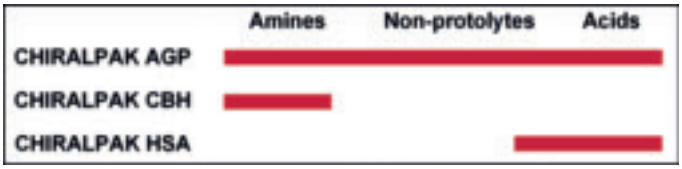
CHIRALPAK® Protein Phase Columns

- Protein-bonded silica
- Reversed-phase applications
- Unique separation characteristics
- Hundreds of applications available

Protein stationary phases were originally developed and manufactured by ChromTech Ltd, but are now manufactured by Chiral Technologies Europe. The range consists of three protein-based columns – CHIRALPAK AGP, CHIRALPAK CBH and CHIRALPAK HSA (previously called CHIRAL-AGP, CHIRAL-CBH and CHIRAL-HSA), where the protein is immobilized on 5µm porous spherical silica particles.

Phase	Chiral Selector	Particle Size (µm)	Applications
CHIRALPAK AGP	α_1-acid glycoprotein	5	Most compound types – amines, acids, alcohols, amides, esters, sulphoxides
CHIRALPAK CBH	Cellobiohydrolase	5	Nitrogen-containing compounds also containing alcohol, phenol, carbonyl, amide, ether or ester group(s)
CHIRALPAK HSA	Human serum albumin	5	Weak and strong acids, zwitterionic and non-protolytic compounds

CHIRALPAK AGP has the broadest applicability of the three chiral phases, separating a wide range of compound types and is often the column of choice for method development. CHIRALPAK CBH has a narrower applicability, preferentially separating compounds containing one or more nitrogen atoms together with one or more hydrogen accepting or donating groups, and is particularly suited for the analysis of very hydrophilic amines. CHIRALPAK HSA is also more suitable for specific applications, particularly very hydrophilic acids.



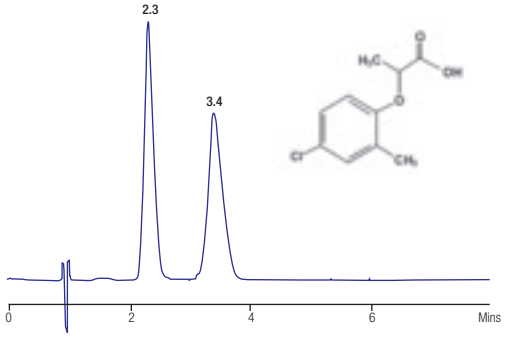
These columns function in the reversed-phase mode, using buffers with low organic content and at moderate pH.

Method Development

Analytes are retained on these protein-bonded phases by a combination of ionic binding (charged solutes), hydrophobic interaction and hydrogen bonding. Consequently, separations are affected by pH and the nature and concentration of aqueous buffer and organic modifier.

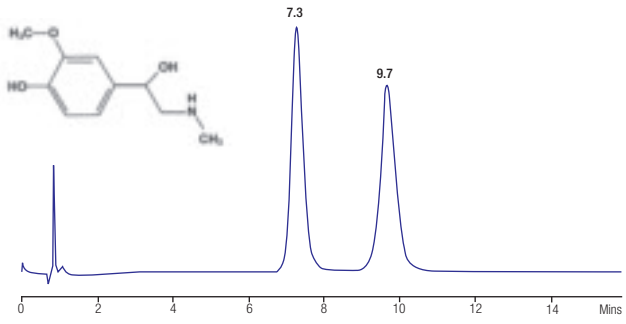
Parameter	Typical Conditions	Effect of Varying Parameter
pH	4 - 7	Variable
Buffer	Phosphate or acetate (0.01 – 0.1M)	Increase in buffer concentration can increase retention and enantioselectivity
Organic Solvent	Propan-2-ol, acetonitrile, methanol (0 – 15%)	Selection of solvent strongly affects enantioselectivity. Higher organic solvent ratios reduce retention time for each phase. For both CHIRALPAK AGP and HSA columns, enantioselectivity is simultaneously reduced, whilst for CHIRALPAK CBH columns it is often increased.

