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Coupling of a microbore column with a column packed with non-porous particles for fast comprehensive two-dimensional high-performance liquid chromatography

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Abstract

To reduce the analysis time in comprehensive two-dimensional HPLC columns packed with 1.5 μm non-porous particles were applied in the second dimension. Preservation of the efficiency under coupling conditions was realized by adapting the peak volumes in the first dimension. To achieve this an instrumental set-up was designed and used which combines a micro-column in the first dimension and a column packed with non-porous particles in the second dimension. A theoretical approach to estimate the optimum column diameter ratio was verified experimentally. As an example a mixture of phenols was separated using a tetrachlorophthalimidopropyl silica in the first dimension and a 1.5 μm RP-18 packing in the second dimension. The total time for the two-dimensional analysis of this mixture with an analysis time of 16 min in the first dimension was 70 min analysing 27 fractions on the second dimension column. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

High-performance liquid chromatography (HPLC) is one of the most important techniques for the analysis of soluble molecules. Because of the different separation principles which can be realized using the same instrument and the high selectivity, sensitivity and robustness of HPLC, it covers a range of analytical problems that is not as readily accessible to other separation techniques.

Unfortunately, resolution and peak capacity in HPLC suffer from a lack of plate numbers. As a result there is only insufficient separation power for multicomponent mixtures. This shortage can partly be overcome by a careful optimization of the selectivity of the separation system.

A much better way out of this dilemma is to enhance the peak capacity of the liquid chromatographic system by the introduction of a second dimension [1–4]. In theory, the maximum peak capacity of a true two-dimensional (2D) system is the product of the peak capacities of the individual columns [3,4]. In practice, this maximum can be approached by an adaption of the characteristics of the column and the mobile phases as reported in a previous paper [5].

The addition of a second dimension separation step for the entire chromatogram of the first dimension in small fractions costs lots of time rendering the total analysis time intolerably long. High-speed separation in the second dimension using short columns packed with fine non-porous particles [6–9]

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would lead to a considerable acceleration of the numerous separation steps necessary for comprehensive 2D separation. However, columns packed with non-porous silica particles (NPS columns) involve some special characteristics which must not be neglected. Otherwise, the results of 2D separations would be completely disappointing.

First, these columns tolerate only small sample volumes if the loss of efficiency caused by the injected volume is to remain within an acceptable range [10,11]. This means a drastic limitation for the fraction volume which can be transferred and hence the volume of the peaks eluting from the first dimension. Second, the low surface of the non-porous particles calls for weak eluents. Thus, a peak-focusing effect by taking advantage of weaker eluents in the first dimension and stronger eluents in the second dimension as demonstrated in Ref. [5] is not possible. Consequently, the requirement of small peak volumes in the first dimension has to be met by the application of small-diameter columns (packed capillaries or microbore columns in the first dimension). Volumes of peaks eluting from such columns range from nanolitres to a few microlitres depending on the column diameter and the retention. A further disadvantage is the limited choice of commercially available non-porous particle phases (mostly RP-18) which means the alternative retention mechanism has to be provided by the column of the first dimension.

In this paper a system for the 2D coupling of microbore columns with NPS columns is introduced and evaluated by the separation of a mixture of some amino-, chloro- and nitrophenols. As alternative retention mechanism to the hydrophobic interactions on the non-porous RP-18 packing a so-called charge-transfer phase was used in the first dimension. It is shown that this column combination offers the possibility of a fast and efficient 2D separation of multi-component mixtures.

2. Experimental

2.1. Apparatus

A scheme of the designed system for fast comprehensive 2D-HPLC is shown in Fig. 1.

