Results and Discussion

Table 1. Triggered MRM acquisition table

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Precursor</th>
<th>Product</th>
<th>Primary Trigger</th>
<th>Threshold</th>
<th>Collision Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF2α</td>
<td>352.2</td>
<td>291.2</td>
<td>0.005</td>
<td>True</td>
<td>3.0</td>
</tr>
<tr>
<td>PGD2</td>
<td>352.2</td>
<td>291.2</td>
<td>0.005</td>
<td>True</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Figure 2: Chromatography in human serum

Figure 3: PGF2α calibration curves in neat solution and human serum

Figure 4: LTBA LLOD in both neat and serum.

Figure 5: Prostaglandins MRM spectra.

Results and Discussion

Triggered MRM (tMRM) method has been applied to this study. Except two primary MRM transitions for each analyte, 4 to 3 more compounds were analyzed and triggered when the primary transitions reach the threshold. These repeats are enabled during tMRM, which only takes about 1 second with 18 concurrent MRMAs. PGD2 and PGF2α are isotopic isomers and baseline separated by LC gradient.

Table 2. Summary

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD (nM)</th>
<th>LOQ (nM)</th>
<th>R²</th>
<th>LOD (ng/mL)</th>
<th>LOQ (ng/mL)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF2α</td>
<td>0.01</td>
<td>0.05</td>
<td>0.9981</td>
<td>0.05</td>
<td>0.05</td>
<td>0.9980</td>
</tr>
<tr>
<td>PGD2</td>
<td>0.01</td>
<td>0.05</td>
<td>0.9986</td>
<td>0.05</td>
<td>0.05</td>
<td>0.9980</td>
</tr>
<tr>
<td>LTBA</td>
<td>0.01</td>
<td>0.05</td>
<td>0.9987</td>
<td>0.05</td>
<td>0.05</td>
<td>0.9980</td>
</tr>
</tbody>
</table>

In the neat solution, the mass resolution of both Q1 and Q2 triple quadrupole acquisition combination MR are 0.7 amu. In the biological matrix, the Q2 resolution is narrowed down to 0.4 amu due to the complex and interference from the matrix, which is simply cleaned up by protein precipitation.

Conclusion

• Baseline separation of four target and other endogenous prostaglandins is achieved under 9 minutes.
• Isotopic isomers, PGD2 and PGF2α, are distinguished by LC separation as well as tMRM method.
• The calibration curve shows excellent linearity (R² > 0.998) with four orders of dynamic range in both neat solution and human serum matrix.
• Great accuracy, precision, reproducibility, and signal stability of LC-MS/MS (Q3D) analyses were observed for all target compounds.
• This fast and simple LC-MS/MS method is suitable for analyzing multiple prostaglandins in biological matrices in a single run.

Mass spectrometry has become an essential tool for small molecule quantitation due to its high sensitivity and specificity, excellent reproducibility and the ability to perform simultaneous analysis of multiple analytes. Prostaglandins can be challenging compounds to analyze due to the low levels in biological matrix relevant to clinical research. In order to address this challenge, a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the simultaneous analysis of four prostaglandins in serum samples has been developed without derivatization.

Bioisosteric of Eicosanoids

Figure 3: LTBA calibration curves in neat solution and human serum

Figure 4: LTBA LLOD in both neat and serum.

Figure 5: Prostaglandins MRM spectra.

MMR acquisition combines MRM with the generation of a product ion spectrum which can then be used for library identification and confirmation. As a result, MRM analysis decreases analysis time, increases throughput, and allows for fast, sensitive, quantitative and qualitative analysis on a single instrument, in a single analytical run. In a future study, a heart-cutting 2D-LC with highest separation power and larger injection volume will be applied to achieve a high sensitive quantitative analysis on challenging biological matrices.

Introduction

Experimental

Sample Preparation:
Sample information: Four prostaglandin standards are purchased from Cayman Chemical.
Calibration curve: The calibration range is from 0.005 to 100 ng/mL. The dilution solvent is acetonitrile.
Serum sample preparation: 250 µL human serum (obtained from UTAX Laboratories, Inc.) was spiked with 500 µL acetonitrile containing 1 nM and centrifuged for 4 min at 10 000 rpm. 500 µL supernatant was transferred and diluted with 500 µL of water. 2 µL is injected onto LC-MS/MS.

LC Method:
Mobile phase: A: 0.1% formic acid in water/acetoniitrile 70/30. B: acetoniitrile/isopropanol 50:50. Flow rate: 0.4 mL/min. Gradient: 0-100% B in 5 minutes and up to 100% B at 0.1 min, hold at 100% B for 2.0 min, post run in 2.0 min.

MS Method: