

# Immobilized Protein Stationary Phases

CHIRALPAK<sup>®</sup> AGP, HSA & CBH Versatile & Validated



# **Immobilized Protein Stationary Phases**

Chiral Technologies is the market leader in enantioselective chromatography and the recognized global provider of novel immobilized chiral stationary phases. We have expanded this portfolio of well-known Daicel chiral stationary phases by adding chiral protein-based phases: CHIRALPAK® AGP, CHIRALPAK HSA and CHIRALPAK CBH.

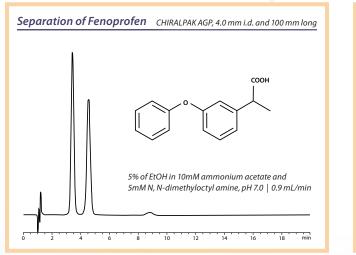
Protein stationary phases (PSPs) were originally developed and manufactured by ChromTech Ltd., U.K. Chiral Technologies Europe acquired ChromTech in 2009, and we are now the only manufacturer of these widely recognized protein stationary phases and columns.

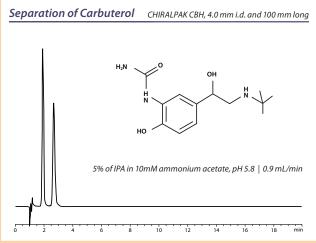
## **Protein Stationary Phases – Chiral Selectors**

Our immobilized protein stationary phases, CHIRALPAK AGP, HSA and CBH, are successfully utilized by scientists for separation and characterization of chiral compounds in a great number of applications, ranging from drug discovery and quality assurance of marketed drugs to environmental monitoring. The three chiral selectors are immobilized on 5-µm spherical silica particles.

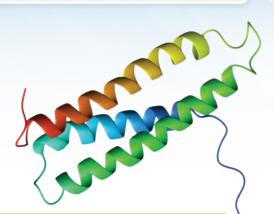
CHIRALPAK AGP –	$\alpha_{i}$ -acid glycoprotein
CHIRALPAK HSA –	human serum albumin
CHIRALPAK CBH –	cellobiohydrolase (stable enzyme)

The CHIRALPAK AGP selector has extremely broad applicability for the separation of a wide variety of chiral compounds such as amines, acids and nonprotolytes. The enantioselectivity of the CHIRALPAK CBH selector is complementary to that of the AGP selector, as shown in the following separations.





In addition, PSP columns are successfully applied to enantiomeric purity analyses of bulk drugs and finished drug formulations. The United States Pharmacopeia (USP), the most widely used compendium of validated test methods, sets standards to ensure the quality and safety of medicines and pharmaceuticals. The USP identifies "L41" for the CHIRALPAK AGP column brand to test *Enantiomeric Purity* of such drugs as montelukast sodium, ropivacaine and tenofovir, as well as the *Stereoisomeric Purity* of galantamine.



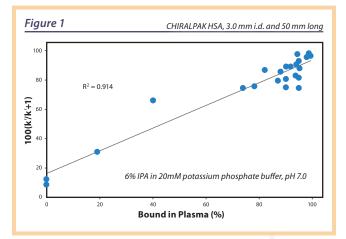
### **PSP Enantioselectivity**

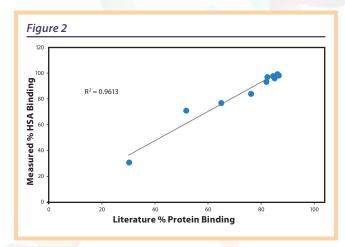
Enantioselectivity can easily be controlled or improved by changes in the mobile phase composition: pH, buffer and organic modifier types and concentrations. The mobile phases, with which these columns are used, are compatible with MS and MS/MS analyses. However, previously established guidelines on method development with PSP columns involved the use of phosphate buffers, which required a lengthy optimization process when attempting to identify a MS-compatible buffer system. A poster on *LC-MS Compatible Reversed-Phase Screening Strategies on Daicel Protein-Based Columns*, presented at the Chirality 2010 meeting by T. Zhang, *et al.*, describes approaches to simplify screening and optimization methodology for separation of diverse chiral compounds on CHIRALPAK PSPs. **To view this poster and learn more about CHIRALPAK PSPs, click here.** 

### **Drug-Protein Binding**

Another application of protein stationary phases, particularly the CHIRALPAK® HSA, is their use in drug-protein binding studies. Proteins found in plasma are responsible for many processes such as the transport, distribution, metabolism and excretion of different molecules. Therefore, it is vital to characterize binding properties between drug molecules and plasma proteins. The degree of drug-protein binding directly affects pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of a drug. Drug potency, therefore, is dependent on the degree of drug interactions with plasma proteins and other blood constituents. Major blood proteins, to which drugs bind, are human serum albumin,  $\alpha_1$ -acid glycoprotein, lipoproteins and globulins. Understandably, the unbound drug is active and exhibits pharmacologic effects. Rational selection of new drug targets is time- and resource-consuming; therefore, better prediction of drug efficacy and safety is vital to support timely go/no-go decisions on which drug candidates move to clinical trials. Although a number of methods are available to measure the degree of the drug interactions with proteins in plasma, HPLC is also a convenient and fast way to determine drug-protein binding properties.

