References

1 Soliman, F. M.; El-Hashash, M. A.; Salman, A. S.: J Serb. Chem. Soc. **56**, 377 (1991)
2 Attia, A.; Abo-Ghalia, M.; Abdel-Salam, O.: Pharmazie **50**, 455 (1995)
3 Moussa, H. H.; Chabaka, L. M.: Egypt. J. Chem. **26**, 267 (1983)
4 Attia, A.; Michael, M.: Pharmazie **37**, 551 (1982)
5 Al-Hajar, F. H.; Sabri, S. S.: J. Heterocyclic. Chem. **19**, 1087 (1982)
6 Ram, V. J.; Pandey, H. K.; Vlietinck, A. J.: J. Heterocyclic Chem. **18**, 1277 (1981)
7 Soliman, F. M.; El-Hashash, M. A.; Sauka, L. M.; Salman, A. S.: Rev. Roum. Chem. **41**, 109 (1996)
8 Kamal-El-Dean, A. M.; El-Kasef, H. S.: Pharmazie **51**, 155 (1996)
9 Ismail, M. F.; Sayed, F. S.; El-Khamry, A. A.; Ali, M. A.; Mansour, M. M.: J. Prakt. Chem. **331**, 399 (1989)
10 Stewart, F. S.; Berwich: Bacteriology, Virology and Immunity for Students of Medicine, 10th ed., p. 68, 1979
11 Goweulock, v. A. H.; Bell, M.: Practical Chemical Biochemistry, Vol. 1, 5th ed. p. 179, 1980

Received February 28, 1998
Accepted May 15, 1998

Dr. Asmaa Said Salem Salman
Chemistry Department
Faculty of Science
Al-Azhar University, Girl's Branch
Nasr City, Cairo,
Egypt

Groningen Institute for Drug Studies (GIDS), Departments of Analytical Chemistry & Toxicology[1] and Medicinal Chemistry[2], University Centre for Pharmacy, State University of Groningen, The Netherlands, Astra Arcus[3], Preclinical R & D, Södertälje, Sweden, and Astra Hässle[4], Division of Analytical Chemistry, Mölndal, Sweden

Temperature effects on the chromatographic behaviour of racemic 2-amidotetralins on a Whelk-O 1 stationary phase in super- and subcritical fluid chromatography

U. Selditz[1], S. Copinga[3], C. J. Grol[2], J. P. Franke[1], R. A. de Zeeuw[1], H. Wikström[2] and O. Gyllenhaal[4]

The chromatographic behaviour of thirty structurally related racemic 2-amidotetralins on a *cis*-(3*R*, 4*S*)-Whelk-O 1 stationary phase was investigated at five different temperatures between 50 and −15 °C using super-or subscritical fluid chromatography (SFC/SubFC). Generally, low selectivity factors were observed for this class of analytes on this stationary phase. In particular, phenyl and benzyl substituted analogues were strongly retained, probably due to strong π−π interactions with the chiral selector molecule, but the enantioselectivity was low. This also counts for the two *p*-iodo substituted methoxy analogues, although here steric effects may be responsible for the high capacity factors. In about 10% of the cases, a decrease in the (surrounding) temperature of the column did not result in an improved selectivity. Only four compounds out of thirty could not be resolved under the given conditions.

1. Introduction

Retention in chromatography is dependent on the free energy of the partitioning process of an analyte between the mobile and the stationary phase. In order to estimate the enthalpic and entropic contributions to this analyte transfer, van't Hoff plots are usually generated, in which the natural logarithms of experimentally determined retention parameters are plotted against the reciprocal of absolute temperature at constant pressure. The slope of such a graph, which theoretically should be linear, yields the enthalpy of the analyte transfer [1, 2]. However, there have also been reports about nonlinear van't Hoff plots where deviations from the theory are explained by alterations of the adsorption or dissociation processes, or by changes in the mobile phase interactions with either the analyte or the stationary phase [2].

In the equilibrium state, the capacity factor k_i' of a certain analyte can be related via the distribution coefficient K_D to the temperature according to equations 1a and 1b, respectively, where in (a) ΔG^0 refers to the change in Gibbs free energy of the analyte transfer between the mobile and stationary phase, R is the ideal gas constant, T is the absolute temperature, and in (b) $V_{m,s}$ are the volumes of the mobile and stationary phases, respectively, $C_{s,m}$ are the respective analyte concentrations in the two phases, and φ is defined as the phase ratio.

$$\Delta G^0 = -RT \ln K_D \tag{1a}$$

$$K_D = C_s/C_m = k_i'(V_m/V_s) = k_i'/\varphi \tag{1b}$$

According to the Gibbs-Helmholtz equation (2), the change in free energy can also be expressed in enthalpic and entropic terms,

$$\Delta G^0 = \Delta H^0 - T\,\Delta S^0 \tag{2}$$

where ΔH^0 and ΔS^0 are the respective changes in enthalpy and entropy of the analyte transfer. When substituting eq. 1a with 1b and combining eqs. 1 and 2, the solvation to $\ln k_i'$ will generate the following relationship:

$$\ln k_i' = -\Delta H^0/RT + \Delta S^0/R + \ln \varphi \tag{3}$$

From this equation it can be concluded, that the enthalpic contribution to retention decreases with temperature, whereas the entropic contribution is independent of temperature. The former parameter can therefore be obtained experimentally by determining the analyte retention as a function of temperature at constant density. The slope of the $\ln k'$ vs. $1/T$ plot represents $-\Delta H^0/R$, whereas the entropy of transfer can be obtained from the y-intercept, if the phase ratio can be estimated [3]. This is, however, not necessary when we look at the selectivity factor α, which is defined as the ratio of the capacity factors of the respective enantiomers. Now, eq. 3 can be rearranged to

$$\ln \alpha = \delta\,\Delta H^0/RT + \delta\,\Delta S^0/R \tag{4}$$

where $\delta\,\Delta H^0$ represents the difference between the interaction enthalpies of the individual enantiomers with the CSP, and $\delta\,\Delta S^0$ the respective entropic interactions. It is assumed that in a chiral recognition process according to a three-point interaction model two interactions between the analyte and the chiral selector are the same for each enantiomer, and one is different. This interaction difference is expressed in $\delta\,\Delta H^0$, whereas the former two interactions contribute to retention, and not to chiral selectivity [1].

In connection with enthalpic and entropic contributions to chiral supercritical fluid chromatography (SFC) separations, the term isoelution temperature (T_{iso}) has been discussed [1, 4]. It represents the balance of both the enthalpic and entropic parameters, which means that at T_{iso} the individual enantiomers will coelute, i.e. $\ln \alpha = 0$. When solving eq. (4) under this condition for 1/T and re-arranging the formed eq. (5a), a relationship between T_{iso} and the change in the enthalpy of transfer, which can be determined from van't Hoff plots, is obtained (5b)

$$1/T_{iso} = \delta\,\Delta S^0/\delta\,\Delta H^0 \qquad (5a)$$

$$T_{iso} = \delta\,\Delta H^0/\delta\,\Delta S^0 \qquad (5b)$$

T_{iso} is different for each analyte and dependent on the type of modifier used for the SFC analysis. Non-alcoholic modifiers seem to lower it, thus affecting it positively for analytical purposes [1].

