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Journal of Chromatography A, 693 (1995) 217–225

JOURNAL OF CHROMATOGRAPHY A

# A simple method for the determination of capacity factor on solid-phase extraction cartridges. I

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First received 30 August 1994; revised manuscript received 9 November 1994

**Abstract**

In this paper a simple experimental method for the rapid determination of capacity factors of phenolic compounds on off-line solid-phase extraction cartridges is described. A model solution of the analytes is brought into equilibrium with the SPE cartridge by recycling in a closed loop. The capacity factors are calculated from the adsorbed amounts which can be determined by a chromatographic method. The method is shown to give comparable results with those of the breakthrough experiments. The simplicity of the experimental arrangement as well as the low cost and unattended operation offer a viable alternative to the conventional methods of the determination of capacity factors on SPE cartridges. Practical limitations of the proposed method are also considered.

## 1. Introduction

Sample preparation plays a vital role in the analysis of water samples. As modern analytical instruments are now powerful and highly sensitive, sample preparation has become the "bottleneck" of water analysis in several respects. It is generally the most laborious, time-consuming and least reliable part of the whole analytical procedure. Solid-phase extraction (SPE) has recently come into the focus of interest and offers a viable alternative to the conventional sample preparation methods. Yet many aspects of the reliability and reproducibility of this technique are awaiting to be clarified. Only a thorough understanding of the critical parameters governing sorption–desorption can secure its efficient and reliable operation.

Although in water analysis there are many steps involved in a complete SPE procedure, one step, sorption from water, is of primary importance. Chromatographically this step of the solid-phase extraction procedure is frontal chromatography. If an aqueous solution of an analyte of $c_0$ concentration is pumped through a conditioned SPE cartridge and the effluent is monitored on-line, the curve recorded is known as frontal chromatogram or breakthrough curve as shown in Fig. 1.

From the breakthrough curve the retention volume of the analyte ($V_R$) can be determined as indicated in Fig. 1. The breakthrough volume ($V_B$) is usually defined as

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0021-9673/95/$09.50 
*SSDI* 0021-9673(94)01121-4

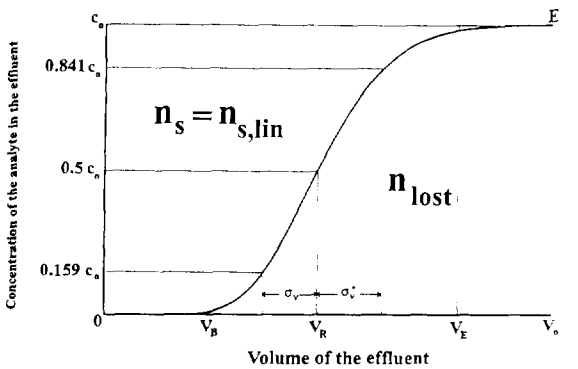


Fig. 1. A frontal chromatogram, the fundamental chromatographic parameters and graphical representation of the SPE preconcentration step.

$$V_B = V_R - 2 \cdot \sigma_V \quad (1)$$

where $\sigma_V$ is the standard deviation of the derivative curve which can be determined graphically from the breakthrough curve as shown in Fig. 1. $\sigma_V$ is in direct relation to the efficiency of the SPE column, i.e. the number of theoretical plates ($N$). Purnell [1] has shown that $N$ can be calculated from the breakthrough curve using the equation

$$N = \frac{V_R \cdot (V_R - \sigma_V)}{\sigma_V^2} \quad (2)$$

Analogously, the equilibrium volume ($V_E$) can be defined as

$$V_E = V_R + 2 \cdot \sigma_V^* \quad (3)$$

where $\sigma_V^*$ can be determined graphically from the breakthrough curve as shown in Fig. 1. Its value is usually different from that of $\sigma_V$ because of the asymmetry of the breakthrough curves on SPE columns. The retention volume ($V_R$) can be expressed analytically by the fundamental equation of chromatography

