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Comparison of liquid and supercritical fluid chromatography for the separation of enantiomers on chiral stationary phases

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

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Abstract

Comparisons of liquid (LC) and supercritical fluid chromatography (SFC) were conducted using commercially available chiral stationary phases (CSPs) bearing three different types of chiral selectors. Chiral compounds of pharmaceutical and agricultural interest were used to probe advantages or limitations of SFC relative to LC for enantiomeric separations. Column equilibration and parameter optimization were generally accomplished more rapidly in SFC than in LC. Although improved resolution was often observed in SFC, analysis times were not always lower in SFC than in LC. In some instances, SFC provided separation capabilities not readily accessible in LC.

Keywords: Liquid chromatography; Supercritical fluid chromatography; Chiral stationary phase; Enantiomers

1. Introduction

Enantioselective analytical methods are now frequently necessary to meet regulatory guidelines for the development and manufacture of chiral drugs [1,2]. Because of rapid progress in the development of chiral stationary phases (CSPs), liquid chromatography (LC) has become the technique of choice for the separation and quantitation of drug enantiomers [3,4]. Although the CSPs currently available can separate many racemates, several problems have hampered implementation of stereoselective analytical methodology. The limited efficiency of many chiral columns causes broad chromatographic peaks and reduces peak resolution [5,6]. Poor peak resolution is particularly detrimental to quantitation of a trace amount of one enantiomer in the presence of a large excess of the other enantiomer, a situation likely to be encountered more frequently as the number of drugs introduced in single enantiomer form continues to grow [7,8]. The complexities of column selection and parameter optimization, coupled with long analysis times, pose tremendous challenges to scientists responsible for chiral method development [9–11].

Although the use of supercritical fluids as mobile phases for chromatography was reported more than 30 years ago [12], the advantages of

* Corresponding author. Tel.: +1 301 9754457; fax: +1 301 9770685; e-mail: klwill@enh.nist.gov

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supercritical fluid chromatography (SFC) for chiral separations on packed columns have only recently been demonstrated [13,14]. Because solutes have higher diffusion coefficients in supercritical fluids than in liquids, the optimum linear velocity is shifted to higher values in SFC than in LC [15,16]. Consequently, higher flow rates can be used in SFC to reduce analysis times without compromising efficiency [17,18]. Rapid column equilibration after changes in chromatographic parameters in SFC also reduces the time required for method development. Although the chiral recognition mechanisms in SFC generally resemble those of LC, in some cases separations can be achieved in SFC that can not be performed on the same CSP in LC [19,20].

Most of the packed column chiral separations by SFC reported in the literature have been performed at or near ambient temperatures and have incorporated modifiers to increase the eluent strength of carbon dioxide [21]. Therefore, the eluent is generally in a subcritical, not supercritical, state. Because no discontinuity occurs in the solute diffusion coefficient when the eluent passes from the subcritical to the supercritical state [22], the term SFC has often been used to encompass both regions.

Enantioseparations in SFC have been reported for several commercially available CSPs, including native and derivatized cyclodextrin [23,24], brush-type [25,26], polysaccharide [19,27], and polymethacrylate phases [28]. These studies have demonstrated the advantages of SFC for chiral separations and the effects of various parameters such as modifier, temperature, and pressure [29]. However, few comparisons of LC and SFC on the same chromatographic columns have been performed [25,30]. As a result, misconceptions about the applicability of SFC for chiral separations persist. In the present work, commercially available columns representative of three major classes of CSPs were utilized for the resolution of a variety of racemates by LC and SFC. Comparison of the chromatographic results for the two techniques provided additional insight into the utility of SFC for chiral separations and revealed areas where SFC provides analytical capabilities not readily accessible with LC.

2. Experimental[1]

2.1. Chemicals

Carbon dioxide (SFC grade) was obtained from Scott Specialty Gases (Plumsteadville, PA, USA). The analytes were obtained as racemic mixtures from Aldrich Chemical Company (Milwaukee, WI, USA), Sigma Chemical Company (St. Louis, MO, USA), and the United States Pharmacopeial Convention (Rockville, MD, USA). For some analytes, the *N*-(3,5-dinitrobenzoyl) derivatives were prepared by reacting the analyte with a stoichiometric amount of 3,5-dinitrobenzoyl chloride in tetrahydrofuran for 15 min at 60°C. The solvent was removed under a stream of nitrogen, and the sample was dissolved in methanol. All solvents and modifiers were HPLC grade. Analytes were dissolved in mobile phase for LC, or methanol for SFC, at a concentration of 2.0 mg ml^{-1}, and additional dilutions were made as needed.

