

Quantitative Method Implementing Fast Polarity Switching Quadrupole Mass Spectrometry for Analysis of Ten Anti-epileptic Drugs in Plasma Samples

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Overview

Purpose: The main objective of this study was to develop a simple and reliable LC-MS method for the determination of anti-epileptic drugs employing fast positive and negative polarity switching which can be implemented in clinical research applications. Collecting both positive and negative data in a single experiment allows for efficient drug identification and eliminates the need for two separate polarity experiments, resulting in time savings and decrease in solvent usage.

Methods: A four minute LC method utilizing Thermo Scientific LC technology coupled with a Thermo Scientific TSQ Quantum Access MAX triple quadrupole mass spectrometer (Figure 1).

Results: Quantitation of 10 therapeutic drugs in human plasma was performed in 4 minutes with a calibration ranges of 0.1-50 µg/mL for 5 compounds, 0.5-50 µg/mL for 1 compound, 0.2-30 µg/mL for 1 compound, 1-30 µg/mL for 1 compound, 2-30 µg/mL for 1 compounds and 5-30 µg/mL for 1 compound.

Introduction and Methodology

Liquid Chromatography – Mass Spectrometry (LC-MS) is an efficient, sensitive, and accurate technique used to analyze a large number of compounds from various classes, including antiepileptic drugs. Faster polarity switching is necessary for screening applications, especially for simultaneous analysis of positively and negatively ionized compounds. The method used in this application utilizes fast polarity switching while still producing high quality quantitative data for the analysis of complex mixtures.

Figure 1. TSQ Quantum Access MAX triple quadrupole mass spectrometer.



Methodology

Internal Standards: Four internal standards were used in the study: phenytoin-d₁₀, topiramate-d₁₂ and gabapentin-d₁₀ for the respective compounds. To the rest of the compounds tolbutamide in respective ionization mode was assigned as the internal standard (Table 1).

Sample Preparation: 100 µL of plasma was diluted with 400 µL of MeOH containing internal standards in concentration of 1µg/mL for phenytoin-d₁₀, topiramate-d₁₂ and gabapentin-d₁₀ and 5 µg/mL for tolbutamide. Sample was vortexed (30sec), centrifuged (10 min @ 13200 rpm) and 10 µL of supernatant was injected onto LC-MS system.

LC Method

Using a Thermo Scientific Accela High Speed LC system:
Solvent A: 2mMol NH₄FA + 0.1% FA in H₂O
Solvent B: 0.1% Formic Acid in MeOH
Analytical Column: Hypersil GOLD™, 50 x 2.1 mm, 3 µm
Flow Rate: 400 µl/min

Time	A%	B%
0.0	95.0	5.0
0.5	95.0	5.0
1.0	5.0	95.0
3.0	5.0	95.0
3.1	95.0	5.0
4.0	95.0	5.0

MS Conditions:

Samples were analyzed using a TSQ Quantum Access MAX™ triple quadrupole mass spectrometer equipped with an APCI Ion Max™ source in SRM data acquisition mode with constant positive/negative polarity switching. Two SRM transitions with scan time of 15 ms were collected for each analyte (Table 1).

Table 1. List of SRM transitions collected for each analyte.

Compound Name	Ionization mode	Precursor Ion	Fragment Ion
Analyte			
Levetiracetam	positive	171.0	69.3, 126.1
Gabapentin	positive	171.9	95.2, 137.0
Primidone	positive	219.0	91.2, 162.0
Carbamazepine	positive	237.0	193.0, 194
Oxcarbazepine	positive	253.0	180.0, 236.0
Lamotrigine	positive	255.9	156.7, 210.6
Tiagabine	positive	376.1	246.9, 278.0
Zonisamide	negative	210.9	118.1, 119.1
Phenytoin	negative	251.0	102.1, 208.0
Topiramate	negative	338.0	78.2, 96.1
Internal Standard			
Gabapentin-d ₁₀	positive	182.0	147.2, 164.3
Phenytoin-d ₁₀	negative	261.0	106.4, 218.3
Topiramate-d ₁₂	negative	350.1	78.4, 95.9
Tolbutamide	positive	271.2	74.4, 155.1
Tolbutamide	negative	268.9	106.4, 170.2

Calibration standards: prepared in house by spiking human plasma with analyte mixture to concentrations of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 30 and 50 µg/mL.

QC samples: prepared in house by spiking human plasma with analytes to concentrations of 5, 10 and 20 µg/mL.

Calibration standards and QC samples: prepared using the aforementioned sample preparation procedure and analyzed with the LC-MS method.

