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

Raghunadha Reddy Seelam
Department of Pharmaceutical Science, School of Pharmacy, University of Maryland, Pine Street, Baltimore, Maryland 21201, USA.

Abstract
A simple, sensitive and reliable method has been developed for simultaneous quantification of Hydroxyurea and Cetirizine in human plasma. The method was validated for estimation of Hydroxyurea and Cetirizine in human plasma samples. The validation parameters such as linearity, sensitivity, specificity, accuracy, precision, dilutional stability, and robustness were investigated. The results obtained indicated that the method is suitable for routine analyses. The proposed method was successfully applied for the analysis of pharmaceutical samples obtained from human plasma. The method is useful and can be applied for clinical drug monitoring.

Materials and Methods

Chemicals and reagents: Hydroxyurea dihydrochloride, Cetirizine dihydrochloride, Quetiapine fumarate and Hydroxyzine pamoate were purchased from Sigma-Aldrich Chemicals. Isopropanol and methanol were obtained from JT Baker (LC-MS grade). Formic acid was obtained from Panchen Chemicals. NaOH and HCl were obtained from A.B. Enterprises. Water (LC-MS grade) was purchased from Fisher Chemicals.

Standard solutions preparation: Approximately 5 mg of Hydroxyurea dihydrochloride or 5 mg of Cetirizine dihydrochloride or 5 mg of Quetiapine fumarate (BSTD) working standard is weighed and transferred to 150 mL volumetric flask, 2 mL of NaOH is added and sonicated and allowed to settle and the final volume is made up with methanol.

Preparation of internal standard solution: The Quetiapine internal standard solution is diluted of about 100 ng/mL from the BSTD stock solution (BSTD stock) using 50% v/v Methanol in Water as the diluent is prepared.

Preparation of calibration curve (CC) standards and quality control (QC) samples: A set of six different concentrations of the analytes were prepared to prepare the working standard solutions for Hydroxyurea and Cetirizine. All the solutions were prepared fresh in a refrigerator between 2 °C and 8 °C and quality control samples, in the range of 0.135 to 1950 ng/mL, of Hydroxyurea and 0.500 to 1500 ng/mL, of Cetirizine were prepared. The precision and accuracy were assessed by spiking 0.5 mL of drug free plasma with appropriate volume of working solution of each analyte and analysis of the samples was performed.

Solutions used for robust on-line sample extraction: SMD Amine Assisted Methanol: Analytical (0.59 mL) in A: Pure methanol in pump C and washing solution in the ratio of 70:25 (methanol:water) in pump D.

Sample preparation: 5 mL of the frozen CC, QC and subject samples from the deep freezer and then in water both maintained at room temperature, vortexed to mix. Removed the caps from the chromatographic vials. Aliquots of 2 ml of sample (with precipitated HPLC vials) were added 50.0 mL of 0.1% Triton X-100 solution (followed) by 50.0 mL of 10 mM Ammonium Acetate buffer of pH 7.4 stock, vortexed to mix and transferred was to an auto sampler. Then, 15 μL aliquot was injected on to the LCMS/MS system. For optimal results, the auto-sampler temperature was set at 4 °C.

Data processing: Chromatograms were acquired on a TSQ tandem mass spectrometry (Thermo Finnegan, San Jose, CA, USA) equipped with Electron ionization (EI) and connected to a PDA system with the standard software Xcalibur 2.1.7 and LC Quan 3.1.5. Mass spectrometric detection was performed on a Triple Quadruple instrument (Thermo, TSQ Quantum Discovery Max).

Conclusion
The method was applied successfully to the analysis of plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence studies of different drugs such as Hydroxyurea and Cetirizine. The established LC-MS/MS method is sensitive and suitable for the study of Hydroxyurea and Cetirizine in plasma. The method is easy to perform and can be applied for clinical drug monitoring.

Acknowledgements
The authors would like to thank N.K. Naidu, S. Venu Gopal, V. Ras Kimbra, S. Santhi Rani and D. Koteswara Rao for their technical assistance.

Table 1: Source specific and composited specific mass spectrometric parameters

Table 2: Accuracy validation of Hydroxyurea and Cetirizine

Table 3: Stability validation of Hydroxyurea and Cetirizine

Table 4: Robustness validation of Hydroxyurea and Cetirizine

Literature Cited