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Multiresidue determination of organochlorines in fish oil by GC–MS: A new strategy in the sample preparation

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ABSTRACT

A rapid, economic and environmentally friendly analytical methodology has been implemented for the determination of α-, β-, γ- and δ-HCH, *p,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE, PCBs congeners #28, #52, #101, #153, #138 and #180 and Hexachlorobenzene in fish oil. 1,2,3,4-Tetrachloronaphtalene was used as internal standard. The sample preparation, consisting of a single step of clean-up and fractionation, took place in a column filled with different layers of neutral and sulphuric acid modified silica. The analytes were eluted by vacuum with of hexane. Significant reduction in terms of solvents, sorbents, and analysis time was achieved in comparison with literature.

Gas chromatography coupled to mass spectrometry was used for the separation and determination of the analytes. The instrumental limits of detection were from 0.1 to 1.3 ng mL^{-1} and the response of the detector was linear up to 200 ng mL^{-1}. The separation proved to be precise (RSD < 3.7% in peak area) and robust in terms of peak area, peak efficacy and resolution. The methodology was validated with two certified reference materials of cod liver oil, BCR 598 and BCR 349, obtaining no statistically significant differences between the concentrations found and certified. For the analytes that were not certified, aliquots of the reference materials were spiked and the recoveries obtained were satisfactory. These results were consistent with those found previously for DDTs by gas chromatography with an electron-capture detector.

The methodology was applied to the analysis of three fish oil pills sold in Spain as a dietary supplement of vitamins and omega-3 fatty acids. The sum of the analytes studied was from 64 to 80 ng g^{-1}. The most abundant compounds are PCBs, followed by DDTs in all samples.

1. Introduction

Persistent organic pollutants (POPs) are toxic chemical compounds that stay stable in the environment over extended periods of time and biomagnify as they move up through the food chain. The use of these compounds was banned [1] or restricted [2] in the European Union in the 1970s due to their low biodegradability, high persistence and toxicity characteristics that include cancer-inducing and endocrine-disrupting properties. Within this group, some organochlorine pesticides (OCPs) and polichlorobiphenyls (PCBs) are of special concern since the Stockholm Convention, in 2001, when they were included in the so-called "dirty-dozen" [3].

Fish oil is a by-product of the fish meal manufacturing industry. Concentrated omega-3 fatty acids can be found in fish oil, whose daily ingestion slows down the progression of coronary artery disease [4]. Since POPs are lipophilic and tend to accumulate through the aquatic food chain [5], they can be found in the lipid compartments of fish and also in the products extracted thereby. Thus, it has been reported that fish oils contain relatively high levels of OCPs, PCBs and other POPs [6–11].

The preferred technique for the determination of volatile pesticides in oils is capillary gas chromatography (GC) due to its high separation efficiency and the variety of selective detection methods that can be used. For the particular case of organochlorine compounds, electron-capture detection (ECD) has been widely used due

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to its high sensitivity and selectivity [12–14]. Nevertheless, analytical problems associated with the analysis of pesticides in fatty matrices are well known and thus the unequivocal confirmation of identity is often compromised. Mass spectrometry (MS), usually in the selected ion monitoring (SIM) mode, is the preferred method of choice [14–17] although confidence in the confirmation of identity may be reduced if one or more of the selected ions are affected by matrix interferences, giving poor spectral information. Alternatively, MS/MS with ion trap [18,19] or triple quadrupole [14,20] can be employed to achieve a high level of selectivity and low detection limits in dirty extracts [21]. Other authors have used high resolution mass spectrometry (HRMS) [17] and reverse-phase liquid chromatography coupled to GC with ECD and nitrogen-phosphorus detection for this purpose [22]. More recently, Hoh et al. have used the comprehensive two-dimensional GC coupled to time-of-flight mass spectrometry (TOF-MS) after direct sample injection for the analysis of fish oils [23,24]. In these papers, not only the authors have determined target analytes, but they also have managed to identify unknown peaks in the traditional one-dimensional GC–MS analysis, thanks to the increase of the power separation and to the mass spectra provided by the TOF-MS. It is evident that all these techniques allow reducing significantly the sample preparation step, but they are either too expensive or too sophisticated to be implemented for routine analysis.

