

REVIEWED

By Britany Wylie at 2:56 pm, Aug 27, 2020

8/27/2020

Worklist: 4471

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2020-1644	2	BLOOD	AM 27 Blood THC Quant by LC-QQQ

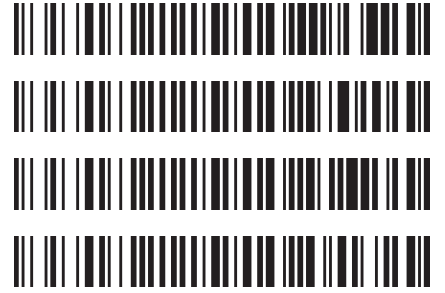


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8/27/2020

Worklist: 4470

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2020-1648	4	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1667	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1669	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1670	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ





AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 8/27/20
Plate lot#: 200303

Analyst: Anne Nord
Plate Expiration: 09-03-2020

Mobile phase A: 0.1% Formic Acid in LCMS Water MTBE
Mobile phase B: 0.1% Formic acid in Acetonitrile Hexane
LCMS Methanol

Blank Blood Lot: 20G20792 **Urine Blank:** 73020 **Column:** UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 69679

Pre-Analytic:

- 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette 1000µL blood (calibrated pipette) Pipette ID: k52558g in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette 500µL 0.1% formic acid in water blood sample, 500 ul saturated phosphate buffer in urine in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer 800µL of blood+acid or urine acid mixture to corresponding wells of SLE+ plate.
- 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right) Manifold ID: 66792
- 8. Wait 5 minutes.
- 9. Add 2.25mL MTBE. *(Add in 3 increments of 750uL)*
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 12. Add 2.25mL Hexane. *(Add in 3 increments of 750uL)*
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
SPE Dry ID: 66819
- 16. Reconstitute in 100µL 100% MeOH and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Create batch and process data.
- 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥ 0.98 for each analyte
- 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less).
Ion ratios must be within +/- 20% of the averaged calibrators
- 4. Case sample response for THC and OH-THC 3ng/mL (quantitative blood), Carboxy-THC: 5 ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- 5. Did all QCs pass for each analyte? (if not is it describe in comments section)
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:

THC-OH not evaluated in urine samples.

Toxicology AM method 27/26 external prep information



working solution 1 ug/ml in meoh C-THC, THC-OH, THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH

Ppd 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

AM 27/26 blood control 100 ul working solution lot () in 9900 ul blood lot ()

		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	
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AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine lot (73020)

out of use

ppd 8/26/20 Exp 7/1/21	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

	1	2	3	4	5	6
a	cal 100 ng	neg blood				QC 1
b	cal 50 ng	1669				cal 100 ng
c	cal 25 ng	1644-2				cal 50 ng
d	cal 10ng	neg urine				cal 25 ng
e	cal 5 ng	urine control				cal 10ng
f	cal 3 ng	1648-4				cal 5 ng
g	cal 1ng	1667-1				cal 3 ng
h	QC 1	1670-2				cal 1ng

c2020-

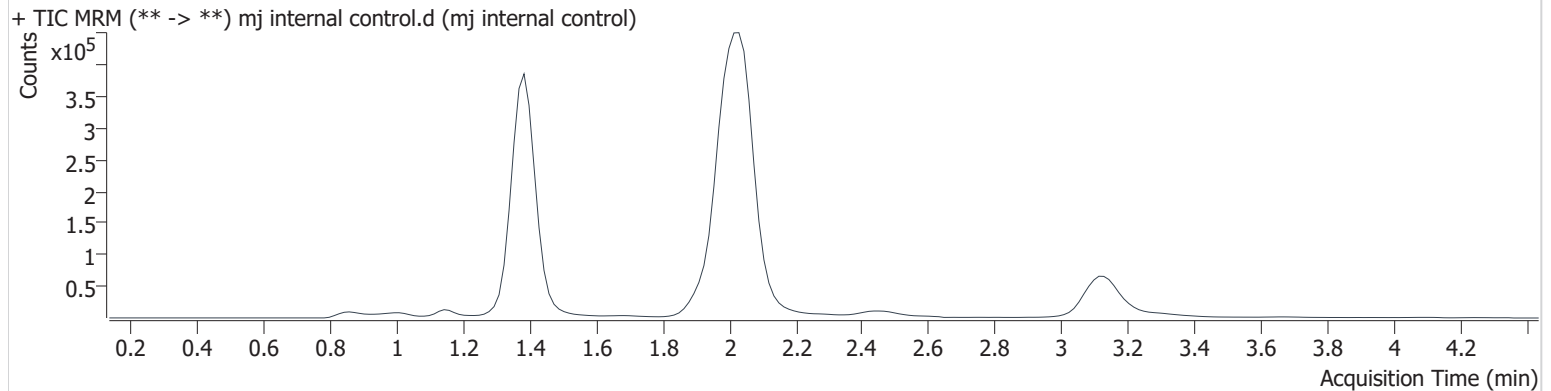
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj internal control.d
Type	QC	Sample	mj internal control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:54:47 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.378	78425	∞	9.5	∞	921448	4.730 ng/ml
THC-COOH	1.401	105961	138776.6	37.9	433.9	613474	14.950 ng/ml
THC	3.134	30987	∞	25.3	519.5	417463	4.244 ng/ml

