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Chiral resolution of diols by capillary electrophoresis using borate–cyclodextrin complexation[1]

T. Jira[a,*], A. Bunke[a], M.G. Schmid[b], G. Gübitz[b]

[a]*Institute of Drug Control, Ernst-Moritz-Arndt-University Greifswald, F.-L.-Jahn straße 17, D-17489 Greifswald, Germany*
[b]*Institute of Pharmaceutical Chemistry, Karl-Franzens-University Graz, Universitätsplatz 1, A-8010 Graz, Austria*

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Abstract

In this study, the chiral separation of compounds with a *cis*-diol structure using an electrolyte containing borate and cyclodextrin (CD) is described. A dual mechanism involving both inclusion of the aromatic moiety into the cavity of the CD and the formation of mixed borate complexes is assumed to be responsible for chiral recognition. It has been shown that the separation is greatly influenced by the structure of the CD, the pH and the concentration of both the CD and borate. The method was optimized with some simple diol compounds and applied to quinazoline-(3H,1H)-4-on-2-thiones and tetrazol-5-thiones of potential pharmaceutical interest.

Keywords: Enantiomer separation; Borate complexation; Diols; Quinazoline-(3H,1H)-4-on-2-thiones; Tetrazol-5-thiones; Cyclodextrins; Borate

1. Introduction

Capillary electrophoresis (CE) has been found to be a powerful alternative to high-performance liquid chromatography (HPLC) for the separation of enantiomers. Methods of enantiomeric separation by CE are ligand-exchange, interaction of chiral analytes with cyclodextrins (CDs) and crown ethers, complexation with oligosaccharides, the use of chiral surfactants or macrocyclic antibiotics, CD-modified micellar electrokinetic chromatography (MEKC) and affinity EKC using proteins as pseudostationary phases. Reviews were given by Terabe et al. [1] and Fanali [2].

The goal of our investigations was to develop a CE method for the chiral resolution of compounds containing the *cis*-1,2-diol structure. *cis*-1,2-Diols are known to form borate complexes [3–5]. This fact has been utilized in HPLC as the principle of "borate affinity chromatography" for the separation of carbohydrates and nucleotides [6].

In CE, borate buffers were used for the separation of carbohydrates, nucleosides and several other biologically active compounds based on borate complexation [7–10]. Tadey and Purdy [11] showed that the combination of borate and β-cyclodextrin (β-CD) resulted in a significant improvement in the separation of nucleotide isomers compared to the use of either CD or borate only. The authors took the interaction of borate with the hydroxyls of both the nucleotides and CD into account.

Recently, Stefansson and Novotny [12] described

*Corresponding author.

[1] Dedicated to Prof. Dr. Beyrich, Greifswald, on the occasion of his 65th birthday.

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the chiral resolution of fluorescently labeled sugars using an electrolyte containing borate and β-CD as the chiral selector system. They describe a dual chiral recognition mechanism. In addition to inclusion of the bulky aromatic groups of the fluorescence label into the cavity of CD, the formation of mixed borate complexes between the sugar and CD is assumed to be responsible for chiral recognition.

In this paper, we describe the use of CDs together with borate for the direct resolution of non-carbohydrate analytes containing a *cis*-1,2-diol structure, e.g. *RS*-phenyl-1,2-ethanediol, *RS*-3-benzyloxy-1,2-propanediol and 1,4-di-o-benzyl-DL-threitol (model compounds) and several quinazoline-(3H,1H)-4-on-2-thiones and tetrazol-5-thiones of potential pharmaceutical interest.

2. Experimental

2.1. Methods

CE was performed using a P/ACE 2100 capillary electrophoresis instrument (Beckman, Fullerton, CA, USA) equipped with an on-column UV detector. Separations were carried out at ambient temperature in a fused-silica capillary tube (57 cm×50 μm I.D.; effective length, 50 cm). Samples were introduced hydrodynamically (0.5 p.s.i., 3 s) into the anodic end of the capillary for an injection volume of about 3 nl. The potential during analysis was 20 kV, unless indicated otherwise. Analytes were detected by UV absorption at 214 or 280 nm. The capillary was flushed with 0.1 *M* NaOH for 2 min prior to each analysis. "Gold" Software (Beckman) was used for data acquisition.

