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Experimental design approach for the optimization of supercritical fluid extraction of chlorophenols from polluted soils

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Abstract

Supercritical fluid extraction and liquid chromatography–electrochemical detection (LC–ECD) were used to the determination of chlorophenols in contaminated soil samples. Full factorial design experiments were used in order to optimize the extraction parameters: pressure, extraction cell temperature and percentage of modifier. Pressure and percentage of modifier (methanol) had statistically significant effects on the recovery of the chlorophenols. Good repeatability (4.9–11.8%) and reproducibility (4.9–12.5%) were achieved and low detection limits (3–150 ng g^{-1}) were obtained. The method was validated by comparing the results with those obtained in a European intercomparison exercise.

Keywords: Experimental design; Soil analysis; Supercritical fluid extraction; Chlorophenols

1. Introduction

Chlorophenols (CPs) have been used for a wide range of domestic, agricultural, and industrial purposes for more than 50 years and they are known to be important pollutants of environmental waters and soils [1–6]. It is well known that these compounds are hazardous to human health, thus making it necessary to identify the occurrence and levels of contamination in the environment, especially for soil reclamation [7]. For the analysis of chlorophenols in solid samples, typical methods for sample preparation usually involving liquid–solid extraction with an organic solvent followed by both clean-up and preconcentration stages are currently used [7]. These methods are time consuming and costly in the amount of solvent required. Greater concern over the disposal of such toxic organic solvents and their effect on the environment has led to a move towards cleaner extraction methods such as supercritical fluid extraction (SFE) [8–16].

The use of SFE as alternative to conventional extraction methods offers several advantages including a minimized sample handling, fairly clean extracts and a reduced use of environmentally aggressive solvents [17,18]. Additionally, in many cases, SFE provides recoveries as good as or even better than those of more conventional solvent extraction techniques [10,19–22], such as Soxhlet extraction. Nevertheless, the optimization of the operating conditions in SFE is still considered a critical step in the development of a SFE sample preparation method for the analysis of real samples. In addition, this technique offers some limitations because the extract efficiency depends on type of matrix, the interactions between the sample matrix and analytes and its concentration in the sample.

Supercritical carbon dioxide is by far the most commonly used fluid in analytical-scale SFE. This is due to its low critical pressure and temperature,

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reasonable price, ready availability, low toxicity, and inertness. However, pure supercritical CO_2 often fails in quantitative extraction of polar analytes such as phenol and chlorophenols from solid matrices. This is caused by the poor solvation power of the fluid and an insufficient interaction between the supercritical CO_2 and the matrix [23]. One way to overcome these problems and increase the extraction efficiency is the addition of organic modifiers to the fluid [24,25] or the use of reagents which react with the functional groups of the analytes and/or interact with the active sites of the matrix [26,27]. Methanol is the most usual choice as a supercritical CO_2 modifier for the extraction of phenol and chlorophenols [7,28–31] because it increases the critical temperature and pressure of the fluid which affects to the solubility of the analytes. However, the proportion of modifier that may be added to the CO_2 is limited by the supercritical region of the CO_2–methanol mixtures [32,33]. Extraction of phenols from soils and sediments has been also performed by in situ chemical derivatization extraction under SFE conditions, forming acetylated derivatives; this approach has been used for gas chromatography analysis of phenols in different matrices [34–36]. Nevertheless, LC–ECD is a selective and sensitive technique for the analysis of chlorophenols without the additional dervatization step [37,38] and their use combined with SFE using modified CO_2 could be a good procedure for the determination of these compounds in soil samples.

Efforts have been made to understand which parameters influence the extraction process and how the extraction process can be optimized [23,28,39]. Nevertheless, the selection of the operating conditions in SFE is still an area of active research that is characterised by much trial and error. Most of the reported SFE methods have been optimised by using one variable at a time, assuming no interaction between variables which can lead to biased results. In order to obtain reliable results in a reasonable time, statistical approaches to SFE can be applied [40]. With factorial designs, parameters showing strong influence on the extraction efficiency can be separated from those with little influence [41]. By combining experimental design of a reduced number of variables with multilinear regression, optimum extraction conditions can be achieved [41,42]. Moreover, the reported methods [43,44] for soil supercritical-fluid extraction have often been optimised by using samples spiked with known amounts of analytes prior to extraction [45]. Unfortunately, the use of spiked samples to evaluate extraction efficiencies can greatly overestimate the recoveries because interactions between the sample matrix and both native and spiked analytes can be different [45], so the use of certified reference materials is recommended.

