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Broadly applicable polysiloxane-based chiral stationary phase for high-performance liquid chromatography and supercritical fluid chromatography

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Abstract

A recently reported broad-spectrum chiral selector and two of its homologs were incorporated into polymethylhydrosiloxane and each of the polymers was immobilized on silica gel to afford chiral stationary phases (CSPs) useful for the separation of the enantiomers of a wide variety of compounds by HPLC and/or supercritical fluid chromatography (SFC). These CSPs are stable toward normal-phase, reversed-phase, and sub/supercritical fluid conditions and are not adversely affected by addition of carboxylic acids or amines as mobile-phase modifiers. These polymeric CSPs show higher efficiencies and shorter analysis times when used with sub/supercritical carbon dioxide then when used in the HPLC mode. Additionally, some separations are achievable using sub/supercritical carbon dioxide but not when the same column is used in the HPLC mode.

1. Introduction

Guidelines for the development of new drugs require the development of efficient methods for the analysis and preparation of enantiomerically pure compounds [1]. In this regard, chiral stationary phases (CSPs) are proving to be extremely useful. CSPs are used to monitor asymmetric syntheses, enzymatic transformations, and classical resolutions. They are used to follow the stereochemical preferences of metabolism and to monitor the concentrations of individual enantiomers in patients undergoing treatment. CSPs not only provide a means for determining enantiomeric purity and absolute configuration, they also provide the means to preparatively resolve racemates. Increasingly, they are being used for the preparation of stereochemically pure compounds, an application which will become commonplace with the adoption of simulated moving bed chromatography, a technique especially well suited to the separation of enantiomers.

Most workers would prefer not to have to derivatize analytes prior to chromatography. As improved CSPs are developed, the need for

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prederivatization is being diminished. For example, a chiral selector developed recently in these laboratories has been used for the resolution of a wide variety of underivatized enantiomers including (but not limited to) α-aryl propionic acids, epoxides, aziridines, sulfoxides, α-aryloxypropionic acids, diols, dihydropyridines, atropisomeric compounds, heterocycles, and a wide variety of esters and amides [2–5]. Simple prederivatization with inexpensive achiral reagents containing chromophores to simplify detection extends the scope of this CSP even further. Recently, the commercial version of this brush-type CSP, the WHELKO-1 [6], has been used with sub/supercritical carbon dioxide to separate the enantiomers of several profens and of several other compounds of medicinal interest [7,8]. In this paper, we report the incorporation of this chiral selector and two of its homologs into polymethylhydrosiloxanes by a well-precedented procedure [9–24]. The resulting chiral polymers have been coated on silica gel, immobilized by heating, packed into HPLC columns by conventional means and used in both the LC and SFC modes.

The use of sub/supercritical carbon dioxide as a mobile phase offers several significant and previously recognized advantages as described in recent reviews [25–28]. Solvent strength can be adjusted by controlling temperature and pressure. Owing to the low viscosity of carbon dioxide, diffusion rates are increased and column efficiencies are increased considerably [6,29]. The low viscosity allows one to use higher flowrates than might be used for liquid chromatography with the same column without giving rise to undue back pressures or to appreciable efficiency loss. This results in shorter analysis times. The low viscosity also allows one to couple several identical columns in series so as to increase the number of theoretical plates available in the case of difficult separations [30]. Coupling dissimilar chiral columns together for the SFC separation of enantiomers has also been demonstrated [31], a subject worthy of discussion in a later paper. Finally, the use of carbon dioxide reduces or avoids the use of mobile-phase components which may be flammable or environmentally harmful. While some investigators have been aware of these advantages for some time, what may not yet be widely appreciated by chromatographers in general is the potential for heretofore unknown scope, selectivity, convenience, and speed when stereochemical analyses are conducted by sub/supercritical chromatography with carbon dioxide on robust, well-designed chiral selectors. It is evident that these selectors can be incorporated into polysiloxanes in such a way as to equal or exceed the levels of chromatographic efficiency typically encountered with brush-type stationary phases.

