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Review

Enantiomer separations on chiral stationary phases in supercritical fluid chromatography[1,2]

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Abstract

Supercritical fluid chromatography (SFC) has proven to be widely applicable for the separation of enantiomers on chiral stationary phases. Many of the chiral selectors utilized in gas and liquid chromatography have also been transferred to SFC. This article provides an overview of the types of separations reported on different classes of chiral stationary phases in SFC and includes comparisons of SFC to other chromatographic techniques. The importance of various chromatographic parameters in obtaining successful enantioseparations in SFC is also discussed. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Chiral stationary phases, SFC; Reviews

Contents

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1. Introduction

The separation of enantiomers on chiral stationary phases (CSPs) has been one of the most successful applications of supercritical fluid chromatography (SFC). The term SFC has been used to encompass the use of mobile phases at temperatures and pressures above the critical point (supercritical), or just below the critical point (subcritical). Both sub- and supercritical fluids have properties that make them uniquely suited to chiral separations. In SFC, lower operating temperatures compared to gas chromatography (GC) reduce the likelihood of racemization of either the analyte or the CSP and often result in improved enantioselectivity [1,2]. The higher diffusivity of the solutes and the reduced viscosity of the eluent in SFC also provide advantages in efficiency and analysis time compared to liquid chromatography (LC) [3–5].

Because supercritical fluids possess both gas-like and liquid-like properties, it is not surprising that research in SFC has tended to parallel either gas or liquid chromatography. As a result, chiral separations in SFC are generally divided into open tubular and packed column approaches. Open tubular columns designed for SFC typically have inside diameters of 50–100 μm. Cyclodextrin derivatives have been the most widely used chiral selectors for open tubular SFC [1,6]. The eluent strength is changed by modifying the density of the supercritical fluid through variations in pressure and/or temperature [7]. Flame ionization detection has often been used in conjunction with open tubular SFC for chiral separations [8,9]. However, most modifiers are not compatible with flame ionization detection. In the absence of modifiers, elution of polar compounds is difficult, if not impossible. In addition, the sample capacity of open tubular systems is quite low [10]. The use of open tubular columns for chiral separations has also been limited by the lack of commercially available CSPs [11].

The use of packed columns in SFC has grown in popularity because of the commercial availability of a wide variety of CSPs for LC that can be transferred directly to SFC [12,13]. The fact that the polarity of carbon dioxide has often been equated with that of hexane has facilitated the transition from normal phase LC to SFC [14]. Because most of the CSPs are silica-based, modifiers are usually necessary to minimize interaction of polar analytes with residual silanols [15]. Modifiers raise the critical temperature and pressure of the eluent. Because near-ambient temperatures are typically used for chiral separations, the eluents are generally in the subcritical state for packed column applications [16]. Detection is commonly performed by ultraviolet (UV) absorbance, although other detection methods, such as mass spectrometry, have also been reported [10].

Because most of the enantiomeric separations achieved using packed column SFC have also been performed by LC, some of the potential of packed column SFC for chiral separations was not immediately recognized. Although the reduced analysis times in SFC improve sample throughput, perhaps the greatest contribution of chiral SFC will be to facilitate chiral method development [17–19]. Increased column efficiency in SFC improves peak resolution and reduces the likelihood of interference from other components in the sample [5]. As a result, quantitation accuracy and method ruggedness in SFC are likely to exceed those of LC. Rapid column equilibration speeds the process of column evaluation and parameter optimization [20].

Although many racemates have been resolved via the open tubular approach [6,21], the use of packed columns has been the dominant technique for chiral separations in SFC [22] and will be the emphasis of this review. This is not intended to be an exhaustive review, but it will focus on the most important developments in the field and identify areas where SFC differs significantly from other chromatographic techniques.

