Talanta 79 (2009) 10–15

Contents lists available at ScienceDirect

Talanta

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

Extraction of polycyclic aromatic hydrocarbons from smoked fish using pressurized liquid extraction with integrated fat removal

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ARTICLE INFO

Article history:
Received 20 October 2008
Received in revised form 1 February 2009
Accepted 20 February 2009
Available online 5 March 2009

Keywords:
Pressurized liquid extraction
Smoked fish
Polycyclic aromatic hydrocarbons
Method validation
Principal component analysis

ABSTRACT

Quantification of polycyclic aromatic hydrocarbons (PAHs) in smoked fish products often requires multiple clean-up steps to remove fat and other compounds that may interfere with the chemical analysis. We present a novel pressurized liquid extraction (PLE) method that integrates exhaustive extraction with fat retention in one single analytical step. The PLE parameters: type of fat retainer, flush volume, solvent composition, fat-to-fat retainer ratio (FFR), and the dimensions of the extraction cells were the most important factors for obtaining fat-free extracts with high recoveries of PAHs. A 100 mL extraction cell filled with 18 g activated silica gel, dichloromethane:hexane (15:85, v/v) as extraction solvent, FFR of 0.025 and 100% flush volume was the best analytical setup for integrated extraction and fat retention.

The one-step procedure provided a more rapid and cost-efficient alternative with minimization of waste generation compared to the standard reference method that is based on a multi-step procedure. Furthermore, the analytical quality of the two methods are comparable, while the new integrated approach for extraction and cleanup is less prone to analytical errors (random and systematic) because of fewer analytical steps.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), of which some are genotoxic and/or carcinogenic, are formed during incomplete combustion processes both spontaneously in nature and owing to fossil fuel combustion. The route to human exposure is manifold, since the PAHs can enter the food chain by both deposition and transfer from soil, air, and water. PAHs may therefore constitute a food safety problem. The most important factor for human exposure—and the factor of interest in this study—is the cooking procedure for food products. PAHs can be generated during drying, smoking, grilling, roasting, and frying. Smoked food products are directly exposed to PAHs during the smoking process, and the amount of PAHs transferred by smoke depends on the temperature of smoke, type of wood, length of time of smoking, and whether the smoking is indirect or direct [1–5].

Lipids interfere with the chemical analysis by gas chromatography–mass spectrometry (GC–MS) and may have severe effects on reproducibility, robustness, and PAH recovery [6]. It is therefore often necessary to remove lipids prior to the chemical analysis, which is typically done in a tiered approach: Extraction of PAHs by Soxhlet [3,7,8]; sonication [7,8]; supercritical fluid extraction [8–10] or pressurized liquid extraction (PLE) [11–13]; gel permeation chromatography (GPC) [12,14,15] and/or solid phase extraction (SPE) [3,11,15] to remove lipids and other interferences; and quantification by GC–MS [10,11,13] or high-performance liquid chromatography (HPLC) [8,9,14].

The large number of analytical steps results in high consumption of hazardous organic solvents, high costs, prolonged analysis time [12,16], an increased risk of analyte losses, decreased reproducibility, and biases. These drawbacks are also experienced in the standard reference method (SRM) for PAH sample preparation used at DTU FOOD, Denmark: PLE extraction, clean-up by GPC and SPE, and quantification by GC–MS [17], although the use of PLE can integrate extraction with fat removal [6,18–20], thereby reducing the number of analytical steps. A one-step PLE method can be achieved by adding a stationary phase (e.g. silica or alumina) directly to the PLE extraction cell. This method has previously been used in the analysis of polychlorinated biphenyls (PCBs) in fish samples [6,18–20].

The aim of this study was therefore to develop and validate a new one-step PLE method that integrates exhaustive extraction of PAHs with fat removal. The new method would be quicker than the SRM and minimize waste generation, while its analytical quality would be comparable to the SRM. Both the levels and patterns of PAHs in food products are of interest, and the method was therefore developed for analysis of 2–6 ringed PAHs. Reproducibility and accuracy of the one-step PLE method was further validated by comparing the

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doi:10.1016/j.talanta.2009.02.048

measured concentrations in 10 smoked fish samples between the two methods.

2. Experimental

2.1. Samples

Pork lard and raw trout were bought from the local butcher and fishmonger, respectively, and samples were then homogenized. Thin layers of the pork lard were scraped off to facilitate homogenization. After removal of head and skin, the fish fillets were homogenized for 10 min using a stainless steel blender (Broendum, Copenhagen, Denmark). In addition, homogenized samples of 10 fish that had been smoked by Danish fishmongers were obtained from the Danish Veterinary and Food Administration (DVFA) in Aarhus. All samples were stored at −18 °C prior to analysis.

