ELSEVIER

Journal of Chromatography A, 746 (1996) 91–101

JOURNAL OF CHROMATOGRAPHY A

Comparison of liquid and supercritical fluid chromatography using naphthylethylcarbamoylated-β-cyclodextrin chiral stationary phases[1]

Karen L. Williams*, Lane C. Sander, Stephen A. Wise

Chemical Science and Technology Laboratory, Analytical Chemistry Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-0001, USA

Abstract

A comparison of liquid (LC) and sub- or supercritical fluid chromatography (SFC) was performed using naphthylethylcarbamoylated-β-cyclodextrin chiral stationary phases (NEC-CD CSPs). Compounds resolved in the normal-phase, reversed-phase and polar organic modes on the NEC-CD CSPs in LC were also separated on the same columns in SFC with a carbon dioxide–alcohol eluent. Advantages of SFC for chiral separations on these CSPs included simple eluents, rapid optimization of selectivity and improved resolution compared to LC results for the same columns. The effect of alcohol modifier was also investigated and the results provided insights into the chiral recognition mechanisms in SFC. The versatility of the NEC-CD CSPs in SFC was demonstrated by performing separations representative of the three distinct mobile phase modes in LC in a single analysis in SFC.

Keywords: Chiral stationary phases, LC; Chiral stationary phases, SFC; Enantiomer separation; Cyclodextrins

1. Introduction

As the awareness of the role of chirality in biological activity and toxicity has increased, the separation of optical isomers has become an important technique in the development of new pharmaceutical and agricultural chemicals [1,2]. Although the evolution of chiral stationary phases (CSPs) for liquid chromatography (LC) has paved the way for the separation of many racemates, the resolution needed for measurement of enantiomeric purity has remained elusive [3,4]. In sub- or supercritical fluid chromatography (SFC), the lower viscosities of the eluents and higher diffusivities of the solutes often translate into improved peak resolution when compared to LC methods [5]. Another consequence of the low eluent viscosity is the ability to use higher flow-rates in SFC without serious losses in efficiency [6]. As a result, analysis time in SFC is often reduced relative to LC [7]. Rapid column equilibration in SFC after changes in chromatographic parameters shortens method development time and ease of mobile phase removal makes SFC attractive for preparative scale separations of enantiomers [8,9]. Because of these potential advantages, the utilization of SFC for chiral separations has recently been the subject of numerous studies [6,10,11]. However, the majority of these studies have focused on columns traditionally used in the normal-phase mode in LC because the polarity of carbon dioxide has often been equated with that of hexane [12,13]. Stationary phases commonly used under reversed-phase conditions have received only cursory exami-

*Corresponding author.

[1]Contribution of the National Institute of Standards and Technology. Not subject to copyright.

0021-9673/96/$15.00 Published by Elsevier Science B.V.
PII S0021-9673(96)00291-9

nations. As a result, some of the potential of SFC for chiral separations has remained untapped.

Of the CSPs currently commercially available for LC, cyclodextrin-based stationary phases are among the most versatile. Modification or derivatization of the cyclodextrin (CD) can be used to vary the selectivity of the bonded phase [14]. Naphthylethylcarbamate derivatives of CD-based CSPs were initially reported for the resolution of enantiomers under normal-phase conditions in LC. Reaction of the CD with either (*R*)- or (*S*)-1-(1-naphthyl)ethyl isocyanate introduced an additional chiral center as well as additional sites for hydrogen bonding and dipole–dipole interactions [15]. In contrast to the native β-CD CSPs, inclusion complex formation was not believed to be involved in the chiral recognition of the naphthylethylcarbamoylated-β-cyclodextrin (NEC-CD) phases in the normal-phase mode. In fact, the configuration of the carbamate substituent was found to dominate the chiral recognition process in many instances, as evidenced by the reversal of elution order when changing from the (*R*)- to the (*S*)-naphthylethyl-carbamate substituted CD CSP [16].

Subsequently, the NEC-CD phases were also shown to be highly effective for reversed-phase LC separations [17]. Compounds that are resolved in the reversed-phase mode generally are not resolved on the same column in the normal-phase mode. This is not surprising because different chiral recognition processes are believed to be operative in the different mobile phase modes. In the reversed-phase mode, the CD is believed to be the primary source of chiral selectivity, although the carbamate substituents clearly play a role because the (*R*)- and (*S*)-naphthylethylcarbamoylated-CD CSPs (*R*-NEC- and *S*-NEC-CD CSPs) often exhibit nonequivalent enantioselectivity and some analytes are resolved on either the *R*-NEC- or *S*-NEC-CD CSP but not on both [18,19].

In the polar organic mode, a third mode of operation of the NEC-CD CSPs, acetonitrile is used in conjunction with small amounts of triethylamine and acetic acid. The addition of methanol is used to control retention. This method of operation extends the applicability of these phases in LC to compounds that can not be resolved in either the normal- or reversed-phase modes. Inclusion complexation is not believed to play a role in chiral selectivity in the polar organic mode. Instead, the analyte is believed to straddle the mouth of the CD in order to maximize hydrogen bonding interactions [20].

