Multivariate Optimization of Supercritical Fluid Derivatization and Extraction of Phenol in Soil Samples

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Abstract

Two-level (Packett–Burman) and three-level (central composite) orthogonal factor designs are used as a formal optimization procedure for the supercritical fluid derivatization–extraction of phenol in soil samples by acetylation. Nine variables are considered: carbon dioxide flow rate, fluid density, extraction cell temperature, static extraction time, nozzle and trap temperatures, amount of derivatizing reagent, pyridine concentration, and time of contact between the derivatizing reagents and sample prior to extraction. The dynamic extraction process is carried out in four steps to simultaneously study the extraction kinetics of the process. The results suggest that only the extraction cell temperature and the amount of derivatizing reagent used are statistically significant to the overall extraction yield, as is the extractant flow rate to the kinetics. The procedure is validated by processing a certified reference material; special attention is paid to developing a test material for this type of experiment.

Introduction

The interest in supercritical fluid extraction (SFE) as a preparative technique for the extraction of organic pollutants from environmental matrices has grown rapidly in recent times. The SFE technique minimizes sample handling, provides fairly clean extracts, expedites sample preparation, and reduces the use (and disposal) of environmentally aggressive solvents (1,2). In addition, SFE provides recoveries as good as or even better than those of conventional solvent extraction techniques in many cases (3–6).

Supercritical CO_2 is by far the most commonly used fluid in SFE; quantitative extraction of polar analytes, however, requires the addition of an organic modifier (7–9). Alternatively, the extraction efficiency for polar analytes can be boosted by adding a derivatizing reagent to the sample matrix. This methodology is known as supercritical fluid derivatization and extraction (10–14). Lee and co-workers (15) developed an SFE method for the determination of chlorophenols in topsoil as acetylated derivatives.

Some developers optimize extraction methods one parameter at a time (16). This sequential univariate strategy, however, is rarely known to be effective for determining the true optimum. Accordingly, formal optimization methods are generally preferred. Factor designs have been used for the simultaneous determination of various analytical SFE parameters including temperature, pressure, CO_2 density, fluid flow rate, and extraction time (17–22). Only two or three variables are considered in most cases, so a reasonably large number of experiments must be performed in order to detect potential interactions between the experimental variables. This process is not affordable when using the classical univariate method. Derivatization–extraction procedures, however, involve a potentially increased number of variables as a result of the need to optimize derivatization as well.

On the other hand, a number of reported methods (23,24) for soil supercritical fluid extractions have been optimized by using samples spiked with known amounts of analytes immediately prior to extraction. In this way, any interactions between the sample matrix and analytes, which may give rise to spurious results when the optimized procedure is applied to real samples, are virtually suppressed (25). Because of the restricted availability of certified reference materials for real contaminated samples, one should bear in mind the need not to suppress analyte–matrix interactions in preparing experimental samples for optimization purposes.

One other frequently overlooked factor in this context is the extraction kinetics. In fact, most early SFE work was primarily aimed at optimizing the extraction yield.

This paper reports the results obtained in the development and optimization of a method for the supercritical fluid derivatization–extraction of phenol in soil samples. Various factor designs were tested to optimize nine experimental variables, namely CO_2 density and flow rate, extraction cell temperature, static extraction time, nozzle and trap temperatures, amounts of acetic anhydride and pyridine, and contact time prior to extraction. The optimization was targeted at both the overall extraction yield and the extraction kinetics; the statis-

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tical significance of each experimental variable studied was thus established in relation to both targets. The procedure was developed by using spiked soil samples prepared in such a way that potential interactions between phenol and the matrix were not suppressed. The phenol recovery was assessed by using a commercially available certified reference material.

Experimental

Analyses were carried out on a Hewlett-Packard 5890 gas chromatograph equipped with a flame-ionization (FID) detector. A fused-silica chromatographic column (60 m × 0.56-mm i.d., 0.2-µm phase thickness) coated with DIIP (diisodecyl phthalate) from Restek (Bellefonte, PA) was used. Chromatographic data were acquired and processed with the aid of the Hewlett-Packard 3365A software package. The chromatographic conditions used were as follows: injection port temperature, 125°C; injection mode, splitless; injection volume, 1 µL; and splitless time, 60 s. The carrier gas used was nitrogen (99.9995% purity) at a flow rate of 5 mL/min; the pressure at the column head was 50 kPa. The oven and FID temperatures were 90 and 150°C, respectively.

Experiments were performed on a Hewlett–Packard 7680A supercritical fluid extractor using standard steel cells of 7.0-mL inner volume; however, the system was altered as described later. The collection trap (7 cm × 5-mm i.d., 540-µL inner volume) was packed with Hypersil ODS of 30-µm average particle size.

