Determination of organochlorine pesticides and polychlorinated biphenyls in post-mortem human lung by matrix solid-phase dispersion with the aid of response surface methodology and desirability function

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ABSTRACT

A matrix solid-phase dispersion (MSPD) method for the simultaneous determination of 20 organochlorine pesticides (OCPs) (aldrin, endrin, dieldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, α-chlordane, γ-chlordane, p,p'-DDE, p,p'-DDT, p,p'-DDD, endosulfan I, endosulfan II, endosulfan sulfate, endrin aldehyde, heptachlor, heptachlor epoxide, endrin ketone and methoxychlor) and six polychlorinated biphenyl (PCB) congeners (PCB 28, 52, 101, 138, 153 and 180) in post-mortem human lung has been developed and validated. Response surface methodology (RSM) and desirability function were employed to optimize the extraction conditions of MSPD. Extraction was carried out using Florisil (2.0 g) as the sorbent material as well as clean-up adsorbent (1.5401 g), n-hexane:dichloromethane (11:89, v/v) as the eluting solvent (15.45 mL) and Na_2SO_4 (2.0 g) as dehydrating agent. Determination and quantification of OCPs and PCBs residues were carried out using a gas chromatograph equipped with an electron capture detector (GC-ECD). A mass spectrometric detector (GC–MS) in the selected ion monitoring (SIM) mode was also used for confirmation purposes. Method detection limits by GC–MS ranged from 0.42 to 0.87 ng g^{-1} and 0.51 to 1.35 ng g^{-1}, for OCPs and PCBs, respectively. Lower detection limits were calculated for GC-ECD ranging between 0.15–0.30 ng g^{-1} and 0.18–0.48 ng g^{-1}, respectively. Relative standard deviations did not exceed 9%. Analytes provided recoveries ranging from 65% to 106%. The proposed method was successfully applied to the analysis of lung tissues from six autopsy cases.

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1. Introduction

Organochlorine pesticides (OCPs) have been extensively used as pesticides in agriculture, while polychlorinated biphenyls (PCBs) as industrial fluids mainly in transformers, capacitors, papers and paints industry. Both groups of these compounds are highly lipophilic, chemically very stable and resistant to environmental degradation, and consequently they are considered to be persistent organic pollutants (POPs) in environment. However, although most of them are banned since 1970 and no longer used, they are globally spread in environment and may be routinely detected in surface waters, air, fish, wildlife, food and even humans. The problem becomes more serious when they bioaccumulate in fatty tissues getting up to the human organism through the diet, especially food of animal origin [1,2]. PCBs have been shown to cause cancer in animals and other non-cancer effects, since they could affect the immune, reproductive, nervous and endocrine system. Studies in human provide evidence for this potential carcinogenetic and non-carcinogenetic effect [3–6]. PCBs are been rated by International Agency of Research on Cancer (IARC) as "probably carcinogenetic to humans" (2A group), while most OCPs were classified as "possibly carcinogenetic to humans" (2B group) [7,8]. It is reported that low chlorinated PCBs may undergo cytochrome P-450 enzyme-catalyzed hydroxylation to form mono-hydroxylated or di-hydroxylated PCBs [9,10], involving arene oxide intermediates [11] while several organochlorine pesticides are metabolized with epoxidation by cytochrome P-450. These processes occur in liver and to a lesser extent, in lungs [12,13]. In addition OCPs and PCBs are suspected for endocrine disrupting activity even at low concentrations. By these means, any analytical method developed to trace these pollutants requires a low minimum detectable value [14].

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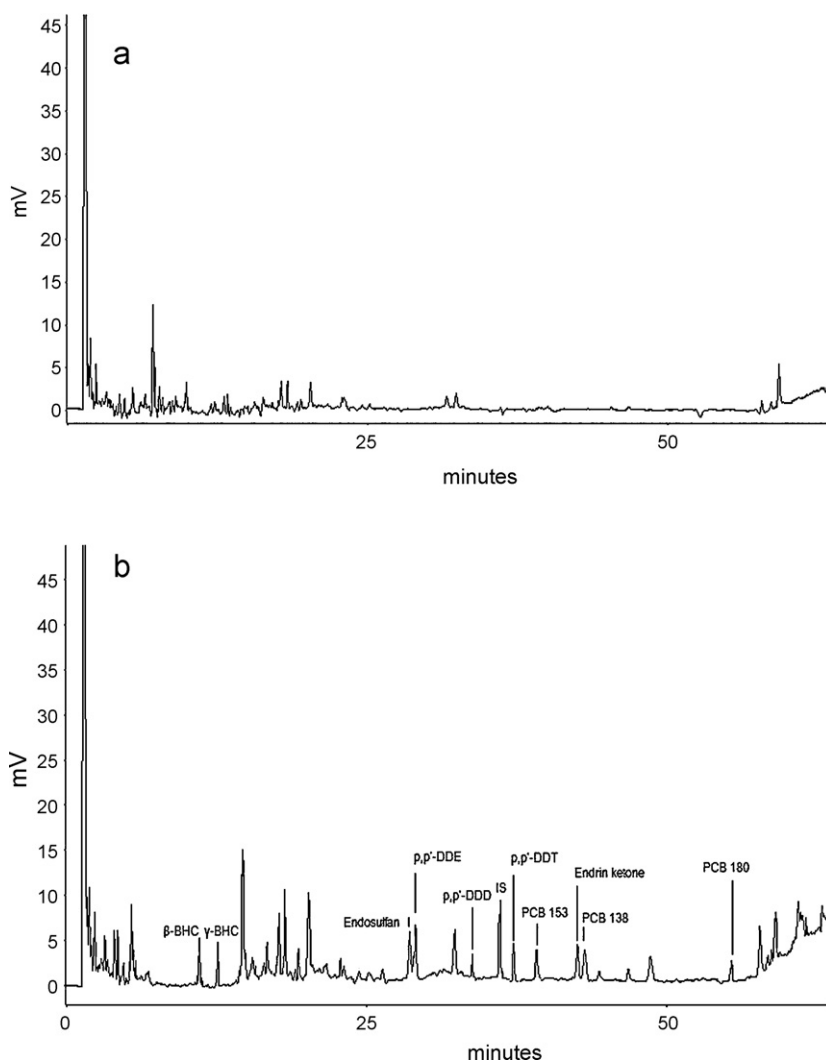E-mail addresses: grallis@cc.uoi.gr (G.N. Rallis), vsakkas@cc.uoi.gr (V.A. Sakkas), vboumba@cc.uoi.gr (V.A. Boumba), tvujuk@cc.uoi.gr (T. Vougiouklakis), talbanis@cc.uoi.gr (T.A. Albanis).
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Fig. 1. MSPD-GC-ECD chromatogram obtained from (a) a blank and (b) a contaminated post-mortem lung tissue (case 4) following the recommended procedure.

