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Quantitative Analysis of 7 Antiepileptic Drugs in Human Serum for Research Using a Two-Channel LC-MS/MS System

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INTRODUCTION

Clinical research laboratories traditionally rely on immunoassay and, more recently, on HPLC for quantitative analysis of antiepileptic drugs (AEDs). We developed a high-throughput analytical research method for the separation, detection and quantification of 7 antiepileptic drugs (Table 1) in donor human blood serum using LC-MS/MS. This method employs protein precipitation followed by dilution before injection into a two-channel LC-MS/MS system, enabling analysis of 7 AEDs in each injection every 2.2 minutes.

Table 1. Analytical ranges of AEDs

Gabapentin	0.30 - 30 µg/mL
Pregabalin	0.25 - 25
Levetiracetam	0.60 - 60
Zonisamide	0.60 - 60
10-OH-Oxacarbazepine	0.60 - 60
Lamotrigine	0.40 - 40
Topiramate	0.30 - 30

METHODS

Samples included serum-based calibrators and quality controls (QCs) purchased from UTAK Laboratories (Valencia, CA, USA) and donor serum specimens. A mixture of AED stable isotopes - Gabapentin-D_{10}, Levetiracetan-D_6 and Topiramate-D_{12} - served as internal standards (IS).

- 100 µL of sample plus 25 µL of IS mixture were mixed with 300 µL of methanol.
- After vortex-mixing and centrifugation, the supernatant was transferred to a micro vial or well of a microtiter plate containing 300 µL of water and mixed.
- 10 µL injections of each sample preparation were made into a 50 x 2.1 mm analytical column (Thermo Scientific™ Accucore™ PFP, 2.6 µm) on either channel of a Thermo Scientific™ Prelude™ sample preparation and liquid chromatography (SPLC) system (Figure 1).
- A mobile phase gradient from water containing 10 mM ammonium acetate to methanol containing 0.1% formic acid separated the AEDs. Table 2 shows LC method details.
- The AEDs were eluted into the heated electro-spray interface of a Thermo Scientific™ TSQ Quantiva™ triple-stage quadrupole mass spectrometer and subjected to positive-ion selected-reaction monitoring (SRM), as shown in Table 3. Ammonium adducts of Topiramate and its D_{12} IS were used as precursor ions. Data acquisition for each AED involved two SRM transitions, one for quantitation and one for confirmation, which were used for ion ratio confirmation (IRC).
- Peak identities were verified by retention time and IRC for each AED and quantitation values were determined using the appropriate internal standard.

Figure 1. Two-channel SPLC-MS system

METHODS

Table 2. LC method parameters

Column: Accucore PFP, 2.6 µ, 50 x 2.1 mm, @ 50°C						
Solvent A: Water + 10 mM ammonium acetate						
Solvent B: Methanol + 0.1% formic acid						
Step	**Start**	**Sec**	**Flow**	**Gradient**	**%A**	**%B**
1	0.00	30	0.5	Step	85	15
2	0.50	60	0.5	Ramp	45	55
3	1.50	30	0.5	Ramp	15	85
4	2.00	45	0.5	Step	2	98
5	2.75	75	0.5	Step	85	15
Start data: 0.2 min Duration: 2.0 min						
Total run time: 4.5 min (includes prestart & refill)						

Table 3. MS/MS method parameters

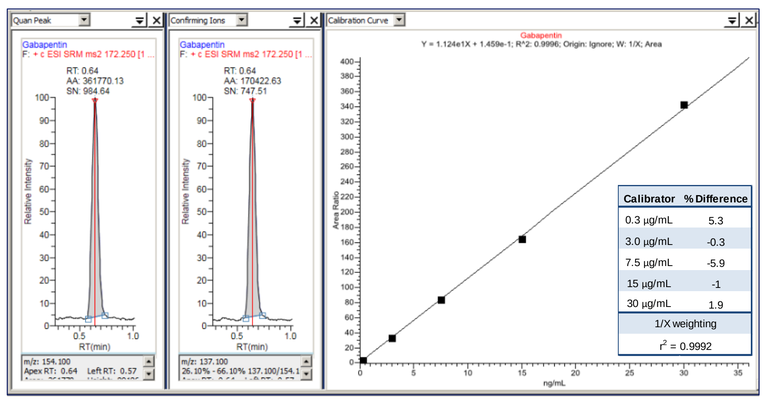
Ion Source: HESI @ +3500 V, Vaporizer @ 400°C, Ion-Transfer @ 350°C					
Sheath gas @ 55, Aux @ 10, Sweep @ 2 arbitrary units					
SRM Transitions: Q1 & Q3 resolutions = 0.7 FWHM, CID gas = 1.5 mTorr					
Analyte	**RT** (min)	**Precursor Ion** (m/z)	**Product Ion** (m/z)	**CE** (V)	**RF** (V)
Gabapentin (confirm)	0.60	172.25	137.10	17	42
Gabapentin (quan)	0.60	172.25	154.10	15	42
Gabapentin-D10	0.60	182.30	147.20	18	45
Pregabalin (confirm)	0.65	160.25	97.10	15	39
Pregabalin (quan)	0.65	160.25	142.20	10	39
Levetiracetan (quan)	0.70	171.25	126.10	15	33
Levetiracetan (confirm)	0.70	171.25	154.00	10	33
Levetiracetan-D6	0.70	177.30	132.10	16	30
Zonisamide (confirm)	1.30	213.15	77.10	30	51
Zonisamide (quan)	1.30	213.15	132.00	16	51
10-OH-Oxcarbazepine (confirm)	1.75	255.20	194.10	21	39
10-OH-Oxcarbazepine (quan)	1.75	255.20	237.00	10	39
Topiramate-NH4 (quan)	1.90	357.20	263.80	15	45
Topiramate-NH4 (confirm)	1.90	357.20	322.10	12	45
Topiramate-D12-NH4	1.90	369.20	270.00	15	47
Lamotrigine (confirm)	1.95	256.10	159.00	31	97
Lamotrigine (quan)	1.95	256.10	210.90	27	97