2. Experimental

Chromatography was carried out using a Hewlett-Packard supercritical fluid chromatograph equipped with an HP 7673 autosampler (5-μl sample loop), an HP 1050 diode-array detector, and an HP Vectra 486/66u personal computer running the HP ChemStation software. The columns were kept at constant temperature in the instrument's column oven. Tri-*tert.*-butylbenzene was used as a void volume marker. Samples (available from prior studies) were dissolved in methanol at a concentration of 0.5 mg/ml. Carbon dioxide was of SFC grade (Scott Specialty Gases, Plumsteadville, PA, USA). The organic modifiers were HPLC grade solvents (EM Science, Gibbstown, NJ, USA).

2.1. Preparation of the csps

The chiral selectors were prepared in the manner described [2,3] and incorporated into the polysiloxane by the method used by Röder et al. [10]. The chiral polymers were coated onto silica gel (5 μm, 300 Å pore size) and immobilized by heating to 100°C for 24 h under reduced pressure. The CSPs were packed into stainless-steel columns (250 × 4.6 mm I.D.) as methanol slurries. Residual silanol groups were end-capped by passing a solution of 2 ml of hexamethyldisilazane in 50 ml of dichloromethane

through a dichloromethane equilibrated column at a flow-rate of 1.0 ml/min.

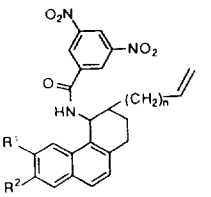
3. Results and discussion

The chiral selector and its two homologs were prepared as the racemates and resolved in the manner described [2]. Incorporation of these selectors into the polymers was done by hydrosilylation of the terminal double bonds with the polymethylhydrosiloxane (Fig. 1). Coating of the chiral polymers onto silica gel of 300 Å pore size, 5 μm particle size, with subsequent immobilization afforded CSPs 1–3. The structural features of the columns are summarized in Table 1. Selectors with different structures were used to study how methyl substituents on the naphthyl of the selector and how the length of the tether influence chromatographic performance. Similar studies have been conducted for brush-type CSPs prepared from these selectors [2–4]. In the reciprocal situation, chromatography of these selectors on an (*S*)-naproxen diallyl amide-derived brush-type CSP has shown that the methyl substituents increase the separation factor of the enantiomers, presumably by increasing their π-basicity [5].

Previous work has shown that incorporation of the chiral selector into a polysiloxane has led to CSPs having decreased retention times relative to those obtained by bonding the same selector to the same support in a brush-type fashion [11–13].

Polymeric CSPs 1–3 are stable under normal-phase and sub/supercritical fluid conditions and do not exhibit any loss of efficiency or selectivity after several months of hard usage. A series of

Table 1
Polysiloxane-based chiral stationary phases 1–3

CSP	Selector[a]			Loading (mmol selector/g CSP)
				
	R^1	R^2	*n*	
1	H	H	3	0.18
2	H	CH_3	3	0.14
3	CH_3	CH_3	9	0.18

[a] The enantiomers having the (*R*,*R*)-configurations were used for the preparation of CSPs 1–3.

α-arylpropionic acids and various other chiral compounds were used to evaluate the performance of these polysiloxane-derived CSPs by HPLC and SFC. The chromatographic results obtained on CSPs 1–3 (Table 1) using a mobile phase of 10% 2-propanol containing 0.2% acetic acid in carbon dioxide at a pressure of 200 bar and a temperature of 25°C are summarized in Table 2. It should be noted that the mobile phase is subcritical under these conditions. Rapid separations of the enantiomers of α-arylpropionic acids on CSP 1 are achieved under these conditions (Table 2). These enantiomers can be separated without an acidic modifier in the mobile phase (Table 3), although addition of such a modifier may improve band shapes somewhat. Using the corresponding brush-type CSP

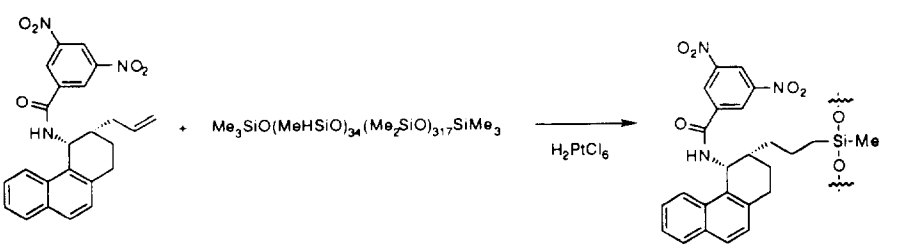


Fig. 1. Formation of the sidechain-modified polysiloxane by hydrosilylation.

Table 2
SFC separations of the enantiomers of arylpropionic acids on CSPs 1–3 using an acidic modifier

Sample		CSP 1	CSP 2	CSP 3
Naproxen	k_1'	1.25	0.84	0.69
	α	2.28	2.21	1.61
Ibuprofen	k_1'	0.09	0.07	0.11
	α	1.44	1.00	1.00
Fenoprofen	k_1'	0.14	0.14	0.21
	α	1.71	1.50	1.00
Flurbiprofen	k_1'	0.18	0.24	0.29
	α	1.39	1.00	1.00
Cicloprofen	k_1'	1.29	0.96	0.81
	α	1.75	1.70	1.37
Pirprofen	k_1'	0.83	0.69	0.73
	α	1.40	1.38	1.00
Carprofen	k_1'	3.09	2.72	3.50
	α	1.79	1.55	1.00
Etodolac	k_1'	0.49	0.51	0.44
	α	1.20	1.14	1.00

Conditions: 10% (0.2% acetic acid) 2-propanol in carbon dioxide, 200 bar, 25°C, 2.0 ml/min, UV detection at 220 nm.

in the HPLC mode, the enantiomers of the α-arylpropionic acids can also be separated without an acidic modifier, but band shapes are poorer than those afforded by the polysiloxane-based CSP. This result either indicates that carbon dioxide suppresses superfluous interactions of the polar analytes with the silica surface or that most of these sites are buried beneath the polymer and are no longer accessible to the analytes.

The separation of several pairs of enantiomers on CSPs 1 and 2 by HPLC using 5% (0.5% acetic acid) ethanol in *n*-hexane are documented in Table 4. Comparison of the chromatographic results obtained on CSP 1 by HPLC (Table 4) and by SFC (Table 2) shows that retention in SFC was greater even though a higher concentration of the polar modifier was used. Furthermore, the enantiomers of all analytes are observed to be separated by SFC, whereas three analytes fail to resolve by HPLC under the indicated conditions. Surprisingly, methyl substituents on the naphthyl system of the chiral selector (CSP 2) do not improve enantioselectivity for these analytes in SFC as they do in HPLC (Table 2). Increasing the tether length is detrimental, for CSP 3 affords separations of just two of these analytes under these same conditions (Table 2). Use of 5% (0.5% acetic acid) ethanol in *n*-hexane as a mobile phase essentially eliminates all retention on CSP 3 (Table 4). The

Table 3
SFC separations of the enantiomers of arylpropionic acids on CSP 1 without use of an acidic modifier

Sample	k'	α
Naproxen	2.35	2.00
Ibuprofen	0.25	1.24
Fenoprofen	0.33	1.42
Flurbiprofen	0.46	1.22
Cicloprofen	2.91	1.58
Pirprofen	1.95	1.31
Carprofen	not eluted after 25 min	
Etodolac	1.64	1.14

Conditions: 5% 2-propanol in carbon dioxide, 200 bar, 40°C, 1.0 ml/min, UV detection at 220 nm.

Table 4
HPLC separation of the enantiomers of arylpropionic acids on CSPs 1 and 2

Sample		CSP 1	CSP 2
Naproxen	k_1'	0.26	0.25
	α	2.27	2.32
Ibuprofen	k_1'	0.14	0.23
	α	2.00	1.00
Fenoprofen	k_1'	0.04	0.06
	α	1.00	1.00
Flurbiprofen	k_1'	0.03	0.21
	α	1.00	1.00
Cicloprofen	k_1'	0.18	0.18
	α	1.83	1.00
Pirprofen	k_1'	0.12	0.21
	α	1.50	1.00
Carprofen	k_1'	0.10	0.49
	α	2.80	1.39
Etodolac	k_1'	0.01	0.06
	α	1.00	1.00

Conditions: 5% (0.5% acetic acid) ethanol in *n*-hexane, 25°C, 2.0 ml/min, UV detection at 220 nm.

values given in Table 4 are those determined, However, one should be aware that, when k_1 is quite small, slight errors in determining the void volume of the column significantly affect the apparent values of k_1 and α. Moreover, when the sample is introduced in a solvent other than the mobile phase (as in this study), the k_1 values of weakly retained substances can be significantly affected [32]. Reducing the concentration of the polar modifier concentration increases retention and one now begins to separate the enantiomers of naproxen, cicloprofen, and carprofen (Table 5).

