A UPLC-MS/MS METHOD FOR THE ANALYSIS OF PLASMA MYCOPHENOLIC ACID FOR CLINICAL RESEARCH

Michelle Wills¹, Gareth Hammond¹, Lisa Calton¹, Gary Chusney²
¹Waters Corporation, Stamford Avenue, Wilmslow, UK. ²Imperial College Renal & Transplant Centre, Hammersmith Hospital, London, UK.

INTRODUCTION

Here we present a UPLC-MS/MS method for the analysis of mycophenolic acid in human plasma for clinical research purposes. An analytically sensitive and precise method was developed using protein precipitation extraction (PPE).

METHODS

Materials
- Calibrators and controls were purchased from Recipe® (Munich, Germany).
- In-house calibrators were prepared using mycophenolic acid certified reference solution from Cerilliant® (Round Rock, TX) and pooled human plasma from Sera Laboratories (Haywards Heath, UK). The calibration range was 0.1–20 µg/mL.
- The stable labeled internal standard (²H₅) of mycophenolic acid was purchased from Cerilliant (Round Rock, TX).
- Method accuracy for mycophenolic acid was determined using external quality assessment samples obtained from an International Proficiency Testing Mycophenolate Scheme (Bioanalytics, UK).
- A comparison was performed using anonymized plasma samples previously analyzed using an independent LC-MS/MS method (n=35).

Methods
- Samples (50 µL) were pre-treated with methanol and zinc sulphate containing the internal standard.
- Following centrifugation, the supernatant was transferred to a 96-well 1 mL plate.
- Using a Waters ACQUITY UPLC® I-Class FTN System, samples were injected onto a 2.1 x 30mm Waters ACQUITY UPLC HSS C18 SB column using a water/methanol/ammonium acetate gradient and analyzed with a Waters Xevo® TQD, using the MRM transitions in Table 1.
- The analysis time per sample was approximately 3.0 minutes injection to injection.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRM Transition (m/z)</th>
<th>Cone (V)</th>
<th>Collision (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycophenolic acid (quantifier)</td>
<td>321.1 &gt; 207.1</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Mycophenolic acid (qualifier)</td>
<td>321.1 &gt; 159.1</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>Mycophenolic acid-²H₅</td>
<td>324.1 &gt; 210.1</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 1. MRM parameters used for the analysis of MPA and the internal standard.

RESULTS

Linearity and sensitivity
- Following CLSI-EP6-A, the method was shown to have a linear fit over the range of 0.1–20.0 µg/mL (n=4).
- Analytical sensitivity investigations indicate the method would allow precise quantification (≤ 20%) at 0.075 µg/mL (see Figure 1).

Figure 1. Example chromatogram of 0.075 µg/mL MPA (quantifier ion) showing the chromatographic separation of the metabolites, mycophenolic acid glucuronide (MPAG) and mycophenolic acid acyl glucuronide (AcMPAG).

Carryover
- No significant carryover was observed from high concentration injections into subsequent blank injections.

Precision
- Five replicates at each QC level were extracted once per day for five days (n=25).
- Total precision of low, mid and high QC samples was 3.6%, 3.5% and 5.3%, respectively.
- Repeatability of low, mid and high QC samples was 3.6%, 2.7% and 3.3%, respectively.

Selectivity
- The bias observed between control and test samples (spiked with high level of interference compound) for the quantitation of mycophenolic acid was < 10% for all interferences tested.
- Interference testing included the metabolites of mycophenolic acid, MPAG and AcMPAG as seen in Figure 1, as well as endogenous compounds (such as cholesterol and creatinine) and exogenous compounds (such as everolimus and tacrolimus).

Accuracy
- Samples were analysed from an International Proficiency Testing Mycophenolate Scheme and the data obtained was compared to the HPLC/MS method mean.
- The determined bias was ≤ 5.1% for all samples.

Method Comparison
- Ordinary linear fit comparison demonstrated r = 0.998. Altman-Bland analysis of the samples demonstrated a mean bias of ~0.05%.
- Comparison with samples analysed by an independent LC-MS/MS method was described by the Deming equation; y=0.90x + 0.13 (Figure 2).

Figure 2. A scatter plot with Deming fit for the reported values against the independent method values.

Matrix Effects
- The normalized matrix factor (using analyte:internal standard response ratio) was 0.97 and 0.96 for low and high QC concentrations respectively.
- Post-column infusion of mycophenolic acid demonstrated minimal ion suppression, shown in Figure 3.

Figure 3. Ion suppression trace of an extracted plasma sample (green), compared to a blank solvent injection (purple). The red trace shows the retention time of mycophenolic acid.

CONCLUSION

- A method for the quantification of mycophenolic acid in plasma using UPLC-MS/MS for clinical research purposes has been developed.
- The method demonstrates good analytical sensitivity, precision and accuracy with no significant carryover and minimal matrix effects.
- Chromatographic separation of the metabolites of mycophenolic acid is achieved.