### Materials and Procedures

**LCMS Systems**

Waters Alliance UPLC-Xevo G2 Q-TOF

Agilent 1100 HPLC/ESI-QQQ triple quadrupole

Cerilliant Stable Isotope Standards Used:

- 25-Hydroxyvitamin D3, Cat# M-131
- 25-Hydroxyvitamin D3 d5, Cat# M-107
- Testosterone, Cat# T-037
- THC-d5, Cat# M-146
- 3,4-MDPV-d3, Cat# M-137
- 9-THC, Cat# T-005
- d5-Testosterone, Cat# T-046
- d5-Mycophenolic acid, Cat# M-106
- d5-THC, Cat# H-074
- d5-25-Hydroxyvitamin D3

**Scrambling Method**

Centrifuged for 4 min at 3000 rpm

Waters Alliance UPLC-Xevo G2 Q-Tof (tandem quadrupole vs. quadrupole time-of-flight), matrix selection, are the most common and prevalent labeled internal standards. Various deuterated compounds are used to help identify scrambling in the clinical sample matrix with expected MS/MS transitions. Some deuterated compounds may yield similar MS/MS transitions in the clinical sample matrix. We used 25-Hydroxyvitamin D3 as a label.

**Evaluation of LCMS MS Deuterium Scrambling in Clinically Significant Small Molecules**

<table>
<thead>
<tr>
<th>Material</th>
<th>Label</th>
<th>Scrambling Method</th>
<th>Transitions</th>
<th>MS/MS</th>
<th>Scrambling Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-Hydroxyvitamin D3</td>
<td>d5</td>
<td>Direct infusion</td>
<td>20CE 21 (0.197) Cm (20:40) TOF</td>
<td>344.30ES+</td>
<td>0.1</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D3</td>
<td>d5</td>
<td>Direct infusion</td>
<td>20CE POS 30 (0.274) Cm (20:40) TOF</td>
<td>345.30ES+</td>
<td>0.2</td>
</tr>
<tr>
<td>Everolimus</td>
<td>d4</td>
<td>Direct infusion</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-(±)-11-nor-9-Carboxy-Delta9-THC</td>
<td>d3</td>
<td>Direct infusion</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JWH-073 3-Hydroxybutyl metabolite</td>
<td>d4</td>
<td>Direct infusion</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

- Scrambling was observed for several of the analytes at select transitions. In all cases, scrambling was mitigated or eliminated by optimizing instrument conditions and transition selection.
- Awareness of potential scrambling is important for proper internal standard selection.
- Scrambling was observed on both the Agilent 6410 triple quadrupole and the Waters Xevo G2 Q-TOF to approximately the same degree. For a specific transition, scrambling ratios were consistent between solvent and solvent. No matrix effects on scrambling.
- Direct infusion can provide rapid and accurate determination of scrambling ratios. Infusion and chromatographic injection results were consistent.
- Scrambling may be mitigated or eliminated by altering instrument conditions and transition selection. However, there is a need to consider potential impact of scrambling on transitions chosen for optimal sensitivity.
- Deuterated internal standards are a viable option for LC/MS/MS analysis with selection of the appropriate transition. Deuterated standards can be more cost effective than 13C labeled internal standards, more readily available and with lower cost per test. 13C labeled internal standards are most effective when deuterium scrambling issues can not be resolved.