Simultaneous Analysis of Newer Antiepileptic Drugs by Rapid Resolution LC/ Triple Quadrupole Mass Spectrometry

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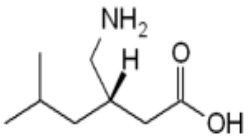
Introduction

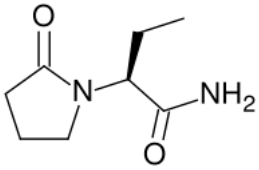
Since the beginning of the nineties, several novel antiepileptic drugs have been introduced. Examples of these are the pharmaceutical compounds lamotrigine, oxcarbazepine, felbamate, zonisamide, gabapentin, pregabalin, tiagabine, topiramate, levetiracetam, stiripentol and rufinamide. In the treatment of epileptic seizures therapeutic drug monitoring (TDM) is an important tool for the physician. Some clinical labs already use LC/MS techniques for monitoring antiepileptic drugs in serum. The established analytical methods often allow only the determination of a single analyte per run. Rapid Resolution LC combined with triple quadrupole mass spectrometry allows a focusing of the separate TDM methodologies into a universal method. The present work describes a new LC/MS/MS method to identify and quantify eleven newer antiepileptic drugs in a single analytical run.

Figure 1) Target compound structures

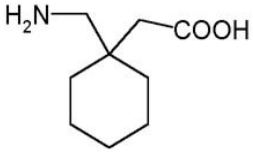
Pregabalin (M_n=159 Da)



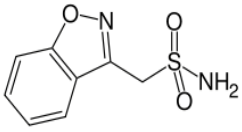
Levetiracetam (M_n=170 Da)



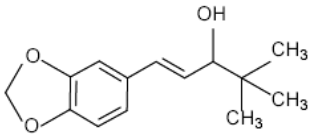
Gabapentin (M_n=171 Da)



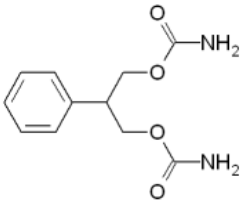
Zonisamide (M_n=212 Da)



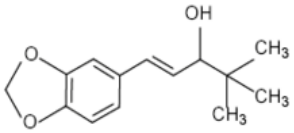
Stiripentol (M_n=234 Da)



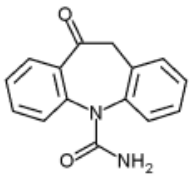
Felbamate (M_n=238 Da)



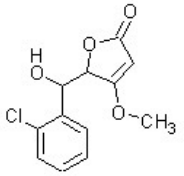
Rufinamide (M_n=238 Da)



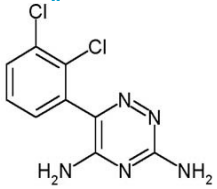
Oxcarbazepine (M_n=252 Da)



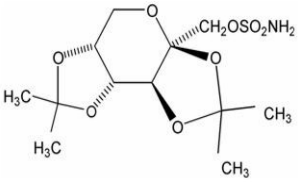
Losigamone (M_n=254 Da)



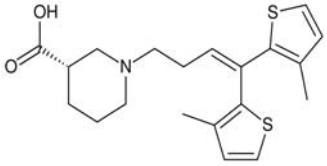
Lamotrigine (M_n=255 Da)



Topiramate (M_n=339 Da)



Tiagabine (M_n=375 Da)



Experimental

Sample Preparation

Two different sample preparation methodologies were evaluated. In addition to the established lab method, consisting of a simple precipitation step, a liquid-liquid extraction procedure was also tested.

Precipitation: 100 µL of serum was diluted with 500 µL methanol, which also contained the internal standard. After centrifugation and reconstitution of the supernatant with the mobile phase, 2 µL of the sample solution was directly injected.

Extrelute Extraction: 100 µL serum and 100 µL internal standard solution were diluted with 800 µl HPLC grade water. The sample was then loaded onto an Extrelute cartridge. After an equilibration step, the analytes were eluted using 5 mL isopropanol/dichlormethane (5:95), followed by evaporation and reconstitution with the mobile phase. The injection volume used was 2 µL.

LC/MS/MS Method

All sample analysis were performed on a LC/MS/MS system consisting of a Agilent 1200 Rapid Resolution Liquid Chromatograph and an Agilent 6140 Triple Quadrupole mass spectrometer, operated with an electrospray ionization source in positive polarity. Determination of the optimal MRM transitions was carried out by flow injection analysis of standards at concentration levels approximating 1ng/µL.

