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

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Background: Waters has developed a clinical research method for the following 18 antiepileptic drugs and metabolites in plasma; 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, phenobarbital, phenytoin, primidone, topiramate and zonisamide (1-100 µg/mL); oxcarbazepine, perampanel, pregabalin and retigabine (0.1-10 µg/mL); tiagabine (0.01-1 µg/mL) and valproic acid (2-200 µg/mL).

Methods: Matrix matched calibrators were prepared using in-house stocks and pooled plasma. Samples (50 µL) were treated with internal standard in methanol. A water/methanol/ammonium acetate gradient was used with a Waters CORTECS C8 column on a Waters ACQUITY UPLC I-Class FTN and Xevo TQD mass spectrometer utilizing polarity switching in a 5 minute run.

Results: No system carryover was observed following analysis of plasma samples containing the highest concentration calibrators.

Analytical sensitivity investigations indicated precise quantification (≤20% CV, ≤15% bias) at concentrations equal to or lower than the lowest concentration calibrator.

Total precision and repeatability were assessed (3 pools, 5 replicates, 5 days; n=25) and determined to be ≤9.5% RSD.

Linearity experiments determined the method was linear for phenobarbital, topiramate and zonisamide and quadratic for the remainder of the panel.

Analytes eluted in regions free of major ion suppression or enhancement. Evaluation of matrix effects at low and high concentrations indicated compensation by the internal standard.

Addition of high concentrations of several endogenous materials did not affect quantification. Full chromatographic resolution of the metabolite carbamazepine epoxide from isobaric oxcarbazepine was established.

Serum external quality assurance samples (n=10-30) for accuracy testing were analyzed, except for oxcarbazepine and retigabine (not included). All samples passed the supplied criteria, with mean deviations ≤10.6% from assigned concentrations.

Conclusions: This quantitative method for clinical research demonstrates excellent precision and accuracy with minimal matrix effects and allows for the multiplexing of 18 antiepileptic drugs and metabolites in plasma.

Key Words: Antiepileptic, LC-MS/MS, mass spectrometry, multiplexing