Despite the obvious versatility of the NEC-CD CSPs, the wide variety of possible eluent systems in LC can present problems for method development. Although examination of structural features of the analyte can provide some guidance in choosing chromatographic conditions [17,21], substantial time and experimentation may be involved in optimizing chiral selectivity. As a result, a system that simplifies the choice of mobile phase composition would reduce the time needed for method development. Recently, the enantioresolution in SFC of racemates typically resolved in either the polar organic or reversed-phase modes in LC was reported [22]. A simple eluent comprised of carbon dioxide with an alcohol modifier was used to resolve compounds having both acidic and basic structural features. Given the multimodal capabilities of the NEC-CD CSPs, it seemed likely that the use of SFC could also be extended to the separation of compounds generally resolved in the normal-phase mode in LC. Therefore, SFC would provide a convenient bridge between the three different mobile phase modes of these CSPs. A comparison of LC and SFC was performed using *R*-NEC- and *S*-NEC-CD CSPs to investigate this possibility. The separation of compounds typically resolved in each of the three distinct mobile phase modes in LC was attempted on the same column in SFC.

2. Experimental

2.1. Chemicals

Carbon dioxide (SFC grade) was obtained from Scott Specialty Gases (Plumsteadville, PA, USA). The analytes were obtained from Sigma (St. Louis, MO, USA), Aldrich Chemical Company (Milwaukee, WI, USA), and the United States Pharmacopeial Convention (Rockville, MD, USA). When available, the individual enantiomers were used to determine elution order. All solvents and modifiers were HPLC grade. The N-3,5-dinitrobenzoyl derivatives of the amines and amino acid esters were

prepared by reacting the analyte with a stoichiometric amount of 3,5-dinitrobenzoyl chloride (Aldrich) in tetrahydrofuran at 60°C for 15 min. The solvent was removed under a stream of nitrogen and the sample was dissolved in methanol. All analytes were initially dissolved in methanol at a concentration of 2.0 mg/ml and additional dilutions in methanol were made as needed.

2.2. Instrumentation[2]

Liquid chromatographic separations were performed at ambient temperature (22°C) and a flow-rate of 1.0 ml/min was used for all experiments. The column eluent was monitored at 254 nm. Sample size was 20 μl. Supercritical fluid chromatography was performed using a commercial chromatographic system consisting of a supercritical fluid pump and a modifier pump. Column temperature was controlled by the column oven and the eluent was monitored with a diode array detector. Samples were introduced by an autosampler with a 5 μl internal loop. Flow-rates for all chromatographic experiments were 2.0 ml/min and the pressure was 15 MPa. Modifier concentrations are given as volume percentages of the total flow-rate.

2.3. Chiral stationary phases

Cyclobond I 2000 RN, (*R*)-naphthylethylcarbamoylated-β-cyclodextrin and Cyclobond I 2000 SN, (*S*)-naphthylethylcarbamoylated-β-cyclodextrin, 25 cm×0.46 cm, 5 μm particle size, were obtained from Advanced Separation Technologies, (Whippany, NJ, USA). The *R*-NEC-CD phase has a higher degree of carbamate substitution (6–7 carbamate substituents per β-CD ring) than the *S*-NEC-CD phase (3–4 carbamate substituents per β-CD ring) [18]. The chromatographic columns were flushed with methanol before connection to the detector of the supercritical fluid chromatograph. Columns were stored in methanol or acetonitrile when not in use.

2.4. Mobile phases

Liquid chromatographic separations under normal-phase conditions were performed using mixtures of 2-propanol in hexane. For reversed-phase separations, a 1% triethylammonium acetate buffer was prepared by adding 1% (v/v) triethylamine to water and adjusting the pH with glacial acetic acid. Mobile phases consisted of various mixtures of acetonitrile in aqueous buffer. In the polar organic mode, eluents were prepared using acetonitrile–methanol mixtures with small amounts of triethylamine and glacial acetic acid added [23].

3. Results and discussion

3.1. Normal-phase mode

Compounds resolved on the NEC-CD CSPs under normal-phase conditions in LC include racemates having a π-acidic group that can interact with the π-basic naphthyl moieties of the CSP. Hydrogen bonding and π-acid–π-base interactions are believed to dominate the chiral recognition process [24]. Nonchiral derivatization of the solute with 3,5-dinitrobenzoyl chloride can be used to strengthen analyte–CSP interactions. To gauge the effectiveness of SFC for separations analogous to those obtained in the normal-phase in LC, the N-3,5-dinitrobenzoyl derivatives of various amines and amino acid esters were prepared and used as test compounds for the *S*-NEC-CD CSP. Liquid chromatographic conditions for the derivatized compounds were chosen based on the results of Stalcup et al. [16]. For SFC, an initial methanol modifier concentration of 10% was used as a starting point. A summary of the results comparing LC and SFC on the *S*-NEC-CD column is provided in Table 1. The chromatographic conditions listed in the table for LC and SFC represent optimized conditions for each technique.

A comparison of the enantioresolution of derivatized phenylalanine methyl ester in LC and SFC is illustrated in Fig. 1. As can be seen from the figure,

[2]Certain commercial equipment, instruments or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1
Comparison of chromatographic results for N-(3,5-dinitrobenzoyl) derivatized analytes in LC and SFC on the *S*-NEC-CD CSP

