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Chiral mobile phases in the enantiomeric analysis and in the evaluation of stability constants of $\pi-\pi$ complexes

Yuri P. Belov

Institute of Physiologically Active Substances, Russian Academy of Sciences, Chernogolovka, Moscow Region 142432, Russia

Abstract - The separation of the enantiomers of N-(3,5-dinitrobenzoyl)amino acid esters on achiral Nitrile column using hexane-isopropanol solution of (S)-N-(1-naphthyl)leucine octadecyl ester as the chiral mobile phase are presented. The general utility of donor type chiral mobile phases in separating the enantiomers of acceptor type compounds is demonstrated. Chromatographic data are used for the calculation of free energies of adsoprtion of the enantiomers and the stability constants of π-π donor-acceptor complexes formed in mobile phase.

INTRODUCTION

With the heightened interest in stereochemistry that presently pervades most branches of chemistry, biochemistry and pharmacology, the need for better methods of ascertaining enantiomeric purities is obvious. An almost ideal solution to this problem is the enantiomeric analysis using chiral HPLC. The successful development of chiral chromatography has had a revolutionary impact upon the synthesis of optically active substances (ref. 1,2). The speed with which this technology has developed is due at least in part to the understanding of chiral recognition mechanism which accounts for the specific interactions leading to chiral discrimination. This is particularly true for the donor-acceptor chiral chromatography (ref. 3,4). Currently donor-acceptor type chiral stationary phases are commercially available.

The method of chiral eluents (ref. 5-7) received widespread attention in the field of enantiomeric analysis. In the present paper we have reported the enantiomeric analysis of the N-(3,5-dinitrobenzoyl)amino acid esters (DNB-AA-OR) on an archiral Nitrile column using donor type chiral mobile phase. Hexane-isopropanol solution of (S)-N-(1-naphthyl)leucine octadecyl ester (chiral selector, CS) was used in this study. Chromatographic data was used for the calculation of the stability constants of the $\pi-\pi$ complexes formed between enantiomers of DNB-AA-OR and CS in the mobile phase.

EXPERIMENTAL

Chromatography was performed using Altex 100A pump, Reodyne 7125 injector with 20 mcl sample loop, LDC/Milton Roy UV-Monitor (254 nm), Kipp and Zonen BD 41 recorder and Nitrile 250x4.6 mm column packed with 5 mcm particles (Regis). All substances used in this study are well known compounds and are commercially available.

RESULTS AND DISCUSSION

Retention of analytes in normal phase chromatography depends on the polarity of the mobile phase. Chromatography of DNB-AA-OR is convenient using 10% solution of isopropanol (IPA) in hexane as eluent (Table 1).

In the presence of CS in the mobile phase the enantiomers of DNB-AA-OR have a different retention (Table 2). Retention of (R)-enantiomers is higher than the retention of (S)-enantiomers.

TABLE 1. $k'_{DNB\text{-}AA\text{-}OR} = f([IPA])$.

Eluent: IPA/Hexane. T = 20°C.

[IPA], %	DNB-Ala-OMe		DNB-Leu-OBu	DNB-NHCH(Oct)-COOOct
	k'	N	k'	k'
0.5	-	-	18.79	12.20
1	27.14	10820	11.89	6.99
2	17.61	10640	8.12	5.60
3	13.53	10880	6.53	4.40
5	9.57	10370	4.83	3.27
10	-	-	3.07	1.97
20	3.13	9830	2.03	1.33
30	2.36	9830	1.71	1.17
50	1.33	8700	0.98	0.70

TABLE 2. $k'_{DNB\text{-}AA\text{-}OR} = f([IPA])$.

Eluent: $5 \cdot 10^{-4}$M CS in 10% IPA/Hexane. T = 20°C.

