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HPLC enantioseparation of potential β-blockers of the aryloxyaminopropanol type

Study of the mechanism of enantioseparation, part VIII

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This paper presents the results of HPLC enantioseparation of derivatives of aryloxyaminopropanols obtained using six chiral stationary phases [macrocyclic antibiotic (vancomycin, teicoplanin, teicoplanin aglycone, and methylated teicoplanin aglycone) and cyclodextrin (β and γ)] and a mixture of methanol/acetonitrile/acetic acid/triethylamine (45/55/0.3/0.2) as the mobile phase. No significant difference was observed in the separation of the enantiomers on the vancomycin and teicoplanin chiral stationary phases. Comparing the separation of enantiomers on teicoplanin-based columns the retention factors were increased in the order: native teicoplanin < teicoplanin aglycone < methylated teicoplanin aglycone. The highest values of resolution were obtained on the column containing carbohydrate moieties. The presence of saccharide moieties in the chiral stationary phase plays an important role, together with charge interactions and steric interactions, in the separation of enantiomers of derivatives of aryloxyaminopropanol.

1. Introduction

β-Blockers of the aryloxyaminoethanol and aryloxyaminopropanol type are drugs with a single stereogenic center and exhibit a chiral structure. From the viewpoint of β-adrenolytic activity, the (−)-enantiomers in both groups are several times more effective and many β-blockers show different therapeutic indications. The absolute configuration in the sense of the Cahn-Ingold-Prelog system exists in (−)-enantiomers in the arylaminoetanol (*R*) and arylaminopropanol (*S*) groups. Of the β-blockers of the arylaminopropanol type used in therapeutic practice, pure enantiomers are (*S*)-(−)-penbutolol, (*S*)-(−)-timolol and (*S*)-(−)-levobutolol (Cižmáriková et al. 1994a; 1994b; 2002). Several chiral stationary phases have been used for the separation of enantiomers of β-blocking drug substances: chiral phases consisting of immobilized proteins (Mikamba 1998; Haginaka 1999; Henriksson 1999), β-cyclodextrin (Park 2000; Matchnett 1996), Pirkle-type phases (Wang 2000; Mislanova 2000), and cellulose-based phases (Sharma 1995; Facklam 1994).

The purpose of this work was to study the enantioseparation of derivatives of aryloxyaminopropanol on various types of chiral stationary phases (macrocyclic antibiotics vancomycin, teicoplanin, teicoplanin aglycone, permethylated teicoplanin aglycone, cyclodextrins-β and γ).

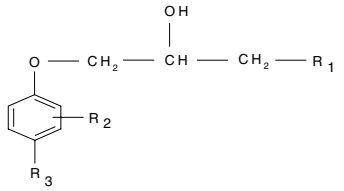
2. Investigations, results and discussion

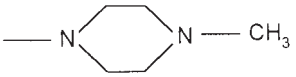
In this study, a number of racemic aromatic potential β-blockers of the aryloxyaminopropanol type (Table 1) were evaluated on macrocyclic antibiotics (vancomycin, teicoplanin, teicoplanin aglycone, and methylated teicoplanin aglycone) and cyclodextrin (β and γ) chiral stationary phases. A common mobile phase consisting of methanol/acetonitrile/acetic acid/triethylamine (45/55/0.3/0.2) was used in all cases. In the mobile phase, the amount of acid is higher relative to the amount of base. Therefore, the ionisation of analytes is assured and ionic interaction of the stationary phase with functional groups (secondary amine group) of aryloxypropanol derivatives is also probable. It is presumed that charge interaction has a positive influence on the separation of enantiomers. Presence of methanol in the mobile phase supports the production of hydrogen bonds, which have an effect on the resolution of enantiomers. The results of the enantioseparations are summarized in Tables 2 and 3. It is evident that there is no significant difference in the separation of the enantiomers on the vancomycin and teicoplanin CSPs with the mobile phase tested. The similar values of retention factors obtained on both chiral CSPs (vancomycin and teicoplanin) indicate similar sorption properties of analytes to the stationary phases. The values of resolution were not significantly higher on the teicoplanin CSP. Comparing the separation on a teicoplanin column containing carbohydrate moieties (Chirobiotic T), a teicoplanin column without carbohydrate moieties (Chirobiotic TAG), and a methylated teicoplanin column without carbohydrate moieties (Chirobiotic TAG-methylated) the retention factors were increased in the order:

$$T < TAG < TAG - \text{methylated}$$

The values of resolution on the three columns tested were in inverse order to the retention factors. It is evident that