Comparison of the chromatographic data obtained for the α-arylpropionic acids in SFC and HPLC clearly shows that the enantiomers of more of the analytes can be separated by SFC with a single mobile phase than is the case for LC. This suggests that enantiomer separation is more likely to be encountered on the first try if SFC is used. Increasing the length of the tether or adding methyl substituents on the naphthyl group of the selector reduces or destroys enantioselectivity for these analytes. In the corresponding brush-type CSPs increased tether length decreases selectivity, whereas the added methyls improve selectivity. The present observation that the ring methyls are detrimental is surprising and no explanation is offered at present. This behavior may prove to be analyte specific for, on this CSP, the chiral recognition process is more complex for the profens than for most other analytes [33] and separation factors

Table 5
HPLC separation of the enantiomers of arylpropionic acids on CSPs 2 and 3 using a lower modifier concentration

Sample		CSP 2	CSP 3
Naproxen	k_1'	0.99	0.67
	α	2.36	1.72
Ibuprofen	k_1'	0.58	0.33
	α	1.00	1.00
Fenoprofen	k_1'	0.57	0.38
	α	1.00	1.00
Flurbiprofen	k_1'	0.01	0.49
	α	7.00[a]	1.00
Cicloprofen	k_1'	0.56	0.94
	α	1.93	1.78
Pirprofen	k_1'	0.40	0.90
	α	1.70	1.00
Carprofen	k_1'	5.32	4.40
	α	1.74	1.37
Etodolac	k_1'	0.33	0.73
	α	1.76	1.00

[a] This separation factor is artificially high due to the very rapid elution of the less retained enantiomer.
Conditions: 1% (0.5% acetic acid) ethanol in *n*-hexane, 25°C, 2.0 ml/min, UV detection at 220 nm.

Table 6
SFC separation of the enantiomers of several test compounds on CSPs 1-3 at a flow-rate of 2.0 ml/min

Sample		CSP 1	CSP 2	CSP 3
trans-Stilbene oxide	k_1'	0.28	0.18	0.09
	α	3.57	3.35	3.5
Styrene oxide	k_1'	0.07	0.05	0.0
	α	1.7	1.00	1.
Phenyl methyl sulfoxide	k_1'	0.92	0.95	0.45
	α	1.41	1.25	1.20
Warfarin	k_1'	3.29	2.41	1.07
	α	2.04	1.85	1.84
Benzoin	k_1'	0.29	0.21	0.09
	α	3.04	2.74	3.0
Abscissic acid	k_1'	0.40	0.37	0.32
	α	1.45	1.30	1.00

Conditions: 10% 2-propanol in carbon dioxide, 200 bar, 25°C, 2.0 ml/min.

Table 7
SFC separation of the enantiomers of several compounds on CSP 1 using a flow-rate of 4.0 ml/min

Sample	k_1'	α
trans-Stilbene oxide	0.26	3.65
Styrene oxide	0.06	1.8
Phenyl methyl sulfoxide	1.43	1.24
Warfarin	3.05	2.05
Benzoin	0.25	3.12
Abscissic acid	0.36	1.45

Conditions: 10% 2-propanol in carbon dioxide, 200 bar, 25°C, 4.0 ml/min.

