THE ANALYSIS OF URINARY FREE CORTISOL BY ON-LINE SOLID PHASE EXTRACTION LC/MS/MS

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ABSTRACT

The objective of this study was to assess the feasibility of using an automated on-line solid phase extraction (SPE) tandem mass spectrometry technique for the analysis of UFC. A method for the direct analysis of cortisol in urine has been investigated using the Symbiosis™ Pharma / Quattro micro™ system. The method demonstrates excellent sensitivity (S/N of 15:1) and the cortisol determination of 10 replicate injections of a spiked 20 ng/mL cortisol standard was all acceptable (mean %RSD -4.4%).  The Passing–Bablok method comparison shows four samples that clearly fall outside the 95% confidence limits, further demonstrating the excellent performance of the Symbiosis™ Pharma / Quattro micro™ system. One of the forty patient samples analysed showed elevated cortisol levels of clinical significance 480 ng/mL (Figure 4).

METHODS

Sample preparation

Calibrators were prepared in urine-like electrolyte solution (24 h urine) over the concentration range 0.5-500 ng/mL. The method used for the analysis of cortisol in urine samples was as described previously. 20 µL of prepared samples were loaded on to the Oasis® HLB SPE cartridge (new cartridge per sample) and washed with 1 mL of water–2% NH₃.  The analytes were eluted from the cartridge using a gradient described in the HPLC Conditions section and identified by MRM tandem mass spectrometry.

RESULTS

Table 1. MRM Transitions and for cortisol and the internal standard.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transitions</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Coefficient (R²)</th>
<th>Limit of Quantification (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>MRM of 2 channels, ES+</td>
<td>325.3</td>
<td>243.1</td>
<td>0.9999</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>Internal standard</td>
<td>MRM of 2 channels, ES+</td>
<td>325.3</td>
<td>243.1</td>
<td>0.9999</td>
<td>10 ng/mL</td>
</tr>
</tbody>
</table>

Table 2. Intra– assay variation of three commercial quality control samples prepared independently.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/mL)</th>
<th>S.D.</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>12.38 ng/mL</td>
<td>0.60</td>
<td>4.83</td>
</tr>
<tr>
<td>37.68 ng/mL</td>
<td>2.48</td>
<td>6.60</td>
<td></td>
</tr>
<tr>
<td>109.46 ng/mL</td>
<td>4.24</td>
<td>3.82</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Inter– assay variation of three commercial quality control samples prepared independently.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/mL)</th>
<th>S.D.</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>12.38 ng/mL</td>
<td>0.36</td>
<td>2.88</td>
</tr>
<tr>
<td>37.68 ng/mL</td>
<td>0.60</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>109.46 ng/mL</td>
<td>3.72</td>
<td>3.39</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Linearity of the data was processed using Quantum™ software, the using the AxpeT™ integration algorithm. A linear fit was applied with a 1/x weighting. The correlation coefficient (R²) for cortisol was 0.9999 (Figure 2) and the correlation coefficient for the internal standard was 0.9999.  The linearity range was from 20 ng/mL to 1,000 ng/mL, using calibrators over the concentration range 0.5-500 ng/mL.

Accuracy

The calculated concentrations and the % deviations for the six QC samples were all acceptable (mean %RSD -4.4%).

Precision

Calibration line for cortisol. X= Calibrator std dev 0.36 0.60 3.72

Limit of Quantification

The limit of quantification (LOQ) was determined to be 10 ng/mL with a S/N of 15:1.

Recovery

Using the Automated Method Development (AMD) functionality of the Symbiosis™ system an extraction recovery experiment was carried out and the recovery was calculated to be 98.2%.

Carry Over

Carry over was determined by analysing a high cortisol standard (1000 ng/mL) followed by a water blank. The area for the water blank was divided by the area for the cortisol standard and expressed as a percentage to give a value of 0.02%.

Ion suppression

The ion suppression was assessed by infusing a solution of cortisol (100 ng/mL) at 50 µL per minute and monitoring the response when using the Symbiosis™ Pharma system.

CONCLUSION

The Symbiosis™ Pharma / Quattro micro™ system is a fast–method for the direct analysis of UFC has been investigated using the Symbiosis™ Pharma / Quattro micro™ system. The method demonstrates excellent sensitivity (S/N of 15:1), sensitivity and precision when injecting only 20 µL of urine.

The method developed was compared with a routine LC/MS/MS method. The Passing–Bablok method comparison shows four samples that clearly fall outside the 95% confidence limits, further demonstrating the excellent performance of the Symbiosis™ Pharma / Quattro micro™ system. One of the forty patient samples analysed showed elevated cortisol levels of clinical significance 480 ng/mL (Figure 4).

DISCUSSION

REFERENCES


Figure 1. Symbiosis™ Pharma / Quattro micro™ system.

Figure 2. Calibration line for cortisol. X= Calibrator

Figure 3. Passing-Bablok method comparison.

Figure 4. Chromatogram to show a patient with an elevated cortisol level (480.6 ng/mL).

Figure 5. Retention time of cortisol and the ion suppression chromatogram for the analysis of water and urine.

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