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Bioanalytical method development and validation for the simultaneous estimation of active metabolite thiocolchicoside and diclofenac in human plasma by LCMS/MS with a special emphasis to bioequivalence study

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The treatment of musculoskeletal disorders as well as in the treatment of pain and inflammation in mammalian organism, a pharmaceutical composition should comprise effective amount of one muscle relaxant and at least one nonsteroidal anti-inflammatory drug. A combination therapy involving thiocolchicoside and diclofenac is introduced in market for the treatment of musculoskeletal disorders.

To obtain optimal drug concentrations (minimum effective concentrations) for pain suppression and avoidance of drug toxicity, monitoring of drug levels has been considered essential to determine drug concentrations in plasma. A new reverse phase high-performance liquid chromatography tandem mass spectrometry assay method was developed for the simultaneous determination of diclofenac (DF) and 3-desmethyl Thiocolchicine (3DMT) (active metabolite of Thiocolchicoside) using aceclofenac (AF) as internal standard in human plasma as well as in pharmaceutical dosage form. The chromatographic separation was performed on a reversed-phase phenomenex gemini C18 with a mobile phase containing methanol : water (containing 0.2% forminc acid) (9:1, v/v). The calibration curves were linear over the range of 1 to 50 ng/ml for 3DMT and 25 to 2500 ng/ml for DF with the lower limit of quantification validated at 0.5 ng/ml for 3DMT and 5 ng/ml for DF. The LC-ESI-MS/MS in MRM mode provided a highly selective method for the determination of 3DMT & DF in human plasma. The positively charged parent and product ion mass spectra of 3DMT, DF & IS (AF) were 402-360, 296-214 & 354-214 respectively. The total run time set for the samples tested was 3.5 minutes. 3DMT, DF & IS (AF) were eluted at retention times of 1.51, 1.87 and 2.28 minutes respectively. The average correlation coefficients obtained was 0.999 or more for both the analytes. The method involves a simple liquid-liquid extraction by ethyl acetate in acidic medium. The extraction recovery was more than 85 % for both the analytes. There is a nominal matrix effect which will not affect the analytes recovery, resolution and detoriation of analysis during the study. The validated LC-ESI-MS/MS method was successfully applied for the evaluation of pharmacokinetic parameters and bioequivalence study of test and reference FDC tablet preparation of thiocolchicoside 8mg and diclofenac sodium 50mg after a single oral administration to all 12 healthy male volunteers and the bioequivalence study report has been accepted and approved by Drug Controller General of India (DCGI).

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