Two PU-980 pumps from Jasco (Gross-Umstadt, Germany), which are capable of delivering mobile phase flow-rates from the microlitre per minute range to a few millilitre per minute with high precision were employed. A micro-injection valve 7520 from Rheodyne (Cotati, CA, USA) with an internal sample loop of 0.5 μl was used for sample injection. To reduce the injected volume additionally and to improve the injection profile, a back-switching technique was applied [12]: the injection valve was switched back to the ''load'' position after 1 s. At the typically applied flow-rate of 5 μl/min only 83 nl of the sample was injected.

The microcolumn was directly connected to the injection valve without any further tubing. The column outlet was connected to a fused-silica capillary with an inner diameter of 75 μm. Detection between the first and the second dimension was performed on this capillary with a Model WellChrom K-2000 UV detector from Knauer (Berlin, Germany) equipped with a cell for capillary electrophoresis.

For fraction transfer a six-port Valco/Vici valve (Schenkon, Switzerland) with an electric actuator was used. The detection after the second (NPS) column was done with a Jasco UV-975 detector equipped with a micro cell (4 μl). The valve for flow interruption was a Model 7000 six-port valve from Rheodyne. The valve was driven by an electric actuator from Besta (Wilhelmsfeld, Germany). The dummy columns produce flow-resistances which are similar to those of the micro-column and the non-porous particle column and protect the pumps from a pressure drop when the valves are switched for fraction transfer and flow interruption.

All parts were connected with polyether ether ketone (PEEK) capillaries with an inner diameter of 125 μm. Data were recorded using the chromatography software Chromeleon from Gynkotek (Germaring, Germany).

2.2. Columns

PEEKsil capillaries (SGE, Ringwood, Australia) of 20 cm×320 μm I.D. served as column material in the first dimension. They were packed in the laboratory [13] with a tetrachlorophthalimidopropyl (TCP) silica, prepared from 5 μm ES silica (Sepserv, Berlin, Germany) according to a procedure by Holstein [14].

In the second dimension a ChromSphere UOP 1.5 μm C_{18} non-porous particle column of 3 cm×4.6

