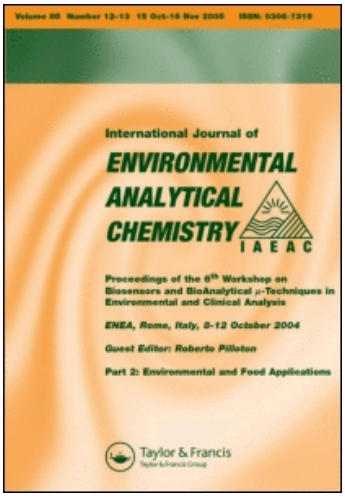

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Microwave-assisted extraction, membrane-assisted solvent extraction combined with gas chromatography/electron-capture detection applied to the analysis of polychlorinated biphenyls in bivalve molluscs

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Microwave-assisted extraction, membrane-assisted solvent extraction combined with gas chromatography/electron-capture detection applied to the analysis of polychlorinated biphenyls in bivalve molluscs

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Microwave-assisted extraction as an initial extraction step and membrane-assisted solvent extraction (MASE) as a clean up–extraction–concentration step were applied to the determination of polychlorinated biphenyls (PCBs) in mussel samples. The MASE conditions, methanol percentage in aqueous extract of sample, extraction time, extraction temperature, shaking speed, and extractant solvent volume, were optimised using a Plackett–Burman factorial design. The purified extract was analysed using gas chromatography/electron-capture detector. The results show that extraction time was statistically significant for PCBs 31, 28, 118 and 180 and shaking speed for PCBs 153, 138 and 156, both factors had positive estimated effects. The other investigated factors were not statistically significant. The extraction efficiency of the whole method was between 74% and 100% and the relative standard deviation ranged from 2% to 15%. The detection limits were about 0.1–0.9 $\mu g\,kg^{-1}$.

Keywords: microwave-assisted extraction; membrane-assisted solvent extraction; polychlorinated biphenyls; Plackett–Burman factorial design; gas chromatography–electron-capture detector; bivalve mollusc

1. Introduction

Polychorinated biphenyls (PCBs) constitute an important group of persistent organic pollutants (POPs) that have been widely used throughout the world. Due to their lipophilic and recalcitrant character, these compounds tend to accumulate in the animal tissues during long periods of time and to concentrate throughout the food chain. POPs have always been associated with negative health implications such as, reproductive toxicity, immunotoxicity, neurotoxicity, endocrine effects and carcinogenicity, so it is important to control PCBs levels in environmental and food samples [1].

The effect of POPs on environmental quality of estuarine and coastal marine systems is usually evaluated and controlled by long-term national or regional monitoring programs. Experience indicates that bivalve molluscs such as, mussels or oysters, are the best indicators of coastal pollution because they accumulate certain microcontaminants to much higher levels than those found in water. They are hardy, sessile, and have a limited capacity for metabolising contaminants [2].

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Measuring PCBs and organochlorine compounds background concentrations in biota requires reliable methods and techniques that provide extremely high sensitivity and specificity. The presence of large amounts of co-extracted lipids interfering the PCBs determination makes that long clean up and trace enrichment steps and selective detectors are needs. These procedures affect to the precision and accuracy of analysis and contribute to its cost. Conventional extraction methods, such as Soxhlet or Soxtec [3,4] and ultrasonication extraction (USE) [5] need large amounts of extractant solvent and long extraction times, are expensive and usually require several clean up steps prior to gas chromatographic determination. The recent techniques, like supercritical fluid extraction (SFE) [6], microwave-assisted extraction (MAE) [7,8], and accelerated solvent extraction (ASE) [9–11] work at elevated temperatures above the boiling point of the extractant solvent, can be automated and reduce the solvent volume, but they require further purification steps. The application of solvent free techniques using a solid adsorbent material, stir bar sorptive extraction (SBSE) [12,13] and solid-phase microextraction (SPME) [14,15], involves the retention and concentration of target analytes from the sample solution. These techniques can integrate sampling, extraction, clean up, and sample introduction into chromatographic system.

Another alternative to conventional and recent extraction devices is the membrane extraction technique [16–20]. In the membrane processes, the separation is the result of differences in the transport rates of the analytes through the interface. The phase from which the transfer occurs is called the donor and the phase that receives the flow is called the acceptor. The synthetic membranes may be of different chemical nature and display different properties. The use of a polymer (dense polypropylene) in membrane-assisted solvent extraction (MASE) allows the diffusion of organic compounds dissolved in an aqueous sample through a hydrophobic non-porous membrane bag into a small amount of organic solvent. Extraction vials are placed in an agitator and shaken at defined time and temperature [21–23]. The advantages of this technique are the low cost, rapidity, analyte preconcentration, interferences elimination, and low volume of extractant solvent (μL).

To date, the application of membranes has only been focused on extraction of organic compounds in liquid samples [18,19,21,23]. The aim of this work is to introduce the polymeric membranes in the purification step of complex solid samples. For this purpose, a procedure for the determination of 10 indicator PCBs recommended for environmental monitoring by International Council for the Exploration of the Sea (ICES) [24] in mussel by using MAE as an initial extraction step and MASE as a clean up–extraction–concentration step prior to gas chromatography–electron capture detector (GC-ECD) determination is described. The MAE conditions optimisation had already been performed by Carro *et al*. in 2000 [25]. The optimisation of MASE is carried out by using a Plackett–Burman factorial design. The optimised factors are methanol percentage in microwave extract, extraction time, extraction temperature, shaking speed, and extractant solvent volume. The developed method, MAE–MASE–GC (ECD), has been applied to certified reference material.

2. Experimental

2.1 *Material and apparatus*

Isooctane, acetone, *n*-hexane, *n*-heptane, and methanol for organic trace analysis were supplied for Merck (Darmstadt, Germany). Sodium hydroxide and anionic detergent, sodium dodecylsulphate (SDS), also were supplied by Merck. Analytical reagent grade

PCB individual congener standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Optimisation experiments were performed on a spiked mussel sample (*Mytilus galloprovincialis*). Sample was collected and immediately freeze dried. The sample (30 g) was spiked by slowly pouring it over 10 mL of methanol containing PCBs compounds (IUPAC number, PCBs 31, 28, 52, 101, 118, 153, 105, 138, 156, and 180). The mixture was manually mixed for 30 min. The sample was then allowed to air-dry for 5 days and stored in the dark for 30 days before analysis. Expected final concentrations were calculated to be from 25 to 130 $\mu g\,kg^{-1}$ for all PCBs, on a dry-weight basis. It was assumed that the contaminants were uniformly distributed in the sample and that, because the sample contained residual moisture from the storage period, any analyte–matrix interactions would have occurred to an extent similar to that in real contaminated sample with identical properties.

The PCBs recovery and method accuracy were determined by using a certified reference material NIST 2977 (mussel tissue) supplied by the National Institute of Standards and Technology (Gaithersburg, MD). The certified contents for PCBs 31, 28, 52, 101, 118, 153, 105, 138, 156, and 180 were showed in Table 1.

Standard stock solutions including the internal standard, PCB155, were prepared by weighing a suitable amount of each standard and diluting to 5 mL with isooctane. Working solutions were made by appropriate dilution of the stock solution in *n*-heptane. Both stock and working solutions were stored at −20°C. The direct calibration of GC-ECD was performed using composite standards of 1–700 $\mu g\,L^{-1}$ in *n*-heptane.

MAE experiments were carried out with a 950-watt MES-1000 System (CEM, Matthews, NC, USA) equipped with Teflon-lined 100 mL extraction vessels. It was operated under closed-vessel conditions at temperature and pressure up to 200°C and 200 psi. One of the vessels was used to control actual temperature and pressure values in the system.

The membrane bag of dense polypropylene (4 cm long, 0.03 mm thick, 6 mm internal diameter) used for MASE was produced by Gerstel (Mülheim, Germany). The extraction cell consisted of a conventional 20 mL headspace vial (Supelco, Bellefonte, PA, USA) with a membrane insert. The membrane sac was attached to a stainless steel funnel with a Teflon ring and placed in a headspace vial. The vial was placed in the stirring hot plate Heidolph, MR 3001 K (Schwabach, Germany).

Table 1. Certified concentrations and expanded uncertainty expressed in $\mu g\,kg^{-1}$ (dry mass basis) for selected PCB congeners in Material Reference NIST 2977.

	Certified value	Expanded uncertainty
PCB31 (TriCB)	3.92	0.24
PCB28 (TriCB)	5.37	0.44
PCB52 (TetraCB)	8.37	0.54
PCB101 (PentaCB)	11.2	1.2
PCB118 (PentaCB)	10.5	1.0
PCB153 (HexaCB)	14.1	1.0
PCB105 (PentaCB)	3.76	0.49
PCB138 (HexaCB)	16.6	1.6
PCB156 (HexaCB)	0.96	0.09
PCB180 (HeptaCB)	6.79	0.67

The purified extracts were analysed by gas chromatography using a Perkin-Elmer Autosystem gas chromatograph equipped with an electron capture detector. A TRB-5 (Teknokroma, Barcelona, Spain) 5% diphenyldimethylsiloxane capillary column (60 m × 0.20 mm i.d., 0.4 μm phase thickness) was used. Carrier gas was hydrogen supplied by Air Liquid (Spain). Chromatographic conditions were as follows: injector temperature (splitless mode, 1.8 min) 270°C, electron capture detector temperature 365°C and column temperature program 90 (3 min) to 215°C (40 min) at 30°C min^{-1} and 275°C (30 min) at 5°C min^{-1}, carrier gas flow at 1 mL min^{-1}.

Data numerical analysis was performed by means of the statistical package, Mini-Tab v.15.

2.2 *Sample preparation-extraction procedure*

2.2.1 *Microwave-assisted extraction*

One gram of freeze dried mussel plus 4 mL of sodium hydroxide solution (5%) was extracted with 20 mL of acetone/hexane during 10 min at 90°C setting the microwave extractor at half power [25,26]. After extraction, the sample vessel was removed from microwave. The organic phase was filtered through anhydrous sodium sulphate and concentrated to dryness. Later it was redissolved in 15 mL of water–methanol.

2.2.2 *Membrane-assisted solvent extraction*

Before use, the membrane bags were conditioned by shaking three times with 20 mL of *n*-heptane at 200 rpm, 30°C, and for 1 h. The membrane bags can be reused up to seven times after cleaning with the extractant solvent without loosing efficiency [21].

Irrespective of the working conditions imposed by the particular experiment in the factorial design, all samples were prepared by following the same procedure. The headspace vial was filled with 15 mL of microwave extract redissolved in water–methanol at a percentage fixed by the factorial design. In the last experiments out of the design frame, 0.2 g of an anionic detergent, SDS, were added to samples before MASE. The membrane bag was attached to the metal funnel with a Teflon ring and the funnel was suspended in the mouth of the vial. Then, the membrane bag was immersed in the MAE extract and filled with a solvent volume fixed by the factorial design. The vial was closed with a metallic crimp cap. The vial was placed in the stirring hot plate, the extraction time and temperature and the shaking speed conditions were imposed by the factorial design. The organic extract was removed from the membrane bag manually by a microlitre syringe and transferred to a 2 mL sampling vial. PCB 155 was added as an internal standard prior to analysis by gas chromatography. Extracts were kept in the fridge until analysis. A 2 μL aliquot was injected into the gas chromatographic system. Unlike other MASE methods developed by different authors [18,19,21,23,27], in this work the GC injection did not require large volumes because the initial microwave extract of sample was sufficiently concentrated.

