ELSEVIER

Journal of Chromatography A, 798 (1998) 275–280

JOURNAL OF CHROMATOGRAPHY A

Use of cationic cyclodextrin for enantioseparation by capillary electrophoresis

Antje Bunke, Thomas Jira*

Institute of Pharmacy, Department of Pharmaceutical/Medical Chemistry, Ernst-Moritz-Arndt-University Greifswald, Friedrich-Ludwig-Jahn-Str. 17, D-17487 Greifswald, Germany

Abstract

The usability of 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin for chiral discrimination of various basic and acidic substances is described. The dependence of chiral separation on cyclodextrin (CD) concentration and pH value was investigated. Altering the pH value the migration order of the enantiomers of acidic analytes could be changed. Due to the quaternary ammonium structure of the CD molecule, a reversal of the electroosmotic flow (EOF) was observed. The direction of the migration of CD molecule was in opposite direction to the EOF because of its positive charge. The influence of CD concentration and pH on the EOF was determined. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Cyclodextrins

1. Introduction

The use of cyclodextrins (CDs) as chiral selectors in capillary electrophoresis (CE) was introduced by Fanali et al. [1] in 1989 and is presumably the widely employed method for enantiomeric separation in CE. With cyclodextrins chiral recognition is realized via hydrophobic interactions of aromatic structures of the analytes with the apolar CD cavity and hydrogen bonding to the hydroxyl groups of the CD [2]. The usage of charged cyclodextrins opens new possibilities for enantioseparation because of additional potential ionic interactions with the analytes.

In contrast to neutral CDs, charged CDs are suitable for the separation of enantiomers of neutral compounds, because they possess electrophoretic mobilities of their own. A growing number of publications discuss the use of charged CDs. Several anionic and cationic CDs were described as chiral additives in CE: carboxymethyl- [3–8]; carboxyethyl-, succinyl-β-CD [4,9]; β-CD-Sulfobutyl ether [8,10–15]; β-CD-sulfoethyl ether [6,8,10]; mono(6-β-aminoethylamino-6-deoxy)-β-CD [16,17]; 6^A-methylamino-β-CD; 6^A, 6^D-dimethylamino-β-CD [18]. Only a few applications of 2-hydroxy-3-trimethylammoniopropyl-β-CD are known from the literature [6,19].

In this study the utilization of cationic 2-hydroxy-3-trimethylammoniopropyl-β-CD (TMA-β-CD) as chiral selector and its ability to reverse the electroosmotic flow in fused-silica capillaries is described. The dependence of chiral separation and electroosmotic mobility on separation conditions like CD concentration and pH value was examined.

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)01197-7

2. Experimental

2.1. Instrumentation

CE was performed using a P/ACE 2100 capillary electrophoresis instrument (Beckman, Fullerton, CA, USA) equipped with an on-column UV detector. 'Gold'-software (Beckman) was used for data acquisition.

Fused-silica capillaries (eCAP capillary tubing 37/30 cm×50 mm I.D.×375 mm O.D. (Beckman) and neutrally coated capillary (eCAP neutral capillary 37/30 cm×50 mm I.D.×375 mm O.D. (Beckman) were used.

2.2. Chemicals

2-Hydroxy-3-trimethylammoniopropyl-β-CD [MS =0.5] was obtained from Wacker-Chemie (Munich, Germany). Na(CH_3COO), NaH_2PO_4, Na_2HPO_4 and NaOH were purchased from Laborchemie Apolda (Apolda, Germany).

p-Brommandelic acid, 2-phenylbutyric acid and 2-phenylpropionic acid were obtained from Fluka (Neu-Ulm, Germany), mandelic acid from Merck-Schuchardt (Hohenbrunn near Munich, Germany), cyclodrine from Ankerwerk (Rudolstadt, Germany). Cyclopentolate was from Cyclopentolat 0.5% eye drops (Alcon Thilo, Freiburg, Germany). 2-Methoxy-2-phenyl acetic acid was purchased from Sigma (St. Louis, MO, USA), norpseudoephedrine–HCl, phendimetrazine bitartaricum and pholedrine sulfate from Isis-Chemie (Zwickau, Germany), terbutaline sulfate from Merckle (Blaubeuren, Germany) and (*R,S*)-tropic acid from Aldrich (Steinheim, Germany). (*R*)-Tropic acid was made by the Institute of Pharmacy of Ernst-Moritz-Arndt-University, Greifswald, Germany. Water was doubly distilled.

