A Novel LC-MS/MS Method for Simultaneous Determination of Ivabradine and its Active Metabolite N-Desmethyl Ivabradine in Human Plasma: Its Pharmacokinetic Application

Vasu Babu Ravi[1,2]*, Venkateswarlu Ponneri[3]

[1]*Research Studies, Rayalaseema University, Kurnool-518 002, India.
[2]Wellquest Clinical Research Laboratories, Ramanthapur, Hyderabad–500 013, India.
[3]Analytical and Environmental Chemistry Division, Department of Chemistry, Sri Venkateswara University, Tirupati-517502, India.

Abstract : The method was new simple, rapid, sensitive and simultaneous liquid chromatography/tandem mass spectrometry assay method for the determination of Ivabradine and N-Desmethyl Ivabradine in human plasma using Ivabradine-d_6 and N-Desmethyl Ivabradine-d_6 as internal standards (IS). Analyte and the internal standards were extracted from the human plasma *via* Solid phase extraction (SPE) using Strata ^{TM} X 33μM polymeric sorbent cartridges (1cc/30mg). The chromatographic separation was achieved on a Kromasil 100-5 C_{18}, 100 x 4.6 mm, 5 µm columns by using a gradient programme at a flow rate of 0.60 mL/min with a total runtime of 3.0 min and the elution was monitored by multiple reaction monitoring modes using electrospray ionization. The calibration curve obtained was linear ($r^2 \geq 0.99$) over the concentration range of 0.20–201 ng/mL for Ivabradine and 0.10–15.14 for N-Desmethyl Ivabradine. Method validation was performed as per FDA guidelines and the results met the within the acceptable limits. The proposed method was found to be applicable to pharmacokinetic studies.

Keywords : Ivabradine, N-Desmethyl Ivabradine, Solid Phase Extraction, LC-MS/MS, Validation, Human plasma, Pharmacokinetics.

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