

## The role of capacity factor in method development for solid-phase extraction of phenolic compounds. II

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### Abstract

Recently, a simple method was developed for the determination of capacity factors of phenolic compounds on solid-phase extraction cartridges [*J. Chromatogr. A*, 693 (1995) 217]. In the present paper the capacity factors determined by this method are used to predict recoveries of phenolic compounds in solid-phase extraction at three different sample volumes. The values are compared with those of recovery tests from both model solutions and spiked surface water samples. Based on calibration data the minimum sample concentrations which can be determined by the SPE procedure are also calculated from the capacity factors.

### 1. Theory

#### 1.1. Calculation of recoveries from chromatographic data

The capacity factors determined by the equilibration method [1] can be used to predict the recoveries of phenolic compounds in solid-phase extraction at any sample volume. The knowledge of the experimentally determined capacity factors should by no means replace the conventional recovery tests required for SPE method development. It, however, guides through the time-consuming procedure of the selection of the appropriate stationary phase and the optimization of sample composition and volume. The fact that the precision of the method is limited for strongly retained solutes, does

not restrict the applicability of the method since optimization primarily focuses on the least retained substances.

In order to predict recoveries from capacity factors the basic chromatographic parameters have to be calculated. The retention volume ( $V_R$ ) is obtained from the basic equation of chromatography

$$V_R = V_M \cdot (1 + k) \quad (1)$$

where  $V_M$  is the void volume of the cartridge and  $k$  is the capacity factor of the solute.  $\sigma_v$  is determined as

$$\sigma_v = \frac{V_M}{N^{1/2}} \cdot (1 + k) \quad (2)$$

where  $N$  is the number of theoretical plates of the cartridge.

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The breakthrough volume ( $V_B$ ) is calculated using the equation

$$V_B = V_R - 2 \cdot \sigma_v \quad (3)$$

Based on the considerations given in Part I, for the solutes having a breakthrough volumes ( $V_B$ ) larger than the sample volume ( $V_0$ ), quantitative recovery (100%) is expected within experimental error. For the solutes for which the sample volume is in excess of  $V_R + 2 \cdot \sigma_v$ , the recovery ( $r$ ) can be calculated as

$$r = \frac{V_M \cdot k}{V_0} \cdot 100\% \quad (4)$$

In case the sample volume falls between  $V_B$  and  $V_R + 2 \cdot \sigma_v$  of an analyte, the recovery cannot be calculated analytically, though error (erf) functions which are available in tabulated form can be used to estimate recoveries. As an alternative to these calculations, a simple computer model based on the theoretical plate concept was developed. It established the breakthrough curve from the input parameters of the capacity factor, void volume and number of theoretical plates of the cartridge. The amount lost was determined by numerical integration then the recovery was calculated. If the calculated recovery is plotted as a function of sample volume normalized to  $V_R$  between  $V_B$  and  $V_R + 2 \cdot \sigma_v$  for three different values of capacity factor, the curves shown in Fig. 1 are obtained.

It is interesting to note that the curves are approaching a single curve by increasing the capacity factor as the column void volume is becoming negligible compared to the retention volume.

Thus the recoveries can be predicted for any sample volume under the conditions of the experiment. These values, however, may at best be rough estimates of experimental recoveries determined from conventional recovery tests and should not be relied on as true values to be used in the analysis of samples. Ideally, they represent the theoretically attainable recoveries under the given conditions. The knowledge of the predicted recoveries, however, may assist in revealing the major sources of error in an SPE pro-

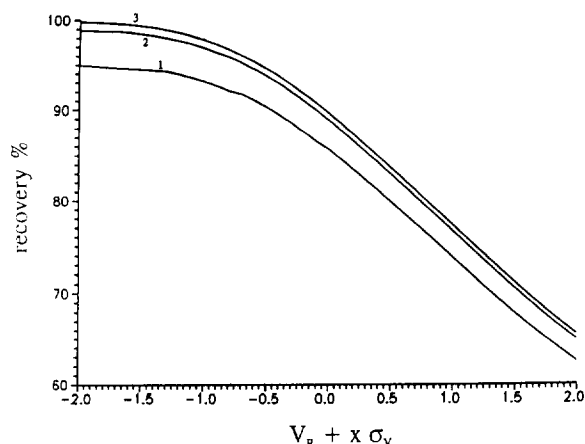


Fig. 1. Recovery as a function of normalized sample volume between  $V_R - 2 \cdot \sigma_v$  and  $V_R + 2 \cdot \sigma_v$  for capacity factors of 10, 100 and 1000. Lines: (1)  $k = 10$ ; (2)  $k = 100$ ; (3)  $k = 1000$ .

cedure. If the recovery tests yield recoveries significantly higher than predicted for a compound, either the capacity factor was incorrectly determined or some matrix constituent interferes. Significant negative departures from the predicted values, on the other hand, may indicate competition between the solutes and the matrix interferences or losses in other steps of the SPE procedure.

