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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

Analysis of Antiepileptic Drugs in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

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User Benefits

- Full solution provided by Shimadzu and RECIPE®
- Fully automated sample preparation
- Verified method for RECIPE® ClinMass® TDM Kit System for Antiepileptic Drugs in Serum / Plasma

■ Introduction

Epilepsy is a chronic neurological disorder which is characterised by recurrent epileptic seizures. For the pharmacological therapy of epilepsy a variety of antiepileptic drugs (AEDs) with different pharmacological properties are available today. The therapeutic drug dose has to be ascertained for the individual patient and subsequently has to be controlled by measuring the drug concentration in blood (Therapeutic Drug Monitoring, TDM)[1].

RECIPE's fully validated analytical method provides the reliable quantification of 27 AEDs (Table 2) for TDM using LC-MS/MS.

By addition of the Shimadzu CLAM (Clinical Laboratory Automated sample preparation Module) in front of the LC-MS/MS system (Figure 1) the required sample preparation could be fully automated which achieves results on a fast and high-precision analytical workflow.

To prove that the automated sample preparation leads to reliable results a method verification procedure was evaluated according to the CLSI Guidelines EP06-A, EP15-A3, EP17-A2.

Fig. 1 CLAM LCMS TQ

■ Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in clinical laboratories. For the described verification, a Shimadzu CLAM-2040 coupled with a Nexera X3 UHPLC system and a LCMS-8060 triple-quadrupole mass spectrometer was used.

27 Antiepileptic Drugs in serum were verified using the ClinMass® TDM Platform (order no. MS9000) in combination with the ClinMass® Add-on Set for Antiepileptic Drugs in Serum / Plasma (order no. MS9200) (RECIPE®,Germany).

Lyophilized, matrix-based calibrator and control samples were reconstituted, aliquoted and stored until use.

Then the samples were loaded directly into the CLAM-2040. It was programmed to perform protein precipitation using Precipitant P including internal standards from the ClinMass® TDM Kit System for Antiepileptic Drugs followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2040 to the LC without human intervention for LC-MS/MS analysis. Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 6.15 min. Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.

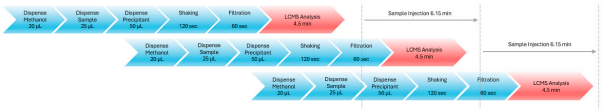

Fig. 2 Scheme fully automated sample preparation and analysis

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060
Ionization	: Electrospray Ionization (ESI)
Interface Voltage	: 5 kV (ESI positive) / -1 kV (ESI negative)
Heating Gas	: 13 L/min
DL Temp.	: 300 ˚C
Interface Temp.	: 400 ˚C
Nebulizing Gas	: 2 L/min
Drying Gas	: 7 L/min
Heat Block	: 400 ˚C
CID	: 270 kPa
UHPLC	: Nexera X3
Column Oven	: 40 ˚C
Injection Volume	: 0.1 µL
Flow rate	: 0.6 mL/min
Time Programme	: Binary gradient

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]
Initial	100	0
0.03	100	0
004	87	13
1.30	87	13
1.31	79	21
2.30	79	21
2.80	50	50
3.30	50	50
3.40	20	80
3.60	20	80
3.70	100	0
4.50	100	0

Table 2 MRM transitions and parameters of the analytes and isotope-labelled substances