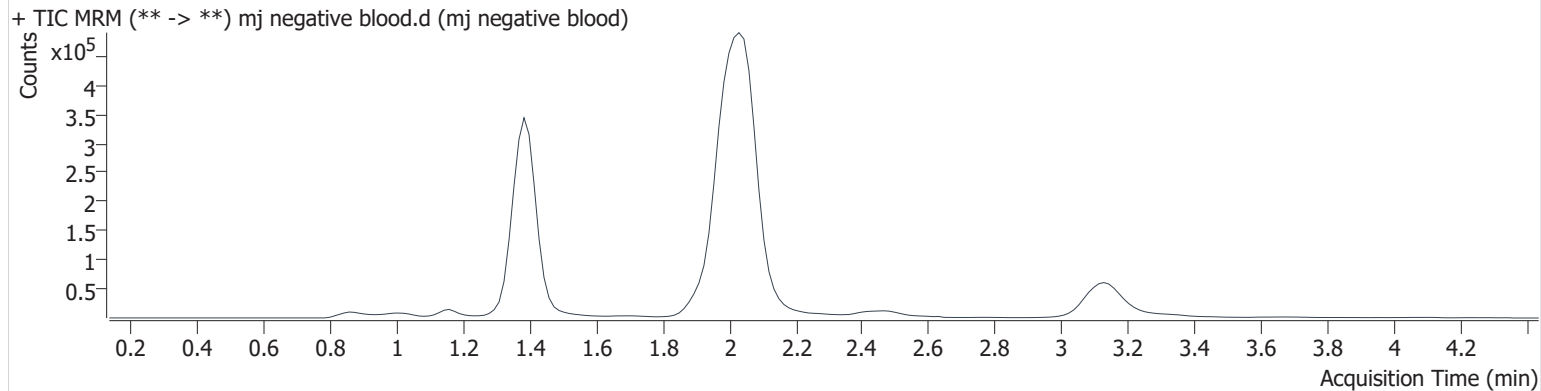
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj negative blood.d
Type	Sample	Sample	mj negative blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 11:02:30 AM		
Sample Info.			

Sample Chromatogram



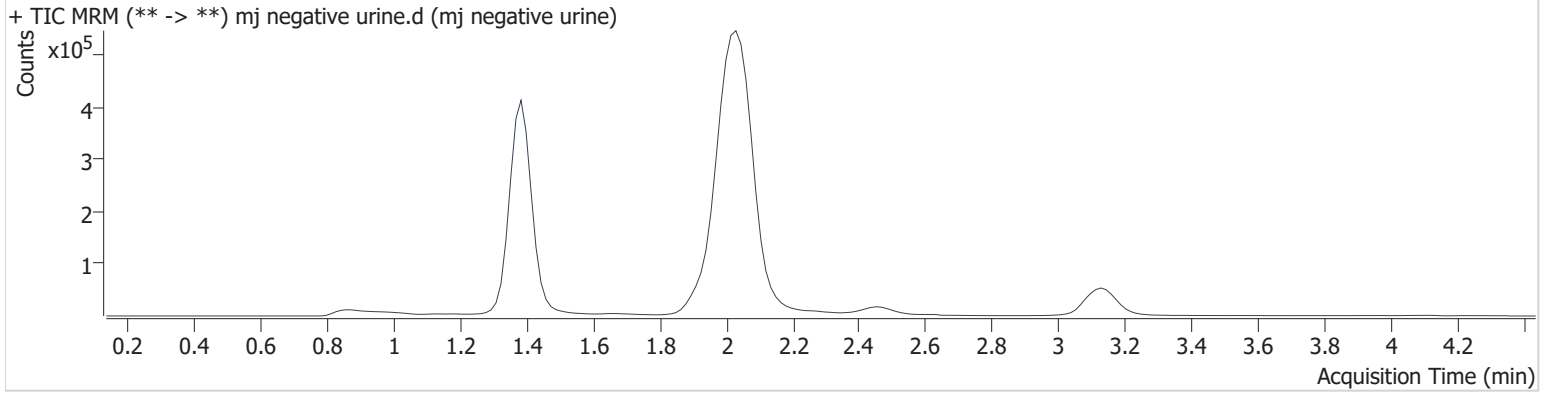
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D2	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 11:40:57 AM		
Sample Info.			