RESULTS

The 4.5-minute method with a 2-minute data window permitted throughputs of 13 samples per hour on a single channel or 26 samples per hour across both channels of the Prelude SPLC. This maximum throughput consumed only 32 mL of solvent A and 22 mL of Solvent B.

Calibration plots for each AED showed excellent linearity within the desired analytical range (e.g., 0.25 to 25 µg/mL for Pregabalin (Figure 2), 0.6 to 60 µg/mL for Zonisamide) with r^2 values greater than 0.99 and differences between calculated and theoretical concentrations for each calibrator were less than +/- 15%.

Figure 2. Example Quan & Confirm peaks and calibration data for Gabapentin



Blanks injected after the highest calibrator showed insignificant peak areas (carryover) for all AEDs.

The analytical accuracies of measuring the 7 AEDs in the UTAK QCs estimated as the percentage bias between nominal and average back-calculated concentrations were between -10% and 17%. The coefficient of variation values for all AEDs were less than 8% for inter-day batches and less than 15% for intra-day batches (Table 4).

RESULTS

Table 4. Inter- & Intra-assay QC results

			Inter-assay			Intra-assay		
AED	**UTAK**	**Nominal (µg/mL)**	**Mean (µg/mL)**	**SD**	**% CV**	**Mean (µg/mL)**	**SD**	**% CV**
Gabapentin	**QC1**	10	9.4	0.3	3.3	9.7	0.8	8.6
	QC2	40	34.7	1.2	3.3	35.9	3.0	8.2
Pregabalin	**QC1**	5	4.7	0.2	3.6	4.7	0.6	13.4
	QC2	15	14.5	0.5	3.4	14.7	1.8	12.0
Levetiracetam	**QC1**	15	14.4	0.5	3.8	14.5	1.0	6.5
	QC2	40	39.2	1.6	4.0	39.6	2.8	7.2
Zonisamide	**QC1**	20	23.7	1.8	7.7	20.3	2.1	10.4
	QC2	60	68.1	4.6	6.8	63.5	5.8	9.2
10-OH-Oxacarbazepine	**QC1**	20	22.3	1.1	5.1	19.4	2.4	12.2
	QC2	60	59.9	3.7	6.2	57.7	6.7	11.6
Lamotrigine	**QC1**	5.5	6.6	1.6	5.8	6.2	0.6	9.8
	QC2	22	27.0	1.6	5.8	25.7	2.9	11.4
Topiramate	**QC1**	15	16.5	0.6	3.7	15.0	1.0	6.5
	QC2	50	49.8	3.1	6.1	50.5	2.3	4.6
			n=10			**n=20**		

CONCLUSIONS

A reliable and rapid LC-MS/MS research method to quantitate 7 antiepileptic drugs in human blood serum was developed and evaluated. Required analytical ranges of each AED repeatedly had linear detector responses and insignificant carryover. The described method met research laboratory requirements for accuracy and precision. Reproducible results across both channels of the Prelude SPLC system permitted a maximum throughput of 26 samples per hour, which is more than an 8.5-fold improvement over a reference method (1).

REFERENCE

1. M. Subramanian, A.K. Birnbaum & R.P. Remmel, High-Speed Simultaneous Determination of Nine Antiepileptic Drugs Using Liquid Chromatography-Mass Spectrometry. Ther. Drug Monit., 2008, 30(3), 347-356.

TRADEMARKS/LICENSING

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