Quantitation of Antiepileptics in Serum by UHPLC-Triple Quadrupole Mass Spectrometry

This study demonstrates a simple, rapid, and reliable method for the simultaneous quantitation of 26 antiepileptics in serum using the Bruker Elute UHPLC coupled to the EVOQ LC-TQ Elite MS/MS system. Sample preparation was performed via protein precipitation using the ClinMass TDM kit system.

Introduction

Antiepileptics, also known as anticonvulsants, are used for the treatment of epileptic seizures which are characterized by convulsions and reduced consciousness. A person undergoing a seizure is at risk of severe injury due to falling or striking other objects. As seizures can happen at any time and often result in significant muscle fatigue, epilepsy causes both psychological and physical strain. Due to the repeating rhythm of the seizures, antiepileptics must be taken permanently, and the best effects are observed at a stable blood concentration. As each individual metabolizes drugs at a different rate, it is important to regularly monitor drug (and often metabolite) concentrations in blood to ensure the correct dosage.

Keywords:
Antiepileptics, serum, quantitation, therapeutic drug monitoring

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The method described in this manuscript focuses on the rapid and reliable quantitation of 26 anti-epileptics in human serum by UHPLC-triple quadrupole mass spectrometry. The drugs quantitated belong to dramatically different substance groups, including carbamates, carboxamides, fatty acids and barbiturates.

Experimental

The analysis was performed on an EVOQ LC-TQ Elite MS/MS system coupled to an Elute UHPLC using the ClinMass® TDM Platform (RECIPE Chemicals + Instruments GmbH, Munich, Germany) which included the mobile phases, autosampler washing solution, and precipitation reagent, as well as the HPLC column with prefilter. The serum calibrators and quality control samples were from the ClinMass add-on set for antiepileptics (MS9200), also provided by RECIPE.

Sample Preparation

Following the protocol of the ClinMass kit, 100 µL precipitation reagent containing the isotopically labelled internal standards were added to 50 µL serum samples and vortexed for 30 seconds. After centrifugation for 5 minutes at 10,000 x g, 50 µL of the supernatant was transferred to an HPLC vial, diluted 1:20 with 950 µL dilution solution D (RECIPE) and analyzed by LC-MS/MS.

Table 1: Mass Spectrometry Method Conditions

Liquid Chromatography	
Instrument	Bruker Elute UHPLC
Column	ClinMass® TDM MS9030 with Prefilter MS9032 (RECIPE)
Mobile Phase A	Included within the ClinMass MS9000 Kit (RECIPE)
Mobile Phase B	Included within the ClinMass MS9000 Kit (RECIPE)
Gradient	0.0 – 0.03 min 0% B 0.03 – 0.04 min to 13% B 0.04 – 1.3 min 13% B 1.3 – 1.31 min to 21% B 1.31 – 2.3 min 21% B 2.3 – 2.8 min to 50% B 2.8 – 3.3 min 50% B 3.3 – 3.4 min to 80% B 3.4 – 3.6 min 80% B 3.6 – 3.7 min to 0% B 3.7 – 4.5 min 0% B
Flow Rate	600 µL/min
Injection Volume	5 µL
Column Oven	40°C
Mass Spectrometry	
Instrument	EVOQ LC-TQ Elite MS/MS system
Ion Source	VIP H-ESI, 4500 V (positive), 4000 V (negative)
Probe Gas	50 units at 350°C
Cone Gas	20 units at 350°C
Nebulizing Gas	50 units
Active Exhaust	on
Collision Gas	Argon, 1.5 mTorr
MRM Transitions	see Table 2

Results and Discussion

Following fast and simple sample preparation, requiring only 50 µL of serum, the chromatographic separation of the 26 antiepileptics was performed within 4.5 minutes using the Elute UHPLC system. Figure 1 illustrates an overlay of the MRM traces for all measured analytes. Due to the fast polarity switching of the instrument, it was possible to analyze compounds in ESI positive and negative mode in the same run.

The quantitation of the analytes was performed using 17 isotopic labelled internal standards. Calibration curves included three calibrator levels and provided excellent linearity with R^2

Table 2: Retention times, MRM transitions, calibration ranges, and coefficient of determination R^2