This paper reports the results obtained in the development and optimization of a method for the supercritical fluid extraction of chlorophenols in a certified soil sample. For the analysis of these compounds, the SFE method was coupled off-line with LC–ECD [46]. The aims of this study were: (i) to optimize the variables which affect the extraction of chlorophenols from soils, such as pressure, temperature and percentage of modifier, using factorial design experiments, (ii) to assess the suitability of SFE for the analysis of chlorophenols in soil samples, (iii) to validate the SFE LC–ECD method for analysing chlorophenols in certified soils.

2. Experimental

2.1. Standards and reagents

The chlorophenols studied were obtained from the following sources: 4-chlorophenol (4-CP) from Carlo Erba (Milan, Italy); 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 2,3-dichlorophenol (2,3-DCP), 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP), 3,4-dichlorophenol (3,4-DCP), 3,5-dichlorophenol (3,5-DCP), 2,3,4-trichlorophenol (2,3,4-TCP), 2,3,5-trichlorophenol (2,3,5-TCP), 2,3,6-trichlorophenol (2,3,6-TCP), 2,4,5-trichlorophenol (2,4,5-TCP) and 2,4,6-triclorophenol (2,4,6-TCP) from Aldrich (Milwaukee, WI, USA); 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP), 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) and pentachlorophenol (PCP) from Chem Service (Chester, PA, USA).

Stock solutions (500 $mg\,l^{-1}$) of the individual chlorophenol standards were prepared in methanol. Calibration standards were prepared by appropriate dilution of the individual stock solutions with LC

mobile phase. Sodium acetate from Fluka (Buchs, Switzerland) and acetic acid from Merck (Darmstadt, Germany) were analytical grade. Acetonitrile and methanol HPLC-grade were purchased from J.T. Baker (Deventer, Netherlands). HPLC-grade water was obtained using a Culligan system (Barcelona, Spain). The solvents acetone and *n*-hexane (residue analysis grade) were supplied by Merck. Formic acid and acetic acid from Merck and phosphoric acid (85%) from Carlo Erba were used for the pretreatment of the soil before SFE extraction. All the solutions were filtered through a 0.45 μm nylon filter before injection into the HPLC system.

2.2. Soil samples

Soil samples were two candidate reference materials supplied by the Bureau Community of Reference (BCR) of the Commission of the European Communities (EC, Brussels). CRM-529 is a sandy soil and CRM-530 is a clay soil; both are contaminated by chlorophenols, chlorobenzenes, chlorinated pesticides (e.g. hexachlorocyclohexane), aromatic carboxylic acids, chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans as a result of industrial processes. For the optimization of the SFE procedure, the clay soil CRM-530 was used. The certified values were: 3-CP 6.80±1.84 μg g^{-1}, 3,4-DCP 7.04±1.73 μg g^{-1}, 2,4,5-TCP 44.41±13.03 μg g^{-1} and 2,3,4,6-TeCP 82.57±17.62 μg g^{-1} (±S.D.).

2.3. Chromatographic conditions

HPLC analysis was carried out on a Hewlett-Packard (Palo Alto, CA, USA) Series 1050 liquid chromatograph with an isocratic pump, a column oven and an automatic injector. The electrochemical amperometric detector was an HP 1049 A (Hewlett-Packard). A D-2500 Chromato-Integrator Merck-Hitachi (Merck) integrator was used. Separations were performed using a Hypersil Green ENV C_8 column from Shandon Scientific (Cheshire, UK) (250 mm×4.6 mm I.D., 5 μm particle size) and a Pelliguard LC-18 (20 μm particle size) precolumn (20 mm×4 mm I.D.) from Supelco (Bellefonte, PA, USA).

A mixture of 30 m*M* sodium acetate–acetic acid pH 4.5–acetonitrile–methanol (60:30:10, v/v/v) was used as the isocratic mobile phase for the chromatographic separation at a flow-rate of 1.5 ml min^{-1}. All the separations were carried out at 30°C and 20 μl was injected into the LC–ECD system. The working potential under these chromatographic conditions was set at +1100 mV between the glassy carbon working electrode and the Ag/AgCl reference electrode. Fig. 1A shows a chromatogram of a standard solution (1 mg l^{-1} of each compound) of the seven-

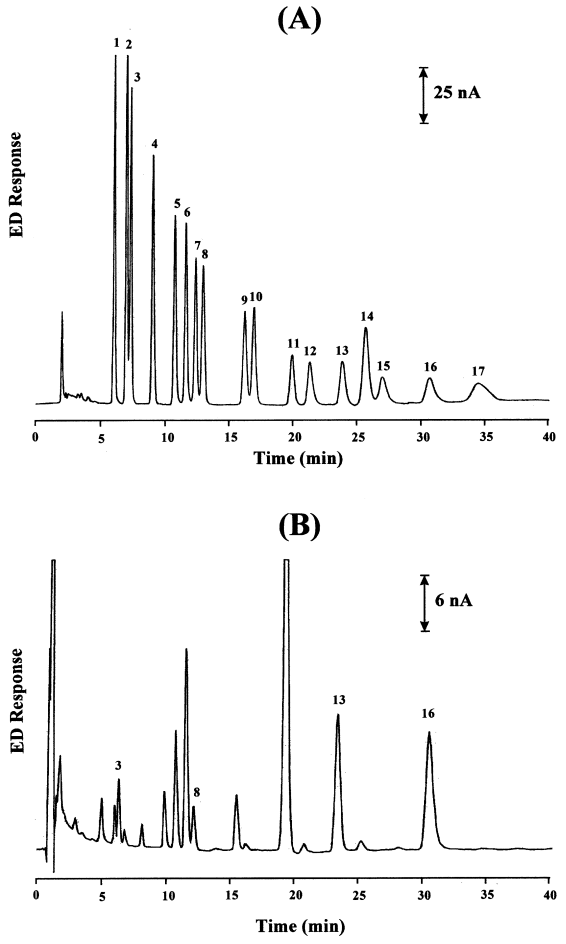

Fig. 1. (A) Chromatograms of a standard solutions of seventeen chlorophenols (1 μg ml^{-1}, 20 μl injected) dissolved in the mobile phase. (B) Chromatograms of the soil extract CRM 530. LC–ECD conditions as in text. Peaks: 1=2-CP; 2=4-CP; 3=3-CP; 4=2,6-DCP; 5=2,3-DCP; 6=2,5-DCP; 7=2,4-DCP; 8=3,4-DCP; 9=2,3,6-TCP; 10=3,5-DCP; 11=2,4,6-TCP; 12=2,3,4-TCP; 13=2,4,5-TCP; 14=2,3,5-TCP; 15=2,3,5,6-TeCP; 16=2,3,4,6-TeCP; 17=PCP.

teen chlorophenols. Quantification was performed by external calibration at eight concentration levels spanning the range 0.001–1.0 mg l^{-1}. Good correlation coefficients ($r>0.999$) were obtained for all the chlorophenols studied.

2.4. Supercritical fluid extraction

All extractions were performed using a supercritical fluid extractor Star SFE PrepMaster (Suprex, Pittsburgh, PA, USA) with a solvent modifier HPLC micropump (Suprex) and the collection system AccuTrap™ (Suprex) with a heated variable restrictor (VariFlow™, Suprex) to control CO_2 flow-rates. Extractions were carried out with high-purity (99.995%) supercritical CO_2 (Praxair, Danbury, CT, USA). All extractions were performed with a 3-ml stainless steel extraction cell which was first packed with a layer of anhydrous sodium sulphate, followed by a weighed amount of soil sample (0.2 g for CRM-530 soil and 1.6 g for CRM-529 soil). The remaining void of the cell was filled with anhydride sodium sulphate. The SFE extractions were carried out using a combination of a 10-min static period and a dynamic extraction step. Nozzle (restrictor) temperature was held at 45°C in all experiments. The flow-rate of the supercritical fluid in the dynamic extraction step was fixed to 1 ml min^{-1} with the help of the variable restrictor. The extract was collected in a trap (69 mm×4.5 mm I.D.) which was filled with different packings and was cooled at −10°C during the extraction with industrial purity CO_2. Hypersil ODS of 30 μm average particle size and dimethyldichlorosilane-treated glass beads of 80/100 mesh (Alltech Associates, Deerfield, IL, USA) were tested as packings for the collection of the analytes. The extracted analytes were eluted from the trap with 5 ml of acetonitrile and collected in a 10-ml vial which contained 1 ml of sodium acetate–acetic acid buffer. The final volume was made up to 10 ml with the buffer solution and the extract was analysed by LC–ECD.

