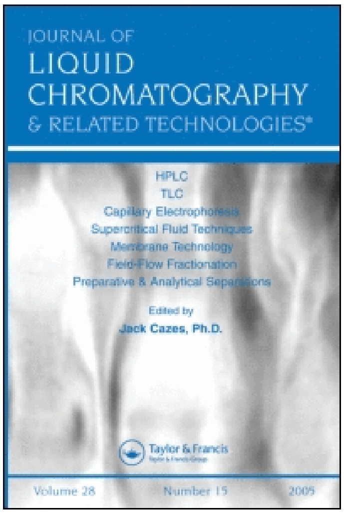

**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Retention Behavior of Some Ring-Substituted Aniline Derivatives on Polyethylene-Coated and Octadecylsilica Columns

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**To cite this Article** Forgács, E.(1993) 'Retention Behavior of Some Ring-Substituted Aniline Derivatives on Polyethylene-Coated and Octadecylsilica Columns', Journal of Liquid Chromatography & Related Technologies, 16: 17, 3757 — 3772
**To link to this Article: DOI:** 10.1080/10826079308019665
**URL:** http://dx.doi.org/10.1080/10826079308019665

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(17), 3757-3772 (1993)

# RETENTION BEHAVIOR OF SOME RING-SUBSTITUTED ANILINE DERIVATIVES ON POLYETHYLENE-COATED AND OCTADECYLSILICA COLUMNS*

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## ABSTRACT

The retention characteristics of 16 aniline derivatives were determined on polyethylene-coated and on octadecylsilica columns in methanol- 25 mM $K_2HPO_4$ mixtures at various organic phase concentrations. Good linear correlations were found on both column between logk$'$ values and the organic mobile phase concentration in the eluent. Stepwise regression analysis proved that not only lipophilicity but electronic parameters of aniline derivatives have significant influence on the retention of aniline derivatives on polyethylene-coated silica column. Principal component analysis indicated that the retention behaviour of PEE column slightly differs from that of ODS column. This discrepancy is probably due to the hydrophobic interactions between the polar substructures of aniline derivatives and the free silanol groups not-covered by polyethylene.

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*This article originally appeared in Volume 16, Number 12, 1993, pages 2483-2500. Due to a printing error which resulted in figures being transposed between two articles, it is being reprinted here in its entirety.

## INTRODUCTION

Reversed-phase chromatography is the most widely used technique in high-performance liquid chromatography. More recently polymer-coated silica have become popular since they combine the mechanical properties of silica with dynamic chemical properties of polymers. For example, poly(alkyl aspartamide) [1], alkyl polyxiloxanes [2], polyvinylpyrrolidone [3], poly(2-sulfoethyl aspartamide) [4], polyethyleneimine [5], polyamine [6], poly-(butadiene-maleic acid) [7], polyvinilimidazole [8] and polypyrrole chloride [9] have been coated on silica. Polymer coating improves not only the selectivity but also the chemical stability of stationary phases. Polymer-coated columns have been used for the separation of various alkaline compounds [10], peptides [11] and proteins [12].

Multivariate mathematical-statistical methods such as stepwise regression analysis [13], principal component analysis (PCA) [14] have been frequently used to extract maximum information from retention data matrices of considerable dimensions. The advantages of PCA in chromatography is that it allows a reduction in the number of variables whilst maintaining the majority of information content. PCA is suitable not only for the calculation of two-two variable relationships, but also for the simultaneous study of all variables relationship.

The objectives of our investigation were to compare the retention behaviour of some ring-substituted aniline derivatives on polyethylene-coated (PEE) and octadecyl-silica (ODS) columns, to evaluate retention data by multivariate mathematical-statistical methods and to find the relationship between the retention characteristics and physico-chemical parameters of aniline derivatives.

