Improving Multiple Reaction Monitoring (MRM) Selectivity for Mesalamine with differential mobility separation device (SelexION®) coupled with SCIEX Triple Quad™ 5500 LC-MS/MS system

SCIEX Triple Quad™ 5500 LC-MS/MS System and SelexION® technology

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Major Challenges for Mesalamine Bioanalytical Quantitation

- Addressing isobaric interferences from complex plasma matrix – Potential interferences originating from isobaric compounds present in complex plasma matrix.
- Improving Signal-to-Noise Ratio – High background noise in MRM mode in processed plasma sample.
- Ensuring better Data quality – Precision and accuracy statistics need to be compromised at lowest level of quantitation.

Key benefits of the differential mobility separation (SelexION®) for Mesalamine Quantitation

- Improved selectivity in MRM mode by eliminating the interfaces.
- Improvement in assay specificity through intelligent removal of isobaric interferences using SelexION® helps in achieving improved Signal-to-Noise Ratio.
- Data exhibits superior accuracy and precision.

SelexION® Technology for Bioanalytical Quantitation

- SelexION® Technology is a planar differential mobility separation device (DMS) that separates compounds based on difference in their chemical and structural properties.
- Planar geometry results in high speed and minimal diffusion losses for maximum sensitivity and UHPLC compatibility.
- SelexION® Technology adds an orthogonal level of separation and selectivity prior to the instrument orifice (Figure 2).
- SelexION® Technology is compatible with fast cycle times required for monitoring multiple MRM transitions combined with narrow HPLC peaks.
- Highly robust, reproducible, and stable for use in regulated bioanalysis.
- Easy to maintain, and can be installed or removed in minutes with no need to break vacuum or use any tools.

Figure 1: SCIEX Triple Quad™ 5500 system with SelexION®

Figure 2: SelexION® Technology on path component
INTRODUCTION

Development of an optimal bioanalytical method that addresses sensitivity, selectivity, and robustness challenges can be extremely challenging. These challenges are furthermore complicated by isobaric matrix interferences or high background noise levels. Resolving the matrix interference and lowering background noise in plasma require extensive sample preparations, which require significant investment of resources in time and cost. These challenges become more complicated if the analytical run times are reduced, which results in co-elution of isobaric interferences. Therefore, there is a strong demand for a technology that can use the power of differential ion mobility and separate analytes that experience isobaric matrix interferences.

In this study, we report the capability of SelexION® technology coupled with Triple Quad™ 5500 LC-MS/MS system for the method development and validation of Mesalamine in human plasma sample. Mesalamine, which is a derivative of salicylic acid is an anti-inflammatory drug used to treat inflammatory bowel disease, colitis, and Crohn’s disease. As a drug, Mesalamine is mainly found localized in gut, and exhibits few systemic side effects. However, as mentioned above quantitation of Mesalamine can be complicated owing to challenges from matrix interferences and hemolysis, which are caused by rupture of red blood cells releasing haemoglobin into plasma.

MATERIALS AND METHODS

Sample Preparation:

Plasma samples were spiked with Mesalamine and deuterated internal standard solution. The samples were derivatised with propionic anhydride at room temperature. The analytes were extracted from plasma by protein precipitation. Organic phase was collected and evaporated to dryness under nitrogen stream. The residue was reconstituted in mobile phase for quantitation using SCIEX Triple QuadTM 5500 LC-MS/MS system equipped with SelexION®.

HPLC Conditions:

The samples were analyzed using the Shimadzu LC system. Chromatographic resolution was achieved on Kromasil C18 (4.6 x 150 mm column, 3.5 µm) using 0.50 m/minute flow rate. Analyte elution was carried out using 5mM ammonium formate (pH: 3.0) and methanol (35:65). Total run time was optimised to 6 min. Injection Volume was 10 µl.

Mass Spectrometry:

An SCIEX Triple Quad™ 5500 system equipped with SelexION® Technology and a Turbo V™ source was used. DMS parameters were optimized for Mesaline in T infusion mode for the interest to maximize signal intensity and reduce the background interference. The mass spectrometry and DMS optimised conditions are given in Table 1.

Software

Data acquisition: Analyst® 1.6 Software

Table 1: Optimised Source, compound and DMS parameters for Mesalamine.

