Ultrafast Simultaneous Analysis of 6 Antiepileptic Drugs in Human Serum Using Online SPE/MS/MS

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Introduction

Clinical research laboratories traditionally rely on HPLC and immunoassay, more recently LC/MS for quantitative analysis of antiepileptic drugs. However, new antiepileptic drugs (AEDs) were introduced to the market recently, which creates a challenge for their analysis using standard quantitative methods. As the number of AEDs increases, there is a need for a fast and robust method that maintains the specificity and sensitivity of traditional technologies. The Agilent RapidFire High-Throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times under 15 seconds. In the present study, we evaluated the ability of this ultra-fast SPE/MS/MS system to quantitate (AEDs) in human serum with very fast sample cycle times and similar analytical results compared to LC/MS/MS.

In the present study, we developed an ultrafast SPE/MS/MS method for simultaneous analysis in human serum of 6 AEDs; MHD (Oxcarbazepine metabolite), Gabapentin, Lamotrigine, Levetiracetam, Pregabalin, and Zonisamide with much faster sample cycle times and similar analytical results compared to LC/MS/MS and HPLC assays. A simple protein precipitation methodology followed by dilute and shoot analysis by RapidFire SPE/MS/MS allows for the accurate and precise measurement of these analytes in human serum over a linear range of (1 - 100 mcg/mL). Samples were analyzed at 14 seconds per sample providing a much higher throughput method of analysis.

Experimental

Sample Preparation
A mixed analyte calibration curve was prepared in drug free human serum from individual stock solutions, followed by a 1:2 protein precipitation with ACN, and a 1:50 dilution in water. The calibration series was 1, 6.25, 12.5, 25, 50, 100 mcg/mL and matrix controls containing AEDs were obtained from UTAK Laboratories, Valencia, CA.USA.

Experimental

Ionization mode	ESI + Agilent 6460
Drying gas temp.	350 °C
Drying gas flow	8 L/min
Sheath gas temp.	400 °C
Sheath gas flow	9 L/min
Nebulizer pressure	45 psi
Nozzle voltage	500 V
Capillary voltage	3000 V

Table. 1. MRM Transitions

Compound	Q1	Q3	Fragmentor	CE
Lamotrigine Quant	256	211	145	26
Lamotrigine Qual	256	108.9	145	57
MHD Quant	255.1	194	85	17
MHD Qual	255.1	165	85	57
Zonisamide Quant	213	132	85	13
Zonisamide Qual	213	51.1	85	61
Gabapentin-d10	182.2	164.1	110	9
Gabapentin Quant	172.1	154.1	110	9
Gabapentin Qual	172.1	137	110	13
Levetiracetam-d6	177.1	132	90	9
Levetiracetam Quant	171.1	126	80	13
Levetiracetam Qual	171.1	69	80	29
Pregabalin–d6	166.2	148.1	105	5
Pregabalin Quant	160.1	55.1	105	13
Pregabalin Qual	160.1	83	105	21

RapidFire Conditions		
Cycle durations (ms)	State #1 aspirate	600
	State #2 load/wash	3000
	State # 3 elute	6000
	State # 4 re-equilibrate	1500
Solvents	Solvent A: water + 0.1% formic acid	
	Solvent B: 50% methanol + 50% isopropanol + 0.1% formic acid	
Column	C18	

Results

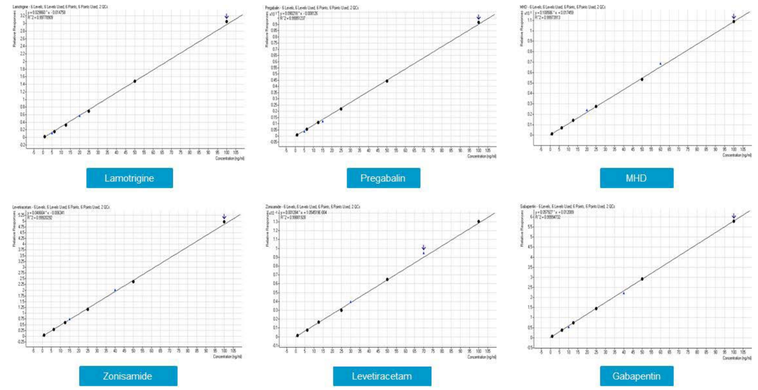

Fig.1. Representative calibration curves showing linear range from 1-100 mcg/mL for each of the six AED analytes. Dark circles are calibrators and blue triangles are QC standards.

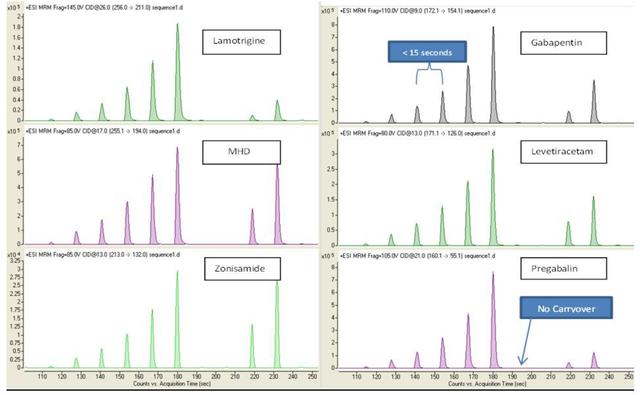

Fig.2. Representative calibration curve data for each of the six AED analytes showing the injection to injection interval of 14 seconds. Carryover assessment using a matrix blank immediately after the highest calibrator for all analytes shows no significant carryover was observed for any of the analytes.

Results

Lamotrigine (mcg/mL)	Interday % Accuracy (n=6)	Interday % Precision (n=6)	Intraday % Accuracy (n=6)	Intraday % Precision (n=6)
4.5	95.1	2.7	99.3	3.1
20	102.6	1.9	101.9	3.5
Gabapentin (mcg/mL)				
10	95.6	0.6	95.2	2.9
40	100.0	0.5	96.6	2.9
Pregabalin (mcg/mL)				
5	92.9	1.2	93.6	4.6
15	94.0	1.1	93.8	2.3
MHD (mcg/mL)				
21	105.7	2.1	100.7	2.3
65	105.1	1.8	102.6	2.1
Levetiracetam (mcg/mL)				
16	105.9	1.3	105.9	4.3
44	105.1	1.0	103.6	1.3
Zonisamide (mcg/mL)				
32	105.5	2.9	100.9	3.8

Table 2. Intraday and interday accuracy and precision data for the QC standards.

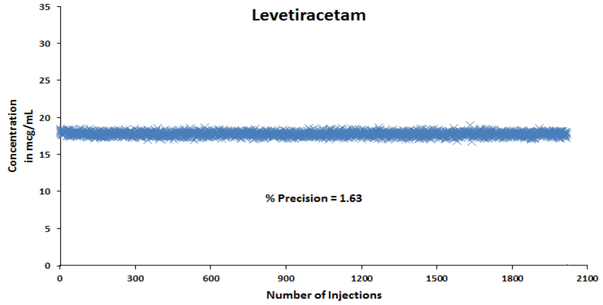

Fig.3. Repeatability evaluation using >2000 sequential injections of Levetiracetam

Discussion

- Six antiepileptic drugs in human serum were accurately and precisely measured using a simple protein precipitation methodology and an ultrafast SPE/MS/MS system
- Samples were analyzed at 14 seconds per sample. This method is capable of analysis throughputs greater than 250 samples per hour.
- The results of this method are comparable to LC/MS/MS, but >20x faster.
- SPE/MS/MS provides a very efficient mode for analyzing AEDs in serum compared to traditional analytical methods.