Table 1
List of mobile phases used for liquid chromatography

Chirex 3022	Hexane-1,2-dichloroethane-ethanol-trifluoroacetic acid (ethanol and trifluoroacetic acid premixed 20:1)
Chiralcel OD	Hexane-2-propanol-diethylamine
Cyclobond I 2000 RN and SN	Hexane-2-propanol
	1% triethylammonium acetate–acetonitrile
	Acetonitrile–methanol–acetic acid–triethylamine

[1] 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, not does imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 2
Chromatographic data for LC and SFC on the Chirex 3022 chiral stationary phase

Compound		k'[a]	α	R_s	Mobile phase
Acebutolol	LC	6.71	1.17	2.1	55:35:10[b]
	SFC	17.60	1.06	1.1	85:153[c]
Atenolol	LC	11.50	1.17	1.4	55:35:10
	SFC	15.78	1.07	0.8	85:15
Clenbuterol	LC	0.97	1.47	2.7	50:35:15
	SFC	6.52	1.27	4.1	85:15
Gallopamil	LC	1.03	1.25	2.2	55:35:10
	SFC	2.81	1.13	2.2	80:20
Isoproterenol	LC	6.61	1.29	1.9	55:35:10
	SFC	12.72	1.12	1.3	85:15
Propranolol	LC	7.02	1.11	1.0	60:35:5
	SFC	8.28	1.07	1.1	85:15
Terbutaline	LC	4.83	1.38	2.3	55:35:10
	SFC	12.29	1.17	2.2	85:15
Verapamil	LC	3.20	1.19	1.3	60:35:5
	SFC	3.37	1.09	1.8	80:20
Warfarin	LC	15.15	1.08	1.4	88:10:2
	SFC	4.59	1.05	1.2	80:20

[a] Capacity factor for the first eluting enantiomer.
[b] Mobile phases for LC are volume ratios of hexane-1,2-dichloroethane–ethanol–TFA.
[c] Mobile phases for SFC are volume ratios of volume ratios of carbon dioxide–methanol–TFA.

2.2. Instrumentation

Liquid chromatographic separations were performed at ambient temperature (22°C) under isocratic conditions. A flow rate of 0.5 or 1.0 ml min^{-1} was used for all experiments, and the sample size was 20 μl. The column eluent was monitored with a variable wavelength detector. Detection was performed at 254 nm unless otherwise noted. Supercritical fluid chromatography was performed using a commercial chromatographic system (Hewlett-Packard G1205A) comprised of a supercritical fluid pump and a modifier pump [31]. Flow rates were 1.0 or 2.0 ml min^{-1}, and the pressure was 15 MPa. Column temperature was maintained by the column oven, and the eluent was monitored with a diode array detector. Samples were introduced using an autosampler with a 5 μl internal loop.

2.3. Chiral stationary phases

Chiralcel OD, a cellulose-based CSP, was obtained from Chiral Technologies (Exton, PA, USA). Chirex 3022, a brush-type phase having π-donor characteristics, was obtained from Phenomenex (Torrance, CA, USA). Cyclobond I 2000 RN, (*R*)-naphthyl-ethylcarbamoylated-β-cyclodextrin, and Cyclobond I 2000 SN, (*S*)-naphthylethylcarbamoylated-β-cyclodextrin, were obtained from Advanced Separation Technologies (Whippany, NJ, USA). Column dimensions for all CSPs were 0.46 cm × 25 cm. Particle size was 5 μm for the Cyclobond I and Chirex CSPs. Particle size for the Chiralcel OD CSP was 10 μm. The same columns were used for both LC and SFC experiments. After completion of the SFC experiments, the columns were flushed with methanol prior to initiating the LC studies. No changes in column performance observed after the use of modifiers and/or additives in SFC.

2.4. Mobile phases

A summary of the eluents used for LC for each of the CSPs is provided in Table 1. For the Chirex 3022 CSP, ethanol and trifluoroacetic acid were premixed at a 20:1 (v/v) ratio. Triethylammonium acetate buffer was prepared by adding 1% (v/v) triethylamine (TEA) to water and adjusting the pH with acetic acid (HOAc). For SFC, carbon dioxide was the primary component of the mobile phase, and alcohol modifiers were added to adjust the elution strength of the mobile phase. For the Chirex 3022 CSP and the Chiralcel OD CSP, small amounts of acidic or basic additives were used to improve peak shape. For the Chirex 3022 CSP, 0.5% (v/v) trifluoroacetic acid was added to the methanol modifier. For the Chiralcel OD CSP, 0.5% (v/v) isopropylamine was added to the methanol modifier.

