A Superior Way to Measure Carryover

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Background

Challenge: Liquid Chromatography (LC) systems and autosamplers must yield sufficiently low carryover to accommodate the sensitivity and dynamic ranges associated with tandem mass spectrometric (MS/MS) detection. The traditional means of using blank samples to measure carryover in an LC-MS/MS system is rather flawed, often yielding results of questionable consistency and accuracy. This situation gets worse the closer the limit of quantitation (LLOQ) is to the actual limit of detection (LOD).

Current Practice: Carryover is typically evaluated by injecting a “carryover blank” sample immediately following injection of a sample at the upper limit of quantitation (ULOQ). Most commonly, bioanalytical SOPs require that target analyte response in a carryover blank sample be less than 20% of the response of a typical sample at the LLOQ concentration level. This practice can be concisely described by an injection order such as the following:

1. Carryover Blank (LUB)
2. ULOQ
3. LLOQ
4. Carryover LLOQ

Objective

We propose to measure carryover with a “carryover LLOQ” instead of a carryover blank, described by the injection order below. Ideally, the carryover LLOQ could be a re-injection of the first LLOQ, given sufficient sample volume, to normalize sample preparation variability.

LLOQ
ULOQ
Mimics LUB
Carryover LLOQ

Major Advantages

Any response due to carryover is within the range of the assay.

Precision and accuracy of the carryover LLOQ response is at least as good as those values corresponding to the LLOQ.

Automated integrations are more reliable near the LLOQ than at five times lower than the LLOQ, especially near the LOD.

A more precise and accurate carryover measurement can save valuable time and effort during development and troubleshooting.

Unbiased and/or automated integrations of such small peaks are challenging to perform consistently.

Imprecise and inaccurate results from a ‘LUB’ measurement can lead to inefficient troubleshooting activities addressing carryover.

Method

Chromatographic carryover can be variable and difficult to generate at a specific level for demonstration purposes. We opted to mimic carryover in a controlled manner. To provide some true baseline noise from bioanalytical sample extracts, we extracted 100 µL aliquots of drug-free human plasma (KEDTA) with 400 µL of acetonitrile via protein precipitation. After drying the blank extracts, we reconstituted with 100 µL of mobile phase containing drug at one of three concentrations (9 replicates each):

- 5.00 pg/mL to mimic an LUB
- 0.90 pg/mL to mimic a carryover blank with response equal to 18% that of the LLOQ
- 5.90 pg/mL to mimic a carryover LLOQ with response equal to 118% that of the LLOQ

We injected 10 µL onto a 2.1 x 50 mm HSS T3 column (Waters) using an Acquity UPLC™ system (Waters) flowing at 1.00 ml/min. Mass spectrometric detection occurred via a TSQ Vantage controlled by Watson TSQ Module 1.0 (Thermo Scientific). The LC gradient resulted in the drug peak eluting with a retention factor of greater than 3. No internal standard was used, as all comparisons were made with drug peak areas.

All integrations were performed in an automated fashion with identical parameters optimized for the LLOQ. The data processing software employed was Watson LIMS 7.4-SP2 (Thermo Scientific).

Results

<table>
<thead>
<tr>
<th>Sample (n=9 each)</th>
<th>Concentration (µg/mL)</th>
<th>%CV</th>
<th>Mean % of LLOQ Mean</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>“LLOQ”</td>
<td>5.00</td>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Carryover Blank” (Mimics LUB)</td>
<td>18% of LLOQ</td>
<td>13.4</td>
<td>21.6</td>
<td>6 of 9 Areas Error Less Above 20.0% of LLOQ Mean.</td>
</tr>
<tr>
<td>“Carryover LLOQ”  (Mimics LUL)</td>
<td>118% of LLOQ</td>
<td>5.8</td>
<td>116.0</td>
<td>2 of 9 Areas Error Above 20.0% of LLOQ Mean.</td>
</tr>
</tbody>
</table>

At the LLOQ, the acceptable level of bias is ± 20%. With our “carryover” level actually set at 18% of the LLOQ, both the LUB and LUL methods of evaluating carryover yielded some areas over 20.0% of the LLOQ mean. However the LUB data was significantly more precise and less accurate than the LUL data.

Conclusion

The proposed LUL approach to measuring carryover is by no means perfect, however it should always be superior to the traditional LUB approach. This is because LUL values are always within the range of the assay, making integration more accurate, consistent, and more easily automated.

Due to imprecision near the LLOQ, it may be advisable to modify the simple LUL approach described here to include additional replicates, depending on the application and how close the LLOQ is to the LOD.