Sample Chromatogram

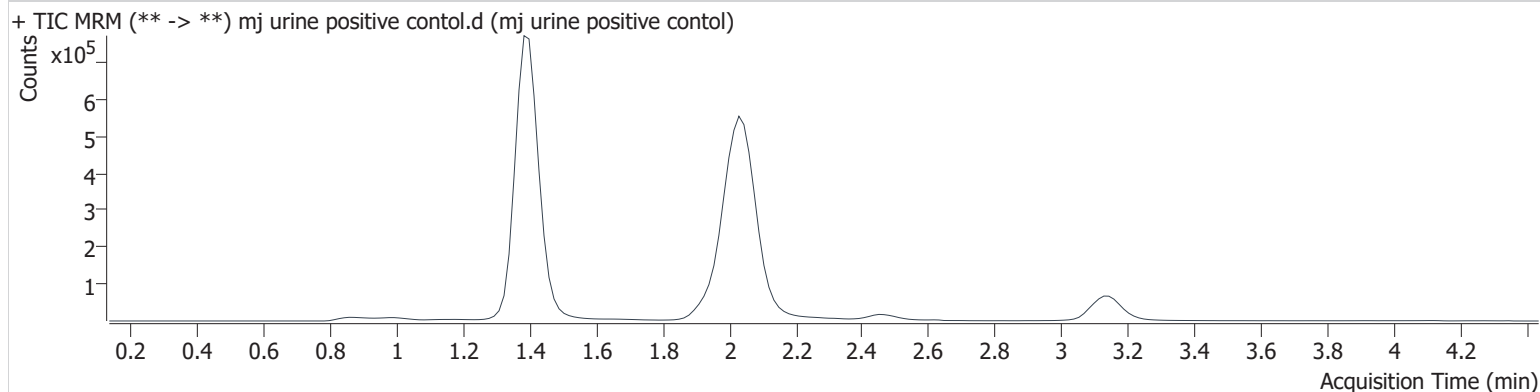


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj urine positive contol.d
Type	Sample	Sample	mj urine positive contol
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E2	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 11:48:41 AM		
Sample Info.			