Analyte / IS, ESI positive	Quantifier MRM		Dwell Time	Q1 Pre Bias	CE	Q3 Pre Bias	used IS
	Precursor [m/z]	Product [m/z]	msec	V	V	V	
10-OH-Carbamazepine	255.1	194.2	10	-18	-45	-13	1
Brivaracetam	213.2	168.1	10	-23	-30	-17	2
Carbamazepine	237.1	165.2	10	-16	-25	-18	3
Carbamazepine-Diol	271.1	180.1	10	-20	-50	-12	10
Carbamazepine-Epoxide	253.1	210.1	10	-12	-25	-14	9
Felbamate	239.1	117.1	10	-17	-36	-22	4
Gabapentine	172.2	137.2	10	-12	-18	-13	5
Lacosamide	251.1	91.1	10	-17	-10	-19	6
Lamotrigine	256.0	58.1	10	-18	-31	-10	7
Levetiracetam	171.2	126.1	10	-12	-30	-26	8
Oxcarbazepine	253.1	180.0	10	-12	-15	-12	9
Phenylethylmalonamide (PEMA)	207.1	91.1	10	-14	-15	-18	10
Perampanel	350.1	247.1	10	-17	-35	-17	11
Phenytoin	253.1	182.1	50	-12	-22	-19	12
Pregabaline	160.2	142.2	10	-11	-15	-14	13
Primidone	219.2	162.2	50	-11	-14	-11	14
Retigabine	304.2	109.0	20	-21	-34	-23	15
Rufinamide*	239.0	127.0	10	-17	-55	-24	16
Stiripentol	217.1	187.1	10	-10	-12	-29	17
Tiagabine	376.1	247.1	10	-27	-21	-11	18
d_4-10-OH-Carbamazepine (1)	259.0	198.1	10	-18	-45	-13	
d_7-Brivaracetam (2)	220.2	175.1	10	-23	-30	-17	
d_{10}-Carbamazepine (3)	247.1	175.1	10	-16	-25	-18	
d_4-Felbamate (4)	243.3	121.0	10	-17	-36	-22	
d_4-Gabapentine (5)	176.1	139.1	10	-12	-18	-13	
d_3-Lacosamide (6)	254.3	91.1	10	-17	-10	-19	
$^{13}C_3$-Lamotrigine (7)	259.0	59.1	10	-18	-31	-10	
d_6-Levetiracetam (8)	177.0	132.1	10	-12	-30	-26	
d_4-Oxcarbazepine (9)	257.3	184.1	10	-12	-15	-12	
d_5-PEMA (10)	212.1	93.1	10	-14	-15	-18	
d_5-Perampanel (11)	355.1	248.1	10	-17	-35	-17	
d_{10}-Phenytoin (12)	263.1	192.1	20	-12	-22	-19	
d_4-Pregabaline (13)	164.0	146.0	10	-11	-15	-14	
d_5-Primidone (14)	224.2	167.2	50	-11	-14	-11	
d_4-Retigabine (15)	308.2	113.0	10	-15	-34	-20	
$^{15}N,d_2$-Rufinamide (16)	242.0	129.0	10	-17	-55	-24	
d_9-Stiripentol (17)	226.0	196.2	10	-10	-12	-29	
d_6-Tiagabine (18)	382.1	253.1	10	-27	-21	-11	
	Qualifier MRM						
10-OH-Carbamazepine	255.1	165.2	5	-18	-25	-11	
Brivaracetam	213.2	196.1	5	-15	-13	-13	
Carbamazepine	237.1	194.1	5	-17	-50	-13	
Carbamazepine-Diol	271.1	210.2	5	-19	-16	-14	
Carbamazepine-Epoxide	253.1	180.1	5	-28	-5	-18	
Felbamate	239.1	65.1	5	-17	-54	-12	
Gabapentine	172.2	55.1	5	-12	-26	-10	
Lacosamide	251.1	74.1	5	-12	-15	-13	
Lamotrigine	256.0	43.2	5	-18	-20	-17	
Levetiracetam	171.2	69.1	5	-12	-40	-13	
Oxcarbazepine	253.1	236.0	5	-18	-22	-16	
Phenylethylmalonamide (PEMA)	207.1	117.1	5	-10	-15	-26	
Perampanel	350.1	219.1	5	-13	-50	-10	
Phenytoin	253.1	77.0	10	-17	-53	-16	
Pregabaline	160.2	97.2	5	-18	-15	-19	
Primidone	219.2	91.1	10	-15	-10	-18	
Retigabine	304.2	230.1	10	-11	-21	-15	
Stiripentol	217.1	159.1	5	-14	-17	-17	
Tiagabine	376.1	149.2	5	-26	-25	-28	
Analyte / IS, ESI negative	**Quantifier MRM**						
Ethosuximide*	140.2	42.1	50	11	24	14	19
N-Desmethylmethsuximide (NDMS)*	188.2	42.2	80	22	26	14	20
Phenobarbital*	231.2	42.0	20	10	22	16	20
Sulthiame	289.0	225.1	50	11	22	15	21
Topiramate	338.2	78.0	20	18	33	10	22
Valproic acid**	143.3	143.1	20	18	11	16	23
Zonisamide	211.3	119.0	20	11	14	22	24
d_3-Ethosuximide (19)	143.2	42.1	20	11	24	14	
d_5-Phenobarbital (20)	236.1	42.1	10	10	22	16	
d_4-Sulthiame (21)	293.0	229.1	20	11	22	15	
d_{12}-Topiramat (22)	350.4	78.0	10	18	33	10	
d_6-Valproic acid (23)**	149.2	149.2	10	18	11	16	
$^{15}N,d_4$-Zonisamide (24)	216.2	123.1	10	11	14	22	
	Qualifier MRM						
Sulthiame	289.0	132.2	10	14	28	27	
Topiramate	338.2	96.0	5	11	23	14	
Zonisamide	211.3	64.0	10	14	53	25	

