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Analytical Methods

Determination of phthalate sum in fatty food by gas chromatography

Ivan Ostrovský [a], Radomír Čabala [b], Róbert Kubinec [a,*], Renáta Górová [a], Jaroslav Blaško [a], Janka Kubincová [c], Lucie Řimnáčová [b], Wilhelm Lorenz [d]

[a] Comenius University, Faculty of Natural Sciences, Institute of Chemistry, Mlynská dolina CH-2, Bratislava 84215, Slovak Republic
[b] Charles University in Prague, Faculty of Science, Department of Analytical Chemistry, Albertov 6, Prague 2, Czech Republic
[c] Technological Department Biocentre, Food Research Institute, Kostolná 5, Modra, Slovak Republic
[d] Martin-Luther University, Halle-Wittenberg, Department of Chemistry, Institute of Analytical and Environmental Chemistry, Halle, Saale, Germany

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ABSTRACT

Presented method for determination of sum of phthalates is based on their alkaline hydrolysis to phthalic acid at 80 °C for 20 h, followed by the selective extraction of lipophilic interferents from acidified hydrolysate at pH 1 with *n*-hexane. Phthalic acid is derivatized to dimethyl phthalate (DMP) with diazomethane in aqueous-chloroform two-phase system. Resulting DMP is absorbed in chloroform and determined by GC-FID. Method calibration resulted in LOD and LOQ of 0.4 (2.1) and 1.2 (6.2) $\mu g\,g^{-1}$ (nmol g^{-1}) DMP, respectively. Real samples of Baltic herring and codfish, butter, pork, goose and duck fats, sunflower, olive, rapeseed and linseed oils were analysed and the background corrected total phthalates content was found in the range from not detected level in duck fat to 12.5 (64.3) $\mu g\,g^{-1}$ (nmol g^{-1}) in butter, respectively.

1. Introduction

Phthalates (PHTs) are by far one of the most ubiquitous chemicals worldwide including environment. Over one million tons of PHTs are produced in Western Europe each year therefore they are regular part of environment and human food chain as well. The food control is limited to some aspects and chemical analysis and toxicological evaluation are often the bottleneck (Grob, 2008). The legislative requirements are often limited to only several mostly used PHTs such as di-2-ethylhexyl phthalate (DEHP), di-*n*-butyl phthalate (DBP), di-*n*-octyl phthalate (DOP) and butylbenzyl phthalate (BBP) in water, food and selected commercial products (Council Directive 88/378/EEC, 1988; Council Regulation EEC 793/93, 1993; Directive 2005/84/EC, 2005; Phthalates Information Centers Europe and USA, 2008). Risk assessments of only several PHTs have been completed where those for diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) show no risks to human health or environment for any current use. Risk assessments for other PHTs remain open as scientific data are still being considered (Official Journal of the European Union, 2006). In other words, there is still a need for their monitoring in the environment and especially in human food chain.

Industry uses mixtures of PHTs in order to provide a wide range of different properties for different uses (Staples, Peterson, Parkerton, & Adams, 1997). Thousands of PHTs isomers are produced by reaction of 1,2-benzenedicarboxylic (ortho-phthalic) and 1,4-benzenedicarboxylic (terephthalic) acid with a mixture of alcohols, typically from methanol up to tridecanol.

There is basic discrepancy between the legislative requirements to determine concentration of several selected PHTs and the concentration of all presented PHTs if their industrial mixtures are applied. Such requirement would underestimate the real content of PHTs. GC method which would determine all or at least several tenths of major PHTs individually would be instrumentally very complicated, laborious and expensive. There is an evident need for analytical procedure which would determine all phthalate forms in one run. Majority of publications deals with phthalate determination in water (Ballesteros, Zafra, Navaloon, & Vilchez, 2006) or biological fluids (Kato, Silva, Needham, & Calafat, 2005), articles about PHTs in samples with fatty matrices (Cortazar et al., 2005; Feng, Zhu, & Sensenstein, 2005) are less frequent. Many kinds of extraction techniques of PHTs from matrices are described in the literature (Cao, 2008; Cortazar et al., 2005; Li, Cai, Shia, Moua, & Jiang, 2008; Psillakis & Kalogerakis, 2003; Tienpont, David, Dewulf, & Sandra, 2005).

