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Analysis of Antiepileptic Drugs in Plasma for Clinical Research

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APPLICATION BENEFITS

- Low volume, simple sample preparation
- One method for the quantification of 18 antiepileptic drugs and metabolites that cover a wide range of polarities

WATERS SOLUTIONS

ACQUITY™ UPLC™ I-Class with Flow Through Needle (FTN)

CORTECS™ C_8 Column

Xevo™ TQD Mass Spectrometer

MassLynx™ Software

TargetLynx™ Application Manager

KEYWORDS

10,11-dihydro-10-hydroxycarbamazepine; carbamazepine; felbamate; gabapentin; lacosamide; lamotrigine; levetiracetam; oxcarbazepine; perampanel; phenobarbital; phenytoin; pregabalin; primidone; retigabine; tiagabine; topiramate; valproic acid; zonisamide; UPLC-MS/MS

INTRODUCTION

Pharmacokinetic interactions between antiepileptic drugs are a known phenomenon, therefore an accurate quantitative method may play a role in researching the pharmacokinetic and pharmacodynamic effects of administration of antiepileptic drugs.

Here we describe a clinical research method using protein precipitation of a plasma sample with internal standards. Chromatographic elution was completed within five minutes using a Waters™ CORTECS C_8 Column on an ACQUITY UPLC I-Class System followed by detection on a Xevo TQD Triple Quadrupole Mass Spectrometer utilizing polarity switching (Figure 1).

Figure 1. The Waters ACQUITY UPLC I-Class System with FTN and Xevo TQD Mass Spectrometer.

EXPERIMENTAL

Sample preparation

Plasma calibrators and quality control materials were prepared in-house using pooled human plasma supplied by BioIVT (West Sussex, UK). Concentrated stock solutions were prepared from certified powders and solutions supplied by Cambridge Bioscience (Cambridgeshire, UK), Fisher (Loughborough, UK), Sigma-Aldrich (Dorset, UK), and Toronto Research Chemicals (Ontario, Canada). Stable labelled internal standards were supplied by Cambridge Bioscience (Cambridgeshire, UK), Sigma-Aldrich (Dorset, UK), and Toronto Research Chemicals (Ontario, Canada). The calibration range was 1–100 µg/mL for all analytes, except for oxcarbazepine, perampanel, and pregabalin (0.1–10 µg/mL), tiagabine (0.01–1 µg/mL), and valproic acid (2–200 µg/mL). In-house quality control samples were prepared in plasma at low, medium, and high concentrations of 2.5, 7.5, and 40 µg/mL for 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, phenobarbital, phenytoin, primidone, topiramate, and zonisamide; 0.25, 0.75, and 4 µg/mL for oxcarbazepine, perampanel, pregabalin and retigabine (0.1–10 µg/mL); 0.025, 0.075, and 0.4 µg/mL for tiagabine; and 5, 15, and 80 µg/mL for valproic acid.

Sample extraction

To 50 µL of sample, 200 µL of internal standard in methanol was added, containing 5 µg/mL of valproic acid-$^{2}H_{6}$, 2.5 µg/mL of carbamazepine-$^{2}H_{2}{}^{15}N$, 10,11-dihydro-10-hydroxycarbamazepine-$^{13}C_{6}$, felbamate-$^{2}H_{4}$, gabapentin-$^{2}H_{4}$, lacosamide-$^{2}H_{6}$, levetiracetam-$^{2}H_{3}$, phenobarbital-$^{2}H_{5}$, phenytoin-$^{2}H_{10}$, primidone-$^{2}H_{5}$, topiramate-$^{13}C_{6}$ and zonisamide-$^{13}C_{2}{}^{15}N$, 0.625 of µg/mL lamotrigine-$^{13}C_{3}$ and oxcarbazepine-$^{2}H_{4}$, 0.5 µg/mL of perampanel-$^{2}H_{5}$, 0.25 µg/mL of pregabalin-$^{13}C_{3}$ and retigabine-$^{2}H_{4}$, and 0.03125 µg/mL of tiagabine-$^{2}H_{6}$. Tubes were placed on a multi-tube vortex mixer at 2500 rpm for 30 seconds, then centrifuged for two minutes at 16,100 g. Fifty microliters (50 µL) of supernatant were transferred to a 1-mL, 96-well plate and 350 µL water added. The plate was then centrifuged at 4,696 g for two minutes prior to analysis.