Due to the large variety of POPs to be determined and also to the complex nature of the matrix, the trace analysis of these compounds requires the development of multiresidue methodologies including sample preparation prior to analytical determination. In general, the sample preparation consists of two separate steps [7,25]. First, the lipids are destroyed by adding concentrated sulphuric acid, and second, a clean-up step by gel permeation chromatography (GPC) and/or mixed silica, alumina or florisil columns is carried out. More recently, other techniques have been used, such as dialysis instead of sulphuric acid followed by a clean-up was carried out with neutral and acid silica gel multilayer column [26] or ultrasonication followed by single drop microextraction [27] but these are still two-step strategies for sample preparation.

These approaches lead to extremely time consuming sample preparations and also large amounts of reagents to be used, which generates considerable liquid and solid wastes. For this reason a single-step strategy with minimal reagent consumption for sample preparation is preferable. In this way, a previous work using this strategy and GC-ECD as analytical technique for the determination of DDTs was carried out with satisfactory results [28]. However, more efforts had to be made to go on decreasing the reagents consumed and the analysis time, and also other analytes of environmental concern had to be studied to widen the applications and mass spectrometry had to be used as detector so as to get a more unambiguous determination.

The aim of this work is to improve the sample preparation in terms of economy, time of analysis and respect for the environment without sacrificing sensitivity, accuracy and precision in fish oil samples, for the determination of α-, β-, γ- and δ-HCH, *p,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE, PCBs congeners #28, #52, #101, #153, #138 and #180 and Hexachlorobenzene in fish oil. To do so, our approach has been to use a single-step sample preparation strategy for both fat removal and clean-up/fractionation.

Moreover, a GC–MS method was validated for the analysis of all these organochlorine compounds (OCs). The methodology was applied to certified reference materials and fish oil samples. The results were compared with those obtained previously by GC-ECD when possible.

2. Materials and methods

2.1. Standards, chemicals and samples

A solid mixture of α-, β-, γ- and δ-HCH isomers (Pestanal™) and a standard solution of DDT at 100 μg mL^{-1} were purchased from Sigma–Aldrich (Seelze, Germany).

Standard solutions of DDE and DDD at 200 and 5000 μg mL^{-1}, respectively, and a solid standard of HCB were obtained from Supelco (Bellefonte, PA, USA).

A standard solution of PCB congeners #28, #52, #101, #153, #138 and #180 at 10 μg mL^{-1} was purchased from Dr. Ehrenstorfer, GmbH (Augsburg, Germany) and kept refrigerated until used.

A stock solution of 1,2,3,4-tetrachloronaphtalene (TCN) (Dr. Ehrenstorfer, GmbH) at 200 ng mL^{-1} was prepared in hexane and kept refrigerated until used as internal standard.

Anhydrous sodium sulphate, silica gel 60 and *n*-hexane were purchased from Panreac Química S.A. (Barcelone, Spain) and Sulphuric acid was from Scharlau Chemie S.A. (Sentmenat, Spain). All these reagents were analytical grade or better.

Helium (Carburos Metálicos S.A., Barcelona, Spain) was Premier X50S quality.

A set of Hamilton™ mycro-syringes of volumes ranging from 10 to 250 μL was used throughout the work.

The certified reference materials of cod liver oil used were BCR 598 and BCR 349, from the Institute for Reference Materials and Measurements (Geel, Belgium).

Cod liver oil pills, "Kromenat" (Kromenat, Spain), and salmon oil pills, "Verdalia" (Naturland I.C.C., Carros, France) and "Arkocaps" (Arkopharma, Carros, France) were purchased in a supermarket as samples. They were codified as CL-1, S-1, and S-2, respectively. The content of three pills of each sample was mixed in a glass vial and then 0.2 g were taken for sample pretreatment.

Glass hypodermic syringes of 5 mL were used as columns for sample preparation (Normax, Lda., Marinha Grande, Portugal).