A consequence of raising the temperature above the isoelution temperature is the reversal of the elution order, and once again an increase in selectivity. Chiral recognitions in this temperature region are said to be "entropically driven" [1]. Such an approach could be helpful in the determination of optical purities as it is favourable to elute the (minor) enantiomeric impurity before the major component.

In the following, the effect of temperature on the chromatographic behaviour of racemic 2-amidotetralins on a Whelk-O 1 stationary phase under SFC or SubFC conditions is presented and discussed. It should be noted that in the temperature range investigated and under the elution conditions chosen (200 bar outlet pressure) the mobile phase is in the supercritical state above 50 °C and in the subcritical state below 30 °C. Yet, for reasons of simplicity we will refer to the methodology as SFC. For some analytes, attempts were made to interpret van't Hoff plots in terms of enthalpy and isoelution temperature.

2. Investigations, results and discussion

Due to the abundance of data, the set of thirty 2-amidotetralins was divided into groups, taking the structural alterations, as compared to the lead compound AH 001 ($R_1 = CH_3$, $R_2 = 8\text{-}OCH_3$), into account. In Table 1, the general structure and the five major groups of 2-amidotetralins to be considered are listed, and how they will be referred to in the following discussion. In addition, the *cis* and *trans* 1-methyl analogues of AH 001 were also analysed, but will be discussed separately.

2.1. Group 1

The selectivities and capacity factors of the most retained enantiomers of the 8-methoxy analytes with alterations in the R_1-position are given in Table 2.

It appears that increasing the steric bulk on the acetyl moiety by *n*-alkyl groups has a negative effect on the selectivity, whereas the capacity factors decrease. However, hardly any changes in either parameter are observed when the length of the alkyl chain is increased by a methylene group, from ethyl to *n*-butyl. An even more decreased selectivity and smaller capacity factors, as compared to AH 001, were observed for the branched alkyl substituted analogues AH 014 and AH 100, respectively, with the *t*-butyl compound not being resolved at all under any condition.

The phenyl and benzyl substituted analogues AH 007 and AH 016, respectively, are strongly retained on the Whelk-O phase, probably due to very intensive $\pi-\pi$ interactions with the aromatic moieties of the chiral selector. As these strong interactions did not result in a high selectivity, it may be assumed that among the required simultaneous three-point interactions, necessary for high selectivity, the non-selective interaction processes are superior to the discriminating interactions and therefore cause the long retention [1].

Introduction of a chlorine atom into a methyl or ethyl group, respectively, hardly affected the selectivity α or the capacity factors. This was different for the trifluoromethyl substituted analogue, which showed a decrease in selectivity and much lower capacity factors than the methyl substituted lead compound AH 001. The strong electron withdrawing features of the former substituent apparently causes a changed electron density distribution on the amide functionality, thus directly influencing the hydrogen bond-accepting and -donating properties of this moiety.

Table 3 shows the resolutions for the enantiomeric separations of the amidotetralins of group 1 and the column efficiencies for the second eluted enantiomers. In Fig. 1 A, plots of the resolution against relative temperature (in °C) for AH 082, AH 001 and AH 016 are pictured, representing a weakly, a medium and a strongly retained analyte, respectively. It is interesting to notice that the resolution seems to pass a maximum value under the given experimental setup for AH 001 and AH 016, and that their individual maxima occur at different temperatures, whereas an almost linear relationship between resolution and temperature was observed for the fast eluted AH 082.

In Fig. 1 B the relationship between efficiency and temperature is given. It is remarkable that substantially higher efficiency values were obtained at elevated temperatures, especially when we consider that the column was not endcapped by the manufacturer. This might be ascribed to the high diffusivity and low viscosity properties of the supercritical fluid, which increase with temperature. However, the efficiency may not increase infinitely, as was reported by Stringham and Blackwell [1]. Around the critical temperature T_c at constant pressure the adsorption of mobile

Table 1: General structure and major groups of substituted 2-amidotetralins within a set of 30 related compounds

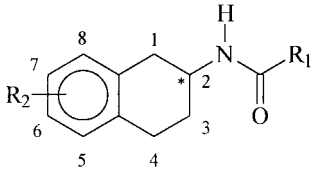
	Structural changes	Referred to as
	R_1 = variable, $R_2 = 8\text{-}OCH_3$	group 1
	R_1 = variable, R_2 = H	group 2
	$R_1 = CH_3$, R_2 = variable	group 3
	$R_1 = CH_3$, R_2-position variable	group 4
	$R_1 = CH_3$, R = disubstituted	group 5

Table 2: Selectivity and capacity factors of R_1-altered 8-methoxy-2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	R_1	α					k'_2				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	CH_3	1.18	1.26	1.36	1.49	1.73	3.00	3.57	3.76	5.40	8.35
AH 002	CH_2CH_3	1.10	1.14	1.19	1.25	1.36	2.73	3.11	3.25	4.51	6.62
AH 013	$CH_2CH_2CH_3$	1.10	1.14	1.18	1.24	1.32	2.69	3.05	3.25	4.51	6.37
AH 111	$(CH_2)_3CH_3$	1.10	1.14	1.17	1.19	1.29	2.73	3.04	3.68	3.97	6.34
AH 014	$CH(CH_3)_2$	1.02	1.03	1.04	1.05	1.06	2.20	2.42	2.50	3.20	4.30
AH 100	$C(CH_3)_3$	1.00	1.00	1.00	1.00	1.00	1.90	2.08	2.55	2.82	4.28
AH 007	C_6H_5	1.20	1.28	1.37	1.49	1.69	6.86	8.86	10.29	15.83	27.41
AH 016	$CH_2C_6H_5$	1.10	1.13	1.17	1.21	1.27	6.17	7.84	9.28	14.04	23.18
AH 017	CH_2Cl	1.14	1.21	1.27	1.36	1.51	2.83	3.37	3.86	5.35	8.24
AH 092	CH_2CH_2Cl	1.08	1.11	1.13	1.16	1.23	3.27	3.54	4.37	4.86	7.20
AH 082	CF_3	1.08	1.13	1.18	1.22	1.35	1.11	1.21	1.34	1.60	2.38