2.2. Materials

Quinazoline-(3H,1H)-4-on-2-thiones and tetrazol-5-thiones were synthesized at the Institute of Pharmaceutical Chemistry, Ernst-Moritz-Arndt-University Greifswald [13]. All reagents were of analytical grade unless indicated otherwise. Sodium borate, urea and methanol were purchased from Laborchemie Apolda (Apolda, Germany); Tris[hydroxymethyl]aminomethane (Tris) hydrochloride and 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS) were from Sigma (Deisenhofen, Germany). α-CD and β-CD were obtained from Serva (Heidelberg, Germany), 2,6-dimethyl-β-CD was from Gyogyszertechnológiai Intézet Hallgatók Laboratóriuma (Szeged, Hungary), hydroxypropyl-β-CD and γ-CD were from Wacker Chemie (Munich, Germany). Sample solutions were prepared by dissolving the analytes (1 mg) in doubly distilled, deionized water or methanol (1 ml); samples and buffer solutions were filtered through a 0.2-μm pore size filter (Schleicher/Schuell, Dassel, Germany) and degassed with helium 5.0. To increase the solubility of β-CD in aqueous buffer, 2.5% (w/w) urea was added.

3. Results and discussion

The chiral separation of different simple 1,2-diols was attempted using a CD-containing borate buffer solution. For our studies, these 1,2-diols with aromatic substituents were investigated as model compounds (I–III in Fig. 1). The influence of the CD-type, the concentration of the CD and the pH and concentration of the buffer on the chiral separation was investigated. The procedure was applied to quinazoline-(3H,1H)-4-on-2-thiones and tetrazol-5-thiones containing the diol structure in their side chains (Fig. 1). In addition to the chiral centre located in the diol group of the side chain, the

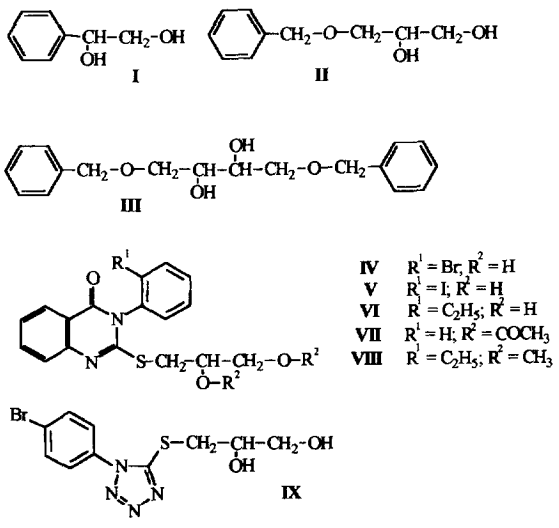

Fig. 1. Chemical structures of the compounds investigated.

quinazolinethiones show axial chirality in the molecule, due to the *ortho*-substitution of the phenyl residue.

3.1. Influence of the type of cyclodextrin

With a view to clarifying the separation mechanism, we investigated, in addition to the native α-, β- and γ-cyclodextrins, several CD derivatives, linear dextrin and D-(+)-raffinose, as chiral selectors. The formation of mixed selector–borate–analyte complexes is imaginable with the three native CDs and the linear dextrin.

Chiral discrimination, however, was only observed with β-CD (Table 1, Fig. 2) and to a lesser extent with hydroxypropyl-β-CD in borate buffer; thus, it must be assumed that stereoselective inclusion is a requirement in addition to the formation of mixed borate complexes. Obviously, only β-CD possesses a suitable cavity.