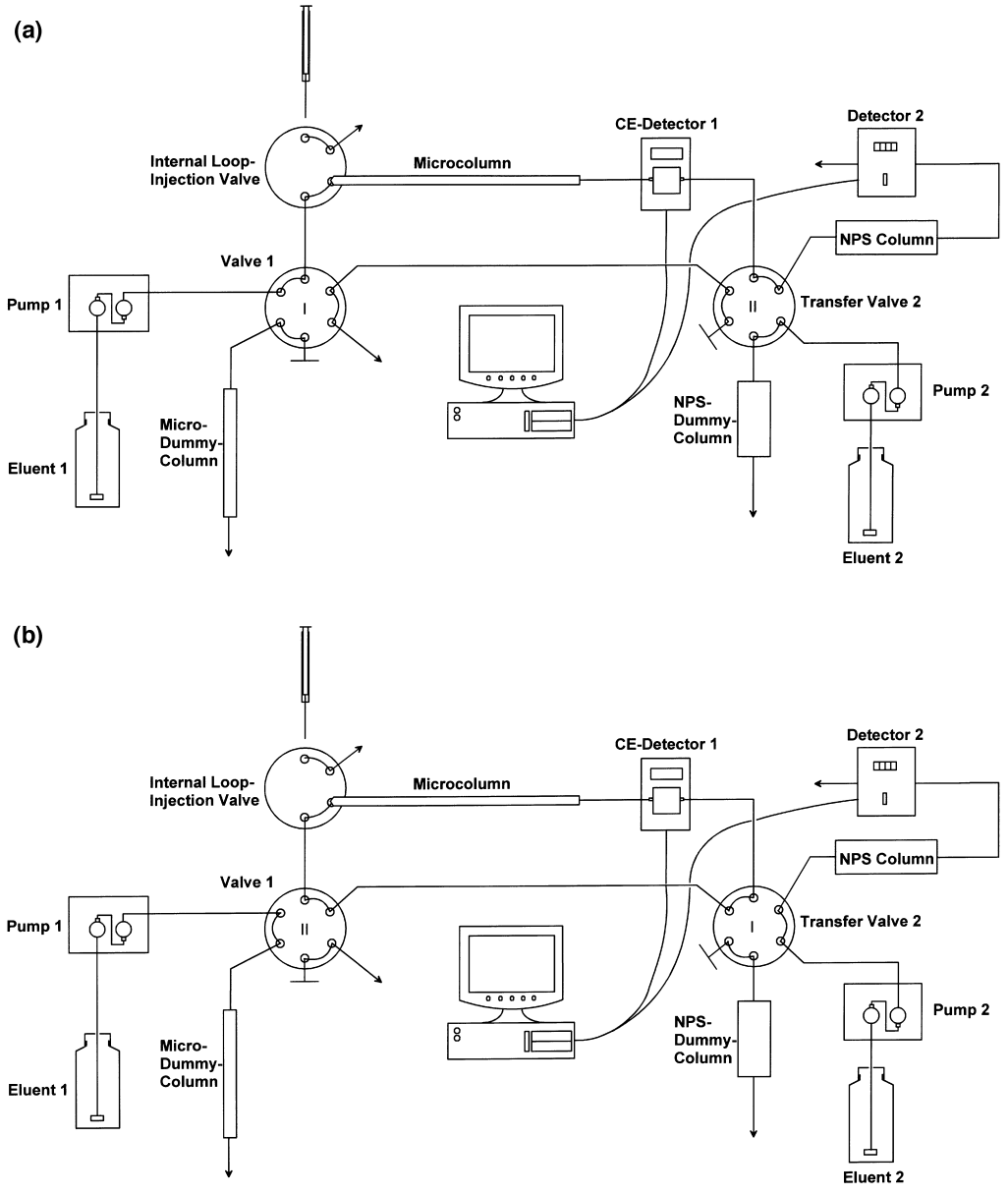

Fig. 1. Schematic of the instrumental set up for fast comprehensive 2D-HPLC. (a) Separation of the sample on the microbore column and transfer of a fraction to the non-porous particle column (for further explanation see Section 2.1). (b) Flow on the microbore column is interrupted while the transferred fraction is separated on the non-porous particle column (for further explanation see Section 2.1).

mm I.D. (Chrompack, Middelburg, Netherlands) was used.

2.3. Chemicals

All solvents were HPLC-grade and purchased from Merck (Darmstadt, Germany). The phenols were obtained from Promochem (Wesel, Germany).

2.4. Application

The 2D system was applied to the separation of a

mixture of phenols. The standard solutions and the mixture contained 3–4 mg phenol per ml in acetonitrile. They were diluted 1:4 with the eluent before injection. The eluent on the first dimension consisted of acetonitrile–phosphate buffer (100 m*M*, pH 2.5) (50:50, v/v), the flow-rate was 5 μl/min. Eluent 2 contained acetonitrile–water (20:80, v/v), the flow-rate on the second column was 1 ml/min.

3. Results and discussion

It has been shown in a previous paper [5] that the efficiency and peak capacity in 2D-HPLC, which in theory is the product of the peak capacities of the single columns is strongly influenced by the volumina transferred from the first column to the second column. In case an entire peak is transferred this volume corresponds to the peak volume V_P, ($V_P = 4\sigma F$, σ in time units). For identical or similar dimensions of the columns being coupled the peak volume eluting off the first column is mostly larger than the injection volume tolerated by the second column if a loss of efficiency of more than 10% is to be avoided. If no focusing effect can be achieved during the transfer process by an appropriate choice of the stationary phases and the eluent strengths as it is described in Ref. [5], the peak volumes resulting from the first dimension have to be adapted to the injection requirements of the second column, or the peaks must be transferred in several fractions. But the more fractions are to be separated on the second column, the more time is consumed for a comprehensive 2D separation without gaining more information. Therefore, the adaption of peak volume and injection volume is the preferred way.