2. Chiral stationary phases

With the exception of the chiral crown ether and protein-based CSPs, chiral separations in SFC on packed columns have been reported on all the major types of stationary phases. A summary of enantiomers resolved by SFC is provided in a recent book on packed column SFC [23], and several papers have provided comparisons of LC and packed column SFC for chiral separations [19,24]. Serial coupling of similar and dissimilar CSPs has also been implemented for enantioseparations [25–27].

2.1. Brush-type CSPs

The first demonstration of packed column SFC on a CSP utilized (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine as the chiral selector and was reported by Mourier et al. in 1985 [3]. Separations of phosphine oxides were obtained using alcohols or alcohol–water mixtures as modifiers. Since that report, enantioseparations on other brush-type CSPs have been obtained for both derivatized and underivatized solutes, including amino acids [28,29], antimalarials [30], pyrethroids [31], and β-blockers [25]. Both β-blockers and β-agonists have been resolved on a brush-type CSP having π-donor characteristics [32]. Although the assumption has often been made that the transition from LC to packed column SFC always results in faster analyses [33], the eluent strength of carbon dioxide–methanol mixtures in SFC was found to be lower than that of hexane–dichloroethane–ethanol mixtures in LC. As a result, retention in SFC was greater than that in LC, despite the fact that a higher flow-rate was used in SFC (Fig. 1). Apparently, the solute–mobile phase and mobile phase–stationary phase interactions were not equivalent for LC and SFC [24,33].

Another dichotomy between LC and SFC using the same column was reported for the separation of β-blockers on ChyRoSine-A, a CSP derived from tyrosine [34]. Although excellent separations were achieved in SFC, poor resolution of the same solutes was observed in LC. Nuclear magnetic resonance and molecular modeling studies suggested that the presence of carbon dioxide in SFC altered the configuration of the analytes [35]. Short (5 cm) columns incorporating this chiral selector were used to resolve β-blockers in less than 2 min [36].

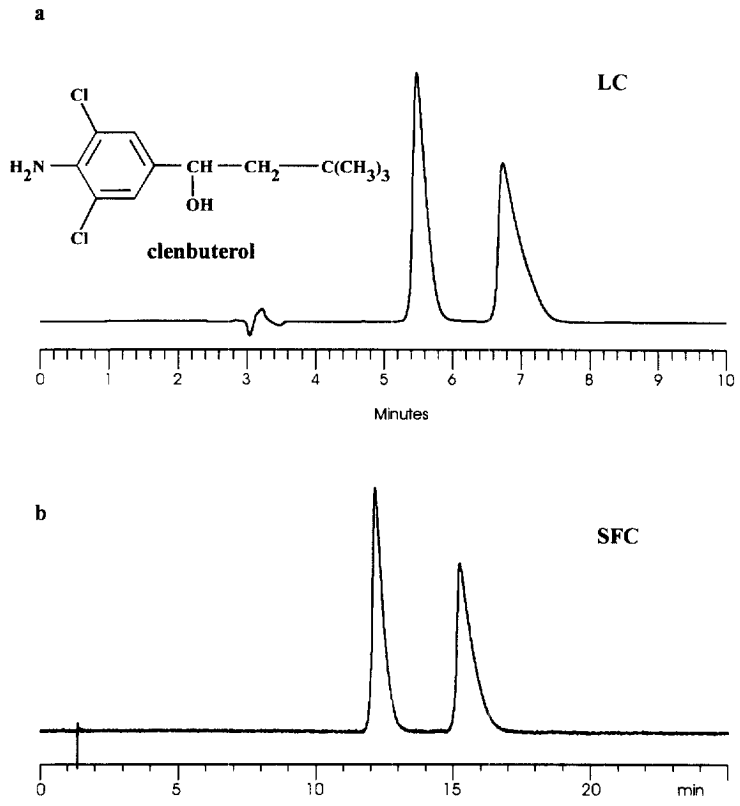

Fig. 1. Comparison of LC and SFC enantiomeric separations of clenbuterol on the Chirex 3022 CSP. Chromatographic conditions: (a) hexane–1,2-dichloroethane–(ethanol–trifluoroacetic acid) (50:35:15, v/v), 1.0 ml/min, 22°C, UV detection at 254 nm; (b) carbon dioxide–methanol (85:15, v/v), 2.0 ml/min, 30°C, 15 MPa, UV detection at 254 nm. From [32].

