A simple and reliable method for simultaneous quantification of antiepileptic drugs in plasma using LC-MS/MS combined with direct injection column

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ABSTRACT

A simple and robust method is presented for the simultaneous quantification of older and newer antiepileptic drugs (AEDs), including lamotrigine, oxcarbazepine, zonisamide, gabapentin, topiramate, levetiracetam, phenytoin, carbamazepine, primidone in plasma by LC-MS/MS. The method involved a rapid sample preparation by dilution with aqueous buffer followed by direct injection into a LC-MS/MS system. The separation is achieved on an Imtakt Cadenza HS-C18 HPLC column using gradient elution with a mixture of methanol and ammonium acetate buffer.

Good linearity (r=0.99, "1/x" weighting) was obtained for all drugs quantified over the range of clinically relevant concentrations in human plasma and the use with two internal standards of sulfamethoxazole (positive ion) and tolbutamide (negative ion) improves accuracy and precision for the analytical procedure. No interference peaks or matrix effects were observed in human plasma. The ruggedness of this method was examined about the reproducibility of column pressure, resolution, retention time and peak area with the greater than 500 injections of diluted plasma sample on a single column.

This method is an excellent approach for the rapid preparation and high throughput of plasma samples containing AEDs drug levels in the ug/ml range or lower, and therefore is the suitable method for daily therapeutic drug monitoring and pharmacokinetic studies of AEDs

INTRODUCTION

A simple and reliable method for simultaneous quantification of antiepileptic drugs (AEDs) in human plasma sample is necessary for efficient therapeutic drug monitoring of patients treated with AEDs polytheraphy. LC-MS/MS assay offers selective and sensitive techniques to quantify the drugs of interest in complex biological samples such as plasma. However, plasma samples still need intensive sample handling and clean-up prior to LC-MS/MS analysis.

Dilute-and-shoot preparation is commonly used for the determination of drugs from biological matrices such as urine, but is not typically used with plasma samples because the amount of protein present in plasma can cause a variety of problems including analytical column failure and matrix effect (mostly ion suppression). In pharmaceutical analysis, pretreatment steps such as automated on-line or off-line solid phase extraction and column switching are often used to remove or minimize matrix effect, but these method development is time-consuming and expensive.

The new direct injection column (Cadenza HS-C18) is containing a silica based hybrid ODS stationary phase with both C18 and hydrophilic groups which is responsible for excluding proteins in biological matrices. It can exclude proteins from biological matrices and also retain drugs by running a linear gradient of the organic solvent with 100% aqueous buffer as the initial solvent. Therefore, it can be used for the direct injection of samples containing body fluids such as plasma using divert valve.

In this work, we developed an LC-MS/MS method combined with direct injection column to quantify nine AEDs in human plasma with constant polarity switching in a single analytical run.

MATERIALS AND METHODS

Sample preparation

10 μL of plasma was diluted with 500 μL 2mM ammonium acetate buffer containing internal standards in concentration of each 1ug/mL for sulfamethoxazole and tolbutamide. Sample was vortexed for 15 sec and centrifuged for 5 min at 13000 rpm and finally 1 μL of supernatant was injected onto LC-MS/MS system.

Liquid Chromatography

LC separation of all analytes was achieved on a Imtakt Cadenza HS-C18 column (3um, 2.0 × 75 mm) at 30 °C using a Agilent 1200 LC system. A gradient condition was used with mobile phase A (2mM ammonium acetate buffer) and mobile phase B (Methanol) as follows : The all analytes were eluted at a flow rate of 350 μL/min.

Mass Spectrometry

Mass spectrometric detection was peformed with an API 4000 triplequadrupole mass spectrometer (MDS SCIEX, Toronto, Canada) operated in multiple reaction monitoring (MRM) mode constant (700ms) positive/negative polarity switching. Ionization of all analytes was carried out using the electrospray ionization (ESI) and Turbolon Spray (TIS) source temperature was maintained at 350 °C. Both the nebulizer (GS1) and TIS (GS2) were set at 50 psi. Mass parameters about analytes were optimized as as shown table 1. Data processing was performed on Analyst 1.5.1 software (SCIEX).

	Total Time (min)	Flow Rate (μl/min)	A (%)	B (%)
0	0.00	350	100.0	0.0
1	2.00	350	100.0	0.0
2	3.00	350	0.0	100.0
3	4.00	350	0.0	100.0
4	4.10	350	100.0	0.0
5	9.00	350	100.0	0.0

RESULTS

Figure 1. Almost all of the plasma protein was already excluded from the column in this conditions. HS-C18 can be used for the direct injection of samples containing body fluids.

