DEVELOPMENT AND VALIDATION OF A LC- MS/MS METHOD FOR DETERMINATION OF NIFEDIPINE AND DEHYDRONIFEDIPINE IN HUMAN PLASMA

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Background: Nifedipine (NIF), a calcium channel antagonist, is metabolized primarily by cytochrome P450 (CYP3A4) to dehydronifedipine (DNIF).

Objectives: To establish and validate a LC-MS/MS method to determine NIF and DNIF in human plasma.

Methods: The LC-MS/MS method is developed to simultaneously determine NIF and DNIF in human plasma. Following Guidance for Industry Bioanalytical Method Validation (FDA), the parameters, i.e., specificity, linearity, accuracy, precision, limit of quantification and stability were validated.

Results: Amlodipine was used as the internal standard (IS). The plasma was extracted by *n*-hexane- dichloromethane (7:3). The samples were separated on a Gemini C6 Phenyl (150 mm × 4.6 mm, 5 μm) column with methanol- ammonium formate 10 mmol pH 6 (67:33, v/v) as mobile phase. Electrospray ionization source was operated in positive ion mode. The precursor → product ion transitions for NIF (*m/z* 347.10 → 314.90), DNIF (344.90 → 284.00) and IS (*m/z* 409.10 → 238.10) were monitored. The lower limits of quantification (LLOQ) were 0.2 $ng.mL^{-1}$ for NIF and 0.15 $ng.mL^{-1}$ for DNIF. The linear calibration curves were obtained over the concentration range in plasma of 0.2-500 $ng.mL^{-1}$ for NIF and 0.15-300 $ng.mL^{-1}$ for DNIF. The intra-day and inter-day precisions were less than 20% for the LLOQ of the two analytes and less than 15% for all quality control samples at concentrations of 0.6, 250, 400 $ng.mL^{-1}$ and 0.3, 150, 240 $ng.mL^{-1}$ for NIF and DNIF, respectively. The intra-day and inter-day accuracies were within 20% for the LLOQ and 15% for all quality control samples of the two analytes. The recoveries of the liquid extraction method were 83.20% for NIF and 81.72% for DNIF. NIF was found to be stable for at least 6 hours at room temperature, 3 freeze-thaw cycles and 60 days at -20 ^{o} C in plasma; DNIF was found to be stable for at least 6 hours at room temperature, 3 freeze-thaw cycles and 45 days at -20 ^{o} C in plasma.

Conclusions: The method is proved to be simple, sensitive, accurate, precise and specific; and can be applied to pharmacokinetic and bioequivalence study of nifedipine.

Keywords: Nifedipine, dehydronifedipine, LC- MS/MS