3. Results and discussion

3.1 *Optimisation of MASE processes: factorial design*

As the number of variable affecting the MASE efficiency is large, a Screening factorial design Plackett–Burman (2^5) type III resolution has been employed for the optimisation

Table 2. Membrane-assisted solvent extraction parameters and factor levels used in the factorial design.

Factor	Key	Fixed	Low (−)	Center	High (+)
Methanol percentage (%)	A	–	10	25	40
Extraction time (min)	B	–	30	75	120
Extraction temperature (°C)	C	–	30	45	60
Shaking speed (rpm)	D	–	200	475	750
Solvent volume (μL)	E	–	200	600	1000

of this system. The aim of factorial designs is to evaluate which of the variables have an influence on the process and which ones do not. The Plackett–Burman designs allow to divide the full factorial design, giving numbers of factor combinations that are a multiple of four. Our particular design Plackett–Burman (2^5) type III resolution with one centre point allowed 12 degrees of freedom which involved 13 randomised runs.

Table 2 shows the lower and upper levels given to each factor. Such values were selected from experience gathered in previous experiments. Table 3 shows the design matrix for experiments and the response values obtained in each one for PCBs 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180.

The numerical analysis of the results in Table 3 led to Pareto Chart of standardised main effects. The factor effect is defined as the difference between the mean value of all measurement at the maximum and the mean value at the minimum of the factor. The standardised effect is obtained by dividing the estimated effect factor or interaction by its standard error. The most significant factors are grouped at the top and the length of each bar is proportional to the absolute value of its standardised effect. The bar that graphically surpass the significance line (95% confidence level) exerts a statistically influence on the results [28]. Figure 1 shows the Pareto Charts for PCBs 31, 28, 118 and 180 where the extraction efficiency only appeared statistically affected by extraction time. In these cases, extraction time had a positive influence suggesting that improved their extraction efficiency. Figure 2 shows the Pareto Charts for PCBs 153, 138 and 156, where shaking speed presented statistical significance (at 95% confidence) and also was affected by a positive sign. PCBs 52, 101 and 105 extraction efficiencies did not present statistically significant variables.

In a Plackett–Burman type III resolution design, the direct evaluation of second order interactions is not allowed because they are confused by the main effects. If we discarded some of the initially considered factors (methanol percentage, extraction temperature, and extractant solvent volume), a more restrictive model fitted by excluding the least significant factors can be evaluated. The reduced model indicated that any second order interactions were not statistically significant in this factorial design.

Table 4 shows the estimated effects of factors. Although extraction time and shaking speed were not statistically significant, they had high positive effects in PCBs 52, 153, 138 and 156 and in PCBs 28 and 118, respectively.

3.1.1 *Impact of extraction time and agitation speed*

The extraction time was varied between 30 and 120 min. Taking into account data analysis of factorial design, 120 min of extraction time provided an higher enrichment of all

Table 3. Design matrix and response values for Plackett–Burman factorial design.

Exp.	A	B	C	D	E	PCB31	PCB28	PCB52	PCB 101	PCB 118	PCB 153	PCB 105	PCB 138	PCB 156	PCB 180
1	+	−	−	−	+	289.1	468.4	312.4	184.8	2146.9	612.8	141.4	415.5	122.0	137.6
2	−	−	−	+	+	970.0	970.0	1777.2	647.8	4681.4	367.6	265.5	111.0	115.1	46.5
3	−	+	+	+	−	3864.3	4242.8	1498.6	460.5	9667.8	1682.8	721.6	1770.0	524.2	797.7
4	−	+	+	−	+	2476.5	2947.0	2262.8	268.9	9835.2	1257.6	518.9	1687.1	354.9	565.1
5	−	−	−	−	−	442.9	526.7	922.4	454.7	3441.4	1362.2	458.7	674.6	202.5	365.1
6	+	+	−	+	+	2007.3	2969.4	2682.2	1391.9	10305	5188.4	898.1	3243.2	1073.9	1708.8
7	+	+	+	−	+	3536.8	2076.1	336.2	250.3	1497.0	1443.7	776.7	1502.0	580.7	1408.7
8	0	0	0	0	0	894.8	2645.6	1742.9	1375.3	7741.8	3309.9	1540.6	2534.9	645.6	914.4
9	−	−	+	+	+	494.4	1145.4	2246.4	644.9	4685.2	3257.8	486.0	1980.2	878.7	1023.8
10	+	−	+	−	−	174.3	158.1	99.9	115.2	199.3	378.9	134.76	208.8	233.9	62.5
11	−	+	−	−	−	1012.9	1012.9	4008.9	1960.0	10069	717.4	552.0	169.0	268.7	545.9
12	+	+	−	+	−	2267.5	2030.5	1374.1	1269.4	2437.6	5058.8	1703.4	3571.7	1342.8	1722.6
13	+	−	+	+	−	897.7	1474.8	1349.7	1123.4	2769.3	2468.5	1260.9	2255.6	664.2	842.6

Notes: − low level of factor; +: high level of factor; 0: centre point; A: methanol percentage; B: extraction time; C: extraction temperature; D: shaking speed; E: solvent volume.

