NOVEL APPROCHES FOR FAST SAMPLE PREPARATION OF POPs IN COMPLEX FATTY (SEMI-)SOLID MATRICES

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Introduction

The developments achieved during the last decades in the field of analytical instrumentation allow nowadays performing determinations of persistent organic pollutants (POPs) at levels that we can hardly imagine years ago[1] while keeping standards of accuracy and selectivity in line with those set in current legislations even with relatively simple bench-top-type instruments[2]. Large volume injection is slowly been accepted as an efficient analytical approach contributing to improve analytes detectability in many application areas. The efforts carried out during the last two-three decades in the area of sample preparation have resulted in a number of novel, and frequently miniaturized, analytical technique that in several instances have facilitated some degree of integration and automation in the treatment of (relatively simple and/or pristine) liquid matrices. The advances achieved in solid-phase extraction and solid-phase microextraction and recent developments in the field of selective sorbents have been key aspects in this context and many of these techniques are nowadays routinely used also in POP analysis. On the contrary, advances in the treatment of (semi-)solid matrices, and of foodstuffs in particular, have been much more limited. The requirement of performing a quantitative extraction of the target compounds from the (usually very complex) matrix in which they are entrapped have typically forced the use of exhaustive (i.e., non-selective) extraction techniques. For this purpose, solid-liquid extraction and Shoxlet extraction are still widely accepted and used for routine applications and/or for reference purposes. The non-selective nature of these techniques makes the subsequent purification of the obtained extracts before instrumental analysis mandatory. These treatments usually involve highly manipulative multistep procedures where automation, or even partial integration, are still more the exception than the rule[3].

In this work, the feasibility of several modern analytical techniques for exhaustive and efficient, but also faster and environmental friendly, extraction of trace micropollutants from fat-containing biological samples is evaluated using selected classes of POPs as model compounds, more specifically polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). Approaches based on the use of matrix-solid phase dispersion (MSPD) with cosorbent and enhanced extraction techniques, such as pressurized liquid extraction (PLE) and ultrasonic assisted extraction (UAE), will be discussed and their relative merits and shortcomings evaluated. Special attention will be paid to the case of USE with ultrasonic tip followed by disposable pipette purification (DPX) due to the rapidity of the approach, minimum amount of reagents consumption and potential for automation.

Materials and methods

Acetone, dichloromethane and isooctane were of pestipur quality and were purchased from SDS (Peypin, France). *n*-Hexane was purchased from Merck (Darmstadt, Germany). Sulphuric acid was of pro-analysis quality (Merck). Anhydrous sodium sulphate was obtained from J.T. Baker (Deventer, The Netherlands) and silica gel 60 from Merck.

The 23 PCB congeners (PCBs 28, 52, 77, 95, 101, 105, 114, 118, 123, 126, 132, 138, 149, 153, 156, 157, 167, 169, 170, 180, 183, 189 and 194) and 15 PBDE studied (PBDEs 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197 and 209) were selected because of their toxicity and relative abundance in environmental samples. Two working stock solutions were prepared from individual PCB and PBDE standards (Ehrenstorfer, Augsburg, Germany) containing 1000 ng/mL of each compound in isooctane. These solution were used for further dilution and, when required, spiking of the samples. 1,2,3,4-Tetrachloronaphthalene (TCN, Ehrenstorfer) and PCB 209 were used as external standards for PCB determination using gas chromatography coupled to an electron capture microdetector (GC–microECD) and added to the final extracts just before the chromatographic analysis. Labelled standards of the 13 most toxic PCB congeners (Wellington Laboratories, Ontario, Canada) and ^{13}C-labelled PBDE 136 (Ehrenstorfer) were added to the extracts before PCB and PBDE confirmation, respectively, by GC-MS-based methodologies previously described[4,5].

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After optimisation of the different experimental parameters affecting the extraction and purification of the target analytes, sample treatment was carried out as follows:

Method A: A representative portion of the homogenised freeze-dried fatty-foodstuff tested (i.e., pork meat, egg or fish purchased in local supermarkets in Madrid), i.e. 1.5 g, was dispersed on 3.0 g of a 1:1 (w/w) mixture of Na_2SO_4 and silica modified with 44% (w/w) sulphuric acid (SiO_2–H_2SO_4). Then, 1.5 g of the resulting homogenized MSPD mixture was packed in an 8 mL glass disposable extraction column (J.T. Baker) on top of an appropriated co-sorbent (i.e., 1.5 g of SiO_2–H_2SO_4 plus 1.0 g of activated SiO_2). After two static 10-min extractions with *n*-hexane, some fresh solvent was eluted through the column to ensure proper purging of the sample and the clean-up sorbent[6].

Method B: A representative portion of the investigated feed sample (which included under development vegetal-based feedstuffs and commercial fish feeds purchased in Madrid), ca. 1.0 g, was mixed with similar amounts of Na_2SO_4 and 44% SiO_2–H_2SO_4 (w/w). A 0.750 g portion of the resulting homogenized MSPD mixture was packed in a 4.75-mL stainless steel extraction cell on top of an adequate co-sorbent and installed in a miniaturized home-made PLE system[7]. A first static PLE was performed for 7 min at 50ºC with *n*-hexane at 10.5 MPa. Afterwards, the solvent was completely replaced by a mixture of *n*-hexane:dichloromethane (1:1, v/v) and a second 7 min static PLE at 50ºC was carried out. Finally, some fresh solvent (i.e., *n*-hexane:dichloromethane, 1:1, v/v) was flushed through the cell to ensure proper purging of the sample, the clean-up co-sorbents and the lines[8].

