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Liquid and subcritical CO_2 separations of enantiomers on a broadly applicable polysiloxane chiral stationary phase

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Abstract

Incorporation of chiral selector **1** into a polysiloxane which is then immobilized on silica affords a chiral stationary phase (CSP 1) capable of resolving a broad array of enantiomers by either high-performance liquid chromatography or supercritical fluid chromatography (SFC). Like its brush-type analog, the commercial version of which is known as the Whelk-O 1, CSP 1, the "polyWhelk-O", is stable to normal and reversed-phase conditions and to a wide range of temperatures, mobile phases and additives. In most cases, the polyWhelk-O affords greater enantioselectivity and less retention than does the brush-type Whelk-O 1 under the same conditions. An extensive collection of separations of the enantiomers of a variety of types of compounds is presented to illustrate the scope and level of performance typically afforded by the polyWhelk-O columns.

Keywords: Enantiomer separation; Polysiloxane phases; Chiral stationary phases, LC; Carbon dioxide

1. Introduction

Since the absolute stereochemistry of a compound typically influences its bioactivity, the development of most present day pharmaceuticals requires methodologies for assessing the enantiopurity of the drug, its precursors and its metabolites. When applicable, chromatography on chiral stationary phases (CSPs) is the method of choice for these determinations. In a number of instances, the use of CSPs also provides a means for obtaining enantiomerically pure substances. While many CSPs have been described, most have been developed empirically and the rational design of chiral selectors is still in its infancy. As CSP design evolves, both the scope and predictability of application can be expected to expand and the need to prederivatize analytes (for purposes of enantiodifferentiation) will diminish, as will the time required for method development.

As an example of design evolution, consider the CSPs derived from selector 1 [1,2]. This selector, designed using mechanistic considerations, was initially tethered to silica with a long tether, a short tether soon being found to enhance performance. Commercialized as the Whelk-O-1, this CSP is capable of separating the enantiomers of many classes of compounds including, but not limited to, arylpropionic acids, epoxides, sulfoxides, diols, dihydropyrimidines, atropoisomeric compounds, aryl phosphonates, aryl carbamates, amides, alcohols and a wide variety of heterocyclics [3]. The next evolutionary step was to incorporate selector 1 into a polymethylhydrosiloxane backbone, the chiral polymer being immobilized on silica to afford CSP 1 [4]. We report herein an extensive study of CSP 1 (henceforth termed the "polyWhelk-O"), compare it

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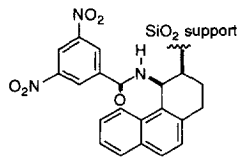


Fig. 1. CSP 1.

to the brush-type analog, investigate its thermal stability and performance by resolving a variety of compounds under a variety of conditions including the use of sub/supercritical carbon dioxide as the bulk mobile phase component (see Fig. 1).

As recognized by the pioneers in the field, there are several distinct advantages in the use of sub/supercritical carbon dioxide as a mobile phase component. The low viscosity of carbon dioxide results in higher column efficiencies and the ability to perform separations at increased flow-rates, thus reducing analysis times. Additionally, longer columns or several shorter columns connected in series may be used without leading to prohibitively high back pressures. Finally, using carbon dioxide as the major mobile phase component eliminates dangerous and/or environmentally undesirable mobile phases, thus simplifying disposal problems, a distinct financial benefit. Despite these advantages, the technique remains underexploited because of the cost of the instrumentation, a presumption of added complexity, and the feeling that the same separations can be obtained by other, more established, means.

In the field of enantiomer separations, supercritical fluid chromatography (SFC) has a great deal to offer, especially as new CSPs are developed which not only have great generality but also have the chemical robustness to withstand the pressures, temperatures and mobile phases employed. The use of CSPs of this character with sub/supercritical carbon dioxide is likely to become the method of choice for many separations of enantiomers due to the ease and speed of method development and to the greater throughput of samples per day.

2. Experimental

Sub/supercritical chromatography was carried out on a Hewlett-Packard supercritical fluid chromatograph equipped with an HP 7673 autosampler (5-μl injection loop) and an HP detector, driven by 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 (25°C and above runs) or in a dewar filled with a frozen slush for the low temperature runs. All flow-rates were a nominal 2 ml/min. All samples were available from prior studies and introduced as methanolic or, in some cases, as EtOAc solutions containing ca. 1–3 mg/2 ml. While many of the analytes used were prepared in these labs, a number have been provided by colleagues over the years. Carbon dioxide was of SFC grade (Air Products). The polymeric Whelk-O-1 phases used in these studies were prepared in-house and packed into columns by Regis Technologies (Morton Grove, IL, USA). The chiral selector was incorporated into the polysiloxane using established procedures [5].