UPLC conditions

System:	ACQUITY UPLC I-Class with Flow-Through Needle (FTN)
Needle:	30 µL
Column:	CORTECS C_{8} Column; 2.7 µm, 2.1 × 50 mm (p/n: 186008349)
Mobile phase A:	Water + 2 mM ammonium acetate
Mobile phase B:	Methanol + 2 mM ammonium acetate
Needle wash solvent:	80% aqueous methanol + 0.1% formic acid
Purge solvent:	5% aqueous methanol
Seal wash:	20% aqueous methanol
Column temp.:	50 °C (precolumn heater active)
Injection volume:	20 µL
Flow rate:	0.50 mL/min
Gradient elution:	(see Table 1)

Time (min)	% Mobile phase A	% Mobile phase B	Curve
Initial	95	5	Initial
0.2	95	5	6
1.5	75	25	6
2.5	75	25	6
4.0	30	70	6
4.01	5	95	6
4.5	5	95	6
4.51	95	5	6

Table 1. Chromatographic elution timetable.

Run time: 5.0 minutes (5.7 minutes injection-to-injection)

MS conditions

System: Xevo TQD

Resolution: MS1 (0.7 FWHM), MS2 (0.7 FWHM)

Acquisition mode: Multiple reaction monitoring (MRM) (see Table 2 for details)

Polarity: ESI positive ionization/ESI negative ionization (ESI+/ESI-)

Capillary: 3.5 kV (ESI+)/0.8 (ESI-)

Source temp.: 150 °C

Desolvation temp.: 500 °C

Cone gas: 100 L/hr

Inter-scan delay: 0.003 seconds

Polarity/mode switch inter-scan delay: 0.020 seconds

Inter-channel delay: 0.003 seconds

Data management

MassLynx Software v4.2 with TargetLynx Application Manager

Function (acquisition time, min)	Analyte	Polarity	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Dwell time (s)
1 (0.65–1.65)	Pregabalin (Quan)	ESI+	160.05	142.1	24	10	0.015
	Pregabalin (Qual)	ESI+	160.05	97.1	24	16	0.015
	Pregabalin-$^{13}C_3$	ESI+	163.1	145.1	24	10	0.015
	Levetiracetam (Quan)	ESI+	171.1	126.1	14	14	0.015
	Levetiracetam (Qual)	ESI+	171.1	69.1	14	28	0.015
	Gabapentin (Quan)	ESI+	172.1	154.1	24	14	0.015
	Gabapentin (Qual)	ESI+	172.1	55.1	24	20	0.015
	Levetiracetam-$^{2}H_3$	ESI+	174.1	129.1	14	14	0.015
	Gabapentin-$^{2}H_4$	ESI+	176.1	158.1	24	14	0.015
	Zonisamide (Quan)	ESI+	212.95	132.1	28	14	0.015
	Zonisamide (Qual)	ESI+	212.95	77.1	28	30	0.015
	Zonisamide-$^{13}C_2{}^{15}N$	ESI+	215.95	134.1	28	14	0.015
2 (1.65–2.22)	Primidone (Quan)	ESI+	219.1	162.1	24	12	0.010
	Primidone (Qual)	ESI+	219.1	91.1	24	32	0.010
	Primidone-$^{2}H_5$	ESI+	224.1	167.1	24	12	0.015
	Felbamate (Quan)	ESI+	239.1	178.1	14	8	0.010
	Felbamate (Qual)	ESI+	239.1	117.1	14	18	0.010
	Felbamate-$^{2}H_4$	ESI+	243.1	182.1	14	8	0.010
	Lacosamide (Quan)	ESI+	251.1	91.1	20	18	0.010
	Lacosamide (Qual)	ESI+	251.1	74.1	20	22	0.010
	Lacosamide-$^{2}H_3$	ESI+	254.1	91.1	20	18	0.015
	Lamotrigine (Quan)	ESI+	255.95	211.1	50	24	0.015
	Lamotrigine (Qual)	ESI+	255.95	145.1	50	38	0.015
	Lamotrigine-$^{13}C_3$	ESI+	258.95	214.1	50	24	0.025
3 (2.12–2.50)	Phenobarbital (Quan)	ESI-	231.1	42.0	28	14	0.075
	Phenobarbital (Qual)	ESI-	231.1	188.1	28	10	0.075
	Phenobarbital-$^{2}H_5$	ESI-	236.1	42.0	28	14	0.075
4 (2.50–3.10)	Valproic acid	ESI-	143.0	143.1	30	5	0.015
	Valproic acid-$^{2}H_6$	ESI-	149.0	149.1	30	5	0.015

Table 2 continued.