Table 1: Structures of racemic β-blockers separated in this study



Compd.	R_1	R_2	R_3	λ_{max}
1	$-NHCH(CH_3)_2$	2-H	4-COC_2H_5	268
2	$-NHCH(CH_3)_2$	2-$COCH_3$	4-CH_2OCH_3	247
3	$-NHCH(CH_3)_2$	2-$COCH_3$	4-$CH_2OC_2H_5$	247
4	$-NHCH(CH_3)_2$	2-$COCH_3$	4-$CH_2OC_3H_7$	247
5	$-NHCH(CH_3)_2$	2-$CH_2O(CH_2)_2OCH_3$	4-$COCH_3$	268
6	$-NHCH(CH_3)_2$	2-$CH_2O(CH_2)_2OCH_3$	4-COC_2H_5	268
7	$-NHCH(CH_3)_2$	2-$CH_2OCH_2CH{=}CH_2$	4-$COCH_3$	268
8	$-NHCH(CH_3)_2$	2-$CH_2OCH_2CH{=}CH_2$	4-COC_2H_5	268
9	$-NHCH(CH_3)_2$	2-CH_2OCH_2(phenyl)	4-$COCH_3$	268
10	$-NHCH(CH_3)_2$	2-CH_2OCH_2(phenyl)	4-COC_2H_5	268
11	$-NHCH_2CH(CH_3)_2$	2-CH_2OCH_3	4-$COCH_3$	268
12	$-NHCH_2CH(CH_3)_2$	2-$CH_2OC_4H_9$	4-$COCH_3$	268
13	$-NHC(CH_3)_3$	2-$COCH_3$	4-CH_2OCH_3	247
14	$-NHC(CH_3)_3$	2-$COCH_3$	4-$CH_2OC_2H_5$	247
15	$-NHC(CH_3)_3$	2-$COCH_3$	4-$CH_2OC_3H_7$	247
16	$-NHC(CH_3)_3$	2-$COCH_3$	4-$CH_2OC_4H_9$	247
17	$-NHC(CH_3)_3$	2-$CH_2O(CH_2)_2OCH_3$	4-$COCH_3$	268
18	$-NHC(CH_3)_3$	2-$CH_2OCH_2CH{=}CH_2$	4-COC_2H_5	268
19	$-NHC(CH_3)_3$	3-$COCH_3$	4-H	247
20		2-$CH_2O(CH_2)_2OCH_3$	4-$COCH_3$	268
21	−NH(cyclohexyl)	2-$CH_2OC_3H_7$	4-COC_2H_5	268

the highest values of resolution were obtained on the column containing carbohydrate moieties. The saccharide parts of the stationary phase influenced the chiral separation of enantiomers:

i) by steric hindrance, where the sugar units are inside the "semirigid basket", which limits the access of other molecules to binding sites, ii) by blocking of possible interaction centers on the aglycone, iii) by offering competing interaction sites, where the three saccharide moieties are chiral and contain hydroxyl-, etheric-, and amide-functional groups (Berthod et al. 2000).

According to the results obtained on the teicoplanin based stationary phase, it might possibly be supposed that a good stationary phase for the separation of enantiomers of derivatives of aryloxyaminopropanols would be a cyclodextrin CSP containing saccharide parts in the molecule. For the enantioseparation of the analytes studied, we tested β- and γ-cyclodextrin CSPs in polar organic mode (mobile phase methanol/acetonitrile/acetic acid/triethylamine 45/55/0.3/0.2; other chromatographic conditions identical to those for the macrocyclic antibiotics CSPs). The assumption was not confirmed and hardly any separation of enantiomers of analytes was obtained on the β- and γ- cyclodextrin CSPs and the retention factors were smaller compared with the teicoplanin CSP. Poor separation of enantiomers ($R_S \sim 0.6$) was obtained using a mobile phase containing less methanol (methanol/acetonitrile/acetic acid/triethylamine 10/90/0.3/0.2), while the values of the retention factors increased and the efficiency of separation decreased (smaller values of the number of theoretical plates). It seems that the compounds studied were enantioresolved by a combination of interactions involving both the aglycone basket and its attached sugar units.