## MATERIALS AND METHODS

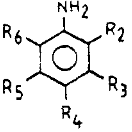
The HPLC system consisted of a Liquopump Model 312 (LaborMIM, Budapest, Hungary) pump, a Cecil CE-212 variable wavelength UV detector (Cecil Instr., Cambridge, England), a Valco injector (Valco Inc.,Houston, Texas, USA) with a 20 $\mu$l sample loop and a Waters 740 integrator (Waters-Millipore Inc., Milford, Massaschusetts, USA). I. column was a polyethylene-coated silica column (further PEE column) prepared in our laboratory (250x4mm I.D.). II. column was a Hypersil ODS column (150x4 mm I.D. particle diameter 5$\mu$m). The flow rate was 0.8 ml/min and the detection wavelength was set to 254 nm. Mixtures of 0.025 M $K_2HPO_4$:methanol were used as eluents. Methanol concentrations ranged from 30-70% (I. column) or 60-80% (II. column) (in steps 5% v/v), respectively. To study effect of buffering the retention times of aniline derivatives were also measured in methanol:water (30:70 v/v) eluent system on PEE column.

The chemical structure of the ring-substituted aniline derivatives are shown in Table I. The retention of compounds 1, 3, 5, 6, 7, 9, 10, 11, 12, 14 and 16 were determined only on polyethylene-coated silica column. The aniline derivatives were dissolved in methanol at the concentration of 0.05 mg/ml. The retention time of each compound in each eluent was determined with three consecutive determinations. As the correlations between the logk' value and the organic phase concentration is generally linear in HPLC we also applied linear equations to describe the dependence of logk' value on the organic mobile phase concentration.

$$\log k' = \log k'_0 + b.C \qquad (1)$$

where: logk' = logarithm of capacity factor; $\log k'_0$ =

TABLE 1
Chemical structure of ring-substituted aniline derivatives



General structure

| No of compounds | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ |
|---|---|---|---|---|---|
| 1. | I | H | H | H | H |
| 2. | Cl | H | H | H | H |
| 3. | $CH_3$ | H | H | H | H |
| 4. | $CH_2CH_3$ | H | H | H | H |
| 5. | H | H | Br | H | H |
| 6. | H | Br | H | H | H |
| 7. | H | H | $CH_3$ | H | H |
| 8. | H | H | I | H | H |
| 9. | H | H | Cl | H | H |
| 10. | H | H | $NO_2$ | H | H |
| 11. | Br | H | Br | H | H |
| 12. | $NO_2$ | H | $NO_2$ | H | H |
| 13. | Cl | H | Cl | H | H |
| 14. | H | Cl | H | Cl | H |
| 15. | H | $CH_3$ | H | $CH_3$ | H |
| 16. | $CH_3$ | H | $CH_3$ | H | H |

logarithm of capacity factor extrapolated to zero concentration of organic component in mobile phase (intercept, related to molecular lipophilicity or retention capacity of solutes) [15]; b = change of logk' value caused by unit change (1 vol %) of organic mobile phase concentration (slope, related to the specific hydrophobic surface area in contact with support) [16], and C = methanol concentration in the eluent (vol %). Eqn.1. was separately applied for each solute and for both columns.

The retention data were evaluated by various multivariate mathematical-statistical methods:

### A. Linear regression analysis

Linear regression analysis was applied to compare the retention behavior of PEE and ODS columns. Dependent variables were slope and intercept values of aniline derivatives determined on ODS column, independent variables were their slope and intercept values determined on PEE column according to eqn 1. To test the structural homogeneity of aniline derivatives linear correlations was calculated between parameters of eqn.1. (slope and intercept values) on PEE column [15].

To study effect of buffering on the retention time on PEE column linear correlation was calculated between the capacity factors determined in buffered or unbuffered eluent systems containing 30% methanol.

## B. Stepwise regression analysis

Stepwise regression was applied to find the molecular characteristics of aniline derivatives influencing significantly their retention on PEE column. The parameters of Eq.1 (dependent variables) were correlated with physicochemical characteristics of aniline derivatives (independent variables). The physicochemical parameters included in the calculation were:

$\pi$ = Hansch - Fujita's substituent constant characterizing hydrophobicity

H - Ac and H - Do = indicator variables for proton acceptor and proton donor properties, respectively

M - RE = molar refractivity

F and R = Swain - Lupton's electronic parameters characterizing the inductive and resonance effect, respectively

$\sigma$ = Hammett's constant, characterizing the electron-withdrawing power of the substituent

Es = Taft's constant, characterizing steric effects of the substituent

B1 and B4 = Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachement.

The acceptance level for the individual independent variables was set to 95% significance level.

