ELSEVIER

Journal of Chromatography A, 911 (2001) 277–283

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Fast separation of pyrimidine derivatives by capillary electrochromatography on ion-exchange/reversed-phase mixed-mode stationary phases

Christian W. Klampfl[a,*], W. Buchberger[a], Paul R. Haddad[b]

[a]*Department of Analytical Chemistry, Johannes Kepler-University Linz, Altenbergerstrasse 69, A-4040 Linz, Austria*
[b]*Separation Science Group, School of Chemistry, University of Tasmania, GPO Box 252-75, Hobart, Tasmania 7001, Australia*

Received 31 July 2000; received in revised form 12 December 2000; accepted 29 December 2000

Abstract

This work describes the use of mixed–mode stationary phases which exhibit both strong ion-exchange (either cation-exchange, SCX, or anion-exchange, SAX) and reversed-phase chromatographic characteristics in capillary electrochromatographic separations of pyrimidine derivatives. Different packing materials, namely C_6, SCX/C_6 and SAX/C_6, were compared and the influence of the composition of the carrier electrolyte (concentration of acetonitrile and pH) on the retention behavior of the selected solutes was investigated. A separation of all eight pyrimidine derivatives could be obtained on a 6.5 cm column packed with the SAX/C_6 stationary phase in less than 3 min, with good peak shapes and efficiencies in the range 39 000 to 81 000 plates per meter.

Keywords: Electrochromatography; Stationary phases, electrochromatography; Mixed-mode chromatography; Pyrimidines

1. Introduction

In recent years capillary electrochromatography (CEC) has received increased attention as a complementary technique to well established electroseparation methods such as capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). CEC shows a high separation efficiency due to the plug-like profile of the electroosmotic flow (EOF) and a reduction of Eddy diffusion effects, but even more importantly it offers the possibility to combine electrophoretic and chromatographic separation mechanisms and hence to achieve unique separation selectivity. However, the literature published so far on CEC has concentrated mainly on the separation of neutral analytes using capillaries packed with reversed-phase (RP) materials. Under these conditions the electrophoretic portion of the separation mechanism is restricted to the use of the EOF instead of a mechanical pump to drive the mobile phase and the analytes towards the detection end of the capillary, so separation selectivities similar to those in RP liquid chromatography have normally been obtained [1–8].

Recently, some studies describing the use of ion-exchange packings [9–16] and so-called mixed-mode

*Corresponding author. Tel.: +43-732-2468-722; fax: +43-732-2468-679.
E-mail address: christian.klampfl@jk.uni-linz.ac.at (C.W. Klampfl).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00515-5

stationary phases [17–29] (i.e., chromatographic supports providing both reversed-phase ands ion-exchange properties) in CEC have been published. The latter type of stationary phase offer remarkable potential for the manipulation of separation selectivity. On one hand the reversed-phase properties of these chromatographic supports allow the separation of non-polar analytes according to their hydrophobicity. On the other hand the ion-exchange functional groups generate an increased cathodic (reversed-phase/strong cation-exchange, RP/SCX) or even an anodic (reversed-phase/strong anion-exchange, RP/SAX) EOF and in the case of charged analytes these ion-exchange sites also influence separation selectivity by either attracting or repelling the analytes, leading to increased or decreased retention compared to conventional reversed-phase materials [28].

Short-end injection methods have been used in CE [30] and recently also in CEC [16,31,32] to reduce separation times. In CEC the reduction of the length of the packed bed (in the present work 6.5 cm compared to more than 25 cm when the conventional injection method is used) does not only reduce analysis times but also simplifies column handling, for example conditioning with a new carrier electrolyte. Due to the lower pressure resistance of these short packed beds, the built-in high-pressure system of the CEC instrument (which permits the application of pressure up to 12 bar) can be used to flush the column and eliminates the need to remove the fragile capillary from the cartridge and connect it to an external high-performance liquid chromatography (HPLC) pump.