It can be concluded:

i) The type of nitrogen substituent (R_1) (branching, cyclic substituent), which is not very close to the stereogenic center (compounds **2**–**4**, **13**–**16**, **21**) had only a small influence on the values of the resolution. When a nitrogen heterocycle was linked as the R_1 substituent, hardly any separation of enantiomers was obtained (compound **20**).

ii) The more effective separations (the highest value of the resolution factor) were obtained for derivatives possessing smaller substituents or without substitution in the 2-position on the aromatic ring (compounds **1**, **19**). In previous studies the highest resolution was shown for enantiomers without any substitution in the 2-position (Čižmáriková et al. 2003; Hroboňová et al. 2001). It appears that the 2-position substituents crowd the environment near the adjacent stereogenic atom and decrease interaction with the stationary phase. Increase in the polarity of the R_2 substituent had a negative effect on the resolution of enantiomers.

iii) Differences in the R_3 substituent have only a small influence on enantioseparations. On increasing of the number of carbon atoms in the alkoxy chain R_3 (compounds **2**–**4**, **13**–**16**) the retention factor decreased. It may be presumed that the separation of the enantiomers studied involves polar interaction. On the basis of the results obtained it is evident that the presence of saccharide moieties in the CSP plays an important role, together with charge interactions and steric interactions, in the separation of enantiomers of aryloxyaminopropanol derivatives.

The chromatograms of the separation of enantiomers of compound no. 17 on vancomycin, teicoplanin, teicoplatin aglycone, methylated teicoplanin aglycone, and β-cyclodextrin CSPs are shown in the Fig.

