A UPLC-MS/MS Method for the Analysis of Plasma Mycophenolic Acid for Clinical Research

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Background: Here we present a UPLC-MS/MS method for the analysis of mycophenolic acid in human plasma for clinical research purposes. An analytically sensitive method was developed using protein precipitation extraction (PPE) which is a fast and cost effective method. Analysis was performed using an ACQUITY UPLC® I-Class system, samples were injected onto an ACQUITY UPLC® C18 column using a water/methanol/ammonium acetate gradient and quantified with a Xevo® TQD mass spectrometer.

Methods: Calibrator and control samples were purchased from Recipe® (Munich, Germany). Certified mycophenolic acid reference material purchased from Cerilliant® (Round Rock, TX) was used to create in-house calibrators, to extend the linear range, in pooled human plasma purchased from Sera Laboratories (Haywards Heath, UK). PPE was carried out by adding methanol and zinc sulphate with the composition of organic allowing for the supernatant to be directly injected without the need for evaporation and reconstitution. Using an ACQUITY UPLC® I-Class system, samples were injected onto an ACQUITY UPLC® C18 column using a water/methanol/ammonium acetate gradient elution profile and quantified with a Xevo® TQD mass spectrometer.

Results: The method demonstrated no significant carryover and was shown to be linear from 0.1 – 20 µg/mL. Coefficients of variation (CV) for total precision and repeatability on 5 separate days for low (0.5 µg/mL), mid (2.4 µg/mL) and high (5.0 µg/mL) QC material were all < 10% (n = 25, days = 5). Analytical sensitivity investigations indicate that this method would allow precise quantification (≤ 20%) at 0.075 µg/mL. Accuracy of the method was assessed by

analysing EQA samples from IPT Mycophenolate scheme (Bioanalytics, UK), the determined bias was ≤ 5.1% for all samples (2013 to 2014). Comparison with samples previously analyzed by an independent LC-MS/MS method demonstrated good agreement using Deming and linear regression (r = 0.998) analysis.

Conclusions: We have successfully quantified mycophenolic acid in plasma using PPE with UPLC-MS/MS analysis, for clinical research purposes. The method demonstrates good analytical sensitivity, linearity and precision with minimal matrix effects.