Application Note : 1903

High-Throughput Screening in Hair for Drugs Using Luxon Ion Source® MS/MS system

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Introduction

Since the hair root is vascularized during its growth, illicit drugs present in the blood stream may enter the hair shaft via the root where they will be sequestered. Therefore, the use of illicit drugs can be revealed by analyzing a small hair sample. To increase the analysis throughput of hair samples, the Luxon Ion Source® coupled to tandem mass spectrometry (MS/MS) was used for the identification and quantification of drugs of abuse.

For this project, we propose to perform a generic extraction method for illicit drug analysis in hair. Screening using the Luxon coupled to a mass spectrometer (Luxon-MS/MS) is chosen as a fast-analytical technique.

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

A pre-wash of the hair is performed to remove external contaminants using Methanol. 10 mg of hair cut into small pieces are transferred in a vial.
2 mL of methanol containing TFA at 0.5% (with internal standard) is added and samples are soaked at 60 degrees Celsius for 1h45. Samples are then sonicated for 15 minutes.
After the extraction, 500 µL of sample are mixed with 200 µL of a solution of KH_2PO_4 (1 mM) / BSA (100 µg/mL) in water.
8 µL of the extract are spotted into 96-LazWell™ plates and evaporated to dryness at 40 degrees Celsius for 8 minutes. Luxon-MS/MS analysis is done after a complete evaporation.

LDTD-MS/MS Parameters

LDTD
Model: Phytronix, Luxon S-960
Carrier gas: 6 L/min (air)
Laser pattern: 3 second ramp to 55% power and hold 2 seconds
MS/MS
Model: Q-Trap System® 5500, Sciex
Ionization: APCI

Table 1 – Mass spectrometer transitions (Positive)

Drugs	Transition	CE
Amphetamine	136 → 119	12
Amphetamine-D_{11}	147 → 98	27
Methamphetamine	150 → 119	15
Methamphetamine-D_{11}	161 → 97	27
MDA	180 → 163	20
MDMA	194 → 163	12
MDMA-D_5	199 → 165	12
MDEA	208 → 163	12
Diethylpropion	206 → 100	35
Diethylpropion-D_{10}	216 → 110	35
Mazindol	285 → 242	35
Mazindol-D_4	289 → 242	35
Morphine	286 → 152	75
Morphine-D_6	292 → 152	75
Codeine	300 → 152	75
Codeine-D_6	306 → 152	75
Cocaine	304 → 182	25
Cocaine-D_3	307 → 185	25
6-Monoacetylmorphine	328 → 165	50
6-Monoacetylmorphine-D_6	334 → 165	50

Table 2 – Mass spectrometer transitions (Negative)

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

Results and Discussion

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% for within-run experiments. Results using the ± 2 STD overlay are plotted. **Figure 3** shows the results of the within-run test for amphetamine. Similar results are obtained for the other drugs.

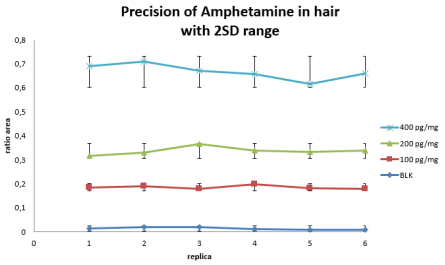

Figure 3 - Within run Precision curves for Amphetamine

For the inter-run precision experiment, each fortified sample sets are analyzed in triplicate on five different days. **Table 3** shows the inter-run precision results.

