Department of Analytical Chemistry[1], Faculty of Chemical Technology, Slovak Technical University, Department of Pharmaceutical Chemistry[2], Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic

Separation of enantiomers of some 1,4-piperazine derivatives of aryloxyaminopropanols on a vancomycin chiral stationary phase

J. Lehotay[1], K. Hroboňová[1], J. Čižmárik[2] and H. Čelková[2]

The behaviour of a vancomycin chiral stationary phase (Chirobiotic V) towards changes in organic and ionic modifiers in the mobile phase was investigated in order to deduce suitable conditions for the liquid chromatographic enantioseparation of a series of 1,4-piperazine derivatives of aryloxyaminopropanols. Acetonitrile and methanol as non-ionic modifiers were tested in the mobile phase while different aliphatic carboxylic acids (formic acid, propionic acid, hexanoic acid, oxalic acid, succinic acid) and bases (triethylamine, trimethylamine, ammonia) were used as ionic modifiers. The influence of the nature and concentration of these modifiers on retention, selectivity and resolution was investigated.

1. Introduction

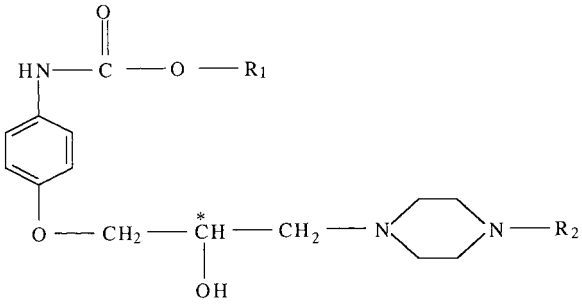
In many publications it has been demonstraed that vancomycin is able to stereoselectively bind enantiomers of widely different character [1–4].

It has been used to treat several staphylococcal infections, particularly when bacterial resistance to other antibiotics has been developed [4]. Vancomycin is a naturally occurring chiral compound. The enantioselectivity of this macrocyclic antibiotic can be attributed to the diversity of its structure. It contains moieties such as sites for $\pi - \pi$ interactions, chiral hydrogen bonding, and peptide and carbohydrate binding. Vancomycin also contains numerous stereogenic centres and various functional groups which are necessary to achieve chiral recognition (e.g., hydrogen bonding groups, hydrophobic pockets, aromatic groups, amide linkage, etc.). An interesting property of vancomycin is that it can be used in both reversed phase and normal phase modes.

β-Adrenoreceptor blocking agents are well known to be strongly affected by chirality. The chiral separation of β-blocking drugs can be performed by means of different chiral stationary phases, with immobilised proteins [5], with CSPs based on the use of cyclodextrins [6, 7] or cellulose derivatives, especially cellulose tris(3,5-dimethylphenylcarbamate) [8, 9]. In preliminary papers the derivatives of aryloxyaminopropanols were studied with respect to their lipophilicity, steric influence and β-blocking activity [10–12].

This paper describes the use of a vancomycin chiral stationary phase for the enantioseparation of 1,4-piperazine derivatives of aryloxyaminopropanols (Table 1). The influence of different parameters on retention and enantioselectivity has been investigated. The effect of the concentration of methanol and acetonitrile and the addition of some ionic modifiers have been studied in order to deduce suitable conditions for the resolution of the racemic derivatives of aryloxyaminopropanols examined.

Table 1: Structures of the tested 1,4-piperazine derivatives of aryloxyaminopropanols



Analyte Nr.	R_1	R_2
1	$-C_2H_5$	H_3C-O
2	$-C_3H_7$	
3	$-C_4H_9$	
4	$-C_2H_5$	
5	$-C_3H_7$	$O-CH_3$
6	$-C_2H_5$	
7	$-C_3H_7$	F
8	$-C_4H_9$	
9	$-C_3$	
10	$-C_2H_5$	
11	$-C_3H_7$	

2. Investigations, results and discussion

2.1. Influence of organic modifiers

Optimisation of reversed phase separations was done by controlling the amount of organic modifiers added. Selectivity was affected by both the type of organic modifier (methanol and acetonitrile). Fig. 1 shows the effect of methanol concentration on the retention of analyte 5 (Table 1). As excepted, the retention is the greatest when eluting with 90% methanol. At a methanol concentration be-