Therefore, OCPs and PCBs raise especial interest in public health epidemiology.

Conventional extraction of organic analytes from biological samples usually begins with a homogenization step, followed by tedious liquid–liquid extraction procedures with one or more clean-up steps, and finally purification of the extract to remove co-extracts before the sample is subjected to chromatographic separation [15,16]. The amount of matrix usually required is large and therefore solvent consumption is high. The past decade has seen many innovations in the analytical procedures that can be applied to sample preparation for extraction and determination of several toxic compounds. Among them, matrix solid-phase dispersion (MSPD) is a relatively recent extraction method, developed by Barker in 1989 for the extraction of solid and semisolids samples [17,18].

MSPD is an extraction method that comprises homogenization of the sample, cellular disruption, fractionation and purification in a single step. The method involves blending a viscous sample with a solid support, isolating target analytes by adsorbing them on the suitable solid adsorbent followed by desorption with a small amount of organic solvent. Therefore, analyte extraction can be easily performed by the use of less toxic reagents and solvents and under mild operation conditions [17–19].

In this manner, the solid support serves the same purposes as the use of sand as an abrasive: the shearing forces of blending with a mortar and pestle disrupt the gross architecture of the sample, breaking the material into smaller pieces. However, the presence of the bound organic provides a further dimension to the process: samples components dissolve and disperse into the organic phase on the surface of the particle, leading to the complete disruption of the sample and its dispersion over the surface. Sample components distribute over the surface based on their relative polarities [18]. MSPD has been largely used for the isolation of numerous organic compounds from various matrices, among them, fruits and vegetables [20,21], milk [22], muscle tissue [23], fish [24,25], biota [26], eggs [14], cattle feed [27], cosmetics [28], indoor dust [29,30], serum [31], porcine tissue [32], tea [33], urine [22], human placenta [34], human hair [35] and butter [36].

With the above in mind, the aim of this study is twofold. Firstly, to examine for the first time – to the best of our knowledge – the analytical utility of matrix solid-phase dispersion in combination with chemometric tools (experimental design, response surface methodology and desirability profile), followed by gas chromatography for the simultaneous determination of OCPs and PCBs in post-mortem human lung from autopsy cases. A Plackett–Burman factorial design was planned in order to define the significant experimental variables affecting the extraction efficiency and afterwards a central composite design (CCD) was employed for the optimization of the extraction process. Finally, to assess the applicability of the proposed method to the analysis of post-mortem

lungs sampled during autopsy at the Department of Forensic Medicine and Toxicology, Medical School, University of Ioannina, in the period of 2010–2011.

2. Experimental

2.1. Chemicals and materials

The organochlorine pesticides examined in this study were aldrin, endrin, dieldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, α-chlordane, γ-chlordane, p,p'-DDE, p,p'-DDT, p,p'-DDD, endosulfan I, endosulfan II, endosulfan sulfate, endrin aldehyde, heptachlor, heptachlor epoxide, endrin ketone and methoxychlor. Fluka standard mix of organochlorine pesticides in toluene:n-hexane (50:50, v/v) was prepared at a concentration of 200 μg mL^{-1}. PCBs standards (PCB 28, 52, 101, 138, 153 and 180) were obtained from Fluka and working solutions were prepared in isooctane at 200 μg mL^{-1}. Isooctane secondary and working calibration standard solutions of OCPs and PCBs were prepared to spike uncontaminated—blank lung tissue samples (Fig. 1a) to the required concentrations. The stability of the stock solutions was checked and no change in concentrations was observed. PCB 118 (1 μg mL^{-1} in isooctane) purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) was used as internal standard (IS).

All solvents used (n-hexane, dichloromethane (DCM), isooctane and acetone), were pesticide residue analysis grade, purchased from Labscan (Dublin, Ireland). Sorbent materials used were Florisil (60–100 mesh) obtained from Fluka (Buchs Switzerland) and LiChroprep RP-18 (40–64 μm) from Merck (Darmstadt, Germany). Florisil was activated at 150 °C for 12 h and then allowed to cool down in a desiccator before use. Anhydrous Na$_2$SO$_4$ (99%) was supplied by Panreac (Barcelona, Spain).

Polyethylene syringe barrels (10 mL capacity) were thoroughly washed with hot water, rinsed with distilled water, n-hexane and air dried for the preparation of MSPD columns. Membrane filters Supor-200 (25 mm, 0.22 μm) obtained from Pall Corp. (Michigan, USA) were used as column frits to retain the column packing. Silanized glass wool, research grade, from Serva (Heidelberg, Germany) was used to plug the MSPD column.