The assumed mechanism for the formation of mixed borate complexes is confirmed by the fact that no separation was obtained with electrolytes containing β-CD but with buffers such as Tris, CAPS or phosphate of equal or different ionic strength, being used instead of borate. Furthermore, if inclusion into the cavity was the only reason for the resolution, separation with other β-CD derivatives would be expected. Partial resolution of all analytes was observed with hydroxypropyl-β-CD, but not with 2,6-dimethyl-β-CD. This fact would argue for a complexation with the diol structure at C-2 and C-3 in β-CD, since in 2,6-dimethyl-β-CD the hydroxyl at C-2 is blocked and only the hydroxyl at C-3 is available. In hydroxypropyl-β-CD with a varying substitution pattern, diol structures are present in limited amounts. However, the hydroxyls at C-2 and C-3 in β-CD are in the *trans*-position and borate complexation occurs favorably with *cis*-diols. NMR investigations of a mixture of β-CD, borate and a sugar derivative, performed by Stefansson and Novotny [12] showed that the H-3 proton was shifted upfield while the H-2 proton was unaffected, leading to the assumption that only the hydroxyl at C-3 is involved in complexation. However, the negative results obtained in our group with 2,6-dimethyl-β-CD, in which the hydroxyl at C-3 is available, do not agree with this interpretation. As an alternative interaction, the formation of hydrogen bonds may be taken into account. We are currently trying to elucidate the details of the mechanism.

3.2. Influence of the pH value, and the concentrations of CD and borate

The pH dependence of the chiral separation factor α ($\alpha = \mu_{eff2}/\mu_{eff1}$) was investigated in the pH range from 8 to 10 (Fig. 3). Generally, resolution was observed for all compounds in a pH range between 8.5 and 9.3. At pH 10, neither the model compounds (I–III) nor the quinazolines were resolved; only the tetrazol derivative IX showed partial resolution, which improved with decreasing pH. Below pH 8.15, the resolution of the quinazolines decreased and for

Table 1
Optical resolution of the compounds investigated

Number	Compound	t_1 (min)	μ_{eff} ($cm^2/V \times min$)	α	R_s
I	*RS*-Phenyl-1,2-ethanediol[a]	9.76	$-3.26 \cdot 10^{-3}$	1.047	0.723
II	*RS*-3-Benzyloxy-1,2-propanediol[a]	9.57	$-2.88 \cdot 10^{-3}$	1.088	1.045
III	1,4-Di-*o*-benzyl-DL-threitol[a]	10.02	$-3.77 \cdot 10^{-3}$	1.000	0.000
IV	3-*o*-Bromphenyl-2-(2,3-dihydroxy-propylthio)-4(3H)-quinazolinone[b]	10.47	$-3.09 \cdot 10^{-3}$	1.013	1.445
V	3-*o*-Iodphenyl-2-(2,3-dihydroxy-propylthio)-4(3H)-quinazolinone[a]	10.63	$-3.29 \cdot 10^{-3}$	1.019	1.431
VI	3-*o*-Ethylphenyl-2-(2,3-dihydroxy-propylthio)-4(3H)-quinazolinone[b]	10.61	$-3.29 \cdot 10^{-3}$	1.015	1.044
VII	2-(2,3-Diacetylpropylthio)-3-phenyl-2-4(3H)-quinazolinone[a]	10.58	$-3.23 \cdot 10^{-3}$	1.000	0.000
VIII	3-*o*-Ethylphenyl-2-(2,3-dimethoxy-propylthio)-4(3H)-quinazolinone[a]	10.36	$-2.95 \cdot 10^{-3}$	1.000	0.000
IX	1-*p*-Bromphenyl-5-2,3-dihydroxypropylthiotetrazol[a]	10.85	$-3.17 \cdot 10^{-3}$	1.021	2.136

Buffer: 1.8% β-CD and 50 m*M* (I–III, V, VII–IX) or 100 m*M* (IV, VI) borate, pH 9.3. Migration time of the first eluting enantiomer; the separation factor α ($\alpha = \mu_{eff2}/\mu_{eff1}$) and the resolution factor R_s are given.
Conditions: [a] 1.8% β-CD, 50 m*M* borate, pH 9.3, U=15 kV, λ=214 nm. [b] 1.8% β-CD, 100 m*M* borate, pH 9.3, U=20 kV, λ=280 nm.