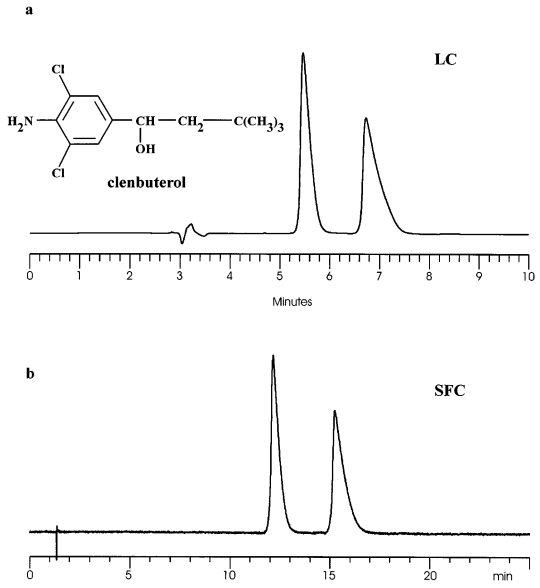

Fig. 1. Comparison of LC and SFC enantiomeric separations of clenbuterol on the Chirex 3022 CSP. Chromatographic conditions: (a) hexane-1,2-dichloroethane–ethanol–TFA (50:35:15, v/v/v), 1.0 ml min^{-1}, UV detection at 254 nm; (b) carbon dioxide–methanol (85:15, v/v), 2.0 ml min^{-1}, 30°C, 15 MPa, UV detection at 254 nm.

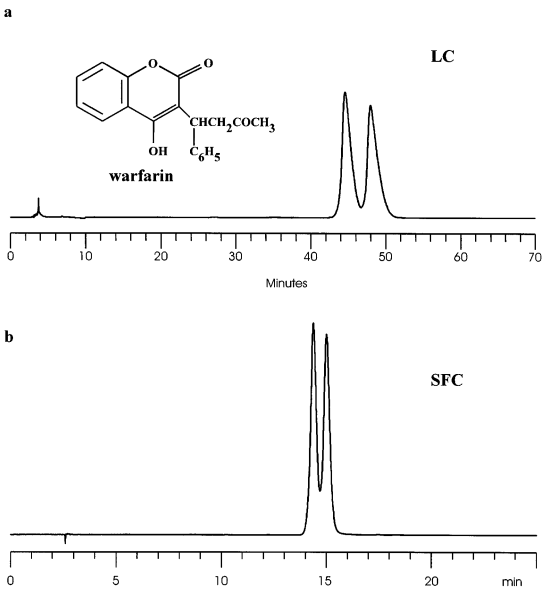

Fig. 2. Separation of the enantiomers of warfarin by LC and SFC on the Chirex 3022 CSP. Chromatographic conditions: (a) hexane-1,2-dichloroethane–ethanol–TFA (88:10:2, v/v/v), 1.0 ml min^{-1}, UV detection at 254 nm; (b) carbon dioxide–methanol (80:20, v/v), 1.0 ml min^{-1}, 30°C, 15 MPa, UV detection at 254 nm.

3. Results and discussion

Because of the diversity of eluent systems used for LC, comparisons between LC and SFC were sometimes performed using very different mobile phase compositions for the two techniques. Conditions listed in the tables were selected to optimize selectivity and resolution. Capacity factors (k') listed in the tables of chromatographic data correspond to the first eluting enantiomer. Selectivity (α) and resolution (R_s) were calculated based on standard equations. All chromatographic analyses were performed in duplicate to verify reproducibility of the enantiomeric separations.

3.1. Chirex 3022

The chiral selector for this CSP consists of (*S*)-indoline-2-carboxylic acid combined with (*R*)-1-(α-naphthyl)ethylamine via a urea linkage [32]. This CSP resolves underivatized β-blockers and

Table 3
Chromatographic data for LC and SFC on the Chiralcel OD chiral stationary phase

Compound		k'	α	R_s	Mobile phase
Acebutolol	LC	2.59	1.07	0.4	80:20:0.1[a]
	SFC	14.56	1.08	1.5	90:10[b]
Atenolol	LC	6.35	1.54	3.4	80:20:0.1
	SFC	3.78	2.20	11.2	80:20
Cromakalim	LC	1.67	1.78	3.4	80:20:0.1
	SFC	0.83	1.37	3.7	80:20
Metoprolol	LC	0.89	2.67	4.8	80:20:0.1
	SFC	1.12	2.77	12.7	80:20
Oxprenolol	LC	1.31	5.70	9.1	80:20:0.1
	SFC	1.27	2.07	9.9	80:20
Pindolol	LC	5.23	7.66	7.5	80:20:0.1
	SFC	1.96	4.49	17.6	70:30
Propranolol	LC	1.95	1.87	5.4	80:20:0.1
	SFC	3.57	1.74	8.6	80:20

[a] Mobile phases for LC are volume ratios of hexane-2–propanol–diethylamine.
[b] Mobile phases for SFC are volume ratios of carbon dioxide–methanol-isopropylamine.