In the present study the retention behavior of a series of pyrimidine derivatives, which are intermediates or by-products in the fabrication of agrochemicals, on reversed-phase as well as two different mixed-mode stationary phases in CEC has been investigated. The combination of hydrophobic and ionic interactions with the stationary phase affects the separation selectivities as well as peak shapes. The influence of the carrier electrolyte composition, in particular the pH and the acetonitrile content, on the chromatographic and electrophoretic behavior of the selected analytes has been studied. This approach allowed the separation of eight pyrimidine derivatives in less than 3 min using a SAX/C_6 stationary phase.

2. Experimental

2.1. Instrumentation

Experiments were performed using a HP^{3D} CE system (Agilent Technologies, Waldbronn, Germany), equipped with a diode array detector and connected to a HP Chemstation (Agilent Technologies) for data processing. A pressure of 10 bar was applied to both ends of the column using helium gas and the column was thermostatted at 25°C during all separations. Samples were injected electrokinetically at the outlet side of the capillary applying +5 kV (SAX/C_6) or −5 kV (C_6 and SCX/C_6), respectively, for 5 s.

2.2. Materials and reagents

Fused-silica capillaries (75 μm I.D.×360 μm O.D.) obtained from Polymicro Technologies (Phoenix, AZ, USA) were used throughout this work. Water was purified using a Milli-Q water (Millipore, Bedford, MA, USA) system. The columns were packed with one of the following stationary phases: (i) 3 μm silica-based mixed-mode strong anion-exchange/reversed-phase material (SAX/C_6, 190 m^2/g, 8 nm pore size, 2.6% carbon, functionalized with *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethyl-ammonium chloride and finally endcapped with C_6 hexyltrimethoxysilane, (ii) strong cation-exchange/reversed-phase material (SCX/C_6, 190 m^2/g, 8 nm pore size, 2.8% carbon, functionalized with propylsulfonic acid and finally endcapped with C_6 hexyltrimethoxysilane, (iii) silica-based reversed-phase material (C_6, 190 m^2/g, 8 nm pore size, 6% carbon). All stationary phases were obtained from Xtec Consultants (Clwyd, UK). All chemicals used were of analytical-reagent grade.

Background electrolytes (BGEs) were prepared from NaH_2PO_4 and titrated to the appropriate pH using 0.5 *M* NaOH before dilution with acetonitrile. All BGE solutions were filtered through a 0.45-μm membrane filter of Type HA (Millipore) and degassed before use.

2.3. Column preparation

Untreated fused-silica capillaries were packed

using a slurry packing technique similar to that published previously [13]. The packed column (25 cm total length, 6.5 cm packed bed) was preconditioned with mobile phase using a HPLC pump for at least 1 h, then mounted in the HP cartridge and conditioned with 10 bar inlet pressure and +10 kV (SAX/C_6) or −10 kV (C_6 and SCX/C_6), respectively, overnight.

3. Results and discussion

3.1. Comparison of the stationary phases

Three different stationary phases, namely a C_6 reversed-phase, a SCX/C_6 mixed-mode (both providing a cathodic EOF) and a SAX/C_6 mixed-mode (providing an anodic EOF) were investigated. A series of pyrimidine derivatives which are a precursor (2-amino-4,6-dimethoxypyrimidine, ADMP) or by-products [2-amino-4,6-dichloropyrimidine (ADCP), 2-amino-4-chloro-6-methoxypyrimidine (ACMP), 2-amino-5-chloro-4,6-dimethoxypyrimidine (5Cl-ADMP), 2,4,6-trimethoxypyrimidine (TMP), 2-amino4-diethylamino-6-methoxypyrimidine (ADAMP), 2,4-diamino-6-methoxypyrimidine (DAMP), dimeric-2-amino-4,6-dimethoxypyrimidine (dim.-ADMP)] in the manufacture of important herbicides and fungicides were chosen as test substances for this study.