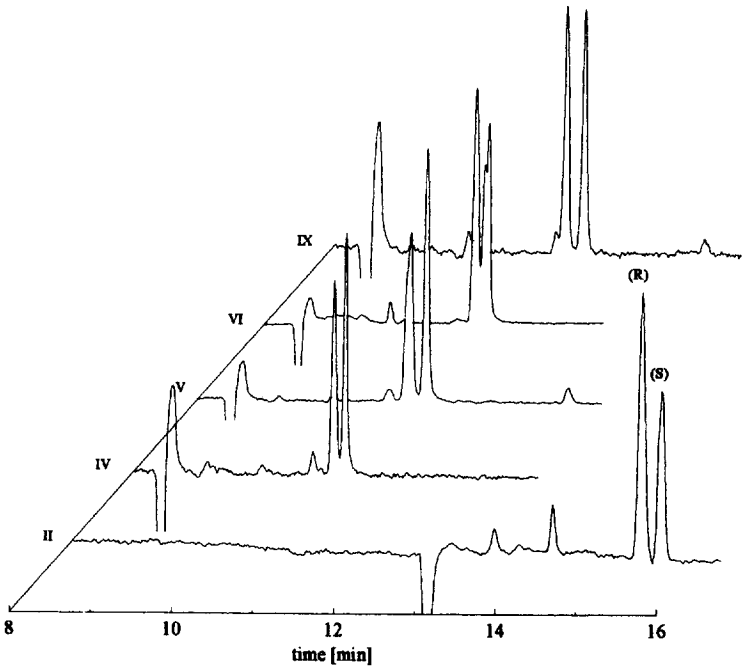

Fig. 2. Electropherogram of the chiral resolution of compounds II, IV–VI and IX. Conditions: II, 1.8% β-CD, 0.1 *M* Tris and 50 m*M* borate, pH 9.3, λ=214 nm, U=15 kV; V, IX: 1.8% β-CD, 50 m*M* borate, pH 9.3, λ=214 nm, U=15 kV; IV, VI: 1.8% β-CD, 100 m*M* borate, pH 9.3, λ=280 nm, U=20 kV.

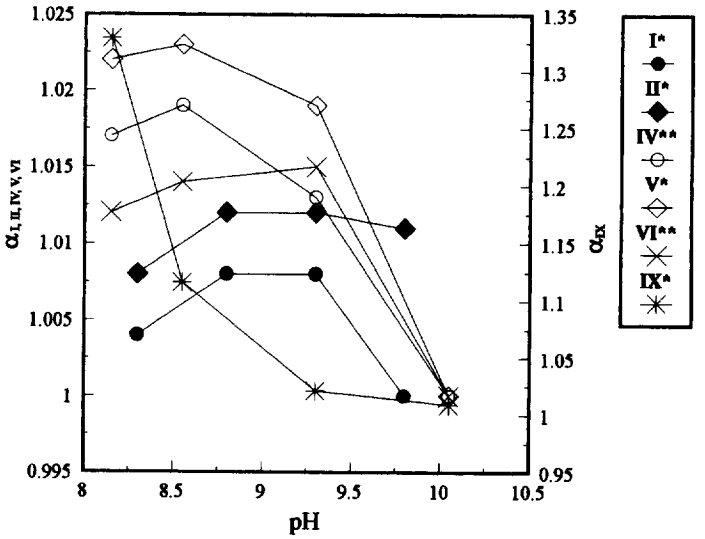

Fig. 3. Dependence of the separation factor α on pH. Conditions: * 1.8% β-CD, 50 m*M* borate (I, II, V, IX); ** 1.8% β-CD, 100 m*M* borate (IV, VI).

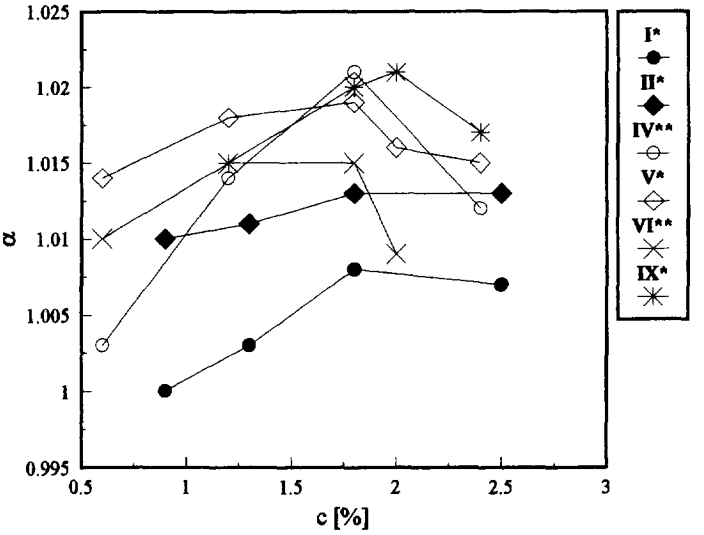

Fig. 4. Influence of the concentration of β-CD on the separation factor α. Conditions: * β-CD, 50 m*M* borate, pH 9.3 (I, II, V, IX); ** β-CD, 100 m*M* borate, pH 9.3 (IV, VI).

compounds I and II, resolution only occurred within a rather narrow pH range.