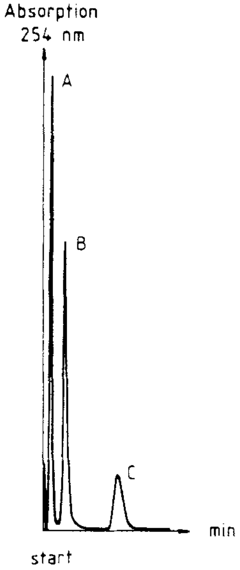


Fig. 1. Separation of aniline derivatives on PEE column. Eluent methanol-25 mM $K_2HPO_4$ (3:7 v/v), flow rate: 0.8 ml/min, detection 254 nm, A = 2-methylaniline, B = 2,4-dimethylaniline, C = 2,4-dibromoaniline.

### C. Principal component analysis

Principal component analysis (PCA) was used to find the similarities and dissimilarities between the retention characteristics and physicochemical parameters of aniline derivatives. The explained variance was set to 99.9%. The parameters of eqn. 1. both for PEE and ODS columns as well as the various physicochemical parameters of aniline derivatives listed above were considered as variables and the aniline derivatives were the observations. The two-dimensional non-linear maps [16] of PCA variables and loadings were also calculated.

## RESULTS AND DISCUSSION

Each aniline derivative showed symmetrical peaks in each eluent system on PEE column (Fig.1.). The retention order of solutes follows the order of their lipophylicity. The less hydrophobic 2-methylaniline derivative elutes earlier than the more hydrophobic 2,4-dibromo derivative. The parameters of eqn.1. are compiled in Tables II. and III. In each instances the relationships between logk′ and the organic phase concentration were linear. In most cases the correlation coefficients were greater than 0.9900 confirming the applicability of eqn.1.

This results further indicates that the retention behaviour of aniline derivatives follows the general rule also on PEE column.

### A. Linear regression analysis

Comparising the slope and intercept values determined on PEE and ODS columns, significant linear correlation was found only between the corresponding intercept values.

$$\log k'0(\mathrm{ODS}) = 0.19 + 0.27(\pm 0.04).\log k'0(\mathrm{PEE}) \qquad (2)$$

$$n = 11 \qquad r = 0.9036 \qquad r_{99\%} = 0.8721$$

The significance level of eqn.2 is higher than 99%. The good correlation indicates that capacities extrapolated to water are strongly related. However, the retention capacity of ODS column is considerably greater than that of PEE column. The fact the regression coefficient is significantly lower than 1, indicates that the selectivity of PEE column exrapolated to water is greater than that of ODS column that is the PEE column is probably more suitable for the separation of strongly hydrophilic compounds using eluents with low concentration of organic modifier. In the case of PEE column no significant linear

TABLE 2.

Relationship between logk′of aniline derivatives and methanol concentration (C) in the eluent on PEE column.

$$\log k' = \log k'_0 + b.C$$

| Number of compound | $\log k'_0$ | $-b.10^{-2}$ | $S_b.10^{-3}$ | r |
|---|---|---|---|---|
| 1. | 1.74 | 4.21 | 2.9 | 0.9952 |
| 3. | 1.37 | 4.52 | 1.3 | 0.9986 |
| 5. | 1.71 | 4.42 | 1.9 | 0.9980 |
| 6. | 1.50 | 4.13 | 4.2 | 0.9843 |
| 7. | 1.49 | 5.23 | 3.8 | 0.9920 |
| 9. | 1.79 | 4.92 | 3.3 | 0.9954 |
| 10. | 0.91 | 3.37 | 1.9 | 0.9931 |
| 11. | 2.11 | 3.82 | 1.5 | 0.9967 |
| 12. | 1.51 | 3.28 | 1.1 | 0.9987 |
| 14. | 2.19 | 4.31 | 3.5 | 0.9999 |
| 16. | 1.41 | 4.14 | 3.8 | 0.9877 |

TABLE 3.