2.2. Instrumentation

The analyses were carried out in an Agilent 6850 gas chromatograph (Agilent Technologies, Tokyo, Japan) coupled to an Agilent 5975B mass spectrometer. A 30 m HP-5MS (J&W Scientific, Folsom, CA, USA) fused silica capillary column (0.25 mm I.D., 0.25 μm film thickness) was used for separation. The following temperature program was applied: 100 °C (2 min) – 10 °C min^{-1} – 180 °C (2 min) – 1.5 °C min^{-1} – 200 °C – 20 °C min^{-1} – 250 °C – 30 °C min^{-1} – 280 °C (4 min). Helium was employed as carrier gas at 1 mL min^{-1}. The injector, ion source and quadrupole were set at 250, 230 and 280 °C, respectively. 1 μL of sample was injected in splitless mode. The ionisation was made by electronic impact at 70 eV.

A Reacti-Therm heating module (Pierce, Rockford, IL, USA) with an evaporating unit was used for preconcentration. The evaporating unit uses nitrogen and can concentrate nine samples simultaneously.

A Visiprep solid phase extraction vacuum manifold system (Supelco, Bellefonte, PA, USA) coupled with a Laboport vacuum pump (KNF Neuberger Inc., Trenton, NJ, USA) was used to facilitate the passage of solvent through the column and expedite the sample preparation.

2.3. Sample preparation

The sample preparation was carried out in glass columns. Glass was preferred to plastic because preliminary experiences showed that recoveries of some analytes when using plastic columns were lower than recoveries obtained when using glass columns. This is

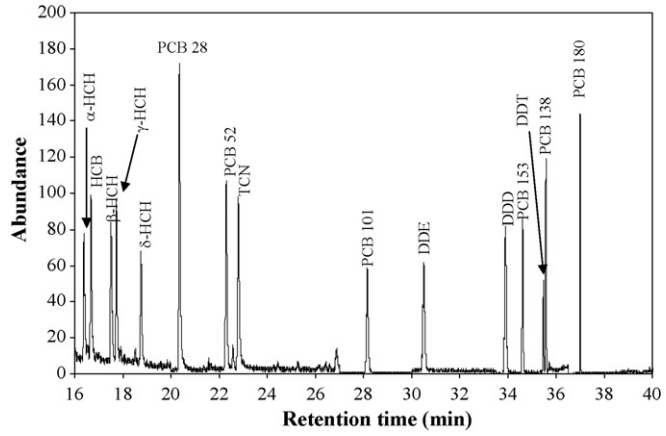

Fig. 1. A total ion current chromatogram of a standard solution of analytes and internal standard at 10 ng mL^{-1} under the optimised conditions.

probably due to some kind of adsorption processes of these analytes in plastic. The columns were 7.6 cm long × 1.3 cm of inner diameter. The columns were filled, from bottom to top, with 0.4 cm of anhydrous Na_2SO_4, 0.8 cm of neuter activated silica, 1.1 cm of sulphuric acid modified activated silica at 22% (w:w), 2.3 cm of sulphuric acid modified activated silica at 44% (w:w), and 0.4 cm of anhydrous Na_2SO_4. This multilayer silica column was bottomed with a polyethylene fritted disk and was washed with 20 mL of hexane prior to use.

Each sample of oil, typically 0.2 g, was accurately weighed in a 5 mL glass vial, dissolved in 1 mL of hexane and added 2.5 μL of TCN at 200 ng mL^{-1}. The sample was loaded into a mixed silica column prepared and activated as explained above and allowed to equilibrate for 5 min. The samples were then eluted with 15 mL of hexane by vacuum, collecting the eluent in test tubes. Later on, this eluent was evaporated, transferred to conical bottom injection vials and evaporated to dryness under a gentle nitrogen stream. Finally, samples were reconstituted with 50 μL of hexane and analysed by GC–MS by triplicate.

3. Results and discussion

Once the column was set up as described in Section 2, placed in the vacuum manifold, and washed, the volume of solvent necessary for the elution of the analytes from the column had to be studied. For this purpose, a stock solution (10 ng mL^{-1}) of the analytes and internal standard was loaded into a mixed silica column prepared and activated as explained above. Hexane was used for the elution of the analytes.

In order to reduce the consumption of both sorbents and eluent and, consequently, the wastes, a downsizing of the columns, in comparison with previous works [28–30], was accomplished. Likewise, in order to reduce the analysis time, the activation of the column and the elution of the analytes were made by vacuum, instead of doing so by gravity.

The elution was carried out with 20 fractions of 1 mL each of hexane. These fractions were collected, evaporated to dryness, reconstituted with 50 μL of hexane and injected in the chromatographic system. The results showed that the compounds were present in the fractions #1 to #15. As a consequence, the elution volume selected was 15 mL.