β-agonists through a combination of $\pi-\pi$, hydrogen bonding, dipole–dipole, and steric interactions. Recommended mobile phases for LC involve hexane-1,2-dichloroethane-ethanol mixtures. A small percentage of trifluoroacetic acid (TFA) is added to improve peak shape [33]. Liquid chromatographic conditions were chosen based on product information and literature results. For SFC, carbon dioxide-methanol mixtures were used as the eluent. A small amount of TFA (0.5%) was added to the methanol modifier for the analysis of most analytes [34]. A comparison of chromatographic results for LC and SFC is summarized in Table 2.

As shown in Table 2, the compounds examined were strongly retained on the CSP in SFC, and a modifier concentration of at least 15% was typically required to elute the compounds. In fact, the capacity factors in SFC were larger than in LC for all the compounds in Table 2 except warfarin. These results indicate that the elution strength of the carbon dioxide–methanol mixtures used is lower than that of hexane-1,2-dichloroethane–ethanol mixtures. Similar results have been reported for comparisons of LC and SFC on other brush-type CSPs [16,20]. The diminished eluent strength in SFC also emphasizes the fact that solute-mobile phase and mobile phase- stationary phase interactions are not equivalent for LC and SFC [35]. Therefore, the enhanced diffusivity of the eluent in SFC does not always guarantee reduced analysis times. Use of higher methanol modifier concentrations in SFC reduced retention but caused asymmetric chromatographic peaks and decreased resolution. Although higher flow rates can also be used to reduce analysis time in SFC, a decrease in resolution would be anticipated to accompany the increase in flow rate [34].

The enantioselectivity (α) observed for the compounds in Table 2 was slightly lower in SFC than in LC, but results for the two techniques tended to follow the same trends. When a high degree of selectivity was achieved for a particular compound in LC, similar behavior was observed in SFC. Although resolution (R_s) in SFC was nearly identical to that of LC for compounds such as propranolol and terbutaline, significant differences in resolution between the two techniques were observed for other compounds such as acebutolol and atenolol.

Separation of the enantiomers of clenbuterol on the Chirex 3022 CSP in LC and SFC is illustrated in Fig. 1. Although a higher flow rate was used in SFC (2.0 ml min^{-1}) than in LC (1.0 ml min^{-1}), the analysis time was longer in SFC. However, resolution was higher in SFC (4.1) than in LC (2.7). Enantioresolution of warfarin using LC and SFC is shown in Fig. 2. For this compound, the analysis time for SFC was reduced substantially relative to that of LC despite the fact that the flow rates were the same for the two techniques. The abbreviated analysis in SFC was accompanied by slightly lower selectivity and resolution in SFC compared with LC.

For the compounds studied on the Chirex 3022 CSP, SFC did not present any clear advantages in terms of analysis time or efficiency. However, the use of SFC did eliminate the need for chlorinated solvents and reduced the time required for column

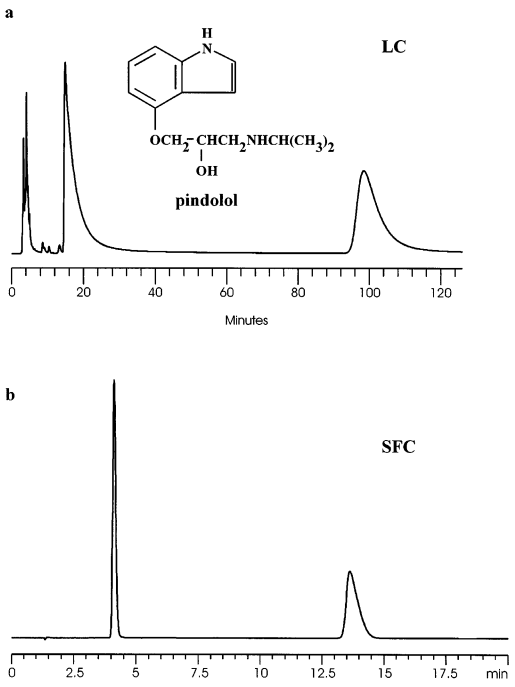

Fig. 3. Comparison of LC and SFC separations of pindolol on the Chiralcel OD CSP. Chromatographic conditions: (a) hexane-2–propanol–diethylamine (80:20:0.1, v/v/v), 1.0 ml min^{-1}, UV detection at 280 nm; (b) carbon dioxide–methanol-isopropylamine (70:30, v/v), 2.0 ml min^{-1}, 30°C, 15 MPa, UV detection at 280 nm.

