

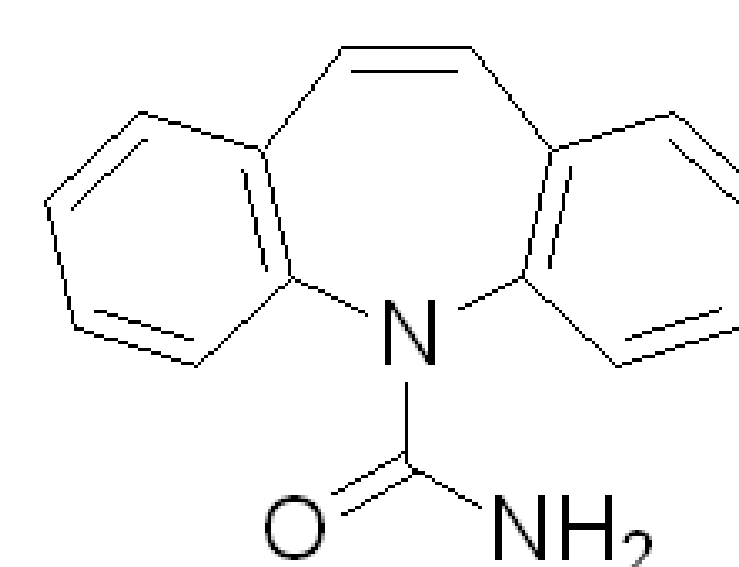
An LC-MS/MS Method for the Quantification of Carbamazepine in Cerebrospinal Fluid Utilizing Methyl-Carbamazepine as an Internal Standard

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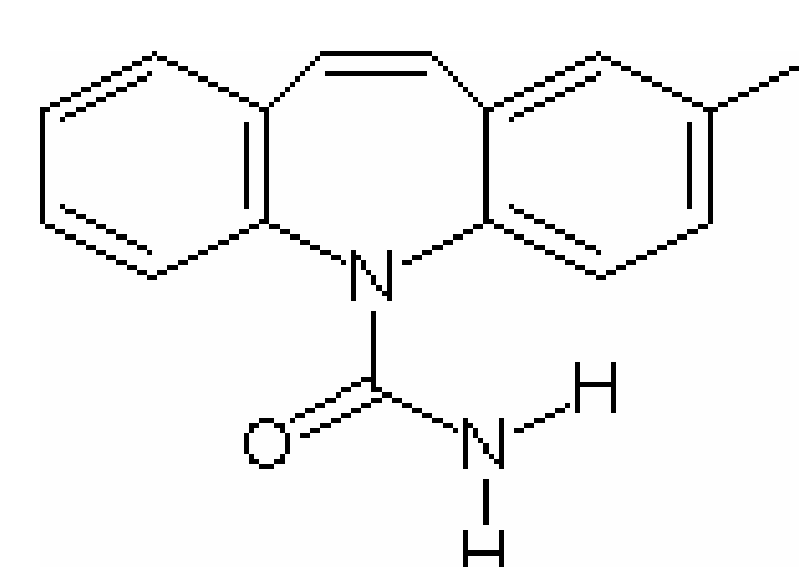


Introduction:

Carbamazepine (CBZ) is an anticonvulsant used for the treatment of generalized tonic-clonic and both simple and complex partial seizures. As its primary site of action is in the brain and spinal cord, the pharmacokinetic profile of CBZ in cerebrospinal fluid (CSF) is of interest. A rapid, sensitive and specific LC-MS/MS method for the determination of CBZ in CSF is described. Methyl-carbamazepine (m-CBZ) was used as an internal standard (IS) in the assay. The data show that the assay has good precision and accuracy over a wide dynamic range (0.05 to 100 ng/mL).



CBZ



m-CBZ

Methods:

Calibration standards and quality control (QC) samples were prepared in artificial CSF (aCSF). Standards and QC samples in aCSF were directly injected onto an LC-MS/MS. The LC-MS/MS system consisted of an Agilent 1100 HPLC system coupled to a Sciex API 4000 tandem quadrupole mass spectrometer equipped with a Turbo IonSpray ionization source. Analyte separation was conducted with a Zorbax XDB-C18 column (4.6 x 30 mm, 3.5 μm). Mobile phase (40:60 10 mM ammonium formate, pH 3.0: methanol) was delivered under isocratic conditions at 0.5 mL/min. Ionization was achieved in the positive ion mode. Nitrogen was used as the nebulizing, collision and exhaust gas. The mass spectrometer was operated in multiple reaction monitoring mode.