Sample Chromatogram



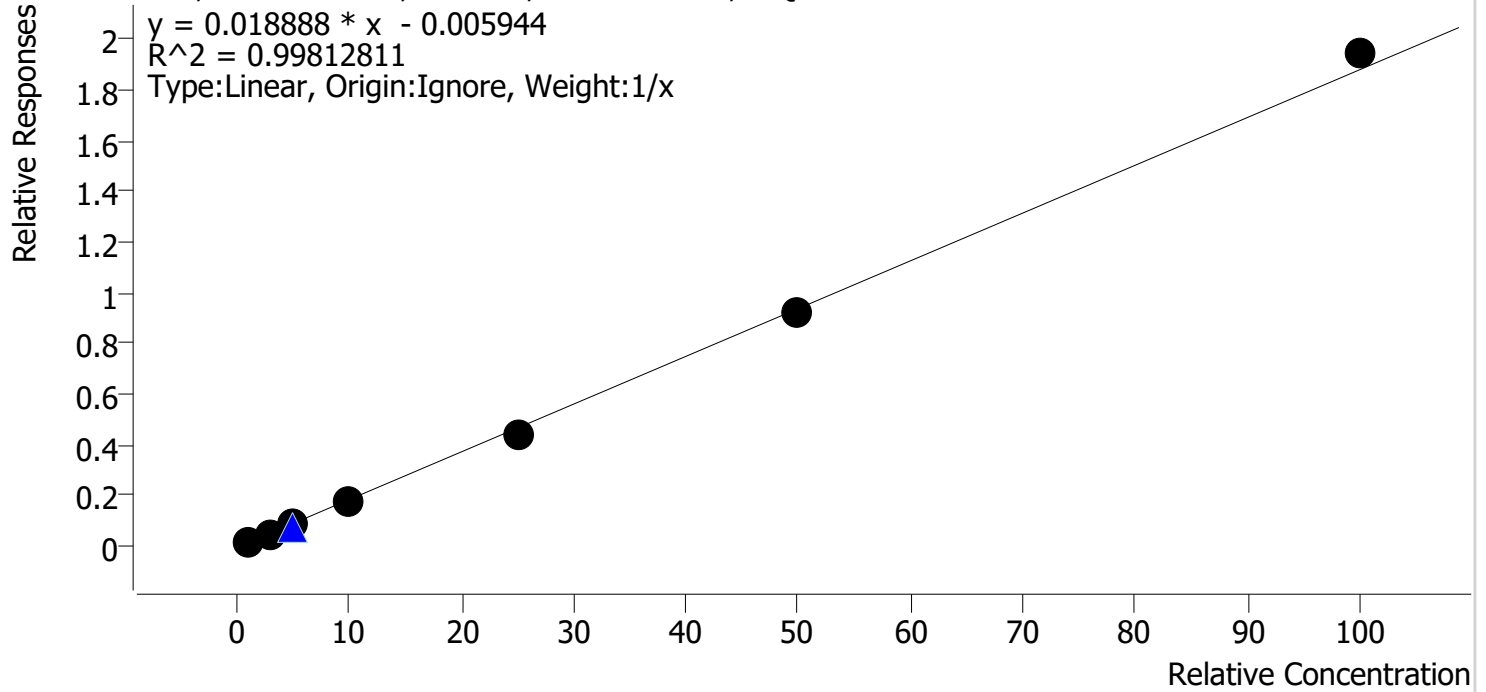
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	1038062	∞	12.5 High	∞	1352841	42.527 ng/ml
THC-COOH	1.416	279700	554.2	36.4	327.6	556037	41.389 ng/ml
THC	3.149	102727	∞	23.7	1840.0	330875	16.752 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Last Cal. Update 8/27/2020 12:50 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



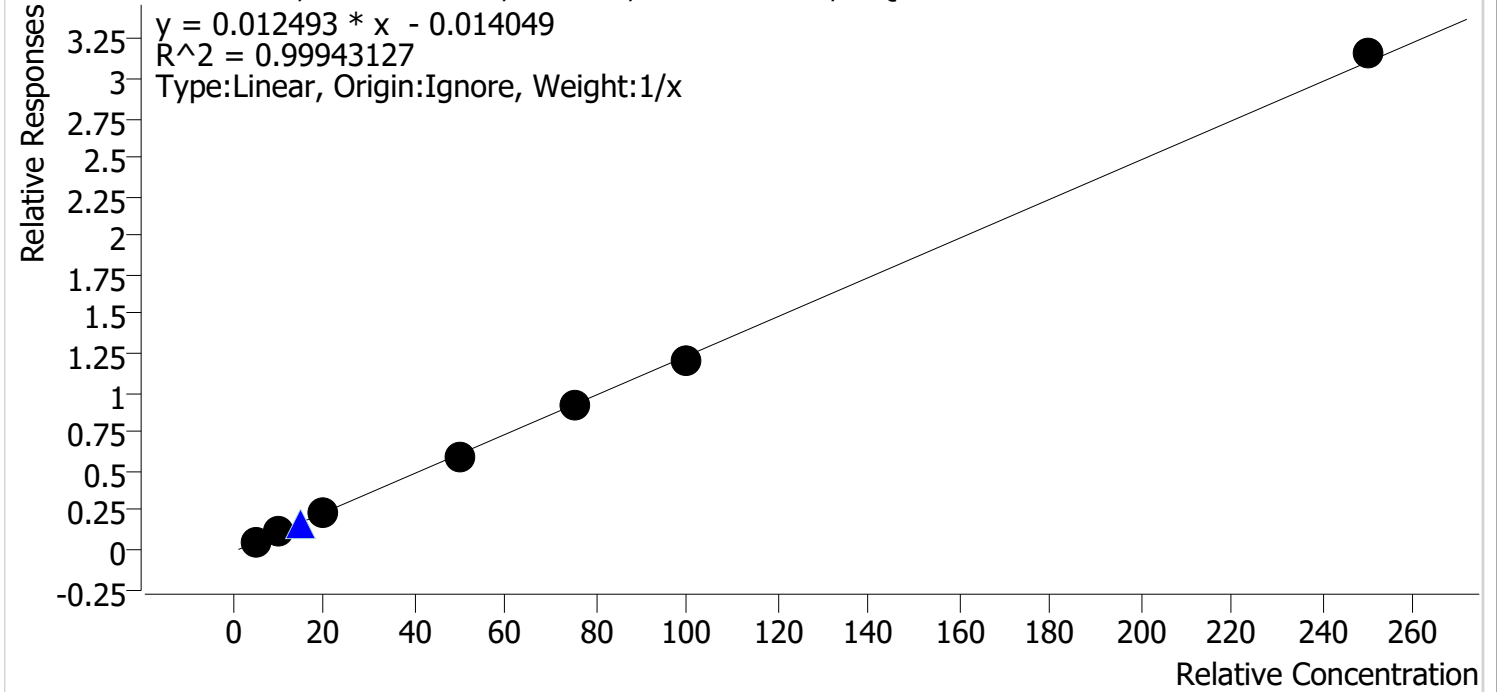
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	1.2	119.0
mj cal2	2	✓	3.0	2.9	96.7
mj cal 3	3	✓	5.0	4.7	94.6
mj cal 4	4	✓	10.0	9.5	94.7
mj cal 5	5	✓	25.0	23.5	94.0
mj cal 6	6	✓	50.0	48.9	97.8
mj cal 7	7	✓	100.0	103.3	103.3