[IPA],%	DNB-Ala-OMe				DNB-Leu-OBu			DNB-NHCH(Oct)-COOOct		
	k'_1	k'_2	α	N	k'_1	k'_2	α	k'_1	k'_2	α
0.5	-	-	-	-	8.56	17.91	2.09	6.24	11.68	1.87
1	17.71	25.61	1.45	9800	7.00	11.32	1.62	5.04	7.55	1.50
2	13.29	16.81	1.26	9900	5.59	7.75	1.39	4.03	5.34	1.33
3	11.12	13.24	1.19	9670	4.99	6.39	1.28	3.53	4.38	1.24
5	8.46	9.36	1.11	9400	4.03	4.73	1.17	2.72	3.18	1.17
10	5.30	5.63	1.06	9900	2.80	3.13	1.12	1.83	2.00	1.09
20	2.93	3.01	1.03	9300	1.64	1.75	1.07	1.09	1.17	1.07
30	1.72	1.77	1.03	8850	1.20	1.24	1.03	0.77	0.79	1.02
50	1.34	1.34	1.00	8900	1.01	1.01	1.00	0.72	0.72	1.00

Enantioselectivity (α) depends on the concentration of IPA: increasing the concentration of IPA leads to decrease of enantioselectivity. In the presence of CS efficiency (N) is slightly lower than in absence of CS (Tables 1 and 2).

Increasing the concentration of CS in mobile phase leads to decrease of the retention of DNB-AA-OR enantiomers and to increase of the enantioselectivity (Table 3).

In the presence of CS in mobile phase DNB-AA-OR enantiomers (E) form π-π complexes in proportion 1:1 (E-CS) and can be adsorbed in form of E and E-CS. Similar to (ref. 8) stability constants of $\pi - \pi$ complexes (K) can be calculated:

$$K = [E\text{-}CS]/[E][CS] \text{ and } [E] = 1/(K[CS] + 1) \qquad (1)$$

In the presence of CS retention of DNB-AA-OR (k') depends on the retention of E (k'_E) and E-CS ($k'_{E\text{-}CS}$):

$$k' = k'_E[E] + k'_{E\text{-}CS}[E\text{-}CS] = k'_E[E] + k'_{E\text{-}CS}(1 - E)$$

$$\text{and } [E] = (k' - k'_{E\text{-}CS})/(k'_E - k'_{E\text{-}CS}) \qquad (2)$$

TABLE 3. $k'_{DNB-AA-OR} = f([CS])$.

Eluent: CS in 10% IPA/Hexane. T = 20°C.

[CS], M	DNB-Ala-OMe			DNB-Leu-OBu			DNB-NHCH(Oct)-COOOct		
	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
0	5.87	5.87	1.00	3.17	3.17	1.00	2.00	2.00	1.00
0.0015	5.87	4.73	1.24	3.17	2.27	1.40	2.00	1.50	1.33
0.0020	5.80	4.37	1.33	3.13	2.07	1.51	2.00	1.33	1.50
0.0030	5.70	3.93	1.45	3.03	1.73	1.75	2.00	1.23	1.63
0.0060	5.50	3.13	1.76	2.93	1.33	2.20	1.87	0.90	2.08
0.0125	5.20	2.30	2.26	2.77	1.00	2.77	1.77	0.70	2.53
0.0250	4.57	1.77	2.58	2.40	0.77	3.12	1.57	0.57	2.75
0.0500	3.73	1.27	2.94	1.97	0.60	3.28	1.23	0.40	3.08
0.1000	2.93	0.93	3.15	1.53	0.47	3.26	0.93	0.29	3.21

From (1) and (2) we have:

$$k'_{E\text{-}CS} = [k'(K[CS] + 1) - k'_E]/K[CS] \quad (3)$$

$$\text{and } K = (k'_E - k')/[CS](k' - k'_{E\text{-}CS}) \quad (4)$$

At room temperature the retention of CS on the surface of Nitrile column is low (Table 5). It seems that chiral recognition of DNB-AA-OR enantiomers at room temperature depends mostly on the retention of π–π complexes formed between enantiomers of DNB-AA-OR and CS in mobile phase.

In comparison with DNA-AA-OR their π-π complexes with CS are more hydrophobic and have less retention on the surface of polar Nitrile column. (S)-enantiomers of DNA-AA-OR form much more stable complexes than (R)-enantiomers (Table 4). It leads to increase of (S)-enantiomers concentration over the concentration of (R)-enantiomers in mobile phase and to decrease of (S)-enantiomers retention in comparison to retention of (R)-enantiomers.

TABLE 4. Stability constants of π-π complexes (K) formed between enantiomers of DNB-AA-OR and CS in 10% IPA/Hexane. T = 20°C.