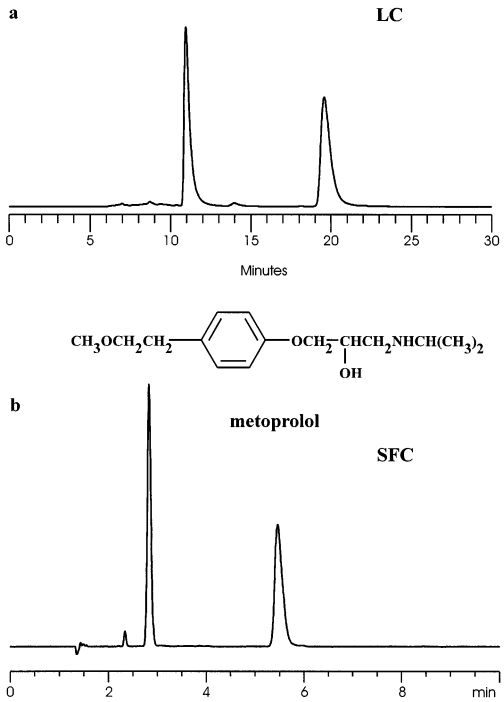

Fig. 4. Enantioseparations of metoprolol on the Chiralcel OD CSP. Chromatographic conditions: (a) hexane-2–propanol–diethylamine (80:20:0.1, v/v/v), 0.5 ml min^{-1}, UV detection at 280 nm; (b) carbon dioxide–methanol-isopropylamine (80:20, v/v), 2.0 ml min^{-1}, 30°C, 15 MPa, UV detection at 280 nm.

equilibration.

3.2. Chiralcel OD

The derivatized polysaccharide phase, Chiralcel OD, incorporates a cellulose carbamate derivative coated on silica gel as the chiral selector. Although this CSP is probably best known for the high selectivities achieved for β-blockers, a wide range of applicability has been demonstrated in LC [11,36]. Because the chiral polysaccharide is not covalently bonded to the silica substrate, some limitations in mobile phase selection exist in LC.

Mobile phases for LC were chosen based on literature results and were comprised of hexane-2-propanol mixtures with 0.1% (v/v) diethylamine (DEA) added to reduce peak tailing [37]. For SFC, carbon dioxide–methanol eluents were used.

Table 4
Comparison of chromatographic results for *N*-(3,5-dinitrobenzoyl) derivatized analytes in LC and SFC on the Cyclobond I 2000 SN CSP

Compound		k'	α	R_s	Mobile phase
Alanine methyl ester	LC	4.12	1.49	3.7	70:30[a]
	SFC	4.23	1.31	4.7	90:10[b]
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	1.79	4.9	70:30
	SFC	2.80	1.43	5.8	90:10
Phenylalanine methyl ester	LC	4.10	1.29	2.3	60:40
	SFC	5.11	1.25	4.0	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	1.23	1.7	80:20
	SFC	6.73	1.45	5.9	90:10
α-Methylbenzylamine	LC	3.29	2.10	6.8	70:30
	SFC	3.55	1.56	7.8	80:20
1,2,3,4-Tetrahydro-1-naphthylamine	LC	2.15	1.92	5.0	70:30
	SFC	3.51	1.46	6.4	80:20

[a] Mobile phases for LC are volume ratios of hexane-2–propanol.
[b] Mobile phases for SFC are volume ratios of carbon dioxide–methanol.

A small percentage (0.5%) of isopropylamine (IPA) was added to the methanol modifier [38]. A summary of results for LC and SFC is given in Table 3.

Retention of both enantiomers of the analytes in Table 3 was reduced in SFC relative to that observed in LC. The decreased retention in SFC can be traced to enhanced diffusion of the solutes in the carbon dioxide–methanol eluent as well as the higher flow rates used in SFC [38].