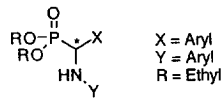
3. Results and discussion

As described earlier [6], selector 1 has a cleft-like active site in which the stereochemistry of analytes having an aromatic group and hydrogen bond acceptor on (or near) a stereogenic center may be "recognized". While the presence of these groups is not always a prerequisite for enantiodifferentiation, selector 1 was designed to utilize interactions involving these groups. Consequently, CSP 1 may not perform satisfactorily in their absence. The analytes used in this study all contain aromatic and hydrogen bond acceptor groups flanking the stereogenic center.

3.1. Comparison to the brush type Whelk-O-1 column

The phosphonates in Table 1 were chromatographed on both the Whelk-O-1 and polyWhelk-O columns using the same mobile phase in order to compare the performance of the two CSPs. The data clearly show that the enantiodifferentiation of the polyWhelk-O is, in these instances, greater than that of the Whelk-O-1. The most striking observation is the greatly reduced capacity factors on the polyWhelk-O column. In all cases, the most retained enantiomer is eluted from the polyWhelk-O column

Table 1
HPLC separations of a series of diethyl N-(aryl)-1-amino-1-arylmethanephosphonates



C-Aryl	N-Aryl	Whelk-O-1			PolyWhelk-O		
		k_1'	k_2'	α	k_1'	k_2'	α
2-Fluorenyl	2-iopropyl	5.87	30.0	5.12	0.45	3.57	7.93
Ferrocenyl	2-isopropyl	4.32	5.79	1.34	0.38	0.59	1.55
4-(N,N)-Dimethylamino	3,5-dimethyl	12.30	24.6	2.00	1.02	2.29	2.25
2-Fluorenyl	2-*tert.*-butyl	5.6	29.2	5.20	0.39	2.71	6.95
1-Naphthyl	3,5-dimethyl	4.58	5.63	1.23	0.37	0.46	1.24
2-Naphthyl	3,5-dimethyl	7.53	18.7	2.54	0.60	1.70	2.83

Conditions: ambient temperature; 5% 2-propanol–95% hexane.

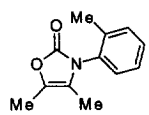
before the least retained enantiomer is eluted from the Whelk-O-1. The implications for saving time and solvent are obvious. In part, the reduced retention results from the reduced surface area of the 300 Å pore silica used to support the polysiloxane. Additionally, the proportion of the selectors in the polymer that are accessible to the analytes will also influence retention. Using identical conditions, retention factors are smaller on the polyWhelk-O than on the Whelk-O-1 column while separation factors are often larger. This is also the case for methanol-modified carbon dioxide under sub/supercritical chromatographic conditions.

3.2. Thermal stability/variable temperature performance of the polyWhelk-O column

In the course of developing a protocol for separating the enantiomers of a given compound, use of a temperature other than ambient is sometimes desirable. Hence, it is advantageous to have CSPs capable of performing over a wide range of temperatures. Table 2 and Table 3 display data for the SFC separation of the enantiomers of two atropoisomers. Although resolution is reduced due to a reduction in selectivity, the polyWhelk-O still performs satisfactorally at 120°C and enables one to study the effect of temperature on the rate at which the enantiomers of these atropoisomers interconvert. The column suffered no degradation in performance as a consequence of having been used at this temperature.

Occasionally, the use of cryogenic temperatures

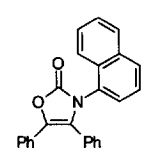
Table 2
Variable temperature SFC separation of the enantiomers of 3-(2-methylphenyl)-4,5-dimethyl-1,3-oxazoliden-2-one on the polyWhelk-O column



Temperature (°C)	k_1'	k_2'	α	R_s
26	1.87	4.42	2.36	14.9
40	1.64	3.57	2.18	11.9
60	1.72	3.26	1.90	9.77
80	1.89	3.18	1.68	9.12
100	2.28	3.48	1.53	8.53
120	2.77	3.87	1.40	6.23

Conditions: 200 bar; 10% 2-propanol–90% CO_2

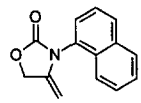
Table 3
Variable temperature SFC separations of the enantiomers of 3-(1-naphthyl)-4,5-diphenyl-1,3-oxazoliden-2-one on the polyWhelk-O column



Temperature (°C)	k_1'	k_2'	α	R_s
26	2.12	8.22	3.88	24.6
32	1.91	7.01	3.67	20.2
40	1.71	5.86	3.13	18.7
50	1.55	4.82	3.11	14.7
60	1.45	4.12	2.84	14.2
70	1.41	3.70	2.62	13.4
80	1.42	3.38	2.28	13.4