Raising the concentration of β-CD resulted in an improvement in the separation of all analytes (Fig. 4), reaching an optimum at about 1.8% (w/w). A further increase in concentration resulted in a decrease in the α-value. The fact that the separation factor passes an optimum that is dependent on the concentration of CD has already been described for other capillary electrophoretic separations of enantiomers [14,15].

Dependence on the concentration of borate was investigated over a range between 10 and 150 m*M* (Fig. 5). While for compounds I, II, V and IX, a

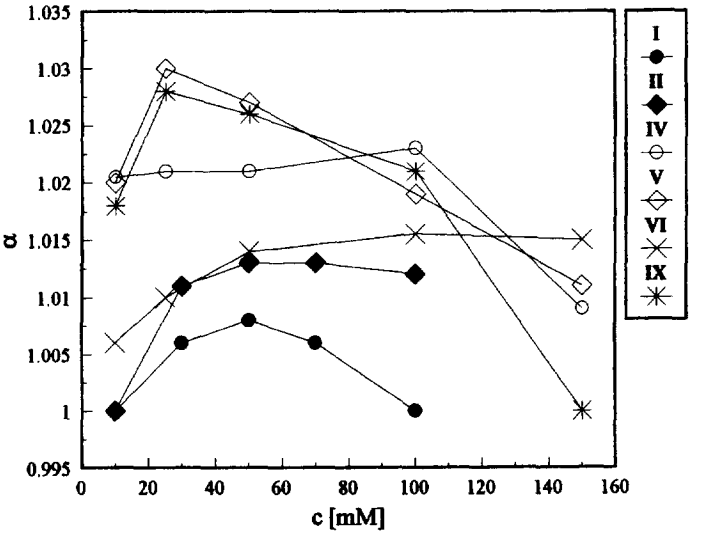

Fig. 5. Influence of the concentration of borate on the separation factor α. Conditions: 1.8% β-CD, borate, pH 9.3.

borate concentration in the range of 25 to 50 m*M* was found to be optimal, an optimum of 100 m*M* was observed for compounds IV and VI. At higher borate concentrations, the separation factor decreased again. By adding 0.1 *M* Tris to the electrolyte, the migration time was increased and compound II was baseline resolved (Fig. 2). No resolution was obtained with compound III, probably due to the position of the hydroxyl groups. The chiral resolution of the tetrazol derivative IX is clearly attributed to the chiral centre in the side chain. To determine whether the resolutions are based on the central chirality or atropisomerism in the case of the quinazolines, the enantiomer of VI with the *R*-configuration in the side chain was investigated. In contrast to the racemate of VI, no separation was obtained in this case. Accordingly, the two peaks observed are related to the *R*- and *S*-enantiomers, based on the chiral centre at the diol group in the side chain. Compounds in which the hydroxyl groups are esterified or etherized were not resolved. These findings are a further indication that complexation of the *cis*-1,2-diol structure with the borate is an essential part of the chiral recognition mechanism.

When the borate concentration was reduced to 25 m*M*, the electropherogram of compound VI showed a third peak (Fig. 6). Since compound VI possesses two chiral elements, four peaks are to be expected. The double peak area of the first peak indicates that two of the four stereoisomers were not resolved. Under identical conditions, analyte VI with the *R*-configuration in the side chain had only one peak. Therefore, the peak with the larger area in the electropherogram of the racemate of VI is related to the isomers *RR* and *RS*, which have the *R*-configuration in the side chain. This has been confirmed by the conformity of the migration times in the electropherograms of VI (racemate) and VI (*R*). The remaining isomers have the *S*-configuration in the side chain and therefore the two smaller peaks correspond to the diastereomeric pairs *SS* and *SR*.

