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Chiral HPLC versus chiral SFC: evaluation of long-term stability and selectivity of Chiralcel OD using various eluents*

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Abstract: The long-term stability of Chiralcel OD columns under varying HPLC and SFC conditions was evaluated. Two new columns from the same supplier lot were procured, and one was installed in an HPLC system, and the other was installed in an SFC system. Enantiomeric mixtures of *trans*-stilbene oxide and carbobenzyloxy phenylalaninol were repeatedly injected in both systems over several days. For HPLC, hexane was the primary solvent used, along with IPA, EtOH, MeOH/IPA, or EtOH/TFA modifier. Carbon dioxide was the primary SFC solvent, together with the similar modifiers as the HPLC. Column performance was monitored by measuring resolution, theoretical plate number, and α between enantiomeric peaks. It was observed that, when the eluent strengths were adjusted to provide comparable retention times, the Chiralcel OD gave superior enantiomeric resolution when used in the SFC than the HPLC. Equilibration in between solvents was faster with SFC. The columns were demonstrated to be quite stable in both systems.

Keywords: *Chiral analysis; HPLC; supercritical fluid chromatography; chiral stationary phase stability; selectivity.*

Introduction

The determination of enantiomeric purity of raw materials, intermediates and finished drug substances has recently increased in importance in the manufacture of chiral drugs. Several chiral stationary phases have been used to analyse chiral drugs by HPLC. However, researchers have often encountered problems: marginal resolution, poor peak shape, poor column stability especially with certain solvents, and difficult method development.

A Hewlett–Packard SFC instrument produced chiral separations that were superior to HPLC for selected drug development samples. As generally recognized, SFC provides more theoretical plates than HPLC for the same column mainly due to the faster mass transfer kinetics in subcritical or supercritical carbon dioxide compared to liquid hexane. However, a review of the literature revealed few reports where chiral analysis was accomplished on an SFC instrument [1–11].

The technique of chiral SFC as an analytical tool for process development of chiral drugs and for quality control during manufacture was investigated. For these uses, column stability and enantiomeric resolution are of major concern.

Experimental

Apparatus

A Hewlett–Packard 1050 HPLC equipped with a variable wavelength UV detector, quaternary pump and a variable injection volume autosampler was used for LC analysis. A Hewlett–Packard G1205A SFC equipped with a multiwavelength UV detector, supercritical fluid pump, modifier pump, and fixed volume internal loop was used for SFC analysis. Both systems were also equipped with an MS-DOS based chem station to facilitate direct comparison of results.

Mobile phases

Liquid mobile phases and SFC modifiers were prepared from EM Science HPLC grade isopropanol, hexane, and methanol. Ethanol 200 proof USP grade, was purchased from Quantum Chemical. SFC grade CO_2 was purchased from Scott Specialty Gases. The

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trifluoroacetic acid (TFA) used was J.T. Baker 'analysed grade'.

Stationary phase

Two Chiralcel OD 25 cm × 0.46 mm i.d. columns were purchased through J.T. Baker from Chiral Technologies. Both columns used in this study were from the same lot of packing material to avoid potential batch to batch differences.

Samples

For each HPLC or SFC run, eight vials were filled with eluent and various solutions of *trans*-stilbene oxide, CBZ-phenylalaninol or CBZ-alanine. The vials were loaded into the autosampler and each was injectd six times.

Methods

Methods using different modifiers and flow rates were developed such that the resolution of enantiomers was optimized while comparable retention times (i.e. analysis times) for the samples were retained. In some situations, the HPLC and SFC modifier concentrations were dissimilar due to practical considerations. Details of the methods are given in Table 1.

Method changes and injection sequences

Over a period of 2 weeks, samples were injected into each Chiralcel OD column, one installed in the HPLC and the other installed in the SFC. The methods (differing mainly in modifier) were changed from day to day. Columns were generally left in the instrument. When switching eluents, equilibration was effectively done by injecting mobile phase repeatedly. This way, improvement in baseline drift and noise could be monitored.

Instrument problems were encountered with the SFC during two of the runs with method C. Although no useful data were collected, samples were injected into the column and this was included in the injection count. For all of the methods, at least 49 injections per day were made onto the column in each chromatographic system.

Performance monitoring

Using standard instrument software, peak retention times (t_R), deadtimes (t_0), and peak widths at half height ($W_{1/2}$) were tabulated in an Excel spreadsheet. Enantiomeric resolution (R), total theoretical plates (n), and separation selectivity factor (α) were calculated from conventional equations for each injection.

Results and Discussion

Test compounds

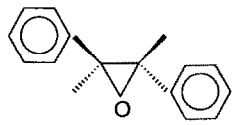
The three compounds studied were chosen out of convenience. *trans*-Stilbene oxide was

Table 1
HPLC and SFC method parameters

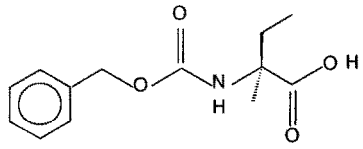
	HPLC	SFC
Instrument	HP 1050	HP G1205A
Column*	Chiralcel OD	Chiralcel OD
Injection volume	5 μl	5 μl
Detection	UV, 210 nm	UV, 210 nm
Temperature	40°C	40°C
Pressure	18–40 bar	200 bar
Eluent	hexane with modifier	CO_2 with modifier
Method A:		
modifier	10% IPA	13% IPA
flow	0.8 ml min^{-1}	1.3 ml min^{-1}
Method B:		
modifier	8% EtOH	13% EtOH
flow	0.8 ml min^{-1}	1.3 ml min^{-1}
Method C:		
modifier	6.4% MeOH, 1.6% IPA†	13% MeOH
flow	0.8 ml^{-1}	1.3 ml min^{-1}
Method D:		
modifier	19.8% EtOH, 0.2% TFA	20% EtOH, 0.04% TFA
flow	0.5 ml min^{-1}	1.0 ml min^{-1}