2.2. Apparatus

Chromatographic analysis of OCPs and PCBs was performed using a Shimadzu 14A capillary gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) at 300 °C. Analytes were separated with a DB-5 column (J&W Scientific, Folsom, CA, USA), 30 m × 0.25 mm I.D., containing 5% phenyl-methylpolysiloxane with a phase thickness of 0.25 μm. GC oven temperature was programmed as follows: initial temperature 150 °C held for 2 min, ramped at 2 °C min^{-1} to 180 °C, ramped 0.5 °C min^{-1} to 184 (held for 2 min), ramped 2 °C min^{-1} to 200 °C (held for 20 min) and finally ramped to 270 °C at 10 °C min^{-1} and held for 2 min (total acquisition program time: 64 min). The injector was set to 250 °C in the splitless mode. Detector temperature was set at 300 °C. Helium was used as the carrier gas at 1.5 mL min^{-1} while nitrogen was used as the make-up gas at 35 mL min^{-1} according to the optimization results of the instrument given by the manufacturer. Identification of peaks was based on the comparison of the retention times of compounds in the standard solutions. Quantification of the analyzed compounds was performed using the method of the internal standard.

A QP 5000 Shimadzu instrument, equipped with a capillary column DB-5-MS, 30 m × 0.25 mm, 0.25 μm, containing 5% phenyl-methylpolysiloxane (J&W Scientific, Folsom, CA, USA) was used at the same chromatographic conditions, mentioned above. The ion

Table 1
Retention times, molecular weights and target ions for the GC–MS analysis of the target compounds.

Compound	t_R (min)	Molecular weight	**Quantifier** and qualifier (m/z)
α-BHC	8.91	288	**181**, 219, 183
β-BHC	10.87	288	**181**, 219, 183
γ-BHC	12.58	288	**181**, 219, 183
δ-BHC	12.78	288	**181**, 219, 183
Heptachlor	14.33	373	**337**, 339, 272
PCB 28	16.71	256	**256**, 258, 260
Aldrin	17.68	362	**263**, 265, 293
PCB 52	19.41	290	**220**, 292, 294
Heptachlor epoxide	20.43	386	**353**, 355, 357
γ-Chlordane	24.35	406	**373**, 375, 377
α-Chlordane	27.11	406	**373**, 375, 377
Endosulfan I	28.47	407	**339**, 341, 279
PCB 101	28.68	324	**326**, 324, 328
p,p'-DDE	28.99	316	**246**, 316, 318
Dieldrin	31.52	378	**263**, 380, 382
Endrin	32.19	378	**263**, 317, 345
p,p'-DDD	33.82	318	**235**, 165, 237
Endosulfan II	34.91	407	**339**, 341, 279
PCB 118	35.97	324	**326**, 324, 328
p,p'-DDT	37.02	355	**235**, 165, 237
Endrin aldehyde	37.10	378	**250**, 173, 345
PCB 153	38.99	358	**360**, 290, 362
Endosulfan sulfate	40.39	420	**272**, 387, 389
Endrin ketone	42.24	378	**317**, 209, 281
PCB 138	42.78	358	**360**, 358, 362
Methoxychlor	47.32	344	**227**, 169, 228
PCB 180	56.34	392	**396**, 392, 394

source and transfer were kept at 290 °C and 240 °C, respectively. In the full-scan mode, electronic ionization mass spectra at m/z of 50–450 were recorded at 70 eV. In the selected-ion monitoring (SIM) mode acquisition, four groups having target ions were monitored at different time windows defined by the corresponding retention times. Three ions of each analyte were chosen, according to the mass spectra characteristic features obtained in the full-scan mode as well as by comparison with the Nist's library. The quality criteria used were the following: retention times of the analytes did not differ more than ±0.1 min from the expected time and the relative intensities of the selected ions did not differ more than 10% from the relative intensities of the same ions acquired from a spiked sample.

Table 1 shows the retention times, molecular weights as well as the target ions of all analytes.

2.3. Sample collection and storage

Post-mortem lung samples were collected from routinely autopsied corpses at the Department of Forensic Medicine and Toxicology, Medical School, University of Ioannina, in the period of 2010–2011. Autopsies were performed 12–18 h after death and refrigeration of the body at 4 °C. The manner of death of the cases included in the study (three males and three females, age range 14–91 yr), was either sudden or violent death and was unrelated to environmental contaminants. The lung tissue was cut into small pieces and homogenized in a commercial blender. Sub-samples of approximately 10 g were placed into polyethylene recipients for autopsy specimens, coded, frozen immediately and stored at −20 °C until analysis.

2.4. Matrix solid-phase dispersion (MSPD) procedure

A representative portion of lung tissue (approximately 0.5 g) was blended thoroughly with 2.0 g Florisil as dispersion sorbent and 2 g Na$_2$SO$_4$ as dehydrating agent in a glass mortar for 15 min using a glass pestle to obtain a dry homogeneous mixture. The

homogenized mixture was quantitatively transferred by using a funnel into a syringe barrel-column (10 mL) containing a 0.22 μm membrane filter, and 1.5401 g of Florisil, acting as a clean-up adsorbent packing at the bottom. A portion of glass wool was placed to the top of the column after the introduction of the homogenized mixture. Finally, this was slightly compressed with the syringe plunger for air removal and for avoiding undesirable channels. 15.45 mL of n-hexane:DCM (11:89, v/v) were added to the column; the sample was allowed to elute by gravity flow and at the end of the elution a slight vacuum was applied. The eluent was collected into a conical tube. The extract was evaporated under a gentle stream of nitrogen till dryness. The residue was re-dissolved with 100 μL of isooctane containing PCB 118 as internal standard. Finally, 1.5 μL of the extract was injected directly into the GC system.