Preliminary Results:

CBZ and m-CBZ produced protonated precursor ions at mass to charge ratios (m/z) of 237.0 and 251.1, respectively, and corresponding product ions at m/z of 194.1 and 208.4, respectively. On the basis of a signal- to-noise ratio of 10, the limit of quantification (LOQ) of CBZ was found to be 0.05 ng/mL (0.25 pg injected on-column). No matrix-related interferences were observed at the retention time of the analyte in either blank aCSF or blank aCSF with IS. The calibration curve was linear with correlation coefficients (r) of >0.998 over the dynamic range.

Table 1. Summary of Inter-Assay Calibration Curve Statistics for Carbamazepine (0.05 – 100 ng/mL) in aCSF

Run ID	Slope	Intercept	Correlation Coefficient (r)
A/P 1	0.107	0.00290	0.9984
A/P 2	0.101	0.00150	0.9984
A/P 3	0.0937	0.00200	0.9983
n	3		3
Mean	0.101		0.9984
SD	0.00666		
% CV	6.62		

Table 2. Calibration Standards Summary for Carbamazepine in aCSF

Test ID	Nominal Concentration (ng/mL)						
	STD 1 0.0500	STD 2 0.100	STD 3 0.500	STD 4 2.50	STD 5 10.0	STD 6 50.0	STD 7 100
	Measured Concentration (ng/mL)						
Test 1	0.0510	0.0980	0.496	2.76	9.98	47.0	98.1
Test 2	0.0500	0.101	0.486	2.76	9.50	48.6	99.5
Test 3	0.0500	---	0.501	2.77	9.84	47.9	94.9
Test 4	0.0490	0.100	0.515	2.62	10.6	46.3	91.1
Test 5	0.0500	0.101	0.510	2.66	10.7	46.8	93.0
n	5	4	5	5	5	5	5
Mean	0.0500	0.100	0.503	2.71	10.1	47.32	95.3
SD	0.000707	0.00248	0.0127	0.0691	0.512	0.920	3.48
% CV	1.41	0.00141	2.53	2.55	5.06	1.94	3.66
% RE	0.000	0.000	0.600	8.56	1.24	-5.36	-4.68

--- denotes rejected due to poor accuracy (>15%).

Table 3. Intra-Assay Accuracy and Precision for Carbamazepine in aCSF

Replicate ID	Nominal Concentration (ng/mL)			
	QC LOQ 0.0500	QC LOW 0.0750	QC MED 6.25	QC HIGH 75.0
	Measured Concentration (ng/mL)			
Rep 1	0.0480	0.0750	7.03	75.3
Rep 2	0.0510	0.0740	6.97	73.3
Rep 3	0.0480	0.0750	6.99	73.5
n	3	3	3	3
Mean	0.0490	0.0747	7.00	73.1
SD	0.00173	0.000577	0.0306	1.10
% CV	3.53	0.773	0.437	1.49
% RE	-2.00	-0.444	11.9	-1.20

Table 4. Inter-Assay Accuracy and Precision for Carbamazepine in aCSF

Assay Day	Nominal Concentration (ng/mL)			
	QC LOQ 0.0500	QC LOW 0.0750	QC MED 6.25	QC HIGH 75.0
	Average(n=3) Measured Concentration (ng/mL)			
Day 1	0.0500	0.0700	6.30	76.5
Day 2	0.0560	0.0770	5.96	72.2
Day 3	0.0460	0.0690	6.08	74.5
n	3	3	3	3
Mean	0.0507	0.0720	6.11	74.4
SD	0.00503	0.00436	0.172	2.15
% CV	9.93	6.05	2.82	2.89
% RE	1.33	-4.00	-2.19	-0.800

Figure 1. Representative Chromatograms and Calibration Curve for CBZ in aCSF