Owing to the reduction in the sorbents used in the columns, the amount of sample that can be purified should be evaluated too. In order to find out this, different amounts of fish oil, from 0.1 up to 0.5 g, were weighed, dissolved in hexane as explained in the sample preparation section, and loaded into the multilayer columns. The columns showed total fat removal efficiency up to 0.2 g of fish oil, whereas remnants of oil were noted in the eluate corresponding to the rest of the samples. Consequently, a maximum of 0.2 g of oil could be purified with these columns. This amount proved to be adequate for the levels of the analytes.

The column washing volume, the total amount of sorbents used and the volume of eluent resulted to be up to 6 times smaller than in our previous work reporting the analysis of fish oils for DDTs [29]. The amount of oil analysed in that work was from 0.2 to 0.4 g, which means that the amount of sorbents and solvents by using the presented methodology would be reduced by one-sixth to one-third, respectively.

The use of vaccum for the conditioning of the column and also for the elution of the analytes allowed these steps go faster than if they had been carried out only by gravity. Thus, the time required from the column conditioning to the reconstitution with hexane was about 60 min. Unfortunately, no data reporting this time was found in the papers mentioned above, but the procedures described in them are likely to take much longer because the elution of the analytes was carried out by gravity and also because the volume of eluate to evaporate was about 10-fold the volume we get in this work.

3.1. Gas chromatography–mass spectrometry conditions

For the optimization of the GC–MS, we used the conditions reported by Papadakis et al. [30] as initial ones. The total GC

Table 1
Retention times, quantification and qualifier ions, calibration parameters and limits of detection and quantification.

Compound	Retention time (min)	Quant./qual. ion (*m*/*z*)	Calibration curve	Slope S.D.	Intercept S.D.	r^2	LOD[a]	LOQ[a]
α-HCH	16.392	181/183	RA = 0.15 +0.0331*C*	0.0005	0.06	0.9987	1.05	3.50
HCB	16.697	284/286	RA = 0.04 + 0.0752*C*	0.0009	0.09	0.9994	0.1	0.33
β-HCH	17.511	181/183	RA = 0.10 + 0.0402*C*	0.0004	0.04	0.9995	0.87	2.90
γ-HCH	17.738	181/183	RA = 0.10 + 0.0284*C*	0.0004	0.04	0.9991	0.93	3.10
δ-HCH	18.759	181/183	RA = 0.05 + 0.0199*C*	0.0002	0.02	0.9993	1.26	4.20
PCB 28	20.351	256/258	RA = 0.04 + 0.138*C*	0.002	0.2	0.9993	0.5	1.67
PCB 52	22.303	290/220	RA = 0.02 + 0.0704*C*	0.0009	0.09	0.9992	0.5	1.67
TCN (i.s.)	22.807	266/264	–	–	–	–	–	–
PCB 101	28.161	326/328	RA = 0.01 + 0.0455*C*	0.0006	0.06	0.9991	0.5	1.67
DDE	30.511	246/248	RA = 0.05 + 0.0428*C*	0.0006	0.06	0.999	0.2	0.67
DDD	33.901	235/237	RA = 0.3 + 0.0707*C*	0.002	0.2	0.9959	0.1	0.33
PCB 153	34.619	360/362	RA = 0.06 + 0.0428*C*	0.0006	0.07	0.9989	0.5	1.67
DDT	35.478	235/237	RA = 0.10 + 0.0125*C*	0.0005	0.05	0.9934	0.5	1.67
PCB 138	35.584	360/362	RA = 0.04 + 0.0399*C*	0.0006	0.06	0.9990	0.2	0.67
PCB 180	37.010	394/396	RA = 0.04 + 0.0351*C*	0.0006	0.06	0.9988	0.2	0.67

RA: relative area = analyte area/internal standard area; *C*: analyte concentration in ng mL^{-1}; S.D.: standard deviation.
[a] In ng mL^{-1}.

run time was 62 min, including a post-run step of 20 min at 300 °C to remove impurities trapped in the column, with 3 min between injections to allow for cool-down, stabilization, and injection.

