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Therapeutic drug monitoring of Antiepileptic drugs in serum: a fully automated approach

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Introduction

Epilepsy is a chronic neurological disorder which is characterized by recurrent epileptic seizures whose frequency and rhythm are mostly not predictable. For the pharmacological therapy of epilepsy a variety of antiepileptic drugs (AEDs) are available today, most of them exhibit a pronounced intra and inter individual variability in pharmacokinetics. For that reason the therapeutic drug monitoring of these molecules needs to be accomplished by extremely accurate techniques (e.g: LC–MS/MS). LC-MS/MS currently lacks in standardization and throughput for routine analysis. We report a method for the quantitation of 26 AEDs (10-OH-Carbamazepine, Carbamazepine, Carbamazepine – Diol, Carbamazepine – Epoxide, Ethosuximide, Felbamate, Gabapentin, Lacosamide, Lamotrigine, Levetiracetam, N-Desmethylmethsuximide NDMS, Oxcarbazepine, Phenylethylmalonamide PEMA, Perampanel, Phenobarbital, Phenytoin, Pregabaline, Primidone, Retigabine, Rufinamide, Stiripentol, Sulthiame, Tiagabine, Topiramate, Valproic acid, Zonisamide) in serum samples by LC-MS/MS analysis. Usually LC-MS/MS analysis of serum samples require manual preparation steps for extraction and protein precipitation before the injection. With the aim to reduce the operator involvement and to increase the throughput and data quality, we completely eliminated the involvement of the operator for sample preparation by the use of a novel automatic preparation unit (CLAM-2000, Shimadzu) (the present work is intended for research use only).

