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Quantitative analysis of seven antiepileptic drugs in human serum using a two-channel LC-MS/MS system for research use

Authors

Mohamed Youssef,[1]
Joe Di Bussolo[2]

[1]Amerispec Diagnostics,
Dubai Science Park, Dubai, UAE
[2]Thermo Fisher Scientific,
San Jose, CA, USA

Keywords

Antiepileptics, anticonvulsants, drug panel, blood serum, multi-channel, monitoring, quantitation, liquid chromatography, tandem mass spectrometry

Method benefits

- Seven antiepileptic drugs measured in a 4.5-minute quantitative method
- Simple sample preparation of serum by protein precipitation and dilution
- Minimization of solvent consumption with innovative liquid chromatography system
- Two-fold sample throughput increase with two-channel LC-MS/MS system

Goal

Development and implementation of a high-throughput analytical research method for the separation, detection and quantification of seven antiepileptic drugs in donor human blood serum using a Thermo Scientific™ Prelude™ sample preparation and liquid chromatography (SPLC) system coupled to a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer.

Introduction

Clinical research laboratories traditionally rely on immunoassay and, more recently, on HPLC for quantitative analysis of antiepileptic drugs (AEDs). An efficient, fast, and accurate LC-MS/MS method with a wide analytical range was developed for the simultaneous quantitation of seven antiepileptic drugs in human serum for research purposes. This method employs protein precipitation followed by dilution before injection into a two-channel LC-MS/MS system, enabling analysis of seven AEDs in each injection every 2.3 minutes. The method produced significant savings by avoiding expensive sample preparation protocols, analysis time and solvent consumption compared to traditional HPLC methods.

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Experimental

Target analytes

The panel of analytes and corresponding concentration ranges, which cover the analytical ranges desired for research purposes, are listed in Table 1.

Table 1. Seven antiepileptic drugs quantitated in human blood serum.

Antiepileptic Drugs	Analytical Ranges (µg/mL)
Gabapentin	0.30–30
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

Sample preparation

Serum-based calibrators and quality controls (QCs), which can be purchased from UTAK Laboratories (Valencia, CA, USA), were subjected to protein precipitation by methanol after adding a mixture of stable isotopes of three AEDs that served as internal standards (IS).

Offline protein precipitation approach: 25 µL of IS mixture and 300 µL of methanol were added to 100 µL of specimen placed in a 1.5 mL micro-centrifuge tube. After vortex-mixing and centrifugation, the resulting supernatant was transferred to a clean 0.5 mL microtiter plate or autosampler vial containing 300 µL of water. After mixing, 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 Prelude SPLC system.

Liquid chromatography

The LC separation was achieved using a Prelude SPLC system with the columns, temperature, and mobile-phase gradient conditions shown in Table 2.

Table 2. LC conditions.

Column:	Accucore PFP, 2.6 µm, 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)						

Mass spectrometry

Analytes and internal standards were detected by positive-ion selected-reaction monitoring (SRM) using a TSQ Quantiva triple quadrupole mass spectrometer with heated electrospray ionization (HESI) and the parameters shown in Table 3. For each analyte, two SRM transitions were monitored—one used for quantification and the other for confirmation. Ammonium adducts of topiramate and its D_{12} IS were used as precursor ions.

Method evaluation

The method performance was evaluated by establishing limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precisions for each analyte. Analytical accuracy was determined in terms of percentage bias between nominal (target) and average back-calculated concentrations of the seven AEDs in two UTAK donor serum controls (Products #72740 and #72741). Intra-day precision was evaluated in terms of percentage coefficient of variation (%CV) using the UTAK serum controls in replicate injections (n=20) within one batch. Inter-assay precision was evaluated using the same controls in two replicate injections in each batch on five different days (n=10).

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 3.3 software.

Table 3. TSQ Quantiva MS/MS parameters.

Ion Source	
HESI:	+3,500 V
Vaporizer temp.:	400 ˚C
Ion transfer temp.:	350 ˚C
Sheath gas:	55 arbitrary units
Aux gas:	10 arbitrary units
Sweep gas:	2 arbitrary units
SRM Transitions	
Q1 & Q3 resolutions:	0.7 FWHM
CID gas:	1.5 mTorr

