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CONFERENCE

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TITLE

Quantification of Sirolimus in Human Whole Blood by LC-MS/MS

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ABSTRACT

Novel Aspect: Sirolimus can be monitored under negative ion mode instead of positive ion mode as an ammonium adduct in previous methods.

Introduction

Sirolimus (also known as rapamycin) is a macrolide and used commonly as an immunosuppressant during organ transplants to prevent rejection during the procedure. Originally developed as an antifungal agent, its use in transplants changed the course of its use. Kidney transplants are the most well-known use, although sirolimus is used during multiple other procedures, including heart, liver and bone marrow transplants. In this work, we report the method developed for the determination of sirolimus in K_2EDTA human whole blood employing protein precipitation supported solid phase extraction and LC-tandem MS detection. Our data shows that the method reported here is rugged, sensitive, selective, accurate, and reproducible.

Methods

Calibration standards and quality controls were prepared in whole blood. Internal standard (tacrolimus) was added to 200 µL of samples was extracted first by a protein precipitation step using water and zinc sulfate as protein precipitation agent followed by acetonitrile as precipitation solvent. The samples were extracted on Waters Oasis HLB cartridge. After evaporating samples to dryness in a 40°C bath under nitrogen, samples were reconsitituted. Samples were injected with gradient of mobile phases water:acetic acid, 100:0.1 and methanol:acetic acid, 100:0.1, v:v with separation by Luna C8, 50x2 mm, 5 µm column. Analyte and IS were detected and quantified by multiple reaction monitoring (MRM) on Sciex API4000 tandem mass spectrometer under negative mode.

Preliminary results

The assay proved to be rugged, sensitive, selective, accurate, and reproducible over the assay range of 0.2-100 ng/mL sirolimus in human whole blood. The analyte and IS showed retention times of ~2.37 and ~2.35 minutes, respectively, with total run time ~5 minutes. The analyte and IS were quantified with parent→daughter ions of m/z 912.7 $(M-H)^-$ →167.1 (for sirolimus) and m/z 802.7 $(M-H)^-$ → 560.5 (for internal standard) in negative ion mode instead of positive ion mode as an ammonium adduct in previous methods. The intra- and inter-day coefficients of variation (%CV) for sirolimus quality controls ranged from 1.2 to 6.6% and 3.5 to 7.7%, respectively, while the intra- and inter-day % relative error to nominal concentration ranged from -7.5 to -2.0% and -8.0 to -1.0%. The stability of sirolimus in human whole blood was also assessed and found to be stable for 7 hours at room temperature, after 6 freeze/thaw cycles at -20°C and after 167 days of storage at -20°C. Overall, sirolimus is stable during storage, processing, and analysis in human whole blood samples and this analytical method has been utilized for the quanitification of sirolimus in human whole blood samples in pharmacokinetic and toxicological studies.