In general the SCX/C_6 material provides a higher EOF and with it higher migration velocities for most of the solutes due to the presence of its anionic functionalities. Fig. 1 shows the velocity differences ($v_{solute} - v_{EOF}$) obtained for these analytes on the SCX/C_6 and the C_6 stationary phases. This parameter allows one to judge the contribution of retention to the migration velocity of the solutes. The chromatographic retention obtained on the two stationary phases can be related to the basic strength of the analytes. The most pronounced migration differences were found for compounds with additional amino groups in position 4, namely ADAMP, DAMP and to some extent also dim.-ADMP. Comparing the pK_a values found in the literature for aminopyrimidines, namely only pK_a 3.5 for the 2-amino group but pK_a 7.3 for an additional amino group in the 4-position, implies that all analytes except these three compounds do not bear significant charge under the selected conditions [33]. Therefore ADAMP, DAMP and dim.-DAMP showed additional interactions with the sulfonic acid moieties of the stationary phase causing not only increased retention but also unfavorable peak distortion which has already been described in previous papers [14,28]. Minor differences in retention could also be observed for the rest of the analytes. Due to its methoxy groups in 4 and 6 positions, which induce a base-strengthening effect, ADMP also shows increased retention on the SCX/C_6 stationary phase. The opposite effect can be

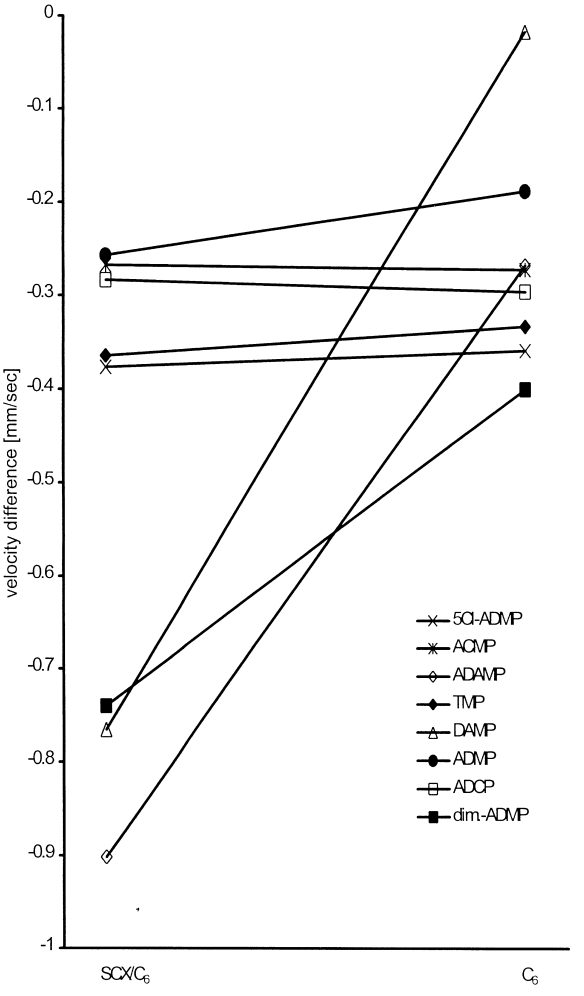

Fig. 1. Comparison of velocity differences ($v_{solute} - v_{EOF}$) obtained for pyrimidine derivatives on a SCX/C_6 and a C_6 stationary phase. Capillary: 25 cm (6.5 cm packed bed)×75 μm I.D. Mobile phase: 10 m*M* aqueous NaH_2PO_4 (pH 6.0 with NaOH) containing 40% acetonitrile. Voltage: −10 kV.

observed for ADCP and ACMP because of their electron-withdrawing chloro groups. TMP (which has no amino group) and 5Cl-ADMP (where the base-strengthening effect of the methoxy group is counterbalanced by the electron-withdrawing effect of its chloro functionality) are almost unaffected by the change in stationary phase [33].