Compound Calibration Report



Batch results	D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin		
Last Cal. Update	8/27/2020 12:50 PM		
Analyst Name	ISP\datastor		
Analyte	THC-COOH	Internal Standard	THC-COOH-d9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



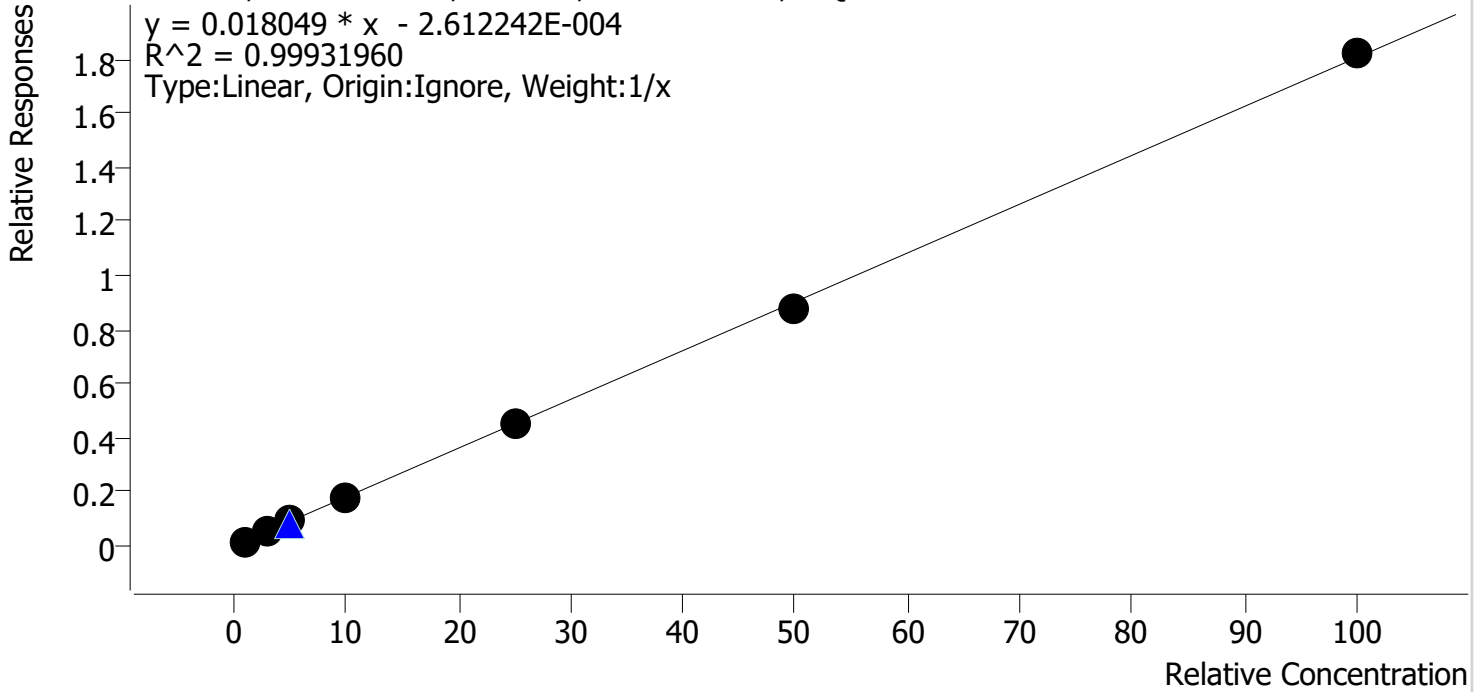
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.1	102.1
mj cal2	2	✓	10.0	10.4	104.1
mj cal 3	3	✓	20.0	19.5	97.3
mj cal 4	4	✓	50.0	49.1	98.2
mj cal 5	5	✓	75.0	74.9	99.8
mj cal 6	6	✓	100.0	96.9	96.9
mj cal 7	7	✓	250.0	254.2	101.7

