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Comparison of solid phase extraction, saponification and gel permeation chromatography for the clean-up of microwave-assisted biological extracts in the analysis of polycyclic aromatic hydrocarbons

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Abstract

The feasibility of different clean-up procedures was studied for the determination of polycyclic aromatic hydrocarbons (PAHs) in biota samples such as oysters, mussels and fish liver. In this sense, once the samples were extracted – essentially with acetone and in a microwave system – and before they could be analysed by gas chromatography–mass spectrometry (GC–MS), three different approaches were studied for the clean-up step: solid phase extraction (SPE), microwave-assisted saponification (MAS) and gel permeation chromatography (GPC). The main aim of this work was to maximise the recoveries of PAHs and to minimise the presence of interfering compounds in the last extract. In the case of SPE, Florisil® cartridges of 1, 2 and 5 g, and silica cartridges of 5 g were studied. In that case, and with oysters and mussels, microwave-assisted extraction and 5 g Florisil® cartridges provided good results. In addition, the concentrations obtained for Standard Reference Material (SRM) NIST 2977 (mussel tissue) were in good agreement with the certified values. In the case of microwave-assisted saponification, the extracts were not as clean as those obtained with 5 g Florisil® and this fact lead to overestimate the concentration of the heaviest PAHs. Finally, the cleanest extracts were obtained by GPC. The method was successfully applied to mussels, oysters and hake liver, and the results obtained for NIST 2977 (mussel tissue) were within the confidence interval of the certified reference material for most of the certified analytes.

Keywords: Microwave-assisted extraction; Biological samples; Polycyclic aromatic hydrocarbons; Solid phase extraction; Saponification; Gel permeation chromatography

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental contaminants originated from different emission sources, mainly related to human activities, such as industrial incineration, transport, uncontrolled spills, surface runoff or atmospheric deposition. Due to the mutagenic/carcinogenic and endocrine disrupting effects of some PAHs, the knowledge of the fate of those compounds, particularly in the aquatic environment, shows an increasing interest [1]. Moreover, they are soluble in fatty and lipid-rich tissues, where they are accumulated. In this sense, the bioaccumulation of such compounds in organisms is an indication of the pollution level in a given environmental compartment and, therefore, they are frequently measured. In order to accomplish this aim, the development of reliable analytical procedures for the determination of these pollutants in complex matrices is of crucial importance [2].

As it happens with many other micro-organic contaminants, the extraction of PAHs from biological tissues is one of the most critical steps in their determination procedure. Although there are many works in the literature that make use of microwave systems for the determination of PAHs in samples such as sediments [3–10], sewage sludge [11,12], particulate matter [13,14], worms [15] and plants [16], only a few report the use of those systems with biological tissues such as oysters, mussel or fish liver for the analysis of PAHs [2,17] or other micro-organic contaminants such as polychlorinated biphenyls [17,18], trichloroben-

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zenes [19] or organochlorine pesticides [20]. In this sense, and due to our experience in the microwave-assisted extraction (MAE) of PAHs and other organic pollutants from matrices such as soils and sediments [10,21–24], it was decided to study the use of MAE for the extraction of PAHs from biota samples such as oysters, mussels or fish liver.

Because of the complexity of the biological matrices mentioned above, the presence of interfering compounds in the extract requires an intensive clean-up before samples can be submitted to the separation and determination step, usually on a gas chromatograph–mass spectrometer (GC–MS) or a liquid chromatograph with fluorescence detector (HPLC-FLD) system. As it is already known, lipidic compounds can significantly reduce the performance of GC–MS due to accumulation in the injection port, column and ionisation source [25], and they can be responsible of artifacts, especially when single ion monitoring (SIM) mode is using. In order to avoid those problems, solid phase extraction (SPE) [17,26–30], saponification [2], gel permeation chromatography (GPC) [26,31–35] or a combination of saponification/SPE [36,37], saponification–GPC [38,39] or SPE–GPC [40–42] can be found in the literature as clean-up steps in the determination of PAHs from biota samples. Therefore, the main aim of this work was to compare the cleaning capability of Florisil® and silica cartridges of different mass, and the use of microwave-assisted saponification (MAS) and gel permeation chromatography as alternative cleaning procedures. The evaluation of the clean-up procedure was done in terms of recovery and the lack of interfering compounds of the final extracts.

