Application Note : 2009

SAMHSA Drug Panel Screening in Oral Fluid:

Drug Screening in Oral Fluid at 8 Seconds per Sample Using Oral-Eze® device and LUXON-MS/MS

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Introduction

In 2019, the US Department of Health and Human Services (via the SAMHSA agency) has established scientific and technical guidelines for federal workplace drug testing programs in oral fluids (Federal Register / Vol. 84, No. 207, 2019).

Our goal for this application note is to use an automated sample preparation method for a drug panel in oral fluid 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. Specific cut-off values were attained for each individual 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

Sample Preparation Method

Sample Collection

Oral fluids were collected using the Oral-Eze® device. After the collection of the oral fluid, the pad is transferred into a tube containing an extraction buffer. During this process, oral fluids are diluted by a factor of 3.

Automated Sample Extraction

An automated process **(Figure 3)** is used to extract the samples using the following conditions:
50 µL of Oral fluid sample
100 µL of Internal standard in acetonitrile

- Vortex

Spot 4 µL of the mixture on a LazWell™96 plate

- Dry 5 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 65% power
- Hold 2 seconds at 65% 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
Cocaine	304.1 → 182.1	25
Cocaine-d_3	307.2 → 185.2	25
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
THC	313.2 → 245.1	-35
THC-d_3	316.1 → 248.1	-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 cut-off

Analyte	Cut-off (ng/mL)
Marijuana (THC)	4
Cocaine	15
Codeine / Morphine	30
Hydrocodone / Hydromorphone	30
Oxycodone / Oxymorphone	30
6-Acetylmorphine	4
Phencyclidine	10
Amphetamine / Methamphetamine	50
MDA / MDMA	50

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 on a LazWell™ plate and dried before analysis.

Following acceptance criteria were used:

- Each concentration must not exceed 20% CV
- 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 Cocaine 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)	7.5	15	30
N	15	15	15
Mean (ng/mL)	7.3	15.6	29.6
SD	0.35	0.59	0.63
%CV	4.8	3.8	2.1
Mean – 2SD (ng/mL)	6.6	14.4	28.3
Mean + 2SD (ng/mL)	8.0	16.8	30.9

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

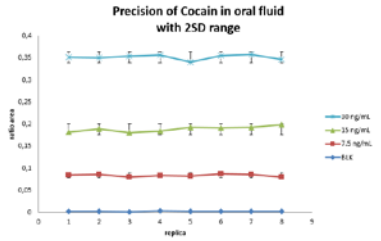

Figure 4 - Intra-Run Precision Curves for Cocaine

Multi-matrix evaluation

Oral fluids are collected on ten different volunteers. Samples are screened to verify the presence of each analyte (all samples were negative). To verify the matrix effect, 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)
THC	-	+	-	+	-	+
Cocaine	-	+	-	+	-	+
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 1 day, sample extracts are spotted on a LazWell™ plate and analyzed. Precision at 50% cut-off standard is reported in **Table 6** for Cocaine. All the results are within the acceptable range (criteria %CV ≤20%) for 1 day 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 Cocaine. All the results are within the acceptable range (criteria %CV ≤20%) for 1 hour 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	1 hour	1 day
Temp. (°C)	22	4°C
Conc. (ng/mL)	7.5	7.5
N	3	3
Mean (ng/mL)	7.7	6.2
%CV	3.9	14.9

Conclusion

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

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