# Simultaneous LC/MS/MS Quantitation of 20 Antiepileptic Drugs in Human Serum

**Lauren E. Frick and Vaughn P. Miller**
Agilent Technologies, Inc; Lexington, MA

MSACL 2017
Poster # 21D

Agilent Technologies

## Introduction

One major strength of liquid chromatography-mass spectrometry (LC/MS/MS) as a detection method is that it allows the concurrent monitoring of multiple analytes in a single injection. Here, an LC/MS/MS analytical method has been utilized to quantify a panel of 20 antiepileptic drugs in human serum. In contrast, historic assays have traditionally monitored a smaller number of compounds due to large concentration discrepancies of the similar analytes. Compounds included in the panel were: Acetylretigabine, Carbamazepine-10,11-Epoxide, Carbamazepine, 10,11-Dihydro-10-Hydroxy-Carbamazepine, Felbamate, Gabapentin, Lacosamide, Lamotrigine, Levetiracetam, Oxcarbazepine, Phenobarbital, Phenytoin, Pregabalin, Retigabine, Rufinamide, Tiagabine, Topiramate, Valproic Acid, Vigabatrin, Zonisamide. The analytical method further utilized the ability of LC/MS/MS to detect compounds over a wide range of concentrations simultaneously, as the calibration concentrations ranged from 12 ng/mL to 200,000 ng/mL for the various analytes. Top concentrations ranged from 1.5 to 200 µg/mL.

Samples were created by spiking drug standards into clean human serum. Samples and controls were prepared for analysis through a simple protein precipitation protocol followed by dilution into water. Injection, separation of analytes, column cleaning, and column reequilibration were accomplished in less than 10 minutes. Two transitions were monitored for each of the 20 compounds of interest, including valproic acid, and 15 isotopically labeled internal standards were included to account for differential suppression and ensure accurate and reproducible quantitation across the chromatogram. A transition for phospholipids was also monitored to verify minimal interference from this class of endogenous molecules.

Calibration curve accuracies were within 20% of the expected concentration at the lowest calibration level, and well within 15% at all higher levels. Reproducibility was good, with all CVs <15% and most well under 10%. $R^2$ values were all >0.997, with some compounds displaying a linear response across their concentration range, and others requiring a quadratic fit.

For Research Use Only. Not for use in diagnostic procedures.

## Experimental

### Sample Prep

Clean human serum was spiked with standards of the 20 drugs to achieve the top concentration sample. Seven lower concentration samples were created by serial 1:2 dilution into clean serum. 40 µL of sample or control were mixed with 40 µL of ISTD solution in methanol and 120 µL of pure methanol. After being vortexed for 30 seconds, samples were spun for 10 min at 10,000 rpm. 50 µL of supernatant were added to 450 µL of water. 4 µL were then injected on to the LC/MS system.

### LC/MS/MS Analytical Method

The LC/MS/MS system consisted of a 1290 binary pump, a thermostatted autosampler, a temperature controlled column compartment and a 6460 triple quadrupole mass spectrometer. Conditions used for the separation of compounds from each other and from regions of phospholipid suppression are given in Tables 1 and 2.

Table 1: LC parameters

| | |
|---|---|
| Guard Column | Agilent Poroshell 120 EC-C18, 2.1 x 5 mm, 2.7 µm |
| Analytical Column | Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm |
| Injection Volume | 4 µL |
| Mobile Phase A | $H_2O$ + 2 mM ammonium acetate |
| Mobile Phase B | Methanol + 2 mM ammonium acetate |
| Needle Wash | 50:50 Isopropanol:Methanol |
| Autosampler Temp | 4 °C |
| Column Temp | 50 °C |
| Flow Rate | 0.4 mL/min |
| Stop Time | 7.5 min |
| Post Time | 1.5 min |

Table 2: Gradient table

| Time (min) | %B |
|---|---|
| 0 | 10 |
| 1 | 10 |
| 5 | 50 |
| 6.2 | 60 |
| 6.3 | 95 |

The 6460 triple quadrupole mass spectrometer was used to detect the 20 compounds in dynamic MRM mode. MS source conditions were as in Table 3 and dMRM acquisition parameters as in Table 4. Positive/negative switching was utilized to monitor compounds of both polarities in a single injection. The total cycle time was ~9.5 minutes injection to injection. Data were acquired with MassHunter Acquisition B.08.00 and analyzed with MassHunter Quantitative Analysis B.08.00 and Qualitative Analysis B.07.00.