Conditions: 200 bar; 10% 2-propanol–90% CO_2

Table 4
Sub/SFC variable temperature separation of the enantiomers of a 3-(1-naphthyl)-4-methylene-1,3-oxazolidin-2-one on the polyWhelk-O column



Temperature (°C)	k_1'	k_2'	α	R_s
60	0.54	0.94	1.74	4.86
40	0.49	0.98	2.00	6.73
28	0.49	1.08	2.20	8.51
0	0.56	1.56	2.79	11.9
−20	0.66	2.24	3.39	13.8

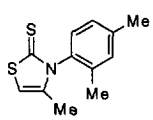
Conditions: 200 bar; 10% 3:1 methanol–acetonitrile + 0.25% diethylamine–90% CO_2.

may be necessary, either because of rapid interconversion of enantiomers or a low level of enantioselectivity. The polyWhelk-O has been used at −40°C and found to perform satisfactorally without undue loss of efficiency, resolution actually being greater at the low temperature due to the increased selectivity (see Tables 4 and 5). Although this situation will vary from analyte to analyte, efficiency at low temperature is usually higher with methanol-modified carbon dioxide than with the more usual hexane–2-propanol mobile phases.

3.3. Comparison of HPLC to SFC chromatographic conditions

Admittedly, it is difficult to directly compare SFC and HPLC methodologies, since the solvent properties of liquid carbon dioxide are pressure- and temperature-dependent and are not strictly comparable to hexane, the major component of most of the HPLC mobile phases used to separate enantiomers. Table 6 and Table 7 display data from the SFC and HPLC separation of a series of *O*-alkyl-1(α-naphthyl)ethylamine carbamates. In this case, the smaller retention factors for the HPLC separations can be attributed to the use of 100% methanol as a mobile phase. While this mobile phase also reduces separation factors and resolution values, these are still large enough for all practical purposes. Because of the use of different mobile phases, the resolution values in Table 6 are much greater than those given in Table 7. However, in most instances where direct comparisons have been made with HPLC, resolution values are greater for the SFC separations. Note the relative insensitivity of the separation factors and resolution values to the nature of the alcohol used to prepare these carbamates.

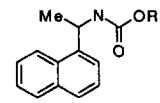
Table 5
Variable temperature separation of the enantiomers of 3-(2,4-dimethylphenyl)-4-methylthiazoliden-2-thione on the polyWhelk-O column



Temperature (°C)	k_1'	k_2'	α	R_s
80	0.62	0.75	1.21	1.52
60	0.41	0.53	1.29	1.68
40	0.37	0.50	1.35	2.08
20	0.37	0.52	1.42	2.34
0	0.37	0.59	1.59	3.52
−20	0.40	0.72	1.80	4.86

Conditions: 200 bar; 10% methanol–90% CO_2.

Table 6
Sub/SFC separation of a series of α-naphthyl-1-ethylamine carbamates on the PolyWhelk-O column



R	k_1'	α	R_s
Ethyl	0.67	6.31	25.2
n-propyl	0.72	6.06	24.9
i-propyl	0.66	6.44	25.5
$CH_2CH_2CF_3$	0.65	6.55	26.9
neo-pentyl	0.63	5.52	19.0
n-octyl	0.87	5.98	25.0

Conditions: 200 bar; 27°C; 20% methanol–80% CO_2.

Table 7
HPLC separation of a series of α-naphthyl-1-ethylamine carbamates on the PolyWhelk-O column

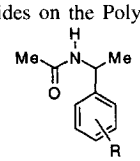
R	k_1'	α	R_s
CH_2CF_3	0.16	4.38	9.71
propyl	0.18	4.17	9.61
i-propyl	0.17	4.47	9.56
pentyl	0.20	4.35	10.4
neopentyl	0.19	4.63	9.85
cyclohexyl	0.23	4.48	10.3
octyl	0.25	4.44	11.5

Conditions: 27°C; 100% methanol.

3.4. Separations of the enantiomers of other classes of compounds

The polyWhelk-O is capable of resolving an array of other compounds encompassing a number of functional groups. Table 8 depicts the chromatographic behavior of a series of α-(aryl)ethylamine acetamides to illustrate the tolerance for variation of the electronic character of substituents on aromatic rings. In most cases, the large resolution values indicate that one could further increase mobile phase polarity to reduce analysis time while still producing baseline separations of the enantiomers. These amines may be acylated with virtually any acylating agent and still afford enantiomers separable on the polyWhelk-O column. Only when both *ortho* positions are substituted does one lose most (or all) of the ability to separate the enantiomers of these analytes. This comes about because two such substituents reduce the ability of an aromatic group to enter the cleft of the selector in such a way as to allow the face-to-face $\pi-\pi$ interaction which is involved in the recognition process. The enantiomers of the pivalamides of these amines exhibit separation factors even larger than those shown for the acetamides.