2.5. Soxhlet extraction

Soil CRM-529 (1.6 g) was weighed and prewetted with 2 ml of H_2SO_4 for 2 h. After this treatment, the sample was extracted in a Soxhlet apparatus with 200 ml of acetone–*n*-hexane (3:2, v/v) for 12 h. The extract was evaporated in a rotary evaporator to ~2 ml and the final volume was made up to 5 ml with the LC mobile phase and the resulting extract was analysed by LC–ECD.

3. Results and discussion

3.1. Optimization of the supercritical fluid extraction.

Initially experiments were conducted to optimize variables such as collection trap material and type and amount of reactive reagents added to the sample before extraction. Hypersil ODS of 30-μm average particle size and dimethyldichlorosilane-treated glass beads were studied as trapping materials for chlorophenols. For this purpose, the SFE conditions were chosen based on the data available in the literature [23,43,47]. The SFE conditions were the following: extraction cell temperature 90°C, SFE pressure 350 atm., nozzle (restrictor) temperature 45°C, trap temperature −10°C, percentage of methanol as modifier 2.5% (v/v), CO_2 flow-rate 1 ml min^{-1}, static extraction time 10 min and dynamic extraction time 30 min. The extractions were carried out in triplicate using anhydride sodium sulphate spiked with the 17 chlorophenols at a concentration of 500 ng g^{-1}. The collected analytes adsorbed in the trap material were eluted with 5 ml of acetonitrile. The recoveries obtained for all compounds were higher than 80% for ODS packing (R.S.D.% 3.2–16.7) and between 47.2 and 99.3% for the treated glass beads (R.S.D.% 5.3–22.5). Due to the higher recoveries and lower relative standard deviations obtained with the solid-phase ODS, this material was chosen as packing for the cryogenic collection trap of the SFE system.

The second variable which was optimised was the addition of reagents to the soil before SFE extraction to increase the extraction efficiency. Formic acid, phosphoric acid and acetic acid were studied as modifiers with the addition of 200 μl of each reagent to different samples of the soil CRM-530 before extraction. Three replicates of each treated soil sample were extracted and the recoveries obtained for the chlorophenols are given in Table 1. The

Table 1
Recoveries (n=3) of the certified chlorophenols in CRM 530 soil adding 200 μl of the different reagents to the sample before extraction

Reagent	Recovery (%)							
	3-CP		3,4-DCP		2,4,5-TCP		2,3,4,6-TeCP	
	Mean	R.S.D.(%)	Mean	R.S.D.(%)	Mean	R.S.D.(%)	Mean	R.S.D.(%)
42.5% Phosphoric acid in methanol	38.4	12.6	65.8	11.2	67.0	9.7	98.8	5.9
25% Formic acid in methanol	47.8	7.2	71.8	8.8	71.1	5.9	98.8	3.9
Formic acid	68.7	4.2	81.7	3.9	85.8	5.6	115.7	10.1
25% Acetic acid in methanol	49.0	11.2	65.6	6.7	76.2	8.3	101.6	5.4
Acetic acid	52.7	11.2	67.7	9.7	78.4	6.1	95.2	5.2

highest recoveries were obtained when formic acid was added to the soil. The low recoveries obtained using phosphoric acid, which contains 15% water, are due to the negative effect of the water on the extraction efficiency [48]. For formic and acetic acids, the amount of water was negligible and the lower pK_a of the formic acid (pK_a 3.74) compared to acetic acid (pK_a 4.75) led to a better efficiency. The amount of formic acid added to the sample was also optimized. Different volumes between 100 and 500 μl were added to soil CRM-530 and the best recoveries were obtained when 500 μl were used.

The effects of temperature, pressure and percentage of modifier on the extraction of the phenols in the certified soil CRM-530 (3-CP, 3,4-DCP, 2,4,5-TCP and 2,3,4,6-TeCP) were studied using an experimental design. In Fig. 1B a chromatogram of the extract of this soil is shown. A full two-level factor design (2^3), which involved a total of eight experiments plus one centered point and the replicates needed for statistical evaluation, was chosen for optimization of the supercritical extraction efficiency. The upper, lower and centered values of each factor were selected from available data in the literature and experience gathered in the above described experiments. The factor levels and the SFE

Table 2
Factor levels, design matrix and recovery values of the selected chlorophenols in soil CRM-530 for the first factorial design experiment.

