A NOVEL UPLC-MS/MS METHOD FOR PLASMA ANTIEPILEPTIC DRUG ANALYSIS FOR CLINICAL RESEARCH

Stephen Balloch, Gareth Hammond, Ben Dugas and Lisa J Calton
Waters Corporation, Stamford Avenue, Wilmslow, UK.

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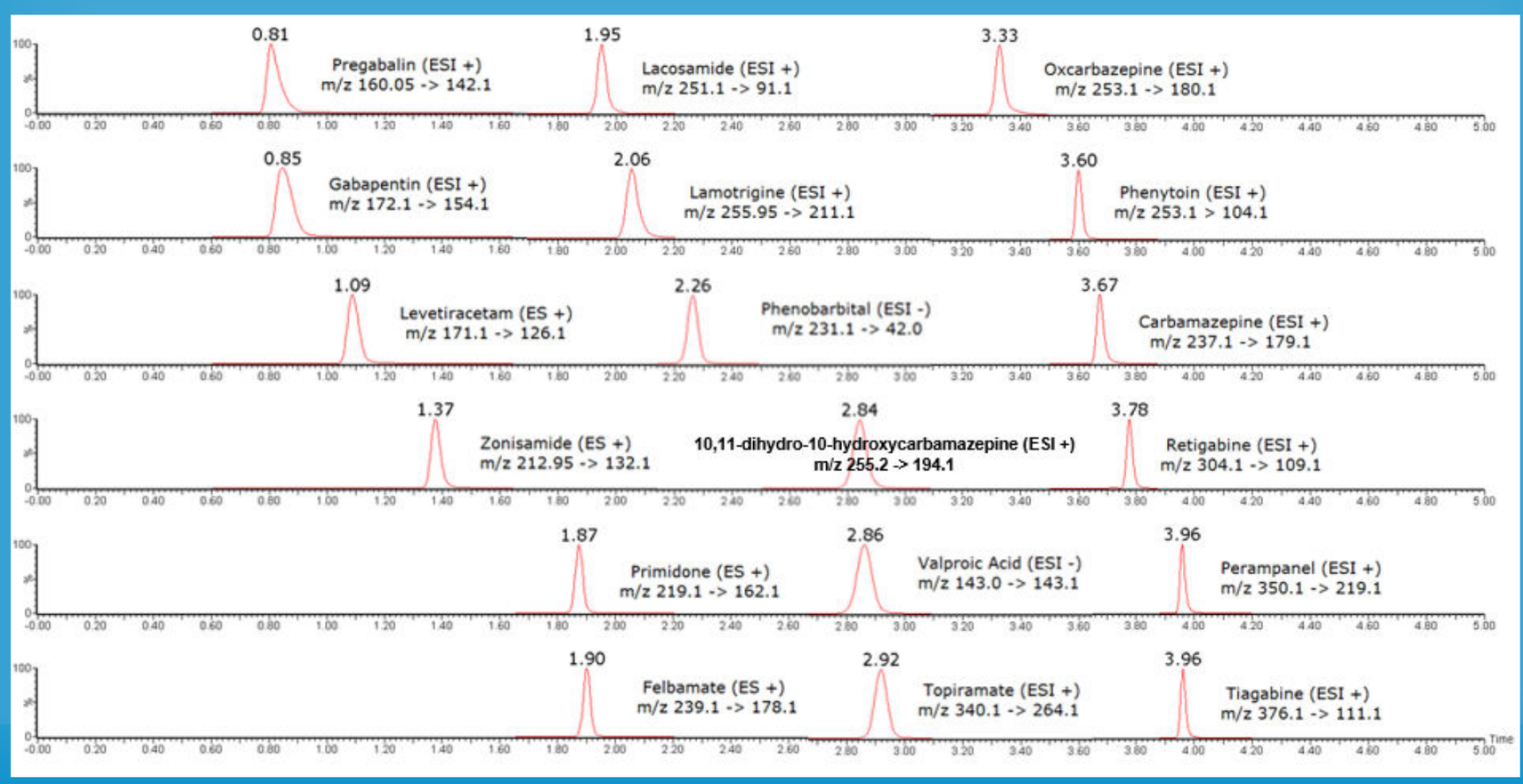
18 AEDS AND METABOLITES ANALYZED IN 5.7 MINUTES



METHODS

Materials

- Matrix matched calibrators and QCs were prepared using in-house stocks and pooled plasma.
- Internal standard, containing stable labeled analogs , was prepared in methanol.