For mass spectrometry detection, the instrument quadrupole was set to 280 °C and operated in electronic ionisation (EI) mode to 70 eV. The MS detector interface temperature was set at 150 °C, source temperature at 230 °C and detector voltage at 1365 V. The filament was switched on 16 min after the injection, which is approximately 1 min before the elution of the first peak of interest, to diminish damage to the mass detector. The MS was operated in the SIM mode in order to improve the signal to noise ratio. Thus, a quantifier and a qualifier ion were selected for each compound as analytical tools, apart from, obviously, the retention time (Table 1).

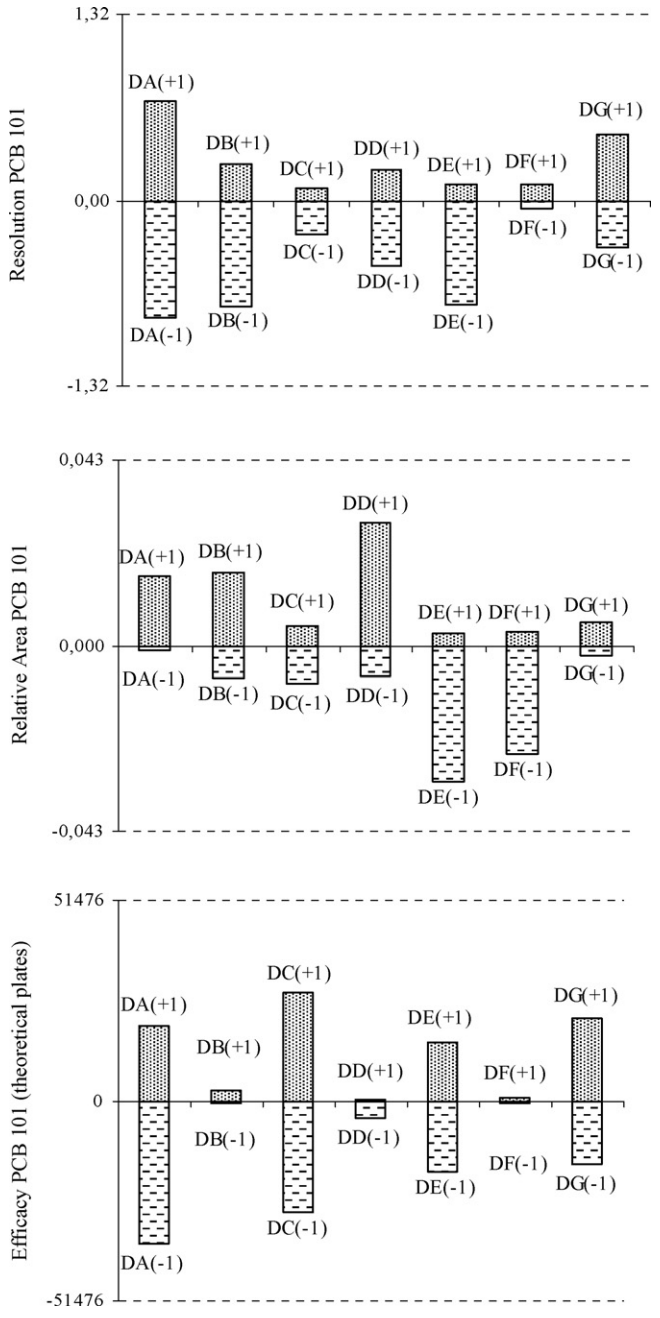

Fig. 2. Variation effects on relative area, resolution and efficacy of PCB #101 as an example of the robustness test.

A chromatogram of a standard solution containing all analytes and the internal standard at 10 ng mL^{-1} is shown in Fig. 1, where good resolution between peaks ($R_s > 1$) and excellent efficiencies can be seen.

3.2. Validation

In order to check the reliability of the GC–MS method, its analytical performance characteristics were evaluated.

3.2.1. Limits of detection and quantification

The instrumental limit of detection (LOD) was estimated in accordance with the baseline noise. The baseline noise was evaluated by recording the detector response over a period about 10 times the peak width. The LOD was obtained as the compound concentration that caused a peak with a height of three times the baseline noise level. Likewise, the instrumental limit of quantification (LOQ) was obtained as the compound concentration that caused a peak with a height of 10 times the baseline noise level.

