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MINIATURISED ONE–STEP SAMPLE PREPARATION METHOD FOR PCB DETERMINATION IN FOOD SAMPLES

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Introduction

Classical methods for the determination of PCBs in fatty foodstuffs are usually laborious and time-consuming multi-step procedures involving a number of steps for quantitative extraction of the target compounds and clean-up of the extracts before final determination of the analytes can be accomplished by gas chromatography (GC) with an appropriate detector. Because most of these successive treatments of the sample are carried out off-line, much manual handling of the extracts is usually required [1], which makes these methods liable to contamination and losses of the analytes. In an attempt to overcome the most pressing shortcomings associated to these classical approaches, speed up the complete analytical process, and increase sample throughput, at-line, or on-line, coupling of the different steps required for sample preparation has become one of the main demands for a number of laboratories. Several examples of on-line clean-up procedures have been described in the literature [2,3]. However, the analyte extraction step is still considered as the main limitation when developing completely on-line and/or automated procedures for solid or semisolid (environmental) samples. This problem has made the number of studies reporting complete on-line sample preparation for the determination of traces pollutants such as PCBs, or the close related organochlorinated pesticides and PCDD/Fs, in fatty foodstuffs to be scarce in the literature [4,5]. Furthermore, the coupling among the various analytical steps have often leaded to rather sophisticated set-ups, which are not always easy to handle and/or maintain, and that occasionally involve amounts of sample, solvents and sorbents in the range of (or larger than) those used in conventional approaches. Thereby, the subsequent coupling of the sample preparation steps with the technique selected for separation–plus–detection of the analytes becomes also difficult. Regarding this latter aspect, miniaturisation of the extraction devices and, if at all possible, no additional clean-up requirements could be considered as key factors when developing complete automated systems.

This paper describes a new miniaturised method for fast determination of PCBs in solid fatty foodstuffs. Once optimised, the analytical procedure allowed the exhaustive extraction of the analytes from the sample and the clean-up of the extracts in a single step with a minimum consumption of solvent and sorbents. The performance of the analytical procedure developed, which was combined at-line with gas chromatography–micro electron capture detection (GC–microECD), was tested for the determination of PCBs in a non-spiked pork meat. The results were compared with those obtained when the same samples was prepared according to a more conventional procedure previously validated in our laboratory [6]. GC with ion trap tandem mass spectrometry (MS/MS) was used for final confirmation of the results.

Materials and Methods

All solvents used were pestipur quality and were purchased from SDS (Peypin, France), except hexane (Merck, Darmstadt, Germany). Sulphuric acid was 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 studied (see Table 1 below) were selected because of their toxicity and relative abundance in environmental samples. A working stock solution was prepared from individual PCB standards (Ehrenstorfer, Augsburg, Germany) containing 1000 pg/µl of each compound in isooctane. This solution was used for further dilution. 1,2,3,4-Tetrachloronaphtalene (TCN, Ehrenstorfer) and PCB 209 were used as external standards for PCB determination by GC–microECD and added to the final extracts just before the chromatographic analysis. Labelled standards of the 13 most toxic congeners were added to the extracts before GC–MS/MS confirmation [7].

The fatty pork meat sample investigated (49% of fat, w/w) was purchased from a supermarket in Madrid (Spain), and conserved at –20º C until analysed.

After optimisation of the different parameters affecting the efficiency of the simultaneous extraction and clean-up procedure proposed (namely the type and amount of sorbent used for dispersion of the sample and for subsequent fat removal, the nature and volume of the extraction solvent, and the number of static extraction cycles), a typical experiment consisted on the dispersion of a representative portion of the freeze-dried meat sample, ca. 1.0 g, on similar amounts of Na_2SO_4 and silica modified with 40% (w/w) sulphuric acid (SiO_2-HSO_4). After blending and homogenisation in a mortar using a pestle (MSPD step), 0.9 g of this mixture was packed in an 8 ml glass disposable extraction column on top of 1.5 g of SiO_2-HSO_4. Hexane was used as extraction solvent. After two static extractions, some fresh solvent was eluted through the column to ensure proper purging of the sample and the clean-up sorbent. Procedure blanks were prepared following the same procedure as for meat but without sample. No background interference was found to be introduced by the methodology proposed. Definitive evaluation of the combined MSPD plus SiO_2-HSO_4 arrangement proposed was carried out by determination of the target compounds in the non-spiked meat tested and subsequent comparison the results obtained with those found using a more conventional procedure for this kind of analysis based on large scale MSPD of the sample and off-line fat removal with SiO_2-HSO_4 plus activated SiO_2 as described elsewhere [6]. Otherwise specified, all experiments were carried out in triplicate.

Determination of the PCBs selected in the final extracts was performed by GC (HP 6890 Series, Hewlett-Packard, Palo Alto, CA) with micro-ECD. Samples were injected in the hot splitless mode (1 µl, 270ºC, splitless time 1.0 min) in a capillary DB-5 column (60 m, 0.25 mm i.d., 0.25 µm film thickness) purchased from J&W Scientific (USA). The column temperature was programmed from 80ºC (2 min) to 185ºC (3 min) at a rate of 30ºC/min, then to 230ºC (10 min) at 1.5ºC/min and then to 270ºC (10 min) at 5ºC/min. Nitrogen was used as carrier gas (constant flow, 1.5 ml/min) and as make-up gas 30 ml/min. The detector temperature was set at 300ºC.