2.3. Methods

The analytes were dissolved in doubly distilled water or 2-propanol (1 mg/ml). Samples were injected hydrodynamically (0.5 p.s.i., 3 s; 1 p.s.i.= 6894.76 Pa). The voltage during the analysis was 20 kV for the analysis of bases and −20 kV for acids. Analytes were detected by UV absorbance at 214 nm. The temperature during the run was 25°C. The uncoated capillary was rinsed with 0.1 *M* NaOH for 2 min prior to each analysis.

The buffer solutions were filtered through a 0.2-mm pore size filter (Schleicher/Schuell, Dassel, Germany) and degassed ultrasonically.

3. Results and discussion

3.1. Effect of TMA-β-CD on the electroosmotic flow (EOF)

Quaternary ammonium salts are known to influence the EOF by their interaction with the capillary wall [20–24]. Cationic surfactants e.g. cetyltrimethylammonium bromide [23] or dodecyltrimethylammonium bromide [20] can be adsorbed at the capillary wall of fused-silica capillaries and influence the EOF in this way. Also short-chain tetraalkylammonium reagents such as tetramethyl- or tetrabutylammonium cations lead to reduction or reversal of the EOF [24].

TMA-β-CD possesses a quaternary ammonium structure in the molecule. In former studies the ability of this CD to reverse the EOF in uncoated capillaries could be demonstrated [19]. By multiple injection of a chirally separated basic and an acidic analyte it was established that the positively charged CD moves to the cathode and does not follow the EOF to the anode [19].

Increasing the CD concentration resulted in an enhanced EOF in anode direction (Fig. 1). This reversal of EOF was already observed at very low concentrations of CD (e.g. 2 m*M*).

The EOF passes through an maximum at pH 3.5 (Fig. 1). This behaviour of EOF in dependence on pH could also be observed using a cationic surfactant, containing quaternary ammonium structure in the molecule as chiral selector in CE [25].

3.2. Chiral separations in the presence of TMA-β-CD

A variety of basic and acidic chiral compounds (Fig. 2) could be separated into enantiomers when TMA-β-CD was added to the running buffer. All resolved analytes contained an aromatic structure for hydrophobic interaction with the CD cavity and

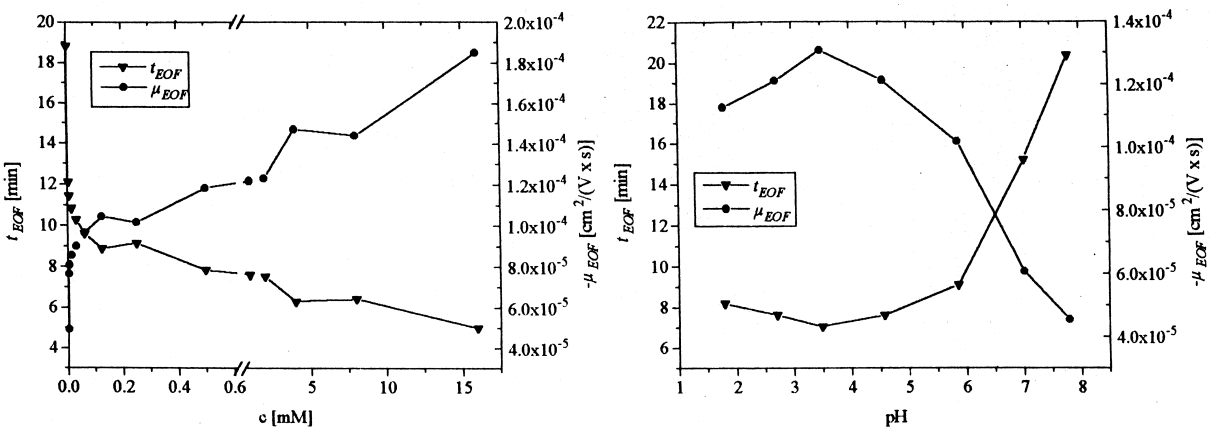

Fig. 1. Dependence of the EOF on CD concentration *c* (left) and pH value (right) marker substance: acetone conditions: 50 m*M* acetate buffer pH 5.0+TMA-β-CD (1) or 25 m*M* acetate buffer+25 m*M* phosphate buffer+16 m*M* TMA-β-CD (2); uncoated capillary.

hydrophilic groups that are responsible for potential hydrogen bonds to the secondary hydroxyl groups at the CD rim. With separated acids, additional ion pairing mechanism is imaginable.