2.2. Chemicals and reagents

Toluene, *n*-hexane (HPLC grade), and sulphuric acid were obtained from Merck (Darmstadt, Germany) and acetone, dichloromethane (HPLC grade), and *n*-pentane from Rathburn (Walkerburn, Scotland). Silica gel 60 (0.063–0.200 mm) was purchased from Merck (Darmstadt, Germany), florisil (60–100 mesh) from Fluka (Buchs, Switzerland), and neutral, basic and acidic alumina (150 mesh) from Sigma–Aldrich (Steinheim, Germany). Pre-rinsed silica gel, alumina and florisil were activated at 130–140 °C over night and stored at 120 °C. Sulphuric acid impregnated silica was prepared by adding sulphuric acid to cold silica gel (2:3, w/w). Anhydrous sodium sulphate was obtained from Merck (Damstadt, Germany), polyacrylic acid from Sigma–Aldrich (Steinheim, Germany), and Ottawa sand (20–30 mesh) from AppliChem (Darmstadt, Germany). Ottawa sand was precleaned by heating at 500 °C for 5 h.

Different spike, recovery, and internal standard solutions were applied, each consisting of PAHs or deuterated PAHs (d-PAHs) purchased from Ehrenstorfer (Augsburg, Germany) and Cambridge Isotope Laboratories (Cambridge, UK). Details on the standard solutions are listed in Table 1 in the supporting information (Table S1). To determine the optimal fat retainer (Section 3.1), PAH quantification was performed using one-point calibrations. To investigate the effects of PLE parameters (Section 3.3) and for validation (Section 3.4), the internal standard method was used, with six calibration solutions ranging from 50 to 250 ng/mL and eight calibration solutions ranging from 0 to 250 ng/mL, respectively.

Table 1
ASE settings for the final one-step PLE method.

Sample preparation	PLE settings	Analysis
100 mL extraction cell	Solvent composition: 85:15 (hexane:dichloromethane)	ASE extracts are evaporated by vacuum evaporator (35 °C) until 2 mL
Sample mixed with 5 g Ottawa sand and 10 g polyacrylic acid	Time of each static cycle: 5 min	The extracts are quantitatively transferred to GC–MS vials and evaporated by nitrogen steam
18 g silica as fat retainer	Number of static cycles: 2	The GC–MS vials are added toluene and the final volume evaporated to 100 μL by nitrogen steam
FFR value: 0.025	Polar solvent: dichloromethane Apolar solvent: hexane Flush volume: 100% Temperature: 100 °C Purge: 60 s Pressure: 1500 psi	

2.3. Fat determination

The fat content of pork lard and fish homogenates was determined in triplicates. For wet trout and smoked fish homogenate, a mixture of 10 g sample, 10 g Ottawa sand, and 20 g polyacrylic acid was added to a 66 mL ASE cell and extracted using an ASE 300 System (Dionex, Sunnyvale, CA) with instrument settings of 100 °C, 2 static cycles of 5 min, 75% flush volume, and an extraction solvent of hexane:acetone (1:1, v/v). The fat content was determined gravimetrically at 70 °C.

2.4. PLE parameters

ASE cells with volumes of 33 mL (ASE 200) and 100 mL (ASE 300) were packed with one cellulose filter at the bottom followed by the fat retainer. For fish homogenates, 2 g of polyacrylic acid was placed on top of the fat retainer to avoid deactivation of the fat retainer by water. Pork lard was ground with sodium sulphate until dry, and 5 g wet trout was ground with 5 g Ottawa sand and 10 g polyacrylic acid. The dried samples were transferred to the ASE cells, and spike standards were added (see Table S1 for details). The void volume was filled with Ottawa sand. The fat retainer, solvents, solvent composition, and flush volume were optimized, while the fat-to-fat retainer ratio (FFR) (0.025), temperature (100 °C), pressure (1500 psi), number of static cycles (2 of 5 min each), and purging time (60 s with nitrogen) were fixed throughout the study. Since the FFR was fixed, the amount of sample weighed for each extraction varied according to the fat content.

2.5. Preparation of extracts

Extracts were evaporated to 80 mL using a rotary evaporator at 35 °C and transferred quantitatively to 100 mL volumetric flasks, which were filled to the mark with pentane. Ten mL was used for fat determination. For quantification, the solvent was further reduced to 2 mL using a rotary evaporator (35 °C) and transferred to GC vials that were placed under a stream of nitrogen. The recovery standard (see Table S1 for details) and 200 μL toluene were added, and the total volume was reduced to 100 μL.

2.6. GC–MS analysis

Extracts were analysed on an Agilent 6890N gas chromatograph that was connected to an Agilent 5975B mass spectrometer with electron ionization. The gas chromatograph was equipped with a 40 m ZB-5 capillary column with special dimensions (0.18 mm id × 0.25 μm film). Helium was used as carrier gas at a flow rate of 0.8 mL/min, and 1 μL aliquots were injected in splitless mode. Injector, ion source, and quadropole temperatures were 330, 230, and 150 °C. The oven programme was: 100 °C (held for 2 min), increased to 230 °C (6 °C/min), and then 1 °C/min to 245 °C, 6 °C/min to 268 °C, 2 °C/min to 300 °C, and 6 °C/min to 330 °C. Selected ion monitoring was used to analyze 18 *m*/*z* values in the range *m*/*z* 128–316, divided into 5 groups with 4–7 ions in each.

2.7. Validation

The new method was validated by comparing its precision (repeatability), accuracy, PAH recoveries, limits of detection (LOD), and limits of quantification (LOQ) to those of the SRM. The determination of these values was based on six extractions of sub-samples of homogenized and dried trout samples spiked with 3 ng of each of the 26 PAHs/g wet tissue (see Table S1) and three method