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

Table 3: Resolution and column efficiency of R_1-altered 8-methoxy-2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	R_1	R_S					N_2/25 cm				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	CH_3	2.78	3.79	4.14	4.33	4.00	11668	9595	6579	3941	1465
AH 002	CH_2CH_3	1.63	2.28	2.46	2.96	2.74	12721	11309	8740	5914	2582
AH 013	$CH_2CH_2CH_3$	1.66	2.18	2.61	2.56	2.58	11832	10090	9748	5363	2838
AH 111	$(CH_2)_3CH_3$	1.68	2.03	2.22	2.18	2.25	12871	9505	6420	5231	2444
AH 014	$CH(CH_3)_2$	0.42	0.58	0.61	0.57	0.61	19742	13077	9202	4494	2661
AH 100	$C(CH_3)_3$	0.00	0.00	0.00	0.00	0.00	8536	5659	3647	3406	1169
AK 007	C_6H_5	3.45	4.58	5.40	4.22	3.65	10398	9676	8673	2870	1314
AH 016	$CH_2C_6H_5$	1.91	2.61	2.50	2.23	2.04	12524	12271	6914	3221	1719
AH 017	CH_2Cl	2.32	3.19	3.70	3.66	3.41	13137	12781	10097	7056	3179
AH 092	CH_2CH_2Cl	1.36	1.71	1.93	1.83	1.70	13377	11397	8571	4688	2019
AH 082	CF_3	1.02	1.58	1.96	2.12	2.46	12695	10034	7640	4877	1813

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

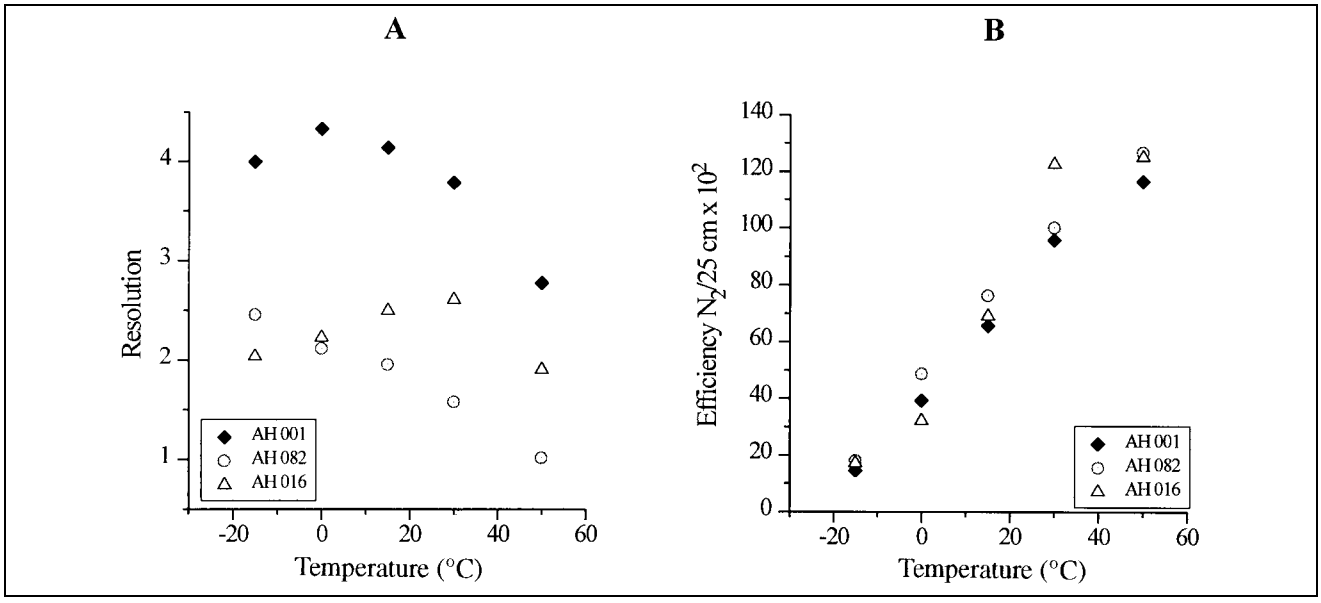


Fig. 1: Correlation between resolution and temperature (A), and efficiency of the second eluted enantiomers and temperature (B) of selected 2-amidotetralins on the *cis*-(3*R*, 4*S*)-Whelk-O 1 stationary phase in SFC experiments (200 bar CO_2, 20% MeOH, 2 ml/min)

phase components by the stationary phase is supposed to reach a maximum value, and then to decrease after the critical temperature has been passed [5]. This change in adsorption is thought to affect the kinetics of binding and, hence, cause a decline in column efficiency. As the intensity of solvent adsorption is very dependent on pressure, the loss of efficiency can be minimized by using pressures above the critical pressure P_c [1].

In our study, the temperature range was not sufficient to observe such an optimum in column efficiency. From Fig. 1B it seems that for the benzyl substituted AH 016 a maximum might be reached close to 50 °C.

Note that at low temperatures the efficiencies of the chlorinated analogue AH 017 are up to 50% increased over AH 001, whereas the substitution with a trifluoro-

methyl group causes only a small increase in column efficiency in that temperature range.

For a discussion of the chiral recognition mechanism, it would be preferable to have retention data of the individual enantiomers at hand. In our case, however, only the single enantiomers of AH 001 were available. The enantioseparation of the racemate as well as of the individual isomers on the Whelk-O 1 column is pictured in Fig. 2.

