Phytronix Breakaway Technologies

Sciex

Toxicology

Application Note : 2512

Ultra-fast analysis of Gabapentin in Oral Fluid:

Gabapentin analysis at 10 Seconds per Sample Using LDTD-MS/MS

Mégane Moreau, Serge Auger, Pierre Picard, and Jean Lacoursière
Phytronix Technologies, Québec, Canada
Keywords: High-Throughput, Gabapentin, Oral Fluid, LDTD-MS/MS

Introduction

Gabapentin, a drug originally prescribed for the treatment of seizures, is now frequently used off-label for pain management. However, its use can cause side effects such as drowsiness and dizziness, which may impair driving and pose significant safety risks. Considering these issues, the National Safety Council's Alcohol, Drugs, and Impairment Division (NSC-ADID) has recently upgraded gabapentin's classification from a Tier II to a Tier I substance, adding it to the standard routine drug testing panel. For this purpose, NSC-ADID established a cutoff concentration of 50 ng/mL in oral fluid.

Our goal for this application note is to use a simple sample preparation method and a fast analysis technique for gabapentin analysis in oral fluid (OF) sample.

The Axino-MS/MS system offers specificity combined with an ultra-fast analysis for an unrivaled analysis method. To develop this application, we focused on performing a simple sample preparation.

Axino Ionization Source

The Axino Ion Source (Figure 1) is the new generation of sample introduction and ionization source based on the LDTD technology for mass spectrometry. The Axino Ion Source uses a Laser Diode to obtain unmatchable thermal uniformity giving more precision, accuracy and speed.

LDTD coupled to the Uplyft flow process begins by shaping a cone out of the well of a Domino plate and the dry samples which are rapidly evaporated using indirect heat. The cone shape improves the flow of the neutral molecules that were thermally desorbed from the surface into the 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 a few seconds sample-to-sample and allows for very small volumes to be used.

Figure 1 - Axino Ion Source®

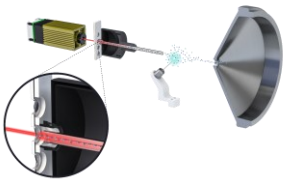

Figure 2 - Axino Ion Source process

Sample Preparation Method

Sample Extraction:

Oral fluid sample collection is performed with the Quantisal device, which is FDA 510(k) cleared for the collection of oral fluid for drug analysis. The calibration curve was prepared in negative OF samples.

- In a 12 X 75 mm borosilicate tubes, 10 µL internal standard solution (gabapentin-d10 at 25 µg/mL in acetonitrile) are added.
- 125 µL of spiked negative OF in Quantisal buffer (1:3) are added.
  - Vortex (1100 rpm/30 seconds).
- 500 µL of extraction solution are added.
- 1000 µL of dilution solution are added.
  - Vortex (1100 rpm/60 seconds).
  - Centrifuge (5000 rpm/5 min.)
- Transfer 50 µL of the aqueous phase (upper layer) in a 0.5 mL microcentrifugation tube.
- In the 0.5 mL microcentrifugation tube, add 150 µL of desorption solution.
  - Vortex (1100 rpm/10 seconds).
- Spot 5 µL of upper layer on a Domino plate
  - Dry with air flow at 40°C for 8 minutes.

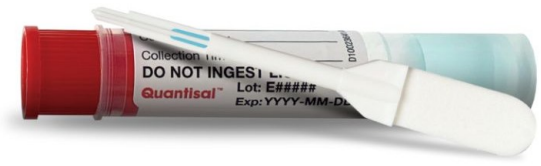

Figure 3 - Quantisal device for oral fluid sample collection

Figure 4 – Domino LazWell sample holder

LDTD®-MS/MS Parameters

LDTD
Model: Axino, Phytronix
Carrier gas: High
Laser pattern: 6-second ramp to 65% power

MS/MS
MS model: Q-Trap System® 5500, Sciex
Ionization: APCI
Analysis Method: Positive MRM mode
CUR: 20
CAD: 8

Table 1 - Positive MRM Transitions for LDTD-MS/MS

Drugs	Transition	CE
Gabapentin	172.0 → 137.0	25
Gabapentin-d_{10}	182.0→ 147.0	25

Results and Discussion

Validation Test

To evaluate method precision and accuracy, a three-point calibration curve was employed (1 x cutoff, 2 x cutoff, and 5 x cutoff). For the screening approach, a single-point calibration at the cutoff level was used, applying a linear regression constrained to pass through the origin. The peak area against the internal standard (IS) ratio was used to normalize the signal.