A new brush-type CSP (Whelk-O-1), bearing both π-acidic and π-basic interaction sites, has been used for both analytical and preparative scale enantiomeric separations of pharmaceutical compounds [19]. Terfloth et al. modified the Whelk-O-1 and analogous chiral selectors by incorporating them into polysiloxanes [37]. The resulting polymers were coated on silica and thermally immobilized. These CSPs resolved several α-arylpropionic acids, including ibuprofen and flurbiprofen. A larger number of compounds was resolved using SFC than LC, and more compounds were resolved with a single mobile phase composition in SFC. These results indicated that successful enantiomer resolution was more likely to be achieved on the first try if SFC were used.

2.2. Cyclodextrin CSPs

Native cyclodextrin-based chiral stationary phases are generally used in the reversed-phase mode in LC and have not been used extensively in SFC. Macaudiere et al. reported the enantioseparation of racemic amides and phosphine oxides on a β-cyclodextrin CSP [38]. When the carbon dioxide–methanol eluent in SFC was replaced with hexane–ethanol in LC, reduced stereoselectivity was observed. The authors theorized that carbon dioxide was more readily displaced from the cyclodextrin cavity than hexane and that this difference permitted inclusion complex formation in SFC. Therefore, SFC was observed to be complementary to LC in terms of the compounds resolved [4].

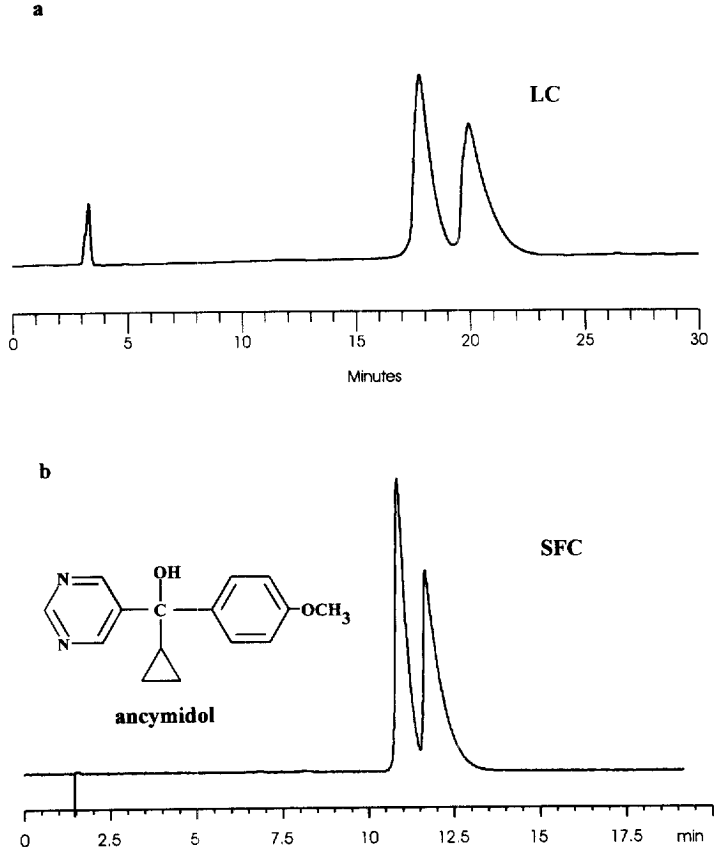

Fig. 2. Comparison of LC and SFC enantiomeric separations of ancymidol on the Cyclobond I 2000 SN CSP. Chromatographic conditions: (a) 1% triethylammonium acetate–acetonitrile (80:20, v/v), pH 7.0, 1.0 ml/min, 22°C, UV detection at 254 nm; (b) carbon dioxide–methanol (90:10, v/v), 2.0 ml/min, 30°C, 15 MPa, UV detection at 254 nm.