$$V_R = V_M \cdot (1 + k) \quad (4)$$

where $V_M$ is the hold-up volume of the SPE cartridge and $k$ is the capacity factor of the solute. In solid-phase extraction there is a fundamental parameter, recovery ($r$) which can be directly related to the frontal chromatogram. By definition, the recovery of an analyte is the amount of the analyte recovered after the SPE procedure, expressed in percentage of the total amount of the analyte originally present in the sample. The knowledge of this parameter allows the concentration of the analyte in the sample to be calculated from the concentration determined in the sample extract. In the case shown in Fig. 1 the total amount of the analyte originally present in the sample is the product of the analyte concentration and sample volume, $c_0 \cdot V_0$, graphically represented by the rectangle $0$–$V_0$–$E$–$c_0$ in Fig. 1. The amount lost ($n_{lost}$) due to breakthrough is graphically represented by the area under the breakthrough curve in Fig. 1. Consequently, the amount taken up by the SPE column ($n_s$) equals to the difference between the total amount and the amount lost, graphically corresponding to the area above the breakthrough curve within the rectangle of the total amount. In this special case it equals to the linear capacity of the column ($n_{s,lin}$) which can be expressed by the equation [2]

$$n_{s,lin} = V_M \cdot k \cdot c_0 \quad (5)$$

As can be seen from Eq. (5) $n_{s,lin}$ is dependent on both the capacity factor and the concentration of the analyte. The linear capacity, however, cannot be increased ad infinitum by increasing the concentration of the analyte because the concept of linear capacity applies only under the conditions of linear ideal chromatography [1], as the total capacity of a solid-phase extraction cartridge is limited by the amount of the stationary phase in the column. As against linear capacity, the total capacity is an ill-defined property of the column which is only referred to in broad qualitative terms. Deviations from linear adsorption isotherms can be observed if the linear capacity approaches the total capacity of the column. Nevertheless, for most environmental samples it is rather unlikely that in practical situations the total capacity of the sorbent is approached.

Provided that the extraction step governs primarily the recovery of an analyte with losses in other steps of the SPE procedure being negligible, the recovery ($r$) of the analyte can be expressed as

$$r = \frac{n_s}{c_0 \cdot V_0} \cdot 100\% \quad (6)$$

Based on the frontal chromatogram, two alternative strategies of solid-phase extraction can be distinguished. The first —probably the more popular— method is to achieve quantitative recoveries (within experimental error) by optimizing the volume and composition of the sample solution. In this case the amount of the analyte determined from the sample extract equals to the amount originally present in the sample. Thus the relatively large uncertainty accompanying the determination of recoveries will not contribute to the overall uncertainty of the whole SPE procedure. This mode of operation, however, is restricted only to volumes smaller than the breakthrough volume ($V_B$) of the least retained compound in the sample. As a result, the linear capacity of other, more strongly retained analytes remain far from being exploited. This mode can be the matter of choice if samples with high level of matrix interference have to be analyzed.

The other strategy of SPE operation, especially for less retained compounds, is to equilibrate the entire column with the sample solution by applying volumes safely in excess of their equilibrium volumes ($V_E$), as shown in Fig. 1. In this mode the recovery of the analyte will not be quantitative but the linear capacity of the column for the analyte will be fully exploited. Having determined the recoveries in separate recovery tests using spiked model solutions of the same volume, the concentrations of the analytes can be calculated.

In this "equilibrium" mode of solid-phase extraction the linear capacity of the column is exploited and the maximum degree of preconcentration is attained. Whichever mode is preferred, the knowledge of equilibrium ($V_E$) or breakthrough ($V_B$) volumes of each analyte is invaluable in method development for solid-phase extraction.

The most straightforward method for obtaining these parameters is to record the breakthrough curves experimentally [3]. This method is, however, quite laborious, time-consuming and costly. Because of the low efficiency of the SPE cartridges, the breakthrough curves of analytes have to be recorded separately, requiring solutions of pure analytes and a number of SPE cartridges. With this method mutual interferences are difficult to characterize. Further difficulties may arise from the fact that the SPE cartridges are not designed to be operated on-line with a pump and a detector. Moreover, if the composition of the sample solution is changed, which is not unusual in the course of SPE method development, a new series of experiments will have to be carried out.

Prediction of retention or breakthrough values is much less laborious than the conventional experimental method. According to Eq. (4), these predictions are usually simplified to the estimation of capacity factors ($k$) of the analytes. To predict $k$ values under the conditions of the preconcentration step, various relationships have been studied, based on the correlation between capacity factors and aqueous molar solubilities or octanol–water coefficients [4–6]. The major drawback of these methods is that these physico-chemical data are only available for pure water. In SPE practice, however, organic modifiers or inorganic salts are often added to the sample solution in order to increase the retention of the analytes. These compositions, unfortunately, cannot be characterized by predictions based on physico-chemical data.