DNB-AA-OR	K, mol^{-1}
(S)-DNB-Ala-OMe	208
(R)-DNB-Ala-OMe	9
(S)-DNB-Leu-OBu	347
(R)-DNB-Leu-OBu	22
(S)-DNB-NHCH(Oct)-COOOct	308
(R)-DNB-NHCH(Oct)-COOOct	10

TABLE 5. Retention of CS on Nitrile column.

Eluent: 10% IPA/Hexane.

T, °C	-55	-40	-20	0	20	40	60	80
k'_{CS}	1.10	0.80	0.47	0.33	0.20	0.13	0.07	0.02

TABLE 6. $k'_{DNB\text{-}AA\text{-}OR} = f(n)$. Eluent: $5 \cdot 10^{-4}$M CS in 10% IPA/Hexane. T = 20°C.

n	DNB-NHCH(CH$_2$)$_n$-H COOEt			DNB-NHCH(CH$_2$)$_n$-H COOBu			DNB-NHCH(CH$_2$)$_n$-H COOOct		
	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
1	3.67	3.81	1.04	3.00	3.13	1.04	2.37	2.47	1.04
2	3.37	3.53	1.05	2.83	2.97	1.05	2.23	2.35	1.06
3	3.03	3.20	1.06	2.63	2.77	1.05	2.03*	2.17*	1.07*
4	-	-	-	2.40	-	-	1.80*	1.93*	1.07*
5	2.77	2.91	1.05	2.23	2.40	1.08	1.73	1.85	1.07
6	2.50	2.71	1.08	2.10	2.25	1.07	1.67	1.79	1.07
7	2.40	2.55	1.06	1.93	2.07	1.07	1.57	1.69	1.07
8	2.23	2.37	1.06	1.87	2.00	1.07	1.47	1.59	1.08
9	2.13	2.27	1.06	1.73	1.85	1.07	-	-	-
10	2.00	2.13	1.07	1.70	1.83	1.08	1.30	1.40	1.08
11	1.90	2.03	1.07	1.60	1.72	1.08	1.28	1.39	1.08
12	1.83	1.97	1.07	1.53	1.63	1.07	1.17	1.27	1.08
13	1.77	1.90	1.07	1.47	1.57	1.07	1.17	1.27	1.08
14	1.70	1.80	1.06	1.43	1.53	1.07	1.13	1.21	1.07
16	1.57	1.70	1.08	1.33	1.43	1.07	1.00	1.08	1.08

* iso-alkyl

TABLE 7. $k'_{DNB\text{-}AA\text{-}OR} = f(n)$.

Eluent: $5 \cdot 10^{-4}$M CS in 10% IPA/Hexane. T = 20°C.

n	DNB-Ala-O(CH$_2$)$_n$-H			DNB-Leu-O(CH$_2$)$_n$-H		
	k'	k'	α	k'	k'	α
1	4.37	4.57	1.05	3.37	3.60	1.07
2	3.67	3.81	1.04	-	-	-
4	3.00	3.13	1.04	2.37	2.53	1.07
8	2.33	2.43	1.04	1.80	1.93	1.07

TABLE 8. $k'_{DNB\text{-}AA\text{-}OR} = a - b\ln(n)$

Eluent: $5\cdot10^{-4}$M CS in 10% IPA/Hexane. T = 20°C.

DNB-AA-OR	a	b	R^2
(S)-DNB-Ala-O$(CH_2)_n$-H	4.36	0.98	1.000
(R)-DNB-Ala-O$(CH_2)_n$-H	4.55	1.02	0.999
(S)-DNB-NHCH[$(CH_2)_n$-H]-COOEt	4.35	1.01	0.995
(R)-DNB-NHCH[$(CH_2)_n$-H]-COOEt	4.59	1.06	0.997
(R)-DNB-NHCH[$(CH_2)_n$-H]-COOEt	4.59	1.06	0.997
(S)-DNB-NHCH[$(CH_2)_n$-H]-COOBu	3.47	0.78	0.996
(R)-DNB-NHCH[$(CH_2)_n$-H]-COOBu	3.74	0.84	0.994
(S)-DNB-NHCH[$(CH_2)_n$-H]-COOOct	2.79	0.64	0.990
(R)-DNB-NHCH[$(CH_2)_n$-H]-COOOct	2.98	0.67	0.989

TABLE 9. $k'_{DNB\text{-}AA\text{-}OR} = f(T)$. Eluent: $5\cdot10^{-4}$M CS in 10% IPA/Hexane.

