

## Columns for drug-protein binding studies

HPLC is a convenient method to use for the determination of the degree of drug/protein binding. ChromTech supply a range of HPLC columns that can be used for these kind of studies:

**CHIRAL-HSA** - human serum albumin

**CHIRAL-AGP** -  $\alpha_1$ -acid glycoprotein

**CHIRAL-RSA** - rat serum albumin

**CHIRAL-DSA** - dog serum albumin

**CHIRAL-MSA** - mouse serum albumin

as well as columns based on albumins from other species.

### Calculation of % protein binding

Retention data ( $k'$ ) is used to calculate the percentage of protein binding. A  $t_m$ -marker is injected (an unretained compound). The retention factor ( $k'$ ) for a drug is calculated by:

$$k' = \frac{t_r - t_m}{t_m}$$

where  $t_r$  = the retention time for the drug

where  $t_m$  = the retention time for the  $t_m$ -marker

The % protein binding (P) is calculated by:

$$P = 100 \left( \frac{k'}{k' + 1} \right)$$

### Mobile phases

Different types of mobile phases can be used. A mobile phase consisting of 5% 2-propanol in 20 mM potassium phosphate buffer pH 7.0 gives data in good agreement with literature data. At the top of the page, to the right, is a table with recommendations on pH and solvent content in the mobile phases. The mobile phase conditions should be chosen to suit the drugs to be tested, ie. for high protein binding drugs a mobile phase with higher eluting strength might be needed in order to reduce the retention times.

	Albumin columns	AGP columns
<b>pH range :</b>	5 - 7	4 - 7
<b>Rec. conc. 2-propanol:</b>	0 - 10% (v/v)	0 - 30% (v/v)* normally 0 - 10%
<b>Rec. conc. acetonitrile:</b>	0 - 10% (v/v)	0 - 30% (v/v) normally 0 - 10%

\*Higher concentrations give high back pressure

### Correlation

It is recommended to include a set of standard drugs to correlate the chromatographic data against published protein binding data. Literature values for plasma protein binding to use for correlation of binding to HSA can be obtained from Goodman A. and Gilman A.G, The Pharmacological Basis of Therapeutics, 9th Edition, McGraw-Hill, New York, p.1712-1792 (1996).

### Article numbers and availability

For these kinds of studies short columns are preferred. **CHIRAL-AGP** and **CHIRAL-HSA** columns are available from stock in standard dimensions. Other dimensions are produced on order (max. 1 week). The other albumin columns are produced on order as special columns (2-3 weeks). When ordering use the article numbers below:

Column	Art.no
CHIRAL-HSA 50x4.0 mm	HSA50.4
CHIRAL-HSA 50x3.0 mm	HSA50.3
CHIRAL-AGP 50x4.0 mm	AGP50.4
CHIRAL-AGP 50x3.0 mm	AGP50.3
CHIRAL-RSA 50x4.0 mm	RSA50.4
CHIRAL-RSA 50x3.0 mm	RSA50.3
CHIRAL-DSA 50x4.0 mm	DSA50.4
CHIRAL-DSA 50x3.0 mm	DSA50.3
CHIRAL-MSA 50x4.0 mm	MSA50.4
CHIRAL-MSA 50x3.0 mm	MSA50.3

Other dimensions are available.  
Other albumins are available.