Phytronix Breakaway Technologies

Sciex

Toxicology

Application Note : 2210

Drug Screening in Whole blood:

Drug Blood Samples Driving Investigation Screen at 8 Seconds per Sample Using LDTD-MS/MS

Pierre Picard, Jean Lacoursière and Serge Auger
Phytronix Technologies, Québec, Canada
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Introduction

Impaired driving is a public health and safety concern. Toxicological testing is a critical part of these investigations. Academy standards board (ASB) published a guideline of the minimum requirements for target analytes and analytical sensitivity (ANSI/ASB standard 120, 1st Ed. 2021). Samples from these investigations are sent to crime labs, however, the analysis time per sample and the volume of requests creates delays. For forensic analysis laboratories, an improvement in the speed of sample analysis must be made. Indeed, in 2014, more than 500,000 samples were backlogged [1].

Our goal for this application note is to use a simple sample preparation method and a fast analysis technique for a drug panel in whole blood. Based on their chemical properties, two panels were used with a specific sample preparation.

The LUXON-MS/MS system offers specificity combined with an ultra-fast analysis for an unrivaled screening method. To develop this application, we focused on performing a simple sample preparation. Panel 1 was extracted using a Salt Assisted Liquid-Liquid Extraction (SALLE) approach and a Liquid-Liquid Extraction (LLE) was used for Panel 2. The drugs present in the first panel have a greater affinity for acetonitrile while the drugs present in the second panel have a greater affinity for MTBE. This is why the drugs under study have been divided into two panels according to their affinity with the extraction solvent.

Each panel was analyzed simultaneously with quantitative screening results obtained in less than 8 seconds per sample and specific cut-off values were attained for each drug.

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 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 Ionization Source

Figure 3 - Automated extraction system

Sample Preparation Method

Sample Collection

Whole blood samples were collected and transferred into barcoded tubes, readable by the Azeo Liquid Handler.

Sample Extraction (SALLE): Panel 1

Each barcoded vial was scanned by the Azeo Liquid Handler and an automatic batch file was created. The Azeo extraction system (Erreur! Source du renvoi introuvable.) is used to extract the samples using the following conditions:

- 100 µL of whole blood sample
- 100 µL of Internal standard in Acetonitrile:Water (1:2)
  - Vortex
- 200 µL of dilution solution (Acetonitrile: Water (1:1))
  - Vortex
- 50 µL of $ZnSO_4$ (1N)
  - Vortex and centrifuge (5000 rpm / 2 min.)
- Mix 300 µL of upper layer, 300 µL of acetonitrile and 300 µL of buffer.
  - Vortex and centrifuge (5000 rpm / 2 min.)
- Mix 90 µL of upper layer with 10 µL of desorption solution
- Spot 6 µL of mixture on a LazWell™96 plate
  - Dry 3 minutes at 40°C

LDTD®-MS/MS Parameters

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

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

MS/MS
MS model: Q-Trap System® 5500, Sciex
Ionization: APCI
Analysis Method: MRM mode

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

Drugs	Transition	CE
Methamphetamine	150.1 → 119.1	15
Methamphetamine-d_5	155.1 → 121.1	15
MDA	180.1 → 133.1	20
MDA-d_5	185.1 → 138.1	20
Tramadol	264.2 → 58.2	50
Oxazepam	287.1 → 241.1	30
Benzoylecgonine	290.1 → 168.2	30
Oxazepam-d_5	292.1 → 246.1	30
Benzoylecgonine-d_3	293.1 → 171.1	30
Temazepam	301.1 → 255.1	25
Temazepam-d_5	306.1 → 260.1	25
Methadone	310.2 → 265.2	20
Methadone-d_9	319.3 → 268.2	20
Lorazepam	321.0 → 275.0	30
Buprenorphine (-H_2O)	450.3 → 418.3	25
Buprenorphine-d_4 (-H_2O)	454.3 → 422.3	25

Sample Extraction (LLE): Panel 2

Each barcoded vial was scanned by the Azeo Liquid Handler and an automatic batch file was created. The Azeo extraction system (Erreur! Source du renvoi introuvable.) is used to extract the samples using the following conditions:

- 100 µL of whole blood sample
- 100 µL of Internal standard in Acetonitrile:Water (1:2)
  - Vortex
- 1000 µL of Methyl tert-butyl ether (MTBE)
  - Vortex and centrifuge (5000 rpm / 2 min.)
- 600 µL of upper layer were transferred to a new deep-well plate and evaporated to dryness.
- Add 100 µL of reconstitution solution.
  - Vortex
- Spot 6 µL of mixture on a LazWell™96 plate
  - Dry 4 minutes at 40°C

LDTD®-MS/MS Parameters

LDTD

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

- 6-second ramp to 65% power
- Hold 2 seconds at 65% power

MS/MS

MS model: Q-Trap System® 5500, Sciex
Ionization: APCI
Analysis Method: MRM mode

Table 2 - Positive MRM transitions for LDTD-MS/MS

Drugs	Transition	CE
Amphetamine	136.1 → 119.1	12
Amphetamine-d_5	141.1 → 96.0	12
MDMA	194.1 → 133.0	25
MDMA-d_5	199.2 → 165.1	20
Carisoprodol	261.2 → 176.2	25
Carisoprodol-d_7	268.2 → 183.2	25
Nordiazepam	271.1 → 140.1	27
Diazepam	285.1 → 154.1	32
Morphine/HYM/Norhydrocodone	286.1 → 152.0	75
Oxazepam-d_5	292.1 → 246.1	40
Morphine-d_6	292.1 → 152.0	75
Codeine/Hydrocodone	300.2 → 152.0	75
Temazepam-d_5	306.1 → 260.1	40
Codeine-d_6	306.2 → 152.0	75
Zolpidem	308.1 → 236.1	35
Alprazolam	309.1 → 274.1	35
Alprazolam-d_5	314.1 → 286.1	45
Clonazepam	316.0 → 214.0	50
Oxycodone	316.2 → 241.0	35
Oxycodone-d_6	322.2 → 247.0	25
Fentanyl	337.2 → 188.1	25
Fentanyl-d_5	342.2 → 188.1	35

Table 3 - Negative MRM transitions for LDTD-MS/MS

Drugs	Transition	CE
THCC	343.3 → 245.2	-45
THCC-d_9	352.3 → 254.2	-45

Results and Discussion

Initial Cut-off Test (ng/mL)

A drug list and screening cut-offs requested by ANSI/ASB standard 120 guidelines can be found in **Table 4**.

Table 4 – Analytes and cut-offs

Analyte	Cut-off (ng/mL)	Analyte	Cut-off (ng/mL)
Carboxy-THC	10	**Benzoylecgonine**	50
Alprazolam	10	**Fentanyl**	1
Clonazepam	15	**Codeine**	10
Lorazepam	15	**Hydrocodone**	10
Diazepam	50	**Morphine**	10
Nordiazepam	50	**Oxycodone**	10
Oxazepam	50	**Amphetamine**	20
Temazepam	50	**Methamphetamine**	20
MDA	25	**MDMA**	25
Carisoprodol	1000	**Zolpidem**	10
Methadone	50	**Buprenorphine**	1
Tramadol	100		

Precision / Accuracy

Three-point screening curves and two QCs (QC-0.5X and QC-2X) were prepared in negative whole blood and used to validate the method. The peak area against the internal standard (IS) ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell™ plate and dried before analysis.

The following acceptance criteria were used:

- Each standard concentration must not exceed 20% CV
- Each standard concentration must be ±20% of the nominal value (%Biais).
- QC-0.5X cut-off must be detected as negative.
- QC-2X cut-off must be detected as positive.

For the inter-run precision/accuracy experiment, each fortified sample set was analyzed in triplicate on five different days. **Table 5** shows the inter-run precision and accuracy results. For Temazepam, the %CV and %Biais was below 20%. All QC-0.5X were detected as negative and QC-2X detected as positive. Similar results were obtained for the other drugs in the panel.

Table 5 - Inter-Run Precision / Accuracy

Temazepam	S1	S2	S3
Conc (ng/mL)	50	100	250
N	15	15	15
Mean (ng/mL)	50.3	99.2	250.5
%CV	8.0	7.8	7.2
%Biais	0.6	-0.8	0.2

Wet Stability of Sample Extracts

Following the extraction, sample extracts were kept at room temperature in closed containers. After 1 day, sample extracts were spotted on a LazWell™ plate, dried and analyzed. Precision and accuracy of standard are reported in **Figure 4 and 5**. All the results are within the acceptable criteria range for 1 day at room temperature.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 75 minutes before analysis. The precision and accuracy results of standard samples are reported in **Figure 4 and 5**. All the results are within the acceptable criteria range for 75 minutes at room temperature.