All of the compounds in Table 3 were enantioresolved using both LC and SFC. Selectivity differences between the two techniques were compound specific. Enantioselectivity for acebutolol, atenolol, and metoprolol was higher in SFC than in LC, but LC yielded higher enantioselectivity for the remaining compounds in Table 3. Bargmann-Leyder et al. [19] compared LC and SFC using propranolol analogues. The results provided evidence that solvation by carbon dioxide of sites on the analyte or the CSP involved in stereoselective interactions may alter the chiral recognition mechanisms from those observed in LC. However, despite differences in selectivity for the two techniques, resolution was higher in SFC for all the compounds in Table 3.

Faster solute diffusion in SFC translated into differences in analysis time between LC and SFC on the Chiralcel OD CSP, as exemplified by the chromatograms shown in Fig. 3 and Fig. 4. Using conditions reported in the literature, chromatographic analysis of pindolol (Fig. 3) required nearly 2 h. In SFC, the same analysis was reduced to less than 20 min while high enantioselectivity and resolution were preserved. Fig. 4 illustrates the separation of atenolol by LC and SFC on the Chiralcel OD CSP. Both selectivity and resolution were higher in SFC than in LC, and the chromatographic separation required less than 8 min.

For the Chiralcel OD CSP, SFC provided significant advantages in terms of analysis time and peak resolution. Column equilibration and parameter optimization were achieved rapidly in SFC, and retention times of the analytes were much less variable in SFC than in LC. Although the pressures and flow rates used in SFC exceeded

Table 5
Comparison of chromatographic results for SFC and reversed phase LC on the Cyclobond I 2000 SN CSP

Compound		k'	α	R_s	Mobile phase
Ancymidol	LC	4.72	1.14	1.3	80:20 (7.0)[a]
	SFC	6.32	1.08	1.3	90:10[b]
Cromakalim	LC	2.19	1.00	0.0	80:20 (4.5)
	SFC	10.25	1.08	1.5	96:4
Ibuprofen	LC	3.26	1.14	0.6	70:30 (4.5)
	SFC	6.14	1.06	1.0	95:5
Mephenytoin	LC	1.29	1.22	1.3	70:30 (4.1)
	SFC	3.15	1.25	3.0	95:5
Piperoxan	LC	1.20	1.15	0.6	80:20 (4.5)
	SFC	3.88	1.08	0.7	90:10
Tolperisone	LC	1.63	1.11	0.9	80:20 (4.5)
	SFC	6.77	1.00	0.0	90:10
Tropicamide[c]	LC	1.56	1.22	1.1	70:30 (4.5)
	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.
[c] Ethanol was used as the modifier for SFC.

the conditions recommended by the column vendor, no deterioration of column performance was observed.

3.3. Cyclobond I 2000 RN and SN

The Cyclobond I 2000 RN and SN CSPs incorporate (*R*)- or (*S*)-naphthylethyl-carbamoylated-β-cyclodextrin covalently bonded to silica. These columns can be used under normal phase, reversed phase, and polar organic mobile phase conditions in LC, and the racemates resolved are generally different for each of the three mobile phase modes [10]. Because of the variety of possible mobile phase systems, choosing the optimum conditions for a desired compound can require substantial time and experimentation. In addition, the *R*- and *S*-naphthylethylcarbamoylated cyclodextrin CSPs often exhibit nonequivalent stereoselectivities.

Compounds for comparison of LC and SFC were chosen to be representative of those known to be resolved in each of the three mobile phase modes in LC. Mobile phases for normal phase LC separations were hexane-2-propanol mixtures [39]. For the reversed phase mode, 1% triethylammonium acetate–acetonitrile mobile phases were used [40]. In the polar organic mode, acetonitrile–methanol–acetic acid–triethylamine mixtures were employed for LC [41]. All enantioseparations in SFC were conducted using carbon dioxide–methanol eluents.

Chromatographic data comparing separations corresponding to each mobile phase mode are shown in Tables 4–6.

Compounds resolved on the Cyclobond I 2000 RN and SN CSPs under normal phase conditions in LC were also resolved in SFC. Table 4 provides a summary of chromatographic data for the two techniques. With the exception of the results for 1-cyclohexylethylamine, selectivities in SFC were lower than those observed with LC for the compounds in Table 4. However, the decrease in selectivity was offset by an increase in resolution relative to LC.

