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HPLC and TLC enantioseparation of the nitro-positioned aryloxysubstituted aminopropanols

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An enantioseparation study of nitro-substituted aryloxyaminopropanols was performed using HPLC on a teicoplanin chiral stationary phase and TLC impregnated with L-aspartic and L-tartaric acid as chiral selectors. The type of substituent on the nitrogen in the hydrophilic part of molecule is essential for excellent separation by HPLC. L-aspartic acid seems to be a suitable chiral selector for enantioseparation by TLC.

Aryloxyaminopropanols are an important group of drugs with a single stereogenic center which exhibit chiral properties. Their (*R*) and (*S*)-enantiomers differ in the pharmacological, pharmacodynamic and toxicological activities. For example from the viewpoint of β-adrenolytic activity, the (*S*)-(-)-enantiomer is several times more effective and many of the enantiomers of the β-blockers show different therapeutic indications (Mehvar and Brocks 2004).

Several current analytical methods have been used for the separation of enantiomers of the aryloxyaminopropanol derivatives. The most widely used technique for the separation and quantification of enantiomers seems to be HPLC (Ward and Farris 2001; Hroboňová et al. 2004; Ilisz et al. 2009) and TLC (Bhushan and Arora 2003; Bhushan and Tawar 2008)

The aim of this paper was the enantioseparation of the prepared aryloxyaminopropanols with an *ortho*, *meta* and *para*-nitro positioned group on the aromatic ring and with an isopropylamino and piperidino group on the basic part of molecule.

The HPLC method used in this study employed a teicoplanin chiral stationary phase and a mixture of methanol/acetonitrile/acetic acid/triethylamine 45/55/0.3/0.2 v/v/v/v as the mobile phase. The results of the enantioseparation are summarised in the Table. There is no significant difference in the separation of the enantiomers on the positioned nitro isomers with isopropyl group on basic nitrogen. The values of the resolution (R_S) for the derivatives studied were in the range of 2.54–3.23. Very poor separation was obtained (R_S = 0.60, Table) if the nitrogen is part of the heterocyclic ring (piperidine). This means that the heterocyclic ring sterically hinders enantioselective interaction with the chiral selector as it was described by Hroboňová et al. (2001).

Separation of enantiomers of three prepared racemic nitrophenoxyisopropylaminopropanols has been achieved using normal-phase TLC on silica gel plates impregnated with L-aspartic and L-tartaric acid as chiral selectors. Different combinations of acetonitrile-methanol-water as mobile phase were found to be successful in resolving of the enantiomers. Spots were detected using iodine vapour and results are summarised in the Table.

Comparing both acids as chiral selectors, better enantioseparation was obtained with L-aspartic acid at a temperature of 15 °C (Table). Using L-tartaric acid at 20 °C resulted in tailing of the resolved spots of enantiomers of the nitrophenoxyisopropylaminopropanols.

Experimental

1. Material

Compounds for this study (Table) were prepared according to the method of Čižmáriková et al. (1985, 2003). All HPLC grade solvents (methanol, acetonitrile) were obtained from Merck (Germany). Triethylamine and acetic acid for analysis were obtained from Lachema (Czech Republic). Ethanol was from Centralchem (Slovakia). L-Aspartic and L-tartaric acid for analysis were obtained from Lachema (Czech Republic).

2. Instruments

The macrocyclic chiral stationary phase was Chirobiotic T (250 × 4.6 mm I.D., 5 μm) (Astec, USA). Experiments were performed with Hewlett Packard (series 1100) HPLC system consisting of a quarternary 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 v/v/v/v). All the separations were carried out at a flow rate 0.7 ml/min and the column temperature was 25 °C. The chromatograms were scanned at the wavelength of 247 or 268 nm depending on the absorption maximum of the studied compound. The injection volume was 20 μl. The analytes were dissolved in methanol (concentration 1 mg/ml), the solvent peak (methanol) was used for determination of the dead time. Fig.

Impregnated thin-layer plates were prepared by spreading of silicagel G in distilled water (60 ml containing 0.3 g of L-aspartic or L-tartaric acid). In each case, a few drops of ammonia solution were added to maintain the pH above the isoelectric points of the amino acids. The plates were dried overnight at 60 °C. The solution of racemic mixtures of nitrophenoxyisopropylamino-

Table: Retention factor of first eluted enantiomer (k_1) and selectivity coefficient (α) of racemic mixtures of compounds studied by HPLC and retention factors (R_f) of compounds studied by TLC

Compound	Substituent	R_{f1}	R_{f2}	k_1	α	R_s
(±) 2-NO_2	isopropylamino	0.08	0.25	4.74	1.13	2.56
(±) 3-NO_2	isopropylamino	0.07	0.20	10.18	1.16	2.54
(±) 4-NO_2	isopropylamino	0.08	0.23	4.76	1.34	3.23
(±) 4-NO_2	piperidino	–	–	3.31	1.05	0.60
Propranolol	–	0.08	0.25	2.48	1.15	1.20

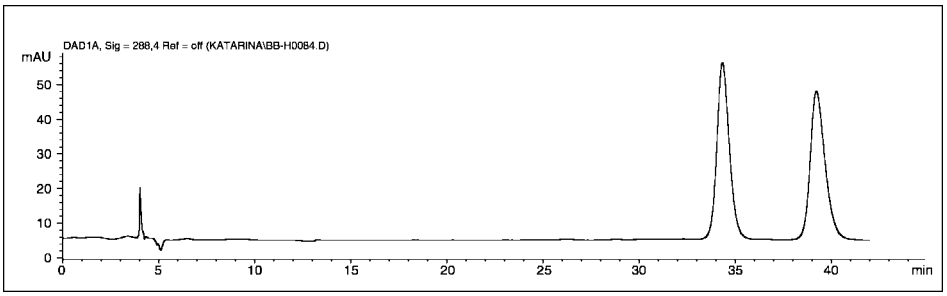

Fig.: HPLC Chromatogram of enantioseparation of compound 1-(isopropylamino)-3-(3-nitrophenoxy)propan-2-ol

propanols were prepared in 70 % ethanol in the same concentration and were applied to the plates at 10 μl level. The spot of each racemic compound were applied side by side on the same plate. Chromatograms were developed at 15 or 20 ± 2 °C in mobile phase acetonitrile/methanol/water (15:3:1 v/v/v). The developed plates were dried at 60 °C for 15 min and spot were located in an iodine chamber.

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