T, K	DNB-Ala-OMe			DNB-Leu-OBu			DNB-NHCH(Oct)-COOOct		
	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
218	-	-	-	-	-	-	1.40	9.00	6.43
233	9.87	25.00	2.53	3.00	13.00	4.33	1.80	7.33	4.07
253	9.13	13.93	1.53	3.40	7.13	2.10	2.17	4.20	1.94
273	7.73	9.27	1.20	3.47	4.90	1.41	2.20	3.00	1.36
293	5.53	5.97	1.08	2.70	3.10	1.15	1.80	2.00	1.11
313	3.87	4.00	1.03	2.00	2.13	1.07	1.27	1.33	1.05
333	2.73	2.73	1.00	1.57	1.57	1.00	0.97	0.97	1.00
353	2.13	2.13	1.00	1.13	1.13	1.00	0 80	0.80	1.00

The retention of DNB-AA-OR enantiomers depends on the length of their alkyl chain. Increasing the alkyl chain length leads to increase of DNB-AA-OR hydrophobicity and decrease of retention on normal phase Nitrile column. Enantioselectivity almost does not depend on the length of DNB-AA-OR alkyl chains (Table 6 and 7).

There is a linear correlation between the retention of DNB-AA-OR enantiomers and the logarithm of the number of methylene units (n) in their alkyl chain (Table 8).

Increasing the temperature leads to decrease in retention of DNB-AA-OR enantiomers and enantioselectivity (Table 9).

A plot of ln(k') verses 1/T (a van't Hoff plot) for (R)-enantiomers of DNB-AA-OR is shown in Table 10. Van't Hoff plots are used for calculation of free energies of adsorption of the (R)-enantiomers of DNB-AA-OR. There is no linear correlation between in(k') and 1/T for (S)-enantiomers of DNB-AA-OR.

TABLE 10. $\ln(k') = -a + b/T$. Eluent: $5 \cdot 10^{-4}$M CS in 10% IPA/Hexane.

DNB-AA-OR	a	b	R^2
(R)-DNB-Ala-OMe	4.03	1694	0.998
(R)-DNB-Leu-OBu	4.44	1632	0.998
(R)-DNB-NHCH(Oct)-COOOct	4.27	1437	0.989

TABLE 11. $\Delta G_S^{293} = \Delta G_R - \Delta\Delta G^{293}$. Eluent: $5 \cdot 10^{-4}$M CS in 10% IPA/Hexane.

DNB-AA-OR	ΔG_R, kJ·mol^{-1}	$\Delta\Delta G^{293}$, J·mol^{-1}	ΔG_S^{293}, kJ·mol^{-1}
DNB-Ala-OMe	-14.29	-187	-14.10
DNB-Leu-OBu	-13.64	-337	-13.30
DNB-NHCH(Oct)-COOOct	-13.04	-257	-12.78

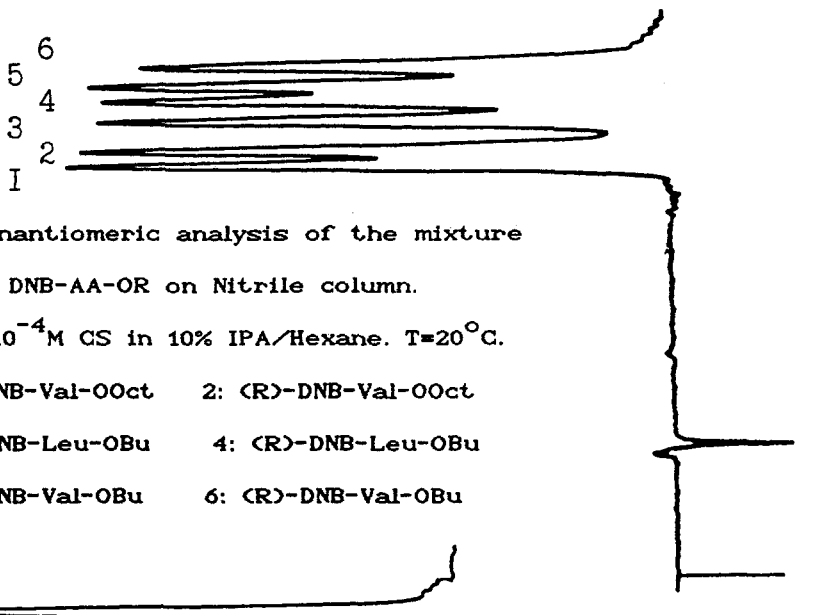

FIGURE 1. Enantiomeric analysis of the mixture of racemic DNB-AA-OR on Nitrile column. Eluent: $1.5 \cdot 10^{-4}$M CS in 10% IPA/Hexane. T=20°C.