Using neutral CDs the mobility of separated enantiomers of a charged compound is between the mobility of the free analyte and the mobility of the fully complexed analyte. The fully complexed analyte would move with the mobility of the CD which in turn moved with the same velocity as the EOF (Fig. 3A). Tait et al. [12] discussed the greater separation time window for the chiral separation of cationic analytes using an anionic CD in comparison with neutral β-CD. Due to the countercurrent mobility of the cationic CD the separation window for anionic analytes seems to be much larger in the presence of TMA-β-CD than in the presence of neutral CDs (Fig. 3B). Movement of a strong complexed acidic analyte in the opposite direction, i.e. to the anode is imaginable. For the separation of

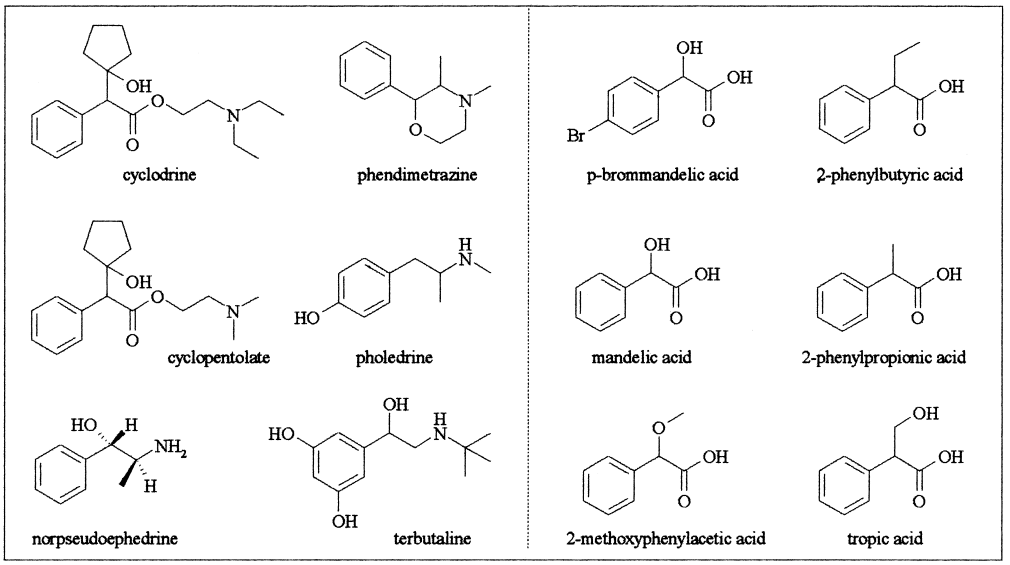

Fig. 2. Structure of separated basic and acidic analytes.