It has turned out that the axial chiral structure can only be resolved if there is an *S*-configuration at the chiral centre of the side chain. The separation of a third peak can also be observed with compound V; however, in this case the first two peaks are partially resolved and the third peak shows a larger peak area. This partial separation might also be related to the

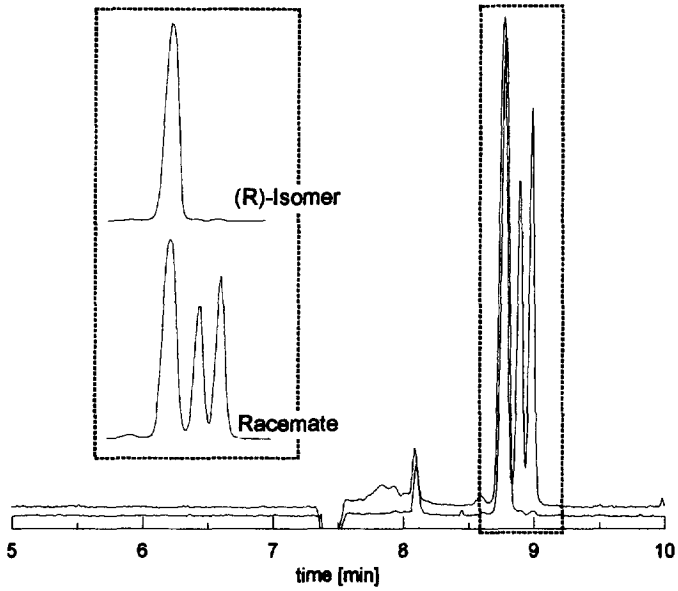

Fig. 6. Electropherogram of the chiral resolution of compound VI. Conditions: 1.8% β-CD, 25 m*M* borate, pH 9.3, λ=280 nm, U=20 kV.

resolution of the axial structure, but this cannot be proved yet, because authentic enantiomers are not available.

4. Conclusion

The combination of β-CD and borate has been shown to be an effective tool for the chiral separation of 1,2-diols. A dual chiral recognition mechanism based on both inclusion into the chiral cavity of the CD and borate complexation with the diol groups is proposed. The postulation of this mechanism is based on three observations: First, no chiral separation was obtained when borate was substituted by other buffers. Second, when CD derivatives with blocked hydroxyl groups were used, no resolution was observed. Third, analytes with esterified or etherized diol groups were not resolved.

The detailed investigation of the mechanism will be the subject of further studies.

Acknowledgments

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References

[1] S. Terabe, K. Otsuka and H. Nishi, J. Chromatogr., 666 (1995) 295.
[2] S. Fanali, J. Chromatogr. A, 735 (1996) 77.
[3] J. Böeseken, Advan. Carbohydrate Chem., 4 (1949) 189.
[4] M. Mazurek and A.S. Perlin, Can. J. Chem., 41 (1963) 2403.
[5] H.B. Davis and C.J.B. Mott, J. Chem. Soc. Faraday Trans., 76 (1980) 1991.
[6] E. Hagemeier, K. Kemper, K.-S. Boos and E. Schlimme, J. Chromatogr., 282 (1983) 663.
[7] S. Honda, S. Iwase, A. Makino and S. Fujiwara, Anal. Biochem., 176 (1989) 72.
[8] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann and H.M. Widmer, Anal. Chem., 63 (1991) 1541.
[9] P.J. Oefner, A.E. Vorndran, E. Grill, C. Huber and G.K. Bonn, Chromatographia, 34 (1992) 308.
[10] J.P. Landers, R.P. Oda and M.D. Schuchard, Anal. Chem., 64 (1992) 2864.
[11] T. Tadey and W.C. Purdy, J. Chromatogr. B, 657 (1994) 365.
[12] M. Stefansson and M. Novotny, J. Am. Chem. Soc., 115 (1993) 11573.
[13] Th. Jira et al., Pharmazie, 51 (1996) 273.
[14] I. Bechet, P. Paques, M. Fillet, P. Hubert and J. Crommen, Electrophoresis, 15 (1994) 818.
[15] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235.