### 1.2. Calculation of minimum sample concentrations

From the experimental capacity factors the minimum sample concentrations which can be determined by the SPE method can also be calculated. These concentrations are usually different for each compound as the limits of detection and the attainable enrichment factors are different. In the calculations it was assumed that the sample volume was selected so that the maximum attainable enrichment factor was exploited for each compound, i.e. the sample volume was in excess of  $V_R + 2 \cdot \sigma_v$  for each solute.

Recovery ( $r$ ) is defined as

$$r = \frac{c_f \cdot V_f}{c_0 \cdot V_0} \cdot 100\% \quad (5)$$

where  $c_f$  and  $V_f$  are the concentration and volume of the final extract, respectively,  $c_0$  is the sample concentration, and  $V_0$  is the sample volume.

By substituting Eq. (4) into Eq. (5) we obtain

$$c_0 = \frac{c_f \cdot V_f}{k \cdot V_M} \quad (6)$$

In an optimized solid-phase extraction procedure  $V_M$  and  $V_f$  are constant and  $k$  is fixed for each compound, therefore  $c_f$  is in direct proportion to the sample concentration ( $c_0$ ). If  $c_f$  represents the minimum concentration which can be quantified in the extract by the chromatographic technique (corresponding to a signal-to-noise ratio of 10:1),  $c_0$  stands for the minimum concentration which can—at best—be quantified by the whole SPE procedure. Similarly, if  $c_f$  is the minimum detectable concentration in the extract (corresponding to a signal-to-noise ratio of 3:1),  $c_0$  represents the minimum concentration in the sample which can—at best—be detected by the whole SPE procedure.

It should be emphasized, however, that  $c_0$  in either case is a theoretical limit under which the analyte cannot be detected or quantified by the given SPE method whatsoever. Its value can be approached for the least retained compounds but can hardly be realized for the most strongly retained solutes.

## 2. Experimental

### 2.1. Conditions

### 2.2. Reagents

Phenol (99.0%), 4-methylphenol (99.4%), 4-chlorophenol (99.0%), 3,4-dimethylphenol (98.0%), 2,4-dichlorophenol (99.0%) and 2,4,6-trichlorophenol (97.0%) were obtained from Supelco (Switzerland). Methanol (HPLC grade) was purchased from ROMIL (UK), *n*-hexane from Aldrich (Germany), hydrochloric acid from Reanal (Hungary). Turbine oil standard was received from the power plant involved. All

reagents were used as received without further purification.

The cartridges used were Waters SEP-PAK C18 Plus, 360 mg sorbent weight, 0.7 ml nominal hold-up volume, purchased from Waters Millipore Division, USA. All cartridges were activated with 3 ml methanol then conditioned with 3 ml of  $10^{-3}$  M hydrochloric acid.

### 2.3. Instrumentation

GC-MS analysis was carried out with a TRIO-1000 instrument of FISON (UK). The column was SPB-1 fused silica, 30 m, 0.32 mm I.D., 0.25  $\mu$ m film thickness, purchased from Supelco. The chromatographic conditions were: injector temperature 280°C, interface temperature 285°C. The temperature program was: 55°C for 1 min, 4.0°C/min to 170°C and 15°C/min to 280°C. 1  $\mu$ l was injected in splitless mode, the split valve was opened 30 s after the injection. The MS was operated in selected-ion recording mode in EI. The ion source temperature was 180°C. The following ions were monitored in the retention windows: 94, 107, 122, 128, 162 and 196 amu for phenol, 4-methylphenol, 3,4-dimethylphenol, 4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol, respectively.

For the determination of potential matrix interferences the following GC conditions were set: injector temperature: 315°C interface temperature 315°C. The temperature program applied was: 45°C for 1 min, 15.0°C/min to 200°C, 5.0°C/min to 310°C, 30 min hold. The MS was operated in EI full scan mode, between 20–300 amu, with 0.9 s scan time and 0.1 s interscan time.

### 2.4. Procedures

A stock solution of phenols was prepared in methanol in a 10-ml calibrated flask. The concentrations of the individual compounds in methanol were: 1.34, 1.03, 0.99, 0.96, 0.97 and 0.89 mg/ml for phenol, 4-methylphenol, 3,4-dimethylphenol, 4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol, respectively.