*The two columns were from the same manufacturer lot.
†IPA was necessary for miscibility of MeOH with hexane.

trans-Stilbene Oxide



CBZ-Alanine



CBZ-Phenylalaninol

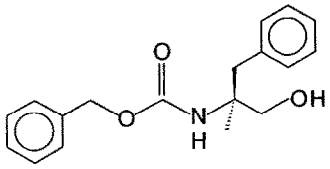


Figure 1
Structures of test compounds.

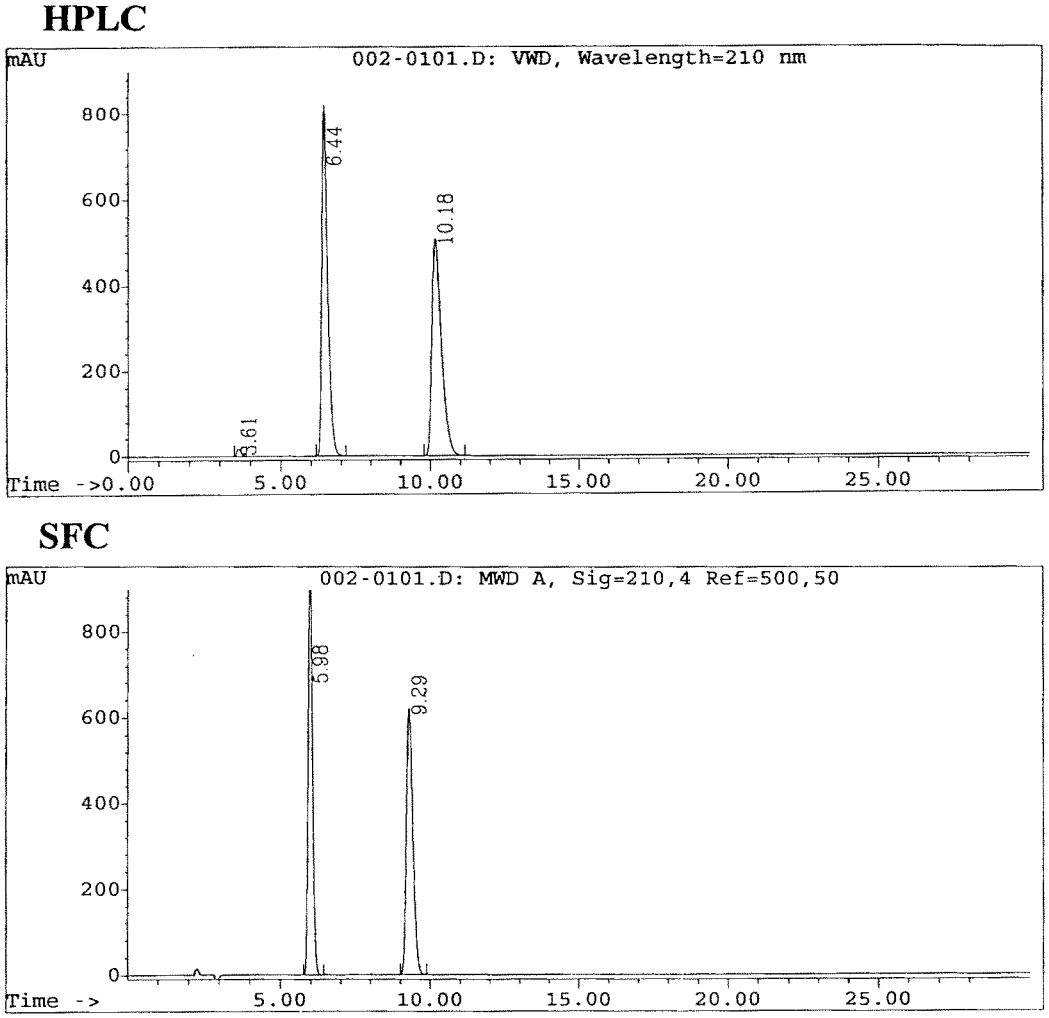


Figure 2
Resolution of *trans*-stilbene oxide enantiomers: method A.

the test compound that came with the columns and CBZ-phenylalaninol and CBZ-alanine were readily available drug synthesis raw materials. The structures (Fig. 1) are such that the compounds have similarities and differences with regard to the groups about the chiral centre.

Resolution, theoretical plates and separation factor

The separation factor, α, for a particular enantiomeric pair and analogous eluent modifier was similar between HPLC and SFC. However, the theoretical plates observed in the SFC system were consistently three to five times greater. As a result, the resolution was better for SFC.

Selectivity

No great difference in selectivity, as measured by α, was observed between techniques. However, the effect of modifier was different for the three compounds (see Figs 2–8).

For *trans*-stilbene oxide, the selectivity using method A (IPA modifier) was clearly best. Method B (EtOH) and method C (MeOH) were comparable. For CBZ-phenylalaninol, the use of IPA versus EtOH and MeOH did not have a significant impact on selectivity for either technique. For CBZ-alanine, the addition of TFA to EtOH was necessary to achieve separation of enantiomers. More TFA was required for HPLC.

