



A simple high-performance liquid chromatographic method for the determination of diclofenac in human plasma: application to a comparative bioavailability study

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Abstract. A rapid, sensitive and specific method to quantify diclofenac in human plasma using indomethacin as the internal standard (IS) is described. Samples were extracted using protein precipitation protocol and analyzed by high performance liquid chromatography coupled to ultra violet detection at 276 nm. Chromatography was performed isocratically with a run time of 8.0 min and the retention time observed for diclofenac and IS was 6.0 and 7.0 min. respectively. The calibration curve was linear over the range 50 – 4,000 ng/ml ($r^2 > 0.9995$). The mean recovery of diclofenac ranged from 88.76 – 99.14% and the limit of quantification was 50 ng/ml. Intra batch precision and accuracy (%CV) of the method ranged from 0.86 – 7.60%, and 99.34 – 103.8%, respectively.

Interbatch precision (%CV) and accuracy ranged from 0.26 – 11.4%, and 92.00 – 105.34%, respectively. This HPLC method was used to determine the relative pharmacokinetics of two diclofenac-cholestyramine 140 mg capsule formulations. The study was conducted using an open, randomized and cross over design with a one-week wash out interval. A single 140 mg dose (equivalent to 70 mg of diclofenac) of each formulation was administered to 26 healthy volunteers (13 males and 13 females) and blood samples were obtained over 12-h interval. The geometric mean of diclofenaccholestyramine/Flotac® ratio was 90.53% for AUC₀₋₁₂ and 100.22% for C_{max}.

Since the 90% CI for C_{max} and AUCs, ratios were all inside the 80 – 125% interval, it was concluded that the diclofenaccholestyramine test formulation is bioequivalent to Flotac® regarding both the rate and the extent of absorption.