A comparison of analyte retention behavior observed on the two mixed-mode stationary phases is shown in Fig. 2. Focusing on the EOF, the SAX/C_6 material provides an anodic EOF of almost the same magnitude as the cathodic one generated by the sulfonated material. As already observed in Fig. 1, a dramatic change in retention was obtained for ADAMP, DAMP, dim.-ADMP and somewhat less

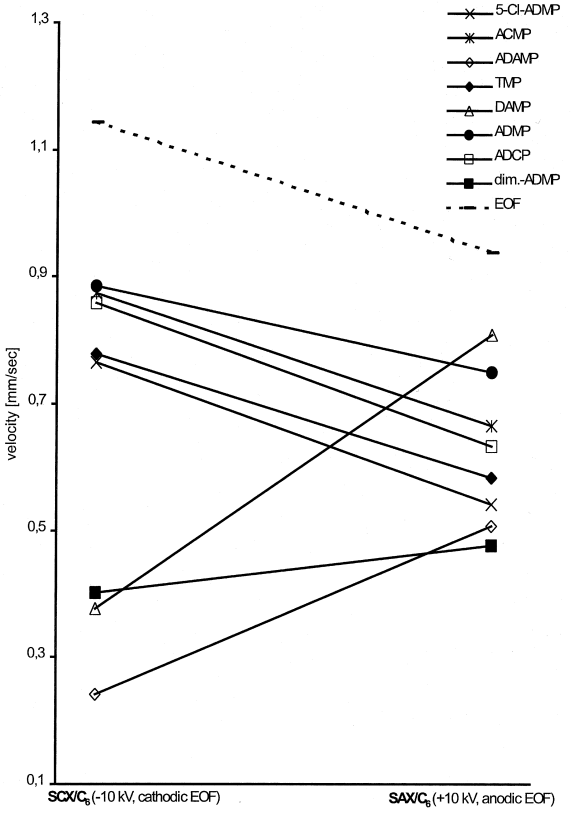

Fig. 2. Comparison of velocity differences ($v_{solute}-v_{EOF}$) obtained for pyrimidine derivatives on a SCX/C_6 and a SAX/C_6 stationary phase. Capillary: 25 cm (6.5 cm packed bed)×75 μm I.D. Mobile phase: 10 m*M* aqueous NaH_2PO_4 (pH 6.0 with NaOH) containing 40% acetonitrile. Voltage: −10 kV (SCX/C_6) or +10 kV (SAX/C_6).

pronounced for ADMP due to their increased basic strength (compared to the other analytes) when switching from the SCX/C_6 to the SAX/C_6 material. The other solutes show similar retention on both stationary phases. A major benefit of the SAX/C_6 material is the slightly better separation of the test solutes that was achieved under the selected conditions Additionally it provided significantly improved peak shapes (not only compared to the SCX/C_6 but also to the C_6 material) due to the screening effect of the positively charged functional groups attached to the stationary phase, which effectively reduced the level of interaction between the basic solutes and the silica base material.

3.2. Effect of the carrier electrolyte pH

Based on a 10 m*M* NaH_2PO_4 carrier electrolyte containing 40% acetonitrile, the influence of pH variations (shown in Fig. 3) was studied within the range of pH 5.0 to 8.0. Whereas the SCX/C_6 material provided a nearly constant EOF over this pH range, the C_6 and the SAX/C_6 materials showed some pH dependence. In case of the latter stationary phase a particularly significant drop in the magnitude of the EOF was observed with increasing pH. This behavior can be explained by the influence of the increasing number of silanol groups (caused by raising the pH) reducing the positive zeta potential provided by the ammonium functionalities of the stationary phase. Apart from the influence on the EOF, changes in the pH of the carrier electrolyte also affected selectivities with the migration order 5Cl-ADMP/dim.-ADMP/ADAMP at pH 5.0 changing to ADAMP/5Cl-ADMP/dim.-ADAMP when the pH 8.0 electrolyte was used. Regarding the tendency of the $v_{solute}-v_{EOF}$ plots, differences between the two compounds with higher pK_a values and the other analytes can be noticed. Whereas the curves obtained for the latter solutes are flat or even decreasing going from pH 5.0 to pH 6.0, those of DAMP and ADAMP show a steady increase over the whole pH range investigated.

