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Multiscreening determination of organic pollutants in molluscs using matrix solid phase dispersion

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ABSTRACT

This work describes the optimisation, validation and application of matrix solid-phase dispersion (MSPD) coupled to gas chromatography mass spectrometry, both single quadrupole (GC–MS) and tandem (GC–MS/MS), for the quantification in molluscs of up to 40 different analytes belonging to several families of priority and emerging organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs) and musk fragrances. The MSPD procedure was fully optimised with a special focus on the clean-up strategy. The best recoveries were obtained using glass syringes, 0.30 g of freeze-dried sample, 0.30 g of Florisil as solid support, 4.00 g of activated silica and 25 mL of dichloromethane as elution solvent. Using GC–MS/MS the method afforded good linearities (r^2, between 0.980 and 0.9996), adequate repeatability and reproducibility (RSD < 17% and 33%, respectively) and low instrumental limits of detection (between 0.010 and 2.74 ng mL^{-1}). The accuracy of the method was evaluated using different approaches, i.e. assessment of spiked fish hatchery samples, laboratory reference material and standard reference material (SRM 2977). Satisfactory apparent recoveries were obtained for all the target analytes after correction with the corresponding labelled surrogate, except for PAHs in the case of SRM 2977, which required the use of the standard addition method. Finally, MSPD was applied to mollusc species collected in Colombia and Nicaragua, where PAHs, PCBs, musks and pesticides were detected at low ng g^{-1} levels.

1. Introduction

Different environmental compartments tend to end up accumulating a huge variety of organic pollutants, primarily resulting from anthropogenic sources. Although these organic pollutants may be present at trace levels, their adverse effects on aquatic life, on animals and even on humans are increasing the concern of society and the scientific community [1,2]. In consequence, the European Union has adopted specific regulations such as the Water Framewok Directive (2013/39/EU), in which the environmental quality standards (EQSs) for 45 priority substances have been included for water and biota samples. The use of aquatic organisms has been pointed as an integrative target sample because they are extensively exposed to hydrophobic contaminants including polycyclic aromatic hydrocarbons (PAHs) [3,4], polychlorinated biphenyls (PCBs) [5], polybrominated diphenyl ethers (PBDEs) [5–8], organochlorine pesticides (OCPs) [8–10], organophosporous pesticides (OPPs) [11] and musk fragances [12]. However, the high fat content that provides the bioaccumulation potential of lipophilic pollutants is one of the major challenges in the analysis of pollutants in biota [13].

In this scenario, where the number of pollutants to be monitorised is getting larger, multi-residual analytical methodologies become an excellent strategy to get this goal [14,15]. In this context, gas chromatography (GC) coupled to single mass spectrometry (MS) [7,16] and, specially, GC coupled to tandem mass spectrometry (MS/MS) [1,17–19] has been the technique of choice to analyse semivolatile and volatile organic compounds, due to its favourable combination of high selectivity and resolution, good accuracy and precision, wide dynamic concentration range and high sensitivity [20]. The use of large volume injection (LVI) in a programmable temperature vaporiser (PTV) can render better limits of detection, but few studies have used this set-up when molluscs have to be analysed [21].

Notwithstanding the advantages of such instrumental techniques, the whole analytical process can be wasted if an unsuitable sample preparation is employed [22]. In the biomonitorisation of coastal environments many extraction techniques have been applied to mollusc samples, such as the conventional Soxhlet extraction [23,24] or sonication-assisted extraction [25]

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which have been replaced by supercritical fluid extraction [26], microwave assisted extraction [27,28], pressurised liquid extraction [16,29,30] or focused ultrasound solid–liquid extraction [31]. In order to solve some operational drawbacks, minimise time, solvent consumption and cost shown by some of the mentioned extraction techniques, matrix solid-phase dispersion (MSPD) has been introduced during the last years [32]. MSPD has been reported as a reliable and simple procedure to extract organic pollutants from several solid samples [4,7,8,18,33–36]. The key factors for the success of MSPD are its feasibility, flexibility, versatility, low cost and rapidity [37,38]. Furthermore, MSPD extraction can isolate and preconcentrate the target compounds and clean up the extract in-line in a single step, which eases the method throughput.

Therefore, this work was focused on the development of a MSPD extraction method followed by GC–MS or GC–MS/MS analysis to determine up to 41 non-polar or slightly non-polar organic pollutants (PAHs, PCBs, PBDEs, OCPs, OPPs and musk fragrances) in molluscs. The suitability of both GC–MS and GC–MS/MS for the multiscreening of such contaminants was also assessed previous to its validation and application to mollusc samples.