The phenol standard used was supplied by Aldrich Chemie (Steinheim, Germany). Acetic anhydride, pyridine, methanol, and *n*-hexane were purchased from Merck (Darmstadt, Germany). A phenol stock solution was prepared by weighing an appropriate amount of the standard and diluting to 10 mL with *n*-hexane. Working solutions were made by appropriate dilution of the stock. All solutions were stored at 5°C in the dark prior to use. For quantitative gas chromatographic determinations, calibration was carried out at four concentration levels spanning the range 1–20 µg/g. For this purpose, a volume of 20 µL pyridine and 50 µL acetic anhydride was added to an overall volume of 0.94 mL phenol standard at each of the four concentration levels in *n*-hexane. Solutions were monitored at 60°C for 30 min prior to injection into the chromatograph.

Extractions were carried out with 99.995% pure carbon dioxide from Carburos Metálicos (Barcelona, Spain). The pump and collection trap were cooled with industrial purity CO_2.

A garden soil sample, the carbon content of which was 2.2%, obtained from the campus of the University of Santiago de Compostela (Galicia, Spain) was used for the optimization experiments. Several kilograms of soil was dried in an oven at 40°C, ground, and sifted to a particle size of less than 60 µm. Sample (200 g) was spiked with 250 mL methanol containing phenol, which was slowly added to form a dough that was mechanically mixed for a few minutes. The sample was then allowed to air-dry for 4 days and was stored in a dry, dark place for 2 months prior to analysis. On the assumption that no phenol loss occurred during drying or storage, the expected final concentration was 11.5 µg/g on a dry-weight basis. We also assumed the contaminant to be uniformly distributed in the sample and that, because the sample contained residual moisture throughout the storage period, any analyte–matrix interac-

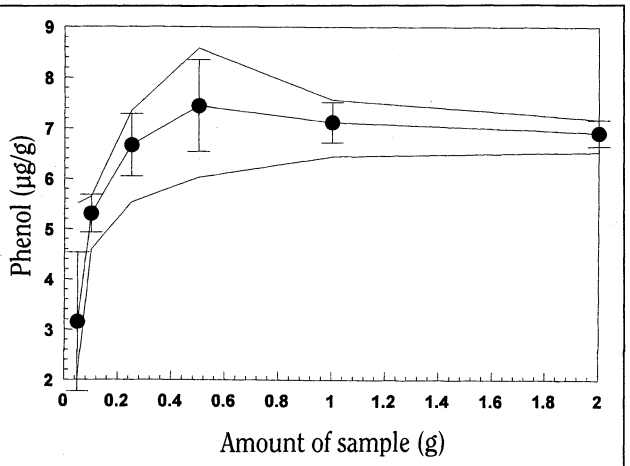

Figure 2. Evaluation of the homogeneity of the material used to optimize the extraction procedure through the variation of the mean values and standard deviations obtained with the amount of sample subjected to extraction and analysis.

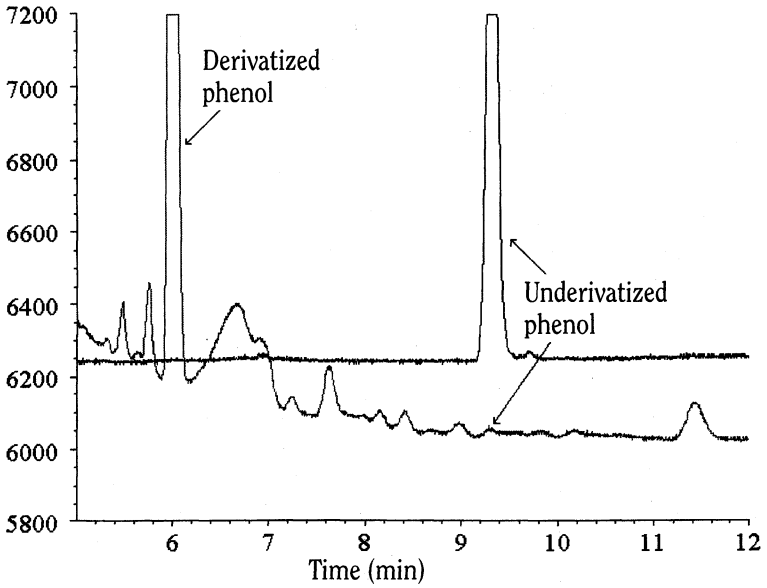

Figure 1. Chromatograms for an underivatized phenol standard and a derivatized phenol standard (containing several other phenolics in lower proportions). Arrowheads point to the position of the peaks corresponding to derivatized phenol and underivatized phenol.

tions would have occurred to a similar extent to those in real contaminated soil of identical properties.

The phenol recovery was determined by using a certified reference material supplied by Environmental Resource Associates (Arvada, CO), (i.e., ERA soil, Lot no. 329), the certified phenol content of which was 9.88 µg/g.

Irrespective of the working conditions imposed by the particular factor design, all samples were prepared by following the same procedure prior to extraction. To minimize contamination and plugging of the sintered disks, the top and bottom caps of the extraction cell were fitted with two filter paper disks of the same diameter as the cap internal diameter. Also, a piece of Teflon tubing of the same outer diameter as the extraction cell internal diameter was placed in the extraction cell to avoid potential interactions between its steel walls and the analyte. The lower half of the tube was packed with Celite and the sample (to which the required amounts of acetic anhydride and pyridine were added), and the upper half was packed with additional Celite to the top. The tube was sealed with the top cap and placed in the extraction chamber. The static and dynamic supercritical fluid CO_2 extraction program was then started under the conditions dictated by the particular factor design tested. Finally, the acetylphenol formed was eluted from the trap with two 1-mL portions of *n*-hexane and collected in two 2-mL vials. The extracts were subjected to direct gas chromatography under the conditions already described.