Thus, the LODs and LOQs obtained for the analytes under these conditions are presented in Table 1. The instrumental LODs are in the low ng mL^{-1} range, as expected for the mass spectrometry detector.

3.2.2. Linearity

The linearity was checked by triplicate injections of 1 μL of a set of seven standard solutions containing increasing concentrations of the analytes from the limits of quantification up 200 ng mL^{-1} under the chromatographic conditions described above. The adequate amount of the internal standard solution was added so that its final concentration was 10 ng mL^{-1}. The calibration curves were obtained by plotting the relative peak area (analyte over internal standard peak area) versus the analyte concentration.

The equations and determination coefficients are summarized in Table 1. In all cases, the calibration curves showed an excellent linear relationship between relative areas and concentrations. The determination coefficients, r^2, were satisfactory in the concentration range assayed. Likewise, the intercepts were found not different from zero according to the Student's test "*t*" ($P = 0.05$).

3.2.3. Precision

The precision of the proposed method is expressed in terms of relative standard deviation (R.S.D.).

The repeatability of the chromatographic method was studied by performing a series of 21 replicates of a standard solution containing 10 ng mL^{-1} of each compound. The results showed that the relative standard deviation of the areas for each and every compound was below 3.70% in all cases.

The reproducibility over time of the chromatographic method was evaluated by separating 21 replicates of a stock solution prepared as explained above, in 2 consecutive days and comparing the standard deviations (below 4.6% in both days for each compound) of the peak areas of each compound. For this purpose, the Snedecor test "*F*" for two tails was used and, as a result, no significant differences between the series for both days were found ($P = 0.05$).

Likewise, the results obtained for the retention times were satisfactory as well, in terms of both repeatiblity and reproducibility.

3.2.4. Robustness

The aim of a robustness test is to identify possible sources of error when changes occur in the specified method conditions [31]. In this work, we evaluated if small changes in the main chromatographic parameters (factors) had a significant influence on relative peak area, peak efficacy and resolution. For this purpose, fractional

Table 2
Certified, found concentrations and recoveries for the analysis of the CRMs. Non-certified analytes added and found, with standard deviation, and recoveries.

Analyte	Certified ($ng\,g^{-1}$)	Found ($ng\,g^{-1}$)	Recovery (% w:w)
BCR 598			
α-HCH	42 ± 3	41.9 ± 3.9	100 ± 12
HCB	55.7 ± 2.0	54.0 ± 1.4	97.0 ± 4.4
β-HCH	16 ± 3	16.7 ± 3.4	104 ± 29
γ-HCH	23 ± 4	24.37 ± 0.32	106 ± 21
DDE	0.61 ± 0.04	0.6333 ± 0.0079	103.8 ± 6.9 (104.0)[a]
DDD	0.40 ± 0.03	0.4066 ± 0.0083	101.7 ± 7.9 (104.8)[a]
DDT	0.179 ± 0.018	0.1761 ± 0.0055	98 ± 10 (108.0)[a]
BCR 349			
PCB 28	68 ± 8	55 ± 8	81 ± 15
PCB 52	149 ± 21	156 ± 5	105 ± 16
PCB 101	372 ± 18	411 ± 42	110 ± 12
PCB 153	940 ± 40	1113 ± 89	118 ± 10
PCB 180	282 ± 23	285 ± 19	101 ± 11
Analyte	Added ($ng\,g^{-1}$)	Found ($ng\,g^{-1}$)	Recovery (% w:w)
δ-HCH[b]	37.5	31.2 ± 0.1	83.5
PCB 138[c]	25.0	21 ± 4	86.0

[a] Recoveries in brackets correspond to the analysis by GC-ECD.
[b] Added to BCR-598.
[c] Added to BCR-349.

factorial designs developed by Plackett and Burman [32], based on balanced incomplete blocks, were used. The factors and levels selected in our case were the following:

Factor A: Flow rate ($0.9_{(-1)}$, $1.0_{(0)}$, $1.1_{(+1)}$) ($mL\,min^{-1}$).
Factor B: Time of the splitless step ($1.8_{(-1)}$, $2.0_{(0)}$, $2.2_{(+1)}$) (min).
Factor C: Temperature for the splitless step ($95_{(-1)}$, $100_{(0)}$, $105_{(+1)}$) (°C).
Factor D: Injector temperature ($240_{(-1)}$, $250_{(0)}$, $260_{(+1)}$) (°C).
Factor E: Injection rate ($280_{(-1)}$, $300_{(0)}$, $320_{(+1)}$) ($\mu L\,min^{-1}$).
Factor F: Detector temperature ($270_{(-1)}$, $280_{(0)}$, $290_{(+1)}$) (°C).
Factor G: Final oven temperature ($270_{(-1)}$, $280_{(0)}$, $290_{(+1)}$) (°C).