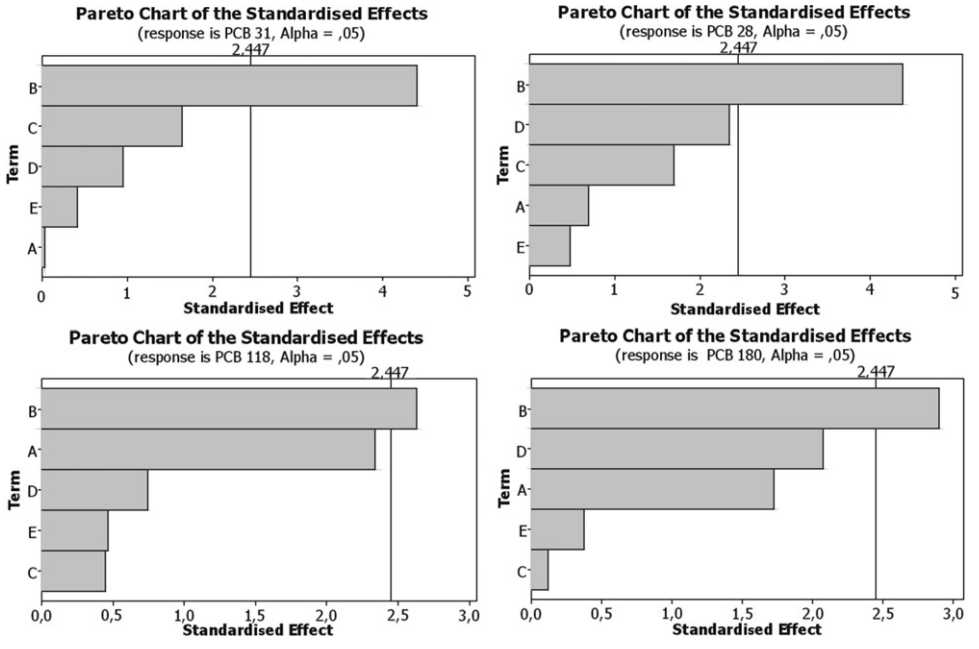

Figure 1. Pareto charts of the standardised effects for the factorial design, obtained by using the PCBs 31, 28, 118 and 180 extraction yields. The vertical lines indicate the statistical significance bound for the effects.

compounds, specially of the congeners that presented the intermediate polarities (PCBs 31, 28, 118 and 180) and the greater number of chlorine atoms (PCBs 153, 138 and 156), because their transport through the membrane material was improved. Some of these results were in agreement with studies reported by other authors [18,27]. However, PCBs and other micropollutants were recovered in only 30 min from simple liquid samples [19,21].

The improvement of analytes transport through membrane would be substantial if the agitation speed also was increased, mainly for the higher chlorinated compounds because the mixing of sample would be more efficient and the boundary layers around the membrane bag would minimise. Schellin and Popp had already found that increasing stirring rates, larger extraction yield of all PCBs (between 25% and 40%) from liquid matrices was attained [21]. The two-factor interaction plot of PCB 156 in Figure 3 shows that the best results were obtained when extraction time and shaking speed were checked at the highest values, 120 min and 750 rpm, respectively for all compounds except for PCB 52. High PCB 52 recoveries were attained at high shaking speed regardless extraction time. However at low agitation speed, the best recovery was obtained at high level of extraction time.

3.1.2 *Impact of methanol percentage*

Although methanol percentage was not statistically significant, it possessed important estimated effects in PCBs 52 and 118 (Table 4). The estimated effects of methanol percentage for the tri-, tetra-, and penta-chlorinated compounds, except for PCB 105,

