

A Simple, Reliable and Rapid LC-MS/MS Method for Simultaneous Determination of Carbamazepine and Carbamazepine-10,11-epoxide in Human Plasma

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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 μ L of reference standards in K₂EDTA human plasma was mixed with 50 μ L of internal reference standards (IS), and then extracted via Protein Precipitation with 400 μ L of ACN:FA/100:0.1 (V:V) in 96-well plates. The plate was vortexed and centrifuged at 3000 rpm for 5-mins, then 50 μ L 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: H₂O:1M Ammonium Acetate / 20:80:0.1 (V:V:V)

LC-MS/MS ANALYSIS

Mass Spec: AB Sciex, API 4000, TIS +
HPLC: Shimadzu Nexera UHPLC
HPLC column: Luna 3 μ C18 (2) 100Å 2.0 \times 30 mm, Phenomenex
Mobile Phase:
A: Water: Formic Acid / 100:0.1 (V:V)
B: Acetonitrile:Formic Acid / 100:0.1 (V:V)
Run time: 2.5 minutes
Assay range: 20-20,000 ng/mL for Carbamazepine
5-5,000 ng/mL for Carbamazepine-10,11-epoxide

Figure 1. Structures of the Tested Substrates

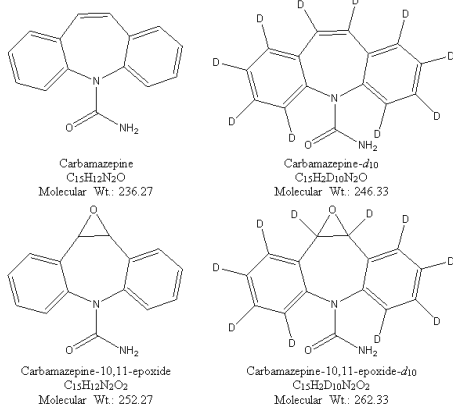


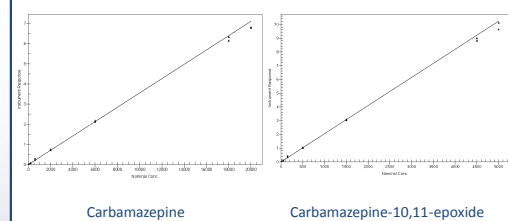
Table 1. LC-MS/MS Parameters

Compound	m/z transition	Dwell Time (msec)	DP (V)	CE (V)
Carbamazepine	237.0 \rightarrow 194.0	80	30	48
Carbamazepine-d ₁₀	247.0 \rightarrow 204.0	80	30	48
Carbamazepine-10,11-epoxide	253.0 \rightarrow 210.0	80	45	24
Carbamazepine-10,11-epoxide-d ₁₀	263.0 \rightarrow 220.0	80	45	24

UHPLC System		Shimadzu Nexera	
Column	Luna C18 (2), 2.0 \times 30 mm, particle size 3 μ m		
Column Temperature (°C)	40		
Injection Volume (μ L)	5		
Flow Rate (mL/min)	0.4		
LC Gradient Time (min)	A: Water/Formic Acid (100/0.1 V:V)	B: ACN/Formic Acid (100/0.1 V/V)	
0.0	80%	20%	
0.4	30%	70%	
1.2	5%	95%	
1.6	5%	95%	
1.7	80%	20%	
2.0	80%	20%	

LC-MS/MS	API 4000, AB Sciex	
Collision Gas (CAD)	8 psig N ₂	
Curtain Gas (CUR)	30 psig N ₂	
Ion Source Gas 1 (GS1)	55 psig N ₂	
Ion Source Gas 2 (GS2)	55 psig N ₂	
Ion Spray Voltage (IS)	5000 V	
Temperature (TEM)	450 °C	
Entrance Potential (EP)	10 V	
Scan duration	2.0 min	

Figure 2. Representative Calibration Curves
Regression Method = Linear, Weighting Factor = 1/X²



RESULTS

Figure 3. Representative Chromatograms

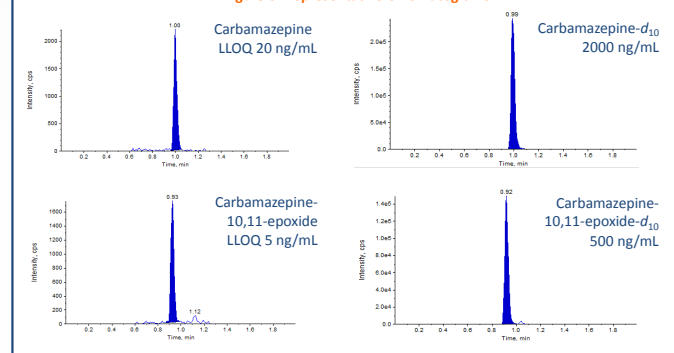


Table 2. Validation Summary Table

Analyte Name	Carbamazepine	Carbamazepine-10,11-epoxide
Average Recovery of Analyte (%)	97.5	97.7
LLOQ QC Intraday Precision Range (%CV)	5.6 to 8.6	3.4 to 12.1
LLOQ QC Intraday Accuracy Range (%RE)	-6.0 to 4.5	-6.4 to 1.4
Analytical QC Intraday Precision Range (%CV)	1.0 to 5.8	0.9 to 4.9
Analytical QC Intraday Accuracy Range (%RE)	-2.5 to 10.5	-7.0 to 8.5
LLOQ QC Interday Precision (%CV)	8.3	8.1
LLOQ QC Interday Accuracy (%RE)	0.5	-2.2
Analytical QC Interday Precision Range (%CV)	2.8 to 6.0	3.6 to 5.2
Analytical QC Interday Accuracy Range (%RE)	-1.3 to 6.4	-3.5 to 4.7

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
- 111 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

CONCLUSIONS

- A simple, high-throughput, reliable and accurate bioanalytical method for simultaneous determination of carbamazepine and its active metabolite carbamazepine-10,11-epoxide with 1000-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.