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Quantification of 11-*nor*-9-Carboxy-THC and Panel of 22 Drugs in Hair using a Hybrid Triple Quadrupole Linear Ion Trap Mass Spectrometer

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INTRODUCTION

Why Hair Testing?

- Investigate past exposure towards xenobiotics (history of use over time)
  - Hair provides an opportunity to identify the drug, or drugs, many months later; even after single dosage
  - Determining recent past drug use as well as examining long-term drug history through segmental analysis
  - Can be used as indication of abstinence
- No active metabolism or excretion occurs within hair structure to remove drugs once they have been deposited
- Sample collection is non-invasive

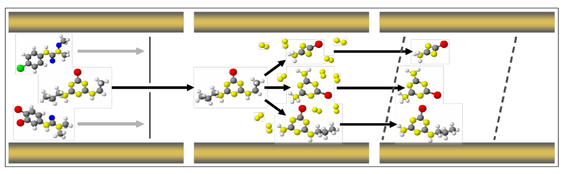
Compound Metabolite Detection and Quantification

- Confirming presence of compounds with confidence
  - Monitoring both parent drugs and their metabolites in hair.
- 11-*nor*-9-Carboxy-Tetrahydrocannabinol (THC-COOH) is the secondary metabolite for Tetrahydrocannabinol with cut off level of 0.2 pg/mg .
  - Requires a unique mass spectrometric analysis (MRM^3) that is only reliably achievable on a QTRAP® instrument.
  - We present a method on a SCIEX QTRAP® 6500+ LC-MS/MS system that reproducibly detects and quantifies 11-*nor*-9-Carboxy-THC levels in hair down to 0.04 pg/mg.

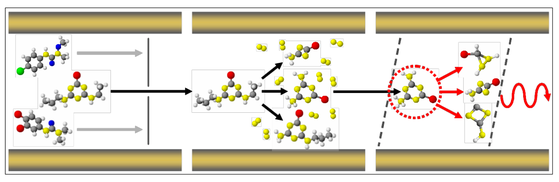
Goal

- To evaluate MRM^3 quantitation method of THC-COOH (in hair) using QTRAP® 6500+ LC-MS/MS system.
- Develop a QTRAP® workflow that enables simultaneous identification and confirmation of compounds from hair through the use of *Scheduled* MRM™ Pro Algorithm –Information Dependent Acquisition – Enhanced Product Ion (sMRM –IDA-EPI).

MRM^3



Selection of Analyte ion in Q1
Fragmentation in Q2
Product ions trapped in Q3 (Linear Ion Trap – LIT)

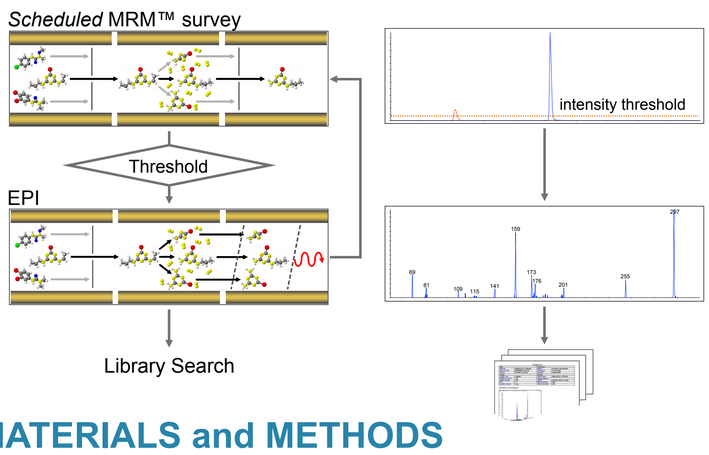


Followed by
Isolation of primary fragment ion in LIT
Fragmentation in LIT
2nd generation of product ions scanned out

Information Dependent Acquisition (IDA)

MRM → EPI

Multi-Target Screening and Quantitation with MS/MS Identification



MATERIALS and METHODS

Compound list and spiking solutions:

Calibrator levels for THC-COOH

Calibrator	[THC-COOH] in hair	[THC-COOH-d9] in hair	Hair weight (mg)	Recon. Vol. (µL)	Injection vol. (µL)	THC-COOH on column (pg)
Cal 1	0.02 pg/mg	10 pg/mg	20	200	20	0.04
Cal 2	0.04 pg/mg	10 pg/mg	20	200	20	0.08
Cal 3	0.1 pg/mg	10 pg/mg	20	200	20	0.2
Cal 4	0.2 pg/mg	10 pg/mg	20	200	20	0.4
Cal 5	0.4 pg/mg	10 pg/mg	20	200	20	0.8
Cal 6	1 pg/mg	10 pg/mg	20	200	20	2