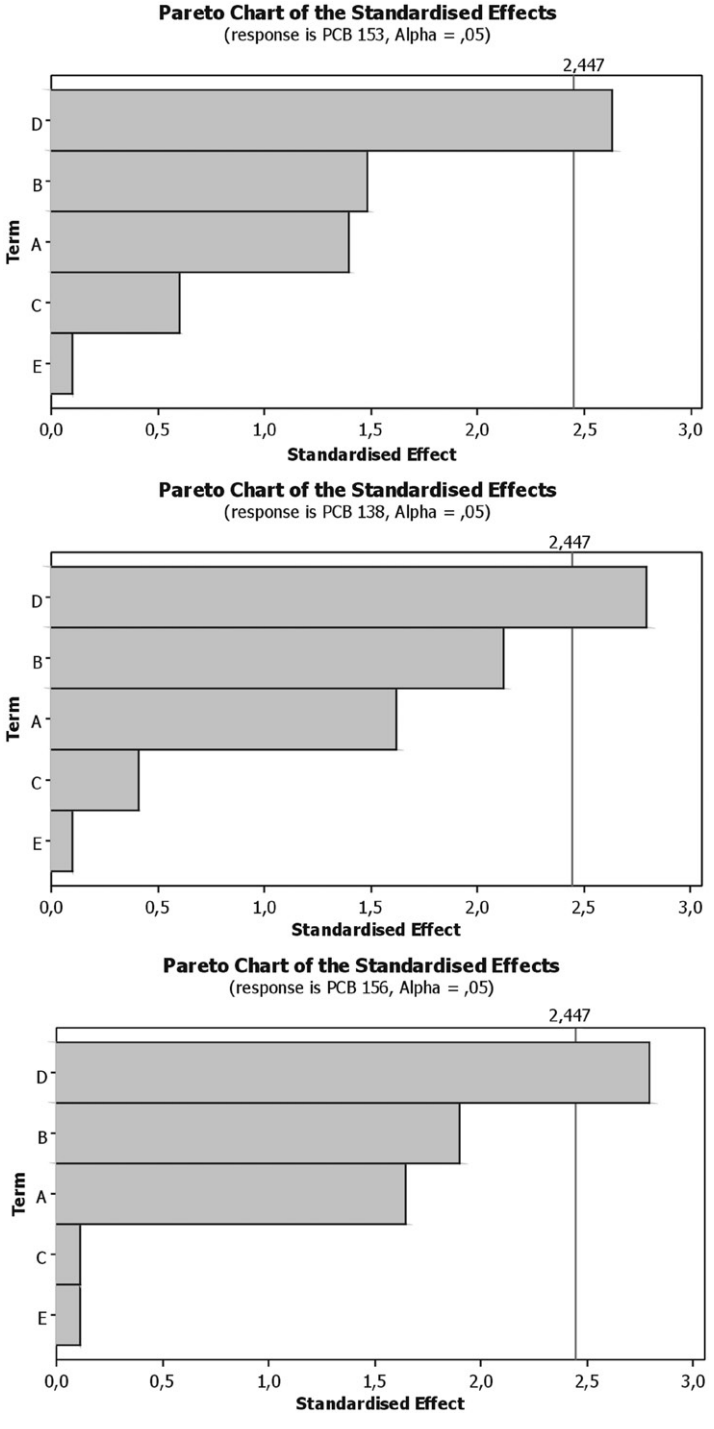

Figure 2. Pareto charts of the standardised effects for the factorial design, obtained by using the PCBs 153, 138 and 156 extraction yields. The vertical lines indicate the statistical significance bound for the effects.

Table 4. Value of estimated effects for PCBs.

Key	PCB31	PCB28	PCB52	PCB101	PCB118	PCB153	PCB105	PCB138	PCB156	PCB180
A	−14.7	−277.9	−1094	−17.0	−3837	1084.3	318.8	800.8	278.9	423.1
B	1982.8	1755.9	909.2	405.0	4315	1150.2	403.9	1049.6	321.5	711.8
C	742.4	677.7	−547.3	−507.6	−738	−469.7	−20.0	203.1	18.6	28.9
D	428.1	940.6	497.6	384.0	1226	2041.9	458.9	1379.1	472.7	509.5
E	185.7	188.4	60.6	−332.4	761	76.6	−290.8	48.2	−18.5	92.3

Notes: A: methanol percentage; B: extraction time; C: extraction temperature; D: shaking speed; E: solvent volume.

presented a negative sign. The PCB 105 and higher chlorinated compounds, hexa- and hepta-chlorinated compounds (PCBs 153, 138, 156 and 180), presented positive estimated effects. The presence of methanol minimises the glass adsorption of analytes [29] and increases the solubility of the more lipophilic compounds in the sample (microwave extract) leading to a slight enrichment of the higher chlorinated compounds in extractant solvent. The same behaviour was observed in other membrane extraction procedures in aqueous samples [21,27]. Figure 4(a) shows the interaction plot for PCB 52, the best recoveries were found at low levels of shaking speed and methanol percentage. For PCB 52, the similar recoveries were obtained at high level of the agitation speed irrespective of the methanol percentage. Another intersection between the two levels of methanol percentage appeared for PCB 153 at the low level of extraction time (Figure 4(b)) where congener recovery is poor regardless of methanol percentage. The highest recoveries of this compound were found at high levels of the methanol percentage and extraction time. PCB 153 is a higher chlorinated and majority compound in environmental bioindicators, and so it needs stronger extraction conditions, mainly and due to its lipophilic nature, those related with the use of methanol modifier.

3.1.3 *Impact of extraction temperature*

In relation to extraction temperature (Table 4), its estimated effect was high and positive for the lower chlorinated compounds (PCBs 31 and 28). The effect was highly negative for PCBs 52, 101, 118 and 153, and it was negligible for the rest of congeners. Optimisation studies were performed at 30–60°C range. Increasing the extraction temperature from 30 to 60°C, a rise in the vapour pressure of the more volatile compounds was produced, this fact improved the transport of the tri-chlorinated congeners through the membrane. Moreover, due to the boiling point of *n*-heptane is 98.4°C, the evaporation processes of solvent did not happen at 60°C minimising the lower chlorinated compounds losses. On the contrary, the recovery of higher chlorinated compounds was improved when the extraction temperature decreased from 60°C to 30°C. In Figure 5(a) it can be seen that in order to obtain PCB 31 quantitative recoveries (recovery $> 50\%$) more agitation was required at 30°C, however at 60°C and 750 rpm, with a rise in its vapour pressure, small losses of analyte were found. The best PCB 31 recoveries were attained at 60°C and at 200 rpm. Figure 5(b) shows that PCB 28 needed more temperature (60°C) to be extracted at 10% of methanol, at 30°C of extraction temperature, 40% of methanol was necessary in order to avoid the PCB 28 glass adsorption. However, PCB 28 extraction was not quantitative at these levels. The higher chlorinated compounds needed more extraction