Analyte	Retention time (min)	Precursor Ion	Product Ion 1	CE 1 (V)	Product Ion 2	CE 2 (V)	Polarity	Calibration Range [mg/L]	R^2
10-OH-Carbamazepine	2.20	255.0	194.0	19	165.1	50	+	2.66 – 43.7	0.9999
Carbamazepine	3.29	237.0	194.1	17	165.1	45	+	1.39 – 20.7	0.9998
Carbamazepine-Diol	1.96	271.0	180.1	28	210.1	12	+	0.560 – 9.35	0.9993
Carbamazepine-Epoxide	2.58	253.0	180.0	24	210.0	11	+	0.588 – 9.41	0.9993
Ethosuximide	1.23	140.1	42.3	19			-	7.47 – 119	0.9996
Felbamate	1.92	239.0	117.1	13	65.2	55	+	6.71 – 106	0.9992
Gabapentin	0.81	172.0	137.0	13	55.1	21	+	1.70 – 26.8	0.9999
Lacosamide	1.70	251.0	91.1	16	74.1	19	+	0.835 – 13.8	0.9991
Lamotrigine	2.09	255.9	58.2	27	43.3	69	+	1.49 – 23.3	0.9983
Levetiracetam	0.89	171.0	126.0	12	69.2	25	+	4.10 – 69.4	0.9989
N-Desmethyl methsuximide	2.53	188.0	42.3	17			-	3.43 – 50.9	0.9978
Oxcarbazepine	2.86	253.0	180.0	29	235.9	10	+	0.291 – 5.14	0.9983
Perampanel	3.84	350.0	218.9	33	246.9	23	+	0.0927 – 1.50	1.0000
Phenobarbital	2.32	231.0	42.3	12			-	3.45 – 51.1	1.0000
Phenylethyl malonamide	1.11	207.0	91.1	23	117.0	22	+	0.753 – 12.3	0.9997
Phenytoin	3.29	253.0	104.1	31	182.1	14	+	1.84 – 27.8	0.9998
Pregabalin	0.81	160.0	142.1	7	97.1	12	+	0.539 – 9.42	0.9996
Primidone	1.49	219.0	91.1	24	162.0	10	+	1.65 – 29.3	0.9994
Retigabine	3.53	304.0	109.0	29	230.0	16	+	0.133 – 2.37	0.9978
Rufinamide	2.06	239.0	127.0	16			+	3.17 – 45.8	1.0000
Stiripentol	4.00	217.0	187.0	8	159.0	12	+	1.18 – 17.8	0.9998
Sulthiamine	1.64	289.0	224.9	16	132.0	24	-	0.915 – 13.1	0.9999
Tiagabine	3.33	376.0	246.9	16	149.0	23	+	0.0203 – 0.332	0.9997
Topiramate	2.94	338.0	78.1	22	96.0	20	-	1.17 – 17.7	0.9931
Valproic Acid	1.75	143.1	143.0	5			-	8.36 – 119	0.9987
Zonisamide	1.76	211.0	119.0	12	147.0	5	-	2.97 – 43.5	0.9998

Table 3: Quantitative results, bias, and relative standard deviation of fourfold measurement of Quality Controls I and II

Sample	QC I				QC II			
Analyte	**Specified Value [mg/L]**	**Actual Value [mg/L]**	**Bias [%]**	**RSD [%]**	**Specified Value [mg/L]**	**Actual Value [mg/L]**	**Bias [%]**	**RSD [%]**
10-OH-Carbamazepine	7.68	8.24	7.3	3.6	19.4	17.5	-9.6	7.4
Carbamazepine	4.06	4.10	1.1	4.0	9.24	9.42	2.0	2.4
Carbamazepine-Diol	1.62	1.74	7.4	2.1	3.85	4.06	5.5	8.0
Carbamazepine-Epoxide	1.66	1.86	11.8	5.0	3.94	4.34	10.0	3.4
Ethosuximide	21.2	20.1	-5.1	3.0	50.7	48.3	-4.7	7.4
Felbamate	18.8	20.5	9.3	1.3	45.4	48.0	5.8	1.6
Gabapentin	4.65	5.05	8.6	2.9	11.0	11.8	6.9	2.3
Lacosamide	2.43	2.57	5.9	1.9	5.66	6.11	7.9	1.7
Lamotrigine	4.17	4.45	6.6	4.0	9.86	10.6	7.4	1.8
Levetiracetam	11.9	12.6	5.7	3.1	29.1	30.2	3.8	0.3
N-Desmethylmethsuximide	8.24	9.35	13.5	0.5	19.7	21.5	9.1	3.4
Oxcarbazepine	0.874	0.936	7.0	4.1	2.16	2.24	3.9	1.7
Perampanel	0.281	0.282	0.2	6.7	0.63	0.69	8.5	5.6
Phenobarbital	9.65	9.76	1.2	1.2	22.7	22.6	-0.6	3.4
Phenylethylmalonamide	2.11	2.23	5.7	2.6	5.03	5.25	4.4	2.9
Phenytoin	4.91	4.50	-8.4	8.3	11.2	10.3	-7.8	14.3
Pregabalin	1.51	1.65	9.2	3.4	4.02	4.04	0.5	1.0
Primidone	4.67	5.08	8.8	3.2	11.3	11.8	4.5	4.4
Retigabine	0.388	0.424	9.1	2.3	0.96	0.99	3.5	2.0
Rufinamide	8.93	9.02	1.0	2.5	20.8	21.0	1.0	3.6
Stiripentol	3.32	3.35	1.0	2.5	7.83	8.12	3.7	3.5
Sulthiamine	2.43	2.50	2.7	3.4	5.74	5.94	3.4	4.0
Tiagabine	0.060	0.061	0.8	1.0	0.140	0.140	-0.7	4.1
Topiramate	3.26	3.39	4.1	4.2	7.74	7.89	1.9	8.0
Valproic Acid	22.6	24.2	7.2	2.5	52.9	53.2	0.5	2.7
Zonisamide	8.29	8.95	8.0	1.6	19.8	20.6	3.9	0.7