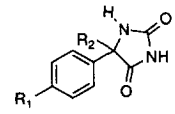
Table 8
Sub/SFC separation of the enantiomers of a series of α-(aryl)ethylamine acetamides on the PolyWhelk-O column



R	Conditions	k_1'	α	R_s
H	A	1.04	2.81	10.2
4-methyl	B	0.56	2.93	8.71
4-ethyl	B	0.55	2.62	6.48
4-Cl	B	0.57	3.88	10.6
4-Br	B	0.64	4.64	15.9
4-OMe	B	0.62	3.58	12.2
4-SMe	B	0.85	4.92	18.8
4-NO_2	B	0.47	3.60	8.61
3,5-dimethyl	B	0.67	3.73	12.5
4-Cl	C	0.41	2.00	2.09
4-SMe	C	0.65	2.42	3.95
3,5-dimethyl	C	0.43	1.93	2.10

Conditions: (A) 200 bar; 27°C; 5% methanol–95% CO_2.
(B) 200 bar; 27°C; 10% methnaol–90% CO_2.
(C) 200 bar; 27°C; 20% methanol–80% CO_2.

Table 9
Sub/SFC separation of the enantiomers of some hydantoins on the PolyWhelk-O column



R_1	R_2	Conditions	k_1'	α	R_s
Cl	H	A	3.04	1.72	9.52
OH	H	B	0.36	3.03	8.46
OMe	H	B	0.43	3.21	12.4
H	CH_3	A	1.50	1.13	1.38
OMe	*i*-propyl	B	0.28	2.79	5.63

Conditions: (A) 200 bar; 28°C; 5% methanol–95% CO_2.
(B) 200 bar; 28°C; 20% methanol–80% CO_2.

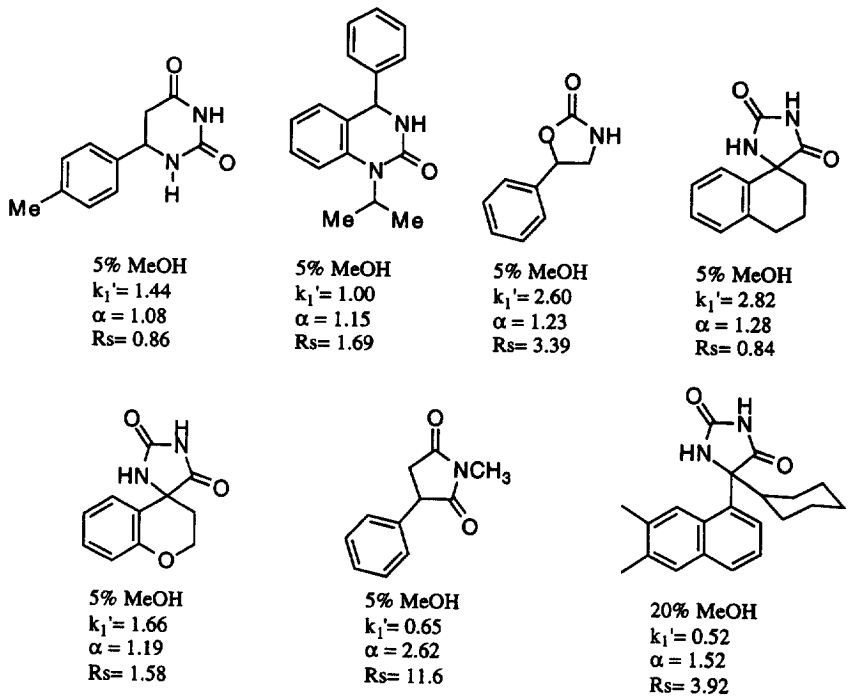
Table 9 and Scheme 1 document the separation of the enantiomers of a variety of hydantoins, nitrogen heterocycles containing amide functionality. Some hydantoins find medicinal application and previously have been used in evaluations of other types of CSPs [7,8]. Although not shown here, alkylation of one or both of the ring nitrogens is found to increase both enantioselectivity and resolution on the polyWhelk-O and Whelk-O-1 columns [9].

The enantiomers of a broad range of arylalkylsulfoxides (Table 10 and Scheme 2) are easily separated on the polyWhelk-O column.

