Phytronix Breakaway Technologies

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Toxicology

Application Note : 2202

SAMHSA Drug Panel Screening in Oral Fluids:

Drug Screening in Oral Fluid at 8 Seconds per Sample Using SALLE method and LDTD-MS/MS

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Keywords: High-Throughput, Oral, LDTD-MS/MS

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.

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 quick and simple sample preparation. Fourteen 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 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®

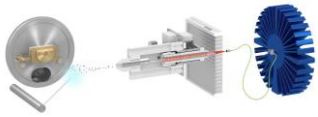

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Sample Collection

Oral fluids were collected using the **Quantisal® 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 4.

Automated Sample Extraction

An automated system (**Figure 3**) is used to extract the samples using the following conditions:

20 µL of Internal standard in acetonitrile
100 µL of Oral fluid sample
- Vortex

100 µL of NaCl (sat): NaOH (1N) / (96:4)
- Vortex

280 µL of acetonitrile
- Vortex and Centrifuge (3000 rpm/2 min.)

Mix 15 µL of the upper layer with 5 µL desorption solution
Spot 8 µL of mixture on a LazWell™96 plate
- Dry 4 minutes at 40°C

Figure 3 - Automated extraction system

LDTD®-MS/MS Parameters

LDTD

Model: Luxon SH-960, Phytronix
Carrier gas: 6 L/min (air) with water infusion at 20 µL/sec
Laser pattern:
6-second ramp to 65% power
Hold 2 seconds at 65% power

MS/MS

MS model: LCMS-8060, Shimadzu
Scan Time: 5 msec
Ionization: APCI
Analysis Method: Positive MRM mode

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

Drugs	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 → 133.0	20
MDA-d_5	185.1 → 138.1	20
MDMA	194.1 → 133.1	25
MDMA-d_5	199.2 → 136.1	25
PCP	244.2 → 159.1	20
PCP-d_5	249.3 → 164.0	20
MOR / HYM	286.1 → 152.0	70
MOR-d_6	292.1 → 152.0	70
COD / HYC	300.1 → 152.0	70
COD-d_6	306.1 → 152.0	70
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
THC	315.2 → 193.1	25
THC-d_3	318.2 → 196.1	25

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 2**.

Table 2 – 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

Data preparation process

Data acquisition systems of mass spectrometers were not designed to deal with signals of only a few seconds per sample. The synchronization sequence adds 6 to more than 15 seconds between each sample. To bypass this, all samples are acquired in a single file (**Figure 4**). To allow the analysis of such data, **Cascade™ software** is designed to detect, split, and integrate every sample peak acquired in a single file.

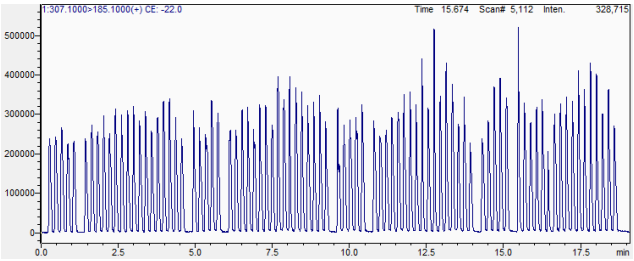

Figure 4 – Single file mass spectrometer data for 96 samples. Cocaine-d_3 transition.

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.

The 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 3** shows the inter-run precision results. No overlapping at the cut-off is observed for THC and the %CV was below 20%. Similar results are obtained for the other drugs in the panel.

Table 3 - Inter-Run Precision for THC

THC	QC-L	CO	QC-H
Conc (ng/ml)	2	4	8
N	15	15	15
Mean (ng/mL)	2.01	3.93	8.02
SD	0.271	0.545	0.909
%CV	13.5	13.7	11.3
Mean – 2SD (ng/mL)	1.47	2.88	6.20
Mean + 2SD (ng/mL)	2.55	5.06	9.84

Wet Stability of Sample Extracts

Following the extraction, the upper layer was mixed with a desorption solution and kept at 4°C in closed containers. After 1 day, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Precision of QC samples are reported in **Table 4**. 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 1 hour before analysis. The precision results of QC samples are reported in **Table 4**. All the results are within the acceptable criteria range for 1 hour at room temperature.

Table 4 - Wet and Dry Stability of THC

Parameters	Dry stability (1 hour / RT)			Wet stability (1 day / 4°C)		
QC	QC-L	CO	QC-H	QC-L	CO	QC-H
Conc. (ng/mL)	2	4	8	2	4	8
N	3	3	3	3	3	3
Mean (ng/mL)	1.93	4.21	7.86	2.10	3.70	8.20
%CV	9.9	5.2	11.0	14.2	3.0	5.8

Multi-matrix validation

Oral fluids are collected from eight different volunteers. Samples are screened to verify the presence of each analyte (all samples were negative). Drugs are spiked at 50% cut-off (QC-L) and 200% cut-off (QC-H) and screened as unknown to verify the method sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy.

		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 5 shows the analysis result of 16 spiked matrices for THC.

Table 5 – THC results

		Spiked sample	
		Yes	No
LDTD-MS/MS	Yes	TP=8	FP=0
	No	FN=0	TN=8

Validation results are reported in **Table 6** for THC. Similar results are obtained for the other drugs.

Table 6 – Validation results for THC

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

Conclusion

Luxon Ion Source® combined to a Shimadzu LC-8060 mass spectrometer system allows ultra-fast (**8 seconds per sample**) screening of drugs panel in oral fluid using a simple and automated sample preparation method.