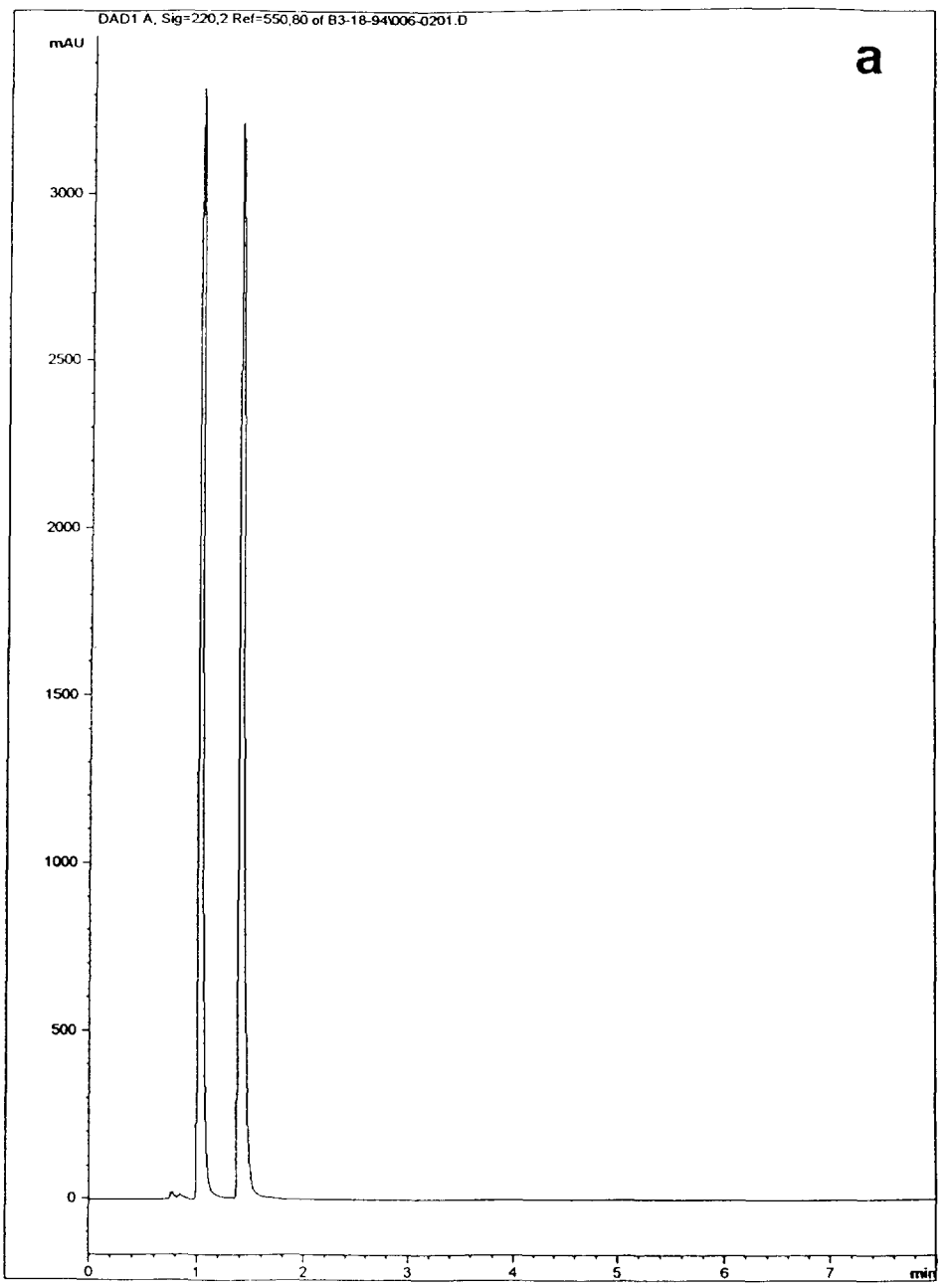


Fig. 2. Chromatograms of (a) *trans*-stilbene oxide, (b) warfarin, and (c) benzoin, obtained using CSP 1 with subcritical carbon dioxide as described in Table 6.