3.3. Effect of the concentration of acetonitrile in the carrier electrolyte

Fig. 4 depicts the influence of the acetonitrile

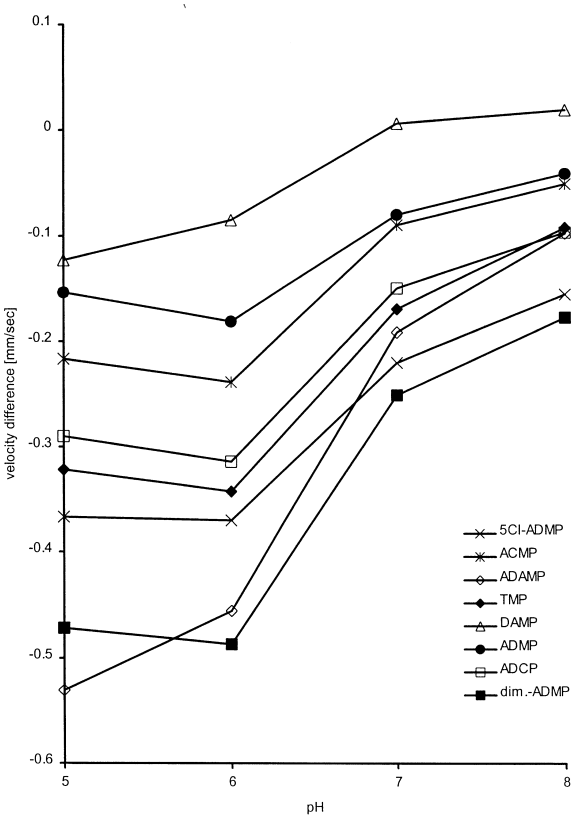

Fig. 3. Dependence of velocity differences ($v_{solute}-v_{EOF}$) obtained for the test analytes on the carrier electrolyte pH. Capillary: 25 cm (6.5 cm packed bed)×75 μm I.D., packed with SAX/C_6. Mobile phase: 10 m*M* aqueous NaH_2PO_4 (pH 5.0–8.0 with NaOH) containing 40% acetonitrile. Voltage: +10 kV.

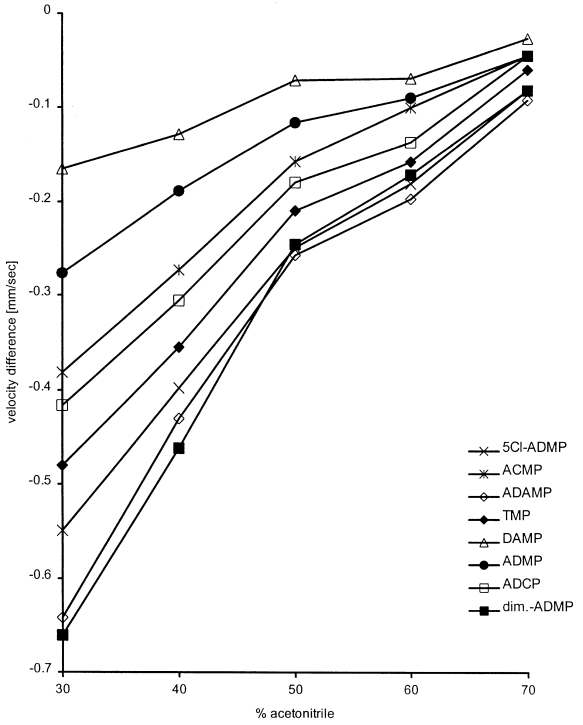

Fig. 4. Dependence of velocity differences ($v_{solute}-v_{EOF}$) obtained for the test analytes on the concentration of acetonitrile added to the carrier electrolyte. Capillary: 25 cm (6.5 cm packed bed)×75 μm I.D., packed with SAX/C_6. Mobile phase: 10 m*M* aqueous NaH_2PO_4 (pH 6.0 with NaOH) containing 30%–70% acetonitrile. Voltage: +10 kV.

content in the carrier electrolyte on the retention of the selected analytes, using the SAX/C_6 stationary phase. As might be expected all solutes showed the same tendency when the concentration of the organic solvent was raised from 30 to 70%. This is a result of decreased hydrophobic interactions with the stationary phase as commonly achieved in RP chromatography. Focusing on the EOF a steady decrease can be observed when the acetonitrile content is raised from 30 to 70%. This may be explained by two different effects: first changes in the viscosity of the carrier electrolyte and second changes in the binding-strength between the quaternary ammonium groups and their counter-ions which are influenced by the amount of organic modifier present in the mobile phase.

