

Chromatography better than ultrafiltration and dialysis for drug-protein binding studies

In a recent publication in *J. Chromatography* the advantages of chromatographic determination of drug protein binding is discussed (*J.Chromatogr. B* 809 (2004) 67-73). The authors, Y. Cheng et al, have compared chromatography, ultrafiltration and equilibrium dialysis. Chromatography using the CHIRAL-HSA column was found to be more reproducible and precise.

The degree of plasma-protein binding has a significant effect on pharmacokinetic and pharmacodynamic properties of a drug in vivo. For example hepatic metabolism rate, renal excretion rate, biomembrane partition rate and steady-state distribution volume area function of unbound/bound concentration ratio. This makes the determination of drug-protein binding an essential part in drug development.

In the article the chromatographic method using the CHIRAL-HSA method is described as generally feasible for medium to high throughput screening. The authors are using the mass spectrometer as the detector, giving high sensitivity and selectivity, however also UV and other detectors can be used.

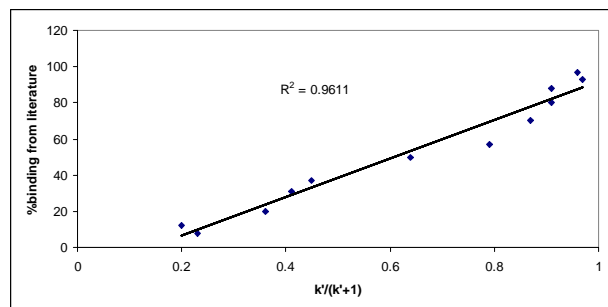
In this study a CHIRAL-HSA 50x2.0 mm column is used to determine protein binding at a flow rate of 0.3 ml/min. A good alternative is to use the CHIRAL-HSA 50x3.0 mm column. Two mobile phases, both based on a 50 mM ammonium acetate buffer pH 7 are used, either with 4 or 20% 2-propanol. The higher 2-propanol concentration was used for compounds with high protein binding.

The percentage of protein binding is calculated from the $k'/(k'+1)$ values. The authors are calculating the k' value from the equation:

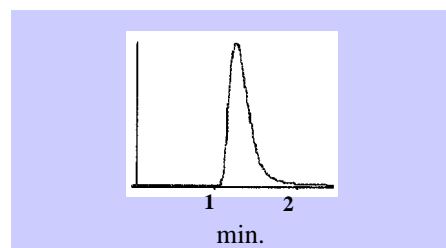
$$k' = \frac{t_r - t_0}{t_0 - t_m}$$

where t_r is the retention time of the drug compound, t_0 is the retention of a drug with no protein binding and t_m is the system void volume time.

Commercial available drugs with known protein binding were chromatographed and k' values were calculated from their retention times. These values were plotted against literature data on protein binding to generate a calibration graph. Compounds with unknown degree of protein binding were chromatographed on the CHIRAL-HSA column and k' was calculated. The calibration graph was then used to obtain the degree of protein binding on these compounds. The results were compared with the degree of protein binding obtained by ultrafiltration or dialysis giving a good correlation. The conclusion is that the chromatographic method with the CHIRAL-HSA column is very suitable for fast and simple screening in early drug discovery.



Calibration graph by linear regression of $k'/(k'+1)$ versus % binding from literature. Mobile phase 4% 2-propanol in 50 mM ammonium acetate buffer pH 7.0



Extracted ion mass chromatogram of quinine. Mobile phase 20% 2-propanol in 50 mM ammonium acetate buffer pH 7

The authors suggest a flow chart for using the CHIRAL-HSA column for fast and convenient determination of drug-protein binding in early drug discovery projects:

