INTRODUCTION

Carbamazepine is one of the most often used anticonvulsant and mood-stabilizing drug. It is primarily used in the treatment of epilepsy and bipolar disorder. Carbamazepine-10,11-epoxide is known as a primary active metabolite of Carbamazepine and the presence of this "epoxide" metabolite can have clinically significant implications in therapeutic drug monitoring of carbamazepine. However, traditional methods such as immunoassays, GC-MS and mathematical conversions are low-throughput and less accurate. Therefore, it is highly desirable to have a simple, reliable, rapid, and accurate bioanalytical method for simultaneous determination of carbamazepine and carbamazepine-10,11-epoxide, which is essential for high sample throughput applications. Here, we report the development and validation of liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for determination of carbamazepine and carbamazepine-10,11-epoxide in human plasma.

METHODS

SAMPLE PREPARATION

50 µL of reference standards in K$_2$EDTA human plasma was mixed with 50 µL of internal reference standards (IS), and then extracted via Protein Precipitation with 400 µL of ACN:H$_2$O/100:0.1 (V:V) in 96-well plates. The plate was vortexed and centrifuged at 3000 rpm for 5 minutes, then 50 µL of the supernatant was transferred to the 96-well Collection Plate on Tomtec Quadra 4 and diluted for 9 times in Reconstitution solvent without drying down for injection onto the LC-MS/MS system.

Recon solvent: ACN: H$_2$O:1M Ammonium Acetate / 20:80:1 (V/V/V)

LC-MS/MS ANALYSIS

<table>
<thead>
<tr>
<th>Mass Spec</th>
<th>AB Sciex, API 4000, TIS +</th>
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</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>Shimadzu Nexera UHPLC</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>A: Water/Formic Acid / 100:0.1 (V/V)</td>
</tr>
<tr>
<td>B: Acetonitrile/Formic Acid / 100:0.1 (V/V)</td>
<td></td>
</tr>
<tr>
<td>Run time</td>
<td>2.5 minutes</td>
</tr>
<tr>
<td>Assay range</td>
<td>20-20,000 ng/mL for Carbamazepine 5-5,000 ng/mL for Carbamazepine-10,11-epoxide</td>
</tr>
</tbody>
</table>

RESULTS

Figure 3. Representative Chromatograms

Carbamazepine LLOQ 20 ng/mL

Carbamazepine-d$_{10}$ 2000 ng/mL

CONCLUSIONS

A simple, high-throughput, reliable and accurate bioanalytical method for simultaneous determination of carbamazepine and its active metabolite carbamazepine-10,11-epoxide with 100-fold assay range using LC-MS/MS was developed and validated under GLP compliance.

The validated method shows adequate selectivity, sensitivity, specificity, accuracy, and reproducibility.

The method has been successfully applied in a Phase I, multiple-dose PK study.

Stability Established in Human Plasma

- 24 hours in plasma at ambient temperature
- 2 hours in whole blood at ambient temperature
- 5 freeze/thaw cycles at -20 & -70 °C
- 113 days solution stock stability in Methanol at -20 °C
- 106 days spiking solution stock stability at 4 °C
- 96 days long-term storage stability in plasma at -20 & -70 °C

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