Quantification of Antimycotics in Human Plasma or Serum by Liquid Chromatography-Tandem Mass Spectrometry for Clinical Research

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Introduction

An analytical method for clinical research for the quantification of eight antimycotics in human plasma or serum is reported. Plasma or serum samples are extracted by offline internal standard addition and protein precipitation. Extracted samples are injected onto a Thermo Scientific™ Vanquish™ Flex Binary system connected to a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer with heated electrospray ionization. Detection is performed by selected reaction monitoring (SRM) using an isotopically labeled internal standard for each target analyte. Method performance was evaluated using the ClinMass® TDM Platform with the ClinMass Add-On Set for Antimycotics from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration ranges, accuracy, and intra- and inter-assay precision for each analyte.

Methods

The reported analytical method includes 5-fluorocytosine, fluconazole, hydroxyitraconazole, isavuconazole, itraconazole, ketoconazole, posaconazole, and voriconazole. Reagents included four calibrators (including blank) and two controls from RECIPE, as well as eight isotopically labeled internal standards for the quantification. Samples of 50 µL of plasma or serum were protein precipitated using 100 µL of precipitating solution containing the internal standards. Precipitated samples were vortex-mixed and centrifuged, and the supernatant was transferred to a clean plate or vial. Chromatographic separation was achieved using an analytical column and mobile phases provided with the kit. Analytes and internal standards were detected by SRM on a TSQ Quantis triple quadrupole mass spectrometer with heated electrospray ionization operated in polarity switching mode. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using quality control samples at two different levels provided by RECIPE, prepared and analyzed in replicates of five on three different days. Intra-assay precision was
evaluated for each day on the same set of runs (control samples at two levels, replicates of five each day, three days) in terms of percentage coefficient of variation (%CV). Inter-assay precision was evaluated on the same controls including all the 15 replicates of the three days. Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results

The method proved to be linear in the calibration ranges covered by the calibrators. The data demonstrated outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the control samples ranging between -8.9% and 1.6%. The %CV for intra-assay precision was always below 12.7% for all the analytes. The maximum %CV for inter-assay precision including all the analytes was 9.4%

Conclusions

A liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of eight antimycotics in human plasma or serum was implemented. The ClinMass TDM Platform with the ClinMass Add-On Set for Antimycotics from RECIPE was used. The method was analytically evaluated on a Vanquish Flex Binary system connected to a TSQ Quantis triple quadrupole mass spectrometer. The method offers the quick and simple offline protein precipitation with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.