

# **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.

## **Method**

Carbamazepine, Carbamazepine-10,11-epoxide, and their deuterated internal standards were extracted from human plasma using protein precipitation extraction with acetonitrile as the extraction solvent. The supernatant was diluted for 9 times in reconstitution solvent without drying down for injection onto the LC-MS/MS system. Chromatographic separation was performed on a Luna C18(2) 3 $\mu$ m, 30x2.0 mm column (Phenomenex) with gradient elution on Nexera LC-30AD UHPLC (Shimadzu). MS/MS detection was performed on a API4000 (AB Sciex) under positive turbo-ion spray ionization. The analytes were quickly eluted within 1 minute with good separation and total analysis per injection is less than 2.5 minutes.

## **Preliminary Data**

The assay was successfully validated for carbamazepine and carbamazepine-10,11-epoxide over a range of 20/5 to 20000/5000 ng/mL in human plasma, respectively. The assay performance was characterized by intra-day precision of 1.0 to 5.8% and 0.9 to 4.9%, intra-day accuracy of -2.5 to 10.5% and -7.0 to 8.5%, inter-day precision of 2.8 to 6.0% and 3.6 to 5.2%, and Inter-day accuracy of -1.3 to 6.4% and -3.5 to 4.7% for carbamazepine and carbamazepine-10,11-epoxide, respectively. The average recovery was 97.5% and 97.7%, and the IS normalized matrix factor was 1.00 and 1.02 for carbamazepine and carbamazepine-10,11-epoxide, respectively. Experiments were conducted to evaluate the stability in human plasma at ambient temperature, through freeze/thaw cycles, in solvent (ambient temperature and frozen), and following long-term storage at -20°C and -70°C, as well as stability in human whole blood and 2% hemolyzed human plasma. Both analytes proved to be stable under all conditions evaluated. This validated assay showed adequate selectivity, sensitivity, accuracy, and reproducibility for the simultaneous determination of carbamazepine and carbamazepine-10,11-epoxide in human plasma.

**Novel Aspect**

Simple extraction procedure, high-throughput, reliable and accurate bioanalytical method using LC-MS/MS