2.5. Experimental design

An experimental Plackett–Burman design was used in the present study to screen the important variables that significantly influenced OCPs and PCBs extraction (extraction yield expressed as average recovery of all analytes). In this design the number of experiments is a multiple of four (4, 8, 12, etc., experiments) and exceeds the number of factors by one. In our case, multivariable approach was based on a 2^{7-4} Plackett–Burman design, applied to evaluate the main effects of the following seven factors: sorbent type, elution solvent, eluting volume, sample to sorbent ratio, Na$_2$SO$_4$ amount, clean-up adsorbent amount as well as grinding time. Each variable was examined at two levels: −1 for the low level and +1 for the high level. Table 2 depicts the variables and their corresponding levels used in the experimental design. The experimental design in total included eight experiments plus three central points in order to estimate the experimental error [23].

A central composite design (CCD) 2^3 with six star points placed at a distance α from the central point was performed in order to determine the optimal conditions of the extraction process using MSPD procedure. The value of α was 1.682 to establish the rotatability condition [22]. Seventeen experiments were required including three central points and they were performed randomly. The conditions set in each experiment are listed in Table 3. Moreover, the profile for predicted values and desirability option was used for the optimization of the extraction process.

In all cases, screening and optimization, STATISTICA 7.0 (Stat-Soft Inc., Tulsa, USA) statistical package was used to generate the experimental matrix and to evaluate the results.

3. Results and discussion

Preliminary experiments were initially performed in order to evaluate the MSPD extraction efficiency (expressed as average recovery of all analytes) for OPCs and PCBs, using n-hexane, DCM and acetone as eluting solvents, as well as Florisil and C$_{18}$ as sorbent materials. Results obtained have shown that mixture of n-hexane:DCM displayed the highest extraction efficiencies of the target analytes. The other parameters were further investigated through Plackett–Burman and central composite design.

3.1. Optimization of the MSPD process

3.1.1. Plackett–Burman design

Analysis of variance (ANOVA) was performed to examine whether the studied experimental factors (Table 2), were significant in the performance of the proposed method. An effect was considered significant when it was above the standard error at the 95% confidence level ($p < 0.05$), which is denoted by the vertical line on the Pareto chart (Fig. 2).

In the studied experimental domain, all variables were statistically significant at 95% confidence level, with the exception of grinding time (non-significant effect, eliminated for further studies). Positive signs indicate that increasing these variables will result in an increase in the analytical signal (average recovery of all analytes). According to the obtained results, the amount of Na$_2$SO$_4$ (2 g), sorbent type (Florisil) and sample to sorbent ratio (1:4, 2 g) were kept fixed, while the eluting solvent, the amount of clean-up adsorbent (Florisil) and eluting volume were evaluated in the CCD for further assessment.

3.1.2. Central composite design

Following the results of the previous design the next step was to optimize the analytical method for the remaining three factors by the employment of a central composite design (CCD) (Table 3).

The main effects, interaction effects, as well as quadratic effects were evaluated through analysis of variance (ANOVA) at spiking concentration level of 10 ng g^{-1}. Moreover, lack of fit, measures the failure of the model to represent data in the experimental domain at points which are not included in the regression [37], was also checked and was shown to be not significant relative to the pure error, indicating a good response to the model. The model regression coefficient (R^2) of 0.92577 is in reasonable agreement with the experimental results, indicating 92.577% of the variability can be revealed by the model and is left with 7.423% residual variability.

Data analysis using the STATISTICA software at 95% of the confidence level permitted to obtain a semi-empirical expression (Eq. (1)) in terms of significant coded factors:

$$
\begin{aligned}
Y = {} & 82.49(\pm 0.30) + 0.72(\pm 0.14)x_2 + 0.70(\pm 0.16)x_2^2 \\
& + 2.29(\pm 0.14)x_7 - 0.93(\pm 0.16)x_7^2 + 1.00(\pm 0.14)x_3 \\
& + 1.47(\pm 0.16)x_3^2 - 1.31(\pm 0.19)x_2x_7 + 0.87(\pm 0.19)x_2x_3 \\
& + 3.42(\pm 0.19)x_7x_3
\end{aligned} \tag{1}
$$

where x_2 is the elution solvent (n-hexane:DCM), x_3 is the eluting volume and x_7 the clean-up adsorbent amount (Florisil).

Positive coefficient indicate that extraction efficiency is favored in the presence of high values of the respective variables within the range studied, while negative coefficients indicate that the reaction is favored in the presence of low values. Positive quadratic coefficients between elution solvent and eluting volume (x_2x_3), amount of clean-up adsorbent and eluting volume (x_7x_3) indicate a synergistic effect, while negative coefficients of elution solvent and amount of clean-up adsorbent (x_2x_7), an antagonistic effect between the variables.

The overall interaction effects are displayed in Fig. 3; a 3D representation of the polynomial (Eq. (1)) obtained from the experimental data, depicting the surface plots of extraction yields versus the significant variables.

As shown in Fig. 3a, the extraction efficiency of MSPD method depends on the amount of Florisil as clean-up adsorbent and the elution solvent (n-hexane:DCM), respectively. Lower extraction yields ($R\%$) were observed in the absence of Florisil or when low adsorbent amounts were used for clean-up. At fixed clean-up adsorbent amount (1.5401 g Florisil), Fig. 3b, one can see that when low amount of eluting volume is introduced, extraction efficiency is decreased irrespectively of the ratio of the eluting solvent system. Highest extraction efficiency was observed when high values of both eluting volume and clean-up adsorbent amount were used, Fig. 3c, fixed elution solvent n-hexane:DCM (11:89, v/v). In general, when n-hexane was used at large amounts the collected fraction contained a large amount of fat as evidenced by the color of the residue obtained. On the other hand, the presence of high ratio of DCM provided cleaner extracts and chromatograms.

Table 2
Plackett–Burman design showing seven variables with coded values as well as response variable (average extraction efficiency—$R\%$).