1: (S)-DNB-Val-OOct 2: (R)-DNB-Val-OOct

3: (S)-DNB-Leu-OBu 4: (R)-DNB-Leu-OBu

5: (S)-DNB-Val-OBu 6: (R)-DNB-Val-OBu

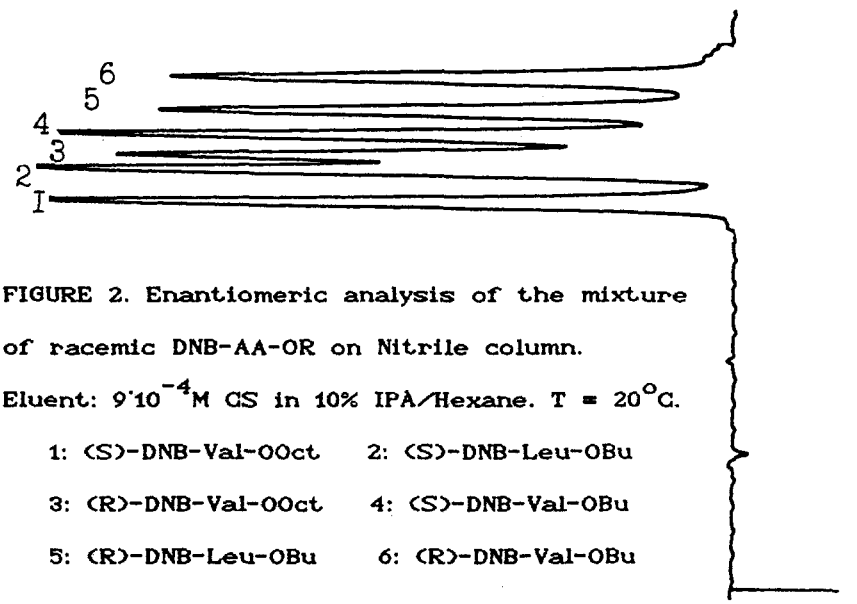

FIGURE 2. Enantiomeric analysis of the mixture of racemic DNB-AA-OR on Nitrile column. Eluent: $9 \cdot 10^{-4}$M CS in 10% IPA/Hexane. T = 20°C.

1: (S)-DNB-Val-OOct 2: (S)-DNB-Leu-OBu

3: (R)-DNB-Val-OOct 4: (S)-DNB-Val-OBu

5: (R)-DNB-Leu-OBu 6: (R)-DNB-Val-OBu

Free energies of adsorption of the (S)-enantiomers at room temperature (293K) are calculated using equation:

$$\Delta\Delta G^{293} = \Delta G_R - \Delta G_S^{293} \text{ (Table 11)}$$

Chiral mobile phases are convenient for enantiomeric analysis of the mixture of racemates. Enantiomeric analysis of the mixture of racemic DNB-AA-OR are shown on Figures 1,2 and 3. The order of elution of analytes easily can be changed using different concentration of CS (cf. Figures 1 and 2).