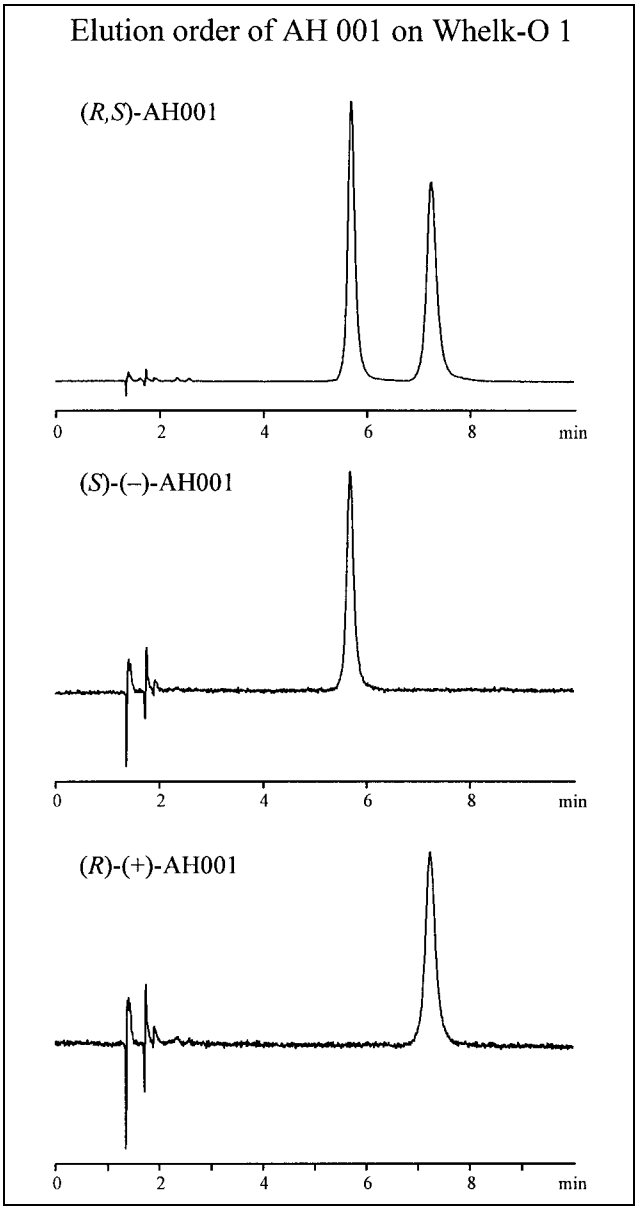


Fig. 2: SFC analysis of racemic AH 001 and its individual optical isomers on a *cis*-(3*R*, 4*S*)-Whelk-O 1 column. Conditions: 200 bar CO_2, 20% MeOH, 2.0 ml/min, 15 °C, 220 nm

In contrast to the Chiralcel OD column, on which the (*R*)-(+) enantiomer of AH 001 is retained least, on the *cis*-(3*R*, 4*S*)-Whelk-O 1 stationary phase the (*R*)-(+)-isomer is retained most.

2.2. Group 2

In this group, the aromatic moiety of the tetralin skeleton remained substituent free, which should affect the π-aromaticity of this part of the molecule. The chromatographic data in Tables 4 and 5 should therefore directly be compared with those of the respective 8-methoxy substituted analytes in Tables 2 and 3, respectively.

Generally, the selectivity and capacity factors, α and k_i', as well as the resolutions for this group of analytes are much lower than those observed for the respective 8-methoxy substituted compounds. This is conceivably caused by much less pronounced charge-transfer interactions of the aromatic rings of the analytes with the π-aromtic moieties of the selector molecule, and is in line with our expectations.

An interesting analyte is compound AH 010. Whereas the selectivity factor α seems to be independent of the temperature, an obvious decline in resolution occurs with decreasing temperature (see Fig. 3). This might be due to the above mentioned phenomenon of approaching the isoelution temperature, at which no resolution can be observed and where a further decrease in temperature would result in a reversal of the elution order. Taking this into consideration, one could also argue that AH 005 is near the isoelution temperature at 50 °C. Unfortunately, no further experiments at more elevated temperatures could be carried out, which might have enlightened this question.

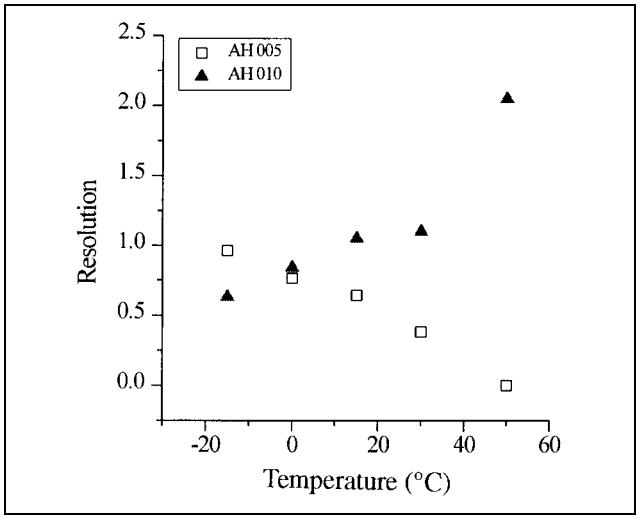


Fig. 3: Correlation between resolution and temperature of AH 005 and AH 010, respectively

Table 4: Selectivity and capacity factors of non-ring substituted 2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	R_1	α					k_2'				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 005	CH_3	1.00	1.02	1.04	1.07	1.12	1.92	2.04	2.02	2.62	3.42
AH 006	CH_2CH_3	1.00	1.00	1.00	1.00	1.00	1.79	1.90	1.85	2.32	2.95
AH 020	$CH_2CH_2CH_3$	1.00	1.00	1.00	1.00	1.00	1.68	1.86	1.88	2.31	2.90
AH 019	C_6H_5	1.02	1.04	1.07	1.09	1.15	4.10	5.07	5.53	6.98	10.88
AH 010	$CH_2C_6H_5$	1.05	1.06	1.07	1.07	1.06	4.17	5.00	5.54	7.85	11.63
AH 011	CH_2Cl	1.02	1.03	1.04	1.05	1.06	2.19	2.42	2.50	3.25	4.30

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

The isoelution temperature T_{iso} can, according to Stringham and Blackwell, be determined from plots of ln α against 1/T [1]. Following their approach, the experimentally obtained data for AH 001 were plotted against 1/T, which is pictured in Fig. 4 A. In Fig. 4 B, the respective results for AH 005 and AH 010 are shown. To determine T_{iso} by means of such a plot, an ordinary least squares (OLS) regression line was fitted through the AH 001-data ($r = 0.987$). For AH 001, this suggests that the two enantiomers will coelute in the temperature range between 80 °C and 90 °C, and that a further increase in temperature would then again result in enantioseparation.

As mentioned before, and evident from Fig. 4 B, AH 005 approaches its isoelution temperature near 50 °C and a further temperature increase would cause the reversal of the elution order. Because the changes in selectivity are only small for AH 010 in the temperature range investigated, the isoelution temperature of this analyte cannot be determined by means of the ln α vs. 1/T plot. In this case the decline in resolution with decreasing temperature can be used to estimate the T_{iso} [4].

Usually, the low thermostability of chiral stationary phases is a limiting factor for temperature studies, however, the Whelk-O selector has been reported to remain intact up to 200 °C, without any signs of racemization, loss of bonded phase or denaturation [1, 2]. Such high analysis temperatures, however, can be avoided by using other bulk fluids, like *n*-hexane with the critical parameters $T_c = 243.5$ °C and $P_c = 30$ bar, respectively, where considerably lower isoelution temperatures were observed as compared to carbon dioxide-modifier bulk fluids [1]. In this context, the term "high-temperature HPLC" was mentioned, which was theoretically examined in the first place by Antia and Horvath [6].

2.3. Group 3

Changing the size or electronic features of the substituent on the aromatic part of the 2-amidotetralin system should affect the chromatographic behaviour of such analogues, as compared to AH 001: Apart from the size of the molecule, also the π-aromaticity of the ring system is influenced. For the direct comparison of the substituent effect on enantioselectivity, all substitutes were placed on the aromatic ring system in the 8-position.

From Tables 6 and 7 it can be seen, that the extension of the alkoxy chain on the aromatic ring system from methoxy to ethoxy affected the chromatographic parameters selectivity, resolution, and capacity factors only slightly.