2. Experimental

2.1. Sampling and sample treatment

Mussels (*Mytilus edulis*) and oysters (*Crassostrea* sp.) were manually collected at different sampling stations located in the estuary of Bilbao and Urdaibai (Basque Country, northern Spain). Samples were collected close to the water line during the low tide, rinsed with the natural water and introduced into plastic bags. Samples were transported on ice in a cool box to the laboratory. Each individual was dissected with a clean scalpel blade to separate the soft tissues from the shells. Around 20 individuals were dissected out, pooled, homogenized and freeze-dried in a Cryodos-50 (Telstar, Spain).

Hakes (*Merluccius merluccius*) were fished in the Bay of Biscay (in front of the Basque coast) and were eviscerated on board and the liver and muscle were kept on N_2 (l) until they were carried to the laboratory.

2.2. Reagents and materials

SS TCL PAH mix (16 PAHs at 2000 $\mu g\,mL^{-1}$ each), 525 fortification solution A (acenaphthene-d_{10}, chrysene-d_{12} and phenanthrene-d_{10} at 2000 $\mu g\,mL^{-1}$ each), corn oil (palmitic, stearic, oleic, linoleic and linolenic acids) and bis(2-ethyl, hexyl) phthalate (DEHP) were purchased from Supelco (Walton-on-Thames, UK). Methoxychlor (Pestanal®), perylene (Oekanal®) and sulfur (Pestanal®) were obtained from Riedel-de-Haën (Seelze, Germany). A mixture containing corn oil (25 $mg\,mL^{-1}$), DEHP (1 $mg\,mL^{-1}$), methoxychlor (2 $mg\,mL^{-1}$), perylene (0.2 $mg\,mL^{-1}$) and sulfur (0.8 $mg\,mL^{-1}$) was prepared following EPA 3640A method [43].

Acetone, dichloromethane (DCM), ethyl acetate (EtOAc), and toluene were purchased from LabScan (Dublin, Ireland) and *iso*-octane and *n*-hexane from Merck (Darmstadt, Germany). 1, 2 and 5 g Florisil® and 5 g silica cartridges were purchased from Supelco and potassium hydroxide and sodium sulphate from Merck. The standard reference material (SRM) NIST 2977 (mussel tissue) was supplied by NIST (Gaithersburg, ND, USA).

2.3. Microwave-assisted saponification

MAS experiments were performed with a MDS-2000 closed microwave solvent extraction system (CEM, Matthews, NC, USA) equipped with a 12-sample tray and pressure feedback/control. Approximately 2 g of biota tissue (freeze-dried oyster or mussel) were weighed in the extraction vessel, 25 μL of 525 fortification solution at 20 $\mu g\,mL^{-1}$ in acetone, 12 mL of saturated methanolic potassium hydroxide and 12 mL of *n*-hexane was added and the vessel was closed, after ensuring that the rupture membrane was in optimal conditions. Extraction was carried out at 21 psi for 15 min and at 80% of the microwave power. Once the irradiation period was over and ambient temperature was obtained, the mixture was shaken for 15 min, centrifuged and the apolar phase was collected. The *n*-hexane phase was filtered through Teflon filters (25 mm, 5 μm, Pall, Ann Arbor, USA) onto a sodium sulfate column. 2 mL of *n*-hexane was added to the methanolic potassium hydroxide phase and the previous procedure was repeated. The two organic fractions were collected and the extract was evaporated to approximately 0.5 mL under a gentle stream of nitrogen in a TurboVap LV Evaporator, after the addition of 1 mL *iso*-octane, in order to minimise the losses of the most volatile analytes [44]. The extracts were finally concentrated to dryness, re-dissolved in 200 μL of *iso*-octane and kept in the freezer at −18 °C until GC–MS analysis.

2.4. Microwave-assisted extraction

MAE experiments were performed also with the MDS-2000 closed microwave solvent extraction system mentioned above. The MAE procedure used was optimised before for the determination of polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs), phthalate esters (PEs) and nonylphenols (NPs) in sediment samples [10]. Briefly, approximately 2.0 g of the biological tissue (mussel, oyster or hake liver) was accurately weighed and quantitatively transferred to the Teflon lined extraction vessel. 25 μL of a mixture of 525 fortification solution at 20 $\mu g\,mL^{-1}$ in acetone and 15 mL of acetone were added to the sample and the extraction vessel was closed. Extractions were performed at 21 psi for 15 min at 80% of the microwave power. When the irradiation period was completed, samples were removed from the microwave cavity and were allowed