Note, in Table 11, how little retention varies as the length of the alkyl substituent is varied. The amount of methanol used as the polar modifier influences the balance between normal-phase and reversed-phase behavior of the analytes.

3.5. Separation of the enantiomers of underivatized amines

Because of their basicity, many amines are strongly retained and afford poor bandshapes on silica-based stationary phases. This problem is usually not as severe on the polyWhelk-O as on its brush-type counterpart but is present nevertheless. Chromatographers often add low concentrations of simple amines to the mobile phase to alleviate such problems. Mobile phase additives such as diisopropyl amine (DIPA) similarly improve the bandshapes of amines chromatographed on the polyWhelk-O column under SFC conditions (Scheme 3).



Scheme 1. Sub/SFC separation of the enantiomers of amide-like heterocyclics on the PolyWhelk-O column.

3.6. Separation of the enantiomers of alcohols

In addition to the enantiomers of underivatized amines, the enantiomers of underivatized aromatic alcohols may be separated on the polyWhelk-O column. Again, because chromatographic efficiency is retained, sub-ambient temperatures may be employed to improve chromatographic performance (Table 12).

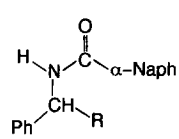
Chlorohydrins, common intermediates in organic synthesis, can be used as precursors to the profen family of nonsteroidal anti-inflammatory drugs. De-

Table 10
Sub/SFC separation of the enantiomers of a series of aryl sulfoxides on the PolyWhelk-O column

R_1	Aryl	Conditions	k_1'	α	R_s
Dodecyl	phenyl	A	1.98	1.35	4.90
i-Propyl	4-methylphenyl	A	1.97	1.24	3.36
i-Propyl	4-methylphenyl	B	0.76	1.08	0.78
t-Butyl	phenyl	A	1.56	1.28	3.67
Methyl	4-chlorophenyl	B	0.81	1.10	1.03
Methyl	perchlorophenyl	A	3.34	1.06	9.67
Methyl	1-naphthyl	A	4.29	1.24	4.10
Methyl	2-naphthyl	A	4.41	1.22	3.99

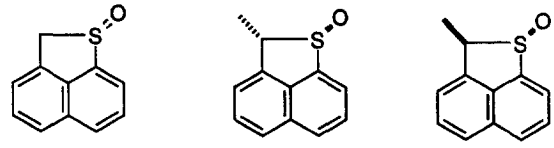
Conditions: (A) 200 bar; 26°C; 5% methnaol–95% CO_2.
(B) 200 bar; 26°C; 10% methanol–90% CO_2.

Table 11
Sub/SFC separations of the enantiomers of a series of O-1-(phenyl)alkyl α-naphthylamine carbamates on the PolyWhelk-O column.