Factors		Levels		
Factors		(−1)	(0)	(+1)
(x_1) Sorbent type		C_{18}	Florisil:C_{18} (1:1)	Florisil
(x_2) Elution solvent		n-hexane	n-hexane:DCM (1:1)	DCM
(x_3) Eluting volume (mL)		5	10	15
(x_4) Sample to sorbent ratio		1:1	1:2	1:4
(x_5) Na_2SO_4 amount (g)		0.5	1.0	2
(x_6) Grinding time (min)		15	22.5	30
(x_7) Clean-up adsorbent amount (g)		0.0	0.5	1.0

Runs	x_1	x_2	x_3	x_4	x_5	x_6	x_7	$R\%$
1	−1	−1	−1	+1	+1	+1	−1	83
2	+1	−1	−1	−1	−1	+1	+1	16
3	−1	+1	−1	−1	+1	−1	+1	95
4	+1	+1	−1	+1	−1	−1	−1	67
5	−1	−1	+1	+1	−1	−1	+1	50
6	+1	−1	+1	−1	+1	−1	−1	71
7	−1	+1	+1	−1	−1	+1	−1	85
8	+1	+1	+1	+1	+1	+1	+1	96
9	0	0	0	0	0	0	0	75
10	0	0	0	0	0	0	0	71
11	0	0	0	0	0	0	0	73

The desirability function for the average extraction efficiency ($R\%$) of target analytes was defined by assigning a desirability value of 0.0 to average $R\%$ below 75.7%, 1.0 above 92.1% and 0.5 of 83.9%. In order to achieve the highest desirability score (desirability 1), software optimized 92% average recovery of the target analytes with calculating the optimized model variables of n-hexane:DCM (11:89, v/v), 1.5401 g Florisil as clean-up adsorbent and 15.45 mL eluting volume (Fig. 4). For validation purposes, duplicate assenting experiments at 10 ng g^{-1} were conducted using the optimized conditions. The results are closely co-related with the data obtained from desirability optimization analysis using CCD, indicating that MSPD combined with CCD and desirability function could be effectively used to optimize the extraction performance of OCPs and PCBs from human lung tissue samples.

Our results are consistent with other works [27,28,34,38] showing the effectiveness of Florisil as clean-up adsorbent in the removal of co-extractives (e.g. polar lipids) in the specific matrix. With regards to the elution solvent system even though non-polar organic solvent like hexane would be better for the target analytes, it lacks the ability to penetrate deep into the tissue [34]. The analytical performance of MSPD was improved when a mixture of n-hexane and DCM was used as the elution solvent. In addition, when this solvent mixture (n-hexane:DCM) was used at a ratio of 11:89 (v/v) cleaner extracts were observed. In a recent work by Moliner-Martinez et al. [38], for the determination of OCPs and PBDEs by MSPD and SPME in biota samples the combination of C_{18} as dispersant and Florisil as fat retainer resulted to numerous advantages compared with previously published works. In our study Florisil was proved to be the most effective dispersion sorbent. This dispersant has been shown to be successfully applied for the extraction of environmental pollutants such as PCBs and pesticides with recoveries over 70% [39–43].

Table 3
Central composite design matrix of three variables in coded units and the response of average extraction efficiency ($R\%$).

Factors		Levels			Star point ($\alpha = 1.682$)	
		Low (−1)	Central (0)	High (+1)	$-\alpha$	$+\alpha$
(x_2) Eluting solvent (n-hexane:DCM)		30:70	20:80	10:90	36.8:63.2	3.2:96.8
(x_7) Clean-up adsorbent amount (g)		0.5	1	1.5	0.16	1.84
(x_3) Eluting volume (mL)		11	13	15	9.64	16.36

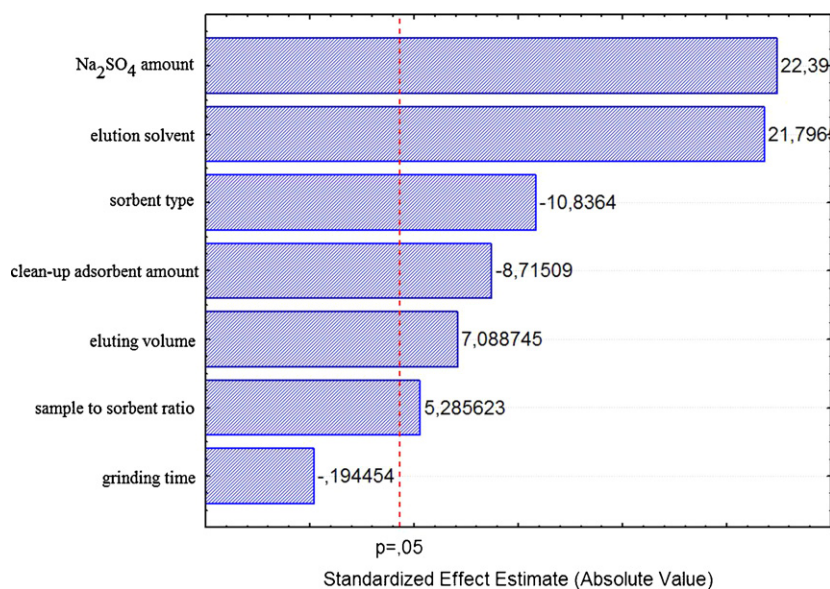| Runs	x_2	x_7	x_3	Average extraction efficiency ($R\%$)
1	−1	−1	−1	81.3
2	−1	−1	+1	78.0
3	−1	+1	−1	83.1
4	−1	+1	+1	90.2
5	+1	−1	−1	85.0
6	+1	−1	+1	81.9
7	+1	+1	−1	78.2
8	+1	+1	+1	92.1
9	$-\alpha$	0	0	83.0
10	$+\alpha$	0	0	86.0
11	0	$-\alpha$	0	75.7
12	0	$+\alpha$	0	84.0
13	0	0	$-\alpha$	87.0
14	0	0	$+\alpha$	86.3
15	0	0	0	82.0
16	0	0	0	82.5
17	0	0	0	83.0

Fig. 2. Standardized main effect Pareto chart for the Placket–Burman design of screening experiment. Vertical line in the chart defines 95% confidence level.