To investigate the extraction kinetics, the dynamic extraction procedure was split into four steps in such a way that extracts were collected at 2, 4, 6, and 16 min for separate analysis.

Table I. Factor Levels in the First (Folded Plackett–Burman) Factorial Design

Factor		Levels		
Variable	Key	Low (–)	High (+)	Center (0)
CO_2 density (g/cm^3)	A	0.4	0.6	0.5
CO_2 flow* (mL/min)	B	0.75	1.25	1.00
Extraction cell temperature (°C)	C	60	90	75
Nozzle temperature (°C)	D	45	60	52.5
Trap temperature (°C)	E	15	30	22.5
Pyridine amount (µL)	F	20	40	30
Acetic anhydride amount (µL)	G	50	100	75
Static extraction time (min)	H	5	15	10
Contact time before extraction (min)	I	0	30	15

* As measured at the pump outlet transducer (as supercritical fluid).

Table II. Design Matrix and Response Values in the First (Folded Plackett–Burman) Factorial Design

Run	A	B	C	D	E	F	G	H	I	Response (total phenol recovery %)
1	0	0	0	0	0	0	0	0	0	61.47
2	+	–	+	+	+	–	–	–	+	61.27
3	–	+	+	+	–	+	+	–	+	84.05
4	–	+	–	–	+	–	+	+	+	57.78
5	–	+	+	–	+	–	–	–	+	48.26
6	–	–	–	+	+	+	–	+	+	53.77
7	+	–	–	+	–	+	+	+	–	58.13
8	–	–	+	+	+	–	+	+	–	62.61
9	–	+	–	+	+	+	–	–	–	49.83
10	+	–	+	–	–	–	+	+	+	69.22
11	+	+	+	–	+	+	–	+	–	59.31
12	+	+	+	–	–	–	+	–	–	73.17
13	+	–	+	+	–	+	–	–	–	61.15
14	+	–	–	–	+	–	–	+	–	61.66
15	+	–	–	–	+	+	+	–	+	54.65
16	–	+	+	+	–	–	–	+	–	59.19
17	–	–	+	–	+	+	+	–	–	84.53
18	+	+	–	–	–	+	–	–	+	39.44
19	–	–	–	+	–	–	+	–	+	62.16
20	–	–	+	–	–	+	–	+	+	39.70
21	–	+	–	–	–	+	+	+	–	52.53
22	+	+	+	+	+	+	+	+	+	71.42
23	–	–	–	–	–	–	–	–	–	63.33
24	+	+	–	+	+	–	+	–	–	59.23
25	+	+	–	+	–	–	–	+	+	49.30
26	0	0	0	0	0	0	0	0	0	64.42

Results and Discussion

Validation of the analytical procedure for phenol by gas chromatography

As noted in the Experimental section, calibration curves were run at four concentration levels using appropriately diluted and derivatized standards. Each concentration level was injected in triplicate. Chromatographic peak areas were fitted by linear regression. The linearity range was 1–20 µg/g with a correlation coefficient of .9999. The repeatability of the chromatographic procedure was assessed by performing nine consecutive injections of a derivatized standard solution and nine individual injections of as many individually derivatized solutions. The between-injection and between-derivatization repeatability data (relative standard deviations) were both 4.8%. Injections of derivatized standards were at the lowest concentration level in the calibration curve. The extent of derivatization of the standards was also determined. The chromatographic system used allowed quantitation of both free and acetylated phenol (see Figure 1) and hence allowed assessment of the extent to which the derivatization reaction proceeded (by performing two separate calibrations for each species at an appropriate concentra-

tion level). The results obtained suggest that approximately 2% of the initial amount of phenol remained underivatized. For direct injections of derivatized standards at signal-to-noise ratios of 3 and 10, the limit of detection was 7.5 ng/g and the limit of quantitation was 25 ng/g.

Evaluation of the homogeneity of the laboratory-spiked soil sample

The homogeneity of the spiked sample with regard to analyte distribution was evaluated after a little more than 2 months storage. The extraction conditions were established empirically from experiments performed to determine the potential original extent of phenol contamination. The tests revealed the absence of a signal for phenol in the original soil; on the other hand, phenol additions carried out immediately before analysis led to the following working conditions. The CO_2 density was 0.5 g/cm^3, and the CO_2 flow rate, as measured at the pump outlet transducer (as supercritical fluid), was 1.0 mL/min. The extraction cell temperature was 70°C, and static extraction time was 10 min. The nozzle (restrictor) and trap temperatures were 45 and 40°C, respectively. Depending on the sample, the amount of acetic anhydride was 10–100 µL. The amount of pyridine used was 20 µL, and the contact time before extraction was 0 min. Such conditions were systematically tested on sample masses from 0.05 to 2.00 g. As can be seen in Figure 2, the results were highly disperse for sample sizes less than 0.25 g. On the other hand, the material was homogeneous with regard to phenol distribution greater than a 0.5-g size; also, the variability for a sample size of 2 g was similar to that obtained for injection replicates of the calibration standards.