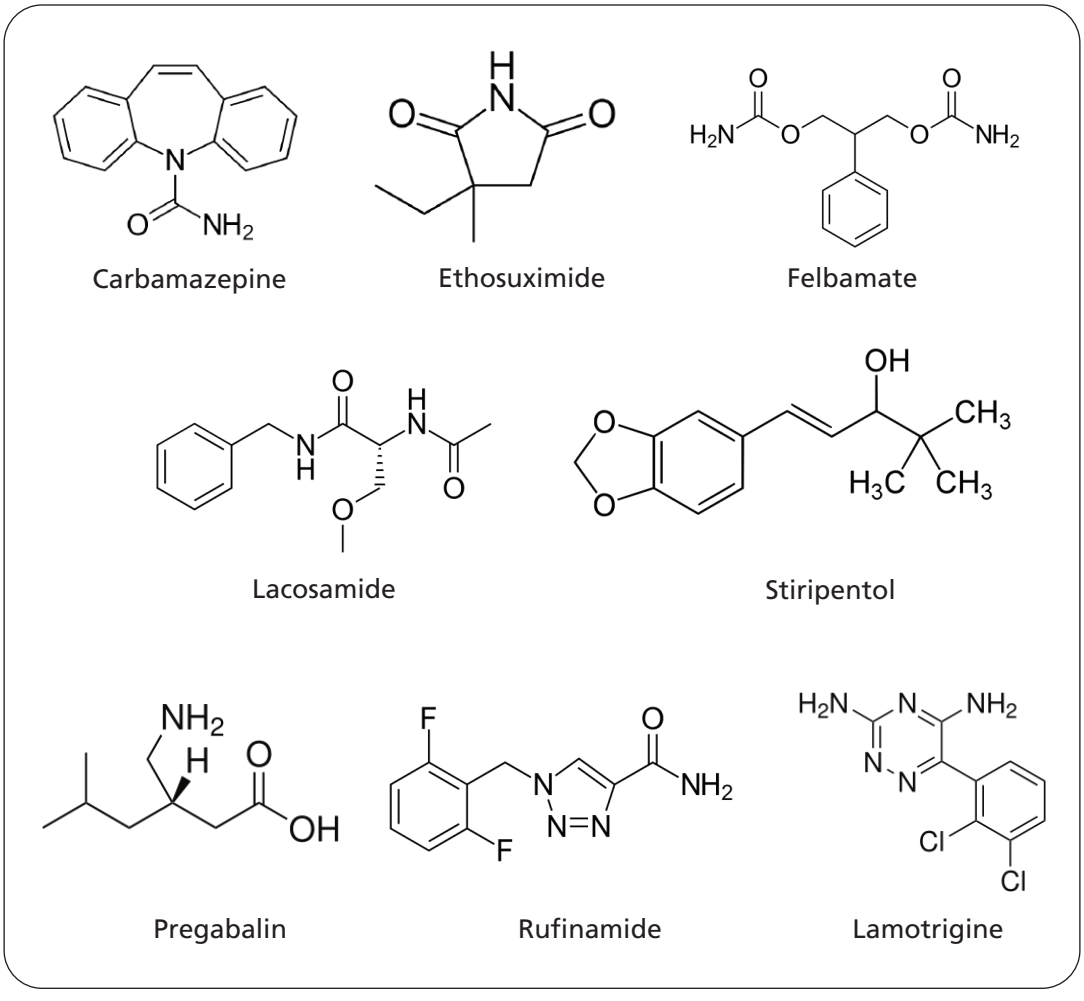

Figure 1: Structure of the most common antiepileptic drugs

Methods and Materials

The analysis of Antiepileptic drugs was performed using a fully automatic LCMS preparation Unit (CLAM-2000, "For Research Use Only. Not for use in clinical diagnostics." Shimadzu) online with a LCMS system (NexeraX2-LCMS8060, Shimadzu) starting from serum samples using the "ClinMass® TDM Kit System" (Recipe, MS9200). Samples, calibrators (Recipe, ClinCal® MS9213) and Internal standard mix (Recipe, ClinMass® MS9212) were loaded onto the CLAM-2000 (refrigerated at 8°C, Figure 2). The quantitation of AEDs in serum samples was performed by LC-MS/MS analysis (Table 1).

Table 1: MRM Transitions for all the analytes.
*SIM analisys (140,00>140,00) was added as additional reference Ion.

Molecule	Quantifier	Qualifier
Gabapentine	171,70>137,10	171,80>55,10
Levitiracetam	170,70>126,00	170,70>69,00
Pregabalin	160,00>142,00	160,00>97,20
PEMA	207,00>91,10	207,00>117,10
D5-PEMA	211,80>93,10	211,80>167,15
Lacosamid	250,80>91,00	250,80>71,10
D3-Lacosamid	253,90>91,15	253,90>108,15
Primidon	219,00>162,10	219,00>91,00
CBZ-Diol	271,00>180,00	271,00>210,00
Felbamat	239,00>117,30	239,20>64,70
Lamotrigin	255,90>43,00	255,90>58,20
10-OH-CBZ	254,60>194,00	254,60>165,00
Rufinamid	238,70>127,15	238,70>100,95
CBZ-Epoxid	252,70>180,00	252,70>236,10
Oxacarbazepin	252,70>180,00	252,70>236,10
CBZ	236,80>165,00	236,80>194,00
Phenytoin	253,10>181,90	253,00>104,10
Tiagabin	376,20>246,90	375,90>149,20
Perampanel	349,70>247,00	349,70>219,00
Retigabin	303,80>109,00	303,80>230,00
D6-Tiagabin	382,10>253,00	382,10>114,00
D4-Retigabin	307,70>112,95	307,70>234,05
Stiripentol	217,00>187,10	217,00>159,00
Ethosuximid	140,00>42,00	140,00>11,0*
Sultiam	288,70>225,30	288,70>132,20
Valpronic acid	143,00>143,00	
Zonisamid	210,80>119,10	210,80>63,90
Phenobarbital	230,90>41,90	230,90>41,90
NDMS	187,90>42,00	187,90>42,00
Topriamat	337,70>78,00	337,80>95,90

Therapeutic drug monitoring of Antiepileptic drugs in serum: a fully automated approach

Figure 2: CLAM-2000 online with Nexera X2 system and LCMS-8060 triple quadrupole mass spectrometer.

Results

Fully Automated sample preparation

The CLAM-2000 was programmed to perform sample extraction and protein precipitation followed by filtration and sample collection. The filtrated sample was then automatically transported using an arm from the CLAM-2000 to the HPLC for LC-MS/MS analysis and no human intervention was required (Figure 3).