<table>
<thead>
<tr>
<th>Source</th>
<th>Compound</th>
<th>DMS</th>
<th>DMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curtain Gas</td>
<td>20</td>
<td>DP</td>
<td>100</td>
</tr>
<tr>
<td>CAD</td>
<td>7</td>
<td>EP</td>
<td>10</td>
</tr>
<tr>
<td>Ion Spray Voltage</td>
<td>5500</td>
<td>CE</td>
<td>19</td>
</tr>
<tr>
<td>Temp</td>
<td>550</td>
<td>CXP</td>
<td>10</td>
</tr>
<tr>
<td>GS1</td>
<td>60</td>
<td>MRM</td>
<td>210.2/192.1, 213.2/194.1</td>
</tr>
<tr>
<td>GS2</td>
<td>70</td>
<td>Dwell Time</td>
<td>200</td>
</tr>
</tbody>
</table>

Results and Discussion

Mesalamine Quantitation in Human Plasma

The mesalamine method was developed using positive electrospray ionisation. The mass spectrometric and SelexION® optimised parameters for analyte and IS are given in Table 1. Mesalamine was derivatised using propionic anhydride to achieve the best sensitivity under room temperature. Protein precipitation technique for sample preparation offers several benefits such as, ease-of-use as it is an extremely popular technique, method portability, and significant monetary savings. During the method development process, lots of background inference peaks were observed and effectively removed with the help of SelexION®. SelexION® also helped in increasing the signal-to-noise (~2X) by reducing the background noise in plasma samples (Fig 6). A linear calibration curve was constructed using the 1/X2 regression. The calibration curve for Mesalamine was linear over a dynamic range of 10.0 – 702.0 ng/mL in plasma sample (Fig 4) with an r value 0.9998. Two precision and accuracy batches were processed to evaluate the developed method. Results of Mesalamine for precision and accuracy in batch-1 were given in Table 2. Different QC level samples (n = 6) were also evaluated for precision and accuracy along with the calibration curve.

The Mesalamine eluted at 3.70 min in a 6 min isocratic chromatographic run time (Fig 5). The signal to noise ratio of Mesaline at LLOQ (10.0 ng/mL) in plasma is 14.2 calculated using sigma standard deviation of the baseline (Fig 5).
Figure 4: Calibration curve of mesalamine in human Plasma from 10.0-702 ng/ml using SelexION®

Table 2: Precision and accuracy statistics data for quality control samples for mesalamine in plasma using SelexION®

<table>
<thead>
<tr>
<th>Data</th>
<th>LLQC</th>
<th>LQC</th>
<th>MQC</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected Conc</td>
<td>10.00</td>
<td>30.10</td>
<td>246.00</td>
<td>526.00</td>
</tr>
<tr>
<td>Number of Values</td>
<td>6 of 6</td>
<td>6 of 6</td>
<td>6 of 6</td>
<td>6 of 6</td>
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<tr>
<td>Means</td>
<td>10.0203</td>
<td>29.3450</td>
<td>251.2695</td>
<td>531.5597</td>
</tr>
<tr>
<td>Stand Dev.</td>
<td>0.6389</td>
<td>0.6569</td>
<td>5.5114</td>
<td>23.5638</td>
</tr>
<tr>
<td>%CV</td>
<td>6.37</td>
<td>2.23</td>
<td>2.19</td>
<td>4.43</td>
</tr>
<tr>
<td>Accuracy</td>
<td>100.20</td>
<td>97.49</td>
<td>102.14</td>
<td>101.05</td>
</tr>
</tbody>
</table>

Figure 5: MRM Quantitation of Mesalamine in human plasma (A) without SelexION® technology (B) with SelexION® technology
Conclusion:

- Differential Mobility Separation (DMS) using SelexION® Technology provides an orthogonal level of selectivity by separating components and interfering ions based on their chemical properties and ion mobility.
- An LLOQ of 10 ng/mL for mesalamine was achieved using protein precipitated extraction method in human plasma.
- Matrix interference and background noise for the mesalamine was significantly reduced to improve selectivity and thus improved the signal to noise ratio.
- Signal to noise ratio was improved ~2X using SelexION® in human plasma.
- Precision and accuracy batch data for quality control (QCs) samples were meeting the requirements for regulated bioanalytical labs.
- DMS can also be used to improve sensitivity and selectivity in MRM when high background or interferences are significantly high.

Reference:

- Application of Differential Ion mobility Mass Spectrometry to Peptide Quantitation. SCIEX Notes Publication number: 9560314-01
- Analysis of Prostaglandin Isomers using the noval SelexION® Ion mobility Device SCIEX Notes Publication number: 4090111-01