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SAMHSA Drug Panel Screening in Urine:

Drug Screening in Urine at 8 Seconds per Sample Using LUXON-MS/MS

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Introduction

The US Department of Health and Human Services (via the SAMHSA agency) has established scientific and technical guidelines for federal workplace programs of drug testing in urine.
Our goal for this application note is to use an automated sample preparation method for a drug panel in urine using a single operation in LUXON-MS/MS.

LUXON-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. Fourteen drugs are analyzed **simultaneously** with **quantitative** screening results obtained in less than 8 seconds per sample. Each drug has been screened based on the SAMHSA guidelines cut-offs.

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 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

Sample Preparation Method

Sample Collection

Urine samples were collected and transferred into barcoded tubes, readable by the Azeo extraction system.

Automated Sample Extraction

Each barcoded vial was scanned by the Azeo liquid handler and an automatic batch file was created. The Azeo extraction system (**Figure 3**) is used to extract the samples using the following conditions:

- 75 µL of urine sample were transferred from the vials to a deep-well plate placed in the Lumo vortexer
- 22.5 µL of Internal standard were added to each sample
  - Mix
- 22.5 µL β-Glucuronidase-RT Enzyme/Hydrolysis buffer were added to each sample
  - Mix and incubate at room temperature for 15 minutes
- 150 µL Extraction buffer and 300 µL Acetonitrile were added into the deep-well plate
  - Mix and wait 1 minute for phase separation
- Spot 4.5 µL of desorption buffer onto a LazWell™96 plate
- Spot 1.5 µL of the upper layer phase onto a LazWell™96 plate
  - Dry 6 minutes at 40°C

Figure 3 - Automated extraction system

LDTD®-MS/MS Parameters

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

- 3-second ramp to 55% power
- Hold 2 seconds at 55% power

MS/MS
MS model: Q-Trap System® 5500, Sciex
Scan Time: 5 msec
Total run time: 8 seconds per sample
Ionization: APCI
Analysis Method: MRM mode

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

	Transition	CE
Amphetamine	136.1 → 119.1	12
Amphetamine-d_5	141.1 → 124.1	12
Methamphetamine	150.1 → 119.1	15
Methamphetamine-d_9	159.1 → 125.1	15
MDA	180.1 → 163.0	20
MDMA	194.1 → 163.1	15
MDMA-d_5	199.2 → 165.1	15
PCP	244.2 → 159.1	15
PCP-d_5	249.3 → 164.0	15
MOR / HYM	286.1 → 152.0	75
MOR-d_6	292.1 → 152.0	75
COD / HYC	300.1 → 152.0	75
COD-d_6	306.1 → 152.0	75
BZE	290.1 → 168.2	33
BZE-d_8	298.1 → 171.1	33
OXM	302.1 → 227.0	40
OXC	316.1 → 241.0	35
OXC-d_6	322.2 → 247.0	35
6-AM	328.1 → 165.0	50
6-AM-d_6	334.1 → 165.0	50

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

	Transition	CE
THCC	343.2 → 245.2	-40
THCC-d_9	352.2 → 254.2	-40

Results and Discussion

Initial Cut-off Test (ng/mL)

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

Table 3 - Analyte cut-offs

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

Precision

Spiked samples around the decision point (50% cut-off: QC-L, cut-off: CO and 200% cut-off: QC-H) and blank solutions are used to validate the precision of the method. The peak area against the internal standard (IS) ratio was used to normalize the signal. Replicate extractions are deposited onto a LazWell™ plate and dried before analysis.

The following acceptance criteria were used:

- Each concentration must not exceed 20% CV
- The mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the cut-off.

For the inter-run precision experiment, each fortified sample set is analyzed in triplicate on five different days. **Table 4** shows the inter-run precision results. No overlapping at the cut-off is observed for BZE, a cocaine metabolite, and the %CV was below 20%. Similar results are obtained for the other drugs in the panel.

Table 4 - Inter-Run Precision

Cocaine	QC-L	CO	QC-H
Conc (ng/ml)	75	150	300
N	15	15	15
Mean (ng/mL)	76.1	147.3	301.5
SD	3.7	4.9	7.4
%CV	4.9	3.2	2.4
Mean – 2SD (ng/mL)	68.6	137.4	286.7
Mean + 2SD (ng/mL)	83.6	157.2	316.2

For the intra-run precision experiment, each fortified sample is extracted and analyzed in 8 replicates. Area ratio results are plotted using the ± 2 STD error bars. **Figure 4** shows the intra-run results for BZE. No overlapping is observed for each concentration and the %CV was below 20%. Similar results are obtained for the other drugs in the panel.

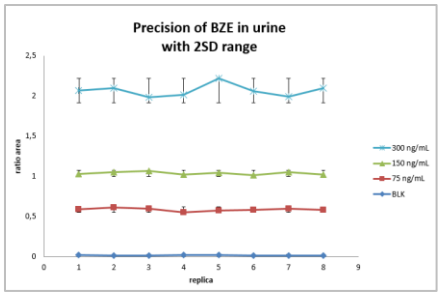

Figure 4 - Intra-Run Precision Curves for BZE

Multi-matrix evaluation

Urine samples were collected from ten different volunteers. Samples are screened to verify the presence of each analyte (all samples showed negative results). To study the matrix effect, the different drugs are spiked at 50% cut-off (QC-L) and 200% cut-off (QC-H) and screened as unknown. **Table 5** shows the screening result of three of them. Samples are spiked at QC-L and QC-H are detected as negative and positive, respectively.

Table 5 – Multi-Matrix Evaluation Results

Analytes	M1 (QC-L)	M1 (QC-H)	M2 (QC-L)	M2 (QC-H)	M3 (QC-L)	M3 (QC-H)
THCC	-	+	-	+	-	+
BZE	-	+	-	+	-	+
COD / HYC	-	+	-	+	-	+
MOR / HYM	-	+	-	+	-	+
OXC	-	+	-	+	-	+
OXM	-	+	-	+	-	+
6-AM	-	+	-	+	-	+
PCP	-	+	-	+	-	+
Amp	-	+	-	+	-	+
Meth.	-	+	-	+	-	+
MDA	-	+	-	+	-	+
MDMA	-	+	-	+	-	+

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 3 days, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Precision at 50% cut-off standard is reported in **Table 6** for BZE. All the results are within the acceptable range (criteria %CV ≤20%) for 3 days at 4°C. Similar results are obtained for the other drugs.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate and kept at room temperature before analysis. Precision at 50% cut-off standard is reported in **Table 6** for BZE. All the results are within the acceptable range (criteria %CV ≤20%) for 30 minutes at room temperature. Similar results are obtained for the other drugs.

Table 6 - Wet and Dry Stability of Cocaine

Parameters	Dry stability	Wet stability
Time	0.5 hour	3 day
Temp. (°C)	22	4°C
Conc. (ng/mL)	75	75
N	3	3
Mean (ng/mL)	76.4	77.1
%CV	5.3	3.8

Conclusion

Luxon Ion Source® combined with Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (**8 seconds per sample**) screening of SAMHSA drug panel in urine using a simple and automated sample preparation method.

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