The following theoretical consideration is helpful to estimate the dimensions of the columns to be coupled.

According to Refs. [10,11,15] the injection volume, V_i, is determined by the tolerated peak broadening due to the injection process, θ, a factor q_i reflecting the quality of the injection profile, the retention factor, k, the square root of the theoretical plate number, N, the reduced plate height, h, the particle diameter, d_p, the square of the column diameter, d_c, and the column porosity, ϵ:

$$V_i = \frac{\pi}{4}\theta q_i (1+k)\sqrt{N} h d_p d_c^2 \epsilon \quad (1)$$

Interestingly, the peak volume, V_P, is determined by the same parameters except $\theta q_i/4$:

$$V_P = \pi(1+k)\sqrt{N} h d_p d_c^2 \epsilon \quad (2)$$

Under coupling conditions the volume of a peak, V_P, intended to be transferred completely onto the second column must not exceed the injection volume, V_i, determined by the column and the retention characteristics of the second column. Or, in other words, the ratio of V_P/V_i should be unity:

$$\frac{V_P}{V_i} = \frac{4}{\theta q_i} \cdot \frac{(1+k_1)}{(1+k_2)} \cdot \frac{\sqrt{N_1} h_1 d_{p1} \epsilon_1}{\sqrt{N_2} h_2 d_{p2} \epsilon_2} \cdot \frac{d_{c1}^2}{d_{c2}^2} = 1 \quad (3)$$

where the indices 1 and 2 denote the first and the second dimension, respectively.

To achieve the best results with 2D separations, the retention mechanisms of the coupled columns should be as different as possible (orthogonal). This means the k values of the analytes on the two columns may differ strongly which affects the peak volume and the injection volume according to Eqs. 1 and 2 and hence the ratio of V_P/V_i. An appropriate parameter to counterbalance strong differences in k values and column performances and to bring this ratio closer to unity is the diameter of the column (Eq. 3). Assuming a rather unfavourable case, where a component elutes with a k value of 10 on the first column and a k value of 1 on the second column and using chromatographic parameters and column characteristics as given below, the necessary column diameter in the first dimension has to be about 15-times smaller than that in the second dimension. This means a micro-column with an inner diameter of about 0.3 mm has to be coupled to a 30×4.6 mm column packed with non-porous 1.5 μm particles if the latter is planned to be used in the second dimension to achieve fast separations. The parameters used for the calculation were derived from experimental results on individual columns. They were as follows: Second dimension: k of the analyte = 1, $N = 3200$, $h = 3$, $d_p = 1.5$ μm, $\epsilon = 0.35$, $d_c = 4.6$ mm, $\theta = 0.32$ (10% loss of efficiency) and $q_i = 1.9$; first dimension: k of the analyte = 10, $N = 4000$, $h = 8$, $d_p = 5$ μm and $\epsilon = 0.8$.

Following the conclusions made above, a 2D separation system was designed and build which contains a commercially available NPS RP-18 col-

umn in the second dimension and a micro-column (20 cm×0.32 mm I.D.) made from PEEKsil tubing [13] in the first dimension (Fig. 1). To test the performance of this system and to verify the considerations given above a mixture of nitro-, amino- and chlorophenols was separated. A different retention behaviour of these compounds on the first column was achieved by employing a laboratory-made TCP silica as packing material. Its alternative selectivity to an alkyl phase is based on its polarity and its charge-transfer interaction capabilities [16].

Fig. 1a shows the flow diagram during the separation of the sample on the first dimension and the transfer of a fraction to column 2 (valve 1 in position I, valve 2 in position II). When the transfer is finished, either after a certain time, or at the peak end (indicated by the monitor detector) the transfer valve 2 and the valve for flow interruption 1 are switched simultaneously. Fig. 1b shows the state, where the flow (and the separation) on the first column is interrupted and the transferred fraction is separated on the second dimension using the second eluent (valve I in position II, valve 2 in position I). In case flow interruption is not desired on the first column during the separation of the transferred fraction on the second dimension, only the transfer valve 2 is switched after the transfer step (i.e., both valves are in position I).

