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SAMHSA Drug Panel Screening in Oral fluid: Development of a Screening Method at 8 seconds per sample using LDTD-MS/MS

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OVERVIEW

Purpose

- Optimization of a screening method of Oral fluid samples (two different types of collection device) using high-throughput analysis with LDTD-MS/MS technology

Method

- Oral fluids are collected with Intercept I2™ and Oral-Eze® device
- Two different automated sample preparation approaches are used.
- Dried sample analyzed by LDTD-MS/MS

Quantification

- Validation: No overlapping at the decision point is observed in the validation run and %CV is below 20%.
- Cross-validation study shows no false positive or false negative results.
- Samples analyzed with a run-time of 8 seconds using LDTD-MS/MS system

INTRODUCTION

In 2019, the US Department of Health and Human Services (via the SAMHSA agency) established scientific and technical guidelines for federal workplace drug testing programs in oral fluids (Federal Register / Vol. 84, No. 207, 2019). Screening various drug classes requires several different immunoassay reagents or an LC-MS/MS method with a longer analysis time per sample. LDTD-MS/MS technology combines speed and the analysis of different drug classes within a single method.

The goal of this presentation is to use an automated sample preparation method for LDTD-MS/MS screening of all compounds in a single operation. Two types of collection devices for oral fluid were evaluated: Intercept I2™ device and Oral-Eze® device.

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. The Luxon Ion Source uses a 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 Ion Source

METHOD

Sample preparation:

Sample collection:

- Oral fluids are collected using the Intercept I2™ and Oral-Eze® devices.
- Negative samples are spiked around the decision point cut-off (**Table 1**). An oral fluid dilution factor of 3 is applied during the spiking process.

Automatic sample preparation: Intercept I2™

- Azeo system (Figure 3) scans the barcodes of the sample vials and generates a sample batch file.
- Robot transfers 100 µL of sample into the extraction plate.
- Add 200 µL Internal standard solution in acetonitrile:Water (1:1).
- 100 µL NaCl (sat) solution
  - Vortex
  - Phase separation by gravity.
- Spot 4 µL of desorption buffer on a LazWell plate
- Spot 4 µL of the upper layer on a LazWell plate
- Dry 4 minutes with convection at 40°C
- LDTD-MS/MS analysis after complete evaporation

Instrumentation

- LUXON S-960 Ion Source
- Sciex 5500 Q-Trap system

LDTD Parameters: Oral-Eze®

- Laser power pattern:
  - Increase laser power to 65% in 3 sec
  - Hold for 2 seconds
  - Decrease laser power to 0%
- Carrier gas flow : 6 L/min (Air)

LDTD Parameters: Intercept I2™

- Laser power pattern:
  - Increase laser power to 55% in 3 sec
  - Hold for 2 seconds
  - Decrease laser power to 0%
- Carrier gas flow : 3 L/min (Air)

MS Parameters

- APCI (+ and -)
- Curtain Gas: 20 (Oral-Eze®)
- Curtain Gas: 10 (Intercept I2™)
- Dwell: 5 msec
- MRM mode (Table 2 and 3)

Table 2 Mass spectrometer transitions (Negative)

Drugs	Transition	CE
THC	313 → 245	-35
THC-D_3	316 → 248	-35

Table 1 Analytes and cut-offs

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

Automatic sample preparation: Oral-Eze®

- Azeo system (Figure 3) scans the barcodes of the sample vials and generates a sample batch file.
- Robot transfers 50 µL of sample into the extraction plate.
- Add 100 µL Internal standard solution in acetonitrile.
  - Vortex
- Spot 4 µL of mixture on a LazWell plate
- Dry 4 minutes with convection at 40°C
- LDTD-MS/MS analysis after complete evaporation

Figure 3 Azeo: Automated extraction system

Table 3 Mass spectrometer transitions (Positive)

Drugs	Transition	CE
Amphetamine	136 → 119	12
Amphetamine-D_5	141 → 124	12
Methamphetamine	150 → 119	15
Methamphetamine-D_9	159 → 125.1	15
MDA	180 → 163	20
MDMA	194 → 163	15
MDMA-D_5	199 → 165	15
PCP	244 → 159	15
PCP-D_5	249 → 164	15
Morphine / HYM	286 → 152	75
Morphine-D_6	292 → 152	75
Codeine / HYC	300 → 152	75
Codeine-D_6	306 → 152	75
Cocaine	304 → 182	25
Cocaine-D_3	307 → 185	25
OXM	302 → 227	40
OXC	316 → 241	35
OXC-D_6	322 → 247	35
6-Monoacetylmorphine	328 → 165	50
6-Monoacetylmorphine-D_6	334 → 165	50

RESULTS

Precision

Spiked samples around the decision point and blank solutions are used to validate the precision of the method. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. The peak area against IS ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell plate and dried before analysis. No overlapping at the decision point is observed for all curves and the CV% was below 15%. **Table 4** and **Table 5** show inter-run precision results for the Intercept I2™ and Oral-Eze ® device, respectively.

