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Chiral supercritical fluid chromatography of phenylpropanols and related compounds

Roger M. Smith*, Li Ma

Department of Chemistry, Loughborough University, Loughborough, Leics, LE11 3TU, UK

Abstract

The relationship between the structures of a number of phenylpropanols and related compounds and their enantioselectivity on supercritical fluid chromatography using Chiralcel OD and Chiralcel OB cellulose based columns has been examined. Changes in the resolution with different temperatures, pressures and proportions of modifier in the carbon dioxide eluent have been determined. © 1997 Elsevier Science B.V.

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1. Introduction

There has been considerable interest in the chiral chromatography of drugs and agrochemicals, because of possible differences in the biological properties of their enantiomers. A wide range of stationary phases has been employed in both high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) [1,2] but it is often difficult to predict which columns will be suitable for a particular analyte. Some of the most widely applicable columns, in terms of the range of different compounds that can be resolved, have been the triesters and tricarbamates of cellulose or amylose [3,4]. In recent years, SFC has attracted attention because it has been reported to offer advantages of speed of separation and in the ease of removal of the eluent in preparative separations [5,6]. For example, Anton et al. [7] reported that subcritical fluid chromatography of 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) using carbon dioxide+10% ethanol at room temperature gave a faster separation with a good resolution (R_s=3.4) than HPLC using hexane–propan-2-ol (90:10, v/v) (R_s=4.7) on a Chiralcel OD column. They suggested that the advantages of SFC resulted not only from the lower viscosity and higher diffusion rates of supercritical fluids but also from changes in the solute–eluent interactions enabling different selectivities to be obtained.

The applications of chiral SFC in the pharmaceutical industry has been surveyed by Kot et al. [8] and more recently by Berger [9]. Most of published studies have concentrated on a comparison of SFC with HPLC separations or on selecting the optimum conditions of temperature and pressure for the separation of a particular analyte. However, few studies have examined the relationship between the structures of related analytes and the resolutions that can be obtained.

In this study, the SFC enantioselectivities of a number of isomeric phenylpropanols and related compounds have been determined on cellulose based columns and compared with their chemical structures. Previous studies by Wainer et al. have examined the chiral recognition of this group of

*Corresponding author.

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analytes by HPLC on a Chiralcel OB column [10] and concluded that there were three major stages of interaction. The formation of a diastereoisomeric complex between the alcoholic hydrogen of the solute and the ester carbonyl of the cellulose benzoate ester group, the stabilization of the complex through insertion of the aromatic portion of the solute into a chiral cavity or ravine on the chiral stationary phase and a discrimination between solutes due to differences in their steric fit into the chiral cavity.

2. Experimental

2.1. Chemicals

Test compounds were from Aldrich (UK) and were used as supplied. Carbon dioxide was industrial grade from BOC (Brentford, UK). Methanol, ethanol and 2-propanol were of HPLC grade from FSA Laboratory Supplies (Loughborough, UK).

2.2. Equipment

Supercritical fluid chromatography was carried out using a Jasco BIP-1 pump (Hachioji City, Tokyo, Japan) with a cooled pump head (~0°C) for carbon dioxide and a PU 4100 pump (Pye Unicam, Cambridge UK) for methanol. The samples as dilute solutions (5–12 mg ml^{-1}) in methanol were injected using a 7125 Rheodyne valve (Cotati, CA, USA), fitted with a 20-μl loop, onto the column which was mounted in a Pye 104 gas chromatography oven. (Pye Unicam). The eluent was pumped at 1 ml min^{-1} and the peaks were detected at 220 nm using an ACS 750/12 variable wavelength detector fitted with a high pressure flow cell (Applied Chromatography Systems, Macclesfield, UK) and recorded using a Hewlett–Packard 3396 integrator. The column back pressure was maintained using a crimped capillary tube mounted in a heated zone in the column oven to prevent eluent freezing. Separations were carried out on Chiralcel OB (cellulose tribenzoate)or Chiralcel OD (cellulose tris(3,5-dimethylphenylcarbamate)) columns (250×4.6 mm I.D.) columns from Daicel (Dusseldorf, Germany).