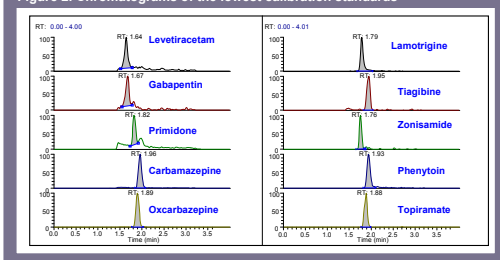
Unknown samples: clinical research samples were provided by John Hopkins Hospital (Baltimore, Maryland) and Cleveland Clinic (Cleveland, Ohio). Samples were prepared using the aforementioned sample preparation procedure before analysis by LC-MS (Tables 5-6)

Intra assay variability: calculated by processing and analyzing 5 replicates of each QC sample.

Inter assay variability: calculated by processing and analyzing 5 replicates of each QC sample in 3 different batches.

Results

Figure 2. Chromatograms of the lowest calibration standards



For each analyte, the following were obtained: calibration range, limit of detection (LOD), limit of quantitation (LOQ), intra- and inter- assay variability with QC samples with concentrations within determined compound specific calibration ranges (Figures 2-3, Tables 2-4).

We also compared the intra- and inter- assay precision data for phenytoin, topiramate and gabapentin when the respective deuterated internal standard was used versus tolbutamide in respective ionization mode.

The rationale behind this comparison was the cost of the internal standard. Currently only three deuterated internal standards out of the ten analyzed antiepileptic drugs are commercially available. We did not find significant differences between the respective deuterated internal standard and tolbutamide. (Tables 2-4).

Figure 3. Examples of Calibration Curves

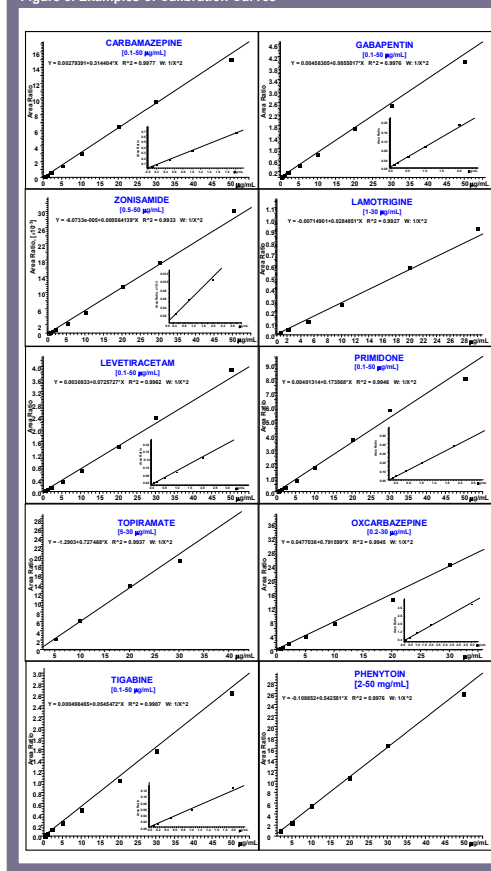


Table 2. Intra assay variability (%RSD) for 10 analyzed compounds.

Compound Name	Internal Standard	QC1 (5µg/mL)	QC2 (10 µg/mL)	QC3 (20 µg/mL)
Levetiracetam	Tolbutamide	9.0	6.2	10.1
Gabapentin	Gabapentin-d ₁₀	6.1	5.3	4.4
Gabapentin	Tolbutamide	7.3	3.5	5.7
Primidone	Tolbutamide	4.8	4.2	6.1
Carbamazepine	Tolbutamide	5.1	8.3	7.0
Oxcarbazepine	Tolbutamide	6.6	8.3	7.2
Lamotrigine	Tolbutamide	5.5	6.0	5.7
Tiagabine	Tolbutamide	7.4	4.0	7.3
Zonisamide	Tolbutamide	6.9	11.2	6.9
Phenytoin	Phenytoin-d ₁₀	8.0	9.8	7.0
Phenytoin	Tolbutamide	9.6	8.2	7.9
Topiramate	Topiramate-d ₁₂	8.2	9.1	9.3
Topiramate	Tolbutamide	7.6	12.3	10.0

Table 3. Inter assay variability (%RSD) for 10 analyzed compounds.