Compound		k'^{a}	α	R_s	Mobile phase
Alanine methyl ester	LC	4.12 (D)	1.49	3.7	70:30[b]
	SFC	4.23 (D)	1.31	4.7	90:10[c]
Alanine ethyl ester	LC	2.70 (D)	1.60	4.2	70:30
	SFC	3.78 (D)	1.31	4.6	90:10
Norleucine methyl ester	LC	2.25	1.65	4.4	70:30
	SFC	3.55	1.31	4.6	90:10
Valine methyl ester	LC	2.58 (D)	1.79	4.9	70:30
	SFC	2.80 (D)	1.43	5.8	90:10
Phenylalanine	LC	4.10 (D)	1.29	2.3	60:40
methyl ester	SFC	5.11 (D)	1.25	4.0	85:15
4-Chlorophenylalanine	LC	3.13	1.25	2.0	60:40
ethyl ester	SFC	5.08	1.14	2.3	85:15
2-Aminoheptane	LC	6.55	1.17	1.2	90:10
	SFC	9.27	1.14	2.6	95:5
1-Cyclohexylethylamine	LC	3.60 (*R*)	1.23	1.7	80:20
	SFC	6.73 (*R*)	1.45	5.9	90:10
α-Methylbenzalamine	LC	3.29 (*R*)	2.10	6.8	70:30
	SFC	3.55 (*R*)	1.56	7.8	80:20
1,2,3,4-Tetrahydro-1-	LC	2.15	1.92	5.0	70:30
naphthylamine	SFC	3.51	1.46	6.4	80:20

[a] Configuration of the first eluting enantiomer is shown in parentheses, when known.
[b] Mobile phases for LC are volume ratios of hexane-2-propanol.
[c] Mobile phases for SFC are volume ratios of carbon dioxide-methanol.

the analysis time was reduced from nearly 25 min in LC to less than 15 min in SFC while selectivity remained almost unchanged. In addition, resolution (R_s) increased from 2.3 in LC to 4.0 in SFC.

Fig. 2 portrays the separation of derivatized 1-cyclohexylethylamine. Although both analyses were completed in less than 20 min, selectivity (α) in SFC exceeded that of LC and resolution improved dramatically.

Retention trends in SFC correlated well with retention behavior observed in LC. Solutes having aromatic moieties, such as phenylalanine methyl ester and 1,2,3,4-tetrahydro-1-naphthylamine, required a higher concentration of polar modifier (2-propanol in LC, methanol in SFC) than the aliphatic compounds such as 2-aminoheptane. Presumably, aromaticity presents additional opportunities for π–π interactions between the analyte and the CSP, resulting in increased retention [25].

Under the conditions employed, the selectivity obtained in SFC was generally lower than that achieved for the same analyte in LC. However, a comparison of alcohol modifiers revealed that enantioselectivity in SFC approached that of LC when 2-propanol was used as a modifier for the analytes in Table 1. The effect of modifier on the enantioresolution of derivatized α-methylbenzylamine is shown in Fig. 3. Although the use of ethanol or 2-propanol as a modifier increased the separation factor and the resolution, the analysis time also increased. Methanol produced the shortest analysis times for the derivatized analytes and resolution in SFC still exceeded that of LC for all of the derivatized compounds investigated, as shown in Table 1.

Because a reversal of elution order often occurs in LC in the normal-phase mode when switching from the *R*-NEC-CD column to the *S*-NEC-CD phase, a comparison of elution order for the two columns was conducted in SFC. The chromatographic results are tabulated in Table 2. With the exception of 1-cyclohexylethylamine, the elution order of all the compounds in Table 2 on the *R*-NEC-CD phase was the opposite of that observed on the *S*-NEC-CD phase. The same elution order patterns for these compounds have been observed in LC [16], suggesting that the chiral separation mechanisms for the two chromatographic techniques are similar. The reversal of elution order also emphasizes the importance of the

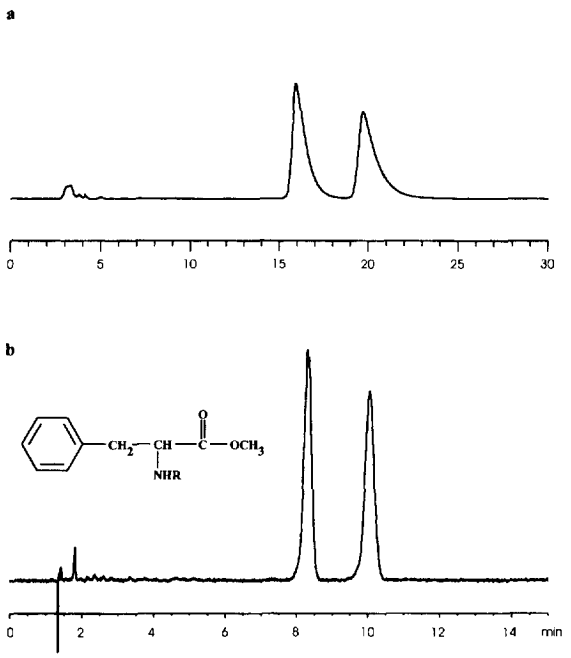

Fig. 1. Separation of N-(3,5-dinitrobenzoyl)-DL-phenylalanine methyl ester on the *S*-NEC-CD CSP. Chromatographic conditions: (a) hexane–2-propanol (60:40), 1.0 ml/min, (b) carbon dioxide–methanol (85:15), 2.0 ml/min, 30°C, 15 MPa.

