TP-073

Antiepileptic Drugs Monitoring in Plasma using the LDTD-MS/MS technique

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OVERVIEW

Purpose

- Optimization of a drug monitoring method in plasma using LDTD-MS/MS

Method

- Protein precipitation of plasma sample
- Samples dried and analyzed by LDTD-MS/MS

Quantification

- Linearity: r^2> 0.99 over the calibration range
- Within-run accuracy between 96 to 110.5% of the nominal value and the precision is lower than 5.6% CV.
- No matrix effect.
- Samples analyzed with a runtime of 8 seconds using LDTD-MS/MS system

INTRODUCTION

The American Academy of Neurology and American Epilepsy Society's (AANAES) guidelines recommend using either standard anticonvulsant drugs such as carbamazepine or phenobarbital, or the newer anticonvulsant drugs gabapentin, lamotrigine, oxcarbazepine or topiramate for patients with newly diagnosed epilepsy. After starting a treatment, Therapeutic Drug Monitoring (TDM) helps in establishing a steady-state baseline concentration for further evaluation of an individual therapeutic concentration.
For this project, a protein precipitation method is developed for the analysis of different antiepileptic drugs (10-OH-Carbamazepine, Oxcarbazepine, Lamotrigine, Levetiracetam, Gabapentin, Topiramate, Primidone and Phenobarbital). A single analysis method using LDTD-MS/MS allows the quantification of all compounds in 8 seconds per sample.

LUXON Ionization Source:

The Luxon Ion Source (Figure 1) is the second-generation sample introduction and ionization source based on the LDTD technology for mass spectrometry. The Luxon Ion Source uses a Fiber-Coupled Laser Diode (Figure 2) to obtain unmatchable thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 Luxon Ion Source

Figure 2 Schematic of the Luxon Ion Source

METHOD

Extraction

The drug-free plasma is fortified with antiepileptic drugs at concentrations that cover the therapeutic range of all drugs (**Table 1**). 20 µL of the plasma sample are mixed with 240 µL of internal standard solution in acetonitrile and mixed to precipitate the protein using an automated system (**Figure 3**).

After centrifugation, 3 µL of the upper layer are deposited onto LazWell96 plates and evaporated to complete dryness before analysis by LDTD-MS/MS.

Figure 3 Azeo: Automated extraction system

Table 1 Therapeutic and calibration range

Drug	Therapeutic range	Calibration range
10-OH-Carbamazepine	3-40 µg/mL	0.5-50 µg/mL
Oxcarbazepine	0.4-2 µg/mL	0.1-10 µg/mL
Lamotrigine	3-14 µg/mL	0.3-30 µg/mL
Levetiracetam	10-43 µg/mL	0.5-50 µg/mL
Gabapentin	2-20 µg/mL	0.25-25 µg/mL
Topiramate	5-25 µg/mL	0.5-50 µg/mL
Primidone	5-15 µg/mL	0.25-25 µg/mL

Instrumentation

- Ion source: Phytronix Luxon SH-960 Ion Source
- Mass spectrometer: Shimadzu, LC-8060

Luxon Parameters

- Laser power pattern:
  - Increase laser power to 55% in 6 sec
  - Hold 2 seconds
  - Decrease laser power to 0%
- Carrier gas flow: 3 L/min (Air)

MS Parameters

- APCI (+ / -)
- Time: 20 msec
- MRM mode

Table 2 MRM transitions parameters

Compound	Transition	CE (V)	Mode
Levetiracetam	171 → 126	16	+
Gabapentin	172 → 137	12	+
Gabapentin-d10	182 → 147	12	+
Primidone	219 → 162	30	+
Oxcarbazepin	253 → 208	30	+
10-OH-Carbamazepin	255 → 194	50	+
Lamotrigine	256 → 157	30	+
Methadone-d9	319 → 268	16	+
Topiramate	340 → 264	10	+
Phenobarbital	231 → 42	25	-
Phenobarbital-d5	236 → 42	25	-

RESULTS

Linearity

Plasma sample is spiked with the drug panel to prepare standards within the calibration range described in **Table 1**. Standards are extracted and used to generate a calibration curve. **Table 3** shows the calibration curve results. Correlation values greater than 0.99 are obtained for all drugs. **Figure 4** shows typical calibration curve results for Gabapentin.

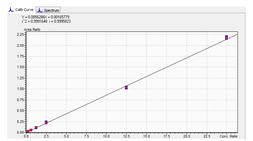

Figure 4 Standard curve for Gabapentin

Table 3 Calibration curve results

Drug	m	b	r^2
Levetiracetam	1.49349	-0.05739	0.99986
Gabapentin	0.08563	0.00166	0.99916
Primidone	0.23423	-0.00228	0.99718
Oxcarbazepin	0.18356	0.00103	0.99672
10-OH-Carbamazepin	0.01544	0.00096	0.99734
Lamotrigine	0.02184	0.00189	0.99956
Topiramate	1.41118	0.02310	0.99788
Phenobarbital	0.60101	-0.02828	0.99959

Accuracy and Precision

Calibration curves are extracted and analyzed. For the intra-run precision and accuracy experiment, each fortified sample set is analyzed in triplicate. **Table 4** show the intra-run results for Gabapentin. Each concentration does not exceed 15% CV and the mean concentrations are within ±15% of the expected value. Similar results are obtained for the other drugs.