*no qualifier MRM available, ** no sufficient fragmentation detectable

■ Results

The trueness was determined by 4-fold analysis of two different quality control (QC) samples in a single analysis sequence. The results (precision in CV% and deviation from the target in % Bias) are summarized in Table 3.

To determine the precision two different levels of QC samples were prepared in 8-fold and analyzed in a single analysis sequence. The intraassay precision for each level is summarized in Table 4.

For determination of the linearity and the lower limit of quantification (LLOQ) several dilutions of the ClinCal® Serum Calibrator Set lyophil. for Antiepileptic Drugs (order no. MS9213, RECIPE® ,Germany) were prepared in 3-fold and analyzed in a single analysis sequence. The results for linearity evaluation and for the LLOQ are summarized in Table 5.

Table 3 Trueness of measurement

Analyte	Sample	Target value [mg/L]	Measured value [mg/L] Mean (n=4)	CV	Bias
10-OH-Carbamazepine	Control Sample Level I	8.19	7.91	1.10%	-3.40%
	Control Sample Level II	18.8	18.9	3.10%	0.60%
Brivaracetam	Control Sample Level I	0.902	0.929	0.80%	3.00%
	Control Sample Level II	2.12	2.12	0.90%	-0.10%
Carbamazepine	Control Sample Level I	4.48	4.59	1.90%	2.40%
	Control Sample Level II	9.96	10.4	0.60%	4.70%
Carbamazepine-Diol	Control Sample Level I	1.68	1.71	1.20%	1.90%
	Control Sample Level II	3.83	3.96	0.70%	3.50%
Carbamazepine-Epoxide	Control Sample Level I	1.91	1.87	1.10%	-2.30%
	Control Sample Level II	4.27	4.11	0.60%	-3.70%
N-Desmethylmethsuximide	Control Sample Level I	9.05	9.21	1.50%	1.80%
	Control Sample Level II	20.7	20.5	1.40%	-1.00%
Ethosuximide	Control Sample Level I	22.4	23.2	1.60%	3.50%
	Control Sample Level II	52.1	52.9	0.50%	1.60%
Felbamate	Control Sample Level I	22.4	21.1	2.20%	-5.90%
	Control Sample Level II	48.2	49.6	2.60%	2.90%
Gabapentine	Control Sample Level I	5.14	5.14	0.50%	-0.10%
	Control Sample Level II	11.8	12.1	0.90%	2.20%
Lacosamide	Control Sample Level I	2.91	2.82	1.30%	-3.10%
	Control Sample Level II	6.69	6.69	1.70%	0.00%
Lamotrigine	Control Sample Level I	4.19	4.08	1.00%	-2.70%
	Control Sample Level II	9.42	9.53	1.00%	1.10%
Levetiracetam	Control Sample Level I	12.7	12.1	1.70%	-4.80%
	Control Sample Level II	28.6	28.7	1.70%	0.40%
Oxcarbazepine	Control Sample Level I	0.92	0.956	1.30%	4.00%
	Control Sample Level II	2.11	2.14	2.20%	1.20%
Phenylethylmalonamide	Control Sample Level I	2.38	2.31	0.70%	-2.90%
	Control Sample Level II	5.66	5.52	1.20%	-2.50%
Perampanel	Control Sample Level I	0.276	0.267	0.40%	-3.30%
	Control Sample Level II	0.631	0.613	0.40%	-2.90%
Phenobarbital	Control Sample Level I	9.35	10.1	3.30%	8.30%
	Control Sample Level II	22.2	22.8	2.00%	2.60%
Phenytoin	Control Sample Level I	4.8	5.11	3.80%	6.50%
	Control Sample Level II	11.4	11.6	5.00%	2.10%
Pregabaline	Control Sample Level I	2.04	2.09	1.20%	2.60%
	Control Sample Level II	5.04	5.02	0.60%	-0.40%
Primidone	Control Sample Level I	5.25	5.32	3.40%	1.30%
	Control Sample Level II	13	13	2.60%	0.10%
Retigabine	Control Sample Level I	0.443	0.445	1.10%	0.50%