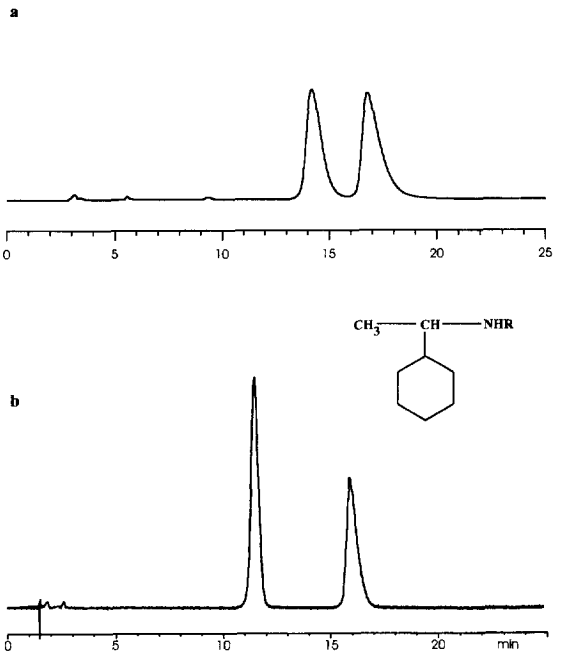

Fig. 2. Separation of (N-3,5-dinitrobenzoyl)-*RS*-1-cyclohexylethylamine on the *S*-NEC-CD CSP. Chromatographic conditions: (a) hexane–2-propanol (80:20), 1.0 ml/min, (b) carbon dioxide–methanol (90:10), 2.0 ml/min, 30°C, 15 MPa.

naphthylethylcarbamate substituent configuration in the chiral recognition process for these analytes.

3.2. Reversed-phase mode

In contrast to separations in the normal-phase mode, enantioseparation on NEC-CD CSPs in the reversed-phase mode in LC is believed to involve inclusion complexation [21]. Recommended mobile phases in LC consist of aqueous buffer–acetonitrile mixtures, and pH is often a crucial parameter in optimizing selectivity [17]. Separation of a number of underivatized analytes was attempted in LC and SFC on the *R*-NEC- and *S*-NEC-CD columns to compare the selectivity and chiral recognition mechanisms. Liquid chromatographic conditions were chosen based on the reports of Armstrong et al. [19]. For SFC, methanol was initially used as the modifier, although other alcohol modifiers were investigated. Chromatographic parameters were chosen to optimize selectivity and resolution for each technique, and a comparison of LC and SFC results for underivatized agricultural and pharmaceutical compounds on the *R*-NEC-CD and *S*-NEC-CD CSPs is shown in Table 3.

Examination of the data in Table 3 reveals that retention, measured by the capacity factor (k′), was higher in SFC than in LC for the compounds studied. An extreme case of this difference is illustrated by the chromatographic data for 5-(4-hydroxyphenyl)-5-phenylhydantoin. Retention in SFC (k′=36.24) was much longer than in LC (k′=8.51). However, it should be noted that, given the nonpolar nature of carbon dioxide, the eluents in SFC were substantially less polar than the mobile phases used in LC. Therefore, it is not surprising to find large differences in retention for the two techniques.

Separation of the enantiomers of bendroflumethiazide in LC and SFC is shown in Fig. 4. In SFC, a simple carbon dioxide–methanol eluent was used and optimization of chiral selectivity was rapidly achieved because equilibration of the column after changes in chromatographic parameters required only a few min. In contrast, equilibration of

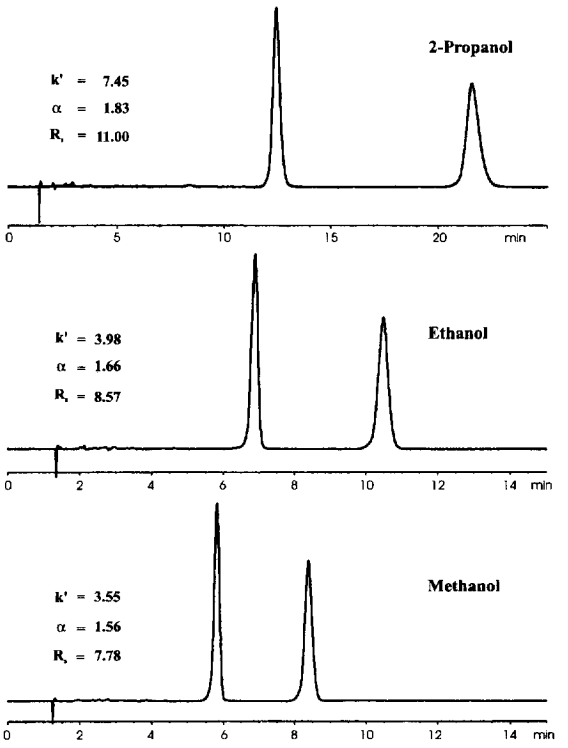

Fig. 3. Effect of modifier on the separation of N-(3,5-dinitrobenzoyl)-α-methylbenzylamine on the *S*-NEC-CD CSP. Chromatographic conditions: carbon dioxide–alcohol (90:10), 2.0 ml/min, 30°C, 15 MPa.

the LC system often required more than 1 h before sample analysis could be performed. Use of aqueous buffers in LC also decreases the column lifetime because of gradual dissolution of the stationary phase, especially at high pH.

Successful enantioseparations of both acidic and basic compounds were readily achieved in SFC and the use of acidic or basic additives to the alcohol modifier was not required. In addition, separations in SFC did not require an aqueous-organic mobile phase, a condition thought to be essential for enantioseparations of the compounds in Table 3 in the reversed-phase mode in LC [14].

Chromatographic results in SFC did not always parallel those of the reversed-phase mode in LC. For example, enantioresolution in SFC of cromakalim is shown in Fig. 5. No separation of this analyte could be achieved in LC, as shown in Table 3. Separation of the enantiomers of tolperisone, which was not possible in SFC, was readily achieved in LC. For the majority of the compounds in Table 3, however, SFC offered enantioselectivity comparable to LC and resolution in SFC equaled or exceeded the resolution in LC.