Table 3: 6460 Agilent JetStream ESI Source Parameters

| | Positive Mode | Negative Mode | Units |
|---|---|---|---|
| Gas Temp | 350 | 350 | °C |
| Gas Flow | 12 | 12 | L/min |
| Nebulizer Pressure | 50 | 50 | psi |
| Sheath Gas Temp | 350 | 350 | °C |
| Sheath Gas Flow | 11 | 11 | L/min |
| Capillary Voltage | 3500 | 2500 | V |
| Nozzle Voltage | 0 | 0 | V |
| Delta EMV | 100 | 800 | V |

## Experimental

Table 4: MS transitions and dMRM acquisition details. Unit resolution was used for both MS1 and MS2.

| Compound Name | ISTD? | Precursor Ion | Product Ion | Ret Time (min) | Delta Ret Time | Fragmentor | Collision Energy | CAV | Polarity |
|---|---|---|---|---|---|---|---|---|---|
| 10,11-dihydro-10-hydroxycarbamazepine | | 255.1 | 237 | 5.64 | 0.92 | 80 | 8 | 4 | Positive |
| 10,11-dihydro-10-hydroxycarbamazepine | | 255.1 | 194 | 5.64 | 0.92 | 80 | 20 | 4 | Positive |
| Carbamazepine | | 237.1 | 194.1 | 6.74 | 0.9 | 146 | 16 | 5 | Positive |
| Carbamazepine | | 237.1 | 193.3 | 6.74 | 0.9 | 146 | 36 | 5 | Positive |
| Carbamazepine 10,11 Epoxide | | 253.1 | 210 | 5.77 | 0.82 | 94 | 12 | 5 | Positive |
| Carbamazepine 10,11 Epoxide | | 253.1 | 180.1 | 5.77 | 0.82 | 94 | 28 | 5 | Positive |
| Carbamazepine 10,11 Epoxide 13C6 | ✓ | 259.1 | 186.1 | 5.77 | 0.78 | 97 | 32 | 4 | Positive |
| Carbamazepine D10 | ✓ | 247.2 | 204.1 | 6.68 | 0.92 | 152 | 20 | 4 | Positive |
| Felbamate | | 178.1 | 117.1 | 4.72 | 0.9 | 71 | 15 | 5 | Positive |
| Felbamate | | 178.1 | 91.1 | 4.72 | 0.9 | 71 | 25 | 5 | Positive |
| Gabapentin | | 172.1 | 154.1 | 2.3 | 1.08 | 106 | 12 | 2 | Positive |
| Gabapentin | | 172.1 | 137.1 | 2.3 | 1.08 | 106 | 16 | 2 | Positive |
| Gabapentin D10 | ✓ | 182.2 | 164.1 | 2.22 | 1.1 | 91 | 12 | 4 | Positive |
| Lacosamide | | 251.1 | 108 | 4.53 | 1.02 | 80 | 4 | 4 | Positive |
| Lacosamide | | 251.1 | 91.1 | 4.53 | 1.02 | 80 | 20 | 4 | Positive |
| Lacosamide 13C D3 | ✓ | 255.3 | 108 | 4.51 | 0.92 | 88 | 4 | 4 | Positive |
| Lamotrigine | | 256 | 210.9 | 5.12 | 1.06 | 154 | 28 | 3 | Positive |
| Lamotrigine | | 256 | 43 | 5.12 | 1.06 | 154 | 40 | 3 | Positive |
| Lamotrigine 13C 15N4 | ✓ | 261 | 46 | 5.12 | 1.02 | 157 | 48 | 4 | Positive |
| Levetiracetam | | 171.1 | 154 | 2.9 | 0.94 | 71 | 4 | 3 | Positive |
| Levetiracetam | | 171.1 | 126 | 2.9 | 0.94 | 71 | 12 | 3 | Positive |
| Levetiracetam D6 | ✓ | 177.1 | 132.1 | 2.86 | 1.06 | 71 | 16 | 4 | Positive |