Column equilibration

In switching from one modifier to another, it was observed that column equilibration occurred much faster with the SFC than HPLC. This was determined by observing the

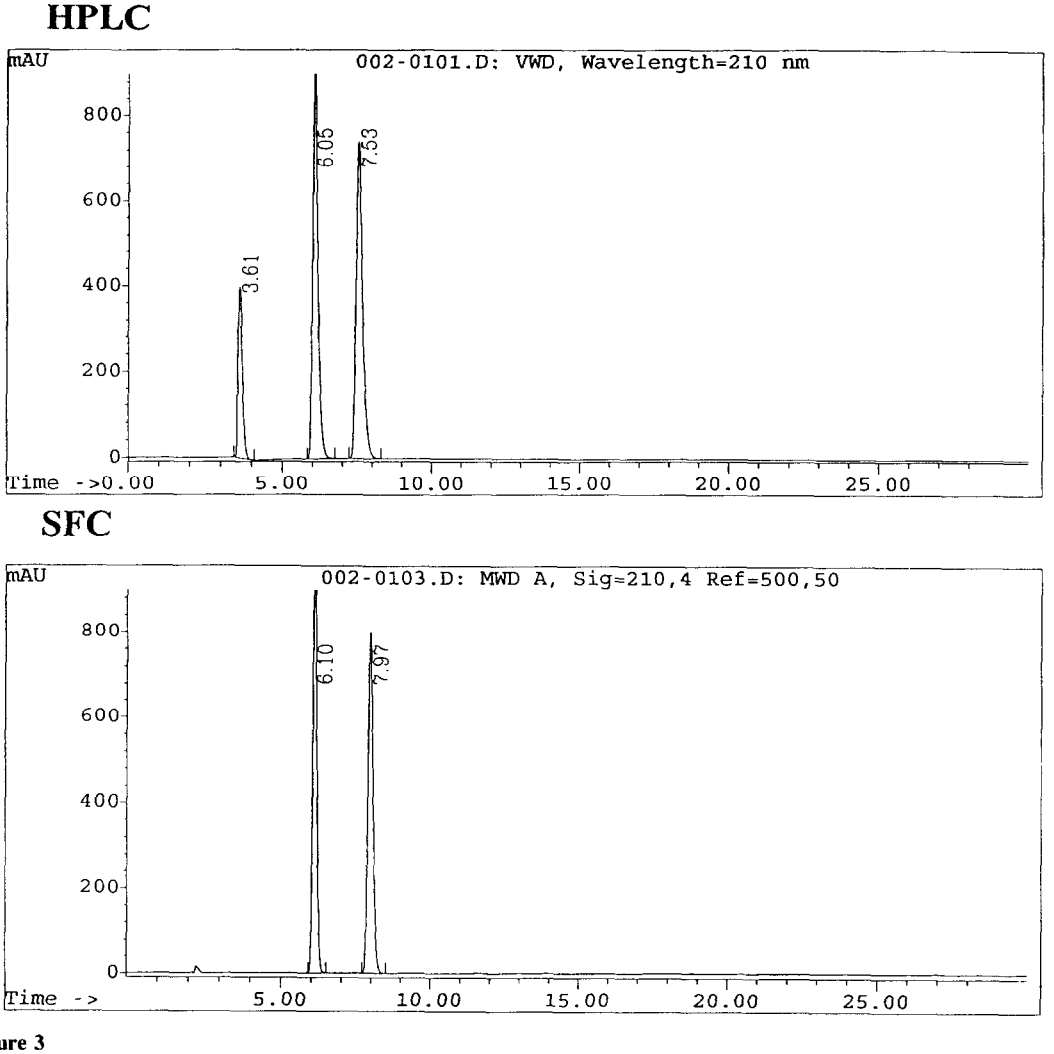


Figure 3
Resolution of *trans*-stilbene oxide enantiomers: method B.