Method C: A 50-mg subsample of the investigated matrix (i.e., common two-banded sea bream, chicken meat, salmon or the corresponding reference material, NIST 1945 and NIST 1947) were placed in an 1.5-mL Eppendorf (Deltalab, Barcelona, Spain) and extracted for 40 s (i.e., 20 pulses of 2 s) with 150 µL of *n*-hexane using a 2-mm ultrasonic titanium probe (130 Vibra Cell, Sonics,Newtown, USA) operated at 130 W and 20 kHz. The supernatant was separated by centrifugation during 2 min at 14,000 rpm (Mini-Spin Eppendorf centrifuge, Eppendorf, Hamburg, Germany) and slowly aspirated with a micropipette (Gilson tipe, Labbox Labware, Mataró, Spain) into a 5-mL polypropylene tip (Labbox Labware) modified to contain the clean-up sorbent (0.8 g of 44% SiO_2–H_2SO_4, w/w). After 10 s, the process was repeated with a new pipette tip[9].

In all cases, the collected extracts were concentrated under a gentle nitrogen stream to a final volume of either 20 µL (Methods A and B) or 50 µL and subjected to instrumental analysis by the corresponding technique without any additional treatment.

Procedural blanks were prepared following the same procedure as for samples but replacing the corresponding matrix by bare sand prewashed with the extraction solvents. No background interferences were found to be introduced by the methodologies proposed.

Fat content in the investigated samples was determined according to the Smedes method[10].

Results and discussion

Method A: Results demonstrated that miniaturised MSPD with a co-sorbent is a valuable sample preparation alternative to more conventional (large-scale) treatment protocols for the determination of PCBs in fat-containing (semi-)solid foodstuffs. Complete sample preparation was done in ca. 40 min with minimum solvent consumption (4.5–10 mL of *n*-hexane, depending on the initial amount of sample) and sample manipulation. The recoveries of the studied endogenous PCBs were in the 81–134% range of those found using a more conventional off-line procedure, even though as small an amount of sample as 0.1 g was used. Detection limits (LODs) by GC–microECD were in all cases below 0.3 ng/g sample (freeze dried basis) and the repeatability of the complete analytical procedure was in general better than 14%. When combined with GC–ITD(MS/MS), the proposed methodology allowed also satisfactory determination of less abundant non-*ortho*-PCBs 77, 126, and 169, even if these congeners were not isolated from the bulk of PCBs.

Method B: A new miniaturized PLE with *in-cell* purification method has been developed for the simultaneous extraction of PCBs and PBDEs from feed matrices of different nature. The proposed methodology allowed quantitative recoveries of the selected PCBs and PBDEs from a model feed matrix and accurate determination of

the target compounds even if only 0.25 g of the sample were used. Sample treatment was completed with only 8 mL of the organic solvents and 3.5 g of sorbents, and 45 min sufficed to obtain ready-to-analysis extracts. The performance of the complete PLE-based method was evaluated at two spiking levels, 0.4 and 4 ng/g wet weight. Recoveries in the range 60–120% were obtained for PCBs, while those of PBDEs ranged from 86% to 114% for most of the target analytes. The relative standard deviations were in general lower than 20%.

Method C: Results obtained for the analysis of endogenous PCBs in naturally contaminated matrices demonstrated the practicality of miniaturized UAE with a tip probe combined with DPX for the determination of the target compounds in a wide variety of solid fat-containing matrices. Sample preparation was complete in ca. 15 min with 1.5 mL of *n*-hexane and less than 1.0 g of SiO_2–H_2SO_4. The performance of the proposed methodology was demonstrated on the base of the mutual agreement observed among the determined endogenous PCB levels in a naturally contaminated internal reference material by using this novel UAE-DPX method and those obtained using a more conventional sample preparation procedure previously validated in our laboratory: recoveries, as compared to levels determined using the latter method, were in the 85–123% range for a large majority of the studied congeners, and the relative standard deviations were in general lower than 14%. Results obtained for the analysis of (internal) reference food samples and certified reference materials NIST 1945 and 1947 demonstrated that, when combined with GC–ITD(MS/MS) for final determination, LODs in the 2–152 pg/g range were obtained for most of the investigated PCB congeners, so allowing their accurate quantization even if as a small amount of sample as 50 mg was used.

The three proposed analytical methodologies represent fast, simple, and cost-effective alternatives to large-scale conventional protocols in use for the determination of different POP families in complex (semi-)solid foodstuffs. In all cases, sample manipulation has been reduced to a minimum, so minimizing the risk of contamination, and ready-for-analysis extracts were collected, which contributes to reduce the possibility of analyte losses. Our experimental results demonstrated that, despite their simplicity and the reduced initial amount of sample considered, methods A and B fulfil the analytical requirements set in current legislation concerning the analysis of dioxin-like compound in fatty matrices, while method C can be considered as a valuable alternative for fast (and virtually automatic) routine analysis/screening of environmentally relevant PCBs in these types of samples. Due to their features, the proposed methodologies are particularly suitable for the treatment of size-limited matrices.

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