3.2. Analytical performance and method validation

With the aim of verifying the suitability and performance of MSPD for the quantitative determination OCPs and PCBs congeners in post-mortem lung tissue, method quality parameters were estimated (Table 4).

The method linearity was evaluated using blank lung tissue samples fortified at a concentration range between 5 and 100 ng g^{-1} (including five concentration levels). Each concentration level was injected in triplicate and the detector response was found to be linear in the range of concentrations studied with coefficients of determination (R^2) ranging from 0.9913 to 0.9999 for all target analytes.

Method accuracy was evaluated by recovery studies calculated according to the following equation:

$$\text{Analytical recovery} = \left\{ \frac{[\text{analyte}]_{\text{found}}}{[\text{analyte}]_{\text{added}}} \right\} \times 100\% \qquad (2)$$

where [analyte]$_{\text{found}}$ is the found analyte concentration in the spiked lung sample and [analyte]$_{\text{added}}$ is the spiked analyte concentration.

Analytical recovery was studied after spiking non-contaminated lung tissue sub-samples at two concentration levels. Table 4 lists the calculated recoveries for each analyte at the fortification levels of 10 and 50 ng g^{-1}, respectively. As can be seen, recoveries were higher than 80% for the most of target compounds.

Table 4
Method linearity, intra-precision, accuracy and limits of quantification (LOQs) of the proposed method.

Compound	R^2 ($n=3$)[a]	Rec ± RSD (%) 10 ng g^{-1}	Rec ± RSD (%) 50 ng g^{-1}	LOQ (GC-ECD) ng g^{-1}	LOQ (GC–MS) ng g^{-1}
α-BHC	0.9994	65 ± 2	71 ± 1	0.9	2.4
β-BHC	0.9913	76 ± 4	80 ± 2	0.5	1.5
γ-BHC	0.9992	94 ± 2	92 ± 2	0.9	2.5
δ-BHC	0.9986	85 ± 4	89 ± 4	0.5	1.4
Heptachlor	0.9999	67 ± 3	72 ± 2	0.6	1.8
PCB 28	0.9992	96 ± 7	96 ± 5	1.4	3.9
Aldrin	0.9993	106 ± 2	104 ± 2	0.6	1.7
PCB 52	0.9998	103 ± 5	99 ± 4	1.6	4.5
Heptachlor epoxide	0.9997	80 ± 1	92 ± 1	0.6	1.6
γ-Chlordane	0.9994	90 ± 3	88 ± 1	0.6	1.7
α-Chlordane	0.9999	69 ± 2	76 ± 2	0.7	1.9
Endosulfan I	0.9963	104 ± 3	101 ± 3	0.6	1.8
PCB 101	0.9992	100 ± 3	104 ± 2	1.1	3.0
p,p′-DDE	0.9996	75 ± 5	82 ± 3	0.8	2.1
Dieldrin	0.9962	81 ± 3	80 ± 2	0.7	1.9
Endrin	0.9997	102 ± 3	96 ± 1	0.7	1.9
p,p′-DDD	0.9989	76 ± 2	81 ± 2	0.7	2.1
Endosulfan II	0.9988	88 ± 1	93 ± 1	0.7	2.0
p,p′-DDT	0.9998	95 ± 3	99 ± 2	0.8	2.2
Endrin aldehyde	0.9998	92 ± 3	89 ± 3	0.9	2.5
PCB 153	0.9966	102 ± 2	97 ± 1	0.9	2.7
Endosulfan sulfate	0.9975	72 ± 1	80 ± 1	0.9	2.7
Endrin ketone	0.9965	104 ± 2	102 ± 2	1.0	2.9
PCB 138	0.9984	105 ± 3	97 ± 2	0.9	2.6
Methoxychlor	0.9972	77 ± 4	84 ± 2	0.7	2.0
PCB 180	0.9998	101 ± 4	95 ± 3	0.6	1.7