Electronic effects on the π-basicity of the aromatic part of the 2-amidotetralin molecule may be used to explain the decreased selectivity and resolution values of AH 095, AH 086, and AH 101, respectively, as compared to the 8-methoxy compound AH 001. The strong retention of the benzyloxy analogue is probably due to non-selective, intensive π−π interactions of this analyte with the chiral selector, in the sense as was discussed before with the R_1-altered analytes. The surprisingly 'normal' capacity factors

Table 5: Resolution and column efficiency of non-ring substituted 2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	R_1	R_S					N_2/25 cm				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 005	CH_3	0.00	0.38	0.64	0.76	0.96	10039	15441	8876	4260	2397
AH 006	CH_2CH_3	0.00	0.00	0.00	0.00	0.00	6960	7808	8450	5422	2046
AH 020	$CH_2CH_2CH_3$	0.00	0.00	0.00	0.00	0.00	10108	10270	9208	4990	1856
AH 019	C_6H_5	0.44	0.80	1.06	1.08	1.10	20494	11019	7452	4154	1494
AH 010	$CH_2C_6H_5$	2.05	1.10	1.05	0.84	0.63	13426	10284	7785	3771	2220
AH 011	CH_2Cl	0.24	0.57	0.60	0.57	0.52	6467	12842	9213	4481	2018

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

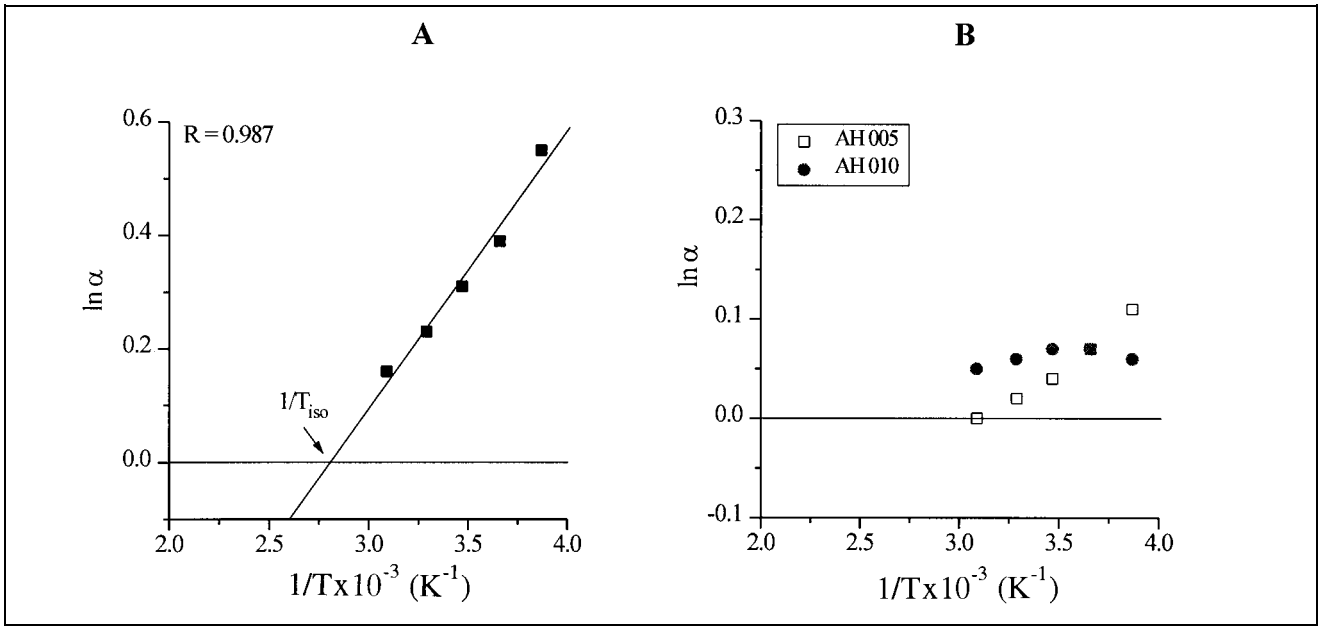


Fig. 4: (A) Plot of ln α vs. 1/T for AH 001. (B) Experimentally determined selectivity factors α plotted as the natural logarithms against the reciprocal of absolute temperature for AH 005 and AH 010

Table 6: Selectivity and capacity factors of R_2-altered 8-substituted-2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	8-Substituent	α					k'_2				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	OCH_3	1.18	1.26	1.36	1.49	1.73	3.00	3.57	3.76	5.40	8.35
AH 087	OCH_2CH_3	1.18	1.28	1.39	1.50	1.84	2.75	3.13	3.72	4.86	8.67
AH 095	$OCH_2C{\equiv}CH$	1.11	1.17	1.23	1.28	1.48	2.96	3.16	3.79	4.21	6.46
AH 086	$OCH_2C_6H_5$	1.13	1.19	1.26	1.33	1.57	4.94	5.67	6.66	8.64	15.91
AH 101	OH	1.12	1.19	1.25	1.30	1.51	3.34	3.42	4.04	4.31	6.36

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

Table 7: Resolution and column efficiency of R_1-altered 8-substituted-2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	8-substituent	R_S					N_2/25 cm				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	OCH_3	2.78	3.79	4.14	4.33	4.00	11668	9595	6579	3941	1465
AH 087	OCH_2CH_3	2.81	3.76	4.58	5.21	6.44	12202	9019	7328	5710	4884
AH 095	$OCH_2C{\equiv}CH$	1.65	2.07	2.72	2.60	2.73	9896	6543	5888	3663	1599
AH 086	$OCH_2C_6H_5$	2.26	2.96	3.37	3.04	3.57	11245	8583	6435	3338	1696
AH 101	OH	2.11	2.57	2.95	2.91	2.78	12406	8357	5895	4193	1384

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

of the 8-hydroxy-2-amidotetralin AH 101 are to be explained in terms of modifier coverage of the silica surface, due to the fairly high concentration of methanol in the bulk fluid. Obviously, the adsorption of modifier molecules is strong enough under the given conditions to resist the competition with the relatively polar analyte.

Interesting are the low efficiencies of AH 095 at elevated temperatures, although almost baseline separations were achieved. Also, high column efficiencies were obtained at lower temperatures for AH 087, whereas only moderate deviations from the results of the other analogues at elevated temperatures were observed.

2.4. Group 4

The characteristic feature of the members of this group lies in the variation of the position of the methoxy group on the aromatic part of the tetralin skeleton. Theoretically, this should not influence the π-aromaticity of this part of the molecule, and, hence, changes in the chromatographic parameters should be mainly due to steric interactions.

From Table 8 it can be seen that, within this group, the 8-methoxy substituted analogue was resolved with the highest selectivity. Since the capacity factors of the second eluted enantiomer of the 5-methoxy analogue AH 018 are in the same order as observed for AH 001, the difference in enantioselectivity must thus be caused by the interaction of the first eluted enantiomer with the chiral selector.