The optimum value is labelled as (0), whereas the values labelled as (−1) and (+1) are the maximum variation in the value of the factor. The average and standard errors (DA, DB, DC, ...) were calculated using the procedures described by Youden and Steiner [33].

The robustness was determined in our case by triplicate injection of solutions of all analytes at 20 $ng\,mL^{-1}$, and TCN at 20 $ng\,mL^{-1}$ as internal standard. The effects of each factor on resolution, efficacy, and relative peak area were calculated. The efficacy was expressed in terms of the number of theoretical plates (N), where the peak width at half height was considered.

The effects of these factors on the relative area, resolution and efficacy of PCB 101 are shown in Fig. 2, as an example. In this particular case, the values of the variations of the seven factors on the efficacy were always within the range calculated using the Youden–Steiner statistical model, which means that the method is robust in terms of efficacy, resolution and relative peak area. Likewise, similar results were obtained for resolution, efficacy and relative peak areas for all compounds.

3.2.5. Accuracy

Two certified reference materials (CRM) of cod liver oil were used for recovery studies. Each CRM was used for the determination of the certified analytes in it. Thus, BCR 598 was used for α-, β- and γ-HCH, HCB, *p,p′*-DDE, DDD and DDT, whereas BCR 349 was used for PCB #28, #52, #101, #153, and #180. Additionally, known amounts of δ-HCH and PCB #138 were added to aliquots of BCR 598 and BCR 349, respectively, in order to calculate the recoveries of both, which are not certified in the CRMs used. Triplicate analyses of the CRMs, typically 100 mg, were conducted for the determination of the analytes, which were quantified by the standard addition method.

Also, another aliquot of BCR 598 was analysed for *p,p′*-DDE, DDD and DDT through the described procedure, but this time using GC-ECD as analytical technique.

The results of the accuracy study are shown in Table 2. The concentrations found for the certified analytes were not statistically different from the certified values, according to the Student's "*t*" test for $P = 0.05$, except for γ-HCH. In this case, the differences were not statistically different for $P = 0.02$. Also in Table 2, it can be seen that the analysis of the spiked CRMs for δ-HCH and PCB #138 showed recoveries close to 100%.

4. Application to real samples

The developed analytical procedure was applied to the determination of the analytes in one cod liver oil (CL-1) and two salmon oils (S-1 and S-2). These fish oils are commercially available in capsule forms as omega-3 fatty acids supplement. As an example, a chromatogram corresponding to the analysis of the cod liver oil is shown as Fig. 3.

The results, summarized in Table 3, showed that the highest total concentration of the analytes was found in S-2, with a 36% more of these compounds than CL-1 and 57% more than S-1.

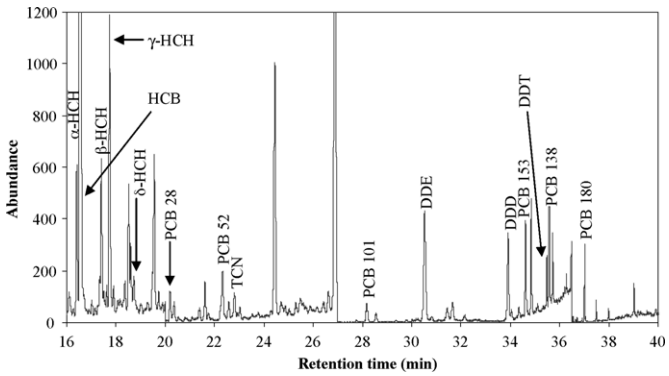

Fig. 3. A total ion current chromatogram of a sample of cod liver oil and internal standard at 10 $ng\,mL^{-1}$ under the optimised conditions.