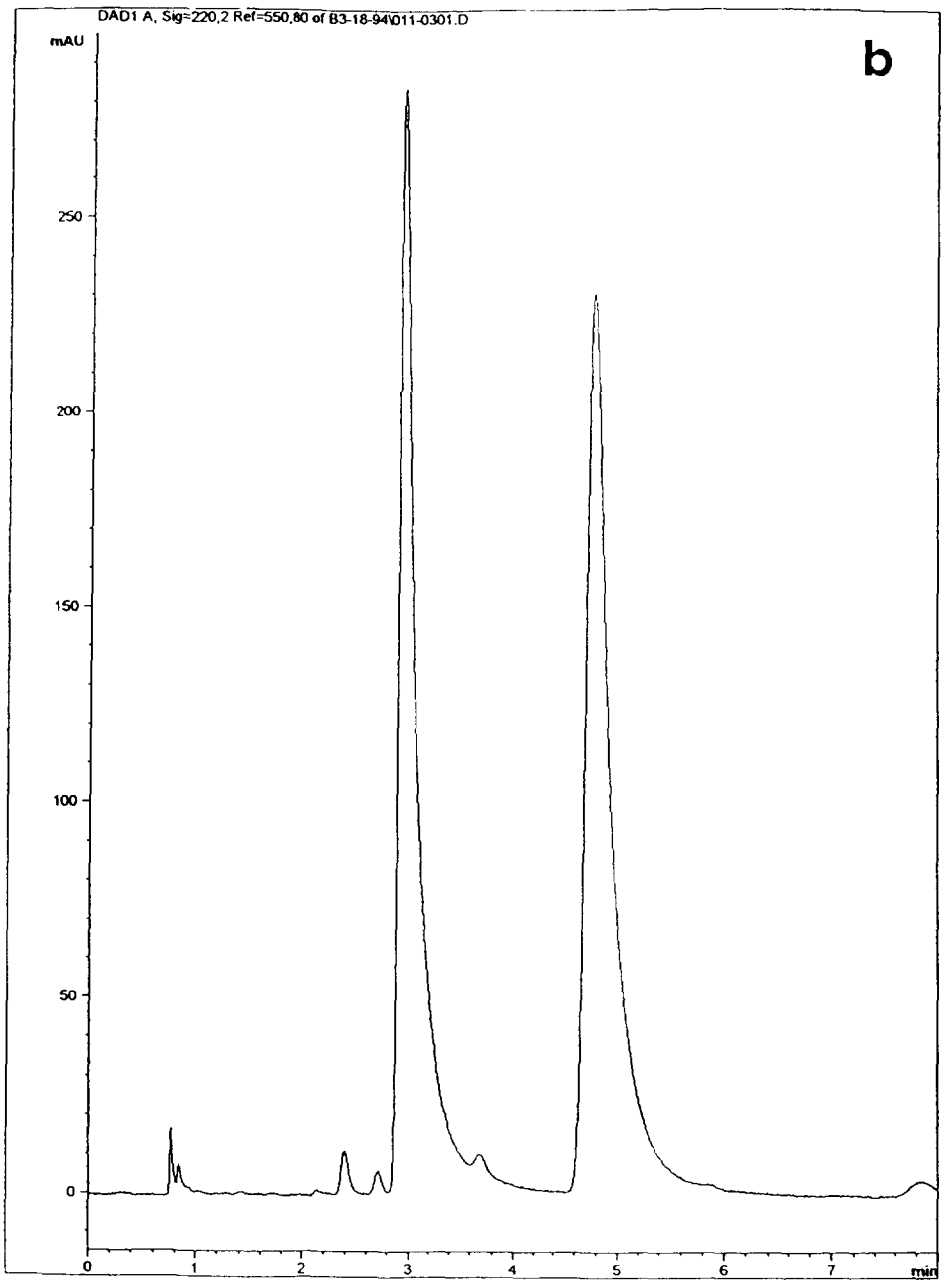


Fig. 2 (*continued*).