The separation of the phenol mixture on the TCP micro-column is shown in Fig. 2. The chromatogram illustrates the ''group type'' character of this separation according to the kind and number of substituents. Most of the peaks represent more than one component. Peak shape, efficiency and resolution are

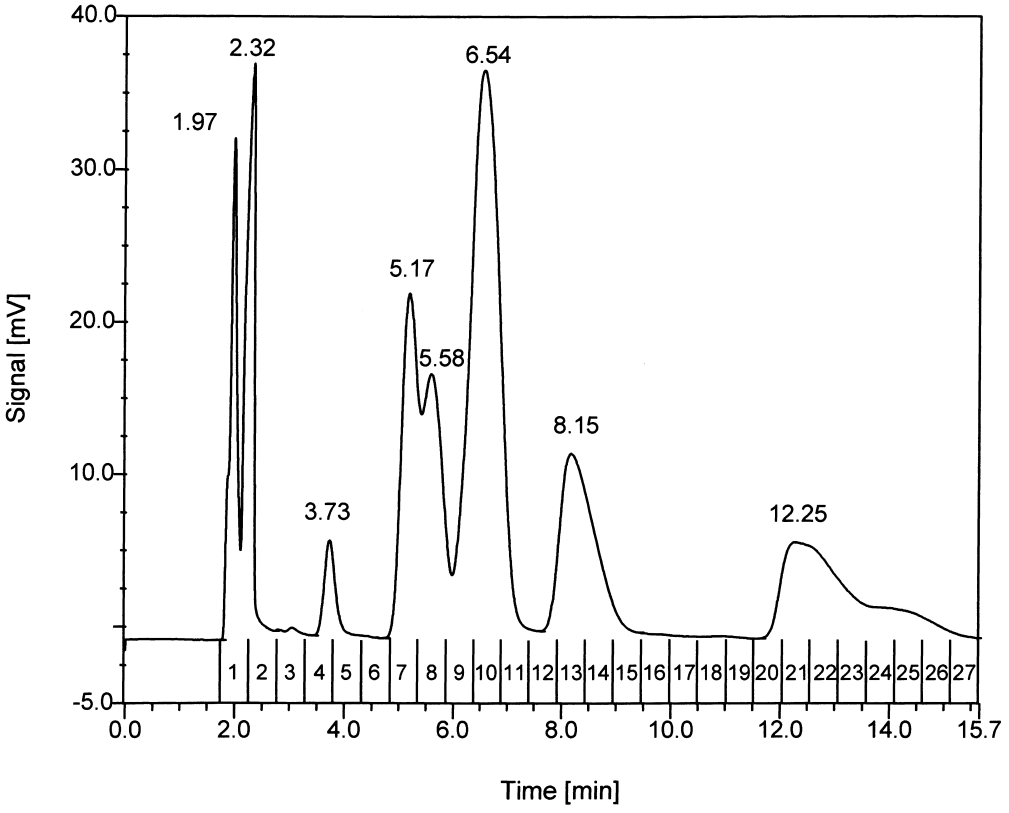

Fig. 2. ''Group type'' separation of a mixture of 12 phenols on a 20 cm×0.32 mm PEEKsil capillary packed with 5 μm tetrachlorophthalimidopropyl silica. Eluent: acetonitrile–100 m*M* phosphate buffer, pH 2.5 (50:50, v/v); flow-rate: 5 μl/min. (Retention time in min) compound: (1.97) 4-aminophenol, (2.32) 2-aminophenol, (3.73) unknown, (5.17) 4-nitrophenol, (5.58) *o*-cresol and 3-chlorophenol, (6.54) 2-methyl-3-nitrophenol, (8.15) 2,3-dichlorophenol, 2,4-dichlorophenol and 2,6-dichlorophenol, (12.25) 2,3,4-trichlorophenol, 2,3,6-trichlorophenol and 2,4,6-trichlorophenol.