Compound Name	Internal standard	QC1 (5µg/mL)	QC2 (10 µg/mL)	QC3 (20 µg/mL)
Levetiracetam	Tolbutamide	8.2	7.1	7.3
Gabapentin	Gabapentin-d ₁₀	4.9	5.1	5.9
Gabapentin	Tolbutamide	6.7	7.6	5.1
Primidone	Tolbutamide	4.4	6.9	8.0
Carbamazepine	Tolbutamide	5.1	8.6	7.0
Oxcarbazepine	Tolbutamide	7.9	12.8	7.9
Lamotrigine	Tolbutamide	4.7	11.2	8.9
Tiagabine	Tolbutamide	5.5	6.1	8.1
Zonisamide	Tolbutamide	6.6	9.9	6.2
Phenytoin	Phenytoin-d ₁₀	9.4	7.1	7.9
Phenytoin	Tolbutamide	9.6	9.7	6.1
Topiramate	Topiramate-d ₁₂	8.2	9.1	9.3
Topiramate	Tolbutamide	7.6	12.3	10.0

Table 4. Calibration Range, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Compound Name	Internal standard	LOD (µg/mL)	LOQ (µg/mL)	Calibration Range, [µg/mL]	Therapeutic Range, [µg/mL]	Ref
Levetiracetam	Tolbutamide	0.1	0.1	0.1-50	5-30	2
Gabapentin	Gabapentin-d ₁₀	0.1	0.1	0.1-50	7-21	1
Gabapentin	Tolbutamide	0.1	0.1	0.1-50		
Primidone	Tolbutamide	0.1	0.1	0.1-50	6-15	1
Carbamazepine	Tolbutamide	0.1	0.1	0.1-50	4-12	1
Oxcarbazepine	Tolbutamide	0.1	0.2	0.2-30	12.5-35	1
Lamotrigine	Tolbutamide	0.1	1.0	1-30	2.5-15	1
Tiagabine	Tolbutamide	0.1	0.1	0.1-50	-	-
Zonisamide	Tolbutamide	0.1	0.5	0.5-50	7-42	1
Phenytoin	Phenytoin-d ₁₀	0.1	2.0	2-50	10-20	1
Phenytoin	Tolbutamide	0.1	2.0	2-50		
Topiramate	Topiramate-d ₁₂	0.5	5.0	5-30	3-20	1
Topiramate	Tolbutamide	0.5	2.0	2-30		

We compared the clinical research samples whose concentrations were determined by HPLC and immunoassay to those measured using LC-MS. The data in Table 5 show the difference is 20% or less in all cases but primidone.

Table 5. Clinical Research Samples

Compound Name	Internal Standard	Reference lab result (µg/mL)	Obtained result by LC-MS (µg/mL)	%Diff
Carbamazepine	Tolbutamide	10.9	9.2	-15.6
Carbamazepine	Tolbutamide	4.4	4.3	-2.3
Carbamazepine	Tolbutamide	7.6	6.4	15.8
Lamotrigine	Tolbutamide	*20.3	24.4	20.2
Levetiracetam	Tolbutamide	*49.2	57.9	17.7
Primidone	Tolbutamide	*9.4	12.3	30.9

*HPLC, *enzyme immunoassay

For phenytoin we also compared the use of respective deuterated internal standard and tolbutamide as internal standard. There is no significant difference in data results (Table 6) concluding that tolbutamide can be used as internal standard if resources are limited.

Table 6. Clinical Research Samples: Internal Standard Comparison for Phenytoin

Compound Name	Internal Standard	Reference lab result (µg/mL)	Obtained result by LC-MS (µg/mL)	%Diff
Phenytoin	Tolbutamide	*12.6	13.4	6.3
	Tolbutamide	2.6	3.0	13.8
	Tolbutamide	20.4	17.8	12.5
	Tolbutamide	13.1	13.8	5.2
	Phenytoin-d ₁₀	*12.6	11.6	-7.8
	Phenytoin-d ₁₀	2.6	3.0	15.2
	Phenytoin-d ₁₀	20.4	17.1	-16.2
Phenytoin-d ₁₀	13.1	13.0	-0.4	

*Obtained by fluorescence polarization immunoassay

Conclusions

An efficient, sensitive, and accurate LC-MS method, with a wide calibration range, was developed for the quantitation of ten antiepileptic drugs in human plasma to support clinical research applications.

The overall method employs protein precipitation sample preparation and fast polarity switching, enabling simultaneous analysis of positively and negatively ionized compounds while saving time and decreasing solvent usage.

References:

- Levy, R.H.; Mattson H.; Meldrum, B.S.; Perucca, E.: Antiepileptic drugs, 5th edition, Lippincott Williams & Wilkins
- http://www.aruplab.com

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