Another approach to the prediction of capacity factors is to correlate chromatographic retention data on an analytical column with the same stationary phase at different isocratic organic–water mobile phase compositions [7,8]. As the relationship between the logarithm of the capacity factor and the volume fraction of the organic modifier ($\Phi$) was found to be linear for phenolic compounds, the capacity factors of these solutes in pure water were determined by measuring their capacity factors at two mobile phase compositions and extrapolating to $\Phi = 0$. The extrapolated values were within 15% of the values determined from experimentally recorded breakthrough curves [8]. For other compounds the method proved to be less successful. The method was, however, applied to on-line trace

enrichment on short precolumns filled with particles of the same diameter as the analytical column. As SPE cartridges cannot be used in analytical elution mode due to their very low separation efficiency, extrapolation from retention data obtained on an analytical HPLC column with the same stationary phase can hardly provide an accurate estimate of the capacity factors of the analytes on SPE cartridges.

In this paper a simple experimental method for the rapid determination of capacity factors of phenolic compounds on off-line solid-phase extraction cartridges is described. The method is capable of providing experimental capacity factors at any sample composition. Theoretical approach is made to reveal the limitations of the method in terms of accuracy and precision. The results obtained are compared with those of independent breakthrough measurements.

## 2. Theory

The method is based on the equilibration of the solutes between the SPE cartridge and the model solution. The scheme of the equilibration process is depicted in Fig. 2. Exactly known volume of aqueous sample solution of known concentration of analytes is pumped through an SPE cartridge and the solution leaving the cartridge is recycled to the stirred solution. The process is finished when steady-state conditions are attained, i.e. the analytes are equilibrated between the stationary phase of the cartridge and the solution. Then the adsorbed amount of each analyte is determined by a chromatographic method. Alternatively, the concentrations of analytes can be determined directly in the solution by HPLC provided that the concentrations are sufficiently high to facilitate direct determination.

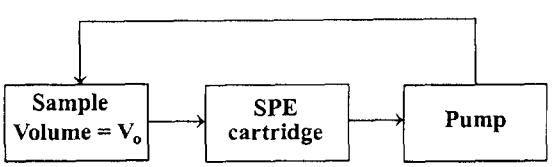


Fig. 2. Scheme of the equilibration process.

In equilibrium, the capacity factor of an analyte on an SPE cartridge can be calculated as follows:

$V_0$ volume of aqueous solution is spiked with $n_0$ moles of an analyte and equilibrated with the stationary phase. If $n_s$ moles of the analyte is adsorbed in the stationary phase of the cartridge and $n_m$ moles is present in the mobile phase of the column hold-up volume $V_M$, the capacity factor of the analyte can be written as

$$k = \frac{n_s}{n_m} \tag{7}$$

If the concentration of the analyte in the mobile phase is denoted by $c_m$, then the overall material balance can be formulated as

$$n_0 = n_s + c_m \cdot (V_0 + V_M) \tag{8}$$

which, after rearrangement, yields

$$c_m = \frac{n_0 - n_s}{V_0 + V_M} \tag{9}$$

$n_m$ can be expressed as the product of the mobile phase concentration and the column hold-up volume

$$n_m = c_m \cdot V_M \tag{10}$$

By substituting Eq. (9) into Eq. (10) we obtain

$$n_m = V_M \cdot \frac{n_0 - n_s}{V_0 + V_M} \tag{11}$$

and finally, substituting Eq. (11) into the expression for the capacity factor (7) we have

$$k = \frac{n_s \cdot (V_0 + V_M)}{V_M \cdot (n_0 - n_s)} \tag{12}$$

This expression for the capacity factor contains only known or measurable quantities: $V_0$, $n_0$, $V_M$ are initially known and $n_s$ is determined by a chromatographic method after elution of the cartridge.

Alternatively, if $c_m$ can be determined directly from the sample solution, the following equation can be derived

$$k = \frac{n_0 - c_m \cdot (V_0 + V_M)}{c_m \cdot V_M} \quad (13)$$

The method is purely an equilibration process and the ultimate goal of this technique is not to achieve separation in the conventional sense but to establish equilibrium of the solutes between the stationary phase of the cartridge and the bulk of the sample solution.