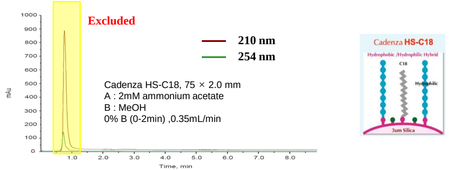


Table 1. MRM transitions and Optimized parameters

Compound Name	Ionization mode	Internal Standard	Q1 (m/z)	Q3 (m/z)	Dwell time (ms)	CE (eV)	CXP (eV)
Primidone	Positive	Sulfamethoxazole	219.1	91	17	38	4
Gabapentin	Positive	Sulfamethoxazole	172.3	137.2	17	22	10
Carbamazepine	Positive	Sulfamethoxazole	237.1	194.2	17	50	10
Oxcarbazepine	Positive	Sulfamethoxazole	253.1	180.1	17	50	10
Lamotrigine	Positive	Sulfamethoxazole	256	211.1	17	35	4
Levetiracetam	Positive	Sulfamethoxazole	171.2	126.1	17	21	6
Zonisamide	Positive	Sulfamethoxazole	213.1	132.1	17	20	10
Sulfamethoxazole	Positive	-	254.2	92	17	38	4
Phenytoin	Negative	Tolbutamide	251.2	102.1	17	-30	-15
Topiramate	Negative	Tolbutamide	338.1	78	17	-50	-4
Tolbutamide	Negative	-	269.2	170	17	-25	-10

Figure 2. TIC chromatogram (The divert valve was switched to the MS at 1 min ~3 min & 4.5 min ~ 6.5min and to waste other time) and MRM chromatograms of all compounds

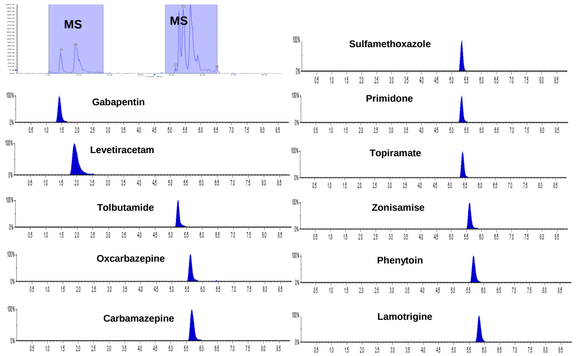


Figure 3. Blank plasma chromatograms.
No interference peaks or matrix effects were observed in human plasma

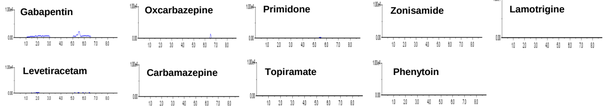


Table 2. Precision and Accuracy of Inter-day (n=3)

Compound name	Internal standard	Pricision (% CV)			Accuracy (%)		
		QL (3ug/mL)	QM (20ug/mL)	QH (40ug/mL)	QL (3ug/mL)	QM (20ug/mL)	QH (40ug/mL)
Primidone	Sulfamethoxazole	3.01	2.88	6.13	113.73	108.42	99.58
Gabapentin	Sulfamethoxazole	10.86	7.66	3.37	95.21	107.08	101.38
Carbamazepine	Sulfamethoxazole	3.05	5.08	4.52	105.15	107.22	102.10
Oxcarbazepine	Sulfamethoxazole	1.48	6.10	4.37	99.73	106.47	98.51
Zonisamide	Sulfamethoxazole	4.28	5.01	3.38	99.50	99.17	103.20
Levetiracetam	Sulfamethoxazole	5.54	4.28	2.54	105.80	105.10	104.30
Lamotrigine	Sulfamethoxazole	7.58	4.33	4.08	99.41	108.34	99.90
Phenytoin	Tolbutamide	7.11	1.92	1.34	102.02	98.02	96.04
Topiramate	Tolbutamide	7.08	5.28	3.60	105.17	102.21	96.07

Table 3. Precision and Accuracy of Intra-day (n=3)