Relationship between logk′ of aniline derivatives and methanol concentration (C) in the eluent on ODS column.

$$\log k' = \log k'_0 + b.C$$

| Number of compound | $\log k'_0$ | $-b.10^{-1}$ | $S_b.10^{-3}$ | r |
|---|---|---|---|---|
| 1. | 0.71 | 3.00 | 2.0 | 0.9985 |
| 2. | 0.63 | 3.36 | 1.9 | 0.9899 |
| 3. | 0.53 | 4.08 | 3.2 | 0.9902 |
| 4. | 0.74 | 1.81 | 2.9 | 0.9993 |
| 5. | 0.66 | 2.76 | 8.2 | 0.9927 |
| 6. | 0.66 | 2.57 | 1.9 | 0.9943 |
| 7. | 0.55 | 4.62 | 3.5 | 0.9999 |
| 8. | 0.78 | 5.08 | 1.1 | 0.9927 |
| 9. | 0.60 | 3.77 | 1.5 | 0.9952 |
| 10. | 0.41 | 4.64 | 3.2 | 0.9927 |
| 11. | 0.79 | 2.70 | 3.7 | 0.9950 |
| 12. | 0.62 | 3.77 | 5.6 | 0.9900 |
| 13. | 0.83 | 3.93 | 1.4 | 0.9974 |
| 14. | 0.75 | 2.78 | 1.3 | 0.9932 |
| 15. | 0.74 | 3.25 | 2.0 | 0.9897 |
| 16. | 0.64 | 4.34 | 2.5 | 0.9982 |

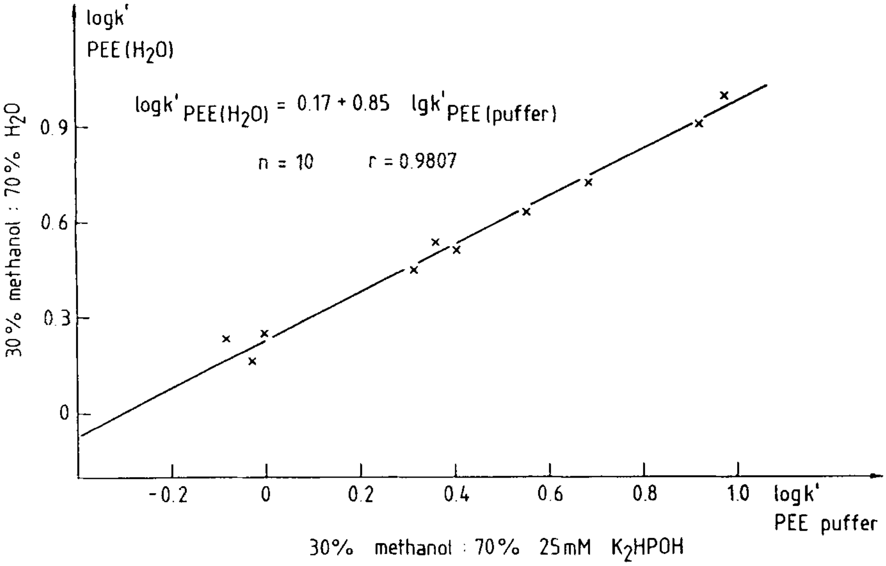


Fig.2. Correlation between capacity factors of aniline derivatives determined in methanol:water (3:7) and in methanol:25 mM $K_2HPO_4$ (3:7) eluent systems.

correlation was found between the slope and intercept values of eqn.1. that is from chromatographic point of view the solutes can not be considered as a homologous series of solutes on PEE column. The relationship between the retention capacities determined in buffered and unbuffered eluent systems is shown in Fig 2. In unbuffered eluent the capacity factors of aniline derivatives are lower than in buffered eluent. This phenomena can be explained by salting out effect of $K_2HPO_4$.

## B. Stepwise regression analysis

Stepwise regression analysis found significant linear correlation between the retention parameters (the slope and intercept values of eqn. 1.) and physicochemical parameters of aniline derivatives.

$$\log k'_0(\mathrm{PEE}) = 1.13 + 0.43.\pi + 0.39.F \qquad (3)$$

$n = 11 \qquad r_2 = 0.7658 \qquad F = 13.08 \qquad F_{99\%} = 9.37$

$$b(\mathrm{PEE}) = -\ 4.70.10^{-2} + 1.00.10^{-2}.F \qquad (4)$$

$n = 11 \qquad r = 0.7046 \qquad r_{95\%} = 0.6319$

Eqns.3. and 4. fit well to the retention parameters the significance level being over 95%. The lipophylicity ($\pi$) (path coefficient 65.20%) and resonance effect (F) (path coefficient 35.80%) of aniline derivatives account for 76.58% of change of the logarithm of capacity factor (see eqn 3). The results indicate that the retention behaviour of PEE column slightly differs from that of ODS (not only lipophylicity ($\pi$) but electronic parameter (F) of aniline derivatives have significant effect on the retention of PEE column). The significant influence of electronic parameter (F) on both retention characterisics (see eqns 3. and 4.) suggest that polar interactions between the hydrophilic substructures of solutes and the silanol groups of silica support not covered by the polyethylene play a considerable role in the retention.