Run	Factor			Recovery (%)			
	A	B	C	3-CP	3,4-DCP	2,4,5-TCP	2,3,4,6-TeCP
1	50	200	0	49.6	40.8	66.7	68.4
2	50	200	10	75.4	71.6	96.4	107.2
3	50	450	0	4.4	1.8	5.9	7.1
4	50	450	10	87.4	78.7	95.7	102.5
5	100	200	0	74.1	68.9	90.4	80.9
6	100	200	10	70.1	68.0	91.2	102.5
7	100	450	0	77.9	77.1	99.2	111.3
8	100	450	10	88.4	81.1	102.0	116.3
9	75	325	5	80.1	66.1	81.9	80.8
10	50	200	0	53.8	46.4	80.3	90.4
11	50	200	10	67.6	65.9	89.8	102.0
12	50	450	0	3.4	2.1	9.3	7.6
13	50	450	10	83.1	72.6	97.1	104.7
14	100	200	0	67.9	70.5	96.6	98.0
15	100	200	10	72.8	73.3	98.2	110.3
16	100	450	0	77.5	74.9	97.5	111.4
17	100	450	10	79.6	70.0	87.7	100.5
18	75	325	5	78.4	72.4	81.2	81.8

A: Extraction cell temperature (°C).
B: Pressure (atm.).
C: Percentage of methanol (%) as modifier.

parameters used in the optimization experiments and the design matrix and the recoveries obtained for the certified chlorophenols in soil CRM-530 are given in Table 2. The experiments were performed in a randomized order to avoid systematic errors. In this first experimental design, the variables were fixed as follows: CO_2 flow-rate at 1 ml min^{-1}, nozzle (restrictor) temperature at 45°C, 500 μl formic acid added to the soil, cryogenic collection trap temperature −10°C and ODS as packing, 10 min static extraction time and 30 min dynamic extraction time. An analysis of the results obtained for the certified chlorophenols (total concentration as Σ of 3-CP, 3,4-DCP, 2,4,5-TCP and 2,3,4,6-TeCP) gave the standardized main effect Pareto chart shown in Fig. 2A. The pressure (factor B), the percentage of methanol used as the CO_2 modifier (factor C) and the interaction between them were statistically significant. In addition, AC (temperature–percentage of methanol) and AB (temperature–pressure) interactions were also significant, although the fact that the extraction temperature (factor A) was statistically nonsignificant at the 95% confidence level, suggested that the significance of the AC and AB interactions was due to the effects of B and C. Nevertheless, it has to be mentioned that the individual Pareto chart showed that changes in temperature had significant effects on the higher chlorinated phenols. The response surface estimated for the model by using two variables, pressure and percentage of methanol, as the only significant factors, is given in Fig. 2B. As can be seen, the extraction efficiency was directly proportional to both factors and the optimum conditions for all chlorophenols were obtained at a pressure and percentage of methanol higher than 300 atm. and 5%, respectively.

In order to obtain a more accurate optimization, a full three-level factor design (3^2) was applied, considering the two main factors which were statistically significant, pressure and percentage of modifier. This second full factor design involved a total of 18 experiments, considering the replicates needed for statistical error evaluation. In this case, the extraction cell temperature was fixed at 100°C to increase the extraction of the low volatile chlorophenols and the factors were studied at the following levels: 325 atm. (low), 387.5 atm. (medium) and 450 atm. (high) for the pressure and 5% (low), 10% (medium) and 15%