| N-Acetylretigabine | | 274.1 | 256.1 | 6.8 | 1.2 | 120 | 12 | 4 | Positive |
| N-Acetylretigabine | | 274.1 | 109 | 6.8 | 1.2 | 120 | 36 | 4 | Positive |
| Oxcarbazepine | | 253.1 | 208 | 6.03 | 0.82 | 120 | 16 | 3 | Positive |
| Oxcarbazepine | | 253.1 | 180 | 6.03 | 0.82 | 120 | 32 | 3 | Positive |
| Oxcarbazepine 13C6 | ✓ | 259.1 | 214 | 6.03 | 0.7 | 120 | 16 | 4 | Positive |
| Phenobarbital | | 231.1 | 188.1 | 5.17 | 0.86 | 91 | 8 | 2 | Negative |
| Phenobarbital | | 231.1 | 42 | 5.17 | 0.86 | 91 | 24 | 2 | Negative |
| Phenobarbital D5 | ✓ | 236.1 | 42 | 5.15 | 0.74 | 91 | 16 | 4 | Negative |
| Phenytoin | | 251.1 | 208 | 6.37 | 0.92 | 105 | 12 | 4 | Negative |
| Phenytoin | | 251.1 | 102 | 6.37 | 0.92 | 105 | 20 | 4 | Negative |
| Phenytoin D10 | ✓ | 261.2 | 105.9 | 6.32 | 0.68 | 105 | 20 | 4 | Negative |
| Phospholipids | | 184 | 184 | 4 | 8 | 250 | 0 | 3 | Positive |
| Pregabalin | | 160.1 | 142.1 | 2.09 | 1.14 | 89 | 8 | 2 | Positive |
| Pregabalin | | 160.1 | 55.1 | 2.09 | 1.14 | 89 | 24 | 2 | Positive |
| Pregabalin D6 | ✓ | 166.2 | 148.1 | 2.04 | 1.02 | 88 | 8 | 4 | Positive |
| Retigabine | | 304.2 | 230 | 6.84 | 0.68 | 123 | 16 | 4 | Positive |
| Retigabine | | 304.2 | 109 | 6.84 | 0.68 | 123 | 36 | 4 | Positive |
| Retigabine D4 | ✓ | 308.2 | 113 | 6.8 | 0.86 | 126 | 36 | 4 | Positive |
| Rufinamide | | 239.1 | 127 | 4.7 | 1.02 | 100 | 20 | 3 | Positive |
| Rufinamide | | 239.1 | 101 | 4.7 | 1.02 | 100 | 50 | 3 | Positive |
| Tiagabine | | 376.1 | 247 | 7.17 | 0.72 | 143 | 16 | 3 | Positive |
| Tiagabine | | 376.1 | 111 | 7.17 | 0.72 | 143 | 32 | 3 | Positive |
| Tiagabine D6 | ✓ | 382.2 | 253.1 | 7.16 | 0.72 | 149 | 16 | 4 | Positive |
| Topiramate D12 | ✓ | 350.1 | 78 | 5.46 | 0.9 | 150 | 20 | 4 | Negative |
| Topiramate | | 338.1 | 96 | 5.51 | 1.02 | 150 | 20 | 4 | Negative |
| Topiramate | | 338.1 | 78 | 5.51 | 1.02 | 150 | 20 | 4 | Negative |
| Valproic Acid | | 225.1 | 143 | 5.3 | 0.92 | 95 | 11 | 3 | Negative |
| Valproic Acid D6 | ✓ | 231 | 149 | 5.3 | 0.41 | 95 | 11 | 4 | Negative |
| Vigabatrin | | 130.1 | 113 | 0.64 | 0.84 | 71 | 8 | 2 | Positive |
| Vigabatrin | | 130.1 | 71.1 | 0.64 | 0.84 | 71 | 16 | 2 | Positive |
| Zonisamide | | 211 | 147 | 3.8 | 0.8 | 86 | 8 | 4 | Negative |
| Zonisamide | | 211 | 118.9 | 3.8 | 0.8 | 86 | 12 | 4 | Negative |
| Zonisamide 13C6 | ✓ | 217.1 | 125 | 3.79 | 0.9 | 83 | 12 | 4 | Negative |