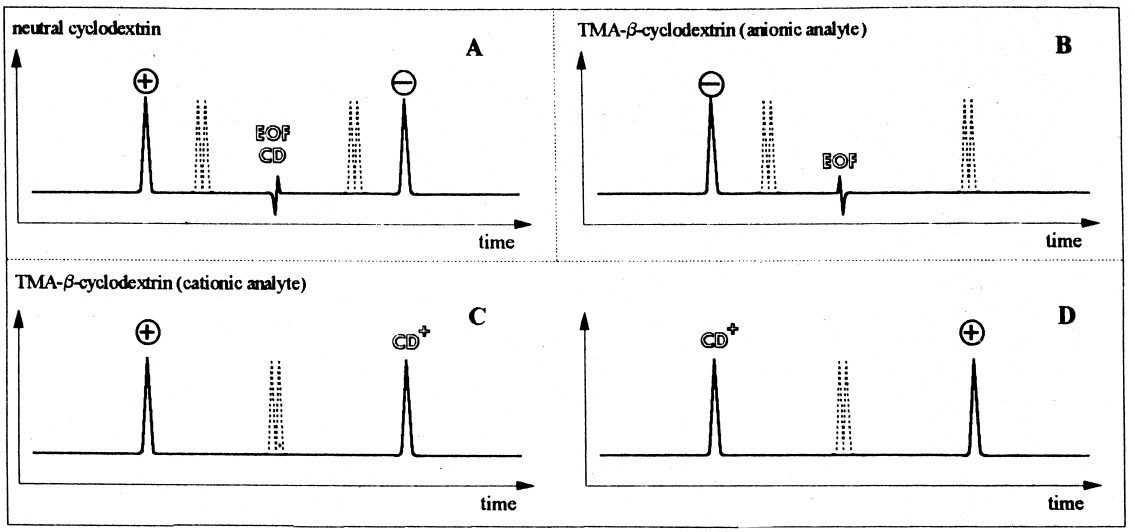

Fig. 3. Theoretical electropherograms for chiral separation of basic (+) or acidic analytes (−) with neutral CD (A) and with TMA-β-CD (B, C, D) in uncoated capillaries. A, C, D: cathode at the capillary outlet; B: anode at the capillary outlet - - - free analyte; – – – separated analyte.

cationic enantiomers, a comparatively small separation window is expected because of the similar moving direction of the CD molecule. Two different cases are possible depending on the fact whether the mobility of the CD is lower or higher than the mobility of the analyte (Fig. 3D, C).

Although the difference of the mobilities of free and complexed cationic substances is presumed to be small, a high selectivity for the separation of basic analytes especially of cyclodrine and cyclopentolate was obtained. The reversed EOF is supposed to contribute to this phenomenon. The mobility of cationic analytes is diminished by the EOF and a higher contact time of the analyte to the chiral selector results. This could be proved by the separation of cyclodrine and cyclopentolate in a coated capillary where the EOF was suppressed. The separation factor was smaller than in the uncoated capillary (Fig. 4). With TMA-β-CD in the coated capillary, better separation than with β-CD was achieved (Fig. 4), indicating that the positively-charged substituent plays an important role in the chiral recognition of cationic analytes.

3.2.1. Dependence of chiral separations on the CD concentration and the pH-value

Raising the CD concentration, an optimum value of the separation factor was observed for all basic and some acidic analytes investigated (Fig. 5). Such behaviour of the separation factor using CDs as chiral selectors was described several times [26–29].

At a CD concentration of about 16 or 25 m*M*, 2-methoxy-2-phenyl acetic acid, 2-phenylbutyric acid, 2-phenyl propionic acid and *p*-brommandelic acid move with the EOF as neutral substances. A reversal of the charge of these analytes at higher CD concentrations was not observed, as is reported for

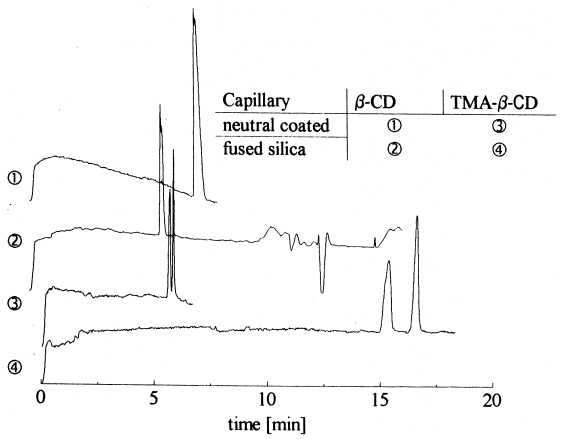

Fig. 4. Electropherograms of cyclopentolate buffer solution: 50 m*M* acetate buffer pH 5.0+16 m*M* CD.