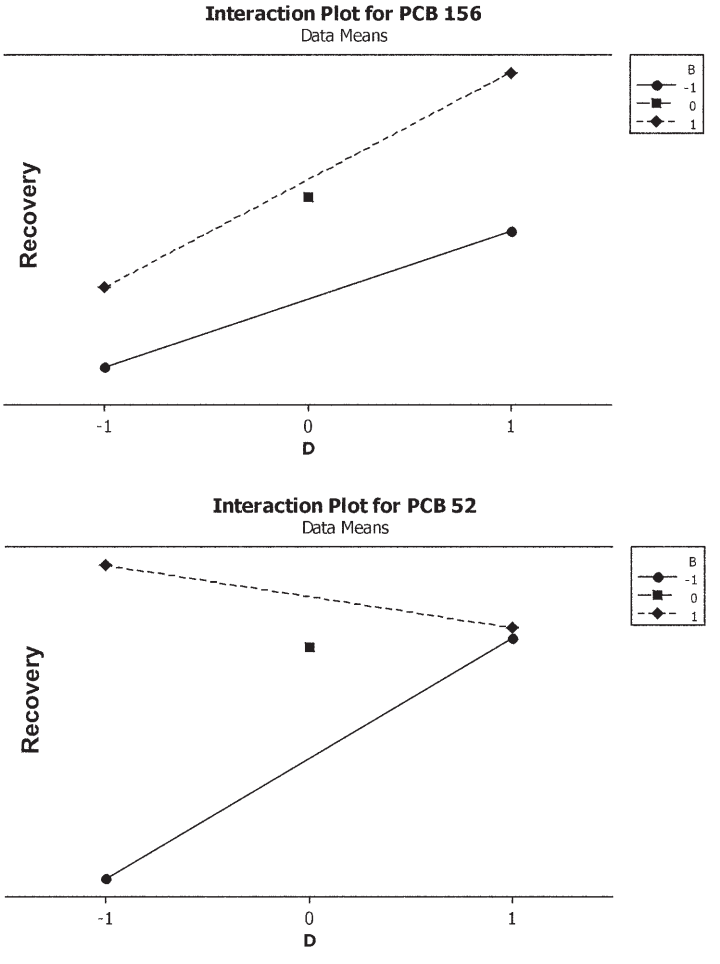

Figure 3. Interactions plot of the two factors B (extraction time) and C (shaking speed) for PCBs 156 and 52.

time at 30°C than at 60°C to reach the equilibrium, this behaviour of PCB 156 can be seen in Figure 5(c).

3.1.4 *Impact of solvent volume*

This parameter has hardly been investigated. In previous MASE investigations, the headspace vial was filled automatically with fixed volume of 800 µL [21,30]. However, Schellin et Popp filled shortened bags with only 100 and 200 µL of extractant solvent in the miniaturised membrane-assisted solvent extraction procedure [31].

In general, in Table 4 it can be seen that the estimated effects of solvent volume were very low. Solvent volume of membrane bag is of minor importance for analytes recovery from methanolic phase and it had a positive estimated effect for the most of compounds. Similar extraction efficiencies were found for the different volumes tested (200, 600 and 1000 µL). As there are no problems about sample sensitivity and in order to avoid potential losses of

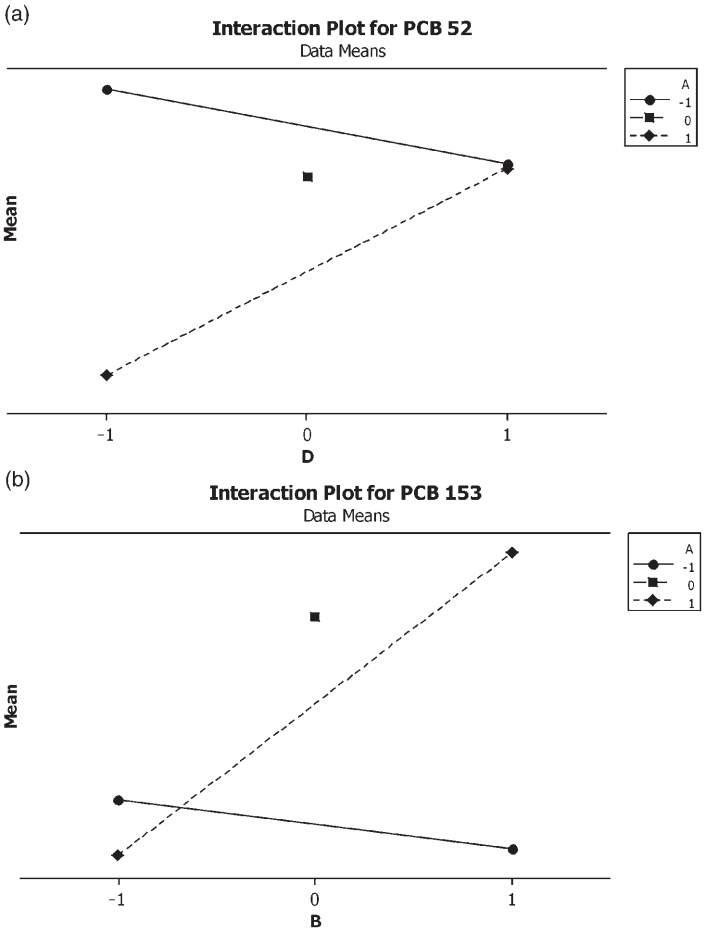

Figure 4. (a) Interactions plot of the two factors A (methanol percentage) and D (shaking speed) for PCB 52. (b) Interactions plot of the two factors A (methanol percentage) and B (extraction time) for PCB 153.

solvent in the moment to transfer to vial and to inject in GC system, 1000 μL of *n*-heptane was considered as the optimal extraction volume of PCB congeners.

3.2 *Further experiments*

The analysis of Plackett–Burman factorial design results suggested that 30°C was considered as an optimal extraction temperature for the most of PCB congeners and 10% of methanol was enough in the microwave aqueous extract. According to these results, a new design shifted in the direction of higher values of some factors, extraction time, shaking speed, and solvent volume, would be desirable. However, solvent volume is not a significant variable and is limited by the membrane bag capacity. Taking these facts into account, further experiments were carried out to fine tune the two main factors, extraction time and shaking speed. With this aim four experiments were performed, two fixing the agitation speed at 750 rpm and varying the values of extraction time between

