

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 unmatched 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 KH₂PO₄ (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.

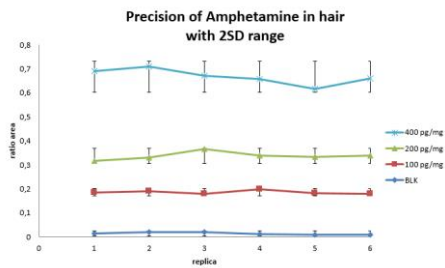


Figure 3 - Within run Precision curves for Amphetamine

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 $\leq 20\%$) 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 $\leq 20\%$) 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 LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>500)	Luxon LCMSMS (>50)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)	Luxon LCMSMS (>200)
1													
2													
3		POS	290				POS	Note 1					
4													
5							POS	475		POS	355		
6													
7													
8													
9													
10													
11													
12													
13	POS	297	758			POS	1365	POS	421	POS	340		
14						POS		POS	3706				
15													
16													
17													
18													
19	POS	239	4616										
20			559										
21													
22													
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24													
25													
26													
27													
28	POS	535						POS	938	POS	384		
29									POS	28452			
30													
31													
32													
33	POS	500											
34													

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