Derivatized cyclodextrin-based CSPs, such as the naphthylethylcarbamoylated-β-cyclodextrin CSPs, can be used under normal phase, reversed-phase, and polar organic mobile phase conditions in LC [39]. Different types of compounds are resolved in each of the three mobile phase modes. Therefore, parameter selection may involve considerable time and experimentation. Williams et al. [40] demonstrated that carbon dioxide–methanol eluents in SFC could be used to achieve separations analogous to those obtained in the reversed-phase mode in LC (Fig. 2). A carbon dioxide–alcohol eluent could also be used to perform separations representative of all three mobile phase modes in LC in a single analysis in SFC (Fig. 3) [18]. This approach streamlined the parameter optimization process compared to LC. The alcohol modifier played an important role in enantioselectivity, and this role was not the same for all analytes. As in LC, more than one chiral recognition mechanism may be operative for the derivatized cyclodextrin CSPs in SFC [40].

2.3. Polysaccharide CSPs

Various derivatives of cellulose and amylose adsorbed on silica gel have proven to have wide applicability in LC [41,42]. However, these stationary phases tend to suffer from low efficiency and long analyses. Dramatic improvements in efficiency and reductions in analysis time have been demonstrated by replacing the hexane–alcohol mobile phases used in LC with carbon dioxide–alcohol eluents in SFC, as shown in Fig. 4 [43]. Separations of β-blocker enantiomers on a cellulose-based CSP (Chiralcel OD) have been reported by several researchers [43–45]. Differences in selectivity between LC and SFC with cellulose-type CSPs tended to be compound specific [32], and an examination of propranolol analogs highlighted differences in enantioselectivity between the two techniques [45]. Benzodiazepines can also be resolved in SFC on the Chiralcel OD CSP (Fig. 5), and this capability was utilized by Wang et al. to determine the enantiomeric