Development of a single GC method for determination of all phthalates in fatty matrices is the goal of this work. Presented method could be applied as preliminary step of quality control for assessing total contamination with PHTs before a more detailed and targeted PHTs analysis. The method is based on alkaline hydrolysis of all phthalates to phthalic acid (PA) followed

* Corresponding author. Tel.: +421 2 602 96 330.
E-mail address: kubinec@fns.uniba.sk (R. Kubinec).

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by selective removal of lipophilic substances and derivatization of PA to dimethyl phthalate and its determination by GC-FID method. Idea to convert all PHTs to PA is not new. Albro, Jordan, Corbett, and Schroeder (1984) published method for total phthalate determination in urine after hydrolysis of PHTs and their metabolites to PA followed by PA esterification to DMP and GC determination. Kato et al. (2005) published isotope-dilution HPLC–MS–MS method of PHTs determination in urine after acidic hydrolysis of PHTs to PA. Both methods are not applicable to samples with high fat content. Innovation in presented method is selective removal of fatty matrix from PA. Fatty matrices have been selected because of the accumulative tendency of phthalates in fats which are an important part of human food chain. Additionally, the determination of low concentration of phthalates in the presence of excess of fats is a challenge for any analytical chromatographer.

2. Materials and methods

2.1. Materials and instrumentation

Methanol (pure) and HCl (p.a.) were from Lachema (Brno, Czech Republic). *n*-Hexane (freshly distiled p.a.), chloroform (p.a.), NaOH and KOH (both p.a.), ethylenglycoldimethylether and diethylether (both p.a.) were purchased from Merck (Darmstadt, Germany). Diazald (N-nitroso-N-methyl-4-toluensulfonamid, 99%) was delivered by Aldrich (St. Louis, MO, USA). pH indicator crystal violet was purchased from Sigma–Aldrich (St. Louis, MO, USA). Dimethyl phthalate (99%, DMP), dibutyl phthalate (99%, DBP) and di-2-etylhexyl phthalate (99%, DEHP) standards were from Lachema (Brno, Czech Republic), whereas didecyl phthalate (for GC, DDP) and diethyl phthalate (for GC, DEP) standards were obtained from Becker Delft N.V. (Delft, Netherland). Hydrogen (electrolytic purity) and helium (99.996%) for GC were from Messer (Bratislava, Slovak republic). Real samples (frozen Baltic herring, codfish in mayonnaise, butter, pork fat, goose fat, sunflower oil, olive oil, rapeseed oil, and linseed oil) were purchased in the local supermarket prepacked in plastic. The duck fat was obtained baking the duck derived from local small-scale farmer in stainless steal baking pan with no possibility of phthalate contamination during the last week of living and preparation.

A GC 6890N from Agilent Technologies (Waldbronn, Germany) equipped with autosampler, split/splitless injector and FID was used for analyses. A GC 6890N equipped with a 5973 N mass-selective detector (Agilent Technologies, Waldbronn, Germany) was used for DMP identification in model samples. The carrier gas was He at inlet pressure of 60 kPa. Temperature of injector and detector was 280 and 320 °C, respectively. Samples of 1 μL volume were injected in splitless mode for 1 min. Temperature program starting at 40 °C with the ramp of 20 °C min^{-1} to 320 °C was used for phthalate separation. DB 5 MS column of 30 m length, 0.25 mm I.D. and 0.25 μm film thickness from J&W Scientific (Agilent Technologies, Palo, Alto, CA, USA) was used. The flows of air and hydrogen for FID were 340 and 40 mL min^{-1}, respectively.

