Enantioselective Reversed-Phase Chromatography with Protein-based Columns

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Excellent reversed-phase separations using a protein-based column, CHIRALPAKAGP, with volatile LC-MS compatible buffer systems have been obtained for a range of basic solutes.

Introduction

Reversed-phase chromatography, being utilized successfully in achiral separations, can also be applied to the separation and analysis of chiral compounds. One important area of such applications is analyses of drugs and their metabolites in physiological fluids and samples of biological origin by the combination of reversed-phase chromatography and LC-MS. This note describes use of a CHIRALPAK AGP column in conjunction with a volatile mobile phase buffer appropriate for analysis and detection by LC-MS.

Experimental

Agilent 1100 and 1200 HPLC systems were used for the study. Columns (150 x 4mm) were packed with CHIRALPAK AGP. Volatile mobile phases were based on 10mM ammonium acetate buffered to a suitable pH with acetic acid and with added 2-propanol as modifier.

Results

An important goal of the work was to simplify the development of separation methods, mainly focusing on selection of mobile phase combinations appropriate for MS detection. Mobile phases based upon ammonium acetate were found to give excellent selectivity for a wide range of solutes. Table 1 shows the retention and selectivity for several basic compounds, mainly beta-blockers and related molecules, using a CHIRALPAK AGP column with 1% 2-propanol in 10mM ammonium acetate at pH 5. Retention and selectivity may be adjusted by changes in pH and the concentration and nature of the modifier.

Table 1. Retention, Selectivity and Resolution

Compound	k_1	alpha	R_s
Alprenolol.HCl	2.60	1.53	3.63
Chlophedianol.HCl	2.40	2.26	6.30
Clenbuterol.HCl	0.89	1.78	3.88
Diperodon.HCl	5.82	1.48	3.31
Hydroxyzine.2HCl	2.73	1.20	1.28
Oxprenolol.HCl	3.87	1.26	1.98
Pheniramine.Maleate	0.32	1.73	2.07
Promethazine.HCl	6.46	1.25	2.05
Propranolol.HCl	6.62	1.28	2.27

Figure 1 shows a typical separation of a mixture of four racemic compounds under these conditions.

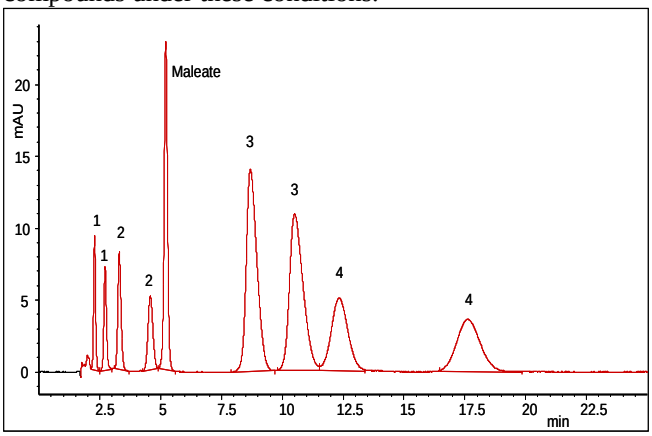

Figure 1. Separation of the enantiomers of a mixture of (1) pheniramine, (2) clenbuterol, (3) oxprenolol and (4) diperodon. *CHIRALPAK AGP (150 x 4 mm), 1% 2-propanol in 10mM ammonium acetate, pH 5. Flow rate 0.8 ml/min, 25°C.*

The chromatogram shows the high performance and the good peak symmetry characteristic of the CHIRALPAK AGP column. Further increase in performance (albeit at the expense of separation time) may be achieved by reduction in flow rate. Reduction of the flow rate by a factor of 2 increased the resolution of the four peak pairs by an average of 34%. Table 2 shows the efficiency (for peak 1) and resolution for the compounds in Figure 1 at 0.8 and at 0.4 ml/min.

Table 2. Effect of Flow Rate on Efficiency and Resolution

	0.8 ml/min		0.4 ml/min	
Compound	N_1	R_s	N_1	R_s
Pheniramine. Maleate	3770	2.59	5663	3.51
Clenbuterol.HCl	3230	4.23	5296	5.50
Oxprenolol.HCl	1894	1.78	2861	2.46
Diperidon.HCl	1554	3.48	2739	4.66

Conclusions

Protein-based chiral HPLC columns may be used in a wide range of reversed-phase applications. The columns in conjunction with ammonium acetate-based mobile phases have been shown to give excellent selectivity together with LC-MS compatibility. Many existing applications using other buffers can be easily modified to use ammonium acetate, thus simplifying development of analytical protocols for the protein-based chiral phases.

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