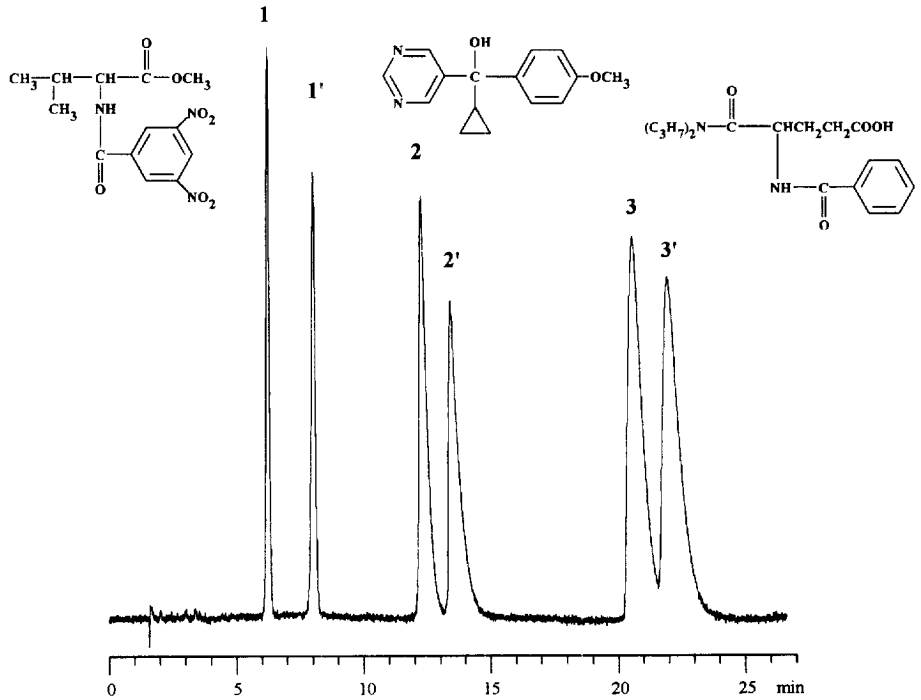

Fig. 3. Separation of the enantiomers of N-(3,5-dinitrobenzoyl)-DL-valine methyl ester (1 and 1'), ancymidol (2 and 2'), and proglumide (3 and 3') on the Cyclobond I 2000 RN CSP. Chromatographic conditions: carbon dioxide–methanol (90:10, v/v), 2.0 ml/min, 30°C, 15 MPa, UV detection at 254 nm. From [18].

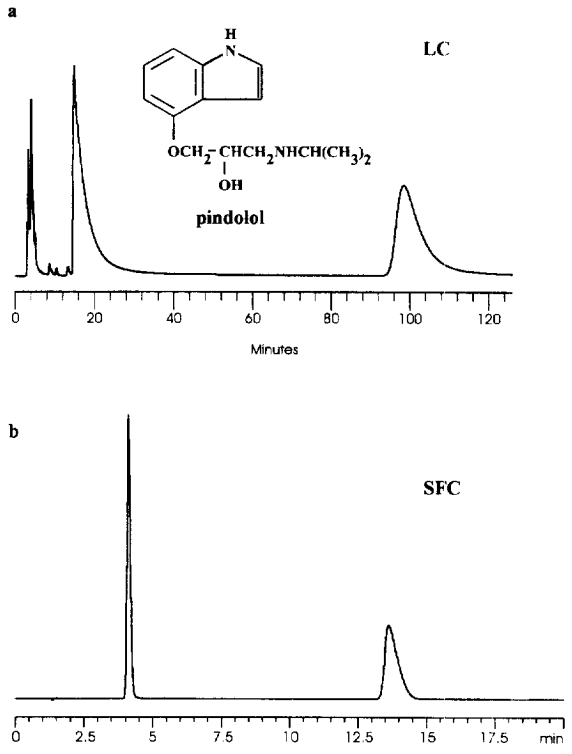

Fig. 4. Comparison of LC and SFC separations of pindolol on the Chiralcel OD CSP. Chromatographic conditions: (a) hexane–2-propanol–diethylamine (80:20:0.1, v/v/v), 1.0 ml/min, 22°C, UV detection at 280 nm; (b) carbon dioxide–(methanol–isopropylamine) (70:30, v/v), 2.0 ml/min, 30°C, 15 MPa, UV detection at 280 nm. From [32].

composition of camazepam and its metabolites [46]. Although amylose-based CSPs have not been studied as extensively in SFC as their cellulose-based counterparts, enantioseparations of non-steroidal anti-inflammatory drugs, such as ibuprofen and flurbiprofen, as well as other compounds of pharmaceutical interest have been demonstrated on an amylose- derived CSP (Chiralpak AD) [25,47].

2.4. Other CSPs

Wilkins et al. adsorbed a chiral anthrylamine derivative on porous graphitic carbon and evaluated the resulting CSP in SFC [48]. Enantioseparations of anti-inflammatory agents and tropic acid derivatives were achieved using a carbon dioxide–methanol eluent. The carbon-based CSP produced superior results when compared to a silica-based CSP having the same chiral selector.

The Chiralpak OT CSP, based on a helical polymethacrylate coated on silica gel, was studied by Macaudiere et al. in SFC [4]. Although successful enantioseparations were obtained, the chromatographic behavior of the CSP in SFC differed from LC results. The eluent used in SFC may have altered the accessibility of sites involved in chiral recognition [49].