Compound name	Internal standard	Pricision (% CV)			Accuracy (%)		
		QL (3ug/mL)	QM (20ug/mL)	QH (40ug/mL)	QL (3ug/mL)	QM (20ug/mL)	QH (40ug/mL)
Primidone	Sulfamethoxazole	4.85	5.51	2.05	96.45	110.80	99.35
Gabapentin	Sulfamethoxazole	9.70	4.09	8.60	104.62	108.10	96.51
Carbamazepine	Sulfamethoxazole	2.28	2.94	5.84	106.84	103.63	100.50
Oxcarbazepine	Sulfamethoxazole	4.07	2.49	6.41	107.49	112.10	100.77
Zonisamide	Sulfamethoxazole	3.67	3.27	4.58	101.52	100.48	99.85
Levetiracetam	Sulfamethoxazole	6.57	7.81	5.68	111.40	108.40	103.50
Lamotrigine	Sulfamethoxazole	3.80	1.91	4.57	112.53	108.03	105.24
Phenytoin	Tolbutamide	1.91	4.53	5.22	107.05	109.34	102.84
Topiramate	Tolbutamide	4.59	3.04	4.96	103.76	109.27	97.88

Table 4. Validation data of calibration samples

Compound name	Linearity (r) ("1/x" weighting)	Nominal Conc. (ug/mL)	Calculated Conc. (ug/mL)	% CV	Calibration curve range (ug/mL)	Therapeutic range (ug/mL)
Primidone	0.9978	3 20 40	3.14 22.19 39.77	10.92 5.46 4.11	1 ~ 50	6 ~ 15
Gabapentin	0.9948	3 20 40	3.09 21.54 39.73	13.44 7.11 6.31	1 ~ 50	7 ~ 21
Carbamazepine	0.9995	3 20 40	3.15 20.41 39.84	5.02 6.15 4.24	1 ~ 50	4 ~ 12
Oxcarbazepine	0.9980	3 20 40	3.11 21.85 39.85	4.71 4.58 4.93	1 ~ 50	12.5~ 35
Zonisamide	0.9974	3 20 40	3.15 22.14 39.84	5.03 5.41 3.89	1 ~ 50	7 ~ 42
Levetiracetam	0.9954	3 20 40	3.20 20.48 39.98	11.41 7.64 5.76	1 ~ 50	5 ~ 30
Lamotrigine	0.9978	3 20 40	3.21 21.05 39.54	7.55 5.82 4.51	1 ~ 50	2.5 ~ 15
Phenytoin	0.9980	3 20 40	3.14 20.74 39.78	5.55 7.00 5.29	1 ~ 50	10 ~ 20
Topiramate	0.9973	3 20 40	3.16 21.14 38.76	7.13 6.99 5.04	1 ~ 50	3 ~ 20

Table 5. Matrix effect (any change in the ionization process of the AEDs)

Compound name	Evaluated Conc. (ug/mL)	ME (%)[a]
Primidone	3 40	-1.6 -0.95
Gabapentin	3 40	3.94 1.55
Carbamazepine	3 40	13.48 0.20
Oxcarbazepine	3 40	2.66 -7.21
Zonisamide	3 40	-0.50 1.24
Levetiracetam	3 40	-14.20 -10.00
Lamotrigine	3 40	2.23 -6.16
Phenytoin	3 40	5.00 -0.11
Topiramate	3 40	5.31 -1.41
Sulfamethoxazole	1	1.1
Tolbutamide	1	1.3

[a] ME (%)= [(Area spiked sample / Area std solution) -1] × 100 (%)

Figure 4. Representative Calibration curves of AEDs

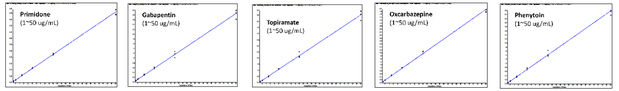
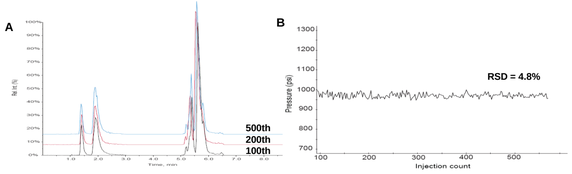


Figure 5. LC-MS/MS chromatogram of TIC (A) show that almost similar peak shape and resolution of all AEDs at injection times above 500 and also pressure change (B) of HS-C18 column is constant.



CONCLUSIONS

1. An efficient, simple and accurate LC-MS /MS method was developed for the quantification of nine AEDs in human plasma to support daily TDM and pharmacokinetic studies.

2. The ruggedness of the dilute-and-shoot method was evaluated with greater than 500 injections on a single column, this method is an excellent approach for the rapid preparation and high throughput of plasma samples containing drug levels in the ng/ml range or higher.

3. HS-C18 column provides excellent separation for low molecular weight analytes especially hydrophobic compounds in protein samples.

REFERENCES

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