	Control Sample Level II	1.02	1.07	0.80%	5.30%
Stiripentol	Control Sample Level I	3.12	3.11	0.60%	-0.30%
	Control Sample Level II	6.89	7.19	0.60%	4.30%
Rufinamide	Control Sample Level I	8.25	7.96	0.60%	-3.50%
	Control Sample Level II	18.8	18.5	1.70%	-1.70%
Sulthiame	Control Sample Level I	2.43	2.47	2.10%	1.80%
	Control Sample Level II	5.41	5.51	2.10%	1.90%
Tiagabine	Control Sample Level I	0.0717	0.0777	1.00%	8.30%
	Control Sample Level II	0.162	0.182	1.90%	12.70%
Topiramate	Control Sample Level I	3.42	3.41	1.00%	-0.40%
	Control Sample Level II	8.07	8.12	2.20%	0.60%
Valproic acid	Control Sample Level I	23.5	24.1	7.00%	2.40%
	Control Sample Level II	52.3	51.8	3.80%	-0.90%
Zonisamide	Control Sample Level I	8.64	8.26	2.20%	-4.40%
	Control Sample Level II	19.7	19.6	1.70%	-0.40%

Table 4 Intraassay results [CV%]

Analyte	Sample	Measured value [mg/L] Mean (n=8)	CV [%]
10-OH-Carbamazepine	Control Sample Level I	7.92	1.5%
	Control Sample Level II	18.6	2.8%
Brivaracetam	Control Sample Level I	0.924	1.4%
	Control Sample Level II	2.12	1.0%
Carbamazepine	Control Sample Level I	4.59	1.5%
	Control Sample Level II	10.3	2.2%
Carbamazepine-Diol	Control Sample Level I	1.72	1.4%
	Control Sample Level II	3.91	1.8%
Carbamazepine-Epoxide	Control Sample Level I	1.85	2.2%
	Control Sample Level II	4.06	2.1%
N-Desmethylmethsuximide	Control Sample Level I	9.19	2.1%
	Control Sample Level II	20.7	2.3%
Ethosuximide	Control Sample Level I	23.0	1.5%
	Control Sample Level II	52.8	0.8%
Felbamate	Control Sample Level I	21.3	3.4%
	Control Sample Level II	48.4	4.0%
Gabapentine	Control Sample Level I	5.13	1.0%
	Control Sample Level II	12.0	1.0%
Lacosamide	Control Sample Level I	2.83	1.3%
	Control Sample Level II	6.73	1.4%
Lamotrigine	Control Sample Level I	4.07	0.9%
	Control Sample Level II	9.46	1.3%
Levetiracetam	Control Sample Level I	12.1	1.2%
	Control Sample Level II	28.7	1.2%
Oxcarbazepine	Control Sample Level I	0.954	1.5%
	Control Sample Level II	2.13	2.0%
Phenylethylmalonamide	Control Sample Level I	2.31	0.6%
	Control Sample Level II	5.50	1.0%
Perampanel	Control Sample Level I	0.267	0.4%
	Control Sample Level II	0.610	0.7%
Phenobarbital	Control Sample Level I	10.0	3.1%
	Control Sample Level II	22.9	2.5%
Phenytoin	Control Sample Level I	5.12	3.7%
	Control Sample Level II	11.8	4.2%
Pregabaline	Control Sample Level I	2.09	0.9%
	Control Sample Level II	5.04	0.8%
Primidone	Control Sample Level I	5.39	4.5%
	Control Sample Level II	13.0	2.9%
Retigabine	Control Sample Level I	0.448	2.2%
	Control Sample Level II	1.08	3.0%
Stiripentol	Control Sample Level I	3.11	0.9%
	Control Sample Level II	7.14	1.0%
Rufinamide	Control Sample Level I	7.98	1.3%
	Control Sample Level II	18.4	1.2%
Sulthiame	Control Sample Level I	2.46	1.8%
	Control Sample Level II	5.47	2.1%
Tiagabine	Control Sample Level I	0.078	1.1%
	Control Sample Level II	0.182	1.8%
Topiramate	Control Sample Level I	3.42	1.3%
	Control Sample Level II	8.08	1.8%
Valproic acid	Control Sample Level I	24.2	5.1%
	Control Sample Level II	53.0	3.5%
Zonisamide	Control Sample Level I	8.23	2.0%
	Control Sample Level II	19.4	1.9%