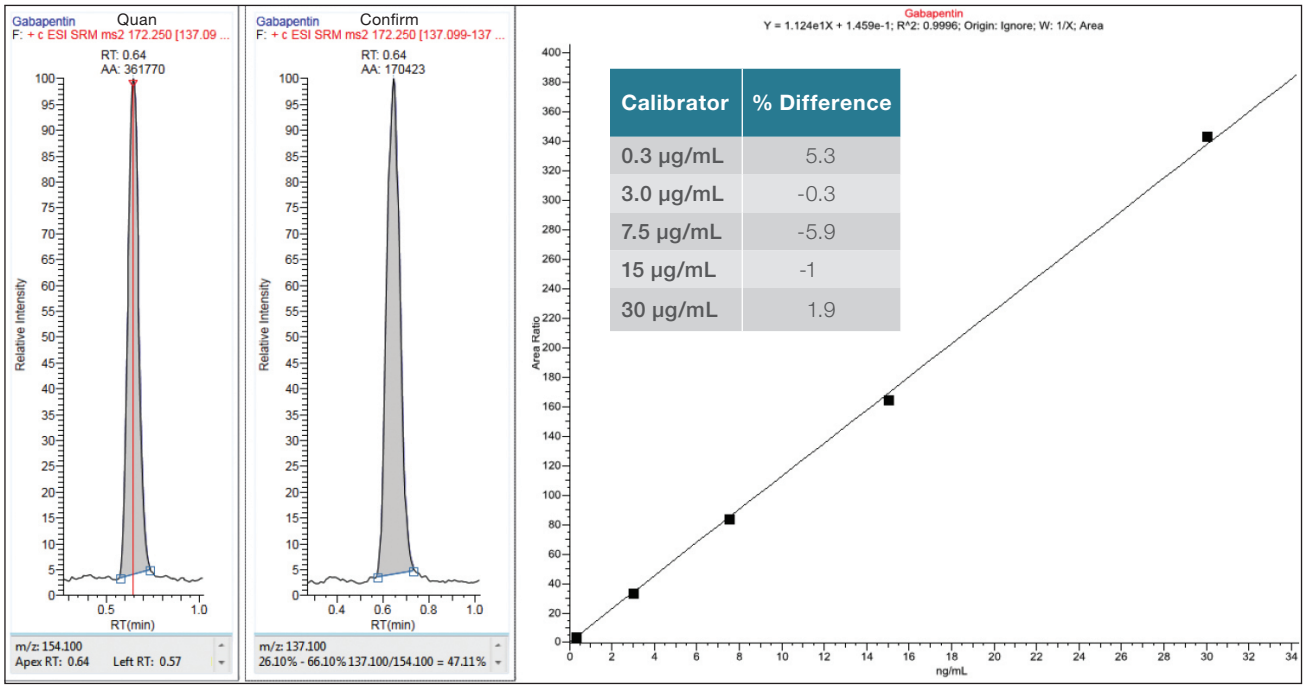
Analyte	RT (min)	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (v)	RF (v)
Gabapentin (confirm)	0.60	172.25	137.10	17	42
Gabapentin (quan)	0.60	172.25	154.10	15	42
Gabapentin-D_{10}	0.60	182.30	147.20	18	45
Pregabalin (confirm)	0.65	160.25	97.10	15	39
Pregabalin (quan)	0.65	160.25	142.20	10	39
Levetiracetan (quan)	0.70	171.25	126.10	15	33
Levetiracetan (confirm)	0.70	171.25	154.00	10	33
Levetiracetan-D_6	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-NH_4 (quan)	1.90	357.20	263.80	15	45
Topiramate-NH_4 (confirm)	1.90	357.20	322.10	12	45
Topiramate-D_{12}-NH_4	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 and discussion

The 4.5-minute method with a 2-minute data window permits 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 consumes only 32 mL of solvent A and 22 mL of Solvent B. A conventional single-channel HPLC system would produce half the throughput and consume 61 mL and 27 mL of solvents A and B, respectively. Hence, this method results in significant savings of solvent while ensuring higher throughput.

Peak identities were verified by retention time and ion-ratio confirmation for each AED and quantitation values were determined using the appropriate internal standard. Calibration plots for each AED showed excellent linearity within the desired analytical range with r^2 values greater than 0.99 and differences between calculated and theoretical concentrations for each calibrator less than ±15%. Blanks injected after the highest calibrator showed insignificant carryover for all AEDs. Figure 1 shows example results for gabapentin.

The analytical accuracies of measuring the seven 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, as shown in Table 4.



Calibrator	% Difference
0.3 µg/mL	5.3
3.0 µg/mL	-0.3
7.5 µg/mL	-5.9
15 µg/mL	-1
30 µg/mL	1.9

Figure 1. Example Quan & Confirm peaks and calibration plot for gabapentin.

Table 4. Accuracy, inter- and intra-assay precisions.

	UTAK	Nominal (µg/mL)	Mean (µg/mL)	Inter-assay		Mean (µg/mL)	Intra-assay	
				SD	% CV		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, robust, sensitive, and rapid LC-MS/MS research method to quantify seven antiepileptic drugs in human blood serum was developed and evaluated. The 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.

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