

High-Throughput Screening in Hair for Drugs:

Screening of Drugs in Hair at 9 Seconds per Sample Using LUXON-MS/MS

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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.

Our goal for this application note is to use an automated sample preparation method for the screen of drugs in hair sample using a single operation in LUXON-MS/MS.

LUXON-MS/MS 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. Drugs are analyzed **simultaneously** with **quantitative** screening results obtained in less than 9 seconds per sample.

Luxon Ionization Source

The Luxon Ion Source® (Figure 1) is the second-generation sample introduction and ionization source based on the LDSTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (Figure 2) to obtain unmatched thermal uniformity providing 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®

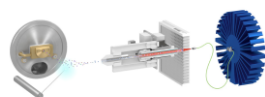


Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Automated Sample Extraction

- 10 mg of hair cut into small pieces are transferred into barcoded tubes, readable by the Azeo extraction system.
- A pre-wash of the hair is performed to remove external contaminants using 1 mL of Methanol, soak 5 minutes and decanted.
- 1 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, vials were transferred to the Azeo system.

- Each barcoded vial was scanned by the Azeo Liquid Handler system (Figure 3).
- 20 µL of desorption solution (KH₂PO₄ (0.1 mM) in water) were mixed with 20 µL of sample.
- 8 µL of mixture were deposited onto a 96-LazWell™ plate.
- Samples were evaporated to dryness at 40 degrees Celsius for 8 minutes.
- Luxon-MS/MS analysis is performed after a complete evaporation.



Figure 3 - Automated Extraction System

LDSTD®-MS/MS Parameters

LDSTD

Model: Luxon SH-960, Phytronix

Carrier gas: 6 L/min (air with 20 µL/min water)

Laser pattern:

- 3-second ramp to 55% power
- Hold 2 seconds at 55% power

MS/MS

MS model: LC-8060, Shimadzu

Ionization: APCI

Table 1 – Mass spectrometer transitions (Positive)

Drugs	Transition	CE
Amphetamine (AMP)	136 → 119	15
Amphetamine-D ₅	141 → 96	15
Methamphetamine (MET)	150 → 119	15
Methamphetamine-D ₅	159 → 125	15
MDA	180 → 133	20
MDA-D ₅	185 → 138	20
MDMA	194 → 163	10
MDMA-D ₅	199 → 165	10
MDEA	208 → 163	12
Diethylpropion (DEP)	206 → 100	25
Diethylpropion-D ₁₀	216 → 110	25
PCP	244 → 159	15
PCP-D ₅	249 → 164	15
Mazindol (MAZ)	285 → 242	20
Mazindol-D ₄	289 → 242	20
Morphine (MOR)	286 → 152	50
Morphine-D ₅	292 → 152	50
Codeine (COD)	300 → 152	50
Codeine-D ₅	306 → 152	50

Cocaine (COC)	304 → 182	20
Cocaine-D ₃	307 → 185	20
6-Monoacetylmorphine (6-MAM)	328 → 165	35
6-Monoacetylmorphine-D ₃	334 → 165	35

Table 2 – Mass spectrometer transitions (Negative)

Drugs	Transition	CE
THC	313 → 245	-30
THC-D ₃	316 → 248	-30

Results and Discussion

Initial Cut-off Test

Table 3 shows the suggested screening cut-offs currently used in the industry.

Table 3 – Analytes and Cut-offs

Analyte	Cut-off
Amphetamine	200 pg/mg Hair
Methamphetamine	200 pg/mg Hair
MDA	200 pg/mg Hair
MDMA	200 pg/mg Hair
MDEA	200 pg/mg Hair
Morphine	200 pg/mg Hair
PCP	200 pg/mg Hair
Cocaine	250 pg/mg Hair
Codeine	200 pg/mg Hair
6-MAM	200 pg/mg Hair
Diethylpropion	200 pg/mg Hair
Mazindol	200 pg/mg Hair
THC	50 pg/mg Hair

Desorption peak

Figure 4 shows a typical desorption peak for a blank sample for Cocaine and Figure 5 shows a typical desorption peak for a cut-off sample for Cocaine. Similar results were obtained for the other drugs.

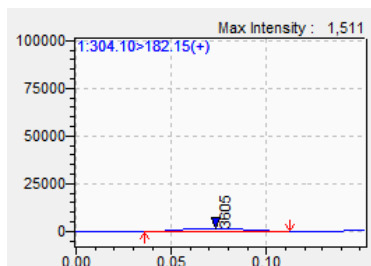


Figure 4 – Desorption peak of blank sample for Cocaine

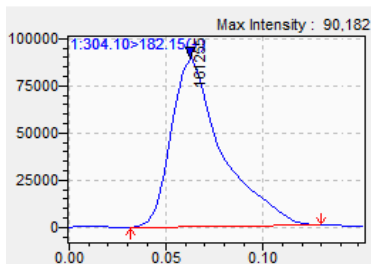


Figure 5 - Desorption peak of cut-off sample for Cocaine

Precision

Spiked samples around the decision point 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 $\pm$ 2 times the standard deviation must not overlap with other concentrations at the cut-off.

Table 4 shows the precision results at the cut-off level.

Table 4 - Inter-run precision

Amphetamine		Methamphetamine	
Conc (pg/mg)	200	Conc (pg/mg)	200
N	6	N	6
Mean	186.7	Mean	223.7
%CV	18.4	%CV	17.7
MDA		MDMA	
Conc (pg/mg)	200	Conc (pg/mg)	200
N	6	N	6
Mean	194.5	Mean	222.9
%CV	19.4	%CV	16.9
Diethylpropion		MDEA	
Conc (pg/mg)	200	Conc (pg/mg)	200
N	6	N	6
Mean	181.1	Mean	215.1
%CV	17.8	%CV	14.4
PCP		Mazindol	
Conc (pg/mg)	200	Conc (pg/mg)	200
N	6	N	6
Mean	217.2	Mean	228.8
%CV	14.7	%CV	14.9
Morphine		Codeine	
Conc (pg/mg)	200	Conc (pg/mg)	200
N	6	N	6
Mean	204.5	Mean	216.0
%CV	15.8	%CV	7.9
Cocaine		6-Monoacetylmorphine	
Conc (pg/mg)	250	Conc (pg/mg)	200
N	6	N	6
Mean	263.8	Mean	218.2
%CV	4.8	%CV	16.4
THC			
Conc (pg/mg)	50		
N	6		
Mean	57.0		
%CV	7.2		

Conclusion

Luxon Ion Source® combined to a Shimadzu LC-8060 mass spectrometer system allows ultra-fast (**9 seconds per sample**) screening of drugs in hair using a simple and automated sample preparation method.