Methods

- 50µL serum samples were pre-treated with internal standard.
- Tubes were shaken on a multi-tube vortex mixer, then centrifuged. Supernatant was transferred to a 1 mL, 96 well plate and water was added prior to injection.
- Using a Waters ACQUITY UPLC™ I-Class FTN System, samples were injected onto a 2.7 µm, 2.1 x 50mm Waters CORTECS™ C_8 column with a water/methanol/ ammonium acetate gradient.
- A Waters Xevo™ TQD detector was used to analyze the samples, using electrospray ionization and polarity switching between positive and negative modes.
- No dilutions or variable injection volumes were necessary; a "one-size-fits-all" approach was used.
- The analysis time per sample was approximately 5.7 minutes injection-to-injection.

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

RESULTS

Linearity, Analytical Sensitivity and Carryover

- Calibration was performed over 1-100 µg/mL for 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, phenobarbital, phenytoin, primidone, topiramate and zonisamide; over 0.1-10 µg/mL for oxcarbazepine, perampanel, pregabalin and retigabine; over 0.01-1 µg/mL for tiagabine and over 2-200 µg/mL for valproic acid..
- Linearity experiments determined the method was linear for phenobarbital, topiramate and zonisamide and quadratic for the remainder of the panel.
- Analytical sensitivity investigations indicated precise quantification (≤20% CV, ≤15% bias) at concentrations equal to or lower than the lowest concentration calibrator.
- No system carryover was observed following analysis of plasma samples containing the highest concentration calibrators.

Matrix Effects and Ion Suppression

- Matrix effect investigations for were evaluated at low and high concentrations for all 18 analytes, using six individual plasma samples.
- Normalized matrix factor calculations, based on the analyte:internal standard response ratio demonstrated that the internal standards compensated for any ion suppression observed, with mean matrix factors in the range 0.91-1.03.
- Post-column infusion experiments revealed that analytes eluted in regions free of significant ion suppression.

Precision

- Low, mid and high concentrations plasma pools were analysed in replicates of 5, on 5 occasions (n=25), to assess repeatability and total precision
- Reproducibility and total precision was determined to be ≤9.5% CV for the entire panel and across the range of concentrations tested.
- In many instances, repeatability and total precision was lower still.

Interference Testing

- Potential interference from endogenous compounds (albumin, bilirubin, cholesterol, triglycerides and uric acid) was assessed at low and high concentrations.
- A substance was deemed to interfere if a recovery range of 85-115% was exceeded; recoveries ranged from 85.1-112.8%.
- Additionally, full chromatographic resolution of the metabolite carbamazepine epoxide from isobaric oxcarbazepine was established.

Accuracy

- LGC (London, UK) provided external quality assurance (EQA) samples in serum; all analytes were present, with the exception of oxcarbazepine and retigabine.
- Waters determinations met the LGC provided acceptable concentration ranges for all EQA samples.
- Mean deviations were ≤10.6% from assigned EQA concentrations.
- Selected results are presented in Table 1, below:

Analyte	Scheme Range (µg/mL)	Number of samples	Mean % deviation from assigned value
10,11-dihydro10-hydroxycarbamazapine	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	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 1. LGC EQA Result Summary

CONCLUSION

- **A clinical research method for the analysis of 18 antiepileptic drugs and metabolites has been developed**
- **All are analyzed simultaneously, from a 50 µL sample, in a little over 5 minutes**
- **Precision goals were met, minimal matrix effects and absence of carryover have been established**
- **Furthermore, strong agreement with an External Quality Assurance scheme was observed**

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