In the recovery tests, distilled water was

spiked with 10  $\mu$ l of stock solution, acidified with 1 M hydrochloric acid to pH = 3.0 then thoroughly homogenized in a calibrated flask of 100, 250 and 500 ml. The sample solution was passed through the cartridge at a flow rate of 3.5 ml/min. The cartridge was dried with nitrogen at a flow rate of approximately 100 ml/min for 15 min then eluted with three times 0.5 ml of methanol. The eluate was collected in a 2-ml calibrated sample vial and filled to the mark with methanol. The extract was then homogenized and injected into the gas chromatograph.

Six calibration solutions were prepared in methanol with 10  $\mu$ l of stock solution in 2-, 5-, 10-, 25-, 50- and 100-ml calibrated vials and flasks. Each solution was injected three times and the calibration curves were recorded. The minimum concentrations of the compounds which can be quantified in the extract were determined as the lowest concentrations which can be quantified in the calibration. The regression coefficients of all curves in the established ranges were above 0.996.

The surface water sample used for the spiking experiments was lake water filtered through a glass microfibre filter GF/D-GF/F. The recovery tests described above were repeated using this filtered water.

The determination of matrix interferences was carried out as follows: 100 ml of filtered lake water was passed through a pre-conditioned SPE cartridge. The cartridge was dried with nitrogen at a flow rate of approximately 100 ml/min for

15 min then eluted with 4.5 ml of *n*-hexane. The eluate was collected in a 5.0-ml calibrated flask and filled to the mark with the *n*-hexane. The extract was then homogenized and injected into the gas chromatograph under the conditions specified above.

For calibration 100 mg of turbine oil standard was weighed into a calibrated flask of 100 ml and dissolved in *n*-hexane. 5, 10 and 20  $\mu$ l of this stock solution were added to distilled water in calibrated flasks of 100 ml. These solutions were subjected to the procedure of solid-phase extraction as described above.

### 3. Results and discussion

#### 3.1. Comparison of calculated and experimental recoveries

The experimentally determined recoveries and their 95% confidence intervals together with the predicted recoveries and their 95% confidence intervals for sample volumes of 100, 250 and 500 ml are summarized in Table 1.

As can be seen from the results, in general good agreement can be observed between the predicted and the experimental values. In some cases the differences are not even statistically significant. This is not surprising in the light of the fact that the recovery tests and the determination of capacity factors were carried out under identical conditions with the exception of sample

Table 1  
Experimental and calculated recoveries for sample volumes of 100, 250 and 500 ml in the experiments with model solutions

Compound	Sample volume (ml)					
	100		250		500	
	$r_{\text{exp}}$ (%)	$r_{\text{calc}}$ (%)	$r_{\text{exp}}$ (%)	$r_{\text{calc}}$ (%)	$r_{\text{exp}}$ (%)	$r_{\text{calc}}$ (%)
Phenol	12.1 $\pm$ 2.4	14.9 $\pm$ 1.1	8.1 $\pm$ 1.8	6.0 $\pm$ 0.7	2.8 $\pm$ 0.9	3.1 $\pm$ 0.3
4-Methylphenol	47.9 $\pm$ 3.1	45.7 $\pm$ 4.9	18.1 $\pm$ 2.3	18.3 $\pm$ 1.9	8.7 $\pm$ 1.1	9.2 $\pm$ 0.9
4-Chlorophenol	70.4 $\pm$ 3.6	68.5 $\pm$ 3.2	25.1 $\pm$ 2.4	27.3 $\pm$ 1.5	14.0 $\pm$ 1.0	13.6 $\pm$ 0.7
3,4-Dimethylphenol	90.1 $\pm$ 4.0	93.1 $\pm$ 3.1	41.7 $\pm$ 3.2	45.2 $\pm$ 5.1	24.5 $\pm$ 1.8	22.6 $\pm$ 2.7
2,4-Dichlorophenol	97.6 $\pm$ 3.2	100	90.2 $\pm$ 3.7	94.7 $\pm$ 2.5	62.3 $\pm$ 3.1	59.0 $\pm$ 6.5
2,4,6-Trichlorophenol	98.3 $\pm$ 2.7	100	98.5 $\pm$ 2.5	99.8 $\pm$ 0.2	91.6 $\pm$ 2.0	95.3 $\pm$ 1.6

Table 2

Experimental and calculated recoveries for sample volumes of 100, 250 and 500 ml in the experiments with spiked surface water

