A bioanalytical method for the quantitation of sulfamethoxazole and trimethoprim concentrations in plasma and urine

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Introduction
Trimethoprim-sulfamethoxazole is a co-formulated antimicrobial that is currently used as first-line prophylaxis and treatment against P. jiroveci pneumonia (PJP) in renal transplant patients. The purpose of this study was to design and validate an LC-MS/MS method suitable for the measurement of sulfamethoxazole and trimethoprim in plasma and urine for application to a clinical pharmacokinetic study of patients administered trimethoprim-sulfamethoxazole.

Methodology

Instrument Parameters: Shimadzu Nexera-8030+ UHPLC-MS/MS.

Chromatography: Kinetex PFP 50 x 2.1 mm (2.6 µm) column with mobile phase consisting of an isocratic (50%-mobile phase B) of 10 mM ammonium formate with 0.4% formic acid (v/v) and 100% acetonitrile with 0.2% formic acid (v/v).

Sample Preparation: A plasma sample (10 µL) was spiked with the internal standards, [2H4]-sulfamethoxazole and [2H3]-trimethoprim and 0.1% formic acid in acetonitrile to precipitate the plasma proteins. An aliquot of 0.5 µL of the supernatant was injected into the LC-MSMS.

Detection: Sulfamethoxazole and trimethoprim were monitored in positive mode electrospray at 253.90→156.00 and 291.00→230.05, respectively. The internal standards [2H4]-sulfamethoxazole and [2H3]-trimethoprim and 0.1% formic acid in acetonitrile to precipitate the plasma proteins. An aliquot of 0.5 µL of the supernatant was injected into the LC-MSMS.

Results
The assay has been successfully developed and validated according to FDA guidelines for bioanalytical method validation. The assay was linear for sulfamethoxazole from 0.5 to 200 µg/mL and for trimethoprim from 0.1 to 40 µg/mL.

The precision and accuracy for sulfamethoxazole was within 6.7% and 106%, respectively in plasma and within 3.6% and 105.5%, respectively in urine.

Precision and accuracy for trimethoprim was within 7.8% and 105.8%, respectively for plasma and within 5.1% and 94.2%, respectively for urine.

Matrix suppression or enhancement found to be limited to less than 6% for sulfamethoxazole and less than 2% for trimethoprim. Both the analytes were found to be stable at room temperature for 4 h and after three freeze-thaw cycles.

Conclusion
An accurate, precise and robust bioanalytical method was successfully developed and validated as per FDA guidelines. This method has been successfully applied to an observational pharmacokinetic study of trimethoprim-sulfamethoxazole in renal transplant recipients at the Kidney Transplant Unit at the Royal Brisbane & Women’s Hospital.