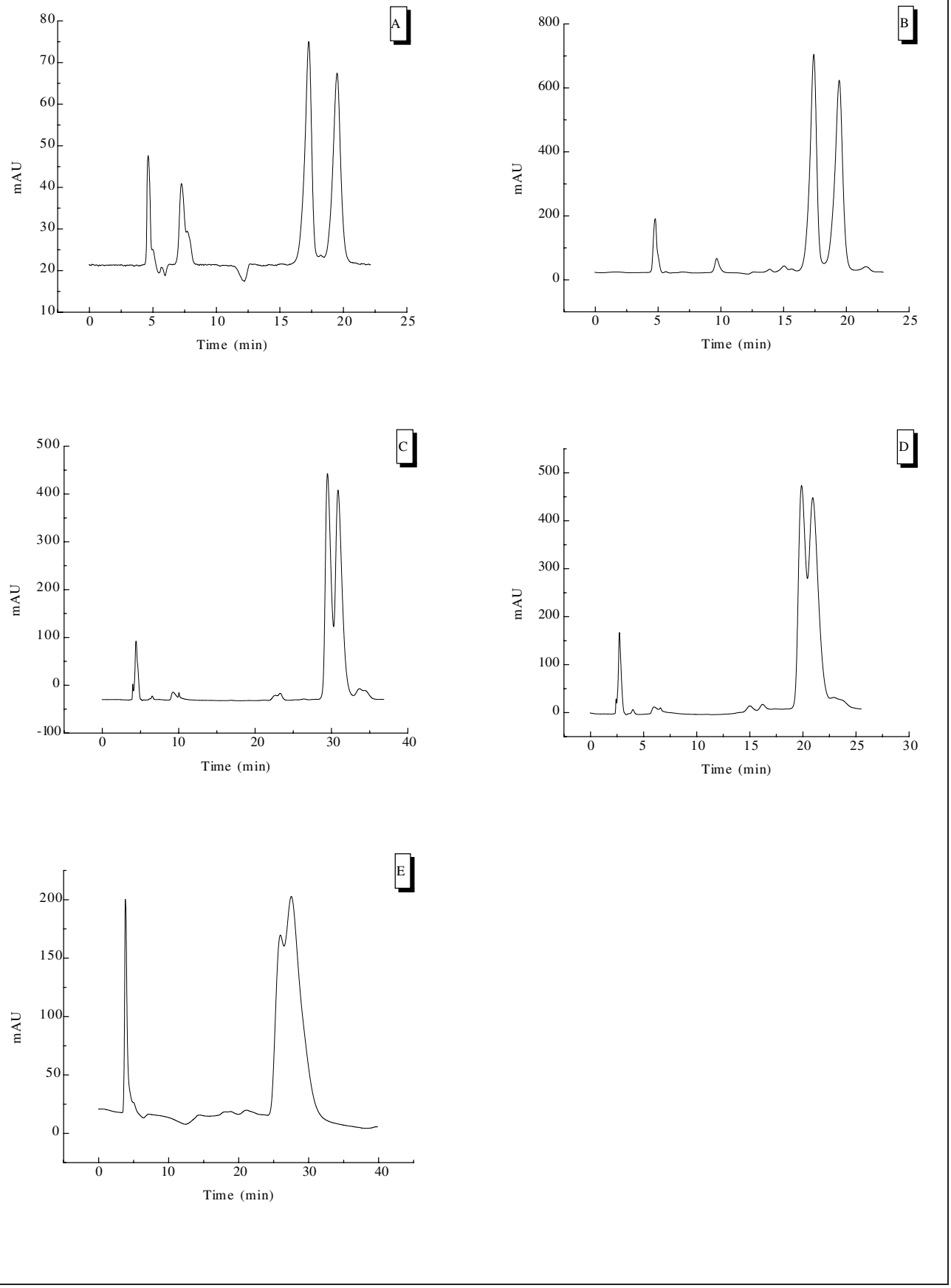


Fig.: Separation of compound **17** in polar organic mode on (A) V, (B) T, (C) TAG, (D) TAG-methylated, and (E) β-CD chiral stationary phases. See Table 1 for phase identification. The chromatographic conditions were identical for all separations: mobile phase, methanol/acetonitrile/acetic acid/ triethylamine 45/55/0.3/0.2 flow rate, 0.7 ml/min column temperature, 25 °C UV detection injection volume 20 µl

Table 2: Retention factors (k_1) and selectivity coefficients (α) of racemic mixtures of compounds studied on macrocyclic antibiotic and cyclodextrin chiral stationary phases in polar organic mode[1]

Compd.	V[2]		T[3]		TAG[4]		TAG-met.[5]		β-CD[6]		γ-CD[7]	
	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α
1	2.57	1.07	3.14	1.13	7.37	1.06	8.40	1.07	–	–	–	–
2	2.37	1.08	3.00	1.07	6.61	1.05	7.81	1.05	1.22	1	0.80	1
3	2.45	1.07	2.98	1.07	6.79	1.07	–	–	–	–	–	–
4	2.54	1.07	2.79	1.16	6.79	1.06	6.91	1.08	0.87	1	1.60	1
5	2.49	1.07	3.04	1.12	7.01	1.05	8.17	1.06	0.89	1	1.40	1
6	2.27	1.07	2.79	1.12	6.48	1.04	7.36	1.04	0.85	1	0.98	1
7	2.14	1.07	2.75	1.11	6.48	1.05	7.31	1.06	1.19	1	0.69	1
8	2.00	1.07	2.56	1.11	6.11	1.04	6.90	1.05	1.15	1	0.57	1
9	2.29	1.06	2.81	1.11	6.96	1.05	7.91	1.06	1.19	1	1.91	1
10	2.17	1.06	2.62	1.10	6.55	1.04	7.39	1.04	1.05	1	1.39	1
11	2.34	1.05	2.70	1.03	6.22	1.03	7.08	1.03	0.87	1	0.70	1
12	1.97	1.04	2.20	1.05	5.16	1.04	5.83	1.03	0.77	1	0.50	1
13	2.14	1.09	2.68	1.12	6.15	1.03	7.16	1.04	1.19	1	0.61	1
14	2.21	1.08	2.67	1.12	6.22	1.06	7.26	1.07	1.09	1	0.62	1
15	1.97	1.06	2.35	1.13	5.39	1.02	6.03	1.03	0.82	1	0.74	1
16	1.88	1.06	2.25	1.13	5.19	1.02	5.66	1.02	0.95	1	0.58	1
17	2.24	1.08	2.70	1.16	6.37	1.05	7.21	1.06	0.80	1	1.03	1
18	1.84	1.08	2.31	1.15	5.61	1.03	6.18	1.04	0.97	1	0.58	1
19	2.23	1.09	2.67	1.18	6.19	1.05	7.19	1.06	1.05	1	0.72	1
20	3.64	1.00	4.11	1.00	7.65	1.00	8.92	1.00	1.39	1	2.30	1
21	2.19	1.07	2.58	1.11	6.61	1.06	7.36	1.06	0.85	1	0.98	1