Function (acquisition time, min)	Analyte	Polarity	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Dwell time (ms)
5 (2.50–3.10)	10,11-dihydro-10-hydroxycarbamazepine (Quan)	ESI+	255.1	194.1	16	18	0.025
	10,11-dihydro-10-hydroxycarbamazepine (Qual)	ESI+	255.1	179.1	16	32	0.025
	10,11-dihydro-10-hydroxycarbamazepine-$^{13}C_6$	ESI+	261.1	200.1	16	18	0.025
	Topiramate (Quan)	ESI+	340.1	264.1	30	8	0.025
	Topiramate (Qual)	ESI+	340.1	184.1	30	18	0.025
	Topiramate-$^{13}C_6$	ESI+	340.1	270.1	30	8	0.025
6 (3.10–3.50)	Oxcarbazepine (Quan)	ESI+	253.1	180.1	30	30	0.050
	Oxcarbazepine (Qual)	ESI+	253.1	208.1	30	20	0.050
	Oxcarbazepine-$^{2}H_4$	ESI+	257.1	184.1	30	30	0.050
7 (3.50–3.85)	Carbamazepine (Quan)	ESI+	237.1	179.1	34	34	0.020
	Carbamazepine (Qual)	ESI+	237.1	165.1	34	34	0.020
	Carbamazepine-$^{2}H_2{}^{15}N$	ESI+	240.1	181.1	34	34	0.030
	Phenytoin (Quan)	ESI+	253.1	104.1	28	34	0.005
	Phenytoin (Qual)	ESI+	253.1	182.1	28	16	0.005
	Phenytoin-$^{2}H_{10}$	ESI+	263.1	109.1	28	34	0.005
	Retigabine (Quan)	ESI+	304.1	109.1	34	34	0.015
	Retigabine (Qual)	ESI+	304.1	230.1	34	18	0.015
	Retigabine-$^{2}H_4$	ESI+	308.1	113.1	34	34	0.025
8 (3.85–4.20)	Perampanel (Quan)	ESI+	350.1	219.1	60	34	0.015
	Perampanel (Qual)	ESI+	350.1	247.1	60	26	0.015
	Perampanel-$^{2}H_5$	ESI+	355.1	220.1	60	34	0.015
	Tiagabine (Quan)	ESI+	376.1	111.1	40	36	0.010
	Tiagabine (Qual)	ESI+	376.1	247.1	40	20	0.010
	Tiagabine-$^{2}H_6$	ESI+	382.1	114.1	40	36	0.010

Table 2. Guideline MRM parameters for analytes and internal standards used in this study.

RESULTS

No system carryover was observed following analysis of plasma samples with 100 µg/mL of 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, phenobarbital, phenytoin, primidone, topiramate, and zonisamide; 10 µg/mL of oxcarbazepine, perampanel, pregabalin, and retigabine; 1 µg/mL of tiagabine; and 200 µg/mL of valproic acid.

Figure 2 shows an example chromatogram for the analysis of the 18 antiepileptic drugs and metabolites.