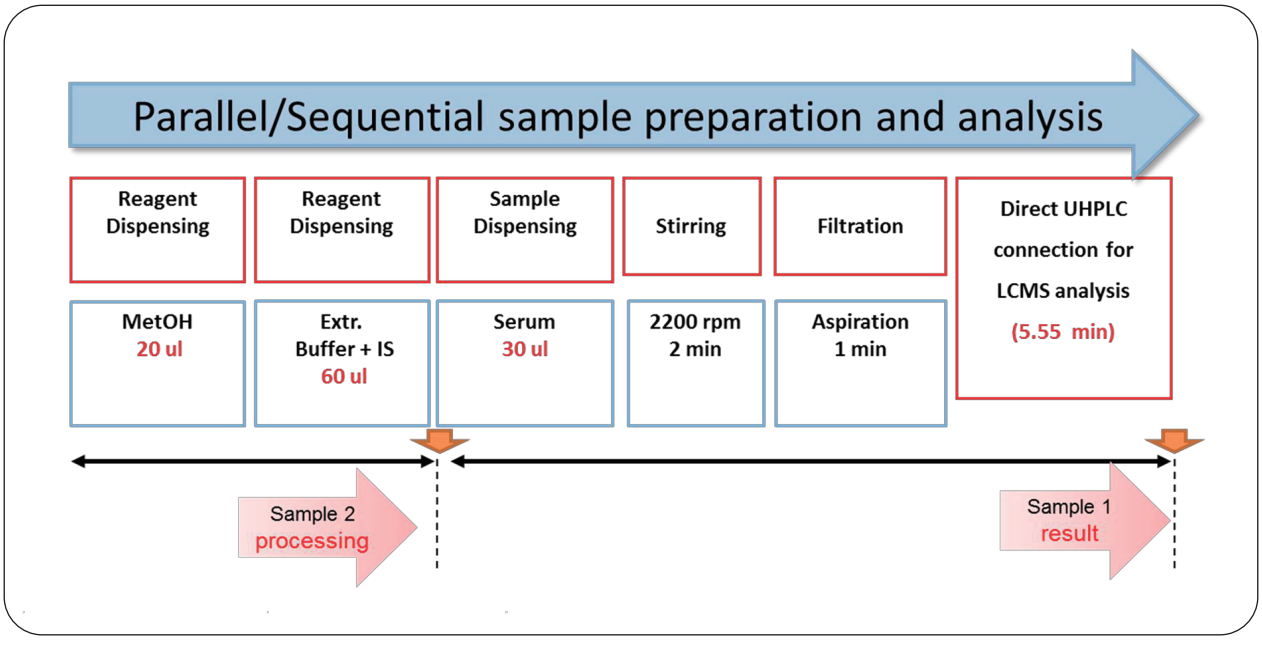

Figure 3: CLAM-2000 fully automated sample preparation and analysis. Due to the overlapped sample preparation the throughput of the instrument was 1 result each 6 minutes for quantification of all Atiepileptics.

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Methods correlation

Fully automated sample preparation method was finally compared with manual sample preparation method by analyzing several serum samples spiked with all the analytes at various concentration levels. The method correlation was evaluated through Passing-Bablok plot (Figure 4).