A fast-gradient HPLC method, using CHIRALPAK HSA, was developed and validated by Dr. Klara Valko – Klara Valko, *et al., Fast-Gradient HPLC Method to Determine Compounds Binding to Human Serum Albumin. Relationships with Octanol/Water and Immobilized Artificial Membrane Lipophilicity* – and published in the Journal of Pharmaceutical Sciences, Vol. 92, No. 11, 2003. To ascertain the percentage of protein binding, HPLC retention data (k') are used. The percentage of protein binding (P) can be calculated as: P = 100(k'/(k'+1)). An excellent correlation of P values with percentage of compound binding to HSA is shown in Figure 1, and the correlation of HPLC-generated values with literature data is shown in Figure 2.





# **List of Available Products**

PART NUMBER	PRODUCT NAME	PARTICLE SIZE (µm)	ID (mm)	LENGTH (mm)	PRODUCT TYPE
CHIR/	ALPAK <sup>®</sup> AGP				
30711	CHIRALPAK AGP (2-pkg)	5	4.0	10	Guard
30712	CHIRALPAK AGP	5	4.0	50	Analytical
30713	CHIRALPAK AGP	5	4.0	100	Analytical
30714	CHIRALPAK AGP	5	4.0	150	Analytical
30733	CHIRALPAK AGP	5	10.0	100	Semi-Prep
30734	CHIRALPAK AGP	5	10.0	150	Semi-Prep
30781	CHIRALPAK AGP (2-pkg)	5	3.0	10	Guard
30782	CHIRALPAK AGP	5	3.0	50	Analytical
30783	CHIRALPAK AGP	5	3.0	100	Analytical
30784	CHIRALPAK AGP	5	3.0	150	Analytical
30791	CHIRALPAK AGP (2-pkg)	5	2.0	10	Guard
30792	CHIRALPAK AGP	5	2.0	50	Analytical
30793	CHIRALPAK AGP	5	2.0	100	Analytical
30794	CHIRALPAK AGP	5	2.0	150	Analytical
CHIR	ALPAK <sup>®</sup> CBH				
33711	CHIRALPAK CBH (2-pkg)	5	4.0	10	Guard
33712	CHIRALPAK CBH	5	4.0	50	Analytical
33713	CHIRALPAK CBH	5	4.0	100	Analytical
33714	CHIRALPAK CBH	5	4.0	150	Analytical
33733	CHIRALPAK CBH	5	10.0	100	Semi-Prep
33734	CHIRALPAK CBH	5	10.0	150	Semi-Prep
33781	CHIRALPAK CBH (2-pkg)	5	3.0	10	Guard
33782	CHIRALPAK CBH	5	3.0	50	Analytical
33783	CHIRALPAK CBH	5	3.0	100	Analytical
33784	CHIRALPAK CBH	5	3.0	150	Analytical
33791	CHIRALPAK CBH (2-pkg)	5	2.0	10	Guard
33792	CHIRALPAK CBH	5	2.0	50	Analytical
33793	CHIRALPAK CBH	5	2.0	100	Analytical
33794	CHIRALPAK CBH	5	2.0	150	Analytical

PART NUMBER	PRODUCT NAME	PARTICLE SIZE (µm)	ID (mm)	LENGTH (mm)	PRODUCT TYPE
CHIR	ALPAK <sup>®</sup> HSA				
34711	CHIRALPAK HSA (2-pkg)	5	4.0	10	Guard
34712	CHIRALPAK HSA	5	4.0	50	Analytical
34713	CHIRALPAK HSA	5	4.0	100	Analytical
34714	CHIRALPAK HSA	5	4.0	150	Analytical
34733	CHIRALPAK HSA	5	10.0	100	Semi-Prep
34734	CHIRALPAK HSA	5	10.0	150	Semi-Prep
34781	CHIRALPAK HSA (2-pkg)	5	3.0	10	Guard
34782	CHIRALPAK HSA	5	3.0	50	Analytical
34783	CHIRALPAK HSA	5	3.0	100	Analytical
34784	CHIRALPAK HSA	5	3.0	150	Analytical
34791	CHIRALPAK HSA (2-pkg)	5	2.0	10	Guard
34792	CHIRALPAK HSA	5	2.0	50	Analytical
34793	CHIRALPAK HSA	5	2.0	100	Analytical

### **PROTEIN-BASED COLUMN ACCESSORIES**

CHIRALPAK HSA

34794

00081	Guard Column Holder	Holder
000D1	Guard Column Coupler	Coupler
000D2	Micro Guard Column Coupler	Coupler
000D3	Column Fittings (5-pack)	Fittings

5

2.0

150

Analytical

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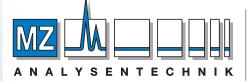
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