## Results and Discussion

### Chromatography

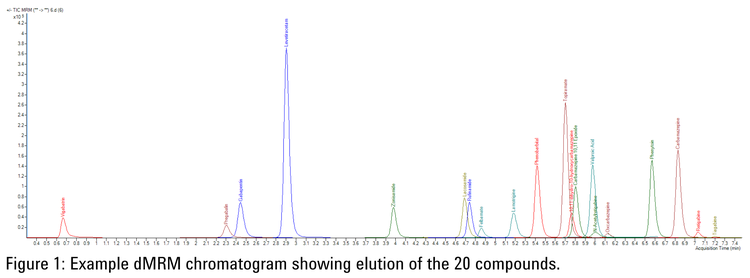


Figure 1: Example dMRM chromatogram showing elution of the 20 compounds.

The method utilized the abilities of LC/MS/MS to detect multiple compounds spanning a wide range of concentrations simultaneously. The calibration concentrations ranged from 12 ng/mL to 200,000 ng/mL for the various analytes. Top concentrations ranged from 1.5 to 200 ug/mL, and are given, along with curve fit parameters, in Table 5. $R^2$ values were all >0.997, with some compounds displaying a linear response across their concentration range, and others requiring a quadratic fit.

Table 5: Top calibration concentrations and curve fit parameters

| Compound | Top Concentration (µg/mL) | Curve Fit | Average $R^2$ (n=3) |
|---|---|---|---|
| Vigabatrin | 180 | Quadratic | 0.9996 |
| Pregabalin | 20 | Quadratic | 0.9996 |
| Gabapentin | 30 | Quadratic | 0.9989 |
| Levetiracetam | 100 | Quadratic | 0.9987 |
| Zonisamide | 40 | Linear | 0.9996 |
| Lacosamide | 20 | Quadratic | 0.9995 |
| Rufinamide | 40 | Quadratic | 0.9995 |
| Felbamate | 80 | Linear | 0.9987 |
| Lamotrigine | 20 | Quadratic | 0.9992 |
| Phenobarbital | 40 | Quadratic | 0.9988 |
| Topiramate | 30 | Quadratic | 0.9994 |
| 10,11-dihydro-10-hydroxycarbamazepine | 40 | Quadratic | 0.9998 |
| Carbamazepine 10,11 Epoxide | 25 | Quadratic | 0.9998 |
| Valproic Acid | 200 | Linear | 0.9974 |
| N-Acetylretigabine | 3.5 | Quadratic | 0.9994 |
| Oxcarbazepine | 5 | Quadratic | 0.9987 |
| Phenytoin | 40 | Quadratic | 0.9990 |
| Carbamazepine | 50 | Quadratic | 0.9980 |
| Retigabine | 5 | Quadratic | 0.9998 |
| Tiagabine | 1.5 | Quadratic | 0.9973 |

## Results and Discussion

### Calibration

All calibration curves employed a 1/x weighting factor.

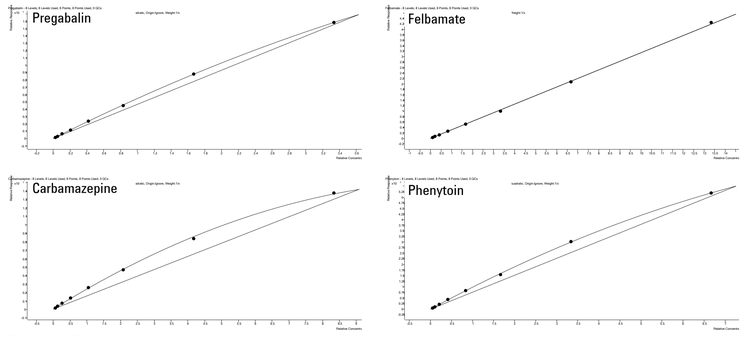


Figure 2: Example calibration curves for compounds dispersed throughout the chromatogram and of both polarities.

