Application Note : 1806

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 dichloromethane and ethanol. 10 mg of hair cut into small pieces are transferred in a vial and then pulverized.

1 mL of methanol (with internal standard) is added and samples are sonicated for 1h. After the sonication process, the solution is transferred into a clean glass tube and evaporated to dryness (no heating to avoid loss of volatile compounds).

A liquid-liquid extraction (LLE) is then performed by adding 800 µL of Methyl-ter-butyl ether (MTBE) and 215 µL of phosphate buffer (1M, pH9).

Finally, 5 µL of the upper layer are spotted into 96-LazWell™ plates and evaporated to dryness. 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 65% power and hold 2 seconds
MS/MS
Model: Q-Trap System® 5500, Sciex
Ionization: APCI (Positive)

Table 1 – Mass spectrometer transitions

Sulfonamides	Transition	CE
Amphetamine	136 → 119	12
Amphetamine-D_5	141 → 124	12
Methamphetamine	150 → 119	15
Methamphetamine-D_9	159 → 125	15
MDA	180 → 133	20
MDMA	194 → 163	12
MDMA-D_5	199 → 165	20
MDEA	208 → 163	12
Morphine	286 → 165	50
Morphine-D_6	292 → 165	50
Codeine	300 → 215	35
Codeine-D_6	306 → 218	35
Cocaine	304 → 182	25
Cocaine-D_3	307 → 185	25
THC	315 → 193	30
THC-D_3	318 → 196	30
6-Monoacetylmorphine	328 → 165	50
6-Monoacetylmorphine-D_6	334 → 165	50

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 other drugs.

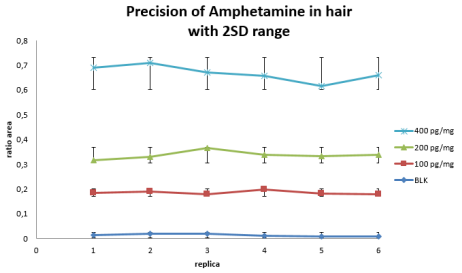


Figure 3 - Within run Precision curves for Amphetamine

For the inter-run precision experiment, each fortified sample sets are analyzed in triplicate on five different days. **Table 2** shows the inter-run precision results.

Table 2 – Inter-run precision

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

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 3** 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 3** 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.

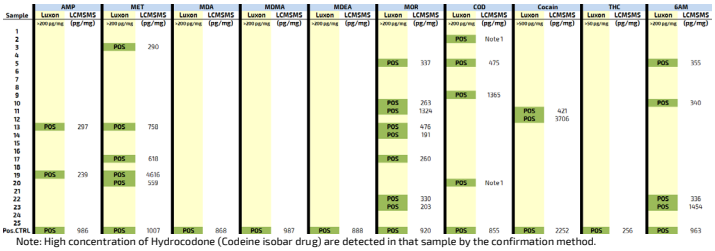
Table 3 - Wet and dry stability Amphetamine

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 4** shows samples' screening and confirmation results. 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.

Table 4 – Sample screen and confirmation results



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

For more information about your specific application, visit www.phytronix.com

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