Compound	Sample volume (ml)					
	100		250		500	
	$r_{\text{exp}}$ (%)	$r_{\text{calc}}$ (%)	$r_{\text{exp}}$ (%)	$r_{\text{calc}}$ (%)	$r_{\text{exp}}$ (%)	$r_{\text{calc}}$ (%)
Phenol	10.2 ± 3.4	14.9 ± 1.1	5.8 ± 1.9	6.0 ± 0.7	2.3 ± 1.3	3.1 ± 0.3
4-Methylphenol	45.5 ± 3.6	45.7 ± 4.9	15.3 ± 2.7	18.3 ± 1.9	6.5 ± 1.6	9.2 ± 0.9
4-Chlorophenol	63.1 ± 3.8	68.5 ± 3.2	20.2 ± 2.4	27.3 ± 1.5	10.3 ± 2.1	13.6 ± 0.7
3,4-Dimethylphenol	86.2 ± 4.7	93.1 ± 3.1	32.0 ± 3.9	45.2 ± 5.1	17.3 ± 2.0	22.6 ± 2.7
2,4-Dichlorophenol	95.1 ± 5.3	100	91.7 ± 4.4	94.7 ± 2.5	51.5 ± 4.5	59.0 ± 6.5
2,4,6-Trichlorophenol	97.1 ± 3.1	100	98.3 ± 3.2	99.8 ± 0.2	88.6 ± 2.9	95.3 ± 1.6

concentrations which varied in the recovery tests.

In order to study the effect of sample matrix on the accuracy of prediction, the recovery tests described above were repeated with spiked surface water. None of the analytes were detected in the blank sample. The experimentally determined recoveries and their 95% confidence intervals together with the predicted recoveries and their 95% confidence intervals —by analogy with Table 1— for each sample volume are summarized in Table 2.

As it can be seen from the data, the experimental values are in nearly all cases lower than the predicted ones implying that the virtual

capacity of the cartridge (the amount of the accessible stationary phase) was reduced. It may be explained by the presence of interfering compounds dissolved in the water which can be more strongly bound to the apolar octadecyl chain than the more polar phenols, decreasing the volume of stationary phase available for the analytes.

In order to determine the possible interfering compounds the total ion chromatogram (TIC) of the filtered lake water sample was acquired which is shown in Fig. 2.

The major compounds identified were homologues of *n*-alkanes and alkylsiloxanes. As the water of the lake, from which the samples were

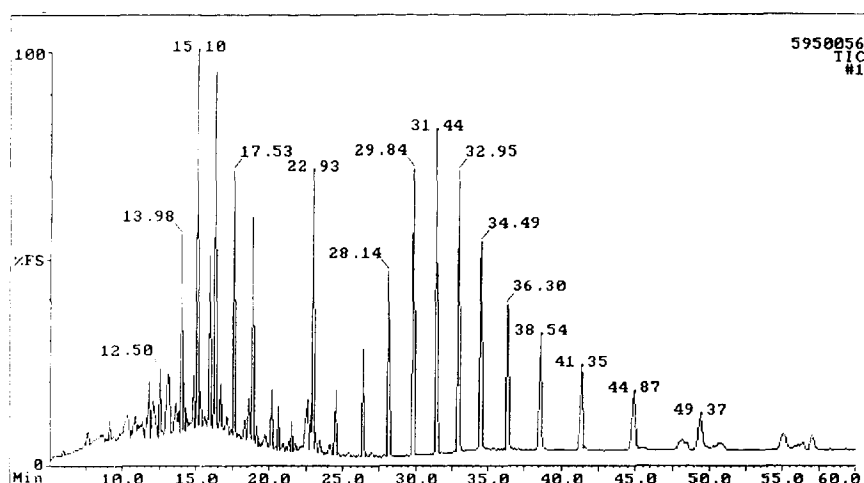


Fig. 2. Total ion chromatogram of the extract of 100 ml lake water.

taken, was suspected to be polluted by oil spillage from a nearby power plant, the experiments were repeated with solutions of the suspected oil standard (see Experimental). The ion chromatograms of 85 amu of the standard and the lake water samples are compared in Fig. 3.

As is obvious from this figure, the major source of pollution was positively identified allowing the estimation of the concentration of the oil in the water without the identification and quantification of the individual compounds. This was accomplished by calibration using the solutions of the oil standard of different concentrations. The calibration and quantification was based on several major peaks in the ion chromatograms. The concentration of the oil in the water sample was found to be  $117 \pm 23 \mu\text{g/l}$  (at 95% level of confidence).