In Fig. 5, van't Hoff plots of the above discussed analytes AH 001 and AH 018 are shown. As mentioned earlier, the slopes of such graphs represent the contribution of enthalpy to the partition of the analyte between the mobile and stationary phase.

The slopes $-\Delta H^0/R$ of the graphs of AH 001, determined by means of ordinary least squares (OLS) regression, are $-0.79 \pm 0.18\ K^{-1}$ for the first eluted enantiomer (correlation coefficient: $r = 0.928$), and $-1.28 \pm 0.22\ K^{-1}$ for the second eluted enantiomer ($r = 0.959$). For AH 018, the re-

Table 8: Selectivity and capacity factors of R_2-position altered 2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	Position R_2	α					k'_2				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	8-OCH_3	1.18	1.26	1.36	1.49	1.73	3.00	3.57	3.76	5.40	8.35
AH 008	7-OCH_3	1.00	1.03	1.06	1.09	1.13	2.79	3.07	3.12	4.13	5.68
AH 030	6-OCH_3	1.02	1.04	1.07	1.10	1.15	2.72	3.12	3.24	4.24	5.74
AH 018	5-OCH_3	1.00	1.04	1.07	1.11	1.17	2.64	3.16	3.86	4.57	8.24

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

Table 9: Resolution and column efficiency of R_2-position altered 2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	Position R_2	R_S					N_2/25 cm				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	8-OCH_3	2.78	3.79	4.14	4.33	4.00	11668	9595	6579	3941	1465
AH 008	7-OCH_3	0.00	0.61	0.84	0.92	0.91	4768	8580	7661	3841	1598
AH 030	6-OCH_3	0.41	0.76	0.96	1.02	1.01	19435	10583	7194	3612	1472
AH 018	5-OCH_3	0.00	0.69	0.94	1.09	1.32	6885	10001	6997	3697	1980

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

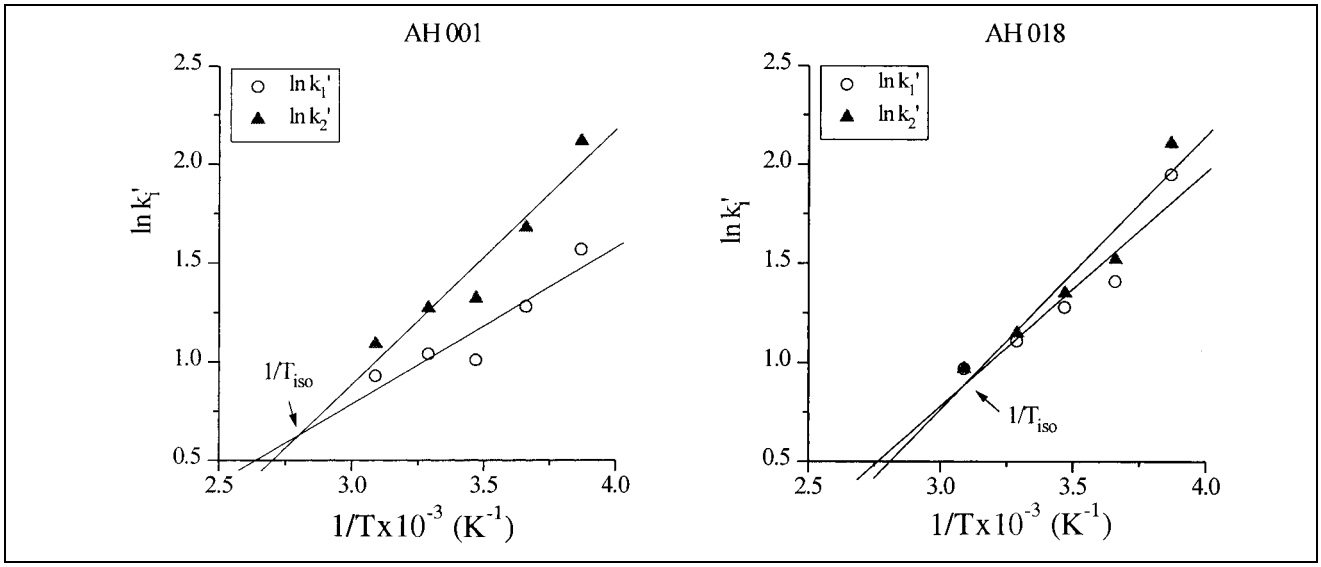


Fig. 5: Van't Hoff plots of AH 001 (left) and AH 018 (right) on basis of experimentally determined retention data. SFC conditions: 200 bar CO_2, 20% MeOH, 2.0 ml/min, *cis*-(3R,4S)-Whelk-O 1 stationary phase

spective slopes for the first and second eluted enantiomers are $-1.18 \pm 0.22\ K^{-1}$ ($r = 0.952$) and $-1.38 \pm 0.22\ K^{-1}$ ($r = 0.963$). Thus, the interaction of the first eluted enantiomer of AH 001 (determined to be the (*S*)-(−)-isomer) is much weaker than that of the first eluted enantiomer of AH 018, resulting in shorter retention times. Unfortunately, we have no retention data on the single enantiomers of AH 018 for a direct comparison. The enthalpic contribution to the interaction of the second eluted enantiomer of AH 018 with the chiral selector is only slightly stronger than that of AH 001, which consequently will result in a greater difference between the enthalpic contributions to the interaction of the individual enantiomers with the CSP for AH 001, as compared to AH 018. In particular, $\delta \Delta H^0/R$ for the 8-methoxy analogue is $0.49\ K^{-1}$, whereas it is about $0.20\ K^{-1}$ for the 5-methoxy analogue. The former value is in line with an earlier calculation from a $\ln \alpha$ vs. $1/T$ plot (see Fig. 4 A, OLSR, slope: $0.49 \pm 0.04\ K^{-1}$, $r = 0.987$).

Van't Hoff plots can also provide information about the isoleution temperature, since the point of the intersection of the respective $\ln k_i'$ graphs indicates coelution, with $\ln \alpha = 0$. From the estimated $1/T_{iso}$ values ($0.0028\ K^{-1}$) in Fig. 4B, an isoelution range between 80 and 90 °C was deduced for AH 001, which is confirmed by the results of the van't Hoff plot in Fig. 5, where approximately the same $1/T_{iso}$ is being determined. Following this approach, an isoelution temperature range of 45 to 55 °C can be predicted for AH 018, giving rise to the assumption that at temperatures higher than 55 °C a reversal in the elution order will take place.

This explanation must also be considered when interpreting the low column efficiencies of AH 008 and AH 018 at 50 °C, calculated for the second eluted enantiomers. Usually, the peaks were only partially resolved, which made a proper determination of the peak width at half heights ($w_{0.5}$) very difficult. The integration error in such cases must be taken into consideration when discussing the efficiency or resolution parameters, as both are related to $w_{0.5}$.