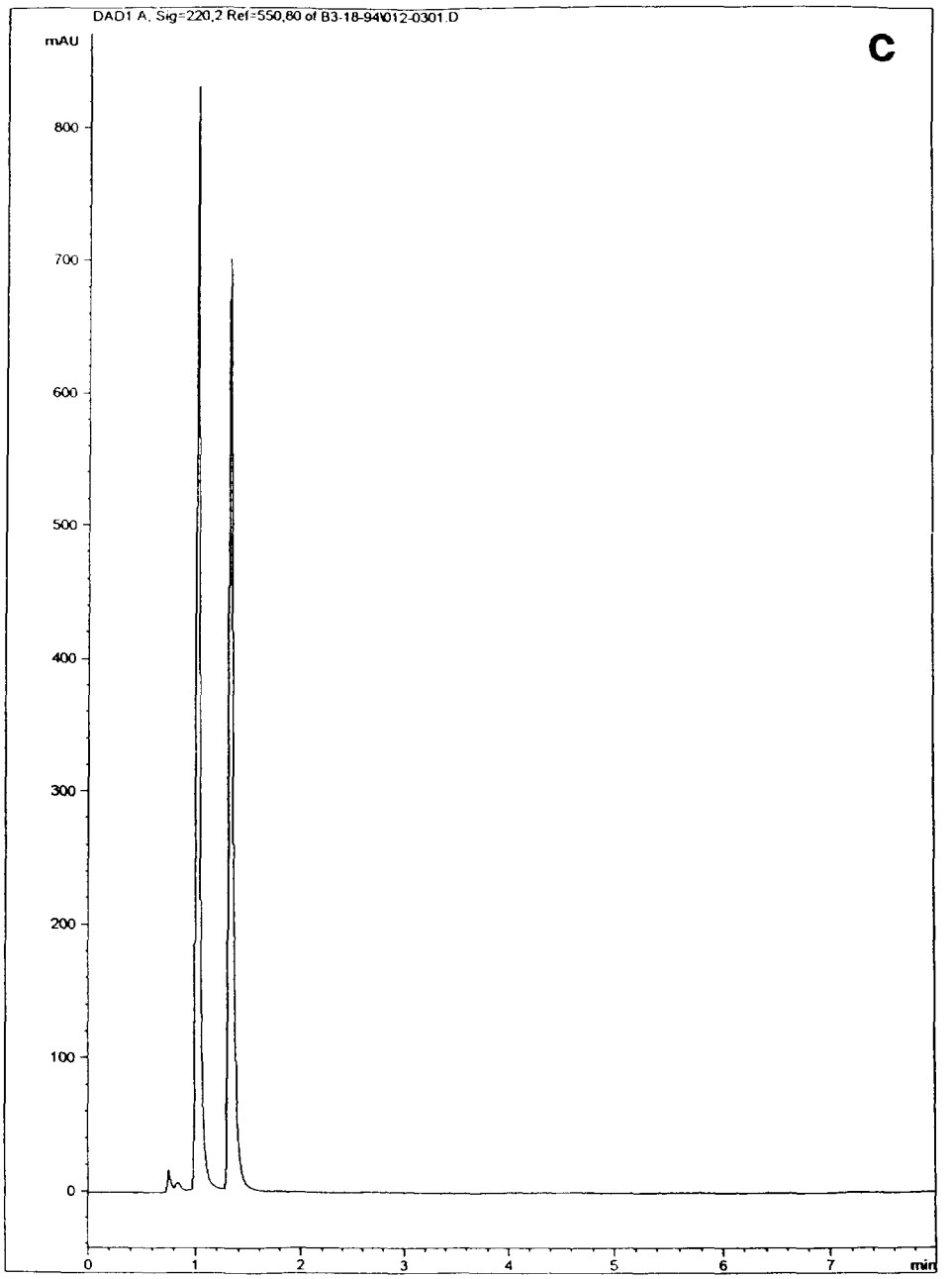


Fig. 2 (*continued*).

are unusually dependent on mobile-phase composition.

The enantiomers of several other racemic analytes were separated on CSPs 1–3 using 10% 2-propanol in carbon dioxide at a pressure of 200 bar and a temperature of 25°C (Table 6). Owing to the low viscosity of the mobile phase, the flow-rate could be increased to 4.0 ml/min to further reduce the time needed for separation (Table 7). Typical chromatograms obtained on CSP 1 are given in Fig. 2.

The use of these polymeric CSPs is not limited to analytical separations. In a preliminary study, a baseline separation of the enantiomers of 5 mg of racemic warfarin was obtained on an analytical column containing CSP 1.

5. Conclusion

Chiral selectors which have proven to be of great scope when used in brush-type CSPs have been incorporated into polysiloxanes and immobilized on silica gel. Columns packed with these materials were used for both the HPLC and SFC separation of the enantiomers of a series of compounds. The use of carbon dioxide as the mobile phase improves column efficiency and reduces analysis times, often to the 2–3 min range. In several instances (to be addressed in a later paper), resolutions were achieved by SFC that could not be achieved by HPLC.

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