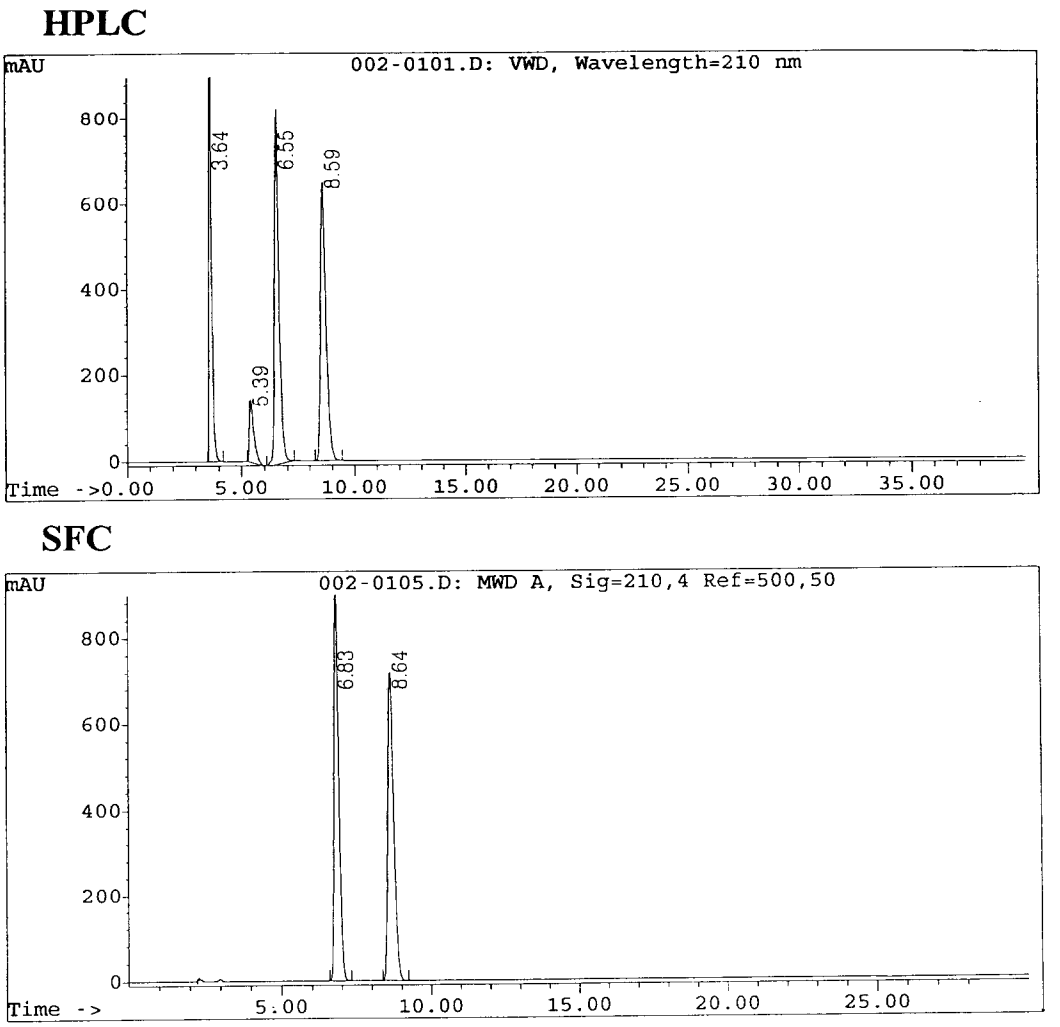


Figure 4
Resolution of *trans*-stilbene oxide enantiomers: method C.

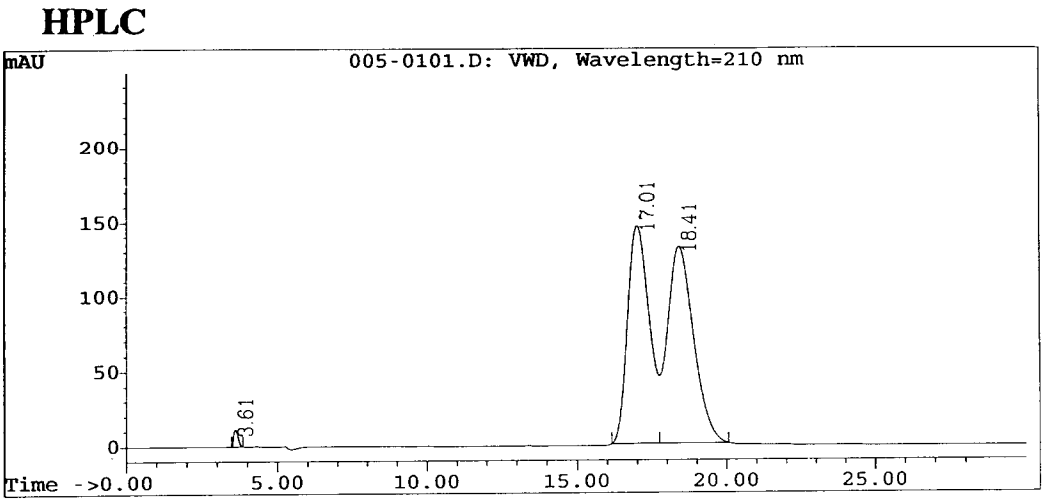


Figure 5
Resolution of CBZ-phenylalaninol enantiomers: method A.