Investigation of different alcohol modifiers provided insight into similarities between the chiral recognition mechanisms in SFC and the reversed-phase mode in LC. The effect of three different

Table 2
Comparison of chromatographic results in SFC for N-(3,5-dinitrobenzoyl) derivatized compounds on the *R*-NEC- and *S*-NEC-CD CSPs[a]

Compound	CSP	k'[b]	α	R_s	% Modifer
Alanine methyl ester	*R*[c]	6.02 (L)	1.21	5.2	10
	S[d]	4.23 (D)	1.31	4.7	10
Alanine ethyl ester	*R*	5.22 (L)	1.18	4.6	10
	S	3.78 (D)	1.31	4.6	10
Leucine methyl ester	*R*	4.51 (L)	1.27	5.8	10
	S	2.79 (D)	1.29	4.1	10
Valine methyl ester	*R*	3.73 (L)	1.37	8.3	10
	S	2.80 (D)	1.43	5.8	10
Phenylalanine methyl ester	*R*	7.22 (L)	1.31	6.3	15
	S	5.11 (D)	1.25	4.0	15
1-Cyclohexylethylamine	*R*	9.44 (*R*)	1.64	11.7	10
	S	6.73 (*R*)	1.45	5.9	10
α-Methylbenzylamine	*R*	5.41 (*S*)	1.28	5.6	20
	S	3.55 (*R*)	1.56	7.8	20

[a] Chromatograhic conditions: carbon dioxide–methanol, 2.0 ml/min, 30°C, 15 MPa.
[b] Confguration of the first eluting enantiomer is shown in parentheses.
[c] (*R*)-naphthylethylcarbamoylated-β-cyclodextrin chiral stationary phase.
[d] (*S*)-naphthylethylcarbamoylated-β-cyclodextrin chiral stationary phase.

Table 3
Comparison of chromatographic results for SFC and reversed phase LC on the *R*-NEC- and *S*-NEC-CD CSPs

Compound	CSP		k'	α	R_s	Mobile phase
Ancymidol	*S*	LC	4.72	1.14	1.3	80:20 (7.0)[a]
	S	SFC	6.32	1.08	1.3	90:10[b]
Bendroflumethiazide	*S*	LC	6.36	1.22	1.9	70:30 (4.5)
	S	SFC	9.95	1.11	1.9	70:30
Cromakalim	*S*	LC	2.19	1.00	0.0	80:20 (4.5)
	S	SFC	10.25	1.08	1.5	96:4
5-(4-Hydroxyphenyl)-5-phenylhydantoin	*R*	LC	8.51	1.10	0.7	80:20 (4.5)
	R	SFC	36.24	1.15	1.5	85:15
Ibuprofen	*S*	LC	3.26	1.14	0.6	70:30 (4.5)
	S	SFC	6.14	1.06	1.0	95:5
Mephenytoin	*S*	LC	1.29	1.22	1.3	70:30 (4.1)
	S	SFC	3.15	1.25	3.0	95:5
Piperoxan	*S*	LC	1.20	1.15	0.6	80:20 (4.5)
	S	SFC	3.88	1.08	0.7	90:10
Tolperisone	*S*	LC	1.63	1.11	0.9	80:20 (4.5)
	S	SFC	6.77	1.00	0.0	90:10
Tropicamide	*S*	LC	1.56	1.22	1.1	70:30 (4.5)
	S	SFC	12.48	1.15	2.1	90:10

[a] Mobile phases for LC are volume ratios of triethylammonium acetate buffer–acetonitrile; pH is given in parentheses.
[b] Mobile phases for SFC are volume ratios of carbon dioxide:methanol.

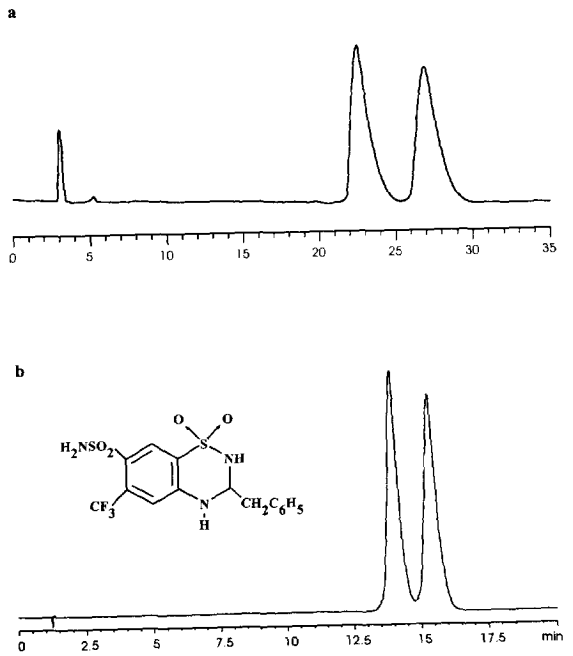

Fig. 4. Separation of bendroflumethiazide on the *S*-NEC-CD CSP. Chromatographic conditions, (a) triethylammonium acetate buffer–acetonitrile (70:30), 1.0 ml/min, (b) carbon dioxide–methanol (70:30), 2.0 ml/min, 40°C, 15 MPa.