We chose 1.0 g as the optimal sample size for subsequent experiments in order to prevent the variability between sample portions from masking the influence of the experimental variables.

Factor designs: evaluation of the response surface

The number of variables potentially affecting the extraction efficiency and kinetics was very large. Nine factors, including CO_2 flow rate, CO_2 density, temperature of the extraction cell, static extraction time, temperature of the nozzle, temperature of the trap, amount of acetic anhydride, amount of pyridine, and contact time between the derivatizing reagents and the sample prior to extraction, were, in principle, influential. A full, two-level factor design (2^9) would involve a total of 512 experiments, in addition to the replicates needed for statistical evaluation of the coefficients for the fitted model and the degree of coincidence of the hyperplane obtained. Therefore, a folded Plackett–Burman ($2^9 \times 3/64$) type IV resolution design that allowed 14 degrees of freedom and involved 24 randomized runs plus two centered points (26) was chosen. This design possesses an alias structure such that main effects are clear of two-factor interactions, but these are partially confounded with other two-factor interactions. Table I lists the upper and lower values given to each factor. Such values were selected from available data and experience gathered in the above-described experiments for the evaluation of sample homogeneity. Table II shows the design matrix for this experiment and the overall phenol extraction yield.

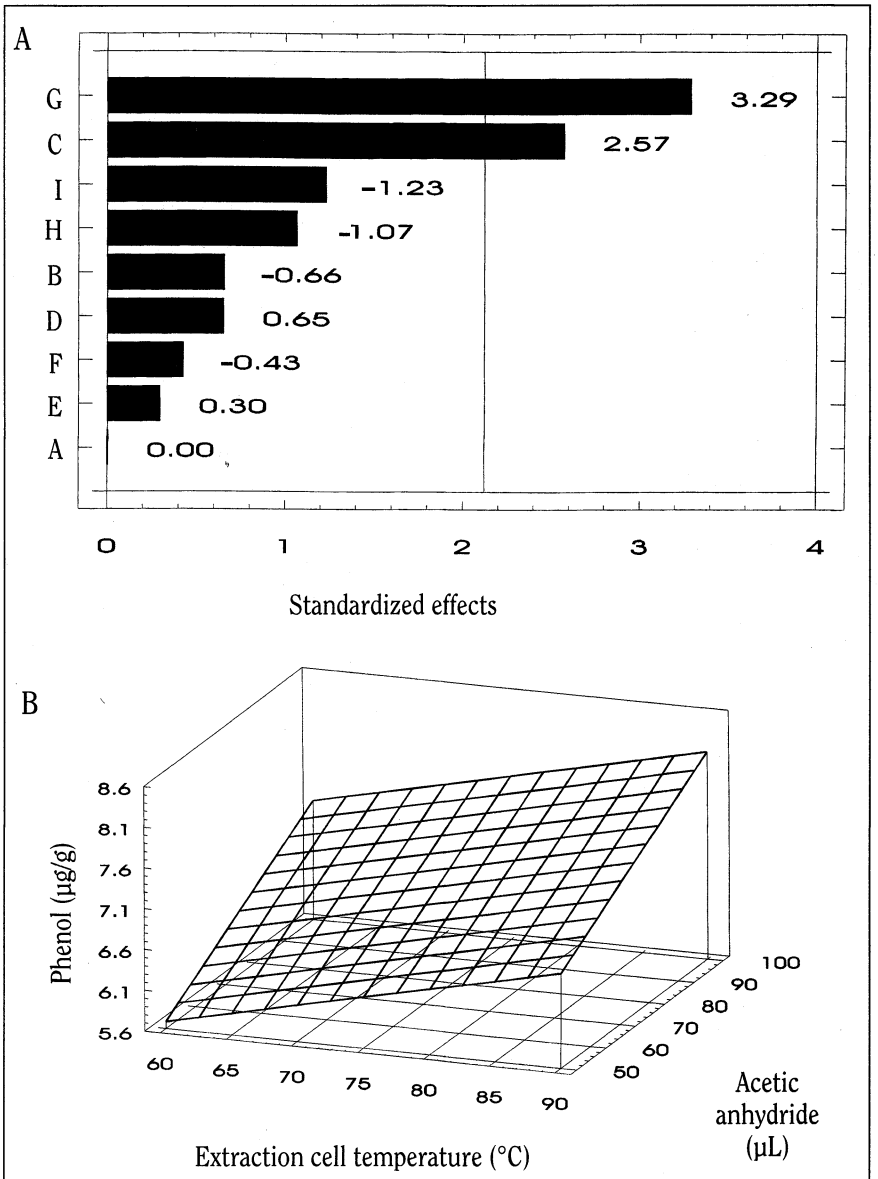

Figure 3. (A) Pareto chart for the standardized main effects in the first factor design (folded Plackett–Burman model). The vertical line indicates the statistical significance bound for the effects. G, amount of acetic anhydride; C, extraction cell temperature; I, contact time; H, static extraction; B, CO_2 flow rate; D, nozzle temperature; F, amount of pyridine; E, trap temperature; and A, CO_2 density. (B) Response surface estimated for the design, obtained by plotting the two statistically significant main factors.