It is interesting to note that the amount of oil loaded onto the cartridge even with the sample volume of 500 ml is far inferior to the total capacity of the cartridge, which is reportedly in the ten milligram range [2]. Nevertheless, significant losses of the analytes can be observed in the recovery tests, especially with the larger sample volumes. This may be accounted for by the presence of other compounds, mainly of biological origin, which were not detected in the sample but may also compete with the analytes.

Table 3

Minimum concentrations of phenolic compounds which can be quantified in the final extract ( $c_{f,\text{min}}$ ) and in the sample by the given SPE method ( $c_{0,\text{min}}$ )

Compound	$c_{f,\text{min}}$ ( $\mu\text{g/l}$ )	$c_{0,\text{min}}$ ( $\mu\text{g/l}$ )
Phenol	270	$38 \pm 3.0$
4-Methylphenol	210	$9.1 \pm 1.0$
4-Chlorophenol	380	$12 \pm 0.6$
3,4-Dimethylphenol	200	$3.5 \pm 0.5$
2,4-Dichlorophenol	390	$2.6 \pm 0.3$
2,4,6-Trichlorophenol	360	$1.2 \pm 0.2$

Whatever is the reason for the differences between the experimental and calculated recoveries, it can be concluded that although the estimation of recoveries can be useful in some cases, the analytical practice should not lack the comprehensive study of matrix effect in method development for solid-phase extraction.

### 3.2. Determination of minimum sample concentration

The minimum concentrations of the compounds which can be quantified from the extract ( $c_{f,\text{min}}$ ) as determined from the calibration are shown in Table 3.

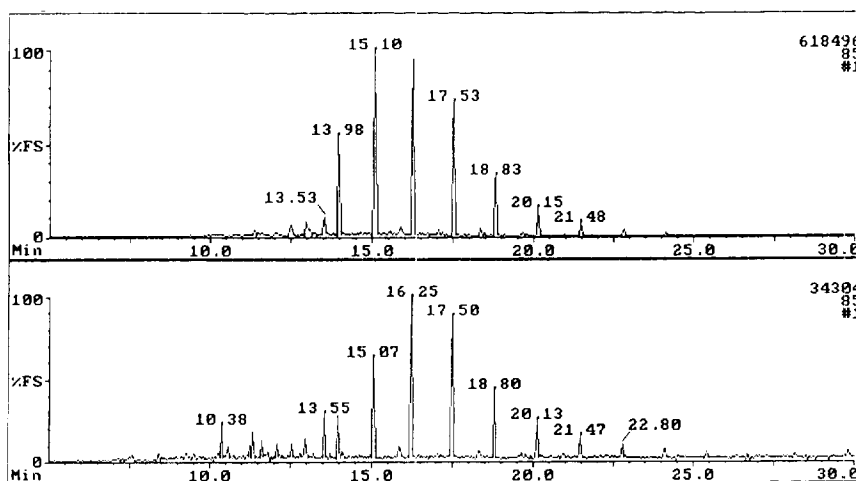


Fig. 3. Ion chromatograms of 85 amu of the extract of 100 ml lake water (top) and the extract of 100 ml oil standard solution of  $100 \mu\text{g/l}$  (bottom).

From these concentrations and the experimental capacity factors determined for the compounds the minimum concentrations which can be quantified in the sample ( $c_{0,\min}$ ) by the SPE method were calculated by Eq. (6). These values are also given in Table 3 together with their 95% confidence intervals.

As can be seen from the table, the minimum concentrations of phenolic compounds in the sample which can be quantified by the given SPE method are in the low  $\mu\text{g/l}$  range. The worsening sensitivities for the compounds of higher molecular mass are compensated by the higher enrichment factors attainable for these solutes.

The sensitivity of the whole SPE procedure can be enhanced either by the evaporation of the solvent (reducing  $V_f$ ) or increasing the sensitivity of detection (increasing  $c_{f,\min}$ ).

In summary we can conclude that the knowledge of the capacity factors allows the estimation of the minimum concentrations of phenolic compounds in the sample which can be quantified by the given SPE method. Although the paper referred only to a limited number of phenolic compounds and a single SPE cartridge, the considerations presented can be applied

—mutatis mutandis— for other types of compounds and cartridges as well. The knowledge of the minimum concentrations in the sample which can be quantified by the given SPE method can save a great deal of research effort and may contribute to the procedure of SPE method development. It should not replace, however, the experimental determination of the limit of determination of the SPE method.

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#### References

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