Table 5 Linearity evaluation, including LLOQ / LOD

Analyte	R^2	Linear Range [mg/L]	LLOQ [mg/L]	LOD [mg/L]
10-OH-Carbamazepine	0.998	2.67 – 85.0	2.67	0.890
Brivaracetam	0.998	0.0708 – 8.24	0.0708	0.0236
Carbamazepine	0.995	0.740 – 43.0	0.740	0.247
Carbamazepine-Diol	0.994	0.287 – 17.8	0.287	0.0957
Carbamazepine-Epoxide	0.995	0.146 – 16.9	0.146	0.0487
NDMS	0.997	3.27 – 87.2	3.27	1.09
Ethosuximide	0.999	4.14 – 242	4.14	1.38
Felbamate	0.997	3.51 – 210	3.51	1.17
Gabapentine	0.999	0.179 – 55.2	0.179	0.0597
Lacosamide	0.998	0.425 – 27.6	0.425	0.142
Lamotrigine	0.996	0.335 – 40.8	0.335	0.112
Levetiracetam	0.999	0.390 – 126	0.390	0.130
Oxcarbazepine	0.998	0.151 – 9.94	0.151	0.0503
PEMA	0.999	0.179 – 23.4	0.179	0.0597
Perampanel	0.999	0.0497 – 2.86	0.0497	0.0166
Phenobarbital	0.998	0.930 – 106	0.930	0.310
Phenytoin	0.997	0.825 – 36.7	0.825	0.275
Pregabaline	0.999	0.671 – 23.0	0.671	0.224
Primidone	0.991	1.63 – 58.2	1.63	0.543
Retigabine	0.999	0.133 – 4.66	0.133	0.0443
Rufinamide	0.999	1.39 – 84.6	1.39	0.462
Stiripentol	0.999	0.525 – 29.2	0.525	0.175
Sulthiame	0.998	0.471 – 24.0	0.471	0.157
Tiagabine	0.999	0.00106 – 0.662	0.00106	0.00035
Topiramate	0.999	0.300 – 36.2	0.300	0.100
Valproic acid	0.992	8.24 – 224	8.24	2.75
Zonisamide	0.998	1.47 – 85.8	1.47	0.488

■ Conclusion

The ClinMass® TDM Kit System for Antiepileptic Drugs in Serum / Plasma (order no. MS9000 and MS9200) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

All 27 analytes passed the acceptance criteria for accuracy (trueness, precision) and linearity.

The lower limit of quantification (LLOQ) was below published clinical reference ranges.

■ References

1. Instruction Manual, ClinMass® TDM Kit System, Antiepileptic Drugs in Serum / Plasma, RECIPE ® Chemicals + Instruments GmbH

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