC [21]. MS detection can generally provide additional specificity to chromatographic techniques. In case of GC–MS (electron impact ionization), all phthalates form the same base peak (m/z 149) and a number of other non-specific ions. Selective determination is only possible for the price of monitoring MS signals of low relative intensity. Such limitation does not occur under electrospray ionization conditions where each isomeric mixture forms a specific base MS peak corresponding to $[M+H]^+$.

Because of the reasons discussed above, HPLC–MS/MS was preferred to GC–MS for the analysis of 2 isomeric mixtures and 6 individual phthalates in this study. Procedural blanks encountered during our initial experiments indicated that there is serious phthalate contamination in our laboratory, so our subsequent research focused mainly on investigating and eliminating the contamination sources. This paper attempts to propose means of handling phthalate contamination in HPLC and summarize them into an optimal method. Although there are many cleanup strategies for fatty matrices, this study attempted to find a balance among cleanup efficiency, cost and complexity by comparing SPE, dSPE and freezing approaches. High-resolution time-of-flight mass spectrometry (HR-TOF-MS) allowed us to effectively control matrix co-extracts within a sample workflow.

2. Materials and methods

2.1. Materials and equipment

Edible oils ($n = 25$) were purchased from local stores in the Czech Republic for monitoring purposes. The samples were stored in original polyethylene terephthalate (PET) or glass bottles. Diethyl phtalate (DEP), dibutyl phthalate (DBP), diisobutyl phthalate (DiBP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), dioctyl phthalate (DnOP), all neat or >99 % purity compounds, were purchased from Sigma-Aldrich or Dr. Ehrenstorfer. Diisononyl phthalate (DiNP, CAS 28553-12-0) and diisodecyl phthalate (DiDP, CAS 26761-40-0) referring to isomeric mixtures of C_9- and C_{10}-dialkyl phthalates, respectively, were obtained from Sigma-Aldrich. Labeled diethyl phtalate-3,4,5,6-d4 (DEP-d4, Sigma-Aldrich) served as an internal standard. High purity grade solvents (Sigma-Aldrich) were double redistilled before use in order to reduce phthalate contamination. Water was purified using Purelab Classic system (ELGA LabWater). Eluent additive grade ammonium acetate (Sigma-Aldrich) was used as supplied. The individual stock solutions of phthalates (1 mg mL^{-1}) were prepared in methanol. The working mixture of analytes (1 μg mL^{-1}), the internal standard solution (10 μg mL^{-1}) and calibration standards (1–300 ng mL^{-1}) were prepared in acetone-methanol 1:4 (v/v).

Glassware, e.g. solvent storage flasks and autosampler vials, was baked at 400 °C for 4 h. All equipment coming into direct contact with the samples, including 15-mL polypropylene extraction tubes, SPE column needles and tubes for sample extracts, was rinsed with double redistilled acetone-methanol 1:2 (v/v) followed by methanol. The same solvents were used for conditioning of SPE cartridges.

2.2. Sample preparation

About 2 mL of oil sample were precisely weighed into the extraction tube. 100 μL of internal standard solution and 6 mL of acetone-methanol 1:2 (v/v) were added and the sample was extracted at 300 r min^{-1} for 5 min using ES-60 incubator shaker (Hangzhou Miu Instruments Co.) at room temperature. After centrifugation (5000 r min^{-1}, 3 min), the raw extract (the upper layer) was subjected to various cleanup experiments (Section 2.3). The optimum cleanup was designed as follows: A 3-mL DSC–18 cartridge (500 mg bed weight, Sigma-Aldrich) was preconditioned using 3 mL of acetone-methanol 1:2 (v/v) and 3 mL of methanol. Then, while collecting the eluent at atmospheric pressure into a glass vessel, 3 mL of raw extract were transferred into the cartridge and 2 mL of methanol were used to completely elute the analytes. The resulting extract (acetone-methanol 1:4, v/v) was ready for HPLC analysis. Concentration and solvent exchange steps were avoided in order to eliminate possible sources of phthalate contamination.

2.3. Cleanup experiments

Freezing, dSPE and SPE strategies were studied for cleaning of edible oil extracts obtained by the LLE procedure (Section 2.2). Cleanup evaluation was based on both analyte recoveries and matrix compound removal (assessed by ESI–HR-TOF-MS, Section 2.5). Freezing experiments were conducted in a refrigerator at −18 °C. After 1 h the liquid phase was removed from the solid precipitate and analyzed.

C18-modified silica, Supelclean ENVI-Carb Supel QuE Z-Sep (Sigma-Aldrich) and EMR-Lipid (Agilent Technologies) sorbents were evaluated in the dSPE experiments. Raw sample extracts (2 mL) were added into polypropylene tubes containing 100 mg of sorbent, the tubes were capped and hand shaken for 1 min. The extracts were ready for analysis after centrifugation (5000 r min^{-1}, 3 min, Universal 320 R centrifuge, Hettich).

The SPE experiments aimed to evaluate the same sorbents in the form of 6 mL SPE cartridges. Supelclean ENVI-Carb and Discovery DSC–18 SPE cartridges (volume 6 mL, bed weight 500 mg) were supplied by Sigma-Aldrich. Z-Sep and EMR-Lipid cartridges were prepared by packing 500 mg of Supel QuE Z-Sep and EMR-Lipid sorbents into 6-mL empty polypropylene cartridges. Cartridge conditioning was performed using 6 mL of acetone-methanol 1:2 (v/v) followed by 6 mL of methanol. Raw sample extracts (6 mL) were loaded while collecting the eluent at atmospheric pressure. Additional 4 mL of methanol were added in order to completely elute analytes from the cartridge.

2.4. HPLC–MS/MS analysis of phthalates

A system consisting of Agilent 1290 HPLC, Jetstream electrospray ionization (ESI) source and Agilent 6490 triple quadrupole MS/MS (Agilent Technologies) was employed for HPLC analyses. Our experiments evaluated several solid core reverse-phase columns, including Kinetex 5 u C18 100A and Kinetex 5 u Phenylhexyl 100A columns (50 mm long, 2.1 mm i.d., 5.0 μm particle size, Phenomenex), Kinetex 2.6 u Phenyl-hexyl 100A and Kinetex 2.6 u Biphenyl 100A columns (150 mm long, 3.0 mm i.d., 2.6 μm particle size, Phenomenex) and a Poroshell 120 EC-C18 column (150 mm long, 3.0 mm i.d., 2.7 μm particle size, Agilent Technologies). Mobile phase consisting of (A) 2 mmol L^{-1} ammonium acetate in ultrapure water and (B) 2 mmol L^{-1} ammonium acetate in double redistilled