Calibrator levels and Analytes Analyzed by the sMRM-IDA-EPI Method

Analyte	Analyte	IS
Amphetamine	Codeine	THC-D3
Methamphetamine	Methadone	Benzoylcgonine-D3
MDA	THC	Codeine-D3
MDMA	6-MAM	6-MAM-D3
Ecgoninemethylester	Buprenorphine	Morphin-D3
MDEA	Cocaethylene	Amphetamine-D5
EDDP	Norcocaine	Methamphetamine-D3
Morphine	Heroin	Methadone-D3
Benzoylcgonine	Anhydroecgonine methyl ester	Buprenorphine-D4
Cannabidiol	Cannabinol	

Level	ng/mg of each compound in hair	Inj. volume (µL)	Amount on column (pg)
Cal 1	0.005	1	0.18
Cal 2	0.05	1	1.8
Cal 3	0.2	1	7.2
Cal 4	1.0	1	36.3
Cal 5	2.5	1	90.9

Sample preparation

For compounds analysed by sMRM-IDA-EPI Method

Wash	Water, methanol, water
Dry	
Weigh	20 mg
Addition of Digest Reagent	0.45 mL acetonitrile/25 mM formic acid (5:95, v/v) + 50 µL internal standard
Centrifugation	
Incubation	37°C for 18 hours
Centrifugation →	Inject 1 µL on to LC-MS/MS

The procedure outlined in *Journal of Analytical Toxicology*, Vol. 32, June 2008; S. Hegstad, H.Z. Khiabani, L. Kristoffersen, N. Kunøe, P.P. Lobmaier and A.S. Christophersen was followed.

THC-COOH Sample Preparation

Wash	Water, methanol, water
Dry	
Weigh	20 mg
Digest	1 M NaOH, 1 mL, 75°C, 1 hour
Extract	Hexane : ethyl acetate (9:1, v/v), 2 mL
Acidify	Acetic acid, 0.2 mL (into aqueous layer)
Extract	Hexane : ethyl acetate (9:1, v/v), 2 mL
Dry	
Recon	50% MeOH/H_2O, 200 µL
Centrifugation →	Inject 20 µL (10%).

LC-MS/MS

Phenomenex Kinetex C18 column were used. Mobile phase A (MPA) was acetic acid in water and mobile phase B (MPB) was acetic acid in methanol. The LC flowrate was 0.5 mL/min and the LC runtime was 8.0 minutes for the MRM^3 method and 6.5 minutes for the sMRM-IDA-EPI method. Data acquisition was done with Analyst 1.6.3 using *Scheduled* MRM™ Pro Algorithn.

QTRAP® 6500+ LC-MS/MS System Conditions used in the Analysis of THC-COOH

- MS Detection
  - Turbo V™ source, **negative** electrospray ionization
- Source/gas
  - IS: -4500
  - Cur: 30 psi
  - TEM: 660°C
  - GS1: 50
  - GS2: 60
  - CAD: 10 (MRM) or High (MRM^3)

Q1 (m/z)	Q3 (m/z)	Time (ms)	Transition	DP	EP	CE	CXP
343.1	245.2	150	THC-COOH 1	-70	-10	-37	-26
343.1	191	150	THC-COOH 2	-70	-10	-42	-20
352.1	248.2	20	THC-COOH-D9	-70	-10	-37	-26

Q1 (m/z)	Q3 (m/z)	Time (ms)	Transition	DP	EP	CE	CXP
352.1	248.2	20	THC-COOH-D9	-70	-10	-37	-26

IS used for MRM^3 method

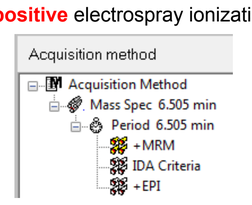
THCA

Scan Type	MS/MS/MS (MS3)
Transition	343.1/299.2/245.2(±1)
Polarity	Negative
1st Precursor	343.10 m/z
DP	-70
CE	-28
2nd Precursor	299.10 Da
Resolution Q1	Unit
Resolution Q3	LIT
Scan Rate	10,000 Da/s
Q0 trapping	Yes
Center/Width	Yes (245.2 / 2 Da)
LIT fix fill time	250.00 msec
Fragmentation	Yes
AF2	0.17 V
Excitation Time	20.00 msec

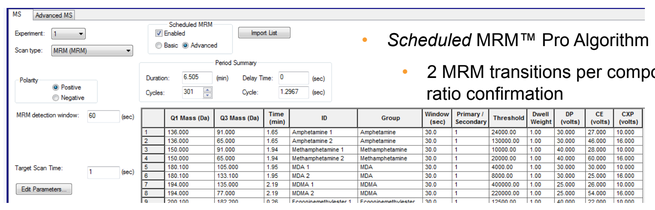
MRM^3

sMRM-IDA-EPI Settings on QTRAP® 6500+ LC-MS/MS System

- MS Detection
  - Turbo V™ source, **positive** electrospray ionization
- Source/gas
  - IS: 4000
  - Cur: 25 psi
  - TEM: 500°C
  - GS1: 90
  - GS2: 70
  - CAD: 10