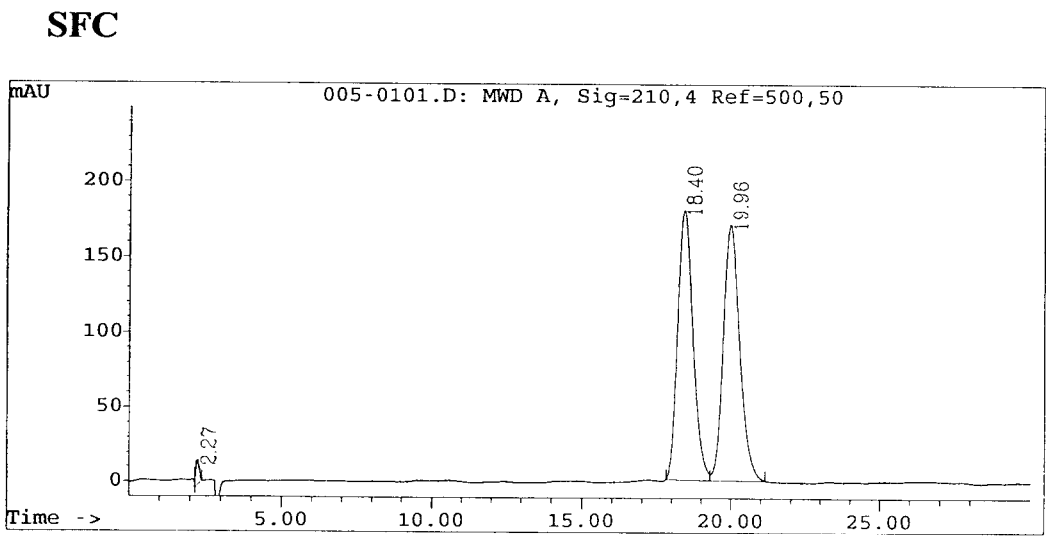


Figure 5
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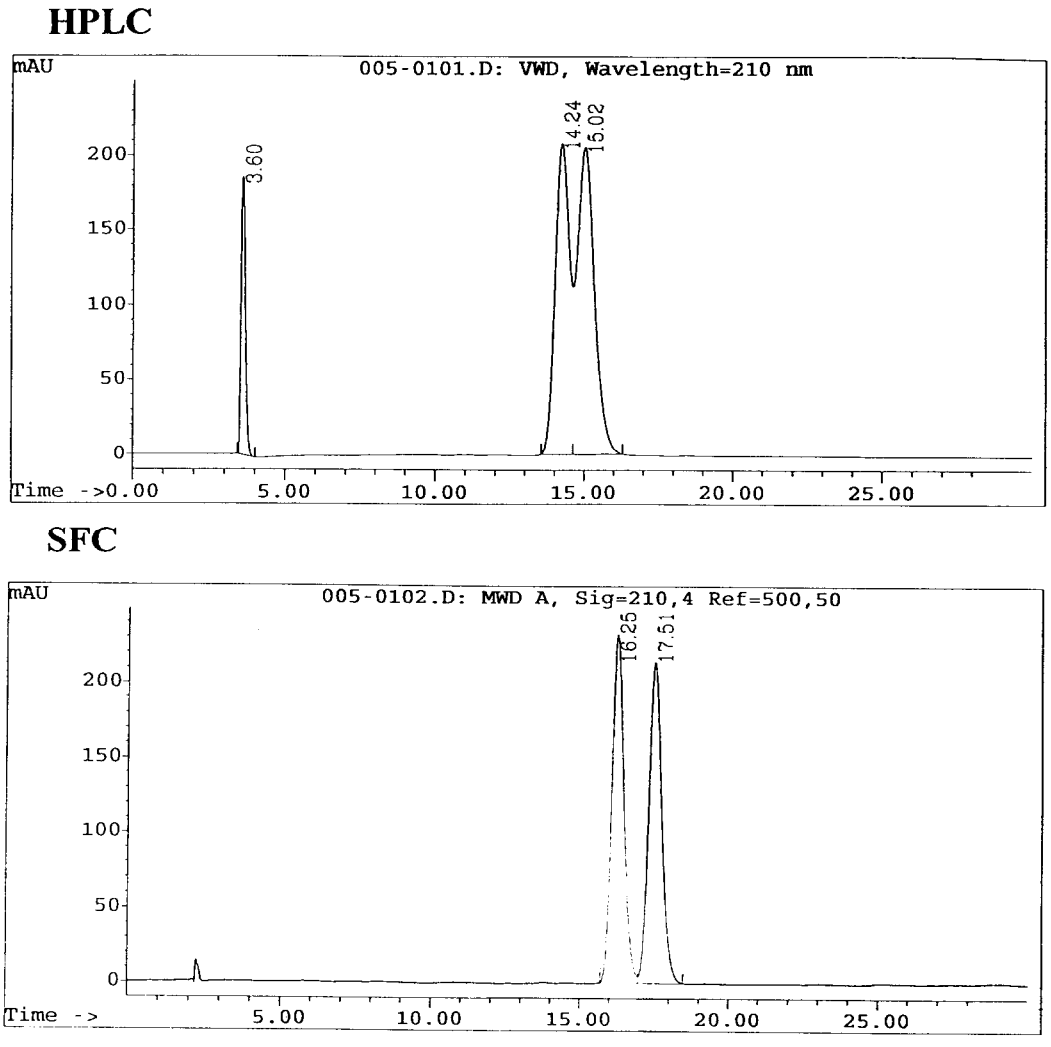


Figure 6
Resolution of CBZ-phenylalaninol enantiomers: method B.