An analysis of the results given in the far right column of Table II produced the standardized main effect Pareto chart shown in Figure 3A; only the amount of acetic anhydride (i.e., the derivatizing reagent, the proportion of which determined the extent to which the derivatization reaction developed and hence the ease with which phenol was extracted by a nonpolar fluid such as supercritical CO_2) and the extraction cell temperature (a major variable in SFE processes) were statistically significant. Figure 3B shows the response surface obtained for the model by using these two variables as the only significant factors. As can be seen, the extraction efficiency was directly proportional to both factors—it peaked at the highest levels tested.

A design of this type does not allow the direct evaluation of interaction terms for two or more factors. Accordingly, some of the initially considered factors were discarded in view of the results. The data in Table II was used to evaluate a more restrictive model. Excluding the less significant factors in Figure 3A (CO_2 density, trap temperature, amount of pyridine, nozzle temperature, and CO_2 flow rate) and keeping the other four allowed two-factor interactions to be evaluated. Based on the Pareto chart in Figure 4, there were significant interactions between factors G (amount of acetic anhydride), I (contact time), and H (static extraction time), as well as between factors C (extraction cell temperature) and H. The results are all logical. Thus, the amount of derivatizing reagent and the contact time must be correlated inasmuch as both influence the formation of acetylphenol. Also, the amount of derivatizing agent and the static extraction time must be mutually influential because not only acetylphenol but also excess derivatizing reagent is included to a variable extent in the supercritical phase, thereby altering the properties of the extraction fluid. Likewise, one may admit interactions between the static extraction time and the temperature of the extraction cell as both factors favor extraction. However, the fact that variables I and H are statistically insignificant suggests that the significance of the G–I, C–H, and C–G interactions arises primarily from the effects of C and G. On the other hand, the adverse effects of the contact time (factor I) and the static extraction time (factor H) should be noted, which is apparent from the effect graph in Figure 5. In fact, a contact time or static extraction time at the high level results in diminished extraction relative to the low levels. This trend is also observed in factors B (CO_2 flow rate) and F (amount of pyridine), yet their coefficients are very close to zero, so they can be ascribed no experimental significance. This reduced model provided the response surface shown in Figure 6 (again based on two main factors, G and C). The overall conclusions are similar to those drawn from the response surface of Figure 3B, except for the influence of the amount of acetic anhydride, which appears to be more influential here, and the extraction cell temperature, which is seemingly only influential with factor G at its high level as a result of an interaction between both.

In any case, the region where the experiment was conducted appeared not to include the optimum. The maximum phenol recovery achieved was approximately 84.5% of the hypothetical content in the spiked samples, based on the assumption that no analyte was lost through drying or storage. Therefore, a new factor design was developed that was shifted in the direction of

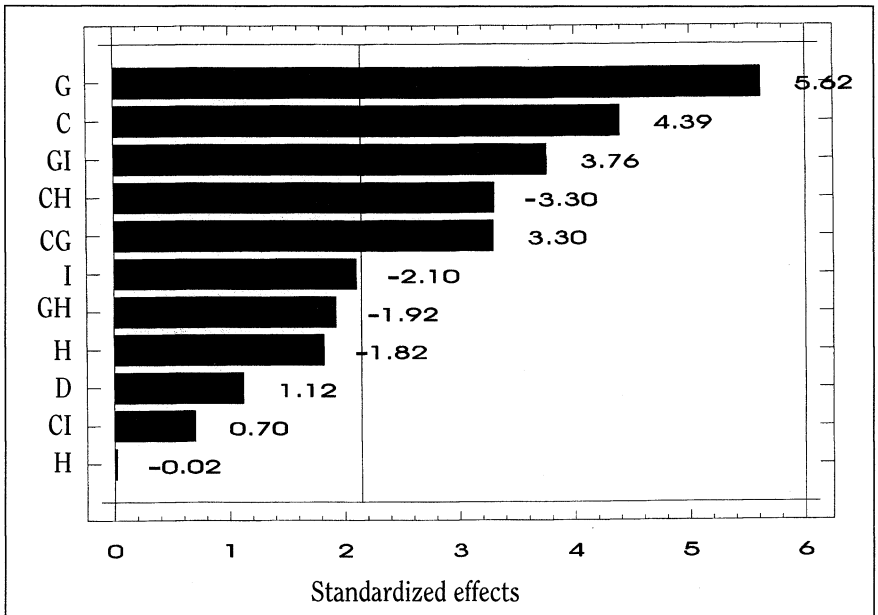

Figure 4. Pareto chart for main effects (after discarding effects A, B, E, and F) and interaction effects between variable pairs. The vertical line indicates the statistical significance bound for the effects. G, amount of acetic anhydride; C, extraction cell temperature; I, contact time; H, static extraction; and D, nozzle temperature.