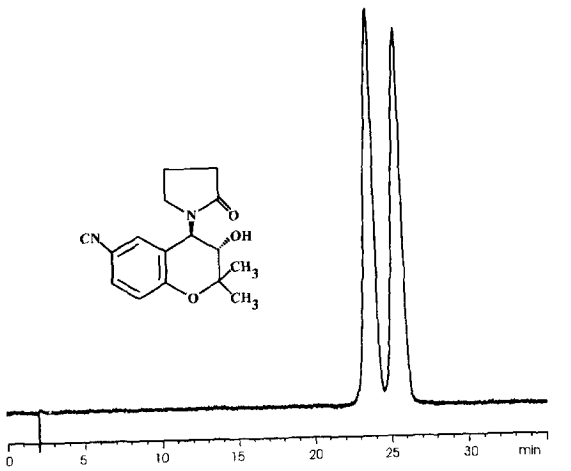

Fig. 5. Enantioresolution of cromakalim on the *S*-NEC-CD CSP. Chromatographic conditions, carbon dioxide–methanol (96:4), 2.0 ml/min, 30°C, 15 MPa.

alcohol modifiers on the enantioresolution of piperoxan in SFC is shown in Fig. 6. Enantioselectivity was reduced significantly when ethanol was used as a modifier and no separation was achieved when 2-propanol was used. The more hydrophobic alcohols interact more strongly with the nonpolar CD cavity and may block inclusion of the

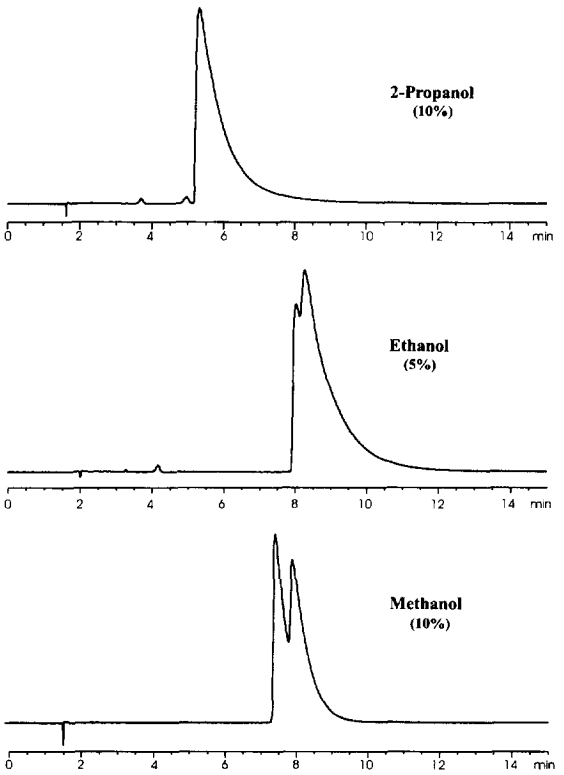

Fig. 6. Effect of modifier on the separation of piperoxan on the *S*-NEC-CD CSP. Chromatographic conditions, carbon dioxide–alcohol, 2.0 ml/min, 30°C, 15 MPa.

analyte [17,26]. Therefore, it appears that inclusion complexation, which is known to be important in the reversed-phase mode in LC [21], may also contribute to enantioseparation in SFC of the compounds in Table 3.

Comparison of Fig. 3 and Fig. 6 reveals an important aspect of the effect of modifier on selectivity in SFC. Although 2-propanol improved the separation of the derivatized analytes in Table 1, the opposite behavior occurred for the underivatized compounds in Table 3. Clearly, as in LC [19], more than one chiral recognition process is possible in SFC.

A comparison of the selectivities of the *R*-NEC-CD and *S*-NEC-CD columns in SFC was performed and the results are summarized in Table 4. In general, for the compounds examined, the *S*-NEC-CD column exhibited higher enantioselectivity than the *R*-NEC-CD column. In fact, three of the compounds in Table 4 were only resolved on the *S*-NEC-CD phase. In LC, it has been suggested that the carbamate substituents affect chiral recognition in the reversed-phase mode predominantly through steric interactions and that the CD is the primary source of chiral selectivity [18,19]. The nonequivalent selectivities of the *R*-NEC- and *S*-NEC-CD CSPs in SFC suggest that analogous chiral recognition processes are operative in SFC.

3.3. Polar organic mode

The polar organic mode can sometimes be used to resolve compounds that are not separated on CD CSPs in either the normal or reversed-phase modes in LC. The presence of two functional groups in the analyte capable of interacting with either the secondary hydroxyl groups or the pendant carbamate moieties of the CD is considered a requirement for successful enantioseparation [27]. Selectivity is altered by changing the ratio of glacial acetic acid to triethylamine in the mobile phase [23]. A comparison between SFC and LC was conducted using compounds known to be resolved on the *R*-NEC-CD CSP in the polar organic mode in LC [20]. Methanol was used as the modifier in SFC. The chromatographic results are listed in Table 5. The mobile phase compositions and chromatographic conditions in Table 5 for LC and SFC yielded optimum selectivity and resolution for the compounds examined.

Fig. 7 illustrates the separation of proglumide in LC and SFC. Analysis time in SFC exceeded that required for LC. In SFC, however, optimization of enantioselectivity was simplified because only the methanol modifier concentration was adjusted. In LC, the addition of both acetic acid and triethylamine is essential to chromatographic separation in the polar organic mode and manipulation of the acid/base ratio is required to obtain and maximize enantioresolution [21].

The effect of different alcohol modifiers on the separation of proglumide was evaluated and the results are summarized in Table 6. Retention increased as the polarity of the alcohol decreased and higher modifier concentrations of ethanol (15%) and 2-propanol (20%) were required to elute the analyte.

