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Application Note : 2305

Screening of SAMHSA Drug Panel in Urine:

Drug Screening in Urine at 8 Seconds per Sample Using Luxon Ion Source®

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Introduction

The SAMHSA Drug Panel was established to evaluate the presence of the most common drugs of abuse and their metabolites in biological matrices. The panel contains stimulants such as cocaine and amphetamines, depressants such as opiates and opioids, and perturbators such as PCP. Urine, a biological matrix strong in information concerning the health of a patient or the consumption of drugs, is the matrix used in this application note.

Our goal with this application note is to develop a rapid screening method for the SAMHSA panel in human urine using the LDTD technology.

The LDTD-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 quick and simple sample preparation. Twelve drugs are 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 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 saturation characterize this type of ionization and is the result of the absence of solvent and mobile phase.

Figure 1 - Luxon Ion Source®

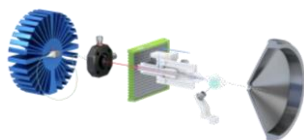

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

- 50 µL of urine is added in a 2 mL Eppendorf.
- 10 µL of internal standard (acetonitrile: methanol; 1:1)
    - Vortex
- 50 µL of B-One enzyme from Kura
- 40 µL of water
    - Mix up and down with a pipette.
- Incubate for 15 minutes at room temperature.
- Add 225 µL of extraction buffer (K_2HPO_4 (500 mM); NaCl (sat); 1:1)
- Add 450 µL of acetonitrile.
    - Vortex
    - Centrifugation
- In a 0.5 mL Eppendorf, mix 100 µL of upper layer with 65 µL of desorption solution (KH_2PO4 at 10 mM and BSA at 100 µg/mL in water).
- Spot 6 µL in LazWell plate
    - Dry at 40 °C for 4 minutes

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix
Carrier gas: 6.0 L/min (air)
Laser pattern:

- 6-second ramp to 55% power
- 2-second hold.

MS/MS – Positive analysis

MS model: QTrap® System 5500, Sciex
IonSpray Voltage: 6000
Temperature, GS1 and GS2 set to zero.
CUR: 20
Scan Time: 3 – 20 msec
Analysis Method: Positive MRM mode

MS/MS – Negative analysis

MS model: QTrap® System 5500, Sciex
IonSpray Voltage: -6000
Temperature, GS1 and GS2 set to zero.
CUR: 20
Scan Time: 3 – 20 msec
Analysis Method: Negative MRM mode

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

	Transition	CE (V)
6-monoacetylmorphine	328.1 → 165.0	45
6-monoacetylmorphine-d_6	334.0→165.0	45
Amphetamine	136.0→119.0	15
Amphetamine-d_5	141.1→124.0	15
Benzoylecgonine	290.1→168.0	20
Benzoylecgonine-d_8	298.0→171.1	30
MDA	180.1→133.1	10
MDA-d_5	185.1→138.3	10
MDMA	194.0→163.0	20
MDMA-d_5	199.2→165.1	20
Methamphetamine	150.1→119.0	15
Methamphetamine-d_9	159.1→125.0	15
Morphine	286.1→152.0	65
Morphine-d_9	292.1→152.0	65
Codeine	300.1→152.0	70
Codeine-d6	306.2→152.0	70
Oxycodone	316.1→241.1	32
Oxycodone-d_6	322.1→247.0	35
Oxymorphone	302.1→227.1	30
Phencyclidine	244.2→159.2	15
Phencyclidine-d_5	249.0→164.1	15

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

	Transition	CE (V)
Delta-9-THCC	343.2→245.1	-35
Delta-9-THCC-d$_9$	352.3→254.1	-35

Results and Discussion

Initial Cut-off Test (ng/mL)

A drug list and screening cut-off suggested in the SAMHSA guidelines can be found in **Table 3**.