Table 4 Intra-run precision and accuracy for Gabapentin

Gabapentin	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6
Exp. Conc (µg/mL)	0.25	0.625	1.25	2.5	12.5	25
Calc. Conc (µg/mL)	0.253	0.607	1.260	2.577	11.995	25.432
N	3	3	3	3	3	3
%CV	9.3	2.7	4.4	5.6	1.1	0.7
%Nom	101.2	97.1	100.8	103.1	96.0	101.7

Matrix effect evaluation

Drugs are spiked in six (6) different plasma matrices and the concentrations are evaluated against a calibration curve. Replicate extractions are deposited on a LazWell plate and dried before analysis. The peak area against the internal standard (IS) ratio was used to normalize the signal. The following criteria are used: Calculated concentration must not exceed 15% CV and the mean concentration must be within 15% of nominal value. Results for all drugs are shown in **Tables 5 to 12** where we see that each concentration does not exceed 15% CV and the mean concentration is within ±15% of the expected value.

Table 5 Matrix effect evaluation for 10-OH-Carbamazepin

10-OH-Carbamazepin	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	5.5	5.5	5.5	5.5	5.5	5.5
Calc. Conc (µg/mL)	5.88	5.58	5.56	6.27	5.22	5.77
N	4	4	4	4	4	4
%CV	7.5	7.2	6.7	5.7	6.2	4.9
%Nom	106.9	101.5	101.1	114.1	95.0	104.9

Table 6 Matrix effect evaluation for Oxcarbazepin

Oxcarbazepin	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	1	1	1	1	1	1
Calc. Conc (µg/mL)	1.10	1.02	1.08	1.15	0.98	1.06
N	4	4	4	4	4	4
%CV	4.7	2.1	4.9	1.1	6.3	2.7
%Nom	110.0	101.7	108.0	115.0	98.1	106.1

Table 7 Matrix effect evaluation for Lamotrigine

Lamotrigine	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	1.2	1.2	1.2	1.2	1.2	1.2
Calc. Conc (µg/mL)	1.23	1.09	1.02	1.17	1.32	1.21
N	4	4	4	4	4	4
%CV	3.7	2.2	5.1	3.8	6.0	3.8
%Nom	102.6	90.9	85.0	97.4	109.6	101.1

Table 8 Matrix effect evaluation for Levetiracetam

Levetiracetam	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	1	1	1	1	1	1
Calc. Conc (µg/mL)	1.08	0.97	0.99	1.00	0.94	0.98
N	4	4	4	4	4	4
%CV	4.3	4.5	1.9	3.2	8.4	3.2
%Nom	107.7	96.6	99.4	99.8	94.2	98.1

Table 9 Matrix effect evaluation for Gabapentin

Gabapentin	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	2.5	2.5	2.5	2.5	2.5	2.5
Calc. Conc (µg/mL)	2.42	2.47	2.44	2.53	2.56	2.63
N	4	4	4	4	4	4
%CV	0.2	1.9	2.9	2.8	2.4	7.2
%Nom	96.6	98.7	97.4	101.1	102.5	105.2

Table 10 Matrix effect evaluation for Topiramate

Topiramate	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	1.2	1.2	1.2	1.2	1.2	1.2
Calc. Conc (µg/mL)	1.21	1.21	1.07	1.07	1.25	1.16
N	4	4	4	4	4	4
%CV	11.8	7.8	6.4	2.7	10.4	2.2
%Nom	100.7	101.0	89.5	89.4	104.0	96.4

Table 11 Matrix effect evaluation for Primidone

Primidone	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	2.5	2.5	2.5	2.5	2.5	2.5
Calc. Conc (µg/mL)	2.59	2.40	2.38	2.76	2.34	2.49
N	4	4	4	4	4	4
%CV	5.4	4.8	9.4	6.1	7.3	5.2
%Nom	103.5	95.9	95.2	110.3	93.7	99.6

Table 12 Matrix effect evaluation for Phenobarbital

Phenobarbital	M 1	M 2	M 3	M 4	M 5	M 6
Exp. Conc (µg/mL)	5	5	5	5	5	5
Calc. Conc (µg/mL)	4.80	4.92	4.91	5.10	5.08	5.04
N	4	4	4	4	4	4
%CV	1.0	1.2	0.4	0.9	0.9	0.8
%Nom	95.9	98.4	98.3	101.9	101.7	100.8

CONCLUSION

- Efficient protein precipitation is used to extract the drugs
- High-throughput analysis using LDTD-MS/MS
- Linearity, accuracy and precision within the acceptance criteria for all compounds
- Sample-to-sample analysis of 8 seconds