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Optimization of a microwave-assisted extraction method for the analysis of polycyclic aromatic hydrocarbons from fish samples

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Abstract

A microwave-assisted extraction (MAE) procedure has been developed and optimized for the extraction of six regulated polycyclic aromatic hydrocarbons (PAHs) from muscle samples of polluted fish. The procedure involves the simultaneous microwave-assisted extraction of PAHs with *n*-hexane and the lipids hydrolysis with potassium hydroxide. Experimental design methodology allows a quick and robust optimization of operational parameters such as the extraction time, temperature, and solvent volumes. In these final optimized conditions, the procedure can be applied to a vast range of fat containing fish samples without significant changes, thus enabling its routine use. Recoveries around 90% for the studied compounds benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, and indene[1,2,3-cd]pyrene and quantification limits (between 0.07 and 0.53 ng/g dry weight) far below the regulated limits, have been obtained. The procedure is applied to several different fish samples. Further, accuracy validation using NIST SRM 2977 reference material was carried out.

Keywords: Polyaromatic hydrocarbons; Microwave-assisted extraction; Fish tissue analysis

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1. Introduction

Polycyclic aromatic hydrocarbons are widespread environmental pollutants having both natural (forest fires and volcanic activity) [1] and anthropogenic origin (incomplete combustion of fossil fuels, coke production, many industrial processes, and so on) [2,3].

Adverse effects of PAHs on living organisms have been studied for decades. Some of them have been classified as carcinogenic and mutagenic [4] and 16 PAHs were listed as priority pollutants by the U.S. Environmental Protection Agency (EPA) [5,6].

Major routes of exposure to PAHs are from inhaled air and especially by the ingestion of contaminated food [7]. PAHs in the atmosphere enter into the aquatic environment being readily taken up and bioaccumulated by aquatic organisms [8]. Because of hydrophobicity of PAHs, marine sediments concentrate the pollutants, especially in coastal waters where fish and molluscs are particularly prone to exposure and accumulation. Oil spills and run-off from land or industrial effluents [9] or the use of creosote-treated wood in mussel aquaculture [10] is an important cause of PAHs pollution in coastal waters. In general, fish have a greater ability to metabolize PAHs than do molluscs, so the compounds tend to persist more in the latter [8]. The ability of fish to metabolize PAHs probably explains why benzo[a]pyrene sometimes is not detected or is found only at very low concentrations in fish from heavily contaminated areas [11,12].

Recently, the European Union established maximum levels for benzo[a]pyrene (2 ng/g wet weight) in muscle meat of fish [13]. Previously, after the Prestige accident, the Spanish government established regulatory levels affecting six PAHs in fish and shellfish tissues [14], on the basis of the same levels established by the French government 3 years before on the occasion of the Erika accident [15]. These levels (on dry weight basis) were 20 ng/g for benz[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), dibenz[a,h]anthracene (DB[a,h]A), and indene[1,2,3-cd]pyrene (I[1,2,3-cd]P) in fish samples and 200 ng/g for crustaceans,

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cephalopods, and molluscs. Although it is common to control the 16 PAHs in US-EPA list to evaluate pollution by the more frequently found PAHs in the environment, those six PAHs were selected on the basis of their potential toxicity regarding human diet.

From the analytical point of view, isolation of fat-soluble analytes, such as PAHs from biological matrices, most often involves the hydrolysis of lipids before the extraction of analytes. Also, more or less complicated clean up procedures need to be applied after extraction to provide extracts ready for the accurate analytical determination [16]. A large number of papers have been published on PAHs extraction from fish material using Soxhlet based procedures [17–19], pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) [20], supercritical fluid extraction (SFE) [21], and matrix solid-phase dispersion (MSPD) [22]. Other techniques, such as the microwave-assisted extraction (MAE), have been used mainly for wood [23], sediment [24], and sludge [25] samples. To our knowledge, MAE has not been reported for the simultaneous extraction of PAHs and hydrolysis of sample lipids.

The aim of the present work was to develop and evaluate a MAE procedure for the extraction of the above mentioned six regulated PAHs from fish samples. Alkaline digestion stage needed to process fatty samples is carried out concomitantly with the extraction itself. To optimize the operational parameters experimental design methodology was applied. In this way a robust procedure was developed that allows the processing of any kind of fish sample no matter what the fat content is. Two types of fish samples representatives from low and high fat content fish (turbot and salmon) were considered for experimental optimization. Other samples of low and high fat content (mussel and lamprey) were also analyzed to verify the applicability of the developed procedure. Accuracy validation was carried out using US National Institute of Standards and Technology (NIST) SRM 2977 mussel tissue reference material. All extracts were analyzed by HPLC using fluorescence detection.