### Accuracy and Reproducibility

Calibration curve accuracies were within 20% of the expected concentration at the lowest level, and well within 15% at all higher levels. Reproducibility was good, with all CVs <15% and most well under 10%. Values for 3 replicate curves run on the same day are given in Table 6, while accuracy average and CV values for 3 replicate curves run on three consecutive days are given in Table 7.

Table 6: Accuracy and reproducibility for curves analyzed on the same day (n=3)

| | Vigabatrin | | Pregabalin | | Gabapentin | | Levetiracetam | | Zonisamide | | Lacosamide | | Rufinamide | | Felbamate | | Lamotrigine | | Phenobarbital | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Level | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV |
| 1 | 89.6 | 3.6 | 94.2 | 3.7 | 88.1 | 4.4 | 87.1 | 2.2 | 105.1 | 6.7 | 92.3 | 3.0 | 91.9 | 2.7 | 92.7 | 7.2 | 97.1 | 4.8 | 101.1 | 2.2 |
| 2 | 101.6 | 1.6 | 97.9 | 1.4 | 98.5 | 2.8 | 98.5 | 1.9 | 96.0 | 2.2 | 98.0 | 2.1 | 99.5 | 1.7 | 100.0 | 4.6 | 97.0 | 3.1 | 93.8 | 1.3 |
| 3 | 104.4 | 2.2 | 103.3 | 2.8 | 106.9 | 1.4 | 106.3 | 0.7 | 96.2 | 3.4 | 105.1 | 0.8 | 104.5 | 2.1 | 101.1 | 1.2 | 101.9 | 0.1 | 101.7 | 5.4 |
| 4 | 104.1 | 2.5 | 103.5 | 1.6 | 105.4 | 1.1 | 107.1 | 0.8 | 99.7 | 1.2 | 103.9 | 1.1 | 103.1 | 0.6 | 101.6 | 3.6 | 101.8 | 1.3 | 101.9 | 4.9 |
| 5 | 102.3 | 1.2 | 103.5 | 1.2 | 104.7 | 2.1 | 104.9 | 0.7 | 101.5 | 2.8 | 103.0 | 2.1 | 103.0 | 2.8 | 103.4 | 5.0 | 105.0 | 7.1 | 102.9 | 3.3 |
| 6 | 99.6 | 0.8 | 99.1 | 1.5 | 99.0 | 1.1 | 99.3 | 1.3 | 101.4 | 2.1 | 99.6 | 0.9 | 99.8 | 1.0 | 102.0 | 4.0 | 97.9 | 0.7 | 99.8 | 4.3 |
| 7 | 97.8 | 1.1 | 97.8 | 0.6 | 96.0 | 0.8 | 95.4 | 1.6 | 100.8 | 1.7 | 97.4 | 0.2 | 97.4 | 0.5 | 100.2 | 3.9 | 99.0 | 2.7 | 98.3 | 5.7 |
| 8 | 100.6 | 0.3 | 100.7 | 0.2 | 101.6 | 0.4 | 101.8 | 0.3 | 99.2 | 1.1 | 100.8 | 0.1 | 100.8 | 0.2 | 98.9 | 3.2 | 100.3 | 0.7 | 100.5 | 1.5 |