In order to ensure that conditions of linear ideal chromatography hold, the concentrations of the analytes have to be carefully chosen. The individual concentrations have to be sufficiently high to facilitate reliable analytical quantification but the total amount of analytes must remain far inferior to the total capacity of the cartridge.

The time —or preferably the volume pumped through the cartridge— required for attaining steady-state conditions in the system is of primary importance. Its knowledge is necessary to decide whether the calculations are valid, i.e. equilibrium conditions hold. The course of the equilibration process is modelled by Purnell's discontinuous model based on the theoretical plate concept [1]. The input parameters of the model are the initial volume of the sample solution ($V_0$), the void volume of the cartridge ($V_M$), the number of theoretical plates of the cartridge ($N$, determined from experimental breakthrough curves) and the value of the capacity factor ($k$). The model calculates the concentration of the analyte in the stirred flask ($c_1$) as a function of the volume pumped through the cartridge. The concentration is expressed in the percentage of the theoretical concentration of analyte ($c_{1,E}$) in the stirred flask as calculated from the input capacity factor assuming equilibrium conditions. The course of the concentration as a function of the volume of the sample solution pumped through the cartridge is depicted in Fig. 3. As it can be seen from the figure, infinite volume would be required for attaining the theoretical (equilibrium) concentration, but a practical equilibrium volume ($V_{PE}$) may be obtained when $c_1(V)$ approaches its theoretical value within a relative error of 2% (see Fig. 3). If $V_{PE}$ is plotted as a function of the capacity factor, the curve shown in Fig. 4 is

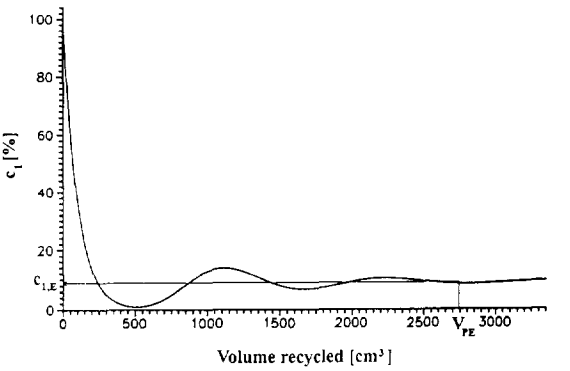


Fig. 3. Modelled course of the concentration of the bulk solution as a function of the volume pumped through the cartridge at a capacity factor of 1250. Sample volume is 100 ml.

obtained. It is obvious from this figure that the application of this technique to compounds with high capacity factors on the cartridge can be impractical due to the excessive volume required to reach equilibrium. The large volume pumped through the cartridge corresponds to prolonged time of the experiment which may result in undesirable loss of analytes due to degradation or other deleterious processes.

The determination of higher capacity factors has another serious limitation. By rearranging Eq. (12) we have

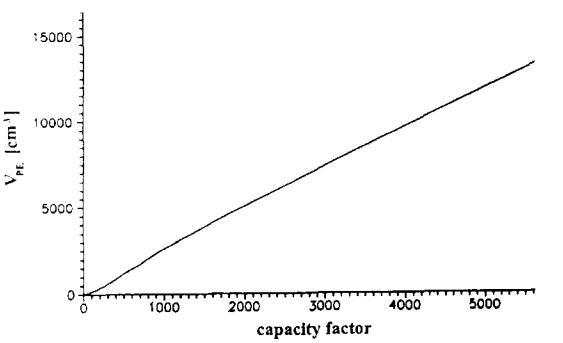


Fig. 4. The calculated volume required to reach equilibrium within a relative error of 2% as a function of capacity factor at a sample volume of 100 ml.

$$n_s \cdot (V_0 + V_M + k \cdot V_M) = k \cdot n_0 \cdot V_M \quad (14)$$

and the ratio of the adsorbed amount to the total amount can be expressed as

$$\frac{n_s}{n_0} = \frac{k \cdot V_M}{V_0 + V_M + k \cdot V_M} \quad (15)$$

Fig. 5 shows this ratio as a function of capacity factor at two different sample volumes. According to Eq. (15) there is only one experimental parameter which can influence the shape of the curve, the sample volume ($V_0$). In the region of small $k$ values the smaller sample volume is advantageous as it gives better sensitivity, i.e. higher slope value. In this case, however, the working range is quite limited by the worsening sensitivity at larger $k$ values. With the larger sample volume the sensitivity is initially lower, but the working range is somewhat broader. The selection of the experimental sample volume requires compromise between sensitivity and working range. As the relative error of the determination of the adsorbed amount is rarely less than 2%, for capacity factors in excess of 1500 the method may at best give rough estimates due to the excessively large standard deviations accompanying the determination of $k$. If, however, $c_m$ can be determined directly from the sample solution with the same precision, this limitation is normally not imposed.