2. Experimental

2.1. Apparatus and reagents

Sample extractions were performed using an Ethos E-320 (1000 W) Microwave Extraction System (Millestone, Leutleirch, Germany) equipped with 12 vessels with a nominal volume of 100 mL. Maximum values for temperature and pressure inside the extraction vessels are 260 °C and 35 bar (500 psi), respectively.

Chromatographic separations were developed in a system composed by a 600E pump, with a gradient controller (Waters, Milford, MA, USA), UV–vis diode array and fluorescence detectors in series (HP Series 1100, Agilent, Waldbrom, Germany). The injector was a Rheodyne Model 7725i (Cotati, CA, USA), fitted with a 20 μL external loop. The temperature precolumn and analytical column was controlled by the means of a MetaTherm 9540 oven, supplied by MetaChem (Torrance, CA, USA). The analytical column was a 250 mm × 2.1 mm I.D. Vydac 201TP52 column, with particle size of 5 μm. A 30 mm × 2 mm guard column Vydac 201TP was employed to protect the analytical column, both purchased from Agilent.

Data acquisition was carried out by the means of Agilent Chemstation Software (Rev. A. 06.03 [509]). Statistical treatments and manipulation were carried out by the means of the Statgraphics V 5.1 statistical package (Statistical Graphics Corp., Rockville, MD, USA) and Nemrod WV 2000D experiment designer software (LPRAI, Marseille, France).

Sample extracts were centrifuged with a Unicen centrifuge (Orto-Alresa, Madrid, Spain), concentrated with a Turbo Vap II nitrogen evaporator supplied by Zymark (Hopkinton, MA, USA).

Acetonitrile and methanol (gradient-grade, Lichrosolv), *n*-hexane, chloroform, and dichloromethane (Suprasolv) were purchased from Merck (Darmstadt, Germany). Ultrapure water was produced by the means of a Milli-Q system supplied by Millipore (Bedford, MA, USA). EPA-610 Polycyclic aromatic hydrocarbons mixture was supplied by Supelco (Bellefonte, PA, USA). Standard reference material (SRM) 2977 (mussel tissue) was purchased from the NIST (Gaithersburg, MD, USA). Potassium hydroxide (Pellets, +85%, AC) was purchased by Sigma–Aldrich (Madrid, Spain).

Durapore filters (Millex GV, 13 mm, 0.22 μm) were supplied by Millipore, and silica (Sep-Pak, 500 mg) and florisil (Sep-Pak, 900 mg) cartridges were obtained from Waters.

2.2. Samples and sample manipulation

To develop the optimization process two types of fish tissue materials were selected to represent low and high fat content fish tissues. Aquaculture turbot (<2% fat) and salmon (>15% fat) were selected to prepare stock materials to carry out the optimization process. One hundred and fifty grams of ground muscle was soaked with 15 mL of a PAHs solution in acetonitrile (B[a]A, B[k]F, and B[a]P and I[1,2,3-cd]P, 102 ng/mL; B[b]F and DB[a,h]A, 202 ng/mL each) stirring mechanically the mixture and then allowing to air dry at room temperature during 24 h with occasional mixing. Aliquots of the non-spiked materials were reserved as sample blanks. All spiked and non-spiked materials were then lyophilized and stored in amber glasses under 0 °C. All sample materials – turbot (Spanish origin), salmon (Norway), mussels, and lamprey (Galician) – were acquired from local markets.

The humidity percentage of fresh fish (76.5% for turbot, 62.9% for salmon) was evaluated gravimetrically. The residual percent of water in lyophilized materials was 8.3% (turbot) and 4.3% (salmon). The humidity of the mussel tissue reference material was 8.2%.

The lipid content of the different samples analyzed in this study was evaluated by the means of the Bligh and Dyer method [26]. The fat content in turbot was 1.7%, 16.6% for salmon, 1.3% for mussel tissue, and 22.4% for lamprey, all based in wet weight.

The method was developed to process 1 g of fresh fish sample (or 0.200 g of lyophilized materials reconstituted with 0.8 mL of ultrapure water). The sample is weighed in the extraction vessel, then 4 mL (although lower volumes may be used depending