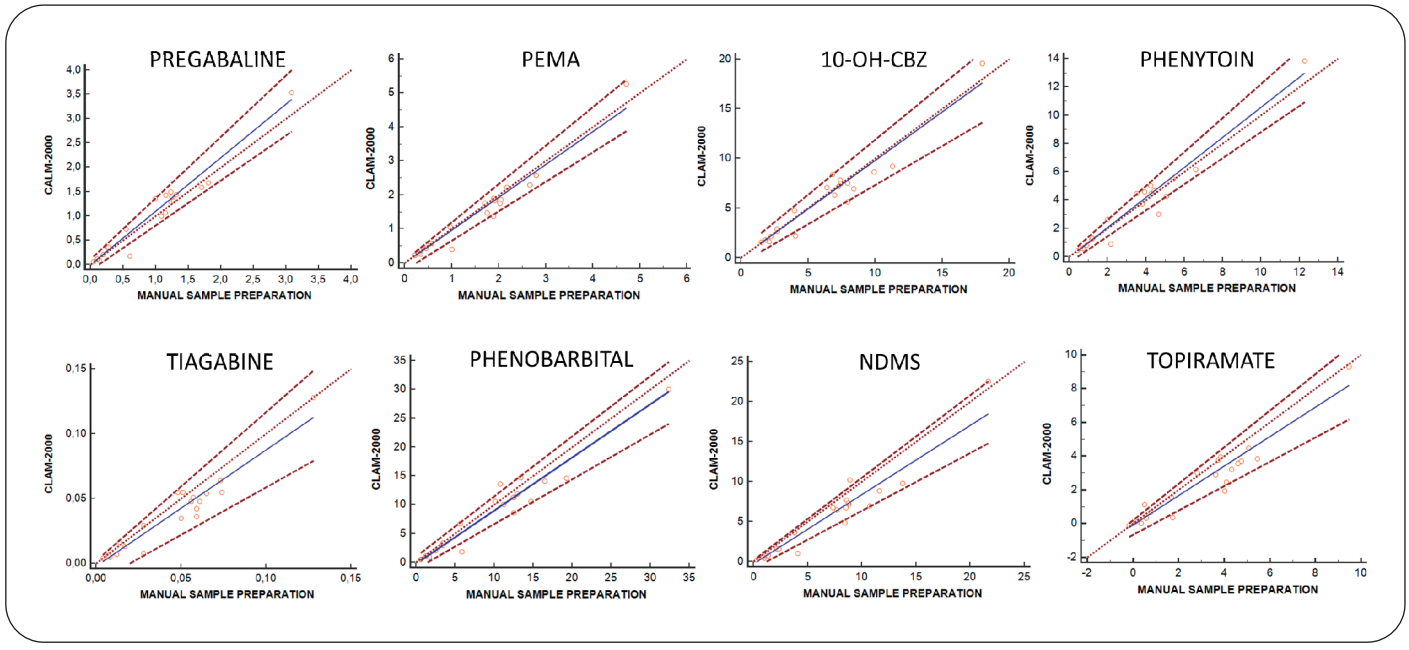

Figure 4: Methods comparison using Passing-Bablok plot.
20 serum samples spiked at different concentrations of all AEDs.

Linearity, Accuracy and Precision

The linearity and accuracy of the method was evaluated using 3 reference serum calibrators levels (Recipe MS9213). For all the analytes linearity and accuracy were within the analytical acceptable range (85%-115%). Furthermore in order to estimate the precision of the method, reference serum samples (Recipe MS9282) spanning from low concentration level to high concentration level were analyzed several times (6 replicates). For all analytes the CV% values were within acceptable analytical ranges.

Therapeutic drug monitoring of Antiepileptic drugs in serum: a fully automated approach

Table 4: Linearity, Accuracy, and precision evaluated using ClinChek ® MS9282 reference serum controls.
*n=6 replicates; **n=3 non-consecutive days.

Compound	R^2	ACCURACY (%)		PRECISION (CV%)				% BIAS	
				INTRADAY		INTERDAY			
		Min	Max	Low	High	Low	High	Low	High
Gabapentin	0.999	100	100	5.6	3.3	7.1	4.7	2.9	9.5
Levitiracetam	0.999	95.9	106	4.2	4.2	3.9	3.9	12.7	14.8
Pregabalin	0.998	94.4	108	3.1	2.2	3.4	2.3	3.3	6.1
PEMA	0.998	94.4	108	1.6	1.2	2.4	1.9	3.1	2.8
Lacosamide	0.999	96.5	105	1.3	3.0	3.6	2.1	5.2	3.1
Primidone	0.999	96.0	106	5.4	4.2	6.8	4.3	8.4	5.1
CBZ-Diol	0.998	95.5	106	4.5	4.2	4.9	4.8	0.5	4.1
Felbamate	0.999	98.1	103	1.5	1.2	4.3	4.5	1.8	2.6
Lamotrigine	0.940	80	123	2.9	2.8	10.4	3.2	6.2	0.3
10-OH-CBZ	0.994	92.9	106	6.2	1.2	5.3	6.2	3.5	9.2
Rufinamide	0.996	95	106	4.2	2.6	3.6	2.5	3.4	3.9
CBZ-Epoxide	0.999	96.2	103	1.5	2.8	2.6	2.4	n.d.	n.d
Oxacarbazepine	0.997	95.5	105	2.2	1.3	3.3	1.7	1.7	6.5
CBZ	0.999	98.8	100	4.0	3.9	4.5	4.5	9.4	1.8
Phenytoin	0.999	94.5	102	6.2	2.2	6.1	5.2	2.8	9.4
Tiagabine	0.999	96.2	101	5.1	5.5	6.0	4.5	3.1	1.4
Perampanel	0.999	97.3	104	6.1	4.6	8.9	4.1	12.7	15.4
Retigabine	0.97	85.9	116	6.8	5.6	8.8	5.3	11.0	0.8
Stiripentol	0.999	96.7	105	4.9	2.2	4.6	3.4	5.2	2.6
Ethosuximide	0.999	96.1	101	7.3	9.6	10.8	11.2	4.3	1.1
Sultiame	0.999	97.3	104	4.2	4.4	4.5	5.5	9.3	8.4
Valpronic acid	0.999	97.7	103	8.8	3.4	6.6	3.9	6.0	2.1
Zonisamide	0.999	97.2	101	3.7	3.4	3.9	2.9	11.6	5.2
Phenobarbital	0.996	94.3	105	6.5	5.1	6.1	4.6	1.5	2.5
NDMS	0.999	97.5	103	11.3	5.3	8.9	5.5	1.0	7.1
Topiramate	0.998	96.2	105	8.2	6.3	13.0	6.3	3.1	3.2

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Conclusions

- Fully Automated sample preparation procedure resulted suitable for the quantitation of Antiepileptic drugs by elimination of all manual preparation steps.
- The automation of the method increases the analytical performance, reduces the risk for human operators and due to the reduced reagent consumption, reduces also the cost of the analysis.

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