Confirmation of the individual PCB congeners investigated was carried out in a GC (CP-3800, Varian, CA, USA) equipped with an ion trap MS detector (Saturn 2000, Varian) working in the MS/MS mode under the experimental conditions described elsewhere [7].

Results and discussion

An off-line large scale method previously validated [6] has now been modified in order to (i) reduce the amount of sample and sorbents used for both MSPD and clean-up of the extracts, and (ii) adapt the methodology for on-line coupling of the extraction and purification steps. Preliminary experiments were carried out to systematically reduce the amount of sample, and consequently those of SiO_2 and Na_2SO_4, required to disperse the meat tested from the 20 g used in the original method to 1.0 g, 0.5 g and, finally, to 0.3 g while allowing a reliable determination of the endogenous PCBs selected. The acetone:hexane (1:1, v/v) mixture eluting form this column was concentrated under a gentle stream of nitrogen, and the fatty extract obtained dissolved in hexane and purified on the silica mutilayer column described above. For obvious reasons, the reduction of the initial sample size used in these experiments permitted of an equivalent reduction of both the amount of sorbents required for purification of the extracts, and the total solvent consumption. However, this approach did not allow a direct coupling between the extraction and the clean-up steps as the acetone:hexane mixture was found to (partially) elute the polar compounds yielded by reaction of the co-extracted material with the sulphuric acid used for fat removal. Thereby, a new set of experiments was carried out to adapt the extraction step for on-line coupling with the clean-up procedure. Firstly, the feasibility of hexane, acetone, and toluene for the on-line coupling of these two analytical steps was tested. Once hexane was selected as extraction solvent, assays were carried out to improve the efficiency of the new extraction procedure proposed. Among the various mixtures of activated silica and silica modified with either 22% or 44% (w/w) of sulphuric acid tested, the latter sorbent was finally selected for subsequent studies as it allowed a preliminary fat removal while providing a performance similar to that of the original large scale method took as reference. Under these experimental conditions, two consecutive static extractions of the sample were preferred to an increase in the solvent volume passed through the column as this approach was found to provide enhanced recoveries of the endogenous analytes while reducing the solvent consumption to a minimum.

Table 1 summarises relevant analytical data relate to the miniaturised method proposed for fast extraction with simultaneous clean-up of endogenous PCBs from real-life fatty foodstuffs. The total analytical procedure developed compared favourably with the more conventional off-line method [6] considered as reference method. Results proved that despite the relative small among of sample used in the analyses, 0.3 g, reliable determination for all analytes was possible by both procedures. Most of the concentration levels calculated using the on-line method were in the range 94-129% of those determined using the off-line procedure. Lower recoveries were obtained only for PCB 194, 59%. The higher levels obtained for PCBs 52, 95 and 101 could be associated to possible losses of these congeners during elimination of the extraction solvent in the off-line procedure, a step avoided in the on-line method. The satisfactory relative standard deviation (RSD, n=3) values obtained (in general, lower than 11%), which were essentially the same than those found using the off-line procedure, proved the accuracy of the method developed. More importantly, the good agreement found among the concentrations of the less abundant non-*ortho* PCBs determined using the miniaturised procedure proposed in combination with GC–ITD (MS/MS) (PCB 77, 297 pg/g fat; PCB 126, 21 pg/g fat; and PCB 169, < 0.90 pg/g fat) with those calculated using a large off-line scale method involving 10 g of sample and GC–HRMS (PCB 77, 276 pg/g fat; PCB 126, 15 pg/g fat; and PCB 169, 0.75 pg/g fat) [8] contributes to fully illustrate the performance of the methodology proposed for the fast determination of PCBs in foodstuffs.

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Table 1. Comparison of relevant analytical data related to the determination of endogenous PCBs in fatty foodstuffs by a large-scale off-line procedure and the new miniaturised on-line extraction plus clean-up method developed.

PCB No.	Concentration (RSD, %) [a] (ng/g freeze dried sample)		Recovery (%) [b]	LOD [c] (ng/g freeze dried sample)
	Off-line method	On-line method		
28	6.6 (7)	7.2 (2)	108	0.2
52	14.3 (6)	19.2 (3)	134	0.2
95	16.32 (5)	24.49 (1)	150	0.09
101	15.2 (6)	22.2 (1)	146	0.1
81	< 0.2	< 0.2	—	0.2
77 (+110)	26.7 (2)	33.4 (3)	125	0.3
123 (+149)	8.2 (9)	9.5 (11)	115	0.1
118	3.9 (6)	5.3 (9)	136	0.1
114	0.061 (0.2)	0.071 (3)	116	0.009
153	8.89 (9)	9.67 (13)	109	0.09
132	3.39 (7)	3.99 (3)	118	0.06
105	0.95 (13)	1.40 (6)	147	0.03
138	7.8 (9)	8.0 (5)	103	0.2
126 (+129+178)	1.26 (11)	1.41 (4)	112	0.02
183	0.95 (22)	0.97 (2)	102	0.02
167	0.82 (3)	0.16 (17)	90	0.02
156	0.25 (14)	0.32 (7)	129	0.02
157	< 0.06	< 0.06	—	0.02
180	3.05 (31)	2.96 (0.7)	97	0.03
169	< 0.01	< 0.01	—	0.01
170	0.10 (9)	0.67 (2)	94	0.02
189	< 0.04	< 0.04	—	0.04
194	0.17 (3)	0.10 (9)	59	0.01

[a] Mean value, n=3; [b] on-line method *vs* off-line method;[c] Limits of detection as determined for a real-life sample using the miniaturised on-line method developed