Explorer Pro model EP 114C (Ohaus, Pine Brook, NJ, USA) analytical weights, mechanical shaker Rotamax 120 (Heidolph, Schwabach, Germany) and Labofuge 200 centrifuge (Heraeus, Buckinghamshire, UK) were used for sample and standard preparation. Two and 4 mL vials closed with PTFE/silicon septa from Agilent Technologies (Palo Alto, CA, USA) were used for analyses and 12 mL glass vial closed with PTFE/silicon septa (Fischer Sci., Pittsburgh, PA, USA) was used for sample preparation. For addition of liquids either syringes of 10, 100, 250 and 500 μL volumes (Hamilton, Sidney, Australia) or micropipettes of 100 and 250 μL volumes (Merck, Darmstadt, Germany) were used.

All laboratory glassware was carefully cleaned by conventional glassware cleaner and heated overnight at 550 °C in oven before use. The cleaned glassware was wrapped into Al-foil to prevent any contamination before analysis.

2.2. Sample preparation

Approximately 1 g of homogenised sample was weighed in 12 ml glass vial and 4 mL of chloroform–methanol mixture (2/1, v/v) was added. Vial was closed and shaked for 1 h at 2 Hz frequency on mechanical shaker. The vial content was centrifuged for 2 min at 1000 RPM to remove any particulates and 2 mL of the clear supernatant (chloroform) was transferred to clean 4 mL vial. To remove methanol from chloroform fraction 1 mL of NaCl solution (9%, w/v) was added to the supernatant and the vial was slightly shaked. After the separation of two fractions the lower chloroform phase was transferred into 4 mL vial and evaporated to dryness at 50 °C. Two millilitres of mixture of NaOH solution (2 mol L^{-1}) with methanol (1:1) was added to the dry residue to hydrolyse PHTs and fats. The vial was closed and placed into oven and heated at 80 °C for selected period of time. After hydrolysis 500–600 μL of concentrated HCl (36%) were added to acidify the hydrolysate to pH close to 1 (at least below 2). Hexane (1 mL) is added to the vial and the content shaked for 20 min to remove the hydrolytic liphophilic products (fatty acids and higher alcohols). After separation of the phases the upper hexane phase was discarded and the lower aqueous acidic fraction was extracted again with 1 mL of chloroform to extract the PA. In the last step the two phase system in the vial was derivatized by diazomethane for approximately 30 min according to Glastrup (1998); the capillary should end in the lower chloroform phase. The vial was shaked several times during the derivatization. After derivatization chloroform fraction containing dimethyl phthalate was taken for GC-FID analysis.

2.3. Standard solutions

Mixed stock solution of DEP, DBP, DEHP and DDP at concentrations of 5 mg mL^{-1} of each phthalate was prepared in chloroform. This solution was used for the optimisation of hydrolysis time. Calibration curve was measured on FID after appropriate dilution of the stock solution with chloroform within the concentration range 1–500 μg mL^{-1}. Another mixed stock solution of DEP, DBP, DEHP and DDP in vegetable oil was prepared at concentration of 20 mg mL^{-1} of each phthalate and used for optimisation of separation of PA from hydrolytic products.

3. Results and discussion

Hydrolysis, selective extraction of liphophilic products and PA derivatization to DMP represent the crucial steps of presented method.

Hydrolysis should guarantee quantitative transformation of all PHTs to PA, therefore, optimisation of its conditions was done with respect to temperature and hydrolysis time. Mixed stock solution (0.2 mL) of DEP, DBP, DEHP and DDP instead of sample was pipetted to empty vial, hydrolysed at 80, 100 °C and laboratory temperature for 24 h and processed further according to Section 2.2. It was found that 80 °C is optimum (data not shown) for hydrolysis because the hydrolysis reaction at laboratory temperature is too slow and at 100 °C there is too high risk of the vial content evaporation to dryness. Similarly, Albro et al. (1984) used for the hydrolysis 1 mol L^{-1} NaOH at temperature 90 ± 5 °C for 90 min and did not found unhydrolysed DMP, diethyl phthalate, DBP and dioctyl phthalate, whereas DEHP was only 60% hydrolysed. Kato et al.