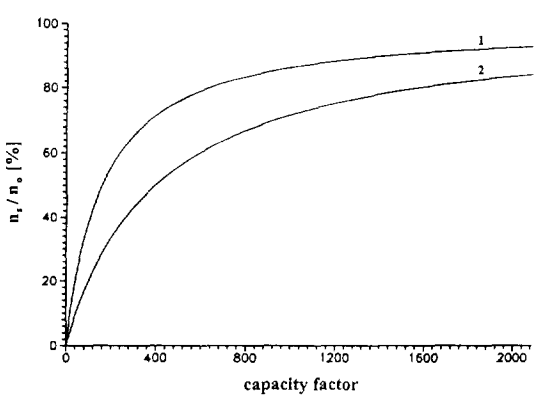


Fig. 5. The ratio of adsorbed amount to the total amount as a function of capacity factor, as calculated from Eq. (15). Lines: (1) $V_0$ = 100 ml; (2) $V_0$ = 250 ml.

## 3. Experimental

### 3.1. Conditions

*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), hydrochloric acid from Reanal (Hungary). All reagents were used as received without further purification.

The cartridge used was 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 of methanol then conditioned with 3 ml of $10^{-3}$ $M$ hydrochloric acid.

*Instrumentation*

Breakthrough curves were acquired with a Jasco PU-980 HPLC pump and a Perkin-Elmer LC-55 UV detector. All measurements were performed at 220 nm. Data acquisition was made by Waters Maxima 820 chromatographic software.

GC–MS analysis was carried out with a TRIO-1000 instrument of FISONS (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.

*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. This solution was used for all experiments except the recording of the breakthrough curves where the stock solutions of the individual compounds in methanol were used at concentrations of approximately 1 mg/ml.

For the acquisition of each breakthrough curve a solution of the individual compound was prepared in $10^{-3}$ *M* hydrochloric acid at a concentration of approximately 1 $\mu$g/ml. The cartridge was fixed in a brass threaded casing. A flow rate of 1.5 ml/min was set and the tubing was filled with the sample solution up to the cartridge inlet. Then the pump was started and measurements begun.

For the equilibration experiments $10^{-3}$ *M* of hydrochloric acid was spiked with 10 $\mu$l of stock solution and thoroughly homogenized in a calibrated flask of 100.0 ml. This sample solution was filled in a 120 ml Teflon flask on top of a magnetic stirrer. The cartridge was connected to the tubing of the proportioning pump then immersed into the sample solution. The outlet end of the proportioning pump was connected with Teflon tubing to the flask containing the sample solution thus forming a closed loop. The flow rate was measured before the start and after the end of the experiment. The system was left running for 16 hours and the volume passing the cartridge was determined as a product of the average flow rate and the duration of the experiment.

The cartridge was dried with nitrogen at a flow rate of approximately 100 ml/min for 15 min then eluted with 3 times 0.5 ml 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 GC–MS.

Four calibration solutions were prepared in methanol with 10 $\mu$l of stock solution in 2-, 5-, 10- and 25-ml calibrated vials and flasks. Each solution was injected three times and the calibration curve was recorded. The regression coefficients of all curves were above 0.998.

The hold-up volume of the cartridges was determined by measuring the weight difference of the dry and conditioned cartridges. Five replicate measurements were made and the cartridge void volume was determined as the average of the five measurements, and was found to be $0.653 \pm 0.023$ ml.

## 4. Results and discussion

Breakthrough curves were obtained for all compounds and retention volumes ($V_R$) as well as $\sigma_V$ values were determined graphically from the curves. Breakthrough volumes ($V_B$), number of theoretical plates ($N$) and capacity factors ($k$) were calculated according to Eqs. (1), (2) and (4), respectively. These parameters are listed in Table 1.