- IDA Criteria
  - Triggers EPI after Dynamic Background Subtraction of MRM Survey Scan



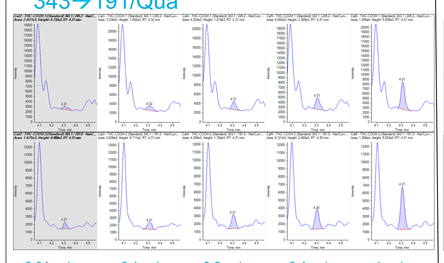
- *Scheduled* MRM™ Pro Algorithm
  - 2 MRM transitions per compound for ion ratio confirmation

RESULTS and DISCUSSION

MRM^3 for quantitative workflows eliminate interference and background, provides high sensitivity MS^3 fragmentation leading to improved LOQ with reproducible % CVs when dealing with tough interferences compared to an MRM approach; demonstrated by the following figures.

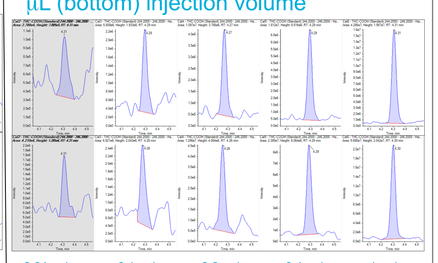
Detection of THC-COOH with MRM compared to MRM^3

MRM: Top/343→245/Quan; Bottom/343→191/Qua



0.04 pg/mg 0.1 pg/mg 0.2 pg/mg 0.4 pg/mg 1 pg/mg

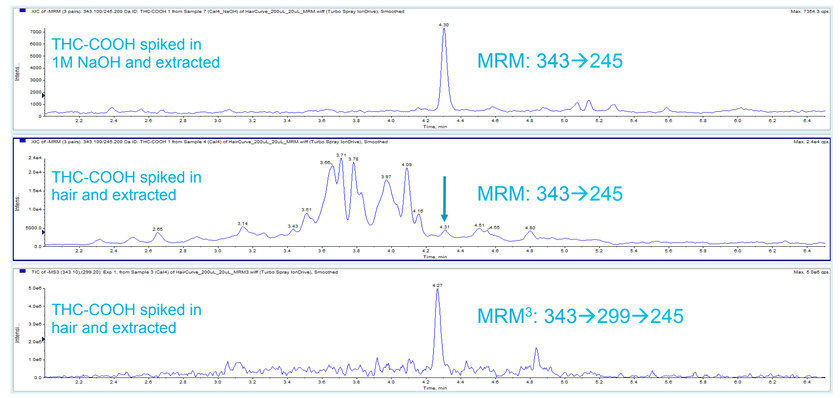
343→299→245±1: 20 µL (top) and 40 µL (bottom) injection volume



0.04 pg/mg 0.1 pg/mg 0.2 pg/mg 0.4 pg/mg 1 pg/mg

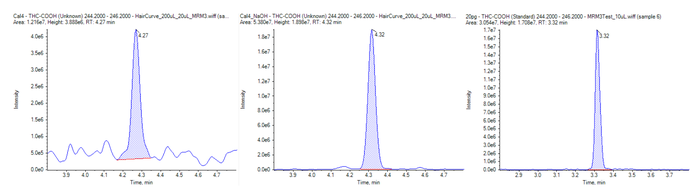
MRM^3 is necessary

0.2 pg/mg THC-COOH (cutoff), 0.4 pg on-column



Accuracy, precision, matrix effect, overall recovery

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	THC-COOH	0.040	2 of 3	0.040	0.004	10.52	99.18
2	THC-COOH	0.100	3 of 3	0.098	0.008	7.94	97.84
3	THC-COOH	0.200	3 of 3	0.206	0.024	11.43	103.12
4	THC-COOH	0.400	3 of 3	0.400	0.020	5.11	99.94
5	THC-COOH	1.000	3 of 3	0.996	0.059	5.93	99.63

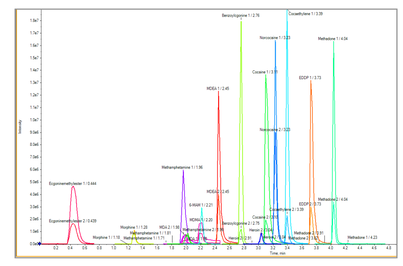


0.2 pg/mg, hair 0.4 pg on column | 0.2 pg/mg, NaOH 0.4 pg on column | Neat 0.2 pg on column