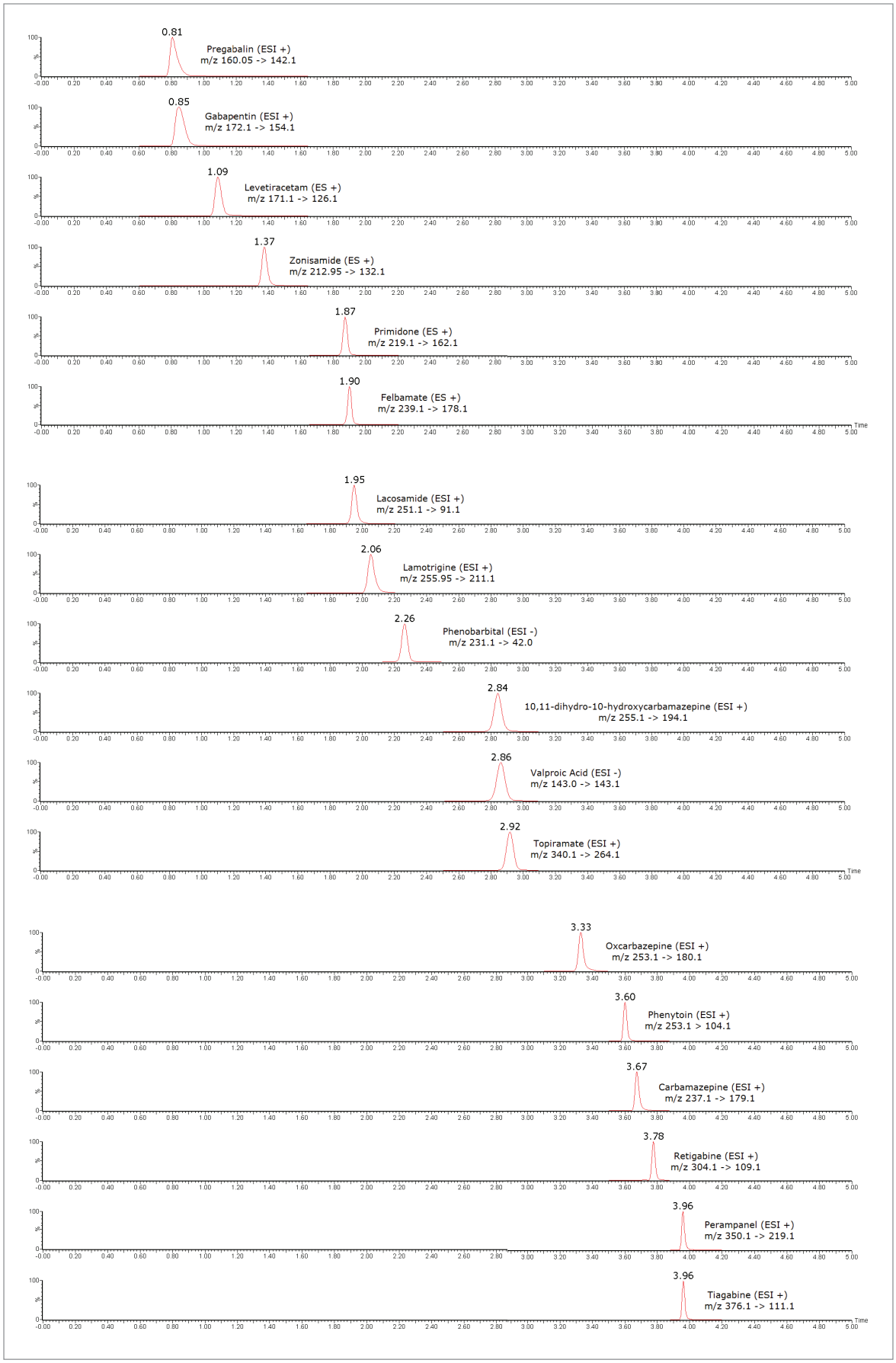

Figure 2. Chromatogram showing the analysis of antiepileptic drugs using the ACQUITY UPLC I-Class/Xevo TQD IVD System.

Analytical sensitivity was assessed by extracting and quantifying 10 replicates of low concentration samples prepared in plasma over five days (n=50). Investigations indicated the method would allow for precise quantification (≤20% CV, ≤15% bias) at the concentrations shown in Table 3.

Analyte	Analytical sensitivity (µg/mL)	Precision (%CV)	Deviation from nominal (%)
10,11-dihydro-10-hydroxycarbamazepine	0.50	13.6	-5.2
Carbamazepine	0.50	12.1	-4.2
Felbamate	0.75	12.7	-13.3
Gabapentin	0.50	16.3	-14.8
Lacosamide	1.00	11.0	-11.5
Lamotrigine	0.90	13.2	-14.8
Levetiracetam	0.50	11.9	-14.4
Oxcarbazepine	0.0750	16.4	-12.4
Perampanel	0.0750	11.3	-7.1
Phenobarbital	1.00	19.6	6.6
Phenytoin	0.50	17.9	-5.8
Pregabalin	0.0750	16.1	-9.1
Primidone	0.50	19.1	-13.6
Retigabine	0.0750	11.5	-13.6
Tiagabine	0.00750	15.8	2.3
Topiramate	0.75	15.4	3.5
Valproic acid	1.50	10.1	6.5
Zonisamide	0.50	17.7	-0.2

Table 3. Analytical sensitivity summary.