3. Results and discussion

In order to be able to examine the structural features which enhance chiral selectivity in SFC, a series of isomeric aromatic alcohols was studied, 1-phenyl-1-propanol, 1-phenyl-2-propanol, 2-phenyl-1-propanol. Their resolutions were compared the separation of the related α-substituted benzyl alcohols: 1-phenyl-1,2-ethanediol, 1,2,3,4-tetrahydro-1-naphthol, ethyl mandelate (ethyl 2-hydroxyphenylacetate) and benzoin. The related epoxide, styrene oxide, and the widely used chiral test compound, 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE), were also tested. With carbon dioxide alone as the eluent, none of these samples was eluted from either a cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD) column or a cellulose tribenzoate (Chiralcel OB) column at 40°C and 125 bar.

When the separations were repeated using 7% methanol in carbon dioxide as the eluent, all the test compounds were eluted from the Chiralcel OB column but only 1-phenyl-1-propanol, 1,2,3,4-tetrahydro-1-naphthol, 1-phenyl-1,2-ethanediol and benzoin were resolved into their enantiomers (Table 1). If the proportion of methanol was reduced successively from 7–2% the retention factors of these compounds increased systematically but the enantioselectivities remained almost constant. However, with the higher retentions the resolutions improved; 1,2,3,4-tetrahydro-1-naphthol increased from R_s= 1.14 with 7% methanol to 1.42 at 2% methanol and benzoin from R_s=0.45 to R_s=0.78 at 3% methanol (Table 1).

Wainer et al. [10] examined a number of these compounds on Chiralcel OB by HPLC using hexane–propan-2-ol (90:10, v/v) as the eluent. They resolved 1-phenyl-1-propanol, 2-phenyl-1-propanol and tetrahydro-1-naphthol, but not 1-phenyl-2-propanol, with higher enantioselectivities than in SFC (Table 1). Similar HPLC results were also noted by the column manufacturer [11] who also resolved 1-phenyl-1,2-ethanediol (R_s=1.34). Anton et al. have [7] compared the separations of benzoin on a Chiralcel OB-H column (an equivalent high-efficiency stationary phase) and reported that a similar baseline resolution could be obtained in 1.5 min by SFC compared to the corresponding HPLC separation in 21 min.

Table 1
SFC separation of phenylpropanols and related compounds on a Chiralcel OB column at 40°C using different levels of methanol in carbon dioxide as modifier

Compound	Eluent (% methanol in carbon dioxide)						HPLC			Ref.
	7%			2%						
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	
1-Phenyl-1-propanol	0.65	1.12	0.32	0.96	1.14	0.50	0.80	1.27		[10]
1-Phenyl-2-propanol	0.60	1.00		0.72(5%)	1.00	-	0.70	1.00	-	[10]
2-Phenyl-1-propanol	1.05	1.00		1.18(5%)	1.00	-	0.77	1.12		[10]
1,2,3,4-Tetrahydro-1-naphthol	1.22	1.24	1.14	2.17	1.25	1.42	3.08	1.82		[10,11]
1-Phenyl-1,2-ethanediol	1.54	1.13	0.63	5.17	1.14	0.74				
Benzoin	1.27	1.11	0.45	2.79(3%)	1.11	0.78	16.9	1.23	3.33	[7]
Ethyl mandelate	0.30	1.00		0.52(3%)	1.00					
Styrene oxide	0.68	1.00								
TFAE	3.68	1.00		5.06(5%)	1.00					

Conditions: flow-rate, 1 ml min^{-1}; temperature 40°C; pressure, 125 bar.
HPLC conditions: eluent, hexane–propan-2-ol (90:10, v/v) [7,10].
k_1=Retention factor of first enantiomer to be eluted.