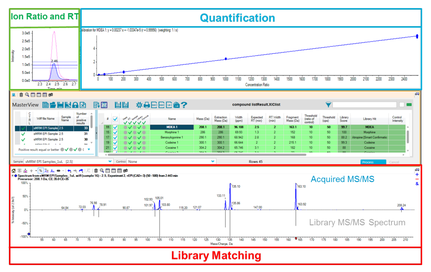
~ 25% suppression recovery >50% extraction recovery

sMRM-IDA-EPI Workflow Results

Elution Profile of 22 Analytes from 1 ng/mg sample (1 µL injection; 36.3 pg on column)

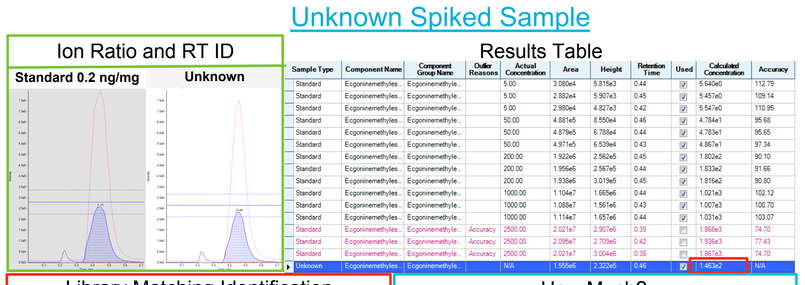


Identification and Quantification - All from a single injection



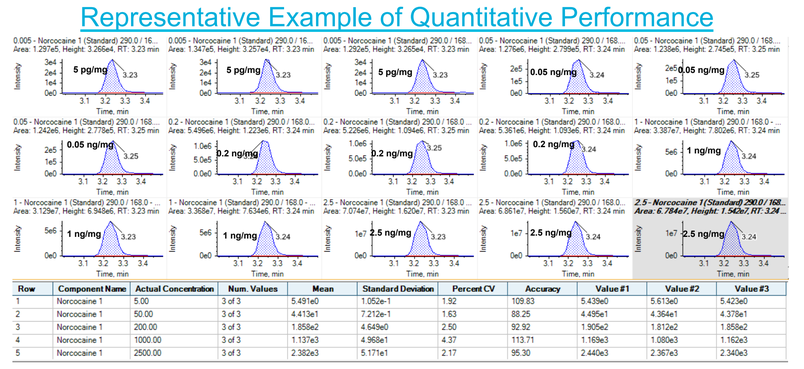
Examples of detection, quantification and simultaneous confirmation by ion ratio, library matching and RT for: Above right, MDEA calibrator series, Below cocaine metabolite from unknown spiked sample.

Unknown Spiked Sample



Name	Mass (Da)	Extraction Mass (Da)	Width (ppm)	Expected RT (min)	RT Width (min)	Fragment Mass (Da)	Found At RT (min)	Threshold (ratio of control)	Threshold (cps)	Library Hit	Library Score
Ecgoninemethylester	200.1	200.1	99.95	0.45	2	82	0.45	10	50	Ecgoninemethylester	97
cocaine	304	304	65.789	3.1	2	182	3.11	10	50	Cocaine	94.6
Benzoylecgonine	290	290	68.966	3.25	2	168	3.25	10	50	Benzoylecgonine	69.1

Representative Example of Quantitative Performance



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	Norcocaine 1	5.00	3 of 3	5.491e0	1.052e-1	1.92	109.83	5.439e0	5.613e0	5.423e0
2	Norcocaine 1	50.00	3 of 3	4.413e1	7.212e-1	1.63	88.25	4.495e1	4.364e1	4.378e1
3	Norcocaine 1	200.00	3 of 3	1.858e2	4.649e0	2.50	92.92	1.905e2	1.812e2	1.858e2
4	Norcocaine 1	1000.00	3 of 3	1.137e3	4.968e1	4.37	113.71	1.169e3	1.080e3	1.162e3
5	Norcocaine 1	2500.00	3 of 3	2.382e3	5.171e1	2.17	95.30	2.440e3	2.367e3	2.340e3

CONCLUSIONS

- QTRAP® technology provides unique advantages in the ability to maximize selectivity when confirming and quantifying low level metabolites in difficult hair matrices.
  - We developed an MRM^3 method that reproducibly detects and quantifies 11-*nor*-9-Carboxy-THC levels in hair down to 0.04 pg/mg
- QTRAP® workflows enable simultaneous identification and confirmation of compounds from hair through the use of *Scheduled* MRM™ Pro Algorithm –IDA – EPI
  - Confirmation via MS/MS library matching (scores >75% for all compounds) and ion ratios (<20%CV for all analytes)
  - Successful quantification of each compound in the panel with high precision.
    - Linearity was from 5.0 pg/mg to 2.5 ng/mg for all compounds except heroin, ecgonine methyl ester (5.0 pg/mg to 1.0 ng/mg) with R^2 values >0.99