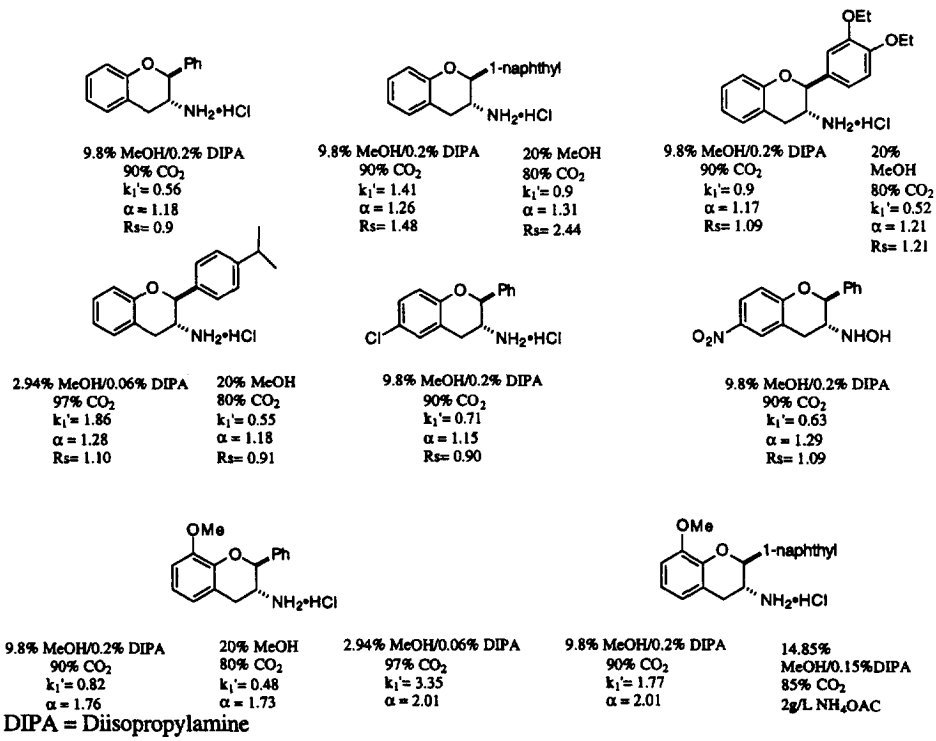


R	k_1'	α	R_s
Ethyl	1.32	2.02	11.2
n-Propyl	1.35	1.92	10.7
n-Butyl	1.40	1.89	10.6
n-Pentyl	1.44	1.90	10.5
n-Hexyl	1.86	1.81	10.6
n-Decyl	2.21	1.79	10.5
n-Undecyl	1.90	1.84	10.6
n-Tridecyl	1.92	1.82	10.7
n-$C_{15}H_{31}$	2.26	1.83	10.4
n-$C_{17}H_{35}$	2.95	1.78	10.3

Conditions: 200 bar; 26°C; 20% methanol–80% CO_2



Scheme 2. Sub/SFC separation of the enantiomers of tricyclic sulfoxides on the PolyWhelk-O column.



Scheme 3. Sub/SFC separation of the enantiomers of *trans*-heterocyclic primary amines on the PolyWhelk-O column.

Table 12
Sub/SFC separation of the enantiomers of a series of α-(aryl)alkanols on the PolyWhelk-O column

Ar	R	k_1'	α	R_s
1-Naphthyl	*n*-butyl	4.87	1.14	2.78
1-Naphthyl	methyl	5.21	1.09	2.12
1-Naphthyl	ethyl	4.85	1.09	1.90
3,4-Dimethoxyphenyl	*i*-propyl	5.25	1.07	1.14
1-(2,3-Dimethylnaphthyl)	methyl	9.64	1.18	3.52
2,4,6-Trimethylphenyl	methyl	1.57	1.13	1.38

Conditions: 200 bar; 0°C; 1% methanol–99% CO_2

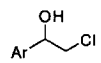
termination of the enantiomeric purity of chlorohydrins and bromohydrins is often possible using the polyWhelk-O column, several such resolutions being shown in Table 13.

Scheme 4 shows a representative collection of underivatized ketones and heterocyclics that are resolvable on the polyWhelk-O column.

3.7. Carboxylic acids

Like amines, the polarity of the carboxyl group often undermines chromatographic efficiency, a

Table 13
Sub/SFC separation of the enantiomers of several chlorohydrins on the PolyWhelk-O column



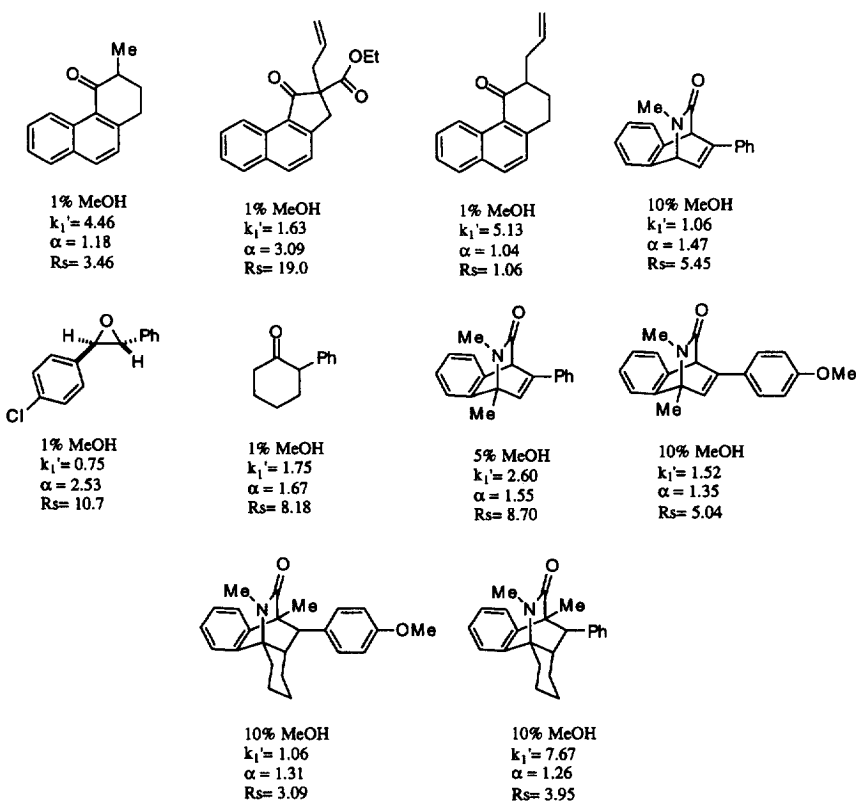
Ar	k_1'	α	R_s
4-Methoxyphenyl	0.74	1.36	2.07
2-(5-Methoxynaphthyl)	1.98	1.62	8.08
2-(4-Methoxynaphthyl)	1.01	1.29	3.66
Phenyl	0.49	1.16	0.78

Conditions: 200 bar; 26°C; 1% methanol–99% CO_2.