[a] Coefficient of determination.
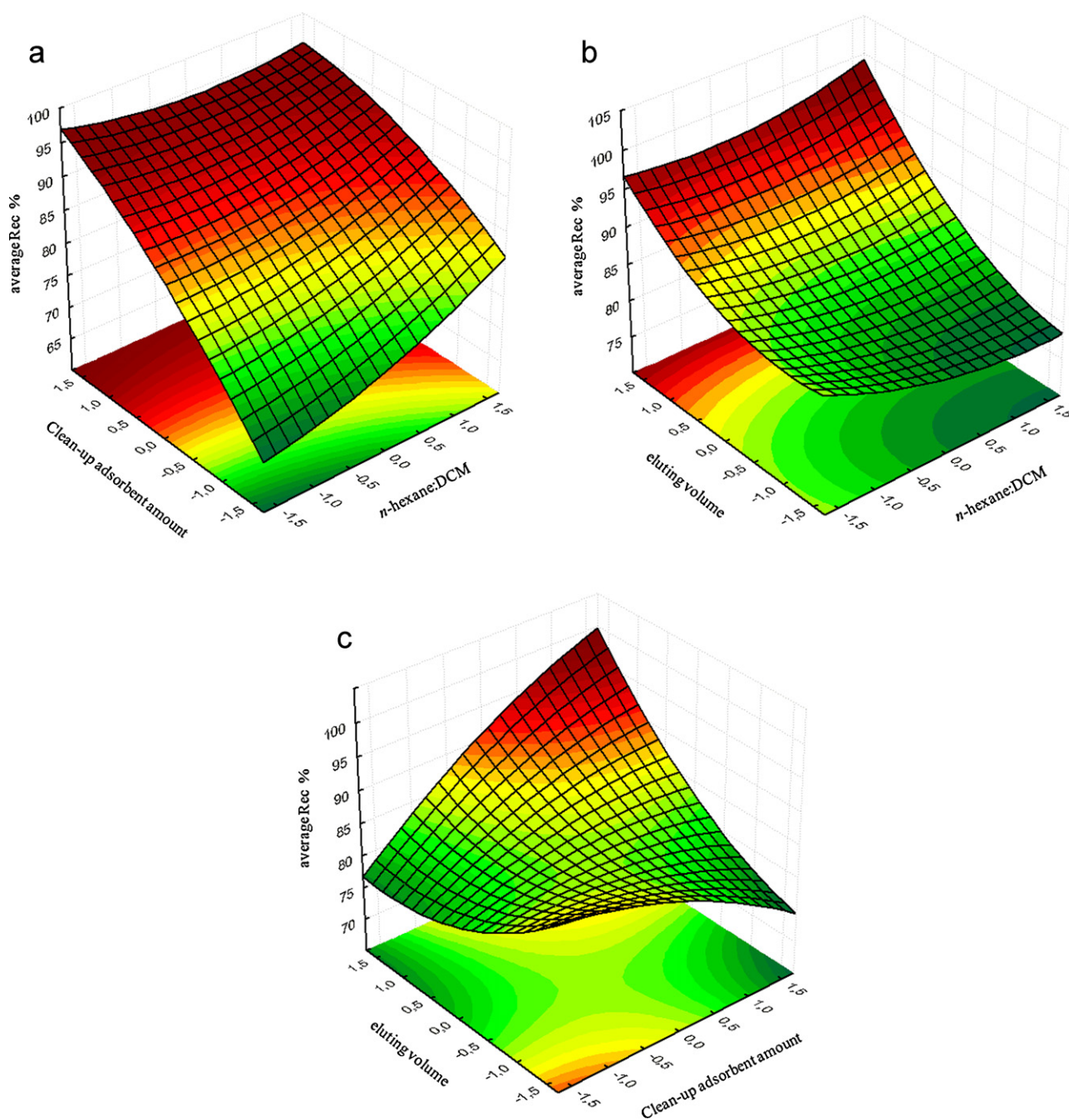
Fig. 3. Response surfaces for the 2^3 central composite design—(a) elution solvent (*n*-hexane:DCM): clean-up adsorbent amount; (b) elution solvent (*n*-hexane:DCM): eluting volume; (c) clean-up adsorbent amount: eluting volume.

Precision was calculated in terms of intra-day repeatability (*n* = 3) and inter-day reproducibility (*n* = 3), on 10 and 50 ng g^{-1} fortification level by calculating the relative standard deviation (RSD%). The intra-day repeatability, ranged from 1% to 7% (Table 4), while the inter-day reproducibility, ranged from 2% to 9%.

Finally, the analytical method proved to be sensitive enough to analyze the expected very small amounts of the target compounds present in lung tissues. The limits of detection (LOD$_S$) and limits of quantification (LOQ$_S$) of the proposed method were evaluated using GC-ECD and GC–MS/SIM at a signal-to-noise ratio of three (S/N = 3) and ten (S/N = 10), respectively. Method detection limits by GC–MS ranged from 0.42 to 0.86 ng g^{-1} and 0.50 to 1.36 ng g^{-1}, for OCPs and PCBs, respectively. Lower detection limits

were calculated for GC-ECD ranging between 0.15–0.30 ng g^{-1} and 0.18–0.48 ng g^{-1}, respectively.