Table 4
SFC separation of underivatized compounds on the *R*-NEC- and *S*-NEC-CD CSPs[a]

Compound	CSP	k'	α	R_s	% Modifier
Ancymidol	*R*	6.90	1.09	1.3	10
	S	6.32	1.08	1.3	10
Bendroflumethiazide	*R*	9.11	1.00	0.0	30
	S	9.95	1.11	1.9	30
Benzoin	*R*	2.70	1.04	0.9	5
	S	2.29	1.06	0.8	5
Cromakalim	*R*	13.29	1.03	0.7	4
	S	10.25	1.08	1.5	4
Ibuprofen	*R*	5.81	1.04	0.5	5
	S	6.14	1.06	1.0	5
Mephenytoin	*R*	4.02	1.09	1.2	5
	S	3.15	1.25	3.0	5
4-Phenyl-2-oxazolidinone	*R*	9.16	1.05	0.6	5
	S	7.07	1.08	1.2	5
Piperoxan	*R*	4.78	1.00	0.0	10
	S	3.88	1.08	0.7	10
Tropicamide[b]	*R*	14.66	1.10	1.3	10
	S	12.48	1.15	2.1	10
Verapamil	*R*	19.29	1.00	0.0	10
	S	10.28	1.05	1.0	10

[a] Chromatographic conditions: carbon dioxide–methanol, 2.0 ml/min, 30°C, 15 MPa.
[b] Ethanol was used as the modifier.

In the polar organic mode in LC, the dominant chiral recognition process is believed to involve hydrogen bonding at the top of the CD cavity rather than inclusion [24]. The fact that the use of less polar alcohols did not compromise enantioselectivity implies that the chiral interactions in SFC are also occurring outside the CD cavity [28]. In addition, the increased retention in SFC when ethanol and 2-propanol were used as modifiers provides evidence of the importance of hydrogen bonding interactions in SFC. As the polarity of the alcohol decreases, interaction of the alcohol with the CD cavity is favored over adsorption on the secondary hydroxyls of the CD [29]. Therefore, the more hydrophobic alcohols have a diminished capacity to displace the analytes from hydrogen bonding sites on the CSP.

Table 5
Comparison of SFC and polar organic LC on the *R*-NEC-CD CSP

Compound		k'	α	R_s	Mobile phase
2-(4-Chlorophenoxy)-	LC	0.87	1.18	2.1	95:5:0.6:0.4[a]
propionic acid	SFC	30.90	1.14	2.0	80:20[b]
Coumachlor	LC	0.33	1.27	1.5	98:2:0.8:0.6
	SFC	19.99	1.06	1.1	85:15
Proglumide	LC	1.07	1.19	1.8	95:5:0.8:0.6
	SFC	15.44	1.10	1.9	92:8
Suprofen[c]	LC	3.23	1.10	1.0	95:5:0.2:0.2
	SFC	21.50	1.05	0.6	80:20

[a] Mobile phases for LC are volume ratios of acetonitrile–methanol–acetic acid–triethylamine.
[b] Mobile phases for SFC are volume ratios of carbon dioxide to methanol.
[c] Ethanol was used as the modifier for SFC.

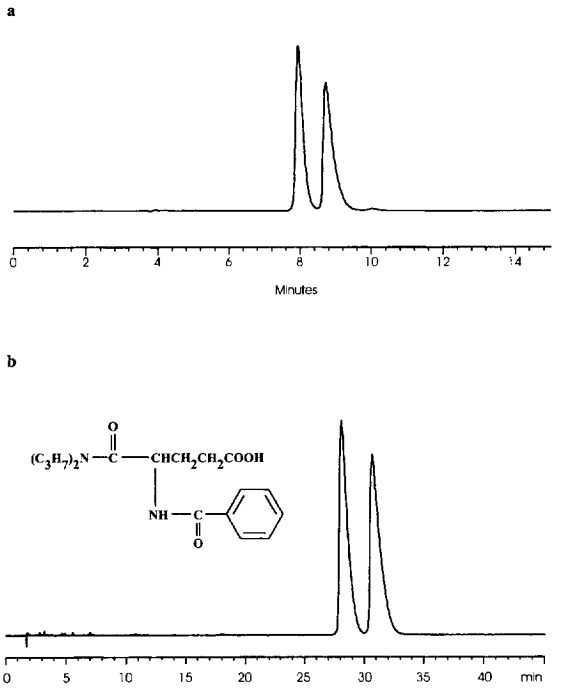

Fig. 7. Separation of the enantiomers of proglumide on the *R*-NEC-CD CSP. Chromatographic conditions, (a) acetonitrile–methanol–acetic acid–triethylamine (95:5:0.8:0.6), 1.0 ml/min, (b) carbon dioxide–methanol (92:8), 2.0 ml/min, 30°C, 15 MPa.

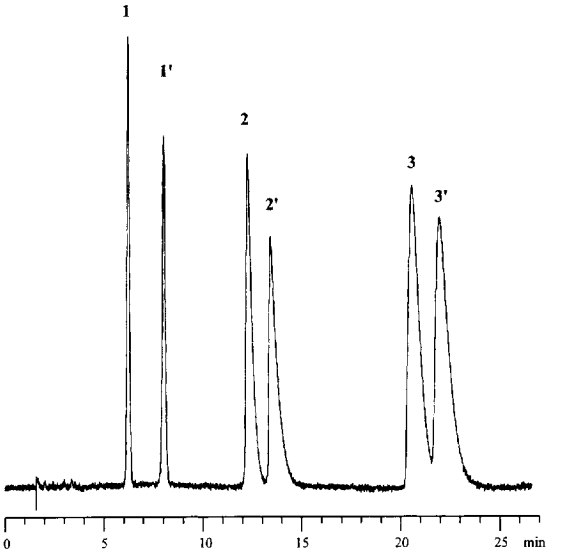

Fig. 8. Separation of N-(3,5-dinitrobenzoyl)-DL-valine methyl ester (1 and 1'), ancymidol (2 and 2') and proglumide (3 and 3') on the *R*-NEC-CD CSP. Chromatographic conditions, carbon dioxide–methanol (90:10), 2.0 ml/min, 30°C, 15 MPa.