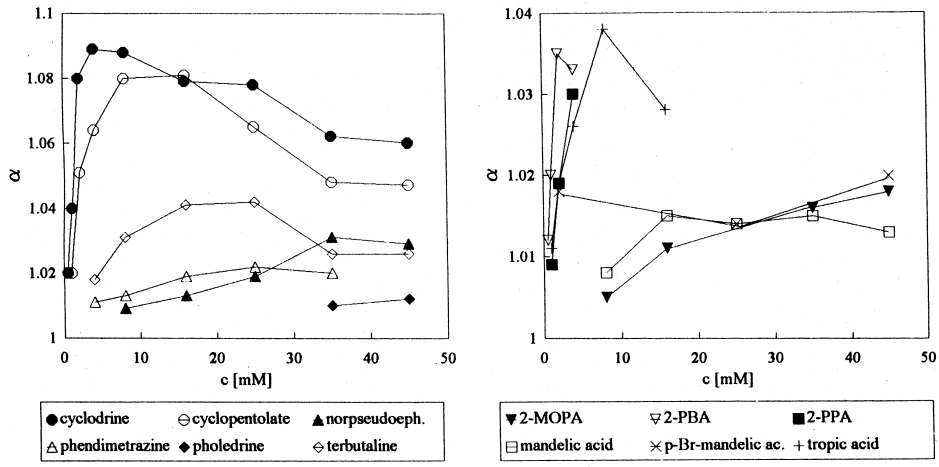

Fig. 5. Separation factor α of basic (left) and acidic analytes (right) depending on the CD concentration *c*. Conditions: 50 m*M* acetate buffer pH 5.0+TMA-β-CD; uncoated capillary.

the separation of cationic analytes with anionic CDs [13].

The separation factors shows that bases are better separated at higher pH and acids at lower pH values (Fig. 6). It is assumed that TMA-β-CD leads to better chiral separation of less charged substances than of higher charged substances because the enantiomers of acids and bases in less ionized form were better separated.

At pH 2.5 the acids exist in undissociated form so that no separation of the enantiomers with neutral CDs is possible. Using TMA-β-CD the acids were moved by the positively charged CD as cations in opposite direction, i.e. to the cathode. The enantiomers of 2-phenylbutyric acid, 2-phenyl propionic acid and tropic acid were also separated under these conditions. The separation of the neutral forms of the acids could only be realized in a coated capillary, in an uncoated capillary the substances were not detected within 45 min due to the prolonged migration times caused by the reversed EOF.

From the possibility to separate the acids via

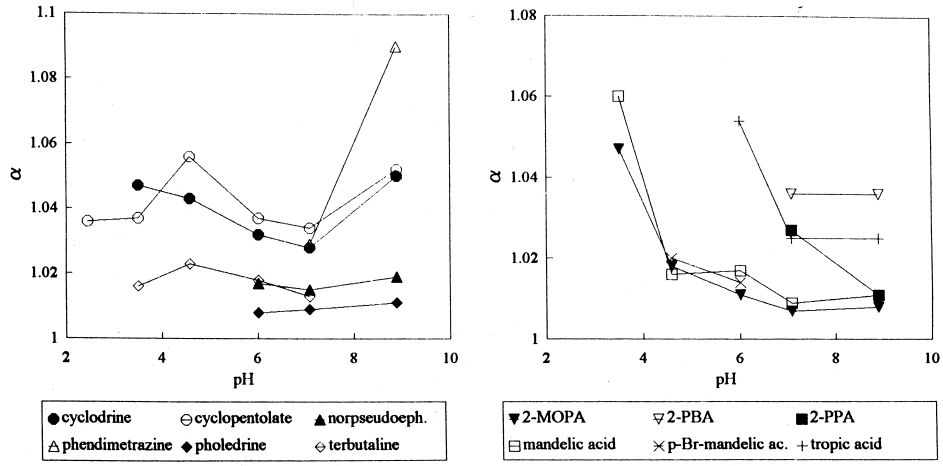

Fig. 6. Separation factor α of basic (left) and acidic analytes (right) depending on the pH value. Conditions: 25 m*M* acetate buffer+25 m*M* phosphate buffer+16 m*M* TMA-β-CD; uncoated capillary.

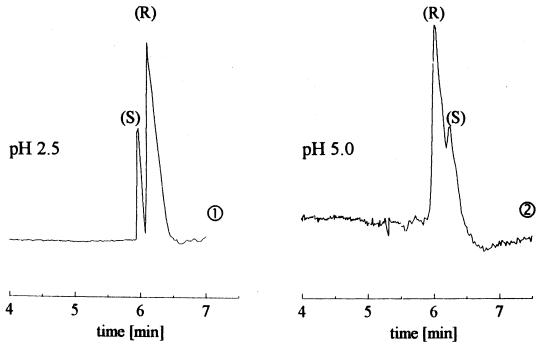

Fig. 7. Electropherograms of tropic acid [racemate spiked with (*R*)-enantiomer] conditions: 25 m*M* acetate buffer+25 m*M* phosphate buffer+16 m*M* TMA-β-CD (pH 5.0 (1) or pH 2.5 (2)); uncoated (1) or coated capillary (2); −20 kV (1) or 20 kV (2).

changing the polarity of the voltage during the analysis results the advantage that the order of the enantiomers in the electropherogram can be changed. The reversal of the enantiomers depending on the pH value of the buffer solution could be shown for tropic acid (Fig. 7).

