Development and validation of mass spectrometry method for estimation of molecule darifenacin in human plasma

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Introduction: Chemically, Darifenacin hydrobromide is (S)-2-[1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidinyl]-2,2-diphenylacetamide hydrobromide. The empirical formula of Darifenacin hydrobromide is C28H30N2O2.HBr. Darifenacin is a muscarinic antagonist indicated for the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency and frequency. Darifenacin has greater affinity for the M3 receptor. M3 receptors are involved in contraction of human bladder and gastrointestinal smooth muscle, saliva production, and iris sphincter function. A novel high performance liquid chromatography using positive ion APC1 ionization Tandem Mass Spectrometry method was developed and validated for quantification of Darifenacin in Human plasma.

Method: The analyte in the plasma was extracted using Solid Phase Extraction method. The analyte was separated using an binary mobile phase on a reserve phase column to meet the demands of the clinical laboratory for speed of analysis and chromatographic resolution. Detection was carried out by MS/MS in the multiple reaction monitoring mode using the respective (M+H)+ ions Q1 m/z 427.3 Q3 m/z 14.6 and Q1 m/z 431.3 Q3 m/z 151.1 for analyte and internal standard. The developed method was validated.

Results: The assay exhibited a linear dynamic range of 0.102-15.066ng/ml for Darifenacin in human plasma. The lower of quantification was 0.102 ng/ml with relative standard deviation of less than 13.03 %. No interference by endogenous substances or matrix effect was observed. The intra-day and inter-day accuracy and precision (% CV) values were in the range of 91.76-107.72% (2.69-8.96%) and 91.18-105.57% (3.84-9.01%) respectively. The spiked plasma samples were found to be stable even after 3 freeze-thaw cycles -70⁰C & -20⁰C. The processed plasma samples were found to be stable in autosampler for 30 hrs at 10°C. A short run time 3.0 min for each sample made it a high throughput method for estimation of clinical samples. The developed and validated method for estimation of Troxipide in human plasma was effectively used to determine plasma concentration profiles in human subjects.

Development and validation of mass spectrometry method for estimation of molecule troxipide in human plasma

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Introduction: Troxipide is a novel gastro protective agent with antiulcer, anti-inflammatory and mucus secreting properties. Chemically, it is 3 - (3, 4, 5-Trimethoxybenzamido)piperidine. It is used in treatment of gastroesophageal reflux disease. Troxipide's mucosal protective effect in gastric ulcer and gastritis is exerted via the inhibition of inflammatory responses and neutrophil-mediated mucosal injury. It promotes ulcer repair by increasing collagen regeneration of the ulcer base and causes healing of peptic ulcer. A novel high performance liquid chromatography positive ion electrospray ionization tandem mass spectrometry method was developed and validated for the quantification of Troxipide in human plasma.

Method: The analyte in the plasma sample was extracted using liquid – liquid extraction. The analyte was separated using an isocratic mobile phase on a reverse phase column to meet the demands of the clinical laboratory for speed of analysis and chromatographic resolution. Detection was carried out by MS/MS in the multiple reaction monitoring mode using the respective (M+H) + ions, m/z 295.20 - 195.20 and 268.20 – 121.40, 132.20 for analyte and the internal standard respectively. The developed method was validated.

Results: The assay exhibited a linear dynamic range of 50.132 – 8021.103ng/ml for Troxipide in Human plasma. The lower limit of quantification was 50.132ng/ml with a relative standard deviation of less than 10.48%. No interference by endogenous substances or matrix effect was observed. The intra-day and inter-day accuracy and precision (% CV) values were in the range of 105.66-110.18% (3.69-4.64%) & 103.94-110.16% (3.69-4.13%).The spiked plasma samples were found to be stable even after 3 freeze-thaw cycles -70°C & -20°C. The processed plasma samples were found to be stable in autosampler for 48 hrs at 10°C. A short run time 2.5mins for each sample made it a high throughput method for estimation of clinical samples. The developed and validated method for estimation of Troxipide in human plasma was successfully used to evaluate plasma concentration profiles in human subjects.