3. Parameter selection

In packed column SFC, the analyst can control flow-rate, pressure, temperature and mobile phase composition. Each of these parameters can affect retention, selectivity, and resolution [23]. Predicting the effect of changes in parameters is often difficult, and effects are often specific to a particular CSP or compound. However, the rapid equilibration of the chromatographic system in SFC after changes in operating parameters speeds the process of evaluating chromatographic conditions.

3.1. Modifier

Nearly all the reports of chiral separations in SFC have included an investigation of the effects of various modifiers and/or additives on enantioselectivity [4,29,40]. The modifier can also impact retention and resolution [18,47]. Because the modifier can interact with both the stationary phase and the analyte, predicting the effect of changing modifier on selectivity is generally not feasible [25]. Also, as shown by Cantrell et al., the physical state of the eluent (subcritical or supercritical) may alter the influence of the modifier on selectivity [50]. Of the alcohol modifiers studied, methanol typically yields better efficiency but does not always produce the highest enantioselectivity [24,33]. Addition of water to the alcohol has been shown to improve efficiency for a brush-type CSP [3].

The addition of small amounts of very polar compounds such as trifluoroacetic acid or isopropylamine to the modifier may also improve peak shape and enhance peak resolution, as shown in Fig. 6. Acidic additives are used for acidic solutes, and

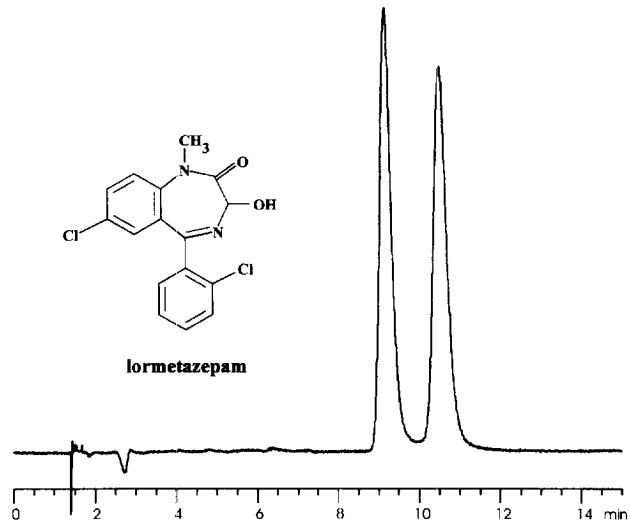

Fig. 5. Separation of lormetazepam enantiomers on the Chiralcel OD CSP. Chromatographic conditions: carbon dioxide–(ethanol–isopropylamine) (85:15, v/v), 2.0 ml/min, 30°C, 20 MPa, UV detection at 240 nm.

basic additives improve the peak shape of basic solutes [23]. The use of additives generally does not compromise enantioselectivity [44,51].

3.2. Pressure

In general, pressure has a greater impact on retention than on enantioselectivity [3,34]. However, noticeable changes in selectivity with pressure have been reported for an amylose-based (Chiralpak AD) CSP [45]. For some analytes, selectivity decreased with increasing pressure, while increased selectivity was observed for other analytes with increasing pressure. The authors postulated that the conformation of the amylose-derived backbone was altered by changes in pressure. These pressure-dependent changes in selectivity were not observed on a cellulose-derived CSP.

3.3. Temperature

Although column efficiency generally improves as temperature increases, the enantioselectivity of the CSP typically decreases at higher temperature. Therefore, most chiral separations in SFC have been performed at temperatures below the critical temperature of carbon dioxide or carbon dioxide–modifier mixtures in order to optimize both selectivity and resolution [14,23]. Predicting the effect of changes in temperature is difficult because even structurally similar compounds may exhibit different temperature-dependent behavior [52]. Stringham and Blackwell demonstrated a novel use of temperature to modify the enantioselectivity of a brush- type CSP [53]. As the analysis temperature increased, the decreased enantioselectivity eventually caused co-elution of the enantiomers. Further increases in temperature led to reversal of elution order ($\alpha<1.0$) for two compounds. These separations were said to be 'entropically driven.' Use of elevated temperatures has the added advantage of high column efficiencies. However, the stability of most CSPs at elevated temperatures has not been demonstrated.