The change of the migration order of enantiomers seems to be useful for quantitative analysis, where it is required to detect the smaller peak before the greater one, because the small peak is less overlayed by the greater peak if it is in the first position. Where peak resolution is only partial the integration of peak areas and the quantitative evaluation is more difficult [30,31].

4. Conclusions

TMA-β-CD is suitable for chiral separation of basic, acidic and neutral substances. The EOF was reversed by TMA-β-CD in fused-silica capillaries.

Due to its positive charge, the CD moves against the EOF to the cathode. The migration order of enantiomers was reversed by altering the pH value.

Acknowledgements

The TMA-β-CD was generously supplied by Wacker-Chemie GmbH (Munich, Germany).

References

[1] S. Fanali, J. Chromatogr. 474 (1989) 441.
[2] F. Bressolle, M. Audran, T.-N. Pham, J.-J. Vallon, J. Chromatogr. B 787 (1996) 303.
[3] S. Terabe, H. Ozaki, K. Otsuka, T. Ando, J. Chromatogr. 332 (1985) 221.
[4] T. Schmitt, H. Engelhardt, Chromatographia 37 (1993) 475.
[5] B. Chankvetadze, G. Endresz, D. Bergenthal, G. Blaschke, J. Chromatogr. A 717 (1995) 245.
[6] G. Endresz, B. Chankvetadze, D. Bergenthal, G. Blaschke, J. Chromatogr. A 732 (1996) 133.
[7] J. Szemán, K. Ganzler, A. Salgó, J. Szejtli, J. Chromatogr. A 728 (1996) 423.
[8] B. Chankvetadze, G. Endresz, G. Blaschke, J. Capill. Elec. 2 (1995) 235.
[9] M.G. Schmid, K. Wirnsberger, G. Gübitz, Pharmazie 51 (1996) 852.
[10] G. Blaschke, B. Chankvetadze, G. Endresz, J. Chromatogr. A 704 (1995) 234.
[11] I.S. Lurie, R.F.X. Klein, T.A.D. Cason, M.J. LeBelle, R. Brenneisen, R.E. Weinberger, Anal. Chem. 66 (1994) 4019.
[12] R.J. Tait, D.O. Thompson, O.J. Stella, J.F. Stobaugh, Anal. Chem. 66 (1994) 4013.
[13] C. Desiderio, S. Fanali, J. Chromatogr. A 716 (1995) 183.
[14] C. Dette, S. Ebel, S. Terabe, Electrophoresis 15 (1994) 799.
[15] B. Chankvetadze, G. Endresz, G. Blaschke, J. Chromatogr. A 700 (1995) 43.
[16] S. Terabe, Trends Anal. Chem. 8 (1989) 129 (Ref. H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245).
[17] N. Egashira, O. Mutoh, Y. Kurauchi, K. Ohga, Anal. Sci. 12 (1996) 503.
[18] A. Nardi, A. Eliseev, P. Bocek, S. Fanali, J. Chromatogr. 638 (1993) 247.
[19] A. Bunke, Th. Jira, Pharmazie 51 (1996) 672.
[20] T. Kaneta, S. Tanaka, H. Yoshida, J. Chromatogr. 538 (1991) 385.
[21] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[22] W.D. Pfeffer, E.S. Yeung, J. Chromatogr. 557 (1991) 125.
[23] H.-T. Chang, E.S. Yeung, Anal. Chem. 65 (1993) 650.
[24] C. Quang, M.G. Khaledi, Anal. Chem. 65 (1993) 3354.
[25] A. Bunke, Th. Jira, Pharmazie, in press.
[26] S.A.C. Wren, J. Rowe, J. Chromatogr. 603 (1992) 235.
[27] S. Pálmarsdóttir, L.-E. Edholm, J. Chromatogr. A 666 (1994) 337.
[28] L.A. St. Pierre, K.B. Sentell, J. Chromatogr. B 657 (1994) 291.
[29] T. Schmitt, H. Engelhardt, J. High Resolut. Chromatogr. 16 (1993) 525.
[30] V.R. Meyer, LC·GC Int. 7 (1994) 94.
[31] V.R. Meyer, J. Chromatogr. Sci. 33 (1995) 26.