Table 3
Concentration of the analytes (ng g^{-1}) ± standard deviation (n = 3) in salmon (S) and cod liver (CL) oil by GC–MS. Values in brackets were obtained by GC-ECD.

Analyte	S-1	S-2	CL-1
α-HCH	<LOD	<LOD	0.5 ± 0.2
β-HCH	2.33 ± 0.09	2.2 ± 0.3	1.7 ± 0.3
γ-HCH	8.6 ± 0.2	9.9 ± 0.8	6.0 ± 0.2
δ-HCH	<LOD	2.2 ± 0.2	1.0 ± 0.3
Sum HCHs	10.9 ± 0.2	14.3 ± 0.8	9.2 ± 0.6
PCB 28	0.9 ± 0.1	1.0 ± 0.1	0.55 ± 0.06
PCB 52	1.47 ± 0.09	1.9 ± 0.2	0.69 ± 0.09
PCB 101	4.9 ± 0.4	7.8 ± 0.9	2.55 ± 0.09
PCB 153	9.5 ± 0.9	18 ± 1	11.5 ± 0.3
PCB 138	9.3 ± 0.8	16 ± 2	11.0 ± 0.4
PCB 180	2.9 ± 0.3	5.1 ± 0.9	5.8 ± 0.3
Sum PCBs	29 ± 1	50 ± 3	32.1 ± 0.6
DDE	13 ± 1 (12.6 ± 0.3)	11 ± 2 (10 ± 1)	12.3 ± 0.6 (11 ± 1)
DDD	6.6 ± 0.5 (3.6 ± 0.04)	3 ± 1 (2.7 ± 0.3)	7 ± 1 (5 ± 1)
DDT	3.5 ± 0.5 (3.4 ± 0.9)	1.9 ± 0.2 (2.0 ± 0.3)	6.2 ± 0.8 (3.9 ± 0.8)
Sum DDTs	23 ± 1 (20 ± 1)	15 ± 3 (15 ± 1)	26 ± 2 (20 ± 2)
HCB	1.54 ± 0.09	0.5 ± 0.2	0.11 ± 0.04
Sum OCs	64 ± 2	80 ± 4	67 ± 2

In general, the levels found are in agreement with the literature [34]. In all cases, the dominant compounds are PCBs, followed by DDTs. The dominant congeners in each chemical family were DDE, PCBs #153 and #138, and γ-HCH, which is also in agreement with literature [34,35]. The prevalence of DDE, which is one of the terminal metabolites in DDT degradation (DDT → DDD → DDE), could indicate that exposure to DDT of all samples was far behind in time [36]. In the particular case of DDTs, the samples had been previously analysed for these compounds by GC-ECD. The results obtained in S-1 and S-2 by GC–MS and by GC-ECD were not statistically different (n = 3, P = 0.05), except for DDD in S-1. This can be explained by the fact that this particular sample provided a high background signal in ECD, which can overlap partially the peak of DDD. This is not likely to occur in GC–MS because a particular ion is selected for a particular compound. The results obtained for DDE and DDD in cod liver oil (CL-1) by GC–MS and by GC-ECD were not statistically different at P = 0.05, whereas for DDT, they were not statistically different at P = 0.02.

The most prominent PCB congeners are #138 and #153, followed by PCB #101, and #180. PCB #28 and #52 normally contribute only minor amounts to the sum of the indicator PCB. This backs the fact that animals can metabolise the lower chlorinated congeners, such as PCB #28, #52 and to a certain extent also PCB #101 [37], so the levels of these congeners are low or eventually below the limit of detection.

5. Conclusions

A multiresidue methodology for the determination of PCBs, DDTs, HCHs and HCB in fish oil was optimised by GC–MS. The sample preparation consisted of a single step including clean-up and fractionation in a small-size multilayer silica column, which was eluted by using a vacuum pump. This sample preparation allowed to save sorbents and solvents up to six times in comparison with previous works as well as to significantly reduce the total analysis time. The full analytical methodology was validated successfully by using two certified reference materials of fish oil.

The application of this methodology to fish oil pills that are sold as omega-3 fatty acids supplement in the diet showed the presence of the analytes at levels of ng g^{-1}. Further work is necessary in order to get a proper study of levels and also to confirm the chemical pattern of the analytes in these oils.

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