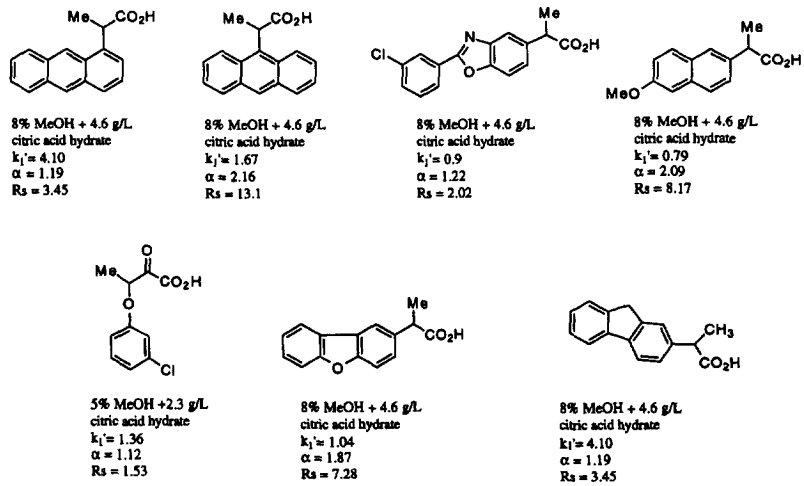
problem often addressed by means of acidic additives to the mobile phase. Addition of citric acid to the methanol used for SFC improves the chromatographic efficiency of acidic compounds on the polyWhelk-O column. Other acids may also be used and, as is typical for the resolution of chiral acids, ester and/or amide derivatives may afford even greater enantioselectivity and chromatographic efficency (Schemes 5 and 6). An assortment of atropisomers are readily resolved (Scheme 7).

3.8. Derivatized amines

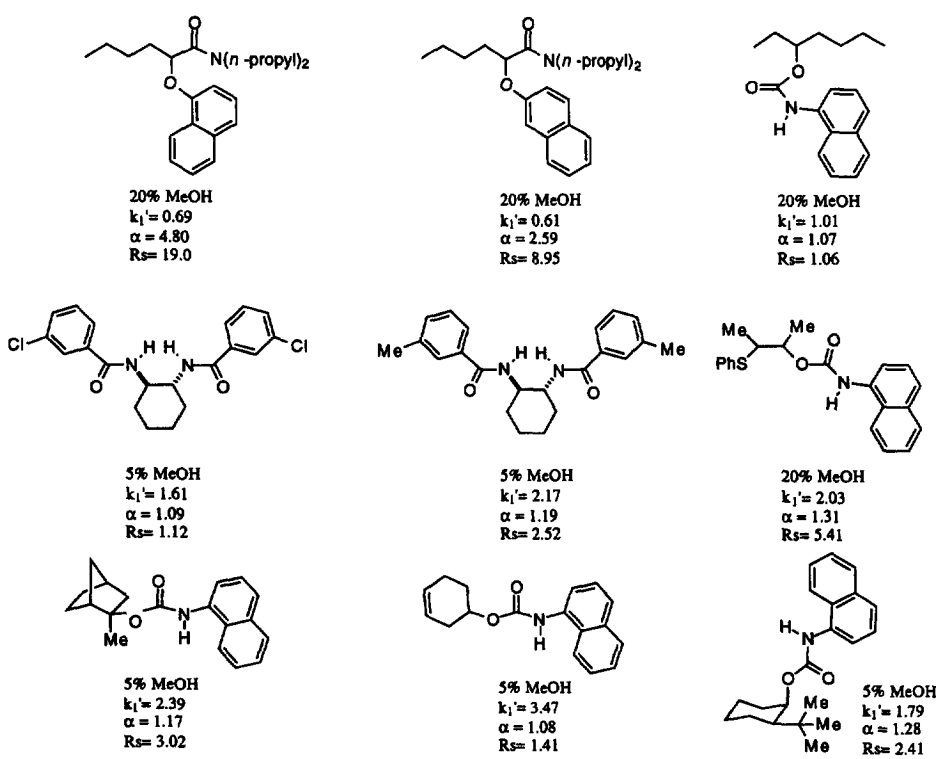
As stated above, the enantiomers of a variety of amines may be separated without derivatization. This is not always the case and derivatization can be required for reasons other than to improve detectability. For some amines, virtually any acylating agent will afford an amide derivative, the enantiomers of which can be separated on the polyWhelk-O column. In these cases, conversion to the 3,5-dinitrobenzamide derivative, although not necessarily the optimal derivative for the polyWhelk-O, is apt to prove adequate and has the dual additional merit of not only facilitating detectability but also of facilitating the separation of the enantiomers on CSPs other than the polyWhelk-O [10]. The separation factors