2. Experimental

2.1. Reagents and materials

The names of the target analytes and the isotopically labelled standards used as surrogates, the abbreviations and the purity of the standards are included in Table 1. PCB Mix-3 (CB 28, CB 52, CB 101, CB 118, CB 138, CB 153 and CB 180) was supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) and the individual standard of CB 52 by Sigma–Aldrich (St. Louis, MO, USA). Bromodiphenyl Ethers Lake Michigan Study mix (BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 138, BDE 153 and BDE 154) was purchased from Isostandards Materials (Madrid, Spain) and the individual standard of BDE 100 from Sigma–Aldrich (St. Louis, MO, USA). SS TCL PAH Mix containing EPAs 16 priority PAHs was obtained from Supelco (Walton-on-Thames, UK). The surrogate standard PAH deut 5 containing 5 deuterated PAHs ([$^2H_8$]-naphthalene, [$^2H_{10}$]-acenaphthene, [$^2H_{10}$]-phenanthrene, [$^2H_{12}$]-chrysene and [$^2H_{12}$]-perylene) was provided by Dr. Ehrenstorfer GmbH. The two polycyclic musks, tonalide (AHTN) and galaxolide (HHCB), were obtained from LGC Standards GmbH (Wesel, Germany), whereas [$^2H_{15}$]-musk xylene was supplied by Dr. Ehrenstorfer GmbH. The four OCPs (o,p′-dichlorodiphenyldichloroethane, p,p′-dichlorodiphenyl-dichloroethylene, o,p′-dichlorodiphenyl-trichloro-ethane, p,p′-dichlorodiphenyl-trichloroethane) and four HCH isomers were supplied by Dr. Ehrenstorfer GmbH. The two OPPs, chlorpyriphos and chlorfenvinphos, as well as the deuterated analogue [$^2H_8$]-1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, were provided by Sigma–Aldrich. [$^2H_{66}$]-n-dotriacontane and [$^2H_{46}$]-n-docosane were acquired from CDS Isotopes Inc. (Sainte-Foy-La-Grande, France).

Individual stock solutions from each solid standard were dissolved to prepare ~1000 μg g^{-1} stock solutions in 2-propanol (HPLC-grade, 99.8%, LabScan, Dublin, Ireland). These solutions were stored in amber vials at −20 °C. 100 mg L^{-1} dilutions were prepared in 2-propanol monthly and more diluted stocks were prepared daily according to the experimentation.

The solvents n-hexane (95%), dichloromethane (DCM; 99.8%), ethyl acetate (EtOAc; 99.8%) and acetone (99.8%) used for elution were provided by LabScan.

Empty polypropylene cartridges (10 mL capacity) were purchased from HSW Norm-Jet (Keltenstrasse, Germany), BD Discardit II (Huesca, Spain) and Omnifix-F (B. Braun Melsungen AG, Germany). Empty glass syringes of 6 mL and 10 mL capacity were acquired from Supelco (Bellefonte, PA, USA) and Rutherford Vintage (Ruthe, Portugal), respectively. 6 mL and 12 mL polyethylene frits were purchased from Supelco.

For dispersion and clean-up purposes, different solid sorbents were used. Diatomaceous earth (acid washed not further calcined, 95% purity), Florisil and silica (high purity grade, 70–230 mesh) were provided by Sigma–Aldrich. Envi-Carb (Supelclean 120/400) was acquired from Supelco, zeolite from Zeolyst International (Conshohocken, USA) and Plexa and octadecyl-functionalized silica (Bondesil-C18) from Agilent Technologies (Lake Forest, USA). Silica and Florisil were activated at 130 °C overnight and maintained in a dry atmosphere until their use for analysis purposes. When necessary, both phases were deactivated with controlled percentages of sulphuric acid (H_2SO_4; 95–97%) purchased from Merck (Darmstadt, Germany).

A Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain) was used to freeze-dry the mollusc samples. Extracted fractions were evaporated at 20 °C in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a gentle N_2 (99.999%, Messer, Vilaseca, Spain) blowdown.

2.2. Material cleaning

All the laboratory material was cleaned with abundant pure water (<0.2 S cm^{-1}, Millipore, Billerica, MA, USA) and without using detergent to avoid possible contamination. The material was sonicated in clean acetone (Q.P., Panreac Química, Spain) for an hour and rinsed with ultrapure water (<0.057 S cm^{-1}, Milli-Q model, Millipore). Finally, the glass material was dried in an oven at 400 °C for 4 h. The glass syringes were sonicated in clean DCM for an hour and rinsed with ultrapure water before drying in an oven at 130 °C for approx. 12 h.

2.3. Samples and sample preparation

For sample preparation, each individual mollusc (*Mytilus edulis, Crassostrea rizophorae, Isognomon radiates*, *Polymesoda solida*, *Anadara tuberculosa* and *Anadara grandis*) was dissected with a clean scalpel blade to separate the soft tissue from the shell. A pool of 20 individuals were dissected out, homogenised and freeze-dried in a Cryodos 50 freeze-drier, which were stored in amber glass bottles at −20 °C in the fridge until analysis.

For optimisation and validation purposes freeze-dried fish hatchery mussels (obtained from a local market), a freeze-dried mussel tissue laboratory reference material (LRM) [39] and the freeze-dried mussel tissue SRM-2977 (National Institute of Standards and Technology, Gaithersburg, MD, USA) were used.

Finally, the method was applied to samples collected in Colombia in October 2012 and in Nicaragua in April 2013. In Cartagena de Indias (Colombia) oyster samples (*C. rizophorae)* were collected in three sampling sites: Contemar, Ecopetrol and Baru in the Natural Park of Corales de Rosario. In Santa Marta (Colombia) oysters (*I. radiates*) were collected in Taganga. In Bluefields (Caribbean Sea, Nicaragua) oysters (*C. rizophorae*) were collected at Half Cay and clams (*P. solida*) at Punta Massaya. In the Natural Park Padre Ramos of Nicaragua (Pacific Ocean) two types of cocles were collected: *A. tuberculosa* and *A. grandis*.

2.4. MSPD extraction and clean-up

Under optimised conditions, 0.30 g of freeze-dried mollusc sample and 0.30 g of Florisil dispersant were manually blended for 2 min in a glass mortar using a glass pestle. A 10-mL glass syringe, containing a polyethylene frit at the bottom, was filled from bottom to top as follows: 0.60 g of deactivated silica, 4.00 g of activated silica and finally the blended material. Isotopically labelled surrogates