2.5. Group 5

This group includes the ring-disubstituted 2-amidotetralins, whose different steric and electronic features should affect the chromatographic behaviour in two ways, as compared to the monosubstituted lead compound AH 001. Firstly, the electron-withdrawing halogen substituents should cause a change in the π-aromaticity of the aromatic part of the tetralin system, reducing the degree of face-to-face charge-transfer interactions with the π-acidic DNB-moiety of the chiral selector. Secondly, in the case of *p*-substitution, π−π face-to-edge interactions with the naphthyl floor are expected to be strongly reduced, as such an interaction is sterically hindered by the *p*-substituent.

The selectivity data listed in Table 10 generally confirm the expected behaviour described above. However, it is interesting to observe that the *o*-chloro substituted compound AH 094 exhibits a significantly lower selectivity than the corresponding *p*-chloro substituted 8-methoxy analogue AH 031. Also, besides the lower selectivity,

Table 10: Selectivity and capacity factors of ring disubstituted 2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

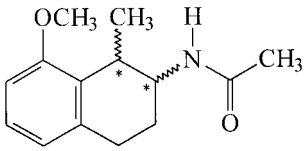
Code	Ring substitution	α					k_2'				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	8-OCH_3	1.18	1.26	1.36	1.49	1.73	3.00	3.57	3.76	5.40	8.35
AH 031	8-OCH_3, 5-Cl	1.13	1.19	1.25	1.30	1.46	3.77	4.48	4.87	6.16	9.85
AH 094	8-OCH_3, 7-Cl	1.07	1.10	1.13	1.16	1.26	2.54	2.55	2.95	3.13	4.24
AH 068	8-OCH_3, 5-I	1.14	1.20	1.26	1.31	1.46	6.22	7.44	8.42	11.06	18.75
AH 081	8-I, 5-OCH_3	1.05	1.09	1.14	1.18	1.32	5.67	6.64	7.49	9.68	16.50
AH 018	5-OCH_3	1.00	1.04	1.07	1.11	1.17	2.64	3.16	3.86	4.57	8.24

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

AH 094 is retained less on the Whelk-O phase than the monosubstituted lead compound AH 001.
When comparing the *p*-chloro and the *p*-iodo substituted 8-methoxy analogues AH 031 and AH 068, respectively, a decrease in selectivity is observed, as compared to AH 001. Since both analytes are resolved on the Whelk-O 1 stationary phase with similar selectivities, it appears that it is not very important, whether the substituent is a chlorine or an iodine.
It is interesting that the iodo-substitution in the 8-position, *para* to the methoxy group, causes a slight increase in selectivity, and an about 50% increase in capacity factors, as compared to the mono substituted analogue AH 018.
As far as the capacity factors are concerned, an increase for all *p*-disubstituted analogues can be observed, as compared to AH 001 (or AH 018), whereas the *o*-disubstituted compound AH 094 is retained least within this series of disubstituted analytes. When taking into consideration the low selectivities of AH 031, AH 068, and AH 081, respectively, it is very likely that the relatively long retentions are caused by nonselective interactions with the CSP, as was explained earlier for the R_1-phenyl and -benzyl substituted analytes.
The resolutions for this group of substances listed in Table 11 are in agreement with the above discussed selectivities. Also listed in Table 11 are the column efficiencies, calculated for the second eluted enantiomers. Except for the value of AH 018 at the highest temperature of investigation, which was dealt with in group 4 already, no irregular behaviour was observed. Through the whole series of disubstituted analytes high efficiencies of the Whelk-O phase were observed, even though it was not endcapped.

2.6. 1-Methyl-8-methoxy-2-acetamidotetralin

The *cis*- and *trans*-isomers of the 1-methyl analogue of AH 001 were available. The chromatographic data obtained on the *cis*-(3*R*, 4*S*)-Whelk-O 1 stationary phase under SFC conditions are given in Table 12.



General structure of *cis*- and *trans*-1-methyl-8-methoxy-2-amidotetralin

Whereas the *trans*-isomer AH 035 showed a low selectivity over the entire temperature range, it steadily increased for the AH 036 analogue. Moreover, within the whole set of racemic 2-amidotetralins, the *cis*-isomer AH 036 was resolved with the highest enantioselectivities on the Whelk-O 1 stationary phase.
Furthermore, the resolution values of AH 035 go through a maximum at 30 °C, whereas for AH 036 this parameter gradually increased with decreasing temperatures.
The differential chromatographic behaviour of the *cis*-isomer, as compared to the *trans*-isomer and also the lead compound AH 001, can be explained by steric effects caused by the pseudo-axial orientation of the methyl group, probably causing a much better presentation of the interaction sites of this analyte towards the chiral selector.

2.7. Conclusions

In general, low selectivity factors were observed for the SFC separation of racemic 2-amidotetralins on the *cis*-(3*R*, 4*S*)-Whelk-O 1 stationary phase. However, these could be positively affected by lowering the system temperature during the analysis. In most cases, the decrease in temperature also caused an increase in retention times as well as an increase in resolution, but sometimes, resolution appeared to give a maximum within the temperature range studied. From plots of $\ln k_i'$ against 1/T, so called van't Hoff plots, energetic contributions to the interactions of the individual enantiomers with the chiral stationary phase could be derived. The difference between the enthalpies of such partitioning processes can be related to

Table 11: Resolution and column efficiency of ring disubstituted 2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

Code	Ring substitution	R_S					N_2/25 cm				
		50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 001	8-OCH_3	2.78	3.79	4.14	4.33	4.00	11668	9595	6579	3941	1465
AH 031	8-OCH_3, 5-Cl	2.28	2.79	3.11	3.05	2.94	12019	8424	6376	4313	1633
AH 094	8-OCH_3, 7-Cl	1.13	1.46	1.77	1.52	1.64	12516	10034	8822	3800	1838
AH 068	8-OCH_3, 5-I	2.59	3.34	3.36	3.01	3.13	11022	9950	5722	3365	1776
AH 081	8-I, 5-OCH_3	0.91	1.54	1.88	1.81	2.17	11876	8742	5984	3291	1555
AH 018	5-OCH_3	0.00	0.69	0.94	1.09	1.32	6885	10001	6997	3697	1980

SFC conditions: 20% MeOH, 200 bar CO_2, 2.0 ml/min

Table 12: SFC data of the *cis*- and *trans*-1-methyl-(8-methoxy)-2-amidotetralins obtained on the (3*R*, 4*S*)-Whelk-O 1 stationary phase

code	α					k_2'				
	50°	30°	15°	0°	−15°	50°	30°	15°	0°	−15°
AH 035	1.11	1.13	1.14	1.15	1.17	1.66	1.79	1.83	2.17	2.81
AH 036	1.41	1.57	1.73	1.86	2.32	3.54	4.45	5.12	6.84	12.07
	R_S					N_2/25 cm				
AH 035	1.58	1.72	1.59	1.46	1.10	12044	10528	7676	4313	1956
AH 036	5.56	6.10	6.66	6.95	7.20	9736	6167	5291	5162	2388