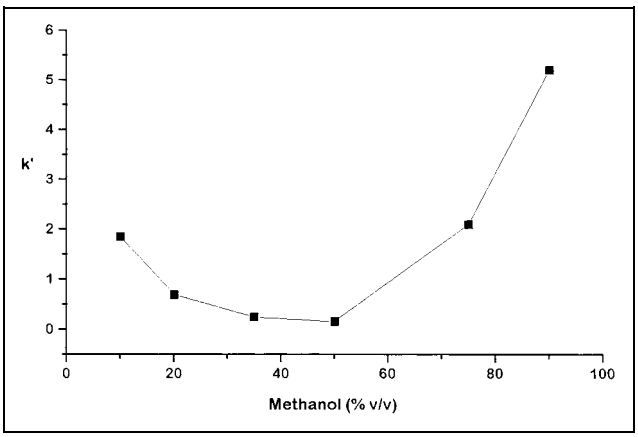


Fig. 1: Reversed-phase retention of the analyte 5 as a function of mobile phase composition. The column was a 25 cm × 0.44 cm (I.D.) vancomycin CSP. The mobile phase consisted of methanol-water. The flow rate was 1.0 ml/min at ambient temperature (22 °C). DAD detection (abs. max. at 240 nm).

tween 90 and 50% (v/v) the retention decreases. Decreasing the concentration of methanol modifier from 50 to 10% (v/v) caused to increase the retention. A major difference in the retention behaviour of the analyte in the presence of acetonitrile was found at high modifier concentrations (>50%). The decrease in retention at high acetonitrile concentrations is thought to be due mainly to suppress hydrogen bonding interactions between the analyte and the chiral stationary phase.

In the reversed-phase mode (the mobile phase is polar and the stationary is less polar) it can be presumed that an increase in lipophilicity (number of carbon atom in alkoxy chain R_1) of the molecule increases the values of retention factors. The measurement indicated (Table 2) the opposite effect of the lipophilicity of the molecules on retention factors (the R_2 substituent is constant). That means, the retention of 1,4-piperazine derivatives of aryloxyaminopropanols (according to R_1 substituent) is not established on the principle of a reversed-phase system. The derivatives containing in the substituent R_2 a fluorine atom are the most polar and their retention is the highest. These observations indicated that the formation of $\pi - \pi$ complexes between the analyte and stationary phase is supported and so the retention factors will be the smallest (Table 2). According to R_2 the retention is given by the reversed-phase mode. These two retention mechanisms on the base of dominant interactions influence the enantioseparation of 1,4-piperazine derivatives of aryloxyaminopropanols on the vancomycin chiral stationary phase.

2.2. Influence of the ionic organic modifiers

The influence of the nature and concentration of ionic modifiers such as aliphatic carboxylic acids and bases was investigated using 1,4-piperazine derivatives of aryloxyaminopropanols. These experiments were performed with methanol as the mobile phase, to which ionic modifiers were added. Acids and bases pH range of mobile phase means that vancomycin has a negative (COO^-) or positive (NH_4^+) charge and it can produce cationic and anionic interactions. The stability of the vancomycin/analyte complexes are dependent on the charge of the analyte. In general, analytes interact more favourably at a pH where they are not ionised.

On addition of aliphatic carboxylic acids (0.01 M, 0.1 M, 1 M), an increase in the concentration of these acids was found to cause a decrease in the values of R_s (Fig. 2). In an acid pH range the studied analytes can exist in nonprotonized form ($pKa_1 = 3.3 - 3.7$) and ion interactions of

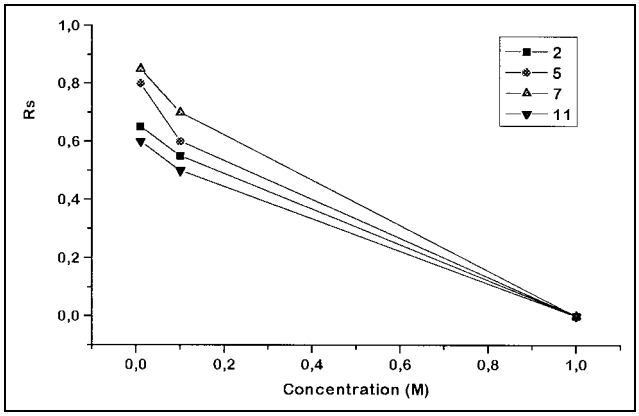


Fig. 2: Influence of the concentration of hexanoic acid on resolution of enantiomers of 1,4-piperazine derivatives of aryloxyaminopropanols (analytes 2, 5, 7, 11). Chromatographic conditions: methanol containing hexanoic acid. All other parameters are as indicated in Fig. 1.