Total precision was determined by extracting and quantifying five replicates of three concentrations of plasma pools over five separate days (n=25). Repeatability was assessed by analyzing five replicates at each QC level. Table 4 presents results of these experiments, where total precision and repeatability at the three concentrations assessed was ≤9.5% RSD.

	Total QC precision (% CV)			QC repeatability (% CV)		
Analyte	Low	Mid	High	Low	Mid	High
10,11-dihydro-10-hydroxycarbamazepine	6.5	6.7	4.1	4.7	4.5	2.9
Carbamazepine	5.2	5.8	4.5	5.0	4.7	3.5
Felbamate	6.8	6.2	4.0	6.2	4.6	4.0
Gabapentin	6.0	6.6	5.4	3.9	4.9	3.7
Lacosamide	4.8	5.9	5.2	4.2	4.8	4.3
Lamotrigine	6.5	5.4	5.7	5.3	5.2	2.9
Levetiracetam	5.4	5.8	4.8	4.3	4.4	3.0
Oxcarbazepine	9.3	8.6	7.4	5.7	6.3	3.6
Perampanel	4.8	5.8	5.7	4.1	4.4	3.1
Phenobarbital	8.4	8.6	7.7	8.4	8.1	4.7
Phenytoin	9.4	9.5	8.9	7.5	9.1	8.3
Pregabalin	6.7	5.3	5.7	6.1	4.6	3.1
Primidone	6.3	6.6	4.4	5.2	4.9	2.8
Retigabine	6.5	6.2	6.1	5.2	4.7	3.5
Tiagabine	8.4	6.1	5.4	7.3	6.1	4.8
Topiramate	5.4	5.7	4.3	3.9	4.6	3.2
Valproic acid	6.9	6.4	6.4	4.5	4.2	3.6
Zonisamide	5.4	6.7	4.5	4.7	4.8	2.9

Table 4. Total precision and repeatability performance.

The method was shown to be linear over the range of 0.752–130 µg/mL for phenobarbital, topiramate, and zonisamide, when low and high pools were mixed in known ratios over the range. Linear fits were used to construct calibration lines. Carbamazepine, 10,11-dihydro-10-hydroxycarbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, phenytoin, and primidone were determined to have quadratic fits over 0.752–130 µg/mL. Similarly, oxcarbazepine, perampanel, pregabalin, and retigabine were quadratic over 0.0752–13.0 µg/mL; tiagabine over 0.00752–1.30 µg/mL; and valproic acid over 1.5–260 µg/mL. Quadratic fits were used to construct calibration lines.

Matrix effects were evaluated at low (QC1) and high (QC3) concentrations in plasma (n=6) taken as a percentage of extracted solvent samples spiked to equivalent concentrations. Calculations using analyte:internal standard response ratio indicated compensation for signal enhancement by the internal standard (Table 5).

