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A Validated UPLC-MS/MS Assay Using Negative Ionization Mode for High-Throughput Determination of Pomalidomide in Rat Plasma

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Abstract : Pomalidomide is a second generation oral immunomodulatory agent, being used for the treatment of multiple myeloma in patients with disease refractory to lenalidomide and bortezomib. In this study, a sensitive UPLC-MS/MS assay was developed and validated for high-throughput determination of pomalidomide in rat plasma using celecoxib as an internal standard (IS). Liquid liquid extraction using dichloromethane as extracting agent was employed to extract pomalidomide and IS from 200 µL of plasma. Chromatographic separation was carried on Acquity BEHTM C18 column (50 × 2.1 mm, 1.7 µm) using an isocratic mobile phase of acetonitrile:10 mM ammonium acetate (80:20, v/v), at a flow rate of 0.250 mL/min. Both pomalidomide and IS were eluted at 0.66 ± 0.03 and 0.80 ± 0.03 min, respectively with a total run time of 1.5 min only. Detection was performed on a triple quadrupole tandem mass spectrometer using electrospray ionization in negative mode. The precursor to product ion transitions of m/z 272.01 → 160.89 for pomalidomide and m/z 380.08 → 316.01 for IS were used to quantify them respectively, using multiple reaction monitoring mode. The developed method was validated according to regulatory guideline for bioanalytical method validation. The linearity in plasma sample was achieved in the concentration range of 0.47–400 ng/mL (r2 ≥ 0.997). The intra and inter-day precision values were ≤ 11.1% (RSD, %) whereas accuracy values ranged from - 6.8 – 8.5% (RE, %). In addition, other validation results were within the acceptance criteria and the method was successfully applied in a pharmacokinetic study of pomalidomide in rats.

Keywords : pomalidomide, pharmacokinetics, LC-MS/MS, celecoxib
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