| | Topiramate | | 10,11-dihydro-10-hydroxycarbamazepine | | Carbamazepine 10,11 Epoxide | | Valproic Acid | | N-Acetyl-retigabine | | Oxcarbazepine | | Phenytoin | | Carbamazepine | | Retigabine | | Tiagabine | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Level | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV |
| 1 | 98.4 | 3.5 | 95.4 | 3.8 | 96.1 | 1.9 | 89.0 | 10.2 | 97.2 | 6.0 | 100.7 | 6.4 | 100.3 | 2.5 | 83.5 | 2.0 | 95.7 | 2.1 | 91.3 | 11.7 |
| 2 | 96.6 | 2.3 | 99.2 | 0.1 | 99.3 | 2.9 | 97.2 | 3.4 | 103.5 | 7.3 | 92.6 | 2.0 | 96.7 | 2.6 | 100.8 | 2.7 | 100.4 | 4.3 | 98.6 | 13.8 |
| 3 | 101.1 | 2.3 | 102.6 | 1.5 | 101.8 | 1.0 | 100.5 | 3.4 | 98.3 | 3.0 | 102.2 | 4.9 | 101.1 | 0.7 | 108.4 | 1.3 | 102.1 | 2.2 | 104.2 | 10.0 |
| 4 | 102.3 | 3.4 | 102.0 | 3.0 | 102.1 | 0.8 | 101.8 | 7.6 | 99.6 | 6.8 | 101.3 | 4.5 | 100.1 | 2.1 | 107.8 | 2.0 | 101.8 | 3.1 | 109.1 | 9.3 |
| 5 | 101.9 | 4.6 | 102.5 | 2.0 | 102.1 | 1.4 | 107.2 | 3.5 | 102.8 | 0.1 | 105.2 | 4.9 | 104.8 | 3.3 | 104.6 | 1.6 | 100.5 | 1.4 | 100.1 | 2.4 |
| 6 | 101.9 | 0.7 | 99.2 | 0.3 | 99.6 | 0.7 | 104.7 | 2.7 | 98.6 | 4.4 | 99.5 | 4.4 | 96.3 | 4.1 | 97.2 | 2.2 | 100.4 | 1.0 | 96.2 | 7.2 |
| 7 | 97.1 | 1.4 | 98.8 | 1.1 | 98.7 | 1.2 | 103.2 | 3.0 | 100.1 | 2.2 | 98.2 | 5.1 | 100.9 | 4.2 | 92.7 | 3.1 | 98.8 | 1.3 | 100.6 | 6.7 |
| 8 | 100.7 | 0.2 | 100.3 | 0.3 | 100.3 | 0.2 | 96.3 | 2.6 | 100.0 | 0.3 | 100.3 | 1.0 | 99.8 | 1.5 | 103.0 | 2.0 | 100.3 | 0.4 | 100.0 | 1.4 |

Table 7: Accuracy and reproducibility for curves analyzed on consecutive days (n=3)

| | Vigabatrin | | Pregabalin | | Gabapentin | | Levetiracetam | | Zonisamide | | Lacosamide | | Rufinamide | | Felbamate | | Lamotrigine | | Phenobarbital | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Level | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV |
| 1 | 89.0 | 2.9 | 93.0 | 5.5 | 87.5 | 5.1 | 84.1 | 4.4 | 101.3 | 8.1 | 91.9 | 4.0 | 88.7 | 5.5 | 92.9 | 6.1 | 92.8 | 8.2 | 97.1 | 6.0 |
| 2 | 101.7 | 1.2 | 100.0 | 2.8 | 100.5 | 5.3 | 101.4 | 3.3 | 101.0 | 3.7 | 99.0 | 3.2 | 100.7 | 5.4 | 101.3 | 4.3 | 98.7 | 5.4 | 99.6 | 5.0 |
| 3 | 104.4 | 2.3 | 103.9 | 3.1 | 106.4 | 1.0 | 106.6 | 0.7 | 98.8 | 1.3 | 105.3 | 1.1 | 104.5 | 2.2 | 100.8 | 1.8 | 102.9 | 0.7 | 103.2 | 7.8 |
| 4 | 104.3 | 2.7 | 101.4 | 2.7 | 103.8 | 2.8 | 107.0 | 2.7 | 96.9 | 2.4 | 103.1 | 1.5 | 104.3 | 2.0 | 103.2 | 3.2 | 102.4 | 2.4 | 98.6 | 5.3 |
| 5 | 102.9 | 0.6 | 103.8 | 2.5 | 105.7 | 2.4 | 104.6 | 1.9 | 98.8 | 4.9 | 103.8 | 1.8 | 105.9 | 2.2 | 104.8 | 4.0 | 107.4 | 7.5 | 103.4 | 2.2 |
| 6 | 99.4 | 0.5 | 99.6 | 1.2 | 98.4 | 1.2 | 99.6 | 0.8 | 101.9 | 1.6 | 98.0 | 1.4 | 97.5 | 1.6 | 98.0 | 2.7 | 97.4 | 0.9 | 98.2 | 2.5 |
| 7 | 97.7 | 1.1 | 97.7 | 2.6 | 96.4 | 2.6 | 95.2 | 2.4 | 102.6 | 2.1 | 98.4 | 1.8 | 97.6 | 1.9 | 98.6 | 0.9 | 97.9 | 3.8 | 99.7 | 3.6 |
| 8 | 100.6 | 0.3 | 100.7 | 0.7 | 101.6 | 0.9 | 101.7 | 0.8 | 98.5 | 0.4 | 100.6 | 0.5 | 100.9 | 0.5 | 100.4 | 1.1 | 100.7 | 1.0 | 100.3 | 1.0 |