3.4. Combined modes in SFC

The tremendous versatility of the NEC-CD CSPs in SFC is highlighted by the chromatogram in Fig. 8. The separation of the enantiomers of N-(3,5-dinitrobenzoyl)-valine methyl ester, ancymidol, and proglumide was achieved in a single run using a carbon dioxide–methanol eluent. The same separations in LC would require three different mobile phases. Clearly, the use of SFC can dramatically reduce the number of eluents that must be investigated in obtaining the desired separation.

Table 6
Effect of modifier on the separation of proglumide in SFC on the *R*-NEC-CD CSP[a]

Modifier	k'	α	R_s
Methanol (10%)	11.68	1.07	1.2
Ethanol (15%)	14.10	1.08	0.9
2-Propanol (20%)	25.72	1.10	0.6

[a] Chromatographic conditions: carbon dioxide–alcohol, 2.0 ml/min, 30°C, 15 MPa.

4. Conclusions

Chiral separations of compounds resolved in the normal-phase, reversed-phase and polar organic modes in LC were performed on NEC-CD CSPs in SFC. Comparisons between LC and SFC demonstrated that, although selectivity was sometimes lower in SFC than in LC, the improved efficiency in SFC resulted in higher resolution in SFC than in LC. Identification of optimum chromatographic parameters was facilitated in SFC and most of the compounds investigated were resolved with a carbon dioxide–methanol eluent. However, the alcohol modifier played an important role in enantioselectivity in SFC and the nature of this role was not the same for all analytes. The feasibility of combining the three LC mobile phase modes in SFC was also demonstrated.

References

[1] P.R. Massey and M.J. Tandy, Chirality, 6 (1994) 63.
[2] W.J. Lough and T.A.G. Noctor, in C.M. Riley, W.J. Lough and I.W. Wainer (Editors), Pharmaceutical and Biomedical Applications of Liquid Chromatography, Elsevier, New York, 1994, p. 241.
[3] E.C. Rickard and R.J. Bopp, J. Chromatogr. A, 680 (1994) 609.
[4] R.W. Stringham, K.G. Lynam and C.C. Grasso, Anal. Chem., 66 (1994) 1949.
[5] D.R. Gere, R. Board and D. McManigill, Anal. Chem., 54 (1982) 736.
[6] A. Kot, P. Sandra and A. Venema, J. Chromatogr. Sci., 32 (1994) 439.
[7] G.J. Terfloth, W.H. Pirkle, K.G. Lynam and E.C. Nicolas, J. Chromatogr. A, 705 (1995) 185.
[8] A.M. Blum, K.G. Lynam and E.C. Nicolas, Chirality, 6 (1994) 302.
[9] K.G. Lynam and E.C. Nicolas, J. Pharm. Biomed. Anal., 11 (1993) 1197.
[10] P. Petersson and K.E. Markides, J. Chromatogr. A, 666 (1994) 381.
[11] W.H. Wilson, Chirality, 6 (1994) 216.
[12] S. Hara, A. Dobashi, K. Kinoshita, T. Hondo, M. Saito and M. Senda, J. Chromatogr., 371 (1986) 153.
[13] P.A. Mourier, E. Eliot, M.H. Caude, R.H. Rosset and A.G. Tambuté, Anal. Chem., 57 (1985) 2819.
[14] K.L. Williams and A.M. Stalcup, in T.T. Ngo (Editor), Molecular Interactions in Bioseparations, Plenum Press, New York, 1993, p. 189.
[15] D.W. Armstrong, A.M. Stalcup, M.L. Hilton, J.D. Duncan, J.R. Faulkner, Jr. and S.-C. Chang, Anal. Chem., 62 (1990) 1610.
[16] A.M. Stalcup, S.-C. Chang and D.W. Armstrong, J. Chromatogr., 540 (1991) 113.
[17] D.W. Armstrong, M.L. Hilton and L. Coffin, LC-GC, 9 (1992) 647.
[18] S.H. Lee, A. Berthod and D.W. Armstrong, J. Chromatogr., 603 (1992) 83.
[19] D.W. Armstrong, C.-D. Chang and S.H. Lee, J. Chromatogr., 539 (1991) 83.
[20] S.C. Chang, G.L. Reid, III, S. Chen, C.D. Chang and D.W. Armstrong, Trends Anal. Chem., 12 (1993) 144.
[21] Application Report LC-93/KIT, Advanced Separation Technologies Inc. (1993).
[22] K.L. Williams, L.C. Sander and S.A. Wise, Chirality, (1996) in press.
[23] D.W. Armstrong, S. Chen, C. Chang and S. Chang, J. Liq. Chromatogr., 15 (1992) 545.
[24] A.M. Stalcup, in G. Subramanian (Editor), A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, New York, 1994, p. 95.
[25] A.M. Stalcup and K.L. Williams, J. Chromatogr. A, 695 (1995) 185.
[26] D.W. Armstrong and W. Li, Chromatography, 2 (1987) 43.
[27] Application Report LC-93/POM, Advanced Separation Technologies Inc. (1993).
[28] D.W. Armstrong, W. DeMond and B. Czech, Anal. Chem., 57 (1985) 481.
[29] P. Macaudière, M. Caude, R. Rosset and A. Tambuté, J. Chromatogr., 405 (1987) 135.