When the test samples were examined on the Chiralcel OD column with methanol as the modifier the pattern of enantioselectivity was different (Table 2). Neither 1-phenyl-2-propanol, 2-phenyl-1-propanol nor 1-phenyl-1,2-ethanediol were resolved, although it was now possible to resolve ethyl mandelate and TFAE as well as 1-phenyl-1-propanol. As the proportion of modifier was reduced the enantioselectivity again remained similar but the resolutions increased. The resolution of TFAE was high, R_s= 12.20, and compared well to a study on a tris(3,5-dimethylphenylcarbamate) cellulose phase by Kaida and Okamoto [4]. They found a resolution of R_s= 4.43 with carbon dioxide–propan-2-ol (96:4) compared to R_s=6.4 using hexane–propan-2-ol (90:10, v/v). They also reported that benzoin was well resolved R_s=3.2 under SFC conditions at 60°C and with a hexane–propan-2-ol (94:4, v/v) eluent under HPLC conditions, R_s=4.45.

As earlier work has shown that the choice of organic modifier can alter SFC separations [4,7], propan-2-ol was examined as an alternative modifier for separations on the Chiralcel OD column. For tetrahydro-1-naphthol and 1-phenyl-1-propanol, the

Table 2
SFC separation of phenylpropanols and related compounds on a Chiralcel OD column at 40°C using different levels of methanol in carbon dioxide as modifier

Compound	Eluent (% methanol in carbon dioxide)					
	7%			3%		
	k_1	α	R_s	k_1	α	R_s
1-Phenyl-1-propanol	1.47	1.20	1.06	1.98	1.22	1.29
1-Phenyl-2-propanol	1.39	1.00	-	-	-	-
2-Phenyl-1-propanol	1.69	1.00	-	-	-	-
1,2,3,4-Tetrahydro-1-naphthol	2.44	1.10	0.78	3.68	1.08	0.77
1-Phenyl-1,2-ethanediol	5.88	1.00	-	-	-	-
Benzoin	—	—	-	-	-	-
Ethyl mandelate	1.10	1.84	-	-	-	-
Styrene oxide	0.72	1.00	-	-	-	-
Ephedrine	0.29	1.00	-	-	-	-
TFAE	11.42	2.12	12.20	12.35 (5%)	2.12	11.21

Conditions: flow-rate, 1 ml min^{-1}; temperature 40°C; pressure, 125 bar.

Table 3
SFC separation of phenylpropanols and related compounds on a Chiralcel OB column at 25°C using different levels of methanol in carbon dioxide as modifier

Compound	Eluent					
	7%			2%		
	k_1	α	R_s	k_1	α	R_s
1-Phenyl-1-propanol	0.68	1.14	0.30	1.10	1.16	0.74
1,2,3,4-Tetrahydro-1-naphthol	1.22	1.31	1.10	2.63	1.30	2.20
1-Phenyl-1,2-ethanediol	1.59	1.15	0.60	5.48	1.18	0.81
Benzoin	1.28	1.15	0.63	2.29 (3%)	1.14	0.83

Conditions as Table 1.

resolution and retention factors at 40°C dropped markedly from $k_1=7.79$, $R_s=1.27$ and $k_1=4.51$ $R_s=0.57$, respectively, at 1.0% propan-2-ol to $k_1=0.91$, $R_s=0$ and $k_1=0.56$, $R_s=0$, at 2% propan-2-ol. A similar effect was found with TFAE but resolution was retained so that at 2% propanol $k_1=2.55$ and $R_s=4.47$.

In many reports the selectivity of analytes in chiral SFC increases as the column temperature is reduced and subcritical column temperatures (below 31.2°C) are often preferred. The separation of the selected test compounds at 25°C (Tables 3 and 4) showed only slightly increased retention factors compared to 40°C (except for TFAE) and often only modest increases in the selectivities and resolutions. For example, with 2% methanol, the resolution of 1-phenyl-1-propanol on Chiralcel OB increased from $R_s=0.50$ at 40°C to $R_s=0.74$ at 25°C and for tetrahydro-1-naphthol the resolution increased from $R_s=1.42$ to $R_s=2.20$. The effects were smaller with higher proportions of modifier. The influence of pressure of the column was also studied at a constant eluent flow rate for the Chiralcel OB column. Although the retention factors increased with reducing pressure the selectivities remained constant.