Table 4 Inter-run precision results Intercept I2 ™ device

	Grand mean (ng/mL)	%CV	Grand mean − 2SD	Grand mean + 2SD		Grand mean (ng/mL)	%CV	Grand mean − 2SD	Grand mean + 2SD
6-MAM					**Metamphetamine**				
2	1.99	6.1	1.75	2.24	25	25.3	3.8	23.4	27.3
4	4.10	7.5	3.48	4.72	50	49.2	3.7	45.6	52.0
8	7.95	4.3	7.27	8.63	100	99.8	4.0	93.8	107.8
Amphetamine					**Morphine / Hydromorphone**				
25	24.7	6.0	21.7	27.7	15	15.3	8.8	12.6	17.9
50	49.9	3.5	46.4	53.4	30	30.2	6.1	26.5	33.8
100	99.8	3.4	92.9	106.6	60	59.1	5.6	52.5	65.6
Cocaine					**Oxycodone**				
7.5	7.6	1.9	7.3	7.8	15	15.0	3.4	14.0	16.1
15	14.9	3.2	14.0	15.9	30	29.8	2.9	28.1	31.1
30	30.0	2.4	28.6	31.5	60	59.8	1.8	58.2	61.9
Codeine / Hydrocodone					**Oxymorphone**				
15	14.8	4.3	13.6	16.1	15	15.1	7.0	13.0	17.2
30	30.6	4.1	28.1	33.1	30	29.2	5.2	26.2	32.2
60	59.1	3.4	55.1	63.2	60	60.1	5.4	53.6	66.5
MDA					**PCP**				
25	25.2	7.2	21.6	28.8	5	5.2	9.3	4.2	6.1
50	49.4	6.5	43.0	55.9	10	9.7	5.5	8.7	10.8
100	100.1	5.1	89.8	110.3	20	20.3	3.9	18.7	21.9
MDMA					**THC**				
25	24.6	3.2	23.0	26.2	2	2.00	8.9	1.65	2.35
50	50.7	2.9	47.8	53.6	4	3.98	5.7	3.53	4.43
100	99.5	2.6	94.3	104.6	8	8.07	6.3	7.04	9.09

Table 5 Inter-run precision results Oral-Eze® device

	Grand mean (ng/mL)	%CV	Grand mean − 2SD	Grand mean + 2SD		Grand mean (ng/mL)	%CV	Grand mean − 2SD	Grand mean + 2SD
6-MAM					**Metamphetamine**				
2	1.97	16.3	1.33	2.62	25	24.1	6.9	20.8	27.5
4	4.22	10.7	3.32	5.12	50	52.9	5.8	46.8	57.5
8	7.93	8.9	6.52	9.35	100	98.8	3.9	93.0	106.6
Amphetamine					**Morphine / Hydromorphone**				
25	24.3	7.5	20.7	27.9	15	14.7	7.8	12.4	17.0
50	51.9	8.3	43.3	60.5	30	31.3	9.5	25.4	37.3
100	99.1	3.6	92.0	106.1	60	59.2	5.2	53.0	65.4
Cocaine					**Oxycodone**				
7.5	7.3	4.8	6.6	8.0	15	14.8	6.3	13.0	16.7
15	15.6	3.8	14.4	16.8	30	31.0	5.3	27.6	33.4
30	29.6	2.1	28.3	30.9	60	59.7	5.0	55.2	65.7
Codeine / Hydrocodone					**Oxymorphone**				
15	14.6	10.2	11.6	17.6	15	15.3	12.8	11.4	19.2
30	31.5	9.3	25.7	37.3	30	31.4	9.3	25.6	37.3
60	58.8	6.1	51.6	66.1	60	61.7	9.9	49.5	73.9
MDA					**PCP**				
25	24.0	10.2	19.1	28.9	5	5.1	13.5	3.7	6.5
50	50.8	6.2	44.5	57.1	10	9.9	11.9	7.5	12.2
100	98.4	5.9	86.8	110.0	20	18.4	12.9	13.7	23.2
MDMA					**THC**				
25	24.4	4.9	22.0	26.8	2	1.98	15.5	1.36	2.59
50	52.2	4.5	47.5	57.0	4	4.12	16.7	2.75	5.49
100	99.3	2.7	93.9	104.7	8	8.05	13.7	5.85	10.25

Cross validation study

Oral fluids were collected from ten different volunteers. Samples were screened to verify the presence of each analyte (all samples were negative). Drugs were spiked at 50% cut-off (QC-L) and 200% cut-off (QC-H) and screened as unknown for the cross-validation study.

The LDTD-MS/MS results were used to evaluate the following validation parameters: the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. A percentage of 100% was obtained for all validation parameters of every drug (**Table 6**) .

In **Table 7 and 8,** THC results are reported for the Intercept I2™ device. Similar results are obtained for the other drugs with both collection devices.

Table 6 Cross validation parameters

		LC-MS/MS	
		Yes	No
Luxon-MSMS	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 THC results for Intercept I2™ device

THC		Spike sample	
		Yes	No
Luxon-MSMS	Yes	TP=10	FP=0
	No	FN=0	TN=10

Table 8 THC validation results for Intercept I2™ device

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

CONCLUSION

Luxon Ion Source combined to a mass spectrometer system allows ultra-fast, 8 seconds per sample, screening of drugs in oral fluid samples using an automated sample preparation method. Two different collection devices were tested. All validation parameters follow the acceptance criteria.