| | Topiramate | | 10,11-dihydro-10-hydroxycarbamazepine | | Carbamazepine 10,11 Epoxide | | Valproic Acid | | N-Acetyl-retigabine | | Oxcarbazepine | | Phenytoin | | Carbamazepine | | Retigabine | | Tiagabine | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Level | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV | Average | CV |
| 1 | 100.4 | 5.9 | 94.4 | 5.6 | 92.7 | 6.2 | 91.6 | 14.6 | 98.2 | 6.9 | 97.5 | 8.7 | 95.7 | 5.9 | 81.8 | 1.9 | 94.4 | 2.7 | 93.0 | 13.6 |
| 2 | 98.6 | 0.9 | 100.2 | 2.1 | 99.1 | 2.6 | 94.6 | 5.1 | 101.3 | 3.6 | 97.6 | 3.2 | 96.3 | 2.7 | 100.0 | 3.0 | 100.6 | 4.1 | 107.1 | 5.5 |
| 3 | 101.0 | 2.1 | 104.6 | 2.8 | 104.8 | 1.7 | 99.9 | 2.4 | 98.0 | 3.0 | 105.0 | 10.0 | 101.3 | 1.8 | 109.7 | 0.7 | 100.9 | 3.2 | 97.3 | 5.8 |
| 4 | 99.1 | 5.9 | 100.4 | 2.3 | 102.7 | 3.1 | 104.4 | 4.7 | 105.0 | 2.8 | 97.6 | 5.5 | 106.6 | 5.8 | 108.0 | 2.4 | 103.6 | 4.3 | 105.9 | 5.4 |
| 5 | 100.8 | 5.0 | 101.7 | 2.2 | 102.8 | 1.4 | 106.1 | 6.9 | 99.3 | 3.1 | 104.1 | 10.4 | 104.4 | 3.3 | 105.8 | 2.3 | 102.8 | 1.5 | 98.9 | 5.4 |
| 6 | 100.7 | 0.9 | 99.4 | 0.4 | 99.0 | 1.3 | 102.6 | 1.6 | 96.4 | 2.6 | 98.3 | 5.3 | 96.2 | 1.0 | 98.2 | 0.5 | 98.5 | 1.6 | 95.4 | 5.5 |
| 7 | 99.3 | 4.0 | 99.2 | 1.7 | 98.5 | 2.1 | 104.7 | 2.5 | 102.1 | 1.8 | 99.7 | 5.9 | 99.2 | 1.9 | 94.6 | 2.0 | 99.0 | 1.7 | 102.9 | 3.8 |
| 8 | 100.2 | 1.0 | 100.2 | 0.4 | 100.4 | 0.5 | 96.1 | 0.9 | 99.7 | 0.4 | 100.1 | 1.1 | 100.7 | 0.8 | 103.4 | 1.4 | 100.3 | 0.4 | 99.5 | 0.9 |

## Conclusions

- An LC/MS/MS analytical method has been developed to quantitate 20 antiepileptic drugs simultaneously in human serum.
- The method is accurate, reproducible, and robust.
- Further work is needed to better understand interferences that would impact the quantitation of any of the members of the panel. Additionally, different sources of mobile phase components and samples from an alternate source will be analyzed.



5991-9194EN