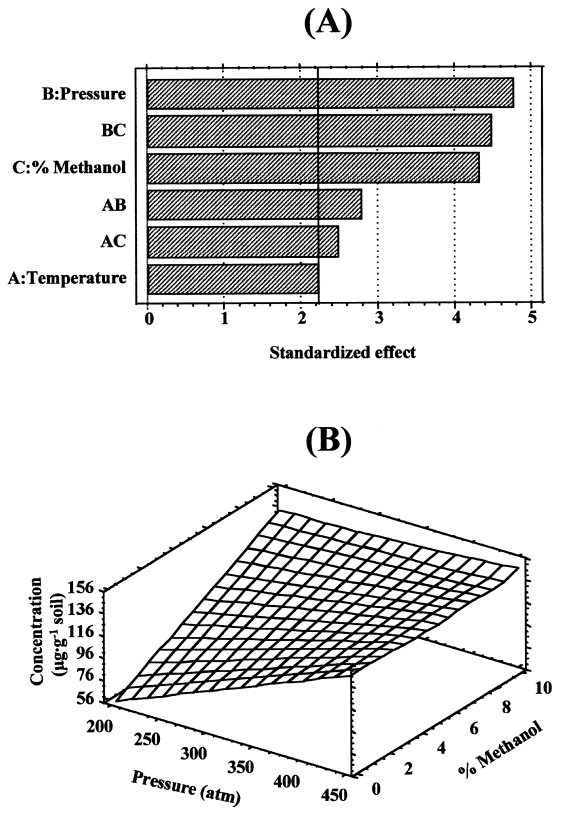

Fig. 2. (A) Pareto chart for the standardized main effects in the first factor design experiment. The vertical line indicates the stastistical significance bound for the effects. (B) Response surface estimated for the design, obtained by plotting two statistically significant main factors, pressure and percentage of methanol (response expressed as Σ concentrations of 3-CP, 3,4-DCP, 2,4,5,-TCP and 2,3,4,6,-TeCP).

(high) for the percentage of modifier. The design matrix of this more restricted model and the recoveries obtained for the selected chlorophenols are given in Table 3. The Pareto chart obtained for the chlorophenols (Σ of 3-CP, 3,4-DCP, 2,4,5-TCP and 2,3,4,6-TeCP) and the response surface estimated from the design are given in Fig. 3. Only pressure had a statistically significant effect. This was consistent with the response surface which showed an increase in the recoveries at high pressure. The influence of the percentage of methanol on the extraction efficiency was independent of the pressure (Fig. 3B) indicating that high pressures and percentages of methanol between 5 and 15% will give higher recoveries. Nevertheless, the low and high

Table 3
Design matrix and recovery values of the selected chlorophenols in the second factorial design experiment

Run	Factor		Recovery (%)			
	Pressure (atm.)	Methanol (%)	3-CP	3,4-DCP	2,4,5-TCP	2,3,4,6-TeCP
1	450	5	83.2	80.4	101.4	107.5
2	450	10	86.8	88.2	112.5	124.5
3	450	15	89.0	97.9	113.6	111.2
4	387.5	5	75.0	69.2	108.5	109.0
5	387.5	10	80.4	72.2	110.2	98.1
6	387.5	15	83.8	77.6	109.3	99.3
7	325	5	72.6	69.2	101.6	91.5
8	325	10	78.4	72.2	108.2	94.2
9	325	15	80.6	77.4	105.0	94.0
10	450	5	81.3	82.2	108.8	122.1
11	450	10	85.4	89.9	112.3	112.8
12	450	15	86.9	88.1	109.4	106.4
13	387.5	5	49.4	72.7	105.5	100.7
14	387.5	10	74.9	76.6	109.1	103.3
15	387.5	15	76.9	80.5	109.2	103.5
16	325	5	79.0	61.2	106.0	96.1
17	325	10	83.1	67.6	102.3	96.8
18	325	15	79.6	72.0	102.3	93.5

chlorinated phenols behaved in a different manner. Whereas the low chlorinated compounds gave higher recoveries at high amounts of modifier, the responses of the high chlorinated compounds increased at low amounts of methanol. This can be seen in Fig. 4, where the response surfaces of 3-CP and 2,4,5-TeCP are given. As a consequence, the percentage of methanol for all chlorophenols was set at 10% for subsequent experiments.

The optimal conditions for the SFE extraction of chlorophenols obtained using soil CRM-530 were: extraction temperature cell, 100°C; SFE pressure, 450 atm.; percentage of methanol, 10%. Finally, the dynamic extraction time was optimized in order to reduce the analysis time. Different extractions were carried out using the optimal conditions and changing the dynamic extraction time between 5 and 30 min. Extraction of 2,4,5-TCP and 2,3,4,6-TeCP was performed in 15 min, while for more polar chlorophenols, such as 3-CP and 3,4-DCP, more time was needed. As a consequence, 30 min was set for the extraction of all the compounds.

3.2. Application

To evaluate the repeatability and reproducibility of the method, three replicate determinations on the same day and three determinations on 3 different days using the CRM-530 soil were carried out, respectively. The values obtained for the certified phenols in the soil are summarized in Table 4. Relative standard deviations (%) ranging from 4.9 to 11.8% for repeatability and from 4.9 to 12.5% for reproducibility were obtained, showing good precision of the method.