Mobile phase: 20% MeOH, 200 bar CO_2, 2.0 ml/min

the selectivity factor α. When plotting ln α against 1/T, the so called isoelution temperature T_{iso} could be assessed on the basis of experimentally determined retention data. Knowing T_{iso} of a certain analyte under given experimental conditions might be helpful in the determination of optical purities, as the elution order will be reversed and other selectivities should be observed when increasing the temperature above T_{iso}. The low selectivities observed for some analytes in these studies appear to be due to the fact that some temperatures chosen were close to this isoelution temperature. The conformationally restricted *cis*-α-methyl analogue of 8-methoxy-2-amidotetralin was best resolved on the Whelk-O stationary phase, whereas the respective *trans*-isomer showed only low selectivites. Over the temperature range investigated, a decline of the column efficiency with decreasing temperature was observed for all compounds. At lower temperatures the kinetics of mass transfer are lower, which will result in peak broadening.

3. Experimental

3.1. Apparatus and column

A Hewlett Packard G1205A supercritical fluid chromatography system (Hewlett-Packard, Little Falls, Wilmington DE, USA), consisting of dual pumps, pressure controller, oven module and autosampler, was used.
The analytes were monitored in the UV range by means of an HP 1050 multiwavelength detector. Chromatograms were generated and evaluated with the HP SFC 2D ChemStation software package, version A.01.02, operated under Windows 3.1. on a Compaq Deskpro 590 personal computer. Low-temperature experiments were carried out by thermostating the column externally in a water-ethylene glycol bath which was cooled to the desired temperature using the LAUDA compact low-temperature thermostat RM 6B (Lauda Dr. R. Wobser GmbH & co. KG, Lauda-Königshofen, Germany). The (3*R*, 4*S*)-Whelk-O 1 column (25 cm × 0.46 cm I.D.) was supplied by Regis (Morton Grove, IL, USA) via Chrompack (Deventer, The Netherlands).

3.2. Materials and sample preparation

Methanol, gradient grade, was purchased from E. Merck (Darmstadt, Germany). Spectroscopic ethanol, supplied by Kemetyl (Stockholm, Sweden), was used for dissolving the analytes. SFC-grade carbon dioxide (99.9%) was supplied by AGA (Lidingö, Sweden).
The racemic 2-amidotetralins were synthesized at the Department of Medicinal Chemistry, University Centre for Pharmacy, Groningen, The Netherlands [7, 8]. About 1 mg of each compound, either as HCl-salts or free bases, was dissolved in 1.5 ml ethanol.

3.3. Chromatographic conditions

The SFC apparatus was operated in the downstream mode at five different temperatures: 50, 30, 15, 0, and −15 °C. The outlet pressure of the supercritical carbon dioxide was set to 200 bar and 20% methanol was added to increase the polarity of the mobile phase. Per run, 5 µl of the prepared solutions were injected. The total flow-rate was set to 2 ml/min. The analytes were monitored by UV-detection at 220 and 272 nm, respectively.
Chromatographic data were calculated as follows: $\alpha = k'_2/k'_1$, $k'_i = (t_i - t_0)/t_0$, whereas the peak of the solvent front was considered to be equal to the dead time t_0 and was taken from each particular run. The resolution was determined according to: $R_S = (t_2 - t_1)/[(w_{0.5})_1 + (w_{0.5})_2]$, where $(w_{0.5})_i$ stands for the peak width at half height of the individual peaks. This value was provided by the ChemStation software. The column efficiency was calculated for the second eluted enantiomer according to $N_2 = 5.54\,(t_2/(w_{0.5})_2)^2$.

Acknowledgement: This SFC investigation was carried out at the Division of Analytical Chemistry, Astra Hässle, Mölndal, Sweden. Financial support by GIDS (Groningen Institute for Drug Studies) is gratefully acknowledged.

References

1 Stringham, R. W.; Blackwell, J. A.: Anal. Chem. **69**, 1414 (1997)
2 Pirkle, W. H.: J. Chromatogr. **558**, 1 (1991)
3 Yonker, C. R.; Smith, R. D.: J. Chromatogr. **351**, 211 (1986)
4 Stringham, R. W.; Blackwell, J. A.: Anal. Chem. **68**, 2179 (1996)
5 Strubinger, J. R.; Song, H.; Parcher, J. F.: Anal. Chem. **63**, 104 (1991)
6 Antia, F. D.; Horvath, C.: J. Chromatogr. **435**, 1 (1988)
7 Copinga, S.; Tepper, P. G.; Grol, C. J.; Horn, A. S.; Dubocovich, M. L.: J. Med. Chem. **36**, 2891 (1993)
8 Copinga, S.: The 2-aminotetralin system as a structural base for new dopamine- and melatonin-receptor agents. Thesis. State University of Groningen, 1994

Received April 28, 1998
Accepted July 5, 1998

Prof. Dr. R. A. De Zeeuw
University Centre for Pharmacy
Antonius Deusinglaan 1
9713 AV Groningen
The Netherlands

Department of Pharmaceutics[1], University of Florida, Gainesville, and Department of Chemistry[2], Lehigh University, USA

A spermine-deoxycholic acid conjugate based lipid as a transfecting agent

P. S. Ajmani[1], S. Attia[1], M. Osifchin[2], S. Regen[2], and J. A. Hughes[1]

Deoxycholic acid-spermine conjugate (DAS), which is composed of natural components (deoxycholic acid and spermine), was incorporated in liposomes and evaluated for its interaction with plasmid DNA (pDNA) and *in vitro* transfection efficiency. Electromicrographs demonstrated that DAS-pDNA complexes are spherical, compact and electronically dense compared to the toroidal shapes formed by the monovalent lipid 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and pDNA. In comparison to the singly charged, non-cholesterol based lipid (DOTAP), the multivalent lipid DAS had similar transfection efficiency in two cell lines. The monovalent sterol, deoxycholic acid propyldiamine conjugate (DAP) was not effective as a transfecting agent. This suggests that multivalent facial amphiphiles such as DAS may serve as excellent candidates for non-viral gene transfer and warrant further study.

1. Introduction

Gene therapy has the potential to be beneficial in the treatment of a number of disorders [1, 2] including cystic fibrosis [3, 4] and stimulation of the immune system for cancer therapy [5]. Non-viral gene delivery [6–11] is an approach which utilizes various delivery systems such as injection of naked plasmid DNA (pDNA) and ligands conjugated to polycationic carriers including polylysine [12] and liposomes [13–15]. The non-viral approach is generally considered to be safer than viral delivery due to the lessened chance of eliciting an immune response. However, when compared to viral vectors, non-viral vectors are