Table 2: Influence pf base nature and concentration on retention, enantioselectivity and resolution of 1,4-piperazine derivatives of aryloxyaminopropanols

Analyte Nr.	Triethylamine			Trimethylamine			Ammonia		
	k′	α	R_s	k′	α	R_s	k′	α	R_s
Concentration: 0.01 M									
1	6.37	1.07	0.61	2.99	1.05	0.56	2.11	1.66	0.51
2	5.89	1.07	0.79	2.93	1.05	0.66	1.85	1.06	0.59
3	5.64	1.07	0.81	2.85	1.08	0.64	1.66	1.05	0.38
4	5.91	1.08	0.93	2.22	1.08	0.61	1.60	1.06	0.31
5	4.98	1.07	0.95	2.00	1.10	0.55	1.51	1.07	0.35
6	4.62	1.14	1.19	1.51	1.10	0.64	1.23	1.07	0.40
7	4.03	1.09	1.30	1.25	1.11	0.57	1.17	1.10	0.34
8	2.84	1.09	0.92	1.20	1.08	0.53	1.15	1.04	0.30
9	4.74	1.06	0.75	2.05	1.05	0.42	1.65	1.03	0.28
10	3.98	1.07	0.80	1.83	1.07	0.45	1.45	1.07	0.36
11	3.77	1.07	0.78	1.62	1.08	0.34	1.36	1.00	0.22
Concentration: 0.01 M									
1	6.32	1.08	0.76	2.75	1.11	0.61	2.45	1.04	0.41
2	5.26	1.08	0.81	2.71	1.11	0.65	2.36	1.05	0.55
3	4.92	1.08	0.82	2.66	1.09	0.53	2.22	1.04	0.53
4	3.95	1.08	0.76	2.23	1.02	0.57	1.75	1.04	0.44
5	3.80	1.09	0.73	1.81	1.12	0.50	1.51	1.06	0.44
6	2.62	1.12	0.85	1.50	1.07	0.62	1.15	1.09	0.56
7	2.50	1.08	0.80	1.25	1.08	0.53	1.11	1.15	0.45
8	1.95	1.15	0.82	1.17	1.05	0.46	0.97	1.03	0.34
9	3.42	1.09	0.59	1.86	1.07	0.40	1.62	1.02	0.30
10	3.02	1.07	0.61	1.68	1.04	0.45	1.40	1.07	0.35
11	2.79	1.07	0.60	1.47	1.04	0.40	1.33	1.05	0.34

k′ = capacity ratio of the first eluting enantiomer; α = enantioselectivity; R_s = resolution
Chromatographic conditions: methanol containing bases. All other parameters are as indicated in Fig. 1.

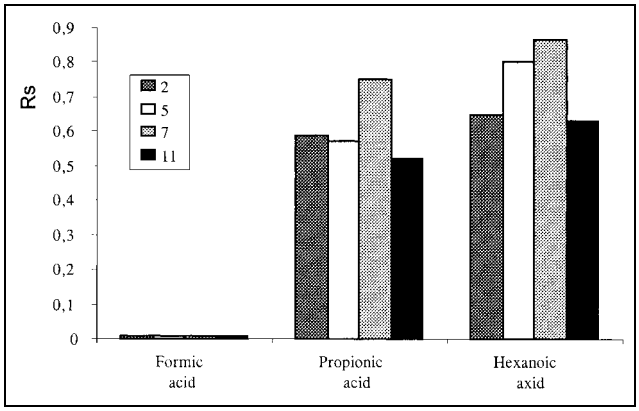


Fig. 3: Influence of the nature of aliphatic carboxylic acids on resolution of some 1,4-piperazine derivatives of aryloxyaminopropanols (analytes 2, 5, 7, 11). Chromatographic conditions: methanol containing an aliphatic carboxylic acid (10 mM). All other parameters are as indicated in Fig. 1.

stationary phases wih functional groups of analytes are suppressed. No chiral separations ($R_s = 0$) were achieved for the tested analytes by the addition of acids in a concentration of 1 M. The influence of the nature of the three carboxylic acids on the chromatographic resolution for 1,4-piperazine derivatives of aryloxyaminopropanols is shown in Fig. 3, with all of the carboxylic acids being added at the same concentration (10 mM). When formic acid was added to the mobile phase, no chiral separations were obtained. For the study compounds, enantioselectivity have a tendency to increase as the length of the aliphatic chain increases. The dicarboxylic acids (oxalic acid, succinic acid (10 mM)) had no significant effect on enantioseparation of the studied analytes. These observations indicate the influence of two factors on enantioseparation:

- strength of acid (pK_a): stronger acids produce stronger ion pair (atom of nitrogen/ion of acid) and the interaction with carboxylic or phenolic group is stronger,
- length of the aliphatic chain of carboxylic acid: steric interactions.