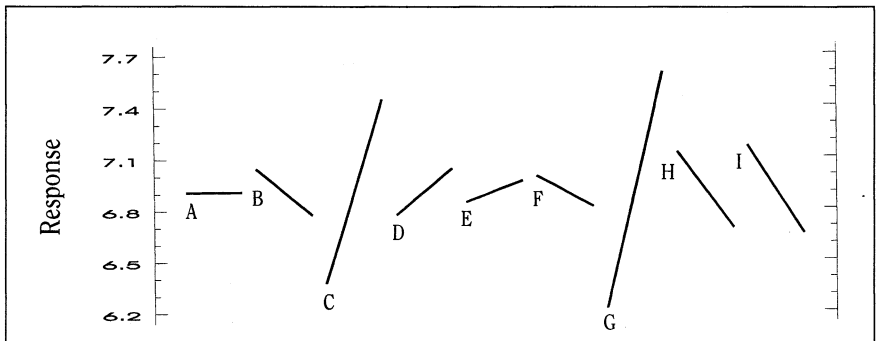

Figure 5. Graph showing the influence of main effects on the extraction of phenol. the lines indicate the magnitude and sign (increase or decrease) of the variation of the extraction efficiency with the factor level (from low to high). A, density; B, CO_2 flow rate; C, extraction cell temperature; D, nozzle temperature; E, trap temperature; F, amount of pyridine; G, amount of acetic anhydride; H, static extraction; and I, contact time.

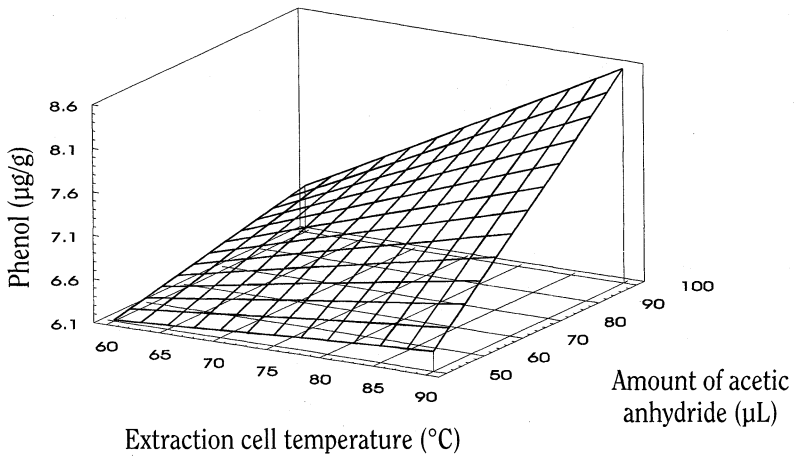

Figure 6. Response function estimated from the first factor design (folded Plackett–Burman model) after discarding factors A, B, E, and F and introducing interaction effects. Function obtained by plotting the two significant main effects and significant interactions.

Table III. Supercritical Fluid Extraction (SFE) Parameters and Factor Levels Used in the Second (Central Composite) Factorial Design and Optimal Values for SFE of Phenol in Soils

Factor	Key	Fixed	Low (–)	High (+)	Axial Center	Axial distance	Optimum
CO_2 density (g/cm³)	A	0.4	–	–	–	–	0.4
CO_2 flow (mL/min)	B	1.2	–	–	–	–	1.2
Extraction cell temperature (°C)	C	–	90	110	100	±1.07809	115
Nozzle temperature (°C)	D	45	–	–	–	–	45
Trap temperature (°C)	E	20	–	–	–	–	20
Pyridine amount (µL)	F	20	–	–	–	–	20
Acetic anhydride amount (µL)	G	–	80	130	105	±1.07809	70
Static extraction time (min)	H	5	–	–	–	–	5
Contact time before extraction (min)	I	0	–	–	–	–	0

Table IV. Design Matrix and Response Values in the Second (Central Composite) Factorial Design

Run	Factor C*	Factor G*	Response (total phenol recovery %)
1	0	0	81.57
2	0	1.07809	80.68
3	+	–	78.70
4	0	–1.07809	85.32
5	–	+	82.24
6	–1.07809	0	73.83
7	–	–	71.23
8	+	+	90.09
9	1.07809	0	91.97
10	0	0	84.31

* Factor C, extraction cell temperature and factor G, acetic anhydride amount.