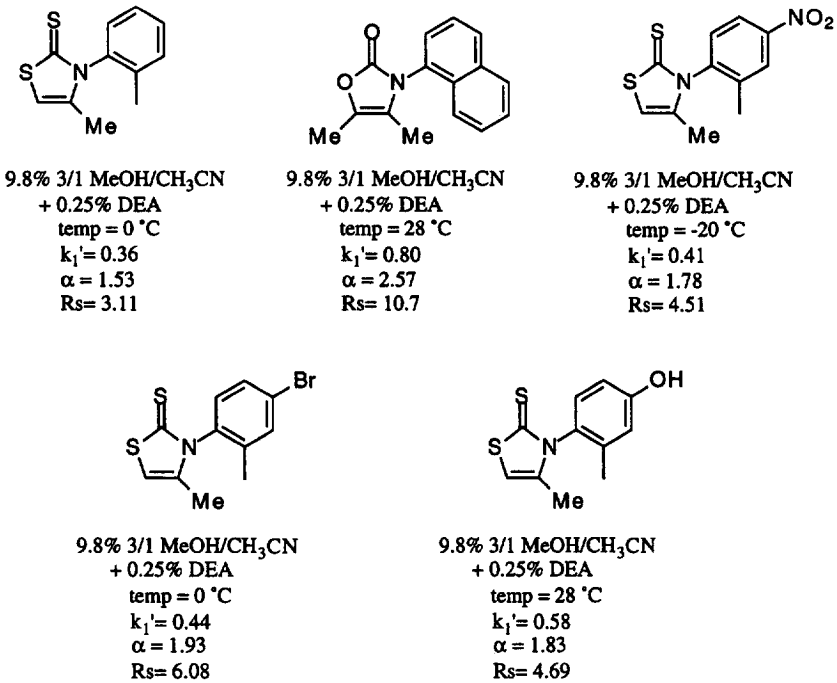
Scheme 4. Sub/SFC enantiomer separations on CSP 1.



Scheme 5. Sub/SFC resolution of carboxylic acids on the PolyWhelk-O column.



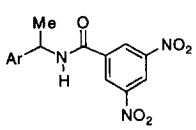
Scheme 6. Sub/SFC separations of the enantiomers of several other amides and carbamates on the PolyWhelk-O column.



Scheme 7. Sub/SFC separation of the enantiomers of heterocyclic atropoisomers on the PolyWhelk-O column.

reported for the 3,5-dinitrobenzamide in Table 14 are not as large as those of the corresponding acetamide or pivalamides (Table 8). Resolution values may be greater, however, due to the larger retention factors.

Table 14
Sub/SFC separation of the enantiomers of some 3,5-dinitrobenzamides on the PolyWhelk-O column



Ar	Conditions	k_1'	α	R_s
p-Methylphenyl	A	0.91	2.47	13.1
p-NO_2-Phenyl	A	0.66	2.08	8.09
o-Methylphenyl	A	0.76	2.07	8.83
3,4-Dichlorophenyl	A	0.90	3.38	20.9
3,4-Dimethylphenyl	A	1.04	3.14	18.9
p-Chlorophenyl	A	0.83	2.92	15.6
3,5-Ditrifluromethylphenyl	B	0.78	2.13	8.79
3,4-Dichlorophenyl	B	0.60	10.1	30.5

Conditions: (A) 200 bar; 26°C; 20% methanol–80% CO_2.
(B) 200 bar; 26°C; 5% methanol–95% CO_2.

4. Conclusion

The polyWhelk-O column has essentially the same scope and attributes as the brush-type analog but often affords greater enantioselectivity, better bandshapes and reduced capacity factors. Extensive testing of this CSP over a wide temperature range and with different mobile phase additives has shown it to be robust and to suffer no decay in performance. In the HPLC mode, the user benefits from the reduced retention factors and the consequent savings in time and eluent. The ability of the polyWhelk-O to perform at low temperature, coupled with the inherent advantages of SFC, puts a powerful tool for enantiomer separations into the hands of the separation scientist.

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