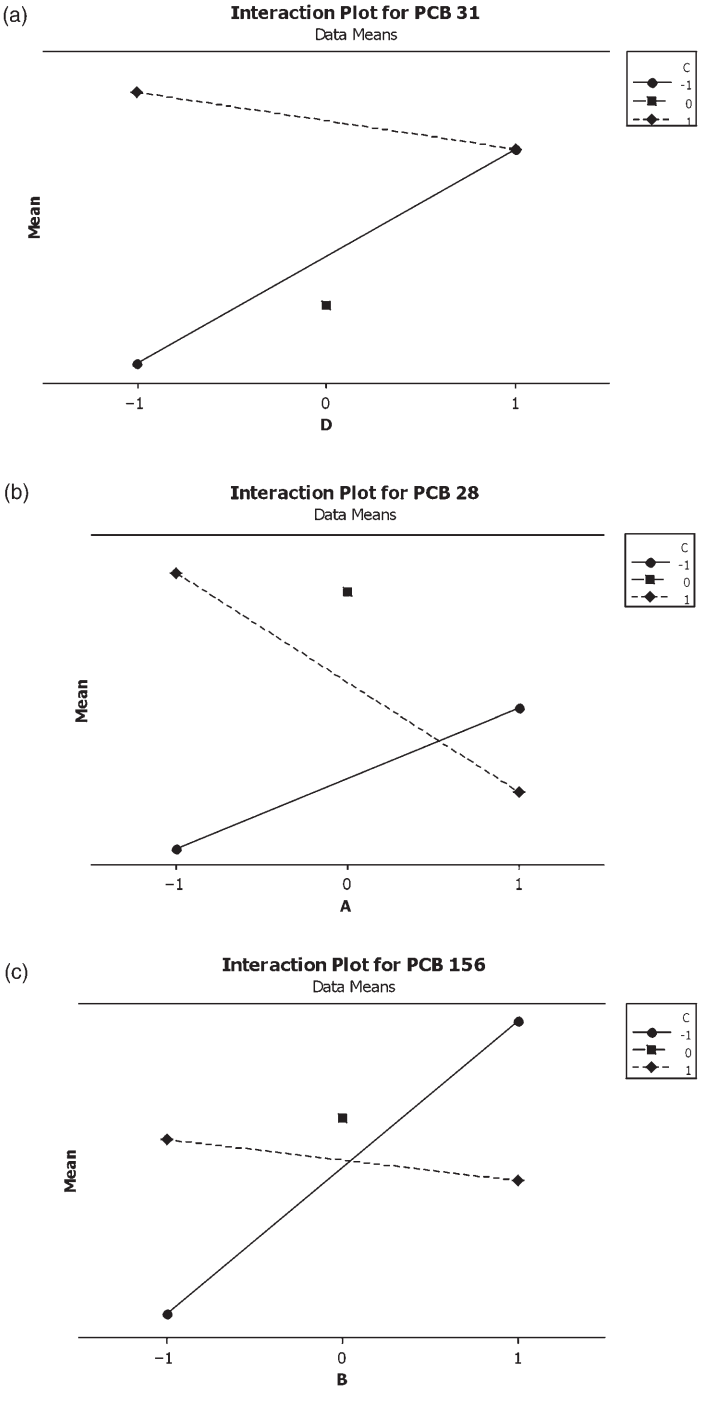

Figure 5. (a) Interactions plot of the two factors C (extraction temperature) and D (shaking speed) for PCB 31. (b) Interactions plot of the two factors C (extraction temperature) and A (methanol percentage) for PCB 28. (c) Interactions plot of the two factors C (extraction temperature) and B (extraction time) for PCB 156.

150 and 180 min. The results of these two experiments showed that 180 min of extraction time did not improve or even damage the extraction efficiency. It could be due to losses of acceptor solvent for evaporation or passage through membrane during the long extraction. Another two experiments were carried out fixing the time at 150 min and varying the agitation speed between 750 and 1000 rpm, this later gave the best extraction efficiency. These results are summarised in Figure 6(a).