### C. Principal component analysis

The results of principal component analysis are summarized in Table 4. Three principal components (background variables) contain the majority of the information content (87.12%) of the 13 chromatographic and physicochemical parameters (slope and intercept values determined on PEE and ODS columns and 9 physicochemical parameters of aniline derivatives). Unfortunately, PCA does not define the background variables as concrete physicochemical units only indicates their mathematical possibility.

The first and second principal component explain a nearly identical ratios of variance (41.52%, 34.19%, respectively). The chromatographic parameters (determined on both columns) of aniline derivatives have great loadings

TABLE 4.

Effect of various physicochemical parameters of aniline derivatives on their retention behaviour on ODS and PEE columns. Results of principal component analysis.

| Eigenvalue | Variance explained% | Total variance explained% |
|---|---|---|
| 5.39 | 41.52 | 41.52 |
| 4.44 | 34.19 | 75.71 |
| 1.48 | 11.41 | 87.12 |
| 0.71 | 5.52 | 92.64 |

| | Principal component loadings<br>1. | 2. | 3. |
|---|---|---|---|
| $\mathrm{log}k'_{0ODS}$ | 0.82 | -0.53 | 0.08 |
| $b_{ODS}$ | 0.63 | -0.41 | 0.53 |
| $\pi$ | 0.48 | -0.82 | -0.07 |
| H-Ac | -0.24 | 0.86 | -0.06 |
| M-RE | 0.88 | 0.09 | -0.14 |
| F | 0.67 | 0.61 | 0.37 |
| R | -0.01 | 0.78 | 0.22 |
| $\sigma$ | -0.43 | 0.62 | 0.60 |
| Es | -0.79 | -0.26 | 0.31 |
| $B_1$ | 0.93 | 0.11 | -0.32 |
| $B_4$ | 0.62 | 0.46 | -0.58 |
| $\mathrm{log}k'_{0PEE}$ | 0.73 | -0.56 | 0.17 |
| $b_{PEE}$ | 0.43 | 0.75 | -0.01 |

in the first and second principal component. This result indicates again the similarity between the retention behaviour of columns (and is in good agreement with the results of the previous calculations).

The two-dimensional non-linear map of principal component loadings is shown in Fig. 3. The proximity of the various chromatographic and physicochemical parameters on the map indicates that they contain similar information. As the physicochemical parameters are inherent characteristics of solutes and the chromatographic ones are measured values, it is reasonable to suppose that the physicochemical parameters near to the chroma-