3.4. Separation of pyrimidine derivatives

From the data in Fig. 3 and Fig. 4, the best mobile phase composition for the separation of all nine analytes was found to be a 10 m*M* NaH_2PO_4 running buffer with 40% of acetonitrile and a pH of 6.0. Fig. 5 shows that the separation of the test mixture could be accomplished in less than 3 min on a SAX/C_6 material using this running buffer and the short-end injection technique. As can be seen from this chromatogram, no peak tailing was observed for DAMP and ADAMP because of the mixed-mode anion-exchange/reversed-phase nature of the packing material. This is an improvement on the results obtained using either the C_6 reversed-phase chromatographic support or a C_{18} stationary phase reported in previous work [34]. Despite the short length of the column used (6.5 cm packed bed) and

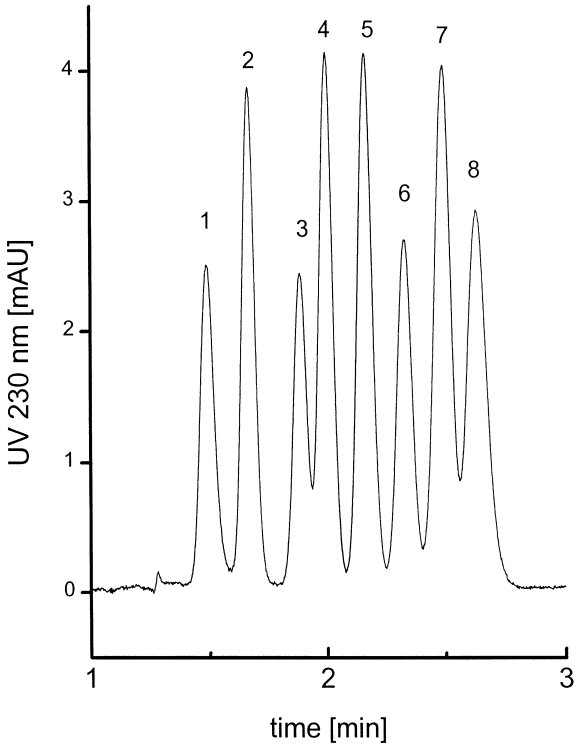

Fig. 5. Separation of pyrimidine derivatives. Capillary: 25 cm (6.5 cm packed bed)×75 μm I.D., packed with SAX/C_6. Mobile phase: 10 m*M* aqueous NaH_2PO_4 (pH 6.0 with NaOH) containing 40% acetonitrile. Voltage: +10 kV. Peaks: 1=DAMP; 2=ADMP; 3=ACMP; 4=ADCP; 5=TMP; 6=5 Cl-ADMP; 7=ADAMP; 8=dim.-ADMP.

the low retention times obtained in this separation, theoretical plate numbers for these peaks were found to be in the range of 39 000 to 81 000 plates per meter.

4. Conclusions

This work has demonstrated the advantages of using mixed-mode ion-exchange/reversed-phase stationary phases for the separation of pyrimidine derivatives. Separation selectivities could be manipulated by changing the stationary phase (reversed-phase, cation-exchange/reversed-phase and anion-exchange/reversed-phase) or the electrolyte composition. The latter was achieved by changing the pH and the amount of acetonitrile present in the electrolyte. Finally a very fast separation of all pyrimidine derivatives investigated could be achieved in less than 3 min employing a SAX/C_6 stationary phase.

Acknowledgements

The authors would like to thank Professor Peter Myers (Xtec Consultants Ltd.) for providing the packing materials used in this study and Agilent Technologies Austria for providing the HP^{3D} CE instrument.