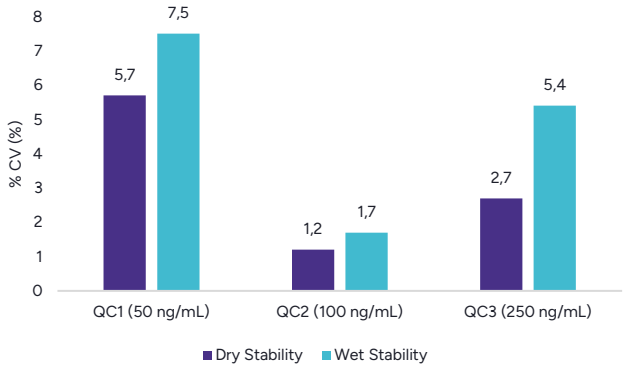

Figure 4 - Precision of sample after stability tests

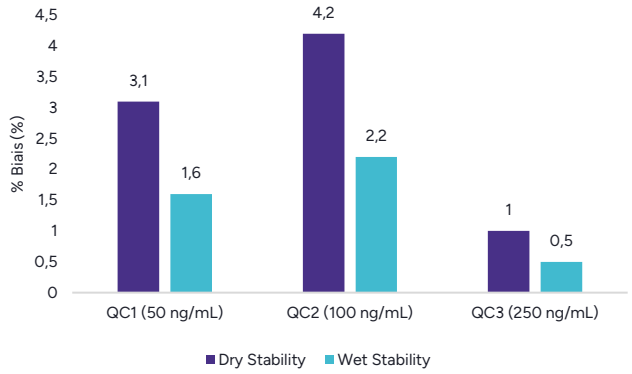

Figure 5 - Accuracy of sample after stability tests

Blank Matrix interference

Seven different blank matrices were extracted and analysed to evaluate the drug signal. The following table (**Table 6**) presents the results of the blank matrix interference. Similar results are obtained for the other drugs.

Table 6 - Evaluation of blank matrix interference for Buprenorphine, Lorazepam and MDA

	Buprenorphine	Lorazepam	MDA
Matrix 1	Negative	Negative	Negative
Matrix 2	Negative	Negative	Negative
Matrix 3	Negative	Negative	Negative
Matrix 4	Negative	Negative	Negative
Matrix 5	Negative	Negative	Negative
Matrix 6	Negative	Negative	Negative
Matrix 7	Negative	Negative	Negative

Multi-matrix validation

Negative whole blood (EDTA-K2) was collected from 6 volunteers. Samples are analyzed unspiked, spiked at QC-0.5x and QC-2X level and screened using LDTD-MS/MS method. The method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy are verified as followed:

		Spiked sample	
		Yes	No
LDTD-MS/MS	Yes	TP (True positive)	FP (False positive)
	No	FN (False negative)	TN (True negative)

Where:

- Sensitivity: (TP / (TP + FN))
- Specificity: (TN / (TN + FP))
- PPV: (TP / (TP + FP))
- NPV: (TN / (TN + FN))
- Accuracy: ((TP+TN) / (TP + FN+TN+FP))

Table 7 shows the analysis results of 18 spiked samples for Temazepam.

Table 7 - Temazepam results

		Spike sample	
		Yes	No
LDTD-MS/MS	Yes	TP=6	FP=0
	No	FN=0	TN=12

Validation results are reported in **Table 8** for Temazepam. Similar results are obtained for the other drugs.

Table 8 - Validation results for Temazepam

Parameters	Temazepam
Sensitivity	1
Specificity	1
PPV	1
NPV	1
Accuracy	1

Conclusion

Luxon Ion Source® combined to a Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (**8 seconds per sample**) screening of drugs in whole blood using a simple and efficient sample preparation method.

References

[1] Durose, M. R., Burch, A. M., Walsh, K., Tiry, E. (2016). *Publicly Funded Forensic Crime Laboratories: Resources and Services, 2014.* U. S. Department of Justice, Office of Justice Programs, Bureau of Justice Statistics, NCJ 250151, 12 p.