A Versatile Workflow to Measure Plasma Renin Activity and Aldosterone for Clinical Research Using Automated On-Line Extraction Coupled to LC-MS/MS

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INTRODUCTION

Researchers studying the renin-angiotensin-aldosterone system need efficient ways to measure renin activity and aldosterone concentration in plasma sample preparations. We report a workflow to accomplish this utilizing automated on-line extraction coupled to LC-MS/MS, which simplifies sample preparation.

METHODS

Plasma Renin Activity

In order to measure angiotensin I (Ang I), donor plasma specimens (100 µL), calibrators and Lyphochek hypertension markers controls (QCs, Bio-Rad Laboratories, Berkeley, CA) were prepared per Carter et al (1) and incubated at 37°C in the autosampler tray of a Thermo Scientific™ Prelude™ SPLC system. 25 µL injections were made at the beginning of the incubation and between 12 and 17 hours later into a Thermo Scientific™ Cycleone™ TurboFlow™ column (0.5 x 50 mm) on the same channel of the Prelude SPLC system. Extracted Ang I and its internal standard were automatically transferred to a 50 x 2.1 mm analytical column – either a Xbridge BEH C18, 5 µm (Waters Corp., Milford, CA) or a Thermo Scientific™ Accucore™ aQ, 2.6 µm column – heated to 30°C for final isolation using a mobile phase gradient from water to methanol, both containing 0.2% formic acid (Figure 1).

RESULTS

Plasma Aldosterone

The analytes were eluted into the heated electro-spray interface of a Thermo Scientific™ TSQ Endura™ mass spectrometer (MS) and subjected to positive-ion selected-reaction monitoring of their +3 charge states (Figure 2).

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Ang I amounts for each sample were measured using a 13C5, 18N1-labeled Ang I internal standard (AnaSpec Inc., Fremont, CA) and calibrators in each batch. Plasma renin activity of each specimen and QC was expressed as the increase in Ang I formed from initial to final incubation (ng/mL/hr) using the data/time stamp of respective data files.

TRADEMARKS/LICENSEING

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REFERENCES


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Table 1. Plasma Renin Activity (PRA) of QCs

<table>
<thead>
<tr>
<th>QC</th>
<th>Initial Ang I (ng/mL)</th>
<th>Final Ang I (ng/mL)</th>
<th>Difference (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC 1</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>QC 2</td>
<td>52</td>
<td>40</td>
<td>12</td>
</tr>
<tr>
<td>QC 3</td>
<td>100</td>
<td>90</td>
<td>10</td>
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</table>

PRA results for donor plasma samples have not yet been compared to those from a reference lab.