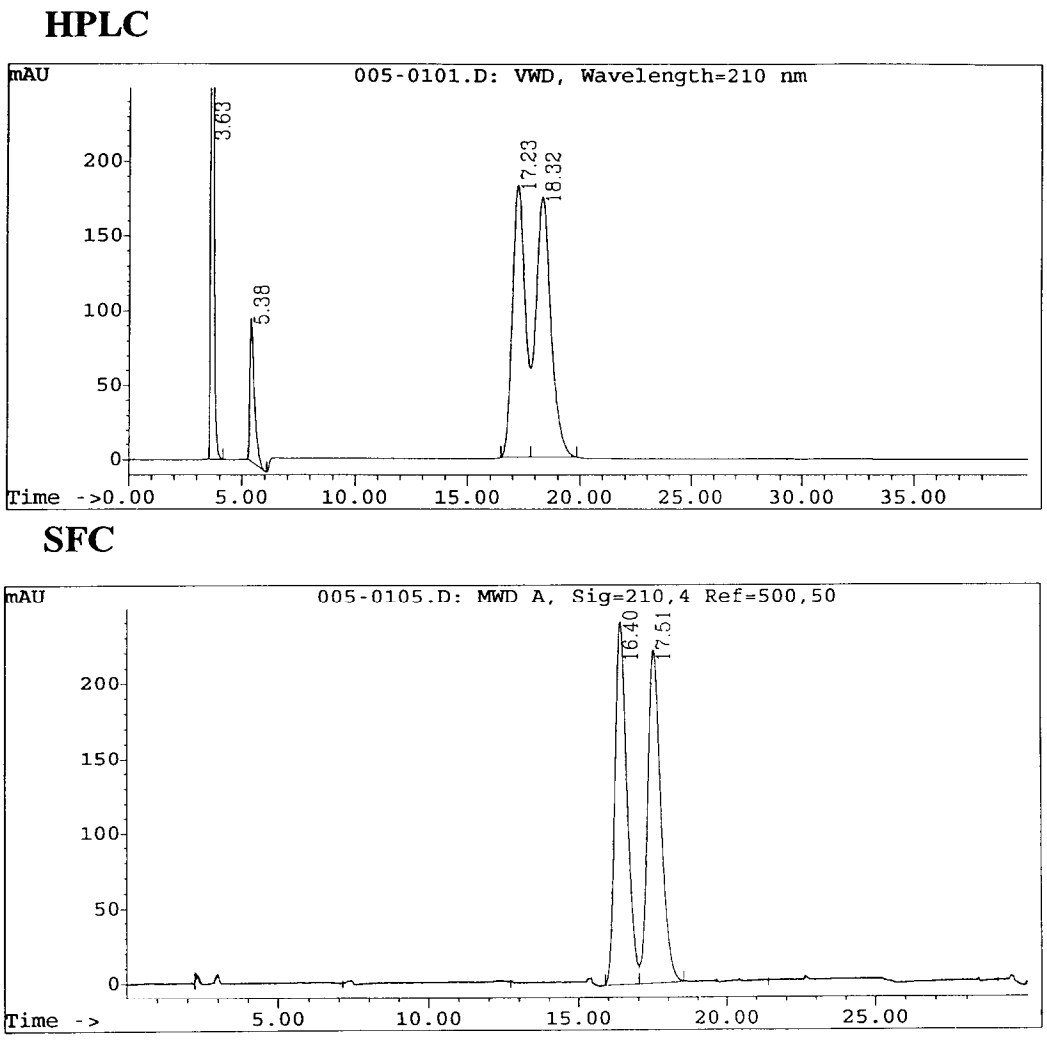


Figure 7
Resolution of CBZ-phenylalaninol enantiomers: method C.

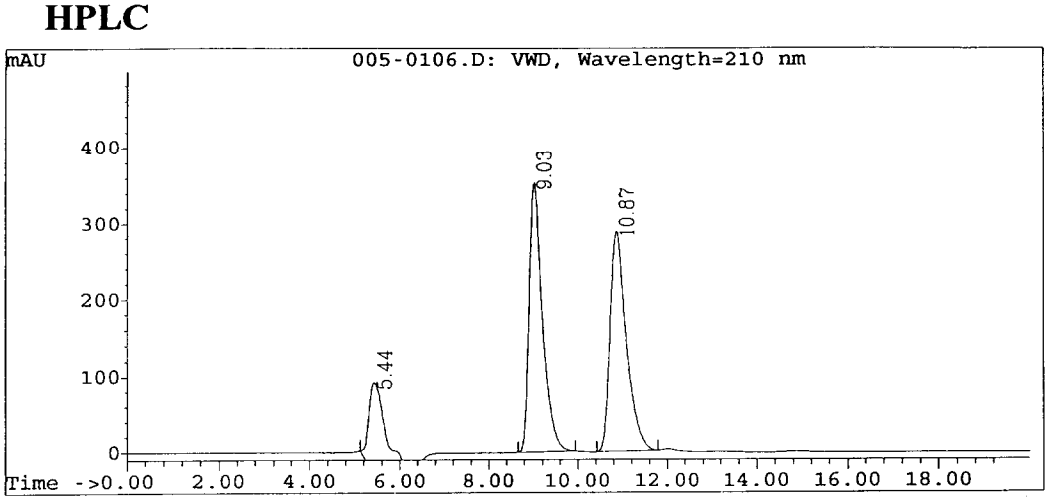


Figure 8
Resolution of CBZ-alanine enantiomers: method D.

SFC

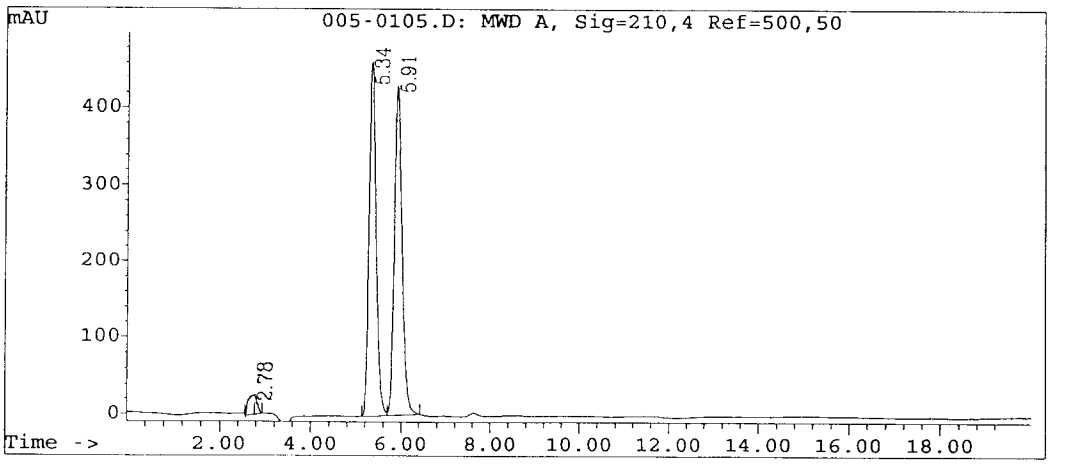
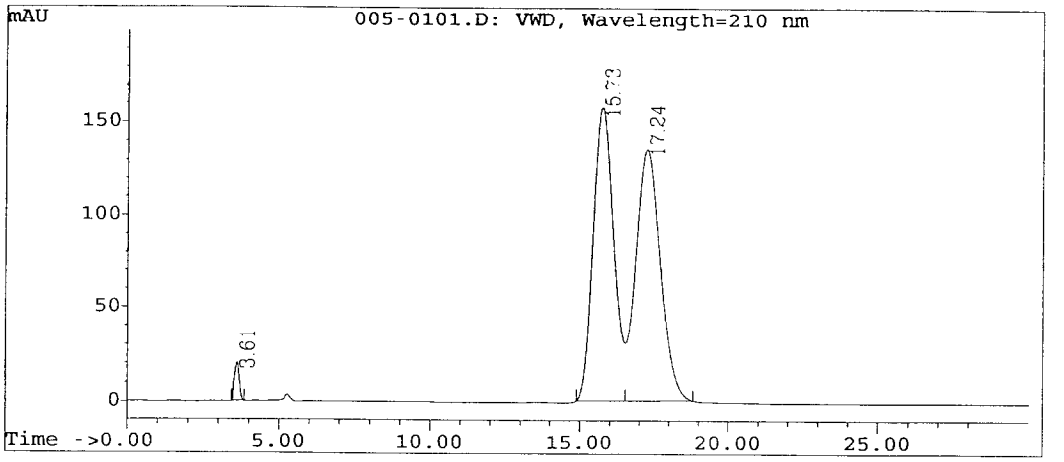


Figure 8
Continued.

Injection # 25



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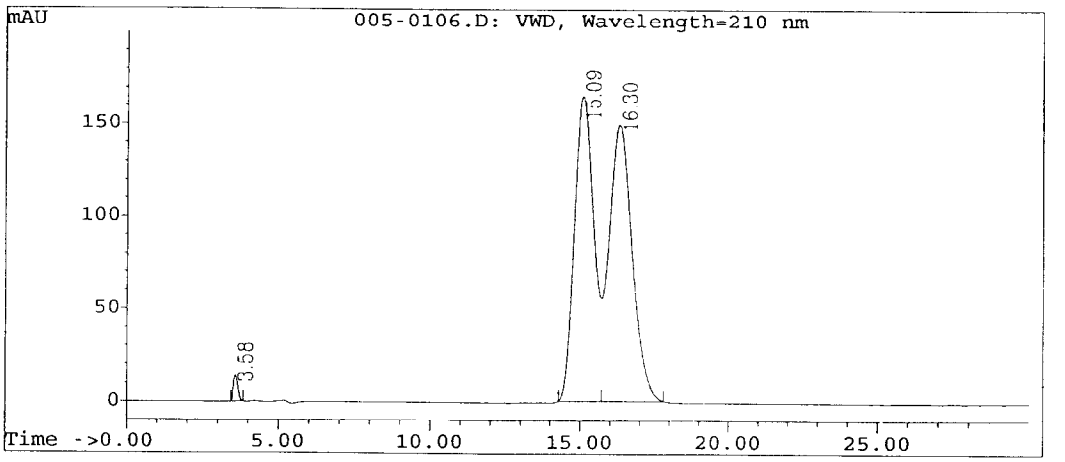

Figure 9
HPLC chromatograms of CBZ-phenylalaninol on Chiralcel OD, method A.

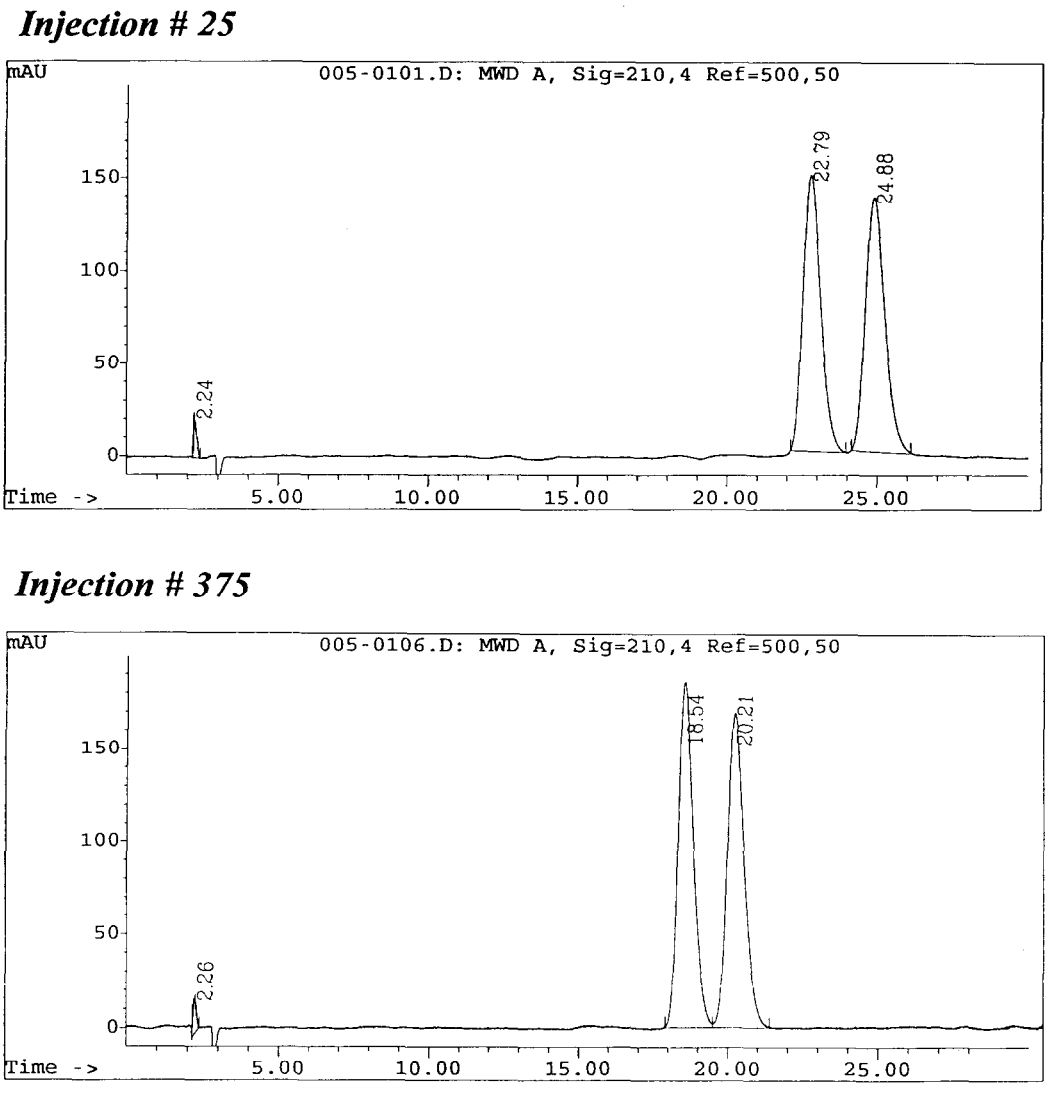

Figure 10
SFC chromatograms of CBZ-phenylalaninol on Chiralcel OD, method A.

Table 2
Resolution, theoretical plates and separation factor for CBZ-phenylalaninol as function of injection number for HPLC and SFC

Inst.	Inj. no.	T_0	T_rA	$W_{½}$A	T_rB	$W_{½}$B	Resolution	α	N_A	N_B
LC	25	3.61	15.732	0.681	17.238	0.850	0.98	1.12	2935	2262
LC	30	3.61	15.715	0.719	17.217	0.852	0.96	1.12	2627	2246
LC	203	3.61	17.008	0.747	18.414	0.880	0.86	1.10	2851	2408
LC	208	3.61	16.992	0.740	18.392	0.887	0.86	1.10	2900	2365
LC	302	3.58	15.096	0.678	16.315	0.792	0.83	1.11	2727	2334
LC	307	3.58	15.086	0.668	16.301	0.791	0.83	1.11	2805	2336
SFC	25	2.24	22.789	0.493	24.881	0.526	2.05	1.10	11752	12306
SFC	30	2.22	22.800	0.503	24.941	0.544	2.04	1.10	11300	11561
SFC	272	2.27	18.400	0.433	19.965	0.466	1.74	1.10	9932	10096
SFC	277	2.30	18.395	0.445	20.011	0.464	1.78	1.10	9398	10230
SFC	370	2.24	18.538	0.425	20.205	0.464	1.88	1.10	10464	10429
SFC	375	2.26	18.541	0.416	20.206	0.465	1.89	1.10	10926	10385

*Method A was used. See Table 1 for experimental conditions.

baseline drift. In SFC, the baseline typically stabilized within 30 min of eluent flow. In contrast, the column installed in the HPLC system required 2–3 h before baseline drift was completely eliminated. Correcting for the higher SFC flow rates, SFC still showed an advantage.

Column stability

Data on resolution, α, and theoretical plates were collected for both *trans*-stilbene and CBZ-phenylalaninol. Either compound was useful in monitoring the change in column performance. HPLC and SFC chromatograms for CBZ-phenylalaninol are shown in Figs 9 and 10, respectively. The data are tabulated in Table 2.

For both columns, there was only a slight drop in performance that was observed after more than 300 injections using the different eluents. Neither HPLC nor SFC demonstrated an advantage with regard to column stability under the conditions used.

Conclusions

The Chiralcel OD column yielded superior enantiomeric resolution on the SFC, relative to HPLC, when the eluent strengths were adjusted to provide comparable retention times. This is important when doing quality control, where resolution and speed are both desirable.

The eluent modifier affected selectivity in the same way in both systems. The effect of modifier was different for each compound tested. This is not inconsistent with expectations, but further work is needed to understand the mechanisms of separation in these systems. Equilibration between solvents was much faster with SFC. This would aid in method development, where changes in mobile phase are quite common.

The columns were demonstrated to be quite stable in both systems. No significant drop in performance was observed over more than 300 injections.

Based on the above, SFC may be a superior alternative to HPLC when performing chiral analysis on Chiralcel OD.

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