Separation of the enantiomers of *N*-(3,5-dinitrobenzoyl)-DL-valine methyl ester on the Cyclobond I SN CSP is illustrated in Fig. 5. Although selectivity was lower in SFC ($\alpha = 1.43$) than in LC ($\alpha = 1.79$), analysis time was reduced

Table 6
Comparison of SFC and polar organic LC on the Cyclobond I 2000 RN CSP

Compound		k'	α	R_s	Mobile phase
2-(4-Chlorophenoxy)-propionic acid	LC	0.87	1.18	2.1	95:5:0.6:0.4[a]
	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–HOAc–TEA.
[b] Mobile phases for SFC are volume ratios of carbon dioxide–methanol.
[3] Ethanol was used as the modifier for SFC.

and resolution was improved in SFC relative to LC.

Although SFC is often compared with normal phase LC [34,38], studies of the Cyclobond I RN and SN CSPs demonstrated that separations achieved under reversed phase LC conditions could be duplicated in SFC using carbon dioxide–methanol eluents. Table 5 lists some of the

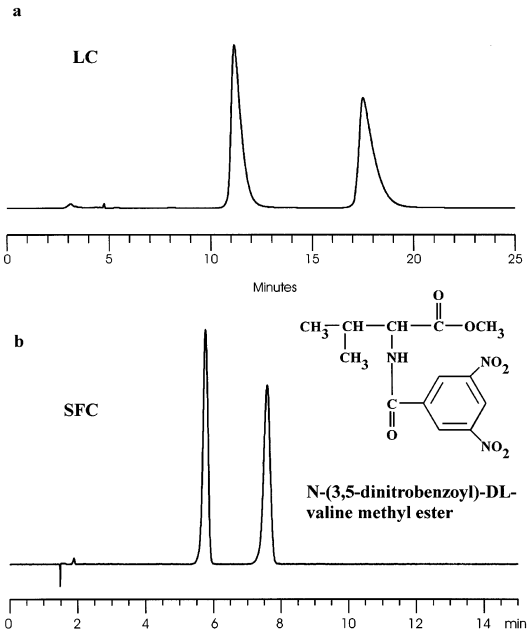

Fig. 5. Enantioseparations of *N*-(3,5-dinitrobenzoyl)-DL-valine methyl ester on the Cyclobond I 2000 SN CSP. Chromatographic conditions: (a) hexane-2–propanol (70:30, v/v), 1.0 ml min^{-1}, UV detection at 254 nm; (b) carbon dioxide–methanol (90:10, v/v), 2.0 ml min^{-1}, 30°C, 15 MPa, UV detection at 254 nm.

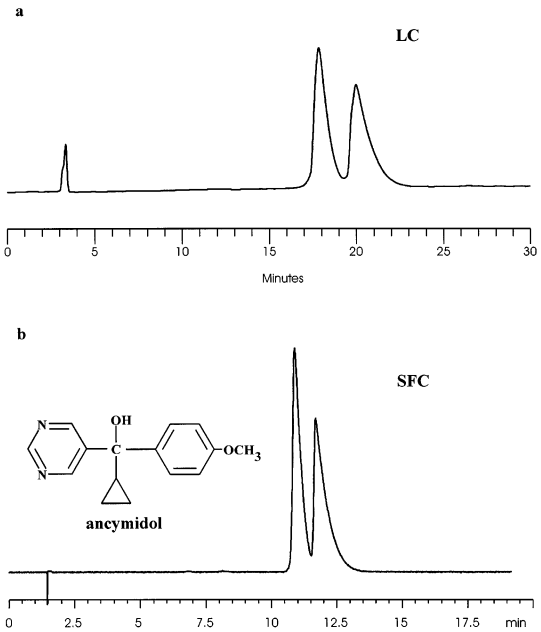

Fig. 6. Separation of the enantiomers of anycmidol on the Cyclobond I 2000 SN CSP. Chromatographic conditions: (a) 1% triethylammonium acetate (pH = 7.0)–acetonitrile (80:20, v/v), 1.0 ml min^{-1}, UV detection at 254 nm; (b) carbon dioxide–methanol (90:10, v/v), 2.0 ml min^{-1}, 30°C, 15 MPa, UV detection at 254 nm.