Analyte	Spiked concentration (µg/mL)	Matrix factor based on peak area mean (range)	Matrix factor based on response mean (range)
10,11-dihydro-10-hydroxycarbamazepine	2.5	1.01 (1.00–1.02)	1.00 (0.99–1.01)
	40	1.00 (0.98–1.01)	1.01 (1.00–1.03)
Carbamazepine	2.5	1.00 (0.98–1.02)	1.00 (0.99–1.00)
	40	1.00 (0.97–1.02)	1.01 (1.01–1.02)
Felbamate	2.5	0.98 (0.98–0.99)	0.97 (0.95–0.98)
	40	0.99 (0.97–1.00)	1.00 (0.98–1.04)
Gabapentin	2.5	0.98 (0.97–0.99)	0.99 (0.98–1.00)
	40	0.99 (0.97–1.01)	1.02 (0.99–1.06)
Lacosamide	2.5	0.99 (0.98–1.00)	0.99 (0.97–1.01)
	40	1.00 (0.99–1.00)	1.00 (0.99–1.01)
Lamotrigine	2.5	1.02 (0.98–1.06)	1.01 (0.97–1.02)
	40	1.00 (0.98–1.01)	1.01 (1.01–1.02)
Levetiracetam	2.5	0.99 (0.98–1.00)	0.98 (0.95–1.01)
	40	1.00 (0.98–1.01)	1.00 (0.99–1.03)
Oxcarbazepine	0.25	0.98 (0.91–1.01)	0.99 (0.97–1.01)
	4	0.98 (0.94–0.99)	1.01 (0.99–1.03)
Perampanel	0.25	0.83 (0.79–0.87)	0.96 (0.95–0.99)
	4	0.89 (0.85–0.92)	1.01 (0.99–1.02)
Phenobarbital	2.5	0.97 (0.90–1.12)	0.97 (0.91–1.09)
	40	0.98 (0.95–1.00)	1.03 (1.01–1.06)
Phenytoin	2.5	0.95 (0.92–1.01)	0.99 (0.96–1.05)
	40	0.97 (0.97–0.98)	1.01 (0.98–1.03)
Pregabalin	0.25	0.99 (0.91–1.03)	0.99 (0.90–1.03)
	4	0.99 (0.96–1.02)	1.03 (1.00–1.05)
Primidone	2.5	1.00 (1.00–1.00)	0.99 (0.97–1.01)
	40	1.00 (0.99–1.02)	1.03 (1.01–1.05)
Retigabine	0.25	1.37 (1.32–1.44)	0.94 (0.91–1.03)
	4	1.17 (1.12–1.21)	0.91 (0.88–0.96)
Tiagabine	0.025	1.02 (0.98–1.08)	0.94 (0.87–1.01)
	0.4	1.05 (1.02–1.09)	1.00 (0.95–1.05)
Topiramate	2.5	1.00 (0.99–1.01)	0.98 (0.97–1.00)
	40	1.00 (0.99–1.00)	1.01 (0.99–1.02)
Valproic acid	5	1.01 (0.96–1.04)	0.99 (0.95–1.03)
	80	1.00 (0.99–1.02)	1.02 (1.00–1.03)
Zonisamide	2.5	0.99 (0.98–1.00)	0.99 (0.96–1.01)
	40	0.99 (0.98–1.00)	1.02 (1.01–1.04)

Table 5. Matrix factor summary.

Potential interference from endogenous compounds (albumin, bilirubin, cholesterol, triglycerides, and uric acid) spiked at high concentrations was assessed by determining the recovery (n=3) from low and high pooled plasma samples (QC1 and QC3 concentrations). Recoveries ranged from 85.1–112.8%. A substance was deemed to interfere if a recovery range of 85–115% was exceeded. Additionally, full chromatographic resolution of the metabolite carbamazepine epoxide from isobaric oxcarbazepine was established.

LGC (Greater London, UK) provided serum external quality assurance samples for accuracy testing, except for oxcarbazepine and retigabine, which were not included in the schemes. All samples passed the criteria of the scheme, with mean deviations ≤10.6% from assigned concentrations. Results are presented in Table 6.

Analyte	Scheme range (µg/mL)	Number of samples tested	Waters mean % deviation from scheme assigned value
10,11-dihydro-10-hydroxycarbamazepine	0–40.76	10	0.7
Carbamazepine	0–38.00	30	-2.0
Felbamate	0–102.49	10	10.6
Gabapentin	0–37.75	10	-2.2
Lacosamide	0–23.87	10	7.0
Lamotrigine	0–34.68	30	-0.3
Levetiracetam	0–100.00	10	0.9
Oxcarbazepine	N/A	N/A	N/A
Perampanel	0–0.592	10	-0.8
Phenobarbital	0–55.00	30	-6.1
Phenytoin	0–36.00	30	5.4
Pregabalin	0–73.06	10	-6.3
Primidone	0–35.15	30	0.6
Retigabine	N/A	N/A	N/A
Tiagabine	0–0.2723	10	-4.4
Topiramate	0–39.3	10	0.7
Valproic acid	0–213.5	30	1.4
Zonisamide	0–48.36	10	-2.6

Table 6. Accuracy summary (N/A = not available).

CONCLUSIONS

The developed method for clinical research demonstrates the capabilities of the sample preparation and UPLC-MS/MS system to quantify 18 antiepileptic drugs and metabolites in plasma. The method demonstrated excellent performance characteristics, including precision and agreement with an external quality assurance scheme, with neither system carryover nor significant matrix effects.

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