3.4. Flow-rate

A direct consequence of the favorable solute diffusion coefficients in SFC is that higher flow-rates can be used to reduce analysis times without seriously sacrificing selectivity or resolution. Kot et al. demonstrated that the selectivity observed for Chiralcel OD and Chiralpak AD CSPs was nearly independent of flow-rate in the range of 0.5–5.0 ml/min. However, a decrease in resolution was observed as the flow-rate increased [25]. The use of

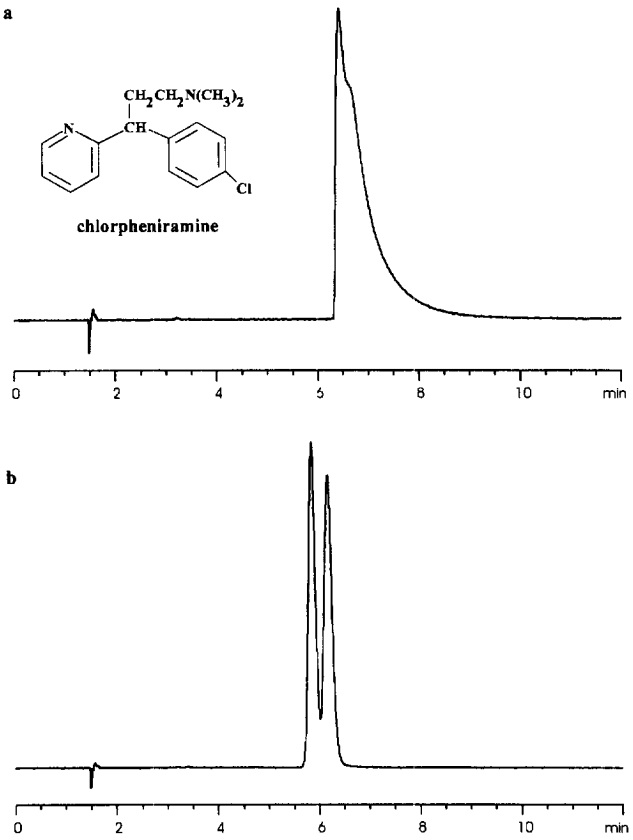

Fig. 6. Comparison of enantiomeric separations of chlorpheniramine on the Chiralpak AD CSP without (a) and with (b) the addition of isopropylamine (0.5% v/v) to the ethanol modifier. Chromatographic conditions: carbon dioxide–ethanol (90:10, v/v), 2.0 ml/min, 30°C, 15 MPa, UV detection at 254 nm.

high flow-rates is also valuable for studying stereolabile compounds [54].

4. Preparative separations

Reduction of solvent waste and the ease of solvent removal make SFC attractive for large scale separations that have traditionally been performed by LC. Preparative scale separations have been limited by the complexity of equipment required, but commercial systems are being developed [55,56]. Blum et al. used a Whelk-O-1 column having dimensions of 25 cm×25.4 mm for the resolution of warfarin and a proprietary drug candidate [19]. Sample loading of 200 mg was possible for warfarin enantiomers. The separation of racemic glibenclamide analogs on cellulose- and amylose-derived CSPs having dimensions of 25 cm×10 mm was reported by Whatley [57]. Improved peak symmetry and resolution allowed higher sample throughput in SFC than in LC.

5. Conclusions

Much of the considerable potential of SFC for chiral separations has only recently been identified, and misconceptions about the applicability of the technique persist. Chiral SFC is broadly applicable to existing CSPs utilized in LC, often resulting in

improved selectivity, resolution, and efficiency. Based on the increasing number of reports of reduced analysis times, simplified method development, and improved method ruggedness, SFC seems destined to become the technique of choice for many enantiomer separations.

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