Phytronix Breakaway Technologies

Sciex

Toxicology

Application Note : 2308

Rapid Screening of Xylazine in Urine:

Screening at 12 Seconds per Sample Using LDTD-MS/MS

Mégane Moreau, Serge Auger, Pierre Picard, and Jean Lacoursière
Phytronix Technologies, Québec, Canada
Keywords: High-Throughput, Urine, Xylazine, Luxon-MS/MS

Introduction

Xylazine, also know as *tranq* or *zombie drug*, is a new muscular relaxant widely used to adulterate drugs on the street[1-3]. This tranquilizer is linked to overdose deaths[1]. In Canada and the United States, xylazine is authorized only in veterinary medicine[2-3]. The presence of this drug on the black market presents a public health threat. In fact, this muscle relaxant is known to cause respiratory distress and cardiac slowing[3]. In addition to causing potentially fatal effects, naloxone, used in cases of overdose, is powerless against xylazine. A rapid analysis of consumers' urine would make it possible to quickly establish the presence of xylazine in certain areas to implement means of control (analysis by infrared spectroscopy in supervised consumption centers) and prevention.

Our goal for this application note is to use a simple sample preparation method for the screening of xylazine in urine using a single operation in LDTD-MS/MS.

LDTD-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled screening method. To develop this application, we focused on performing a quick and simple sample preparation. Xylazine screening results were obtained in less than 12 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. Luxon Ion Source uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing 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 12 seconds sample-to-sample and allows working with very small volumes.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Hydrolysis

- 22.5 µL of Internal standard is added into a Micro Centrifuge Tube of 1.5 mL using an automatic pipet.
- 75 µL of Urine sample were transferred to the 1.5 mL Micro Centrifuge Tube (containing IS) using an automatic pipet.
  - Mix at 1000 rpm for 30 seconds.
- 22.5 µL of β-Glucuronidase-RT Enzyme/Hydrolysis buffer (1:2) is added to each sample using an automatic pipet.
  - Mix at 1000 rpm for 30 seconds and incubate at room temperature for 15 minutes.

Salt-Assisted Liquid-Liquid Extraction

- 150 µL Extraction buffer (1 M K_2HPO_4: NaCl (sat) (1:1)) is added into to the Micro Centrifuge Tube 1.5 mL using an automatic pipet.
- 300 µL Acetonitrile is added into the Micro Centrifuge Tube 1.5 mL using an automatic pipet.
  - Cap and mix at 1000 rpm for 30 seconds.
  - Centrifuge at 13500 rpm for 3 minutes for phase separation.
- Transfer 15 µL of upper layer phase into a new Microcentrifuge tube using automatic pipet and add 45 µL of desorption buffer (5 mM KH_2PO_4, 25 mg/mL BSA in water).
  - Vortex
- Spot 6 µL of mixture onto a LazWell™96 plate using an automatic pipet.
  - Dry 8 minutes at 40°C using the Aura LazWell Dryer

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix
Carrier gas: 6 L/min (air).
Laser pattern:

- 6-seconds ramp to 85% power.
- Hold 1 second at 85% power.

MS/MS

MS model: Q-Trap System® 5500, Sciex
Total run time: 9 seconds per sample
Ionization: APCI
Analysis Method: Positive MRM mode

Table 1 - MRM transitions for LDTD-MS/MS

ID	Q1	Q3	Time (msec)	DP (V)	CE(V)
Xylazine	221,1	90	50	80	30
Fentanyl-d_5	342,2	188,1	50	80	35

Results and Discussion

Screening Range (µg/mL)

The screening range for xylazine can be found in **Table 2**.

Table 2 – Xylazine calibrators concentration

Analyte	Cal 1 (ng/mL)	Cal 2 (ng/mL)	Cal 3 (ng/mL)
Xylazine	25	50	125

Validation Test

For the screening of xylazine in urine, a complete validation was carried out. Linearity, precision, accuracy, stability, and blank interference were evaluated.