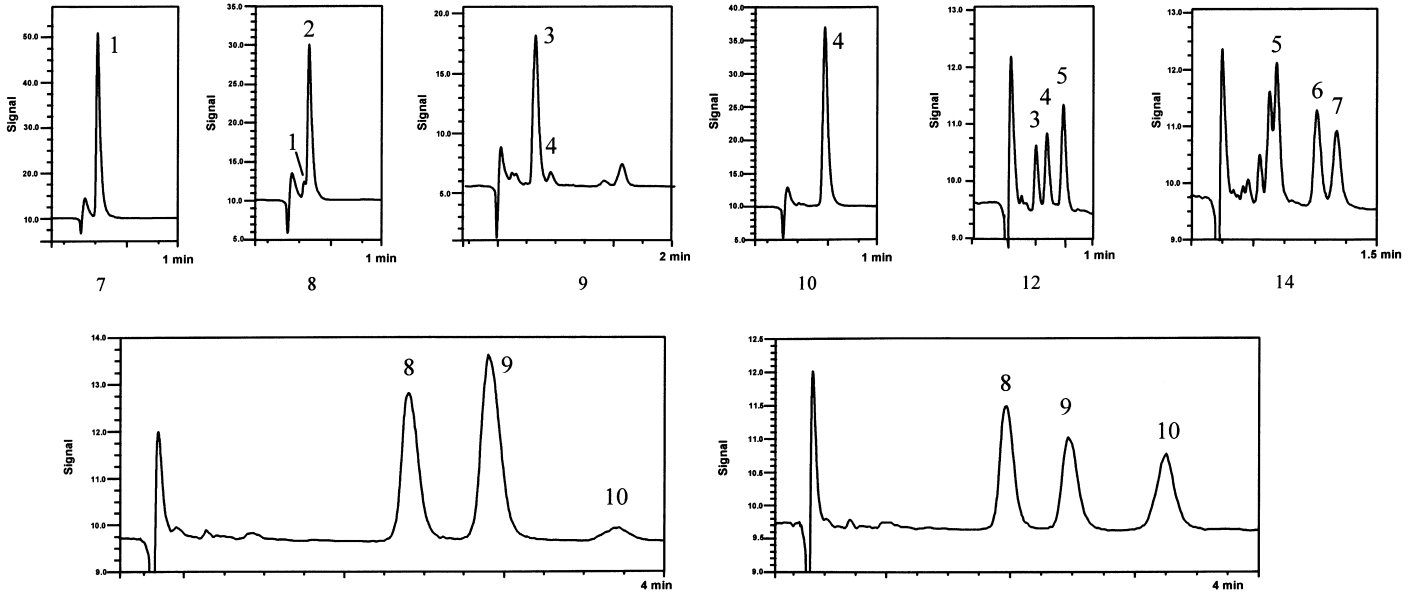

Fig. 3. Separation of the transferred fractions 7–10, 12 and 14, and 23 and 24 (see Fig. 2) on a 30×4.6 mm ChromSphere UOP 1.5 C_{18} column. Eluent: acetonitrile–water (20:80, v/v), flow-rate: 1 ml/min. Components: 1=4-nitrophenol, 2=*o*-cresol, 3=3-chlorophenol, 4=2-methyl-3-nitrophenol, 5=2,6-dichlorophenol, 6=2,4-dichlorophenol, 7=2,5-dichlorophenol, 8=2,3,6-trichlorophenol, 9=2,4,6-trichlorophenol, 10=2,3,4-trichlorophenol.