Figures 17 to 19 show typical applications on CHIRALPAK protein phase chiral columns.



Column: CHIRALPAK AGP (100 x 4.0mm)
Eluent: 5% 2-Propanol in 10mM ammonium acetate pH 5.8
Flow rate: 0.9ml/min

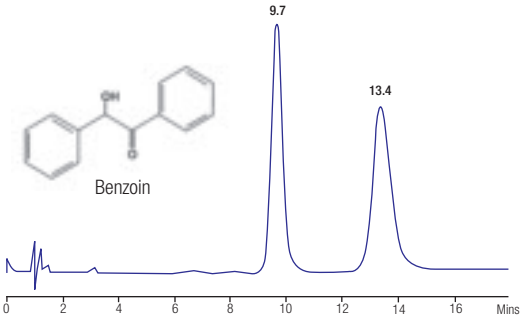
Figure 17. Mecoprop



Column: CHIRALPAK CBH (100 x 4.0mm)
Eluent: 5% 2-Propanol in 10mM Na phosphate, pH 7 + 50µM Na_2 EDTA
Flow rate: 0.9ml/min

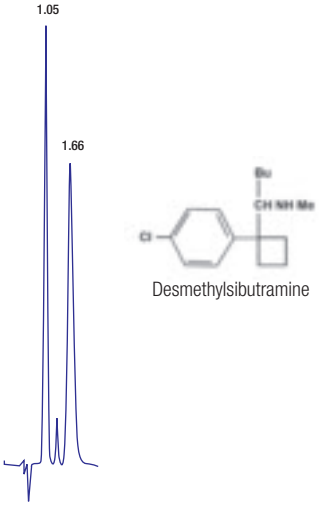
Figure 18. Metanephrine

CHIRALPAK Protein Phase Columns (continued)



Column: CHIRALPAK HSA (100 x 4.0mm)
Eluent: 2% CH_3CN in 100mM Na phosphate pH 7.0
Flow rate: 0.9ml/min

Figure 19. Benzoin



Column: CHIRALPAK AGP (50 x 4.0mm)
Eluent: 5% CH_3CN in 10mM ammonium acetate buffer, pH 4.1
Flow rate: 0.9ml/min

Figure 20. Fast chiral separation suitable for MS detection

Columns for LC-MS

Shorter CHIRALPAK protein phase columns are available for rapid analysis and LC-MS applications. In order to convert from a UV to LC-MS method, in addition to decreasing column dimensions, phosphate buffers are replaced with ammonium acetate and the concentration of buffer and organic modifier reduced. Figure 20 shows the rapid separation of desmethylsibutramine using a short CHIRALPAK AGP column.

Drug – Plasma Protein Binding Studies

Another application of these protein-based columns is their use in drug-plasma protein binding studies. As the degree of drug-protein binding directly affects pharmacokinetic and pharmacodynamic characteristics of a pharmaceutical compound, a drug's potency may be dependent on the degree to which it binds to the plasma proteins and other blood constituents. HPLC analysis using CHIRALPAK AGP and CHIRALPAK HSA columns has been shown to be useful in drug binding studies.

Ordering Information

CHIRALPAK Phase	Column Dimensions (mm)						Guard Cartridges[1] (2/pk)	
	50 x 2.0	100 x 2.0	150 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	10 x 2.0[2]	10 x 3.0[3]
AGP	30792	30793	30794	30782	30783	30784	30791	30781
CBH	33792	33793	33794	33782	33783	33784	33791	33781
HSA	34792	34793	34794	34782	34783	34784	34791	34781

CHIRALPAK Phase	Column Dimensions (mm)					Guard Cartridges[1] (2/pk)
	50 x 4.0	100 x 4.0	150 x 4.0	100 x 10.0	150 x 10.0	10 x 4.0[4]
AGP	30712	30713	30714	30733	30734	30711
CBH	33712	33713	33714	33733	33734	33711
HSA	34712	34713	34714	34733	34734	34711

[1] Use with free-standing holder 00081
[2] For use with 2.0mm i.d. columns and column coupler 000D2
[3] For use with 3.0mm i.d. columns and column coupler 000D2
[4] For use with 4.0mm i.d. columns and column coupler 000D1