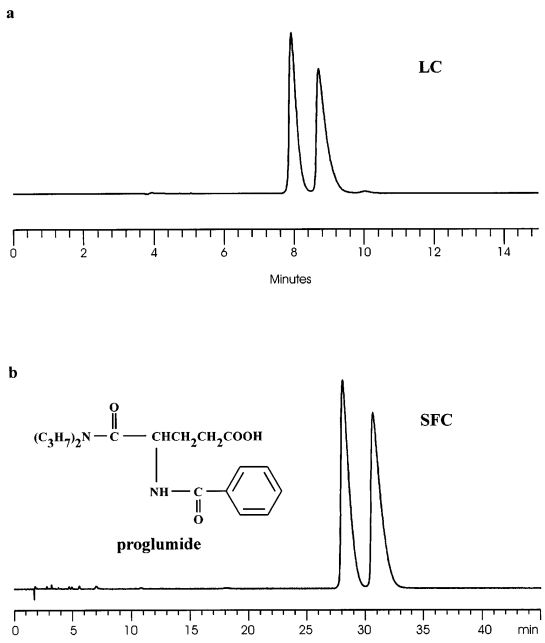

Fig. 7. Separation of the enantiomers of proglumide by LC and SFC on the Cyclobond I 2000 RN CSP. Chromatographic conditions: (a) acetonitrile–methanol–HOAc–TEA (95:5:0.8:0.6, v/v/v/v), 1.0 ml min^{-1}, UV detection at 254 nm; (b) carbon dioxide–methanol (92:8, v/v), 2.0 ml min^{-1}, 30 C, 15 MPa, UV detection at 254 nm.

compounds resolved. Although aqueous–organic eluents were required to enantioresolve these compounds in LC, no additives or aqueous buffers were required for SFC, as demonstrated by the separation of the enantiomers of ancymidol shown in Fig. 6.

The discrepancies in selectivity observed for cromakalim and tolperisone (Table 5) indicate that LC and SFC can not be considered interchangeable for the compounds examined. However, SFC provides a rapid and convenient method of evaluating CSPs for a desired separation. Elimination of aqueous buffers in the eluent system is also likely to extend column life.

Compounds representative of those resolved in the polar organic mode in LC were also resolved in SFC. Substantial differences in retention for the two techniques were observed, as shown Table 6. Enantioseparation of proglumide by LC and SFC is shown in Fig. 7. Although the time required for the SFC analysis exceeded that needed for LC, optimization of selectivity in SFC was limited to adjusting the modifier concentration, and column equilibration occurred within a few minutes. In LC, manipulation of the acetic acid/triethylamine ratio is often required to obtain and optimize the desired separation.

The ease of method development in SFC is exemplified by the chromatogram in Fig. 8. The enantioseparation of *N*-(3,5-dinitrobenzoyl)-DL-valine methyl ester, ancymidol, and proglumide was achieved in a single analysis on the Cyclobond I RN CSP using a simple carbon dioxide–methanol eluent. Similar results were also achieved with the Cyclobond I SN CSP. In LC, each of the three compounds would require a unique set of mobile phase conditions.

4. Conclusions

SFC was applied successfully to the enantioresolution of a wide variety of analytes on commercially available CSPs bearing three different types

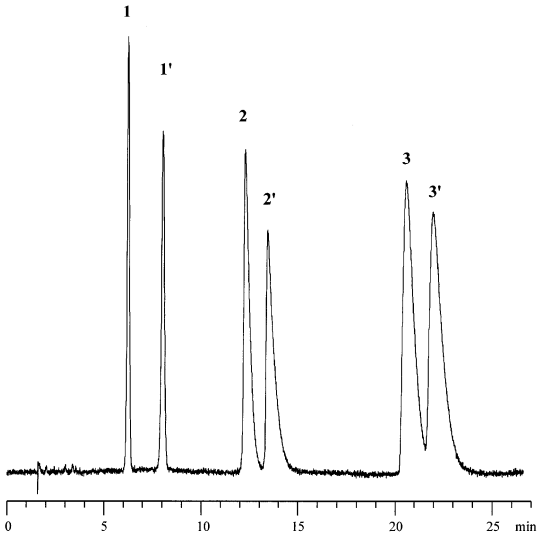

Fig. 8. Separation of the enantiomers of *N*-(3,5-dinitrobenzoyl)-DL-methyl ester (1 and 1′), ancymidol (2 and 2′), and proglumide (3 and 3′) on the Cyclobond I 2000 RN CSP. Chromatographic conditions: carbon dioxide–methanol (90:10, v/v), 2.0 ml min^{-1}, 30°C, 15 MPa, UV detection at 254 nm.

of chiral selectors. Advantages or limitations of SFC relative to LC depended upon the analyte and the CSP. In general, column equilibration and parameter optimization occurred much more rapidly in SFC. In many instances, improved resolution was observed in SFC, but analysis times were not always lower in SFC than in LC. Although SFC has traditionally been compared with normal phase LC, separations performed under reversed phase conditions in LC were also achieved using SFC. Based on these observations, SFC offers tremendous potential for simplifying the chiral method development process and improving the ruggedness of chiral analyses.

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