therefore not as good as for single component peaks. Because of the insufficient resolution the beginning and the end of the peaks are not clearly defined, which makes it problematic to cut and transfer entire peaks to the second dimension. In addition, peak volumes of the later eluting peaks are too large. Therefore, the entire chromatogram of the first dimension was cut into fractions of 30 s as is indicated by the lines underneath the chromatogram, each fraction corresponding to a volume of 2.5 μl (at the chosen flow-rate of 5 μl/min). It was not advisable to increase the fraction volume in order to diminish the total analysis time further because the loss of efficiency on the second column is considerable (more than 10%) if the volume of the transferred fractions exceeds 2.5 μl. It can be seen in some exemplary chromatograms in Fig. 3 that the fast separations on the second NPS column display good efficiencies which are not affected negatively by the fraction transfer and the different elution strengths of the eluents used in the first and in the second dimension. The total time for the entire 2D analysis is the sum of all individual separations in the second dimension and the analysis time in the first dimension. The analysis times for the separations in the second dimension were 1 min for fractions 1–12, 1.5 min for fractions 13–20, and 4 min for fractions 21–27. If all 27 fractions are transferred systematically, the complete two-dimensional separation lasts about 70 min. In the case that peaks eluting off the first column are known to represent pure components only, or if parts of the chromatogram do not contain any components at all (e.g., fractions 6 and 16–19 in our test mixture), such fractions can be omitted entirely. This reduces the total analysis time for the given mixture to less than 1 h.

In conclusion, the total analysis time in comprehensive 2D-HPLC could be drastically reduced by the acceleration of the separations in the second dimension. This goal was realized by the application of a short column packed with 1.5 μm non-porous particles in the second dimension. The requirements of this column with respect to the injection volume were met by the use of a column with much smaller diameter in the first dimension. A theoretical approach to estimate the optimum ratio of the column diameters in fast comprehensive 2D-HPLC could be verified in practice by the successful combination of a micro-column in the first dimension with the non-porous particle column in the second dimension.

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References

[1] J.C. Giddings, in: Unified Separation Science, Wiley, Chichester, New York, 1991, p. 131.
[2] J.M. Davis, Adv. Chromatogr. 34 (1994) 109.
[3] J.M. Davis, J.C. Giddings, Anal. Chem. 55 (1983) 418.
[4] V.R. Meyer, T. Welsch, LC·GC Int. 9 (1996) 670.
[5] A.P. Köhne, U. Dornberger, T. Welsch, Chromatographia 48 (1998) 9.
[6] T. Welsch, G. Mayr, N. Lammers, in: O. Kaiser, R.E. Kaiser, H. Gunz, W. Günther (Eds.), Chromatography, InCom Verlag, Düsseldorf, 1997, p. 349.
[7] T. Welsch, G. Mayr, J. Chromatogr. A. 845 (1999) 155.
[8] M. Hanson, K.K. Unger, LC·GC Int. 9 (1996) 650.
[9] M. Hanson, K.K. Unger, LC·GC Int. 9 (1996) 741.
[10] B.L. Karger, M. Martin, G. Guiochon, Anal. Chem. 46 (1974) 1640.
[11] H. Colin, M. Martin, G. Guiochon, J. Chromatogr. 185 (1979) 79.
[12] M. Schmid, M. Habram, T. Welsch, in: 19th International Symposium on Column Liquid Chromatography and Related Techniques, Innsbruck, 28 May–2 June 1995, Abstracts, Vol. 1, 1995, p. 108.
[13] M. Schmid, F. Bäuml, A.P. Köhne, T. Welsch, J. High Resolut. Chromatogr., submitted for publication.
[14] W. Holstein, Chromatographia 14 (1981) 468.
[15] J.C. Gluckman, M.V. Novotny, in: M.V. Novotny, D. Ishii (Eds.), Microcolumn Separations – Columns, Instrumentation and Ancillary Techniques, Journal of Chromatography Library, Vol. 30, Elsevier, Amsterdam, 1984, p. 57.
[16] L. Nondek, J. Chromatogr. A 373 (1986) 61.