LC Conditions

Column:	Agilent Zorbax SB-Aq; RR-HT (100*2,1 mm, 1.8 um)
Column temp:	60°C
Mobile phase:	A: 5mMol Ammonium acetate in water (pH=2.9)
	B: Methanol / Acetonitrile (50:50,v:v)
Flow rate:	0.4 ml/ min
Gradient:	15 % B at 0 min; 30% B at 1 min
	65 % B at 5 min; 15% B at 7min
Stop time:	11 min

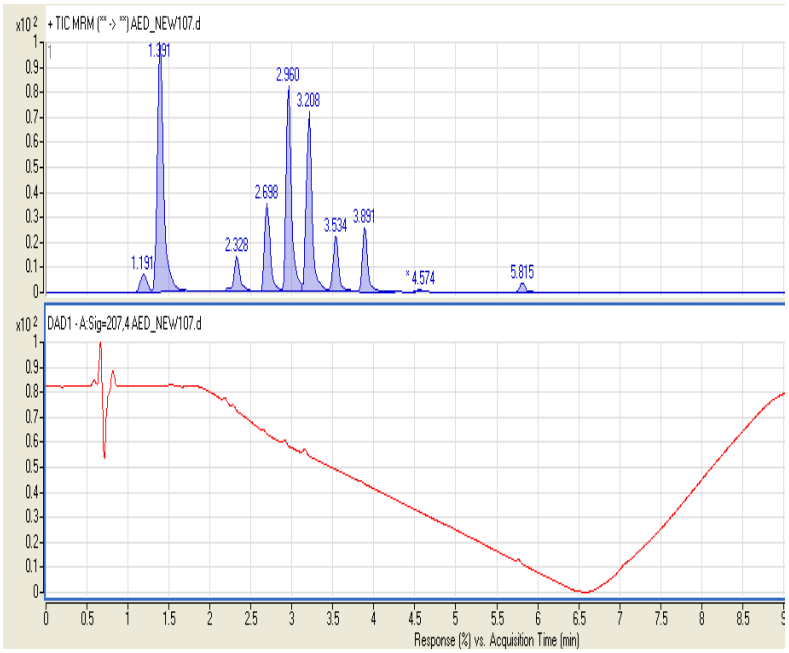
MS Conditions

Mode:	ESI positive
Drying gas flow:	12l / min
Drying gas temp:	350°C
Vcap:	3500 V
MRM:	23 transitions
	(see table 1)
Dwell time:	50 msec

Table 1) Data Acquisition Parameters for MRM transitions

Compound	RT[min]	Precursor $[M-H]^+$	Frag(V)	CE1(V)	Product Ion 1 [m/z]	CE2(V)	Product Ion 2 [m/z]
Pregabaline	1.20	160	80	10	142	10	97
Gabapentine	1.23	172	80	15	154	25	95
Levetiracetam	1.41	171	90	10	126	25	69
Zonisamide	2.24	213	70	20	132	30	77
Lamotrigine	2.34	256	100	20	159	25	145
Felbamate	2.71	178	90	15	117	25	91
Rufinamide	2.97	239	80	20	127	n.a	n.a
Oxcarbazepine	3.21	255	100	20	194	5	237
Topiramate	3.53	357	70	10	264	15	184
Losigamone(ISTD)	3.90	255	90	10	115	10	141
Tiagabine	4.57	376	80	20	247	30	149
Stiripentol	5.82	217	100	15	145	15	159