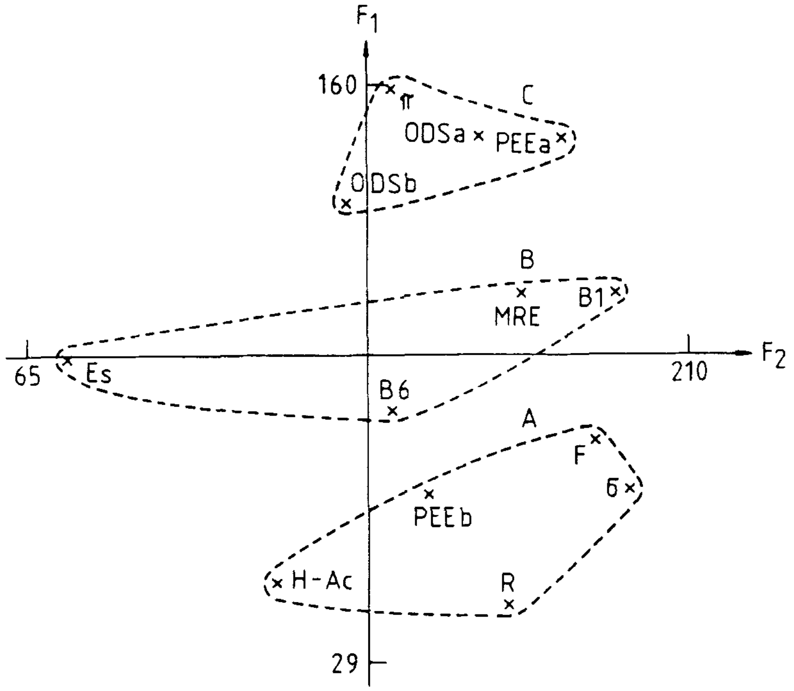


Fig. 3. Two-dimensional non-linear map of principal component loadings. Number of iterations: 208, maximum error: $4.12.10^{-2}$ Symbols: logarithm of capacity factor extrapolated to zero concentration of organic component in mobile phase determined on PEE column = $a_{PEE}$, logarithm of capacity factor extrapolated to zero concentration of organic component in mobile phase determined on ODS column = $a_{ODS}$ and other symbols see Material and Methods.

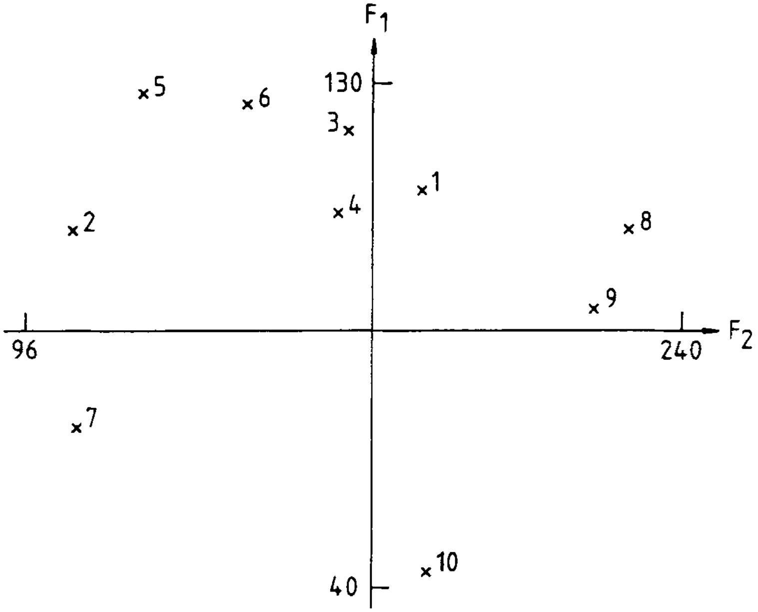


Fig. 4. Two-dimensional non-linear map of principal component variables. Number of iterations: 130, maximum error: $2.00.10^{-2}$. Numbers refer to aniline derivatives in Table I.

tographic parameters are the determinants of chromatographic behaviour. The specific hydrophobic surface area of anilines determined on PEE column forms a cluster with the electronic parameters (see cluster A). This results suggests that their interaction with the surface of polyethylene-coated silica is influenced by electronic interactions. Separation of steric parameters from the chromatographic ones indicate that they do not have a considerable effect on the retention behaviour of PEE or ODS columns (cluster B). Cluster C contains chromatographic parameters (slope and intercept values) of ODS column, intercept values determined on PEE column and lipophilicity of aniline derivatives. This results indicates that lipophilicity of aniline derivatives has an marked effect on the retention characteristics of both columns that is the separation mechanism is in both cases is a reversed-phase one.

The two-dimensional non-linear map of principal component variables is shown in Fig. 4. Aniline derivatives do not form clusters neither on the bases of the nature of substituents nor on the bases of substituent position. This finding indicates that retention behaviour of anilines is influenced in similar degree by the quality and position of substituents.

It can be concluded from our data that ring-substituted aniline derivatives can be well separated on the polyethylene-coated silica column. Various multivariate-statistical calculations indicate that the retention behaviour of PEE column sligthly differs from that of ODS column and the electronic parameters of substituents also influence the retention of ring-substituted anilines on PEE column.

## ACKNOWLEDGEMENT

This work was supported by the Grant OTKA 2670 of the Hungarian Academy of Sciences.

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Received: December 23, 1992
Accepted: January 13, 1993