References

[1] H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[2] A.L. Crego, A. Gonzalez, M.L. Marina, Crit. Rev. Anal. Chem. 26 (1996) 261.
[3] M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 744 (1996) 63.
[4] L.A. Colon, Y. Guo, A. Fermier, Anal. Chem. 461A (1997).
[5] L.A. Colon, K.J. Reynolds, R. Aliceamaldonado, A.M. Fermier, Electrophoresis 18 (1997) 2162.
[6] K.D. Altria, N.W. Smith, C.H. Turnbull, Chromatographia 46 (1997) 664.
[7] K.D. Altria, N.W. Smith, C.H. Turnbull, J. Chromatogr. B 717 (1998) 341.
[8] M.G. Cikalo, K.D. Bartle, M.M. Robson, P. Myers, M.R. Euerby, Analyst 123 (1998) 87R.
[9] N.W. Smith, M.B. Evans, Chromatographia 41 (1995) 197.
[10] S. Kitagawa, A. Tsuji, H. Watanabe, M. Nakashima, T. Tsuda, J. Microcol. Sep. 9 (1997) 347.
[11] D.M. Li, H.H. Knobel, V.T. Remcho, J. Chromatogr. B 695 (1997) 169.
[12] W. Wei, G.A. Luo, C. Yan, Am. Lab. 30 (1998) 20C.
[13] E.F. Hilder, M. Macka, P.R. Haddad, Anal. Commun. 36 (1999) 299.
[14] C.W. Klampfl, P.R. Haddad, J. Chromatogr. A 884 (2000) 277.
[15] M. Ye, H. Zou, Z. Liu, J. Ni, J. Chromatogr. A 887 (2000) 223.
[16] E.F. Hilder, C.W. Klampfl, P.R. Haddad, J. Chromatogr. A 890 (2000) 337.
[17] I.S. Lurie, T.S. Conver, V.L. Ford, Anal. Chem. 70 (1998) 4563.
[18] T. Adam, S. Ludtke, K.K. Unger, Chromatographia 49 (1999) S49.
[19] N. Smith, M.B. Evans, J. Chromatogr. A 832 (1999) 41.
[20] K. Walhagen, K.K. Unger, A.M. Olsson, M.W. Hearn, J. Chromatogr. A 853 (1999) 263.
[21] M. Zhang, Z. El Rassi, Electrophoresis 19 (1998) 2068.
[22] M.Q. Zhang, Z. El Rassi, Electrophoresis 20 (1999) 31.

[23] N.M. Djordjevic, F. Fitzpatrick, F. Houdiere, G. Lerch, J. High Resolut. Chromatogr. 22 (1999) 599.
[24] P.Q. Huang, X.Y. Jin, Y.J. Chen, J.R. Srinivasan, D.M. Lubman, Anal. Chem. 71 (1999) 1786.
[25] T. Adam, M. Kramer, Chromatographia 49 (1999) S35.
[26] Q. Tang, M.L. Lee, J. Chromatogr. A 887 (2000) 265.
[27] M. Zhang, G.K. Ostrander, Z. El Rassi, J. Chromatogr. A 887 (2000) 287.
[28] C.W. Klampfl, E.F. Hilder, P.R. Haddad, J. Chromatogr. A 888 (2000) 267.
[29] F. Steiner, B. Scherer, Proceedings of the 23rd International Symposium on Capillary Chromatography, Riva del Garda, 2000.
[30] A.J. Zemann, J. Chromatogr. A 787 (1997) 243.
[31] M.R. Euerby, C.M. Johnson, M. Cikalo, K.D. Bartle, Chromatographia 47 (1998) 135.
[32] X. Cahours, Ph. Morin, L. Agrofoglio, M. Dreux, J. High Resolut. Chromatogr. 23 (2000) 138.
[33] A.R. Katrizky, C.W. Rees, Comprehensive Heterocyclic Chemistry, Vol. 3, Pergamon Press, Oxford, 1984.
[34] W. Ahrer, I. Hagenauer, W. Buchberger, Monatsh. Chem. 131 (2000) 155.