Results and Discussion



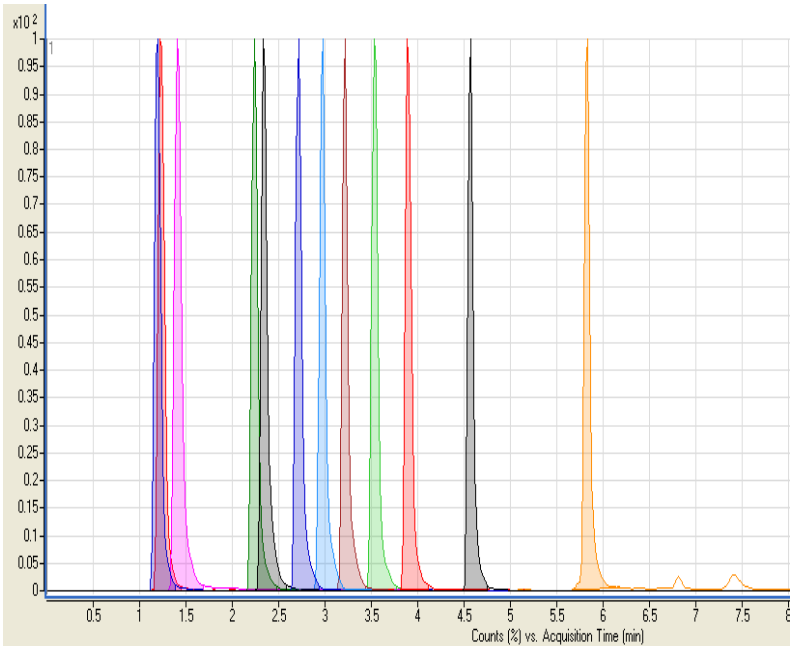


Figure 3) Results of a sample from combination therapy

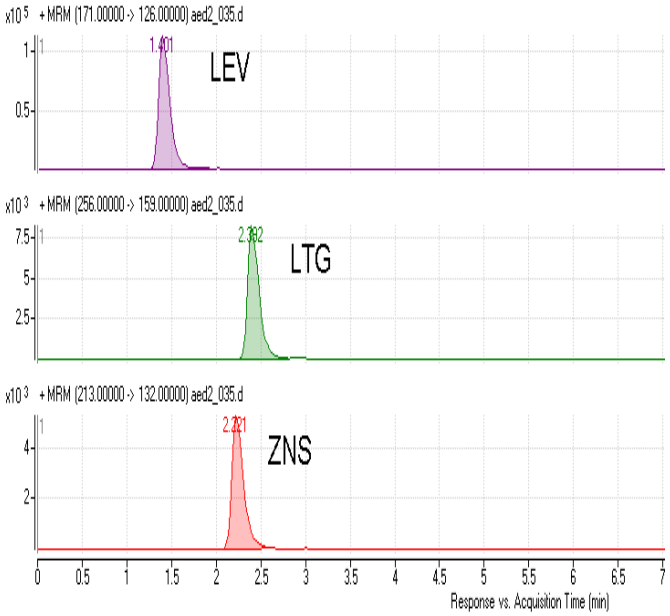


Table 2) Performance Data of the LC/MS/MS method (including the sample preparation step)

Compound	Recovery %	Precision %	Linearity r^2	Correlation with SQ r^2
Pregabalin	97	3.1	0.9971	0.987
Gabapentin	97	1.4	0.9983	0.992
Levetiracetam	99	1.8	0.9997	0.998
Zonisamide	99	3.5	0.9952	0.995
Lamotrigine	98	1.9	0.9969	0.998
Felbamate	98	1.0	0.9993	0.997
Rufinamide	102	1.4	0.9997	0.990
Oxcarbazepine	99	0.8	0.9990	0.992
Topiramate	98	2.9	0.9973	0.994
Tiagabine	97	3.0	0.9993	0.989
Stiripentol	101	2.2	0.9972	0.969

Results and Discussion

The LC/MS/MS method presented here, combining Rapid Resolution LC and Triple Quadrupole mass spectrometry, allows the simultaneous determination of eleven newer antiepileptic drugs in human serum in less than seven minutes (total run time 11 minutes).

For sample preparation the precipitation method yielded the best results. The liquid/liquid extraction led to poor recovery rates for the antiepileptic drugs pregabalin and gabapentin. The precipitation method is simple, fast, cost-effective and generates sample extracts without significant losses of the analytes of interest (**Table 2**). In combination with an optimized chromatographic separation, the matrix interferences could be totally excluded.

The TIC and UV trace for the antiepileptic drugs are shown in **Figure 2a**, an overlay of the eleven EIC's is displayed in **Figure 2b**. It can been seen that the early eluting analytes pregabalin, gabapentin und levetiracetam are sufficiently separated from the matrix eluting with the solvent front. This is essential for the performance characteristics, which are summarized in **Table 2**.

The recovery for all eleven AED's is nearly optimal when using the described precipitation procedure. The method proved to be precise; with the intra-day precision ranging from 0.8 to 3.1%. An ISTD calibration was created over the concentration range, which was relevant for the TDM of the specific drug (Levetiracetam: 0.05-50 µg/mL, Tiagabine: 0.0005-0.5 µg/mL). All calibration curves were linear without special weighting or curve treatment (r^2 values between 0.9971 and 0.9990). External quality control was ensured through participation in interlaboratory tests, organized by Bioanalytical Service (Cardiff, UK).

With this newly developed LC/MS/MS method, 250 samples were analyzed. An example is given in **Figure 3** which summarizes the analysis results of a sample from a combination therapy. Additionally, the LC/MS/MS results were compared to those generated by the hereto established single quadrupole method. The achieved correlation of the different data sets, as expressed by the r^2 values of a two dimensional concentration plot, was generally greater than 0.99, with only the values for pregabaline (0.987) and stripentol (0.969) found to be lower (**Table 2**).

Conclusions

The presented LC/MS/MS method, combining RR-LC and Triple Quadrupole mass spectrometry, allows the simultaneous determination of eleven newer antiepileptic drugs in human serum. The method is fast, selective, sensitive and robust. The selectivity of the method has allowed for the complete amalgamation of multiple assays used in therapeutic drug monitoring without compromising the data integrity. The time and cost saving benefits are significant. The method can also be used for screening purposes.