Development and Validation of LC-MS/MS Methods for the Quantification of Biomarkers Tryptophan, Kynurenine and 3-Hydroxykynurenine in Human Plasma

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INTRODUCTION

- Tryptophan is an essential amino acid known for its crucial role in protein synthesis and as the precursor for several biologically active compounds such as kynurenine.
- Several pathological conditions are associated with the metabolism of tryptophan and the generation of its metabolites, and are linked to the development of a variety of diseases.
- Cancer patients tend to have tryptophan concentrations lower than normal and kynurenine concentrations higher than normal due to the increasing activity of enzyme indoleamine-2,3-dioxygenase (IDO) which catalyzes the first-limiting step of tryptophan degradation along the kynurenine pathway.
- The success of this work seeks to establish reliable robust accurate methods for the quantification of tryptophan, kynurenine, and 3-hydroxykynurenine in human plasma with limits of quantitation below the endogenous concentrations found in a healthy population.
- Quantification of abnormally low endogenous concentrations in human plasma required that calibration standards and low level QC be prepared in surrogate matrix (ultrapure water or 4X charcoal stripped plasma).

RESULTS

- For 3-OH kynurenine, analysis was performed using a Waters Acquity UPLC HSS T3 (100 x 2.1 mm)
- Positive ions generated by a TurboIonSpray source were monitored in the multiple reaction-monitoring (MRM) mode.
- Separate analytical methods were developed for tryptophan/kynurenine and 3-OH kynurenine.

QC Inter-Batch Accuracy Range (% Bias)
- 7.7 to 0.0%  -1.2 to -0.3%
- 6.7 to -1.3%

QC Inter-Batch Precision Range (% CV)
- 4.3 to 11.8% 2.8 to 5.1% 4.7 to 8.6%

QC Intra-Batch Precision Range (% CV)
- 1.7 to 10.9% 1.1 to 5.6% 1.7 to 16.2%

Figure 6. 3OH-Kynurenine Low Plasma QC

CONCLUSION/NOVEL ASPECT

Abnormally low concentrations of tryptophan, kynurenine and 3-hydroxykynurenine in human plasma can be measured accurately and precisely using robust and sensitive LC-MS/MS methods.

Table 1

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean Basal</th>
<th>150 µM</th>
<th>500 µM</th>
<th>Mean Basal</th>
<th>150 µM</th>
<th>500 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level +</td>
<td></td>
<td></td>
<td></td>
<td>Level +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3OH-Kynurenine Low Conc.</td>
<td>spike</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.100 µM</td>
<td>15.0 µM</td>
<td></td>
<td></td>
<td>11.30 nM</td>
<td>21.0 µM</td>
<td>29.0 µM</td>
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<tr>
<td>0.115 µM</td>
<td>15.0 µM</td>
<td></td>
<td></td>
<td>11.50 nM</td>
<td>21.1 µM</td>
<td>29.1 µM</td>
</tr>
<tr>
<td>0.230 µM</td>
<td>15.0 µM</td>
<td></td>
<td></td>
<td>11.70 nM</td>
<td>21.2 µM</td>
<td>29.2 µM</td>
</tr>
</tbody>
</table>

By utilizing a 3-period method the ratio data for tryptophan at low concentrations became reasonably acceptable even though the peak area variability increased.