For the study of the effects of nature and concentration of bases on enantioseparations, triethylamine, trimethylamine and ammonia (0.01 M, 0.1 M, 1 M) were used as ionic modifiers. In a basic pH range the studied analytes can exist in protonized form and ion interactions of stationary phases with functional groups of analytes are favoured. Better separation of aryloxyaminopropanol derivatives (higher values of R_s) were obtained than with acid modifiers. The influence of concentration of basic modifiers is demonstrated in Table 2. The addition of bases in a concentration of 1 M did not lead to the enantioseparation of aryloxyaminopropanol derivatives, while with the mobile phase modified by 0.1 M or 0.01 M bases, a resolution of analyte enantiomers was obtained. For triethylamine, the smallest concentration (0.01 M) seems to be the most favourable for the enantioseparation. The different interaction of two enantiomers (R and S) with the stationary phase leading to chiral discrimination can be expressed as the difference in the free energy $-\Delta_{RS}\Delta G^{\circ}$ calculated from the separation factor α. The results given in Table 3 show the very small energy difference that is needed for a chromatographic resolution of enantiomers of aryloxyaminopropanol derivatives.

Table 3: Free energy differences necessary for enantioseparation of derivatives of aryloxyaminopropanols

Analyte Nr.	$-\Delta_{RS}\Delta G^{\circ}$ (J/mol)
1	175
2	177
3	160
4	182
5	178
6	232
7	210
8	207
9	151
10	167
11	170

Chromatographic conditions: methanol containing 10 mM triethylamine (99.8/0.2 v/v). All other parameters are as indicated in Fig. 1.

3. Experimental

3.1. Chemicals and reagents

Methanol and acetonitrile of HPLC grade were obtained from Merck. All other chemicals were of analytical reagent grade. The 1,4-piperazine derivatives of aryloxyaminopropanols were synthesised by standard procedures [13]. Structures of the compounds are shown in Table 1.

3.2. Apparatus

The chromatographic system consisted of a Waters model 501 pump, injection valve Valco with a 10 µl injection loop and a Waters 990 photodiode array detector. The experimental data were collected and analysed on a NEC PowerMate 2 data system. Chirobiotic V column (250 × 4.5 mm I.D. 5 µm) were obtained from Astec (USA).

3.3. Chromatographic conditions

The experiments were carried out at 22 °C. A flow rate of 1 ml/min was used. The chromatograms were scanned at 240 nm. The sample concentrations were 0.1 mg/ml. The void time was determined by injection of distilled water.

3.4. Preparation of mobile phases

Mobile phases containing organic modifiers (methanol, acetonitrile) and solutions of carboxylic acids (formic acid, propionic acid, hexanoic acid, oxalic acid, succinic acid) or bases (triethylamine, trimethylamine, ammonia) in water (concentration 1 M, 0.1 M, 0.01 M).

Acknowledgement: We thank to Prof. D. W. Armstrong for a vancomycin chiral column.

References

1 Armstrong, D. W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, Ch.; Chen, J. R.: Anal. Chem. **66**, 1473 (1994)
2 Armstrong, D. W.; Hilton, M.; Coffin, L.: LC · GC **9**, 646 (1992)
3 Chen, S.; Liu, Y.; Armstrong, D. W.; Victory, P.; Martnez-Teipel, B.: J. Liq. Chrom. **18**, 1495 (1995)
4 Armstrong, D. W.; Rundlett, K. L.; Chen, J. R.: Chirality **6**, 496 (1994)
5 Hermansson, J.; Grahn, A.: J. Chromatogr. A **694**, 57 (1995)
6 Chang, S. C.; Reid III, G. L.; Chen, S.; Chang, C. D.; Armstrong, D. W.: Trends Anal. Chem. **12** (4), 144 (1993)
7 Armstrong, D. W.; Ward, T. J.; Armstrong, R. D.; Beesley, T. E.: Science **232**, 1132 (1986)
8 Ekelund, J.; Arkens, A.; Bronnon-Hansen, K.; Fich, K.; Olsen, L.; Vibholm Petersen, P.: J. Chromatogr. A **708**, 253 (1995)
9 Falcklam, C.; Modler, A.: J. Chromatogr. A **664**, 203 (1994)
10 Smith, L. M.: J. Med. Chem. **20**, 705 (1997)
11 Seki, T.; Takezaki, T.; Ohuchi, R.; Ohuyabu, H.; Tanimoto, Y.; Yamaguchi, T.: Chem. Pharm. Bull. **43**, 247 (1995)
12 Čelková, H.; Čižmárik, J.; Mlynárová, R.; Hroboňová, K.; Lehotay, J.: Acta Poloniae – Drug Research **55**, 449 (1998)
13 Hlaváčová, H.; Csöllei, J.; Seginko, J.: Česk. a Slov. Farm. **44**, 261 (1995)

Received November 25, 1998
Accepted March 23, 1999

Assoc. Prof. Ing. J. Lehotay, DrSc.
Department of Analytical Chemistry
Slovak Technical University
Radlinskeho 6
81237 Bratislava
Slovakia