Linearity

For inter-run results, linearity was evaluated. Correlation coefficient (r) must be higher than 0.97. Linearity obtained was higher than 0.991.

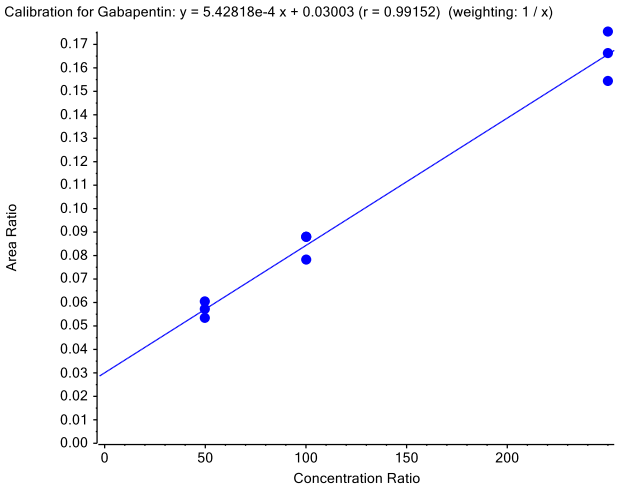

Figure 5 – Three-point calibration curve

Inter-run Precision and Accuracy

Spiked samples around the decision point (Cal-1X, two times cut-off: Cal-2X, and five times cut-off: Cal-5X) and blank solutions are used to validate the precision of the method. For quality control samples, these samples are spiked in real negative matrices. For QC-0.5X, 50% cut-off are spike in sample, and for QC-2X, 200% cut-off are spiked in sample. Replicate extractions are deposited on a Domino LazWell™ plate and dried before analysis.

- Each concentration must not exceed 20%CV for 66.6% of samples.
- Each concentration must not exceed 20%Bias for 66.6% of samples.

For the inter-run precision experiment, each fortified sample set is analyzed in triplicate. Table 2 shows the inter-run precision and accuracy results for gabapentin. %CV and %Bias were below 20%.

Table 2 - Inter-Run Precision / Accuracy

Gabapentin	Cal-1X	Cal-2X	Cal-5X
Conc (ng/mL)	50	100	250
N	6	6	6
Mean (ng/mL)	49.2	102.0	248.7
%CV	9.7	7.5	5.1
%Bias	1.5	-2.0	0.5

Run Acceptance Criteria for Intra-run

Intra-run validation tests were carried out using a one-point calibration curve and linear through zero regression. Cutoff and Qcs were analyzed in triplicate. Table 3 shows intra-run results.

- Blank samples must be detected as negative.
- At Cal-1X concentration, %CV must not exceed 20% for at least 66.7% of this standard.
- At QC-0.5X, at least 66.7% of samples must be detected as negative.
- At QC-2X, 66.7% of samples must be detected as positive.

Table 3 - Intra-Run Precision / Accuracy

Gabapentin	Run 1	Run 2
Blank	Negative	Negative
Conc cutoff (ng/mL)	50	50
N	3	3
Mean (ng/mL)	48.8	50.0
%CV	6.7	6.4
QC-0.5X	Negative (3/3)	Negative (3/3)
QC-2X	Positive (3/3)	Positive (3/3)

Multi-matrix study

Real OF samples (N=4) have been tested with this method. These samples were readily known to be exempt from gabapentin. All samples were determined to be negative by Axino-MS/MS analysis. Results are reported in Table 4

Table 4 – Multi-matrix Results

	Axino	Real
M1	Negative	Negative
M2	Negative	Negative
M3	Negative	Negative
M4	Negative	Negative

Conclusion

Axino Ion Source® combined to a Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (**10 seconds per sample**) for screening gabapentin in oral fluid using a simple and efficient sample preparation method.