3.3. Application to real samples

The proposed method was successfully applied to the analysis of lung tissues, from six routinely autopsied cases. Among the target analytes, eight OCPs and three PCBs were detected (Table 5). β-BHC is known to be the most persistent and metabolically stable BHC isomer and was detected in four samples (cases 1–4). On the other hand, α-BHC was not detected possibly due to faster degradation in the body [44]. The high frequency and concentration of γ-BHC (cases 1–5) can be explained by the wide use of lindane
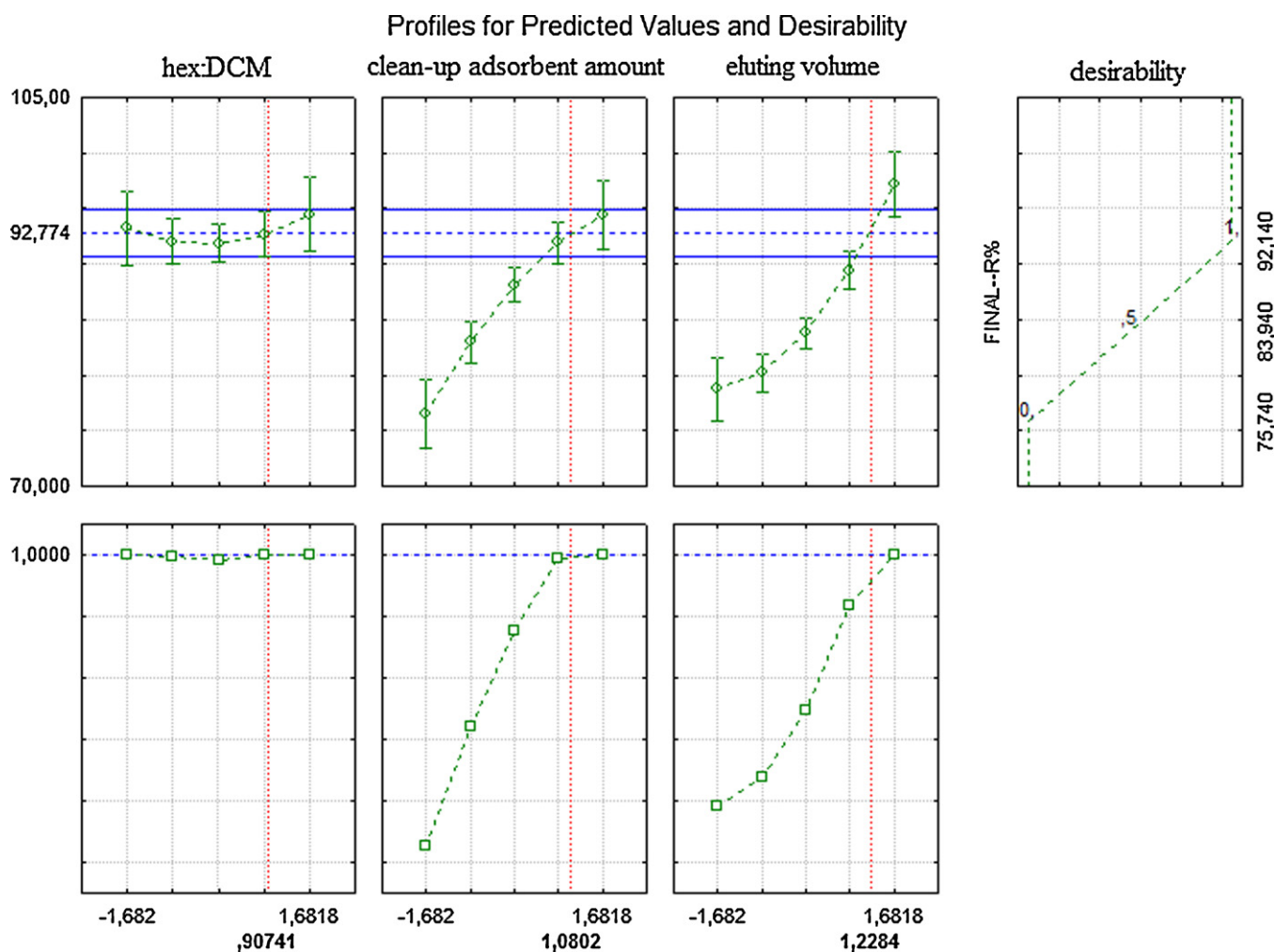
Fig. 4. Profiles for predicted values and desirability function for the average recovery $R\%$ of all target analytes. Dashed lines indicate the optimization values.

currently being one of the main sources of BHC pollution [44]. The presence of BHCs at children or young adults (case 5, age 24 yr), can be explained by their tendency to be easily transformed from mother to child [45]. Endosulfan is one of the most frequent contaminants occurred in food, soil and water in Europe [46], and was detected in three samples (cases 2–4) at concentrations ranging from 1.84 to 4.04 ng g^{-1}. The most abundant pesticide residue was p,p'-DDE (cases 1–5), the major metabolite of

p,p'-DDT. In case 5, only the main metabolite p,p'-DDE was detected at 2.15 ng g^{-1}. The absence of the parent compound (p,p'-DDT) suggests that young adult was probably exposed to the metabolite rather to the commercial pesticide, that was banned since the 1970s [47]. Endrin ketone was detected in only one sample (case 4, Fig. 1b).

PCB 153, 138 and 180 were the dominant congeners found in the selected cases. High-chlorinated PCBs congeners that lack

Table 5
Target compounds found in post-mortem human lung tissues from autopsy cases (nd: not detected; nq: not quantified).

Compound	Case 1[a] (ng g^{-1})	Case 2[b] (ng g^{-1})	Case 3[c] (ng g^{-1})	Case 4[d] (ng g^{-1})	Case 5[e] (ng g^{-1})	Case 6[f] (ng g^{-1})
β-BHC	3.19	3.81	nq	5.12	nd	nd
γ-BHC	2.81	3.71	4.63	4.99	1.57	nd
Endosulfan I	nd	2.46	1.84	4.04	nd	nd
Endosulfan sulfate	nd	3.32	nd	nd	nd	nd
p,p'-DDE	5.94	2.78	2.16	4.85	2.15	nd
p,p'-DDD	nd	nd	1.46	1.42	nd	nd
p,p'-DDT	4.89	2.36	3.34	3.91	nd	nd
Endrin ketone	nd	nd	nd	4.41	nd	nd
PCB 153	3.06	nd	nq	3.00	nd	nd
PCB 138	nq	2.76	3.21	6.31	nd	nd
PCB 180	nq	1.73	2.10	4.67	nd	nd

[a] Male 53 yr.
[b] Male 83 yr.
[c] Female 91 yr.
[d] Female 91 yr.
[e] Female 24 yr.
[f] Male 14 yr.

unsubstituted meta-para positions are better candidates for bioaccumulation and human exposure occurs mainly by food chain. Therefore the higher concentrations of high-chlorinated congeners and the absence of low-chlorinated congeners in human tissue samples indicate that the principal source of contamination with PCBs results from diet and not from direct exposure [44].

4. Conclusions

In this study, for the first time, matrix solid-phase dispersion method (MSPD) coupled to GC-ECD and GC–MS has been evaluated for the enrichment, separation and simultaneous determination of suspected OCPs and PCBs in post-mortem human lungs from autopsy cases. Extraction of analytes and clean-up were carried out in a single step without additional purification. Experimental design, RSM and desirability profile were used to optimize the parameters of matrix solid-phase dispersion (MSPD) and to investigate the interaction effect of different factors. This simple and rapid extraction method provides good repeatability and reproducibility range, high extraction efficiency and short time compared to other methods. Therefore, the proposed analytical protocol is a promising trend that could be fully exploited in the forensic toxicology field to assess other POPs, pharmaceuticals and metabolites.

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