For the model calculation of the practical equilibrium volume ($V_{PE}$), the average number of theoretical plates (15) was considered. Its value for the most strongly retained compound, 2,4,6-trichlorophenol, was determined from Fig. 4 and was found to be 2500 ml. To make assurance double sure, a 16-hour equilibrium period was chosen for the experiments with an average flow rate of 3.3 ml/min, corresponding to a volume of 3200 ml. Three parallel equilibration experiments were carried out with a sample volume of 100.0 ml. The cartridges were then eluted and extracts were analyzed by GC–MS. Each extract was injected in triplicate. The average of the adsorbed amounts, in percentage of the spiked amounts and their relative standard deviations, the calculated capacity factors and their 95% confidence intervals together with the capacity factors determined in the breakthrough experiments are summarized in Table 2.

The results of the equilibration experiments are fairly close to the values determined from breakthrough curves implying that the prerequisites for calculations were met. A somewhat larger difference can be observed for 2,4,6-tri-

Table 1
The basic chromatographic parameters of the compounds obtained in breakthrough experiments

| Compound | $V_R$ (ml) | $\sigma_V$ (ml) | $V_B$ (ml) | $N$ | $k$ |
|---|---|---|---|---|---|
| Phenol | 15.2 | 3.8 | 7.6 | 12 | 22.3 |
| 4-Methylphenol | 48.9 | 10.8 | 27.3 | 16 | 73.9 |
| 4-Chlorophenol | 62.9 | 13.5 | 35.9 | 17 | 95.3 |
| 3,4-Dimethylphenol | 132 | 29.0 | 74 | 16 | 201 |
| 2,4-Dichlorophenol | 259 | 53.4 | 152 | 18 | 395 |
| 2,4,6-Trichlorophenol | 720 | 174 | 372 | 13 | 1100 |

chlorophenol, a compound with relatively large capacity factor. The equilibration experiment seems to underestimate its value by 20%. This finding, however, is in accordance with the theoretical considerations, i.e. at such a large capacity factor any small inaccuracy of the determination of the adsorbed amount will introduce considerable bias into the calculated value of the capacity factor. In spite of the fact that the R.S.D.% values of the adsorbed amounts of analytes are considerably smaller for the more strongly retained solutes, the same parameters of their calculated capacity factors are larger as a result of the lower sensitivity in the region of large capacity factors.

For practical purposes, the precision attainable by the proposed method even for the 2,4,6-trichlorophenol is satisfactory. On the other hand, the values obtained from breakthrough experiments may also be subject to small variations, but their statistical evaluation is quite tedious. Nevertheless the good agreement between the results of the two independent experiments suggests that the equilibration method can provide reliable estimates of capacity factors of phenolic compounds on solid-phase extraction cartridges. There is an implication that the method —mutatis mutandis— can be used for other types of compounds as well. The simplicity of the experimental arrangement as well as the low cost and unattended operation may offer a viable alternative to the conventional methods of the determination of capacity factors on off-line SPE cartridges.

## Acknowledgements

The authors are grateful to Lajos Gáspár and Péter Ágh for their indispensable technical assistance. The financial support from OTKA A 167 for purchasing the GC–MS and from OTKA 2561 for financing the project is also gratefully acknowledged.

Table 2
The average adsorbed amounts of analytes in percentage of the total amounts and their relative standard deviations obtained in equilibration measurements, the capacity factors with their 95% confidence intervals calculated from the adsorbed amounts and the capacity factors determined in breakthrough experiments.

| Compound | $n$, (R.S.D.%) | $k_{equilibration}$ | $k_{breakthrough}$ |
|---|---|---|---|
| Phenol | 12.4 (5.6) | 21.8 ± 1.7 | 22.3 |
| 4-Methylphenol | 30.9 (6.1) | 69.0 ± 7.5 | 73.9 |
| 4-Chlorophenol | 38.0 (2.6) | 94.5 ± 4.9 | 95.3 |
| 3,4-Dimethylphenol | 52.7 (4.4) | 172 ± 21 | 201 |
| 2,4-Dichlorophenol | 74.3 (2.2) | 450 ± 50 | 395 |
| 2,4,6-Trichlorophenol | 85.2 (1.5) | 894 ± 124 | 1100 |

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