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On-line robotic high-throughput liquid chromatography–tandem mass spectrometric method for simultaneous quantification of Hydroxyzine and Cetirizine in human plasma

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A simple, sensitive and reliable method have been developed for simultaneous quantification of Hydroxyzine and Cetirizine in human plasma by using on-line robotic HTLC–MS/MS method. Quetiapine was used as an internal standard(IS). A typical two-column setup featuring two six-port switching valves was employed for method development and Turbo flow on-line technique was applied for separation of analyte from plasma sample. The analytical procedure involves on-line coupling of sample extraction with Cyclone P (50 mm × 0.5 mm 50 µm) HTLC column by injecting 15µL sample and chromatographic separation is performed with Inertsil ODS-3(5 µm ,4.6 x100 mm.). To achieve required chromatograms with consistency we have performed different combinations of the solvents and gradient system. Finally we succeeded with the solution combinations of 5mM Ammonium Acetate:Methanol: Acetonitrile (5:5:90) in pump A, Pure methanol in pump B, 0.1% formic acid in pump C and washing solution in the ratio of 70:25:5 (methanol:water:IPA) in pump D and analyzed more than 150 samples with out overloading of the chromatographic columns with improved real throughput efficiency. Detection was performed at transitions of m/z 375.300→201.100 for Hydroxyzine, m/z 389.200→201.100 for Cetirizine and m/z 384.200→253.100 for Quetiapine by positive electro-spray ionization (ESI+) in multiple reaction monitoring (MRM) mode using tandem mass spectrometry. The calibration curves were linear over a concentration range of 0.100 ng/mL to 150.000 ng/mL of Hydroxyzine and 0.500 ng/mL to 500.000 ng/mL of Cetirizine for quantification with the correlation coefficients demonstrating good linearity (0.994-0.999). The total run time of analysis was 5 min and the lower limits of Quantification were 0.100 ng/mL for Hydroxyzine and 0.500 ng/mL for Cetirizine, respectively. The method validation was carried out in terms of specificity, sensitivity, linearity, precision, accuracy and stability. The validated method was successfully applied in bioavailability and bioequivalence study.

Biography

S Raghunadha Reddy has completed his PhD from Jawaharlal Nehru Technological University, Anantapur and currently doing Postdoctoral studies from Department of Pharmaceutical Science, School of Pharmacy, University of Maryland. Previously he was worked as Head of Quality Assurance and Regulatory Affairs at Clinsync Clinical Research Pvt. Ltd. He has published 17 papers in reputed journals and has been serving as an Editorial Board Member of *Journal of Comprehencive Pharmacy*. He has extensive experience in Good Clinical Practice-ICH, Good Laboratory Practice, QMS (ISO9001-2008), Bioanalytical method Development and validation, Computer System Validations (21 CFR Part-11) and Regulatory Affairs.

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