Determination of Serum Carbamazepine by Tandem Mass Spectrometry

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History

• Carbamazepine was developed by chemist Walter Schindler in Switzerland (1953) (1).

• It was first used for treatment of trigeminal neuralgia in 1962 and its anticonvulsant effect was discovered in 1963.

• Carbamazepine was approved by the FDA in 1974 for the treatment of epilepsy and is still one of the most widely prescribed antiepileptic drugs (2).
The molecule of carbamazepine (5H-dibenz(b,t)azepine-5-carboxamide) consists of two benzene rings, one seven-membered ring, one double bond and one amide group.

That is, carbamazepine is an iminostilbene derivative (3).

Carbamazepine is structurally different from other antiepileptics and it is structurally similar to the tricyclic antidepressant imipramine (4).
Carbamazepine used in the treatment of:

- Partial seizures,
- Generalized tonic-clonic seizures,
- Trigeminal neuralgia and other neuropathic pain syndromes
- Bipolar disorders (5)
• Similar to many antiepileptic drugs, therapeutic drug monitoring (TDM) of carbamazepine is routinely used to optimize dosing, with a recommended therapeutic range (6) (https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/37037).
• The quantitation of carbamazepine and its metabolite was performed using human matrices, like DBS, plasma, serum, urine, brain homogenates.
• Carbamazepine levels were measured with methods such as immunoassays, capillary electrophoresis (CE), micellar electrokinetic capillary chromatography (MEKC), high performance liquid chromatography, (HPLC) gas chromatography mass spectrometry (GC-MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) (7).
• In clinical laboratories, therapeutic drug monitoring of carbamazepine is usually conducted using commercially immunoassays on a suitable automated analyzer.

• However, these methods can suffer with non-specific interferences coming from related compounds, metabolites or matrix effects.

• Carbamazepine is metabolized by the liver to carbamazepine-10,11-epoxide (CBZE) and this metabolite lead to cross-reactivity with various immunoassays (8).
McMillin et al. reported significant discordance between carbamazepine concentrations determined by the ADVIA Centaur assay and the PETINIA assay. The cross-reactivity of epoxide is as high as 94% with the PETINIA immunoassay (Siemens Diagnostics). Such discordance may cause confusion in interpreting serum carbamazepine levels (9).
• Furthermore, CBZE is pharmacologically active and potentially toxic metabolite of carbamazepine.
• In general, epoxide represents 10-15% of carbamazepine concentration but epoxide concentration may be significantly elevated if carbamazepine is used in combination with phenytoin, phenobarbital, primidone, or valproic acid (10).
• Thus, CBZE monitoring is recommended;

• Concomitant administration of other drugs that induce hepatic oxidizing enzymes (eg, most antiepileptic drugs [with the exception of valproic acid and the benzodiazepines], propoxyphene)

• Concomitant administration of drugs that inhibit its breakdown such as valproic acid, felbamate, and lamotrigine

• High-dose carbamazepine therapy, especially in combination with the above conditions (6).

• However, there is no immunoassay to measure the level of epoxy metabolite (9).
• LC-MS/MS methods offer an improved specificity, sensitivity and have shown to be more accurate and precise.

• Consequently, they are considered as the “gold standard”.

• In addition, they allow measurement of metabolite levels.

• Our aim in this study was to develop a LC-MS/MS method to measure the levels of carbamazepine and its epoxy metabolite.
Material and Methods

API 3200 triple quadrupole mass spectrometer equipped with an electrospray ionization interface was used (Applied Biosystems/MDS Sciex) as detector.

Separation was carried out using a Phenomenex C18 HPLC column (50 mm x 4.6 mm, part no: 00B-4041-E0).
LC (Liquid Chromatography) Parameters

- The mobile phase A was containing 0.1% formic acid and HPLC grade water and the mobile phase B was containing 0.1% formic acid and acetonitrile.
- The flow rate was 1 mL/min.
- The column temperature was adjusted to 40 °C and the injection volume was adjusted 40 μl.
Table 1. MRM table for carbamazepine and CBZE.

<table>
<thead>
<tr>
<th></th>
<th>Q1</th>
<th>Q3</th>
<th>Time</th>
<th>DP</th>
<th>EP</th>
<th>CE</th>
<th>CXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>237</td>
<td>194</td>
<td>400</td>
<td>30</td>
<td>10</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>Carbamazepine 10,11-epoxide</td>
<td>253</td>
<td>210</td>
<td>400</td>
<td>40</td>
<td>10</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>324.3</td>
<td>110.1</td>
<td>400</td>
<td>30</td>
<td>10</td>
<td>25</td>
<td>6</td>
</tr>
</tbody>
</table>

DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential, Q1: precursor ion m/z, Q3: product ion m/z values.

Table 2. Other LC-MS/MS Parameters for carbamazepine and CBZE.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CUR (Curtain gas)</td>
<td>10</td>
</tr>
<tr>
<td>CAD (Collision activated dissociation)</td>
<td>5</td>
</tr>
<tr>
<td>IS (Ionspray voltage)</td>
<td>5500</td>
</tr>
<tr>
<td>TEM (Temperature)</td>
<td>600</td>
</tr>
<tr>
<td>GS1 (Ion Source Gas 1)</td>
<td>40</td>
</tr>
<tr>
<td>GS2 (Ion Source Gas 2)</td>
<td>60</td>
</tr>
</tbody>
</table>
Sample Preparation

• 100 μL of the internal standard (gliclazide) and 500 μL of acetonitrile included 0.1 % formic acid was added on a standard solution or sample then vortexed for 30 s.

• This mixture was centrifugated at 12 000 rpm for 10 min. The supernatants were taken into glass tubes and evaporated with nitrogen gas. The residue was dissolved in 200 μL of in the mixture of acetonitrile:water (50:50;v/v) then injected into LC-MS/MS system.
Results

- The calibration curve was administered at a range of 0.15 to 80 µg/ml for carbamazepine.
- Detection limit (LOD) and quantitation limit (LOQ) were 0.15 µg/ml and 0.3 µg/ml, respectively.
- The retention time was determined as 2.50 min for carbamazepine.
- Total run time was 5 minutes.
XIC of +MRM (12 pairs): 259.300/214.000 Da ID: ADMA from Sample 19 (019) of carbamazepine kiyas 1 03092019.wiff (Turbo Spray) Max. 5.0 cps.

XIC of +MRM (12 pairs): 324.300/110.100 Da ID: glik from Sample 19 (019) of carbamazepine kiyas 1 03092019.wiff (Turbo Spray) Max. 1.3e5 cps.

XIC of +MRM (12 pairs): 253.000/210.000 Da ID: epoks from Sample 19 (019) of carbamazepine kiyas 1 03092019.wiff (Turbo Spray) Max. 5.4e4 cps.

XIC of +MRM (12 pairs): 237.000/194.000 Da ID: karb from Sample 19 (019) of carbamazepine kiyas 1 03092019.wiff (Turbo Spray) Max. 1.6e5 cps.
Conclusions

• In the following period, the validation studies of the active metabolite and carbamazepine will be completed by obtaining the standard of the active metabolite.

• After completion of all these studies, we think that we will contribute to routine drug level monitoring by measuring both carbamazepine and active metabolite levels.
References


6- https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/37037


