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Analytical Methods

Determination of polychlorinated biphenyls in fish: Optimisation and validation of a method based on accelerated solvent extraction and gas chromatography–mass spectrometry

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ABSTRACT

A simple and robust method for the determination of 18 polychlorinated biphenyls (PCBs) in fish was developed and validated. A mixture of acetone/n-hexane (1:1, v/v) was selected for accelerated solvent extraction (ASE). After the digestion of fat, the clean-up was carried out using solid phase extraction silica cartridges. Samples were analysed by GC–MS in selected ion monitoring (SIM) using three fragment ions for each congener (one quantifier and two qualifiers). PCB 155 and PCB 198 were employed as internal standards.

The lowest limit of detection was observed for PCB 28 (0.4 ng/g lipid weight). The accuracy of the method was verified by means of the Certified Reference Material EDF-2525 and good results in terms of linearity ($R^2 > 0.994$) and recoveries (80–110%) were also achieved. Precision was evaluated by spiking blank samples at 4, 8 and 12 ng/g. Relative standard deviation values for repeatability and reproducibility were lower than 8% and 16%, respectively.

The method was applied to the determination of PCBs in 80 samples belonging to four Mediterranean fish species.

The proposed procedure is particularly effective because it provides good recoveries with lowered extraction time and solvent consumption; in fact, the total time of extraction is about 12 min per sample and, for the clean-up step, a total solvent volume of 13 ml is required.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of toxic and highly persistent organic compounds that consist of 209 congeners differing in the number and position of chlorine atoms on the two coupled biphenyl rings. Long-term chronic exposure to PCBs has been linked to severe injury to the nervous, endocrine, reproductive and immune systems in birds, fish and mammals (Skaare et al., 2000; Toft, Edwards, Baatrup, & Guillette, 2003). PCBs were produced for many years and used as additives in pesticides, paints, sealants or plastics as well as heat exchange fluids, in electric transformers and capacitors (Llobet, Martí-Cid, Castell, & Domingo, 2008).

The marine ecosystem was largely affected by PCBs impact and it still plays a fundamental role in the fate of these compounds. Unlike other contaminants, such as organotin compounds which are still used in various countries in spite of the 2008 ban by the International Marine Organisation (Magi et al., 2008), the production and use of PCBs has come to a complete stop, after they were banned during the seventies; nevertheless, PCBs concentration in marine matrices is still relevant, due to their persistency (Olsovska, Kresinova, Flieger, & Cajthaml, 2010). PCBs are lipophilic, therefore fishes can absorb, retain and concentrate them, particularly in fatty tissues (Storelli, Giacominelli-Stuffler, Storelli, & Marcotrigiano, 2003); bioaccumulation and biomagnification of these contaminants in fish and marine mammals were reported by several authors (Andersson et al., 2001; Smith & Gangolli, 2002): a recent study on the occurrence of dioxins, furans and PCBs in various edible fishes from the Adriatic Sea showed that PCBs were the dominant chemicals with concentration levels up to 1980 ng per gram of lipid weight (Storelli, Barone, Perrone, & Giacominelli-Stuffler, 2011). Other papers, during the last decade, highlighted that considerable quantities of PCBs are still present in the Italian marine environment (Stefanelli et al., 2004; Trocino et al., 2012).

In human exposure, dietary intake is the main route: fish, shellfish and animal fats contribute mainly to the PCB intake in human beings (Bocio, Domingo, Falcó, & Llobet, 2007). Several studies demonstrated a clear correlation between the frequency of fish consumption and the level of organochlorine compounds in human

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tissues, serum and milk (Fitzgerald et al., 2004; Fromberg, Granby, Hojgard, Fagt, & Larsen, 2011).

The determination of PCBs in real matrices is generally considered a challenging task: (van Leeuwen & de Boer, 2008) co-extracted substances can interfere with the analysis, leading to unreliable results also when the powerful instrumental technique of gas chromatography coupled to mass spectrometry is employed. Sample preparation is then a crucial step prior to the analytical determination of these pollutants.

The aim of the present work was to develop a simple and effective method for the detection of 18 congeners of PCBs (N°IUPAC 28, 52, 95, 99, 101, 105, 110, 118, 138, 146, 149, 151, 153, 170, 177, 180, 183, 187) in edible marine species from the Mediterranean Sea. The chosen PCBs included the six indicator congeners (N°IUPAC 28, 52, 101, 138, 153, 180) set by the European Commission as those recommended for quantification purpose because they cover a wide degree of chlorination and their sum comprises about half of the amount of non-dioxin-like PCBs present in food and feed (EFSA, 2005).

The proposed procedure is based on accelerated solvent extraction (ASE), solid-phase extraction (SPE) and gas chromatography–mass spectrometry (GC–MS). Each step of the method was optimised to improve recovery of analytes, accuracy and sensitivity. Quantification was performed using two internal standards: PCB 155 (hexa-chlorobiphenyl) for congeners with a degree of chlorination up to six and PCB 198 (octa-chlorobiphenyl) for the heptachloro derivatives, that proved to be an effective alternative to the more expensive labelled compounds.

This simple and reliable procedure can be easily employed in routine analysis of PCBs. In fact, the developed method was validated and applied to the determination of polychlorinated biphenyls in eighty edible fish samples collected in the Mediterranean Sea: obtained results are presented and discussed.

2. Experimental

2.1. Chemicals and solutions

Acetone and *n*-hexane were supplied by BDH-Prolabo (VWR International s.r.l., Italy); 2,2,4′-trimethylpentane (isooctane) was purchased from Labscan (Ireland). All these solvents were "organic trace analysis" grade. Sulphuric acid (98%) was obtained from Merck (Darmstad, Germany).

Diatomaceous earth was supplied by Phenomenex Inc. (Torrance, CA, USA).

For extractive and clean-up step tests, Extrelut-NT3 cartridges (1–3 ml sample) were purchased from Merck and SPE cartridges Bond Elut SI (SiOH, 6 ml/500 mg) were purchased from Varian, Inc. (Palo Alto, CA, USA).

PCB-Mix 37, containing all the investigated congeners at 10 mg/l with a purity higher than 97%, was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Stock standard solution (1 mg/l) of PCB-Mix 37 and all the intermediate standard solutions (200–100–50 ng/ml) were prepared in isooctane by dilution and stored at −20 °C in the dark for a maximum of 12 days. Standard solutions at 10 ng/ml and 5 ng/ml were prepared daily.

An internal standard mixture (I.S.) with a concentration of 1 mg/l was prepared by dissolving PCB 155 and PCB 198 (purity higher than 98%, Dr. Ehrenstorfer GmbH) in isooctane and stored in a freezer as described for PCB-Mix 37.

Quantification was based on a five-point calibration curve in the concentration range 5–200 ng/ml with I.S. added at 50 ng/ml.

The Certified Reference Material EDF-2525 was supplied by Cerilliant Corporation (Texas, USA) and contained all the investigated PCBs except for PCB 95.

2.2. Sample extraction

Four different fish species were considered (sea bass, black sea bass, sea trout, sole) for a total number of 80 fishes collected along the Italian coasts of the Mediterranean Sea between December 2008 and September 2009.

Fillets of fish were freeze–dried for 12 h after removing the skin; about 1 g of each was mixed with diatomaceous earth and transferred into the 33 ml stainless-steel extraction cells of the accelerated solvent extractor (ASE 200, Dionex Corp., Sunnyvale, CA, USA) for PCB extraction. To prevent the metal frit of the extraction cell from clogging, disposable cellulose filters were placed into the cells outlet. The optimised ASE parameters used for the final method were the following: acetone/*n*-hexane (1:1, v/v), one extraction cycle at 100 °C (5 min heating and 5 min static time) and 10 MPa. After the extraction cycle, each extraction cell was flushed with solvent (60% of the cell volume), purged with nitrogen (90 s) and the extracts were transferred to standard collection vials for clean-up. The collected solvent volume was about 13 ml and the extraction time was 12 min per sample.

2.3. Sample clean-up

The clean-up step was performed by solid phase extraction (SPE). The ASE extract was transferred into a previously calibrated flask and evaporated till dryness using a rotary evaporator (bath temperature 60 °C, reduced pressure, average evaporation time 5 min per sample). The extracted lipid content was determined gravimetrically using an analytical balance (ARS 220-4M, Kern & Sohn Gmb, Balingen, Germany).

A maximum of 0.40 g of fat, spiked with a fixed amount of I.S. (at 50 ng/ml), was first dissolved in *n*-hexane and then transferred into an Extrelut-NT3 cartridge; the diatomaceous earth of the cartridge acted as solid support for 3 ml of concentrated sulphuric acid, which was used to digest the fat (Attard Barbini, Stefanelli, Girolimetti, Di Muccio, & Dommarco, 2007). To achieve a good clean-up in terms of removal of interferences, an SPE silica cartridge, previously conditioned with 5 ml of *n*-hexane, was positioned under the Extrelut-NT3. The PCBs were eluted from the combined cartridges with 13 ml of *n*-hexane.

The extract was evaporated until dryness using a rotary evaporator, and then the residue was dissolved with 2 × 500 μl of isooctane and evaporated with a gentle stream of nitrogen. The residue was reconstituted with 100 μl of isooctane and analysed by GC–MS.

2.4. GC–MS equipment

Instrumental analysis was performed with a gas chromatograph coupled to a mass spectrometer equipped with a single quadrupole mass analyser (Focus DSQ, Thermo Scientific, Austin, TX, USA). Separation was accomplished on a Rtx-5MS fused silica capillary column (30 m × 0.25 mm I.D. Restek, Bellefonte, PA, USA) coated with 5% diphenyl 95% dimethyl polysiloxane. A sample volume of 1 μl was injected in splitless mode; the injector temperature was 250 °C.

The oven temperature was programmed as follows: 100 °C for 1 min, from 100 to 190 °C at 20 °C/min (held for 2 min), then to 250 °C at 3 °C/min and finally to 300 °C at 50 °C/min (held for 10 min).

Helium was employed as carrier gas (purity ⩾99,999%) with a constant flow of 1.0 ml/min.

The mass spectrometer was operated with an EI source (250 °C) and the electron energy was 70 eV. The MS transfer line was held at 270 °C and the quadrupole mass analyser was set at 150 °C. Mass spectrometric determination was carried out in Selected