Introduction

In this research study, a robust, sensitive and relatively fast analytical method was developed for the quantitation of free testosterone in serum using a miniature Ultivo Triple Quadrupole LC/MS. Ultivo has been designed to address many challenges faced by routine analytical laboratories and this research study was conducted in order to assess how this novel triple quadrupole mass spectrometer (MS) could perform with a typical endogenous analyte of research interest. Innovative technologies within Ultivo allow us to reduce its overall physical footprint, while generating a comparable analytical performance level to similar, but physically larger MS systems presently on the market. Instrumentation innovations, such as VacShield, Cyclone Ion Guide, Dodecapole Vortex Collision Cell and small Hyperbolic Quads were designed to maximize quantitative performance from within a miniature package and also to enhance instrument reliability and robustness.

Moreover, Ultivo reduces the need for user intervention for system maintenance, making the system operation and maintenance manageable for non-expert MS users. MassHunter Software simplifies data acquisition, method set up, data analysis and reporting, which results in the fastest possible acquisition-to-reporting time, increasing lab productivity. Herein, this research study aims to outline typical confirmation performance of free testosterone in human serum using the Ultivo Tandem LC/MS. Lower limits of quantitation, chromatographic precision and calibration linearity, range and accuracy will be outlined.

Experimental

MS Method:
Agilent Ultivo triple quadrupole mass spectrometer
Ion mode: AJS positive
Gas temperature: 300 °C
Drying gas (nitrogen): 8 L/min
Nebulizer gas (nitrogen): 50 psi
Sheath gas (nitrogen): 380 °C
Sheath flow: 12 L/min
Capillary voltage: 3000 V
Nozzle voltage: 0 V
Cell Accelerate Voltage: 9 V
Q1/Q2 Resolution: 0.7/0.7 unit

Table 1. MRM acquisition table

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRM</th>
<th>Dwell (msec)</th>
<th>Fragments (V)</th>
<th>CE (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone-d3</td>
<td>292.2&gt;97.0</td>
<td>75</td>
<td>130</td>
<td>17</td>
</tr>
<tr>
<td>Testosterone</td>
<td>289.2&gt;109.1</td>
<td>75</td>
<td>130</td>
<td>18</td>
</tr>
<tr>
<td>Testosterone</td>
<td>289.2&gt;97.0</td>
<td>75</td>
<td>130</td>
<td>17</td>
</tr>
</tbody>
</table>

Results and Discussion

Sensitivity

Figure 1. Triplicates of serum blank and 1 pg/ml spiked

In this study, the d3 isotopically labelled internal standard of testosterone wasn’t premixed with calibrators. The above injector program was applied at the beginning of each injection. 19 μL of standard spiked calibrators were drawn, followed by needle wash. The injector then moved to the ISTD solution (pre-spiked in serum matrix) and drew 1 μL of the solution followed by needle wash again. The mixture was injected onto the column. To test the accuracy of this program, %RSD was calculated based on the response of ISTD MRM transition as 2.51.

Conclusions

This research project demonstrates that the performance of the novel Ultivo Triple Quadrupole LC/MS with the analytical methodology described herein generated excellent linearity, precision and analytical sensitivity across the range of 1 pg/mL through 200 ng/mL for free testosterone in human serum within an analysis cycle time of 6 minutes.

Future work will be required to assess potential interferences for this analytical method over a range of serum and blood matrices sourced from different suppliers and prepared for LC/MS analysis via a range of further sample preparation techniques.

References


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