For a number of the analytes, individual enantiomers were available and the order of elution could be identified by spiking the racemic mixtures (Table 5). The order of elution of the 1,2,3,4-tetrahydro-1-naphthol enantiomers was opposite on the Chiralcel OB and OD column suggesting a different interaction. For 1-phenyl-1-propanol at 25°C the order on the Chiralcel OB column was *S* then *R*. A reversal in the elution order of enantiomers between 15°C and 55°C has been reported previously for HPLC on a Chiracel OD column by Balmer et al. [12]. A similar change has also been noted for SFC separations on a Chiralcel OD column by Smith et al. [13]. They found a convergence of retention factors as temperatures increased for some analytes and a divergence for others.

Thus in the present study, when a chiral separation is successful it appears to satisfy the same principal requirement that the phenyl and hydroxyl groups should be substituted directly on the chiral centre that was proposed by Wainer et al. [10] for the

Table 4
SFC separation of phenylpropanols and related compounds on a Chiralcel OD column at 25°C using different levels of methanol in carbon dioxide as modifier

Compound	Eluent					
	7%			3%		
	k_1	α	R_s	k_1	α	R_s
1-Phenyl-1-propanol	1.94	1.32	1.53	3.27	1.77	3.22
1,2,3,4-Tetrahydro-1-naphthol	2.85	1.14	0.95	3.30	1.14	1.21
TFAE	6.18	2.62	12.57	12.50 (5%)	2.51	13.35

Conditions as Table 2.

Table 5
SFC identification of the order of elution of selected analytes by spiking studies with individual enantiomers

Test enantiomer	Chiralcel OB			Chiralcel OD		
	MeOH (%)	k_1	Order	MeOH (%)	k_1	Order
(a) Conditions: 40°C and Table 1						
(*R*)-(−)-Tetrahydro-1-naphthol	7	1.22	(*R*)(*S*)	7	2.44	(*S*)(*R*)
(*R*)-(−)-1-Phenyl-1,2-ethanediol	5	2.30	(*R*)(*S*)			
Compound	Chiralcel OB					
	MeOH (%)	k	Order			
(b) Conditions: 25°C and Table 1						
(*R*)-(+)-1-Phenyl-1-propanol	5	0.70	(*S*)(*R*)			
(*R*)-(−)-Tetrahydro-1-naphthol	5	1.52	(*R*)(*S*)			
(*R*)-(−)-1-Phenyl-1,2-ethanediol	3	3.44	(*R*)(*S*)			

HPLC separation. 1-Phenyl-1-propanol and 1,2,3,4-tetrahydro-1-naphthol both gave good separations. However, the separation of 1-phenyl-1,2-diol, benzoin and ethyl mandelate was unpredictably column dependent suggesting that other factors were also influencing the resolution. As expected from this proposed mechanism 1-phenyl-2-propanol and 2-phenyl-1-propanol were unresolved (although the latter was resolved in the HPLC study).

It is also interesting to note the differences in the interaction of the benzoate OB column and the carbamate OD columns. In the latter case the presence of the 3,5-dimethyl groups probably enhances the $\pi-\pi$ interaction of the phenyl group on the analyte with the stationary phase. In HPLC separations, this interaction is usually stronger with the alkylphenyl-substituted derivatised celluloses compared to the unsubstituted phenyl derivatives [3]. For example, cellulose tri-4-methylbenzoate has also been shown to have much stronger chiral selectivity than cellulose tribenzoate. The cellulose tri-4-methylbenzoate stationary phase also had a higher chiral selectivity in SFC than HPLC [4] unlike the carbamate phases whose selectivity was less than in HPLC.

This importance of the interaction of the phenyl and hydroxyl groups to the enantioselectivity agrees with a report by Oguni et al. [14] who examined the interaction between tris(4-methylbenzoate) cellulose and 1-phenyl-1-ethanol by ^{13}C nuclear magnetic resonance spectroscopy. They found that the presence of the ester restricted the movement of the *R* enantiomer more than of the *S* enantiomer and that the effect was greatest at the C_1 carbon.

4. Conclusions

The separations on SFC largely mirror those in HPLC and it seems that a similar discrimination mechanism may be occurring. For good selectivity the phenyl and hydroxyl groups both need to be substituted on the chiral centre Reducing the temperature slightly improved the separations. The mechanism of the separation differed between the columns and although the Chiralcel OD column appeared to be more versatile it could not resolve 1-phenyl-1,2-ethanediol.

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