CHIRAL RESOLUTION OF NOMIFENSINE ENANTIOMERS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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The impact of drug chirality on pharmacokinetic behaviour and the therapeutic implications have received little attention, largely because routine chiral analysis of drugs and metabolites has been impracticable. The recent advent of new chiral stationary phases (CSPs) for high-pressure liquid chromatography (HPLC) permitting the facile resolution of chiral drugs and metabolites, has renewed interest in methods for chiral quality control and for stereoselective metabolism. In the present report, the factors affecting chiral resolution on a new α_1-acid glycoprotein (α_1-AGP) column 100 x 4 mm; 'Enantiopac',LKB-Produkter, Sweden)have been assessed, with reference to nomifensine as a model system.

A modular liquid chromatograph was used (215 nm, 0.04 AUFS). Separation depends on: concentration of organic modifier; buffer pH; ionic strength of electrolyte. Flow rate and temperature exert minor effects. Initial experiments confirmed propan-2-ol as a satisfactory organic modifier (Hermansson, 1984), NaCl as a suitable electrolyte and that no useful separation was observed below pH 6.4 (using NaH_2PO_4 as buffer). The maximum recommended pH for the column is 7.4. Enantiomer separation was optimised by sequential adjustment of each of the three parameters. This gave 5-8 values for each parameter over the range in the Table, the other two parameters being constant at a central value (cf Table). Flow rate (0.3 ml/min) and temperature (23°C) were constant throughout. Chiral separation was assessed in terms of: mean theoretical plates/metre (N); resolution (R_s); selectivity factor (α); and retention time (t_R, min) for the retarded enantiomer. The enantiomers were characterised by circular dichroism. Peak A: ΔOD_{240} = positive, Peak B: ΔOD_{240} = negative. The CD spectra for the two peaks showed a baseline intersection, confirming that they were enantiomorpic entities.

Table: Performance of Parameters for Chiral Resolution of Nomifensine

	propan-2-ol (%)			pH			NaCl (M)		
	1.0	3.5	7.5	6.4	7.0	7.3	0.00	0.10	0.20
N	4,670	8,380	5,290	8,660	8,380	8,960	7,760	8,380	6,970
R_s	2.43	1.61	0.72	0.89	1.61	1.94	1.45	1.61	1.02
α	1.63	1.40	1.18	1.26	1.40	1.50	1.35	1.40	1.25
t_R	43	33	18	20	33	39	40	33	29

The data generated yielded an optimum eluent composition of: propan-2-ol-NaH_2PO_4 (3.5:96.5, v/v) at pH 7.0 and containing 0.1 M NaCl. For replicate 20-µl injections of 1 µg nomifensine, RSD in t_R for the first and second peaks was 1.4% and 1.6% (n=7), respectively. In the range 0.02-2.00 µg, the peak height (y) of each enantiomer regressed linearly with concentration (x): (peak 1) y = 1.28 x + 4.1 (r = 0.9982; n = 6); (peak 2) y = 0.87 x +3.7 (r = 0.9926; n = 6). Resolution was not observed in absence of organic modifier; it was highest at low concentrations, but this corresponded to excessive t_R. R_s and t_R increased with pH, being optimum at pH 7.0 for t_R = 33 min. 0.1 M NaCl gave good resolution for a reasonable t_R. Optimisation can be more effectively carried out using the modified simplex algorithm of Berridge (1982). The high sensitivity of α_1-AGP to these parameters requires careful equilibration of the eluent for chiral quality control. These observations can be interpreted to suggest that the chiral selectivity of α_1-AGP may be attributable to the spatial configuration of the protein residues themselves.

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