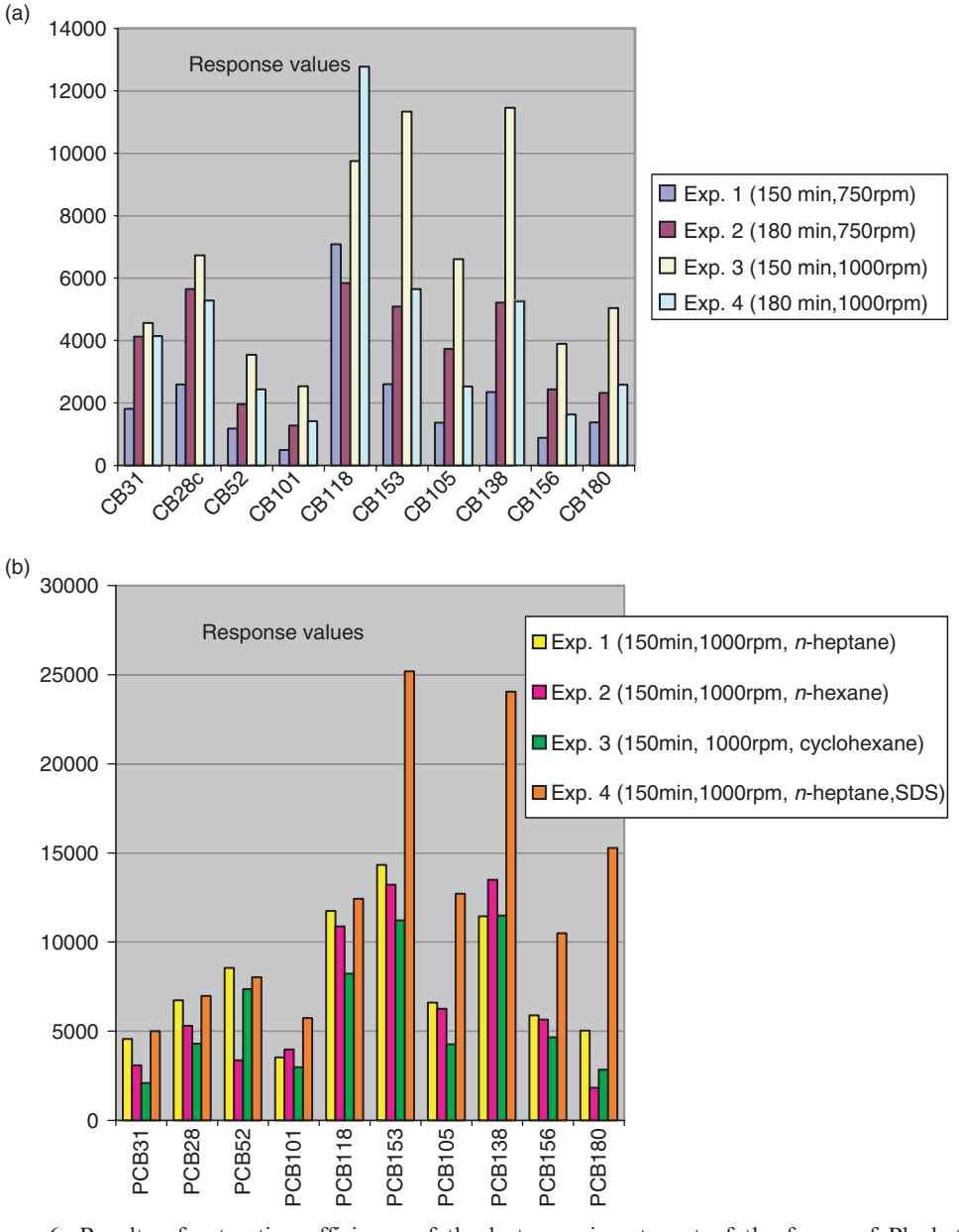

Figure 6. Results of extraction efficiency of the last experiments out of the frame of Plackett–Burman design.

The last experiments of optimisation have been performed fixing the MASE variables at their optimal value, 10% of methanol, 150 min of extraction time, 30°C of extraction temperature, 1000 rpm of agitation speed, and 1000 μL of solvent and varying the extraction solvent, *n*-heptane, *n*-hexane, and cyclohexane (Figure 6(b)). As the injection volume in our chromatographic system is 1 μL, the extraction solvent nature (boiling point) would only affect to the extraction step not to the split outlet during the large volume injection, it allows testing more solvents. The best results were obtained by using *n*-heptane as extractant solvent because it displayed an enrichment of almost all target congeners. *n*-Hexane and cyclohexane demonstrated a lower selectivity for these compounds. The last proofs were carried out by adding an anionic detergent, SDS, to samples before MASE procedure. As can be seen in the Figure 6(b), the addition of SDS detergent to microwave extract-sample prior to MASE led to an increase in the enrichment of PCB congeners. The addition of SDS to complex samples in MASE procedure decreased their high surface tension, improved the wettability of membrane by the sample, and reduced the matrix binding of organic analytes. This behaviour was different to that found in water samples (simpler matrixes) for the higher organochlorine compounds. In this case, the more lipophilic analytes recoveries were 10–30% lower with SDS than without SDS [27].

3.3 *Validation of the whole procedure*

MAE–MASE procedure was performed by using MAE conditions previously optimised by Carro *et al*. [25] and using the MASE conditions optimised in this work, they were: 10% of methanol, 150 min of extraction time, 30°C of extraction temperature, 1000 rpm of agitation speed, 1000 μL *n*-heptane as extractant solvent, and 0.2 g of SDS.

All optimisation was based on peak height measurements (counts) versus the peak weight counts of the internal standard.

Calibration curves of MASE, constructed at four concentration levels ranging from 0.1 to 200 μg L^{-1} (0.1, 50, 100 and 200 μg L^{-1}) of each compound and internal standard in 15 mL of water–methanol, presented a very good linearity, the correlation coefficients were of 0.991–0.998.

The detection limits were calculated as three times the standard deviation of the peak height counts for 30 determinations of the blank and were between 0.1 and 0.9 μg kg^{-1} for studied PCBs. These detection limits, μg kg^{-1}, were very high in comparison with those achieved by Popp *et al.* (ng L^{-1}). The low detection limits attained by Popp *et al.* were directly related to large injection volumes, from 100 to 400 μL, injected in the chromatographic system [21,27]. In the present work, the chromatographic injection volume only was 1 μL.

The recovery percent and reproducibility expressed as relative standard deviation (RSD) of the method were evaluated by extracting six samples of the certified reference material, NIST 2977, in several days. Table 5 shows that these results varied between 73.7% and 100% for the recovery and between 2% and 15% for RSD.

4. Conclusion

The MAE technique used as an initial extraction step combined with the MASE technique utilised as a clean up–extraction–concentration step offers a rapid, precise, and accurate

Table 5. Validation data in terms of extraction efficiency and relative standard deviation of optimised MAE–MASE procedure for PCB congeners in certified reference material, NIST 2977.

	MAE–MASE procedure $n = 6$	
Compound	Mean recovery (%)	RSD (%)
PCB31	100	8
PCB28	92.8	11
PCB52	93	2
PCB101	73.7	15
PCB118	82.7	8
PCB153	93.8	8
PCB105	92.6	15
PCB138	84.9	3
PCB156	80.3	10
PCB180	88.4	2

method for gas chromatographic determination of PCBs in mussel samples. The MASE conditions are optimised by using a Plackett–Burman factorial design. Five extraction variables were optimised (methanol percentage, extraction time and temperature, shaking speed, and solvent volume). The extraction time and shaking speed were the only statistically significant variables. Due to selective enrichment of the analytes in the organic phase of MASE procedure, good sensitivity and selectivity are achieved avoiding large solvent volumes. Moreover, polypropylene membranes have the advantages of easy handling and low cost. For certified reference materials quantitative recoveries around 75–100% with RSD from 2% to 15% are achieved.

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