Montgomery County Coroner's Office / Miami Valley Regional Crime Laboratory - Toxicology

Confirmation and Quantitation of Antiepileptic and Acidic Drugs by LC/MS/MS

Summary of Validation

The toxicology laboratory validated a new protein crash extraction and LC/MS/MS method for confirming and quantitating 14 antiepileptic/acidic drugs in blood, serum, urine and tissue homogenate. All calibrators, controls and interference studies were prepared from NIST-Traceable reference standards with certificates of analysis. Analytes include pregabalin, gabapentin, levetiracetam, lamotrigine, zonisamide, topiramate, butalbital, meprobamate, carisoprodol, phenytoin, oxcarbazepine, carbamazepine, Carbamazepine-10,11-epoxide, and (+/-)-10,11-dihydro-10-hydroxycarbamazepine.

The method was validated using 7 blood calibrators and low, medium, and high controls in blood, serum, urine and tissue homogenate. The analytical work was done by Philip Carter, Treena Wiebe, Elizabeth Kiely, Kialee Bowles, and Brian Simons and reviewed by Heather Antonides.

This validation started on April 16, 2020 and ended on April 24, 2020. LCMSMS instruments #2 and #3 were used during the method validation and all data was combined. LCMSMS#1 cannot be used for this method due to fast polarity switching in the same time segment.

The method was determined to be acceptable for the qualitative and quantitative determination of all analytes listed above.

The validated parameters are shown below:

Parameter	**Acceptance Criteria**	**Results**
Bias	Maximum of +/- 20%	All biases (within and between day) were less than 20%
Carryover	A negative specimen following the highest calibrator is a true negative	No carryover was seen in negative specimens following the highest calibrator
Interference	No interfering signal from matrix or drugs used in assay	No interfering signals were seen in the matrices or with related and unrelated drugs commonly seen in this laboratory. The internal standard does not interfere with the analytes and the analytes do not interfere with either internal standard.
Limit of Detection	At least 1 ng/mL	All analytes employ quadratic regression for the curves. All LODs are administratively set to the lowest acceptable calibrator. See Note in SOP for lamotrigine.
Precision	The % coefficient of variation for	The % coefficient of variation for all

	controls (within and between runs) not to exceed 20%	controls (within and between runs) was less than 20%
Stability on the autosampler	Determine stability post extraction	Gabapentin was found to be stable on the instrument for four days post extraction using Gabapentin13C3 as the internal standard. All other analytes, based on the tolbutamide IStd must be reinjected with calibrators and controls in order to report a quantitative result from a reinjection.
Recovery	N/A	Most analytes exhibited a recovery of about 70%. Tolbutamide exhibited the lowest recovery at 55%.
Matrix Effect	N/A	Due to the nature of the protein crash/extraction matrix effects could not be evaluated.
Uncertainty of Measurement	N/A	The UOM was set for all analytes (k=2.17). The UOM will be evaluated again in one year.