the maximum gradient relative to the previous one; only three value levels and those factors that were previously found to be actually significant were tested in order to evaluate the curvature of the response surface. A central 2^2+ star, orthogonal composite design involving 10 randomized runs with four error degrees of freedom was used; the factors considered were assigned low and high levels, and all other experimental variables were given the values listed in Table III. The results thus obtained are shown in Table IV, together with the corresponding design matrix. As can be seen from the Pareto chart in Figure 7A, only one factor, namely, the extraction cell temperature factor, was statistically significant; all other interactions between factors and quadratic terms were not. This is consistent with a very slightly curved response surface; in Figure 7B, the amount of acetic anhydride was only influential when the extraction cell temperature was at its low level. Because the extraction cell temperature was restricted to a maximum value of 120°C in the extractor used, it was concluded that the experimental field contained no clear-cut maximum, so the conditions given in the second column of Table III was adopted as optimal for extraction of phenol from the material tested. The maximum recovery thus achieved (again, based on the assumption that no phenol was lost in the material preparation procedure) was 92%. However, the result was validated by means of a series of extractions from a certified reference material (ERA soil, Lot no. 329) that provided an average recovery of 74.2 ± 7.9%. This reference material, which, in fact, is certified on the basis of the spiked amounts of analytes and not by means of intercomparison analysis rounds, shows a clear heterogeneous particle size distribution with no indication of homogeneity level. Thus, it was suspected that particle size distribution was influencing the obtained recovery. A portion of this material was ground to an approximately 60-µm particle size and reanalyzed. Under these conditions, recovery for phenol was 81.4 ± 6.2%, which is in good agreement with the expected value for the extraction procedure.

A series of six consecutive extractions performed on the same day and another six carried out on different days were used to evaluate the repeatability and reproducibility of the experimental procedure. All these extractions were carried out using the laboratory-spiked soil. The average recoveries obtained were 82.6 ± 3.8% (same-day extractions) and 83.1 ± 5.2% (different-day extractions).

Extraction kinetics: effect of experimental factors

The extraction kinetics were studied in all experiments by

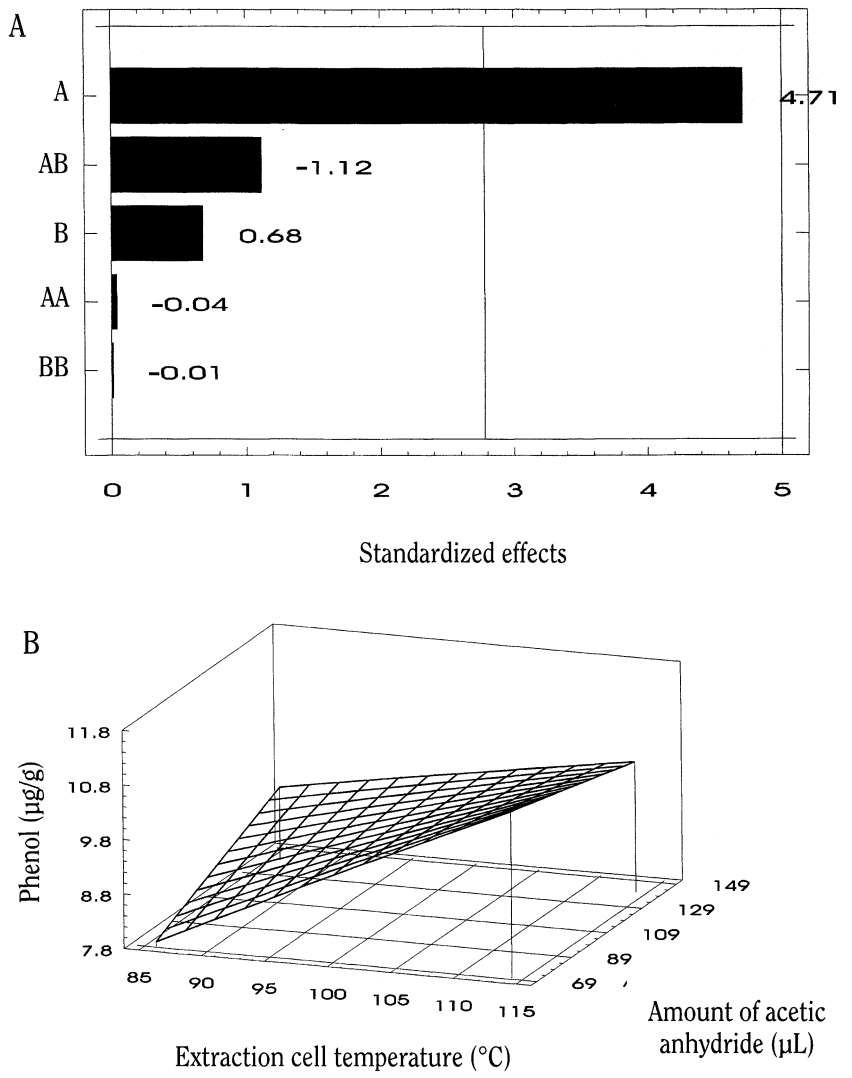

Figure 7. (A) Pareto chart of standardized effects for the second factor design (central composite model). The vertical line indicates the statistical significance bound for the effects. A, extraction cell temperature and B, amount of acetic anhydride. (B) Response surface estimated from the design.