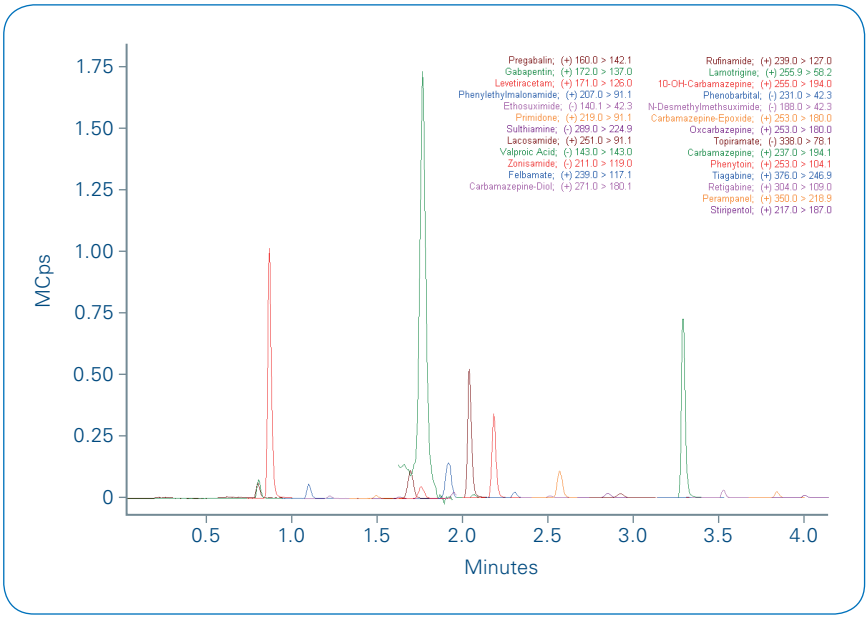

Figure 1: Overlaid MRM traces of all analytes (lowest calibrator level)

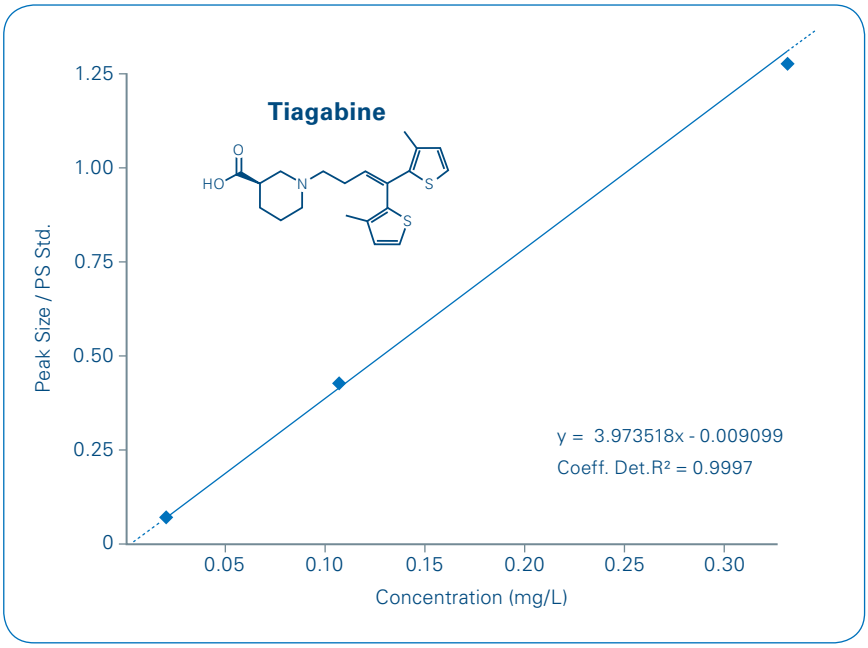

Figure 2: Calibration curve of tiagabine. Calibration range 0.0203 – 0.332 mg/L

values from 0.9931 – 1.0000 (Table 2). The calibration curve of tiagabine is shown as an example in Figure 2. Quality controls (QC) in serum with a low (QC I) and a high (QC II) concentration were measured four times. The RSD of < 9% for all analytes (with the exception of phenytoin QC II, which had an RSD of 14.3%) underlines the very good precision of the instrumentation used. The experiments also showed a high accuracy with a bias within ± 10% for most of the measured analytes (highest value 13.5%). Detailed results are presented in Table 3. The values for precision and accuracy are well within the range required by common guidelines for quantitative results.

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Conclusion

- The Bruker Elute UHPLC coupled to the EVOQ LC-TQ Elite MS/MS system and the ClinMass TDM kit provide a quick and reliable method to easily detect and quantitate 26 antiepileptics in serum.
- Low sample requirements (50 µl serum), easy preparation, and short run time (4.5 minutes) support high sample throughput. Linearity of calibration, precision, and accuracy were outstanding, supporting the use of this combined system in clinical research workflows.

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