Abstract

Shortly after the invention (1978) and commercialization (1980’s) of triple-quadrupole (QqQ) mass spectrometers, the instruments quickly became the reference platform for targeted quantitation of compounds in complex mixtures. Advantages of modern QqQ instruments include a multitude of ionization sources and scan modes (including timed-MSM), and relative ease of use and low cost. However, QqQ instruments are limited by the need to select specific precursor-to-product ion transitions for targeted quantitation. High-Resolution, Accurate-Mass (HRAM) instruments are able to be quantitative while scanning large mass ranges and provide an alternative strategy for quantitative metabolomics. Here, we compare HRAM-based quantification of specific metabolites using an Orbitrap Fusion mass spectrometer and an Agilent 6460 QqQ mass spectrometer as the reference method.

Experimental Highlights

• Culture Media: 5% fetal bovine serum and 2 mM glutamine were added to HyClone RPMI culture media to produce R5 media
• Metabolomic Cell Culture: OCIAR9/GADR ovarian cancer cells were incubated in R5 media at 37°C at 90% relative humidity and 5% CO2
• Culture Media Samples: Aliquots of R5 media were collected pre-seeding and at 24-hr, 48-hr, and 72-hr intervals post-seeding
• Metabolites Studied: 2-hydroxyglutarate, 2-oxoglutarate, aspartate, glutamate, glutamine, lactate, pyroglutamate, and pyruvate
• Internal Standards: [13C5]-glutamine, [13C3]-lactate
• Neat Calibration standards: 0.0244, 0.0977, 0.390, 1.56, 6.25, 25.0, and 100 µM
• Calibration range includes only those neat standards that had a S/N>5:1 for each metabolite.

Results

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Chromatographic Retention Time (RT), curve range, regression model and weighting factor, and detection limits for eight individual metabolites present in neat calibration standards.</th>
<th>10x Dilution Factor</th>
<th>LOD (mcM)</th>
<th>QqQ (mcM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-oxoglutarate</td>
<td>14.5</td>
<td>(0.0977 - 100) Quadratic (1/x2)</td>
<td>0.012</td>
<td>0.004</td>
</tr>
<tr>
<td>glutamine</td>
<td>11.5</td>
<td>(0.0977 - 100) Linear (1/x)</td>
<td>0.009</td>
<td>0.031</td>
</tr>
<tr>
<td>lactate</td>
<td>4.22</td>
<td>(0.0977 - 100) Quadratic (1/x)</td>
<td>0.016</td>
<td>0.059</td>
</tr>
<tr>
<td>pyroglutamate</td>
<td>8.61</td>
<td>(0.0977 - 100) Quadratic (1/x)</td>
<td>0.016</td>
<td>0.059</td>
</tr>
<tr>
<td>glutamine</td>
<td>11.5</td>
<td>(0.0977 - 100) Linear (1/x)</td>
<td>0.009</td>
<td>0.031</td>
</tr>
<tr>
<td>pyruvate</td>
<td>4.22</td>
<td>(0.0977 - 100) Quadratic (1/x)</td>
<td>0.016</td>
<td>0.059</td>
</tr>
</tbody>
</table>

Figure 1: Lactate and glutamine levels were above the limit of quantitation (ALQ) for all samples at the 10x dilution factor used for this study. Better correlation between the measurements probably could have been achieved by diluting the samples further for the Orbitrap Fusion analysis.

Future Directions

• Investigate the influence of source parameters on the Orbitrap Fusion and their effects on reliable quantitation.
• Evaluate additional metabolites using the existing workflow.
• Test performance across a variety of matrices including whole blood, plasma, urine, and tissue.

Acknowledgments

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References