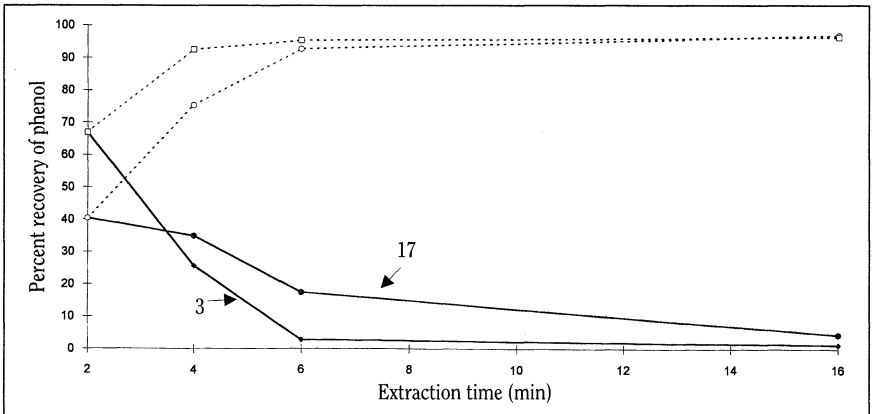

Figure 8. Phenol extraction kinetics in two experiments representative of the first factor design (folded Plackett–Burman model). The run numbers 3 and 17 correspond to the sequential numbers in Table II. Solid lines represent the proportions of phenol recovered in each successive extraction at the stated time value. Dashed lines indicate the percent cumulative recovery of phenol during the consecutive extractions.

splitting the dynamic extraction step and collecting extracts at 2, 4, 6, and 16 min. Logically, the extraction-versus-time plots obtained in the different experiments performed with the first factor design tested were widely divergent. In some cases, the extracts collected at the fourth time (16 min) contained no phenol. On the other hand, some samples exhibited much slower kinetics. Figure 8 gathers some typical results (the corresponding experiment number, as the sequential number in Table II, is given in each case). The optimal extraction conditions were those producing the maximum extraction yield at the shortest possible extraction time. In this sense, the second factor design (three-level design) provided very similar kinetic curves that corresponded to rapid extractions (less than 6 min).

To determine which experimental variables affected the extraction kinetics, we substituted the overall recoveries (the cumulative amounts of phenol obtained in the four dynamic extraction portions) in the far right column of Table II with those obtained for the first two portions (2 and 4 min) and reevaluated the model statistically. Figure 9 shows the Pareto chart obtained under these conditions; the only significant variables were the extraction cell temperature (also significant to the overall extraction recovery) and the extractant flow rate. The influence of the latter variable on the extraction kinetics is quite logical, even though it exerted no significant effect on the overall extraction yield (after 16 min). In fact, a flow rate of 1.2 mL/min (the optimal condition reflected in Table III) allowed the quantitative extraction of phenol from the samples within 5 min.

Conclusion

Because several factors have to be considered in the development of derivatization SFE analytical procedures, formal optimization techniques should be applied. Factorial designs have proved to be excellent tools in revealing which experimental factors are really influential in the overall analyte's recovery and extraction kinetics. Plackett–Burman designs allow for screening and evaluation of approximate response surfaces and factors' selection. Three-level orthogonal central composite

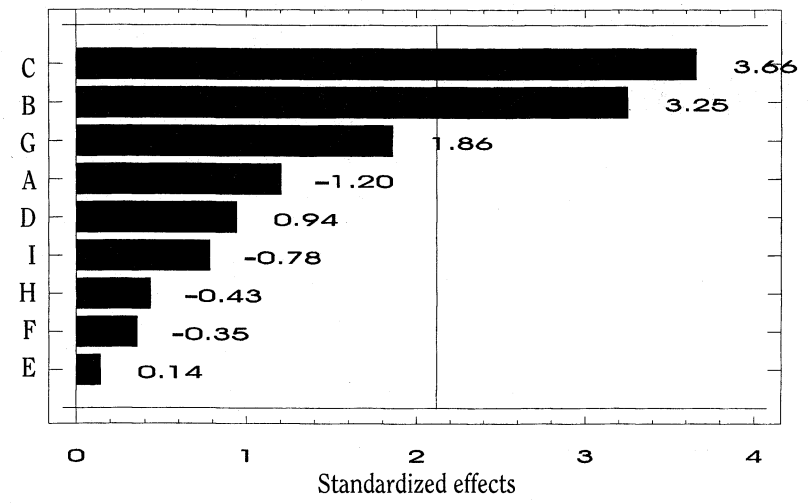

Figure 9. Pareto chart of main effects for the first factor design (folded Plackett–Burman model), obtained by using the phenol recoveries after two consecutive extractions (2 and 4 min) as response values. The vertical line indicates the statistical significance bound for the effects. C, extraction cell temperature; B, CO_2 flow rate; G, amount of acetic anhydride; A, CO_2 density; D, nozzle temperature; I, contact time; H, static extraction; F, amount of pyridine; and E, trap temperature.

designs must be used to obtain a fine adjustment of the response surface. Here, the analytical derivatization–extraction of phenol in soil samples was optimized by means of these types of factorial designs. Results suggest that only the extraction cell temperature and the amount of derivatizing reagent used are statistically significant to the overall extraction yield. Regarding extraction kinetics, extraction cell temperature plays a larger role, followed by supercritical fluid flow rates. Using the optimal conditions established, 81 ± 6% of the present phenol can be extracted from different soil types.

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