ThP-079

Development of a Screening Method for 11-nor-9-carboxy-delta 9-tetrahydrocannabinol Drugs in Hair Using LDTD-MS3 at 8 Seconds per Sample

Pierre Picard[1], Serge Auger[1] and Jean Lacoursière[1]
[1]Phytronix Technologies, Québec, CANADA

OVERVIEW

Purpose

- High-throughput screening analysis of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in hair using LDTD-MS/MS/MS

Method

- Hair digested in Methanol-TFA
- KH_2PO_4 salt addition and LazWell spotting
- Samples dried and analyzed by LDTD-MS/MS/MS

Quantification

- Linearity: r^2> 0.99 over the calibration range
- 2-time standard deviation (2SD) minimal difference between the cut-off and blank
- Samples analyzed with a runtime of 8 seconds using LDTD-MS/MS/MS system

INTRODUCTION

Hair roots are vascularized during their growth which allows the tetrahydrocannabinol metabolite (11-nor-9-carboxy-delta 9-tetrahydrocannabinol, THCC) present in the blood stream to enter the hair shaft via the root where they are then sequestered. Therefore, the use of illicit drugs can be revealed by analyzing a small hair sample. To increase the analysis throughput and the specificity of hair samples, the LDTD technology coupled to Q-trap mass spectrometry (MS3) is used for the identification and quantification of THCC. For this project, we propose to perform a generic extraction method for illicit drug analysis in hair. Screening using the LDTD coupled to a Q-trap mass spectrometer Q-trap (LDTD-MS3) is chosen as a fast-analytical and specific 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. The Luxon Ion Source uses a 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 Ion Source

METHOD

Sample preparation:

- 10 mg of Hair sample (cut in 1-2 mm length) are pre-washed with 1 mL of dichloromethane followed by 1 mL of ethanol
- 2 mL of Methanol (0.5% TFA) are added, and the digestion is done as follows:
  - Incubation 60°C for 1h45
  - 15 minutes in sonication bath
- After digestion, 200 µL of solution are mixed with 200 µL KH_2PO_4 (1 mM) Water:Acetonitrile (1:1))
  - Mix
- 8 µL are transferred to a LazWell plate and dried 8 minutes at 40°C (convection)
- LDTD-MS/MS analysis after complete evaporation

Instrumentation

- Luxon S-960 Ion Source®
- Sciex 5500 QTrap® system

LDTD Parameters

- Laser power pattern:
  - Increase laser power to 65% in 6 sec
  - Hold 3 seconds
  - Decrease laser power to 0%
- Carrier gas flow : 4.5 L/min (Air)

MS Parameters

- APCI (-)
- Curtain Gas: 15
- MS/MS/MS

Period 1:

Scans in Period: 34
Relative Start Time: 0.00 msec
Experiments in Period: 2

Period 1 Experiment 1:

Scan Type:	MS/MS/MS (MS3)
Polarity:	Negative
Scan Mode:	Profile
Ion Source:	Turbo Spray
# Scans to Sum:	1
1st Precursor:	343.10 Da
2nd Precursor:	299.20 Da
Resolution Q1:	Low
Resolution Q3:	UT
Scan Rate:	1000 Da/s
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	15.0000 msec
Q0 trapping:	Yes
MCA:	No
Center/Width:	Yes
UT fill time:	250.00 msec
Q3 Entry Barrier:	8.00 V
Fragmentation:	Yes
Excitation Time:	25.00 msec
Step Size:	0.10 Da

Start (Da)	Stop (Da)	Time (sec)	Param	Start	Stop
244.28	246.28	0.0021	AF3	0.47	0.47
			EXB	151.76	151.72

Period 1 Experiment 2:

Scan Type:	MS/MS/MS (MS3)
Polarity:	Negative
Scan Mode:	Profile
Ion Source:	Turbo Spray
# Scans to Sum:	1
1st Precursor:	352.20 Da
2nd Precursor:	308.20 Da
Resolution Q1:	Low
Resolution Q3:	UT
Scan Rate:	1000 Da/s
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	15.0000 msec
Q0 trapping:	Yes
MCA:	No
Center/Width:	Yes
UT fill time:	150.00 msec
Q3 Entry Barrier:	8.00 V
Fragmentation:	Yes
Excitation Time:	25.00 msec
Step Size:	0.10 Da

Start (Da)	Stop (Da)	Time (sec)	Param	Start	Stop
253.28	255.28	0.0021	AF3	0.48	0.48
			EXB	151.58	151.53

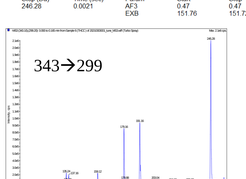

Figure 3 Carboxy THC-MS^3 spectra

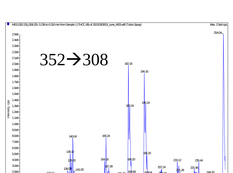

Figure 4 Carboxy THC-D9-MS^3 spectra

RESULTS

Linearity

The negative hair sample extracted is spiked to get the following calibration range (1, 2.5, 5 and 50 pg/mg of hair). **Figure 5** shows the calibration curve results.

Precision

Spiked samples around the decision point and blank solutions are used to validate the precision of the method. Cutoff concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with blank level at the decision point. The sample peak area against the 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 2.5 pg/mg of hair and the CV% was below 20%. Results using the ± 2 STD error bar are plotted. **Figure 6** shows visual separation at 2.5 pg/mg concentration.

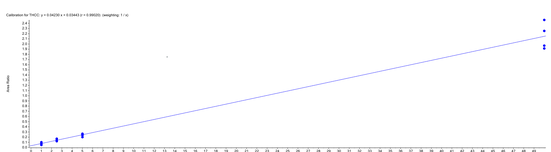

Figure 5 THCC calibration curve (pg/mg of hair)

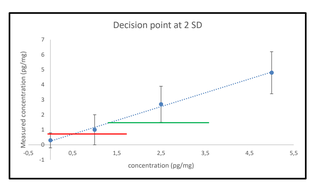

Figure 6 Determination of decision point with 2SD separation

Observations:

- The main advantage of using MS^3 over MRM is the increased selectivity. The THC carboxylic acid can be measured as the background decrease significantly.
- Cycle time required by the Trap limit the number of points per peak. This is reflected as an increase of variability compared to MRM.
- Limit of detection (LOD) of 1 pg/mg of hair is obtained.
- Analytical screening cutoff value of 2.5 pg/mg of hair.
- The total on plate quantity desorbed at the LOD is 40 femtogram.

Table 1 THCC Standard measurement reproducibility and accuracy

Actual Concentration (pg/mg)	Num. Values	Measured values (pg/mg)	Standard Deviation	CV (%)	Accuracy (%)
1	8 of 8	1	0.5	56.8	96.3
2.5	8 of 8	2.7	0.6	20.3	107.1
5	8 of 8	4.8	0.7	14.7	96.6
50	8 of 8	50	6.1	12.2	100.1

CONCLUSION

- Simple-extraction procedure for Carboxy-THC in hair sample
- High-throughput screening of Carboxy-THC in a single LDTD-MS/MS/MS run
- Good linearity, accuracy and precision
- Screening cut-off value of 2.5 pg/mg of Hair
- Sample-to-sample analysis in 8 seconds