Limits of detection (LOD) for the seventeen chlorophenols studied, based on a signal-to-noise ratio of 3:1, were calculated using soil CRM-530 cleaned using successive SFE extractions. This clean soil was spiked at low ng g^{-1} levels and the limits of detection obtained for the 17 chlorophenols are given in Table 5. All LOD values were in the ppb level, showing that the method can be used to the determination of chlorophenols in soils with low levels of pollution.

To demonstrate the applicability of the proposed method for the analysis of chlorophenols in soils, a sandy certified soil CRM-529 of different characteristics from the clay soil CRM-530 was analyzed. The chromatogram of a extract of soil CRM-529 obtained using SFE is given in Fig. 5. The results obtained using SFE and Soxhlet methods and those reported by all the laboratories participating in the European certification exercise are given in Table 6.

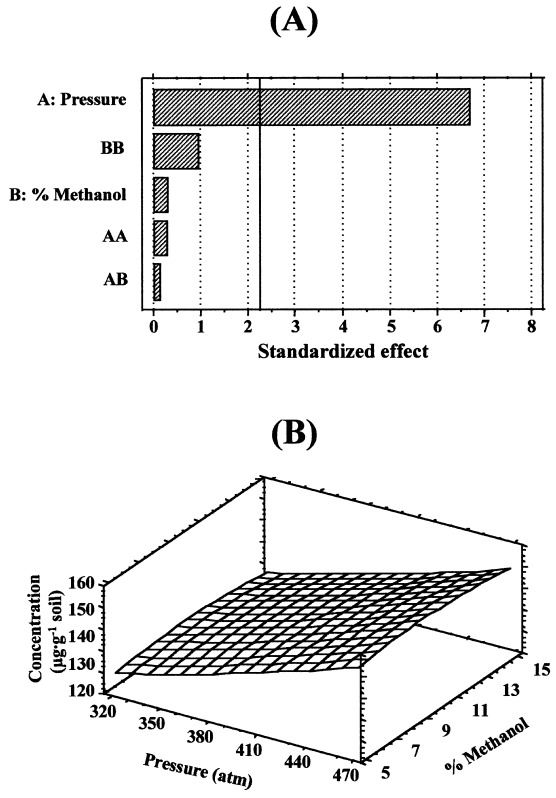

Fig. 3. (A) Pareto chart for the standardized main effects in the second factor design experiment. The vertical line indicates the stastistical significance bound for the effects. Response surface estimated for the design, obtained by plotting the pressure and percentage of methanol factors; (response expressed as Σ concentrations of 3-CP, 3,4-DCP, 2,4,5,-TCP and 2,3,4,6,-TeCP).

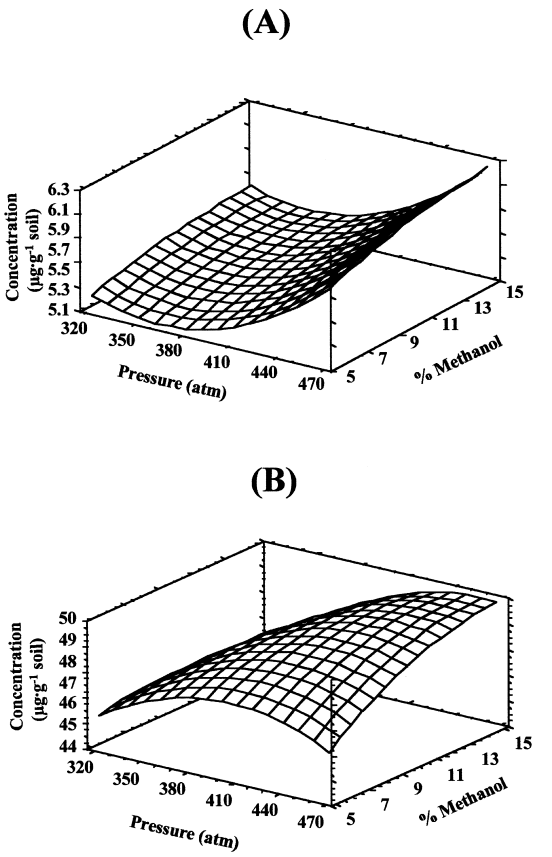

Fig. 4. Response surface estimated for 3-CP and 2,4,5,-TCP for the second factor design experiment (response expressed as concentration).

The results obtained using SFE agreed with the mean of all laboratories and a good precision (R.S.D.% between 4.8 and 9.7%) was achieved, while for the Soxhlet extraction worse precision was obtained. These results showed that the SFE can be considered as a good alternative for the analysis of chlorophenols in polluted soils.