Table 3 - Inter-run precision

	Grand mean (pg/mg)	Grand mean – 2SD	Grand mean + 2SD		Grand mean (pg/mg)	Grand mean – 2SD	Grand mean + 2SD
Amphetamine (pg/mg)				**Codeine (pg/mg)**			
100	102.2	90.9	113.5	**100**	101.7	82.9	120.4
200	198.0	170.3	225.7	**200**	196.8	170.1	223.5
400	390.7	351.8	429.7	**400**	397.9	348.3	447.5
Methamphetamine (pg/mg)				**Cocaine (pg/mg)**			
100	104.8	96.9	112.7	**250**	261.5	228.0	295.1
200	193.9	172.5	215.3	**500**	492.9	457.9	527.8
400	384.7	362.1	407.3	**1000**	960.4	856.9	1063.9
MDA (pg/mg)				**THC (pg/mg)**			
100	105.3	84.0	126.5	**25**	22.7	14.8	30.6
200	192.8	161.5	224.0	**50**	52.1	45.3	58.9
400	390.1	342.5	437.8	**100**	103.6	90.3	116.8
MDMA (pg/mg)				**6-MAM (pg/mg)**			
100	103.3	89.9	116.7	**100**	103.3	93.9	112.8
200	196.4	179.8	212.9	**200**	200.9	183.3	218.6
400	389.2	361.1	417.3	**400**	378.0	337.2	418.8
MDEA (pg/mg)				**Diethylpropion (pg/mg)**			
100	102.8	87.4	118.2	**100**	101.8	77.3	126.4
200	200.6	161.0	240.2	**200**	196.7	168.3	225.1
400	386.9	361.4	412.3	**400**	413.2	341.6	484.8
Morphine (pg/mg)				**Mazindol (pg/mg)**			
100	103.4	80.3	126.5	**100**	94,0	78,5	109,4
200	203.7	151.3	256.1	**200**	226,9	163,7	290,2
400	374.3	296.0	452.5	**400**	397,6	362,6	432,7

Wet stability of sample extracts

Following the extraction, sample extracts are kept at 4°C in closed containers. After 4 days, sample extracts were spotted on a LazWell™ plate and analyzed. Precision at 50% cut-off standard is reported in **Table 4** for Amphetamine. All the results are within the acceptable range (criteria %CV ≤20%) for 4 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 4** for Amphetamine. All the results are within the acceptable range (criteria %CV ≤20%) for 2 hours at room temperature. Similar results are obtained for the other drugs.

Table 4 - Wet and dry stability Amphetamine

Parameters	Dry stability	Wet stability
Time	2 hours	4 days
Temp. (°C)	22	4
Conc. (pg/mg)	100	100
N	6	6
Mean (pg/mg)	101.4	102.1
%CV	3.6	3.9

Luxon-MS/MS: Sample screen

Sample specimens are extracted and analyzed using a Luxon-MS/MS method. After a fast desorption, specimens, fortified and blank samples are evaluated using peak area ratio. All samples having a concentration higher than the cut-off standard are classified as drug positive samples. **Table 5** shows the screening and confirmation results of the samples. All samples are analyzed using LC-MS/MS confirmation method for cross validation. No false positives or false negatives are observed using the Luxon-MS/MS screening method.

Conclusion

Luxon Ion Source® combined to Q-Trap 5500 mass spectrometer system allows ultra-fast (**8 seconds per sample**) screening of drugs in Hair sample using a generic sample preparation.

Table 5 – Sample screen and confirmation results

	AMP (pg/mg)		MET (pg/mg)		MDA (pg/mg)		MDMA (pg/mg)		MDEA (pg/mg)		MOR (pg/mg)		COD (pg/mg)		Cocaine (pg/mg)		THC (pg/mg)		6AM (pg/mg)		PCP (pg/mg)		Mazindole (pg/mg)		Diethylpropion (pg/mg)	
Sample	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>500)**	**LCMSMS**	**Luxon (>50)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**	**Luxon (>200)**	**LCMSMS**
1																										
2													POS	Note 1												
3			POS	290																						
4																										
5											POS	337	POS	475					POS	355						
6																										
7																										
8																										
9													POS	1365												
10											POS	263							POS	340						
11											POS	1324			POS	421										
12															POS	3706										
13	POS	297	POS	758							POS	476														
14											POS	191														
15																										
16																										
17			POS	618							POS	260														
18																										
19	POS	239	POS	4616																						
20			POS	559									POS	Note 1												
21																										
22											POS	330							POS	336						
23											POS	203							POS	1454						
24																										
25																										
26															POS	938										
27															POS	1100	POS	384								
28	POS	535																								
29															POS	28452										
30																	POS	1070								
31																	POS	731								
32																	POS	1304								
33	POS	500													POS	1525										
34											POS	799			POS	667										

Note: High concentrations of Hydrocodone (Codeine isobar drug) are detected in that sample by the confirmation method.

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