Binding study of isoflavones and human serum albumin using high-performance frontal analysis

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Quantitative investigation of drug-binding is essential in pharmacokinetic study and clinicial applications. Equilibrium dialysis and ultrafiltration followed by HPLC analysis methods have been widely used for this purpose. However, there conventional analytical methods suffer some limitations. To overcome this problem, high-performance frontal analysis(HPFA), a chromatographic method which allows simple and easy determination of unbound drug concentrations after direct sample injection has been reported. Here we present the protein analysis of Isoflavones by HPFA. The analysis was performed on a Develosil 100-Diol-5 (10 cm x 4.6 mm I.D.) column, phosphate solution (pH 7.4, ionic strength 0.17) was used as the mobile phase at a flow rate of 1 ml/min. Optimum ultrawavelength was set on 260 nm. Injection volumes were chosen to ensure the drug to be eluted as zonal peal with a plateau. By Scatchard analysis, it was found that the binding constant (K) and binding affinity (nK) of isoflavones to HSA were: K=1.581105 [M^{-1}], nK=0.77104 [M^{-1}] for daidzein, K=1.082105 [M^{-1}], nK=0.32104 [M^{-1}] for genistein, and K=3.533105 [M^{-1}], nK=0.70104 [M^{-1}] for genistin, respectively.