[1] The mobile phase for all the separations was 45/55/0.3/0.2 (v/v/v/v) methanol/acetonitrile/acetic acid/triethylamine. The flow rate was 0.7 ml/min. The detection wavelength was 247 or 268 nm depending on the absorption maximum of the compound and the operating column temperature was 25 °C.
[2] V = Chirobiotic V (250 × 4.6 mm) column with vancomycin as chiral stationary phase, $t_0 = 4.40$ min
[3] T = Chirobiotic T (250 × 4.6 mm) column with teicoplanin as chiral stationary phase, $t_0 = 4.70$ min
[4] TAG = Chirobiotic TAG (250 × 4.6 mm) column with teicoplanin aglicon as chiral stationary phase, $t_0 = 4.00$ min
[5] TAG-methylated = Chirobiotic TAG-methylated (125 × 4.6 mm) column with methylated teicoplanin aglicon as chiral stationary phase, $t_0 = 2.42$ min
[6] β-CD = LiChrocart ChiraDex (250 × 4 mm I.D. 5 μm) column with β-cyclodextrin as chiral stationary phase, $t_0 = 3.85$ min
[7] γ-CD = LiChrocart ChiraDex Gama (250 × 4 mm I.D. 5 μm) column with γ-cyclodextrin as chiral stationary phase, $t_0 = 3.90$ min

Table 3: Resolution values (R_s) for enantiomeric separation of compounds under study using macrocyclic antibiotics as chiral stationary phases in polar organic mode*

Nr.	R_S			
	V	T	TAG	TAG-met.
1	1.30	1.67	1.00	0.60
2	1.11	0.87	0.75	0.56
3	1.27	0.87	1.13	–
4	1.30	0.89	1.24	1.00
5	1.18	1.55	0.85	0.99
6	1.11	1.49	0.70	0.53
7	1.15	1.28	0.92	0.49
8	1.10	1.31	0.69	0.65
9	1.18	1.38	0.88	0.60
10	1.03	1.31	0.64	0.42
11	0.69	0.41	0.54	0.26
12	0.59	0.52	0.57	0.25
13	1.28	1.52	0.50	0.37
14	1.29	1.27	0.88	0.77
15	1.05	1.47	0.47	0.26
16	1.07	1.46	0.33	0.20
17	1.33	1.78	0.83	0.62
18	1.27	1.70	0.59	0.35
19	1.54	2.14	0.52	0.60
20	0	0	0	0
21	1.12	1.31	0.96	0.65

* The same conditions as in Table 2

3. Experimental

3.1. Materials

The analytes separated in this study (Table 1) were prepared according to the method of Čižmáriková and coworkers (Čižmáriková et al. 1994a; 1994b; 1985; 2003). All HPLC grade solvents (methanol, and acetonitrile) were obtained from Merck (Germany). Triethylamine and acetic acid were obtained from Lachema (Czech Republic).

3.2. Instruments

The macrocyclic chiral stationary phases Chirobiotic T (250 × 4,6 mm I.D. 5 μm) (Astec, USA), Chirobiotic V (250 × 4,6 mm I.D. 5 μm) (Astec, USA), Chirobiotic TAG (250 × 4,6 mm I.D. 5 μm) (Astec, USA), Chirobiotic TAG-methylated (150 × 4,6 mm I.D. 5 μm) (Astec, USA), LiChrocart ChiraDex (250 × 4 mm I.D. 5 μm) (Merck, Germany), and LiChrocart ChiraDex Gama (250 × 4 mm I.D. 5 μm) (Merck, Germany) were used.

Experiments were performed with a Hewlett Packard (series 1100) HPLC system consisting of a quaternary pump equipped with an injection valve (Rheodyne), diode array detector and thermostat. The mobile phase was a mixture of methanol and acetonitrile to which acetic acid and triethylamine were added (methanol/acetonitrile/acetic acid/triethylamine 45/55/0.3/0.2). All the separations were carried out at a flow rate of 0,7 ml/min and the column temperature was 25 °C. The chromatograms were scanned at a the wavelength of 247 or 268 nm depending on the absorption maximum of the compound studied. The injection volume was 20 μl. The analytes were dissolved in methanol (concentration 1 mg/ml), and filtered with a 0,45 μm filter when necessary. The retention time of the solvent peak (methanol) was used for determination of the dead time for all types of column.

3.3. Chromatographic characteristics

The selectivity coefficient was expressed as

$$\alpha = k_2/k_1$$

where k_2, k_1 are the retention factors for the first and second eluting enantiomer. The retention factors k_1 and k_2 were calculated as follows:

$$k_1 = (t_1 - t_0)/t_0 \text{ and } k_2 = (t_2 - t_0)/t_0$$

where t_0, t_1, and t_2 are the dead elution time and the elution times of enantiomers 1 and 2, respectively.

The resolution (R_s) of the first and second eluting enantiomers was calculated by the ratio of the difference between the elution times t_1 and t_2 to the arithmetic mean of the two peak widths w_1 and w_2.

$$R_s = 2(t_2 - t_1)/(w_1 + w_2)$$

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