Linearity

The calibration curves were plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criteria was used:

- Linear regression (r) must be ≥ 0.99

Table 3 – Three points calibration curve linearity (r)

Run 1	**0.99533**
Run 2	**0.99533**
Run 3	**0.99407**
Run 4	**0.99213**

Precision and Accuracy

Inter-run validation tests were carried out using a tree points calibration curve. Each standard was analysed in triplicate.

- For the precision acceptance criterion, %CV shall not exceed 20% for at least 66.7% of each standard.
- For the accuracy acceptance criterion, %Bias shall not exceed 20 % for at least 66.7% of each standard.

Table 4 - Inter-Run Precision and Accuracy

	Cal 1	Cal 2	Cal 3
Nominal (ng/mL)	25	50	125
N	12	12	12
Mean (ng/mL)	24.8	51.9	123.9
%CV	12.2	8.7	7.9
%Bias	-0.6	3.8	-0.9

Run acceptance criteria for Intra-run

Intra-run validation tests were carried out using a one-point calibration curve and linear through zero regression. Each standard was analyzed in triplicate.

- 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 sample must be detected as negative.
- At QC-2X, 66.7% of sample must be detected as positive.

Results are presented in **Table 5**.

Table 5 – Intra run results for Xylazine samples.

		Run 1	Run 2	Run 3	Run 4
Blank		Negative	Negative	Negative	Negative
Cal-1X	Conc (ng/mL)	25	25	25	25
	N	3	3	3	3
	%CV	8.1	12.8	9.0	7.8
QC-0.5X		Negative	Negative	Negative	Negative
QC-2X		Positive	Positive	Positive	Positive

Blank Interference Evaluation

10 blank matrices were analyzed to evaluate the blank interference.

- At xylazine signal, all matrices must be, and were detected as negative.
- At fentanyl-d_5 (Internal standard) signal, all matrices must have an interference peak lower than 20% (peak area) of the Cal-1. All matrices blank interference at IS signal were lower than 2.6%.

Stability

Dry stability: Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 1 day before analysis. Acceptance criteria are the same as the run acceptance criteria. Results are presented in **Table 6**. All the results are within the acceptable criteria range for 1 day at room temperature.

Wet stability: Following the extraction, sample extracts are kept at 4°C in closed containers. After 14 days, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Acceptance criteria are the same as the run acceptance criteria. Results are presented in **Table 6.** All the results are within the acceptable criteria range for 14 days at 4°C.

Table 6 - Wet Stability for Xylazine samples

		Dry stability (1 day/RT)	Wet stability (14 days/4°C)
Blank		Negative	Negative
Cal-1X	Conc (ng/mL)	25	25
	N	3	3
	%CV	2.4	3.1
QC-0.5X		Negative	Negative
QC-2X		Positive	Positive

Conclusion

The Luxon Ion Source® combined with the Sciex QTrap® 5500 mass spectrometer system enables the rapid analysis of xylazine in urine. This analysis method can thus be used in toxicological applications to assess intoxication and the presence of xylazine in the territory.

Reference

1. Centers for Disease Control and Prevention (CDC), 2023. What You Should Know About Xylazine, [Online], URL: https://www.cdc.gov/drugoverdose/deaths/other-drugs/xylazine/faq.html
2. Government of Canada, 2023. Health Canada Drug Analysis Service. Spotlight: The Emergence of Xylazine in Canada. Longueuil (QC), 2023. [Online], URL: https://www.canada.ca/fr/sante-canada/services/publications/vie-saine/emergence-xylazine-canada.html#t1
3. Drug Enforcement Administration, U.S. Department of Justice, 2022. DEA Joint Intelligence Report – The Growing Threat of Xylazine and its Mixture with Illicit Drugs, [Online], URL: https://www.dea.gov/sites/default/files/2022-12/The%20Growing%20Threat%20of%20Xylazine%20and%20its%20Mixture%20with%20Illicit%20Drugs.pdf

© 2024 Phytronix Technologies and Sciex. The trademarks mentioned herein are the property of Phytronix Technologies or Sciex or their respective owners.

For R&D use only, not for diagnostic purposes