

# ChromTech Chiral Columns for Drug-Protein Binding Study

## Columns for Drug-Protein Binding Study

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

|                    |                               |
|--------------------|-------------------------------|
| <b>CHIRAL-HSA:</b> | human serum albumin           |
| <b>CHIRAL-AGP:</b> | $\alpha_1$ -acid glycoprotein |
| <b>CHIRAL-RSA:</b> | rat serum albumin             |
| <b>CHIRAL-DSA:</b> | dog serum albumin             |
| <b>CHIRAL-MSA:</b> | mouse serum albumin           |

Albumins from other species also available, please contact Chrom Tech for pricing.

## 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:

$$k' = \frac{t_r - t_m}{t_m} \quad \text{where } t_r = \text{retention time for the drug}$$

$$\quad \text{where } t_m = \text{retention time for the } t_m \text{-marker}$$

The % protein binding (P) is calculated by:

$$P = 100(k'/(k' + 1))$$

## Mobile Phases

Different types of mobile phases can be used. A mobile phase consisting of 5% 2-propanol in 20mM potassium phosphate buffer pH 7.0 gives data in good agreement with literature data. **Table 1** lists recommendations on pH and solvent content in the mobile phases for chiral columns. 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.

**Table 1:**

### Recommended Chromatography Mobile Phase Conditions

|                              | Albumin columns | AGP columns                    |
|------------------------------|-----------------|--------------------------------|
| pH range:                    | 5-7             | 4-7                            |
| 2-propanol<br>(rec. conc.)   | 0-10% (v/v)     | 0-30% (v/v)*<br>normally 0-10% |
| Acetonitrile<br>(rec. conc.) | 0-10% (v/v)     | 0-30% (v/v)*<br>normally 0-10% |

\*Higher concentrations give high back pressure.

## Drug Protein Binding Columns Ordering Information

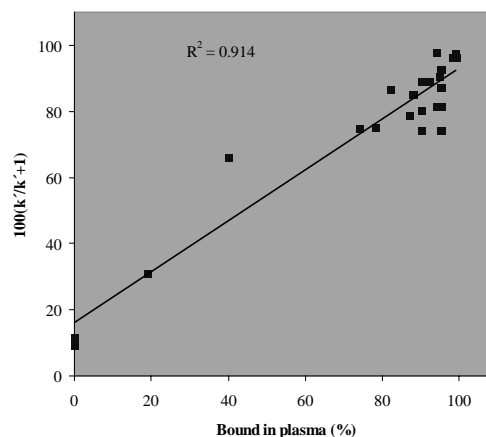
| Cat.No.         | Description                                |
|-----------------|--|
| <b>CT-20054</b> | Chiral-AGP, 4.0 x 50mm, 5 $\mu$ m          |
| <b>CT-20053</b> | Chiral-AGP, 3.0 x 50mm, 5 $\mu$ m          |
| <b>CT-20052</b> | Chiral-AGP, 2.0 x 50mm, 5 $\mu$ m          |
| <b>CT-29054</b> | Human serum albumin, 4.0 x 50mm, 5 $\mu$ m |
| <b>CT-29053</b> | Human serum albumin, 3.0 x 50mm, 5 $\mu$ m |
| <b>CT-29052</b> | Human serum albumin, 2.0 x 50mm, 5 $\mu$ m |
| <b>RSA504</b>   | Rat serum albumin, 4.0 x 50mm, 5 $\mu$ m   |
| <b>RSA503</b>   | Rat serum albumin, 3.0 x 50mm, 5 $\mu$ m   |
| <b>MSA504</b>   | Mouse serum albumin, 4.0 x 50mm, 5 $\mu$ m |
| <b>MSA503</b>   | Mouse serum albumin, 3.0 x 50mm, 5 $\mu$ m |
| <b>DSA504</b>   | Dog serum albumin, 4.0 x 50mm, 5 $\mu$ m   |
| <b>DSA503</b>   | Dog serum albumin, 3.0 x 50mm, 5 $\mu$ m   |

## Correlation of Chromatographic Data

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).

Below is a plot with results obtained from chromatography of standard drugs on a Chiral-HSA column. In **Table 2** the results are correlated to data from Goodman & Gilman. All data is shown in the table.



**Table 2: Chromatographic Results vs. Literature Values for Plasma Protein Binding**

| Drug Compound | % Bound in Plasma** | % Protein Binding |
|---------------|---------------------|-------------------|
| Isaniazid     | 0                   | 11.8              |
| Ethosuximid   | 0                   | 9.2               |
| Primidone     | 19                  | 31                |
| Folinic acid  | 40                  | 65.9              |
| Carbamazepine | 74                  | 74.8              |
| Diltiazem     | 78                  | 75.3              |
| Desipramine   | 82                  | 86.8              |
| Propranolol   | 87                  | 78.8              |
| Budesonide    | 88                  | 85.4              |
| Indometacin   | 90                  | 80.2              |
| Verapamil     | 90                  | 74.4              |
| Imipramine    | 90.1                | 88.9              |
| Nortriptyline | 92                  | 89.2              |
| Sulindac      | 94                  | 97.9              |
| Fluoxetine    | 94                  | 81.7              |
| Amitriptyline | 94.8                | 90.8              |
| Propafenone   | 95                  | 81.4              |
| Carvedilol    | 95                  | 92.6              |
| Paroxetine    | 95                  | 87.3              |
| Omeprazole    | 95                  | 74.2              |
| Nitrendipine  | 98                  | 96.4              |
| Nicardipine   | 98.8                | 97.5              |
| Ketoconazol   | 99                  | 96.3              |

\*\*Values from Goodman A. and Gilman A.G., The Pharmacological Basis of Therapeutics, 9th Edition, McGraw-Hill, New York, p. 1712-1792 (1996). The percent protein binding values were calculated from the chromatographic data obtained on the Chiral-HSA, as described.