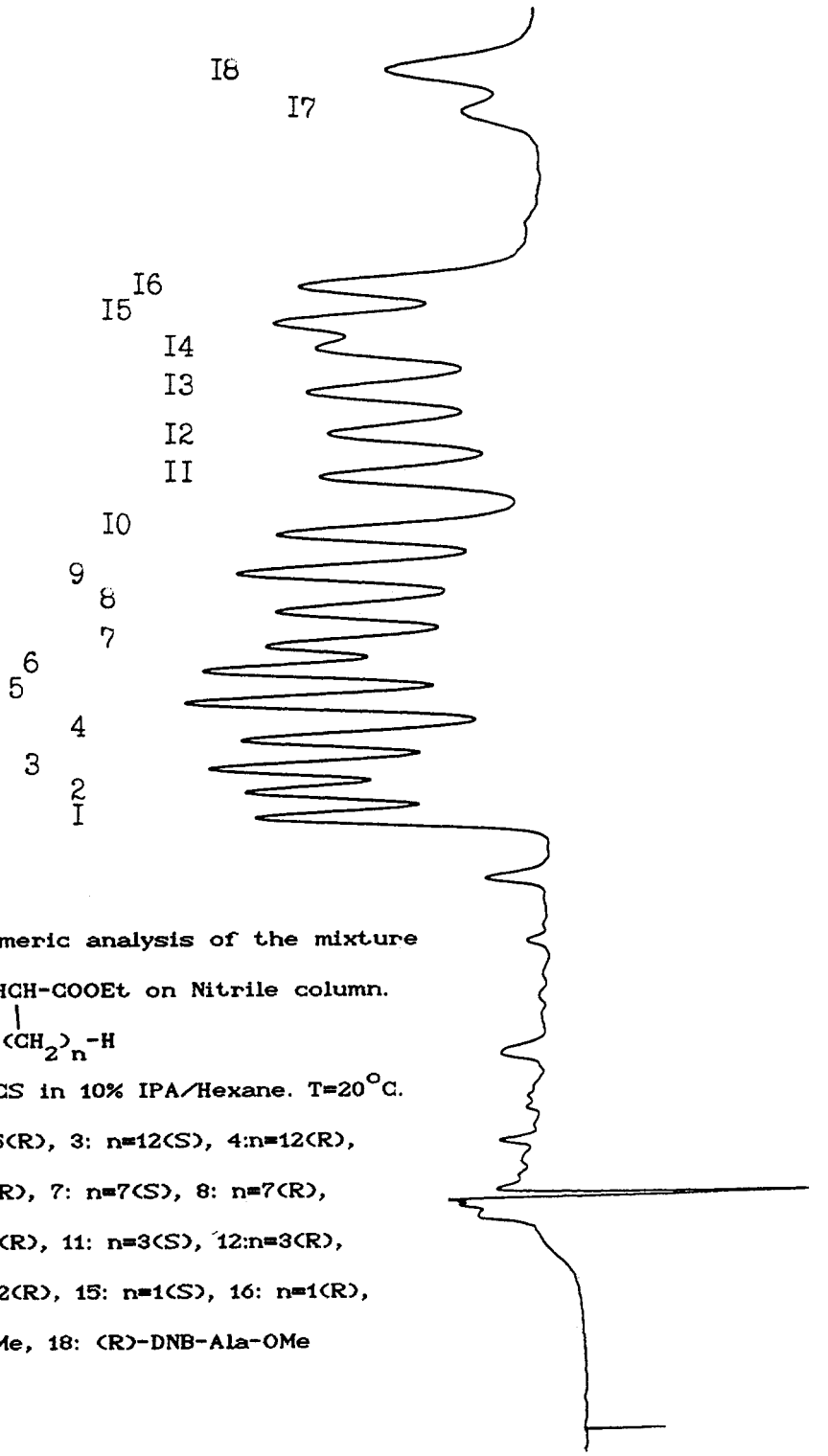

FIGURE 3. Enantiomeric analysis of the mixture of racemic DNB-NHCH-COOEt on Nitrile column.
$(CH_2)_n$-H

Eluent: $2.4 \cdot 10^{-4}$M CS in 10% IPA/Hexane. T=20°C.

1: n=15(S), 2: n=15(R), 3: n=12(S), 4:n=12(R),

5: n=9(S), 6: n=9(R), 7: n=7(S), 8: n=7(R),

9: n=5(S), 10: n=5(R), 11: n=3(S), 12:n=3(R),

13: n=2(S), 14: n=2(R), 15: n=1(S), 16: n=1(R),

17: (S)-DNB-Ala-OMe, 18: (R)-DNB-Ala-OMe

CONCLUSION

π-Donor type chiral mobile phase has been used for HPLC enantiomeric analysis of π-acceptor type analytes. π-π Donor - acceptor interaction, dipole-dipole stacking, hydrogen bonding and Van der Waals interactions seem to contribute for the enantiomeric discrimination.

Chromatographic data can be used for the calculation of stability constants of π-π donor-acceptor complexes.

Donor-acceptor type chiral stationary phases are well known and widely used for analytical and preparative separations of racemates. Donor-acceptor type chiral mobile phase are very promising for the enantiomeric analysis of a wide range of chiral organic compounds.

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