Table 3 – Analytes and cut-offs

Analyte	Cut-off (ng/mL)
Marijuana (delta-9-THCC)	50
Cocaine (Benzoylecgonine)	150
Codeine / Morphine	300
Hydrocodone / Hydromorphone	300
Oxycodone / Oxymorphone	100
Heroin (6-Acetylmorphine)	10
PCP (Phencyclidine)	25
Amphetamine / Methamphetamine	500
MDA / MDMA	500

Precision and Accuracy

Three-point screening curve and two QCs (QC-0.5X and QC-2X) were prepared in synthetic urine and used to validate the method. The peak area against the internal standard 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 is analysed in triplicate on five different days. **Table 4** shows the inter-run precision and accuracy results. For MDMA, the %CV and %Bias was below 20 %. All QC-0.5X were detected as negative and all QC-2X were detected as positive. Similar results were obtained for the other drugs on the panel.

Table 4 - Inter-Run Precision and Accuracy for MDMA

MDMA	1 X	2 X	5 X
Conc (ng/mL)	500	1000	2500
N	25	25	25
Mean (ng/mL)	520.6	945.0	2534.3
%CV	4.3	4.5	2.6
%Bias	4.1	-5.5	1.4

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 1 day, sample extracts are spotted on a LazWell™ plate, dried and analyzed by LDTD-MS/MS. The precision and accuracy results of QCs are reported in **Figure 3.** All the results are within the acceptable criteria range for 1 day at 4°C.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 2 hours before analysis. The precision and accuracy results of QCs are reported in **Figure 3**. All the results are within the acceptable criteria range for 2 hours at room temperature.

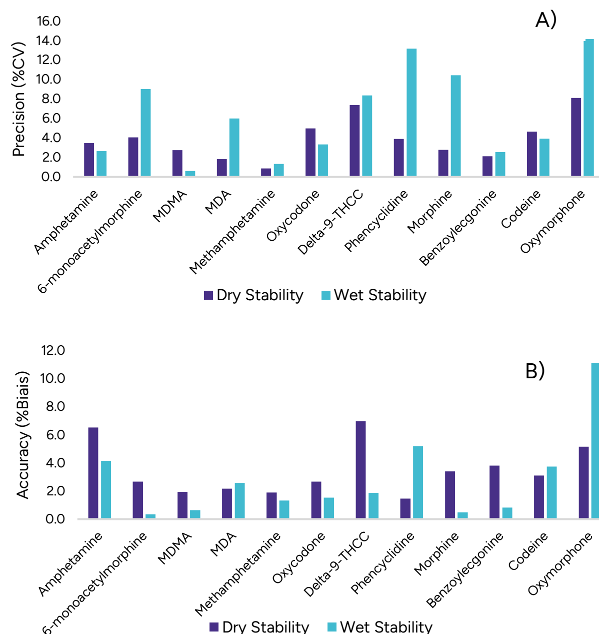

Figure 3 - Wet and Dry Stability A) Precision of the calibrator 1X; B) Accuracy of the calibrator 1X

Multi-matrix Validation

Urines are collected from twenty different volunteers. Samples are screened to cross-verify with LC-MS/MS to assess the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy.

		LC-MS/MS	
		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 5 shows the analysis results of 23 spiked matrices for THCC.

Table 5 – THCC results

		LC-MS/MS	
		Yes	No
LDTD-MS/MS	Yes	TP = 2	FP = 0
	No	FN = 0	TN = 21

Validation results are reported in
Figure 3 - Wet and Dry Stability A) Precision of the calibrator 1X; B) Accuracy of the calibrator 1X for THCC. Similar results are obtained for the other drugs. No false negative was observed.

Table 6 – Validation results for THCC

Parameters	THCC
Sensitivity (%)	100
Specificity (%)	100
PPV (%)	100
NPV (%)	100
Accuracy (%)	100

Conclusion

The Luxon Ion Source® combined with the Sciex QTrap® 5500 mass spectrometer system enables high-throughput analysis of SAMHSA panel in urine.

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