4. Conclusions

To study the usefulness of a full experimental design for the optimization of pressure, temperature and percentage of modifier for the SFE extraction of chlorophenols in soil samples, a certified soil (CRM-530) was used. The statistical approach has proved to be an excellent tool in revealing which experimental factors were really influencing the overall recoveries. Pressure, percentage of methanol and the first order interaction between these variables were statistically significant. Optimum SFE conditions for chlorophenols were 450 atm., 100°C and 10% of percentage of modifier (methanol), using 500 μl of formic acid as the reagent.

Good repeatability (4.9 to 11.8%) and reproducibility (4.9 to 12.5%), and low detection limits (3 to 150 ng g^{-1}) were achieved. The method was also applied to the analysis of a sandy soil candidate to reference material, CRM 529. The results showed

Table 4
Quality parameters of the SFE and LC–ECD method for the certified chlorophenols in the soil CRM-530

	Repeatability			Reproducibility		
	Mean (n=3) ($\mu g\ g^{-1}$ soil)	S.D.	R.S.D. (%)	Mean (n=9)[a] ($\mu g\ g^{-1}$ soil)	S.D.	R.S.D. (%)
3-Chlorophenol	5.476	0.647	11.8	5.401	0.676	12.5
3,4-Dichlorophenol	5.644	0.482	8.5	5.642	0.562	9.9
2,4,5-Trichlorophenol	43.959	2.181	4.9	43.267	2.120	4.9
2,3,4,6-Tetrachlorophenol	100.694	7.202	7.1	97.383	6.152	6.3

[a] Nine determinations=3 replicates×3 different days.

Table 5
Limits of detection for chlorophenols in soil samples

Compound	Limit of detection ($ng\ g^{-1}$ of soil)
2-Chlorophenol	3
3-Chlorophenol	3
4-Chlorophenol	6
2,3-Dichlorophenol	6
2,4-Dichlorophenol	9
2,5-Dichlorophenol	6
2,6-Dichlorophenol	3
3,4-Dichlorophenol	6
3,5-Dichlrophenol	9
2,3,6-Trichlorophenol	9
2,4,6-Trichlorophenol	10
2,3,4-Trichlorophenol	29
2,3,5-Trichlorophenol	31
2,4,5-Trichlorophenol	15
2,3,4,6-Tetrachlorophenol	92
2,3,5,6-Tetrachlorophenol	62
Pentachlorphenol	150

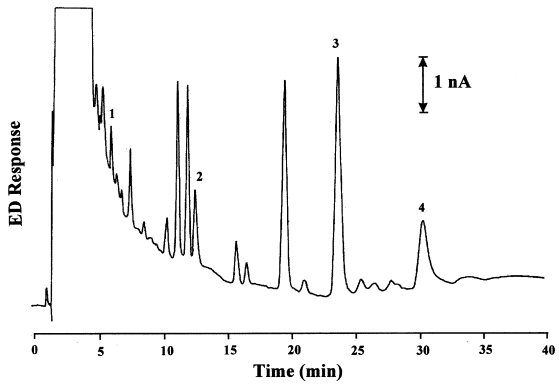

Fig. 5. Chromatograms of the soil extract CRM 529 using SFE. Peaks: 1=3-CP; 2=3,4-DCP; 3=2,4,5,-TCP; 4=2,3,4,6-TeCP. For chromatographic conditions see Section 2.

that the SFE with LC–ECD was a rapid and clean procedure, and that it can be used for the analysis of chlorophenols in contaminated soils with the advantage of a reduction in solvent consumption and elimination of clean-up steps. With SFE instead of the conventional Soxhlet extraction the total time required for the entire analytical method is reduced from about 2 days to 1.5 h.

Table 6
Analysis of soil CRM-529 by SFE and Soxhlet with LC–ECD

Compound	Concentration ($\mu g\ g^{-1}$)		
	SFE (n=3)	Soxhlet extraction (n=6)	Certification exercise (n=14)
	Mean±S.D.	Mean±S.D.	Mean±S.D.
3-Chlorophenol	0.034±0.003	0.040±0.007	0.048±0.011
3,4-Dichlorophenol	0.197±0.010	0.257±0.039	0.271±0.070
2,4,5-Trichlorophenol	1.541±0.074	1.382±0.191	1.659±0.404
2,3,4,6-Tetrachlorophenol	1.440±0.140	0.965±0.165	1.229±0.290

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