Compound Calibration Report



Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Last Cal. Update 8/27/2020 12:50 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	0.8	84.5
mj cal2	2	✓	3.0	3.4	111.7
mj cal 3	3	✓	5.0	5.2	104.6
mj cal 4	4	✓	10.0	10.2	101.6
mj cal 5	5	✓	25.0	24.8	99.0
mj cal 6	6	✓	50.0	48.9	97.7
mj cal 7	7	✓	100.0	100.8	100.8

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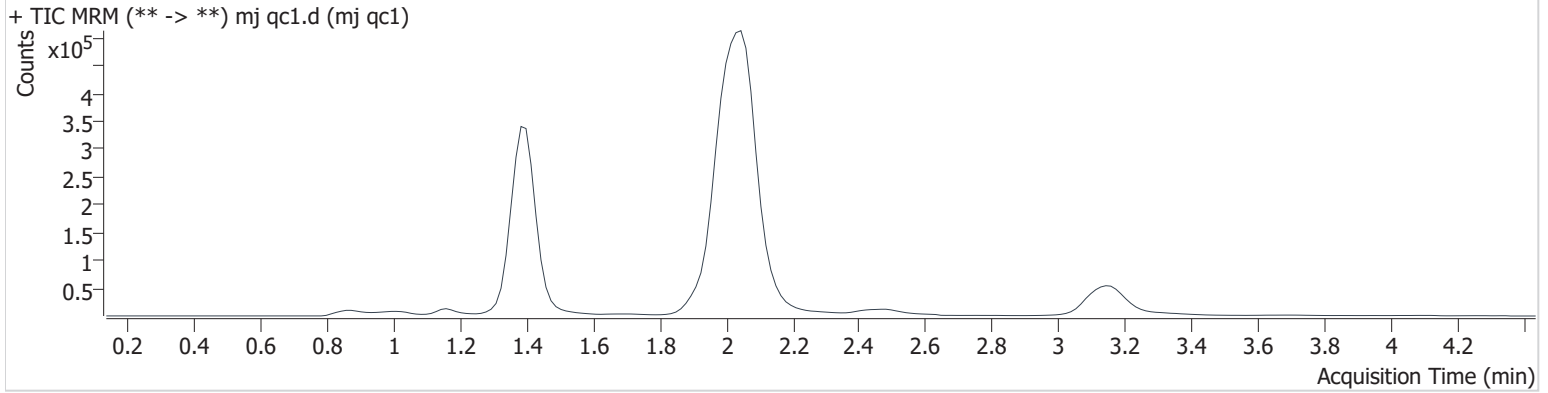
AM #27 Cannabinoids

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Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj qc1.d
Type	Cal	Sample	mj qc1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:00:43 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	14883	∞	12.1	∞	992417	0.845 ng/ml	Low
THC-COOH	1.416	32780	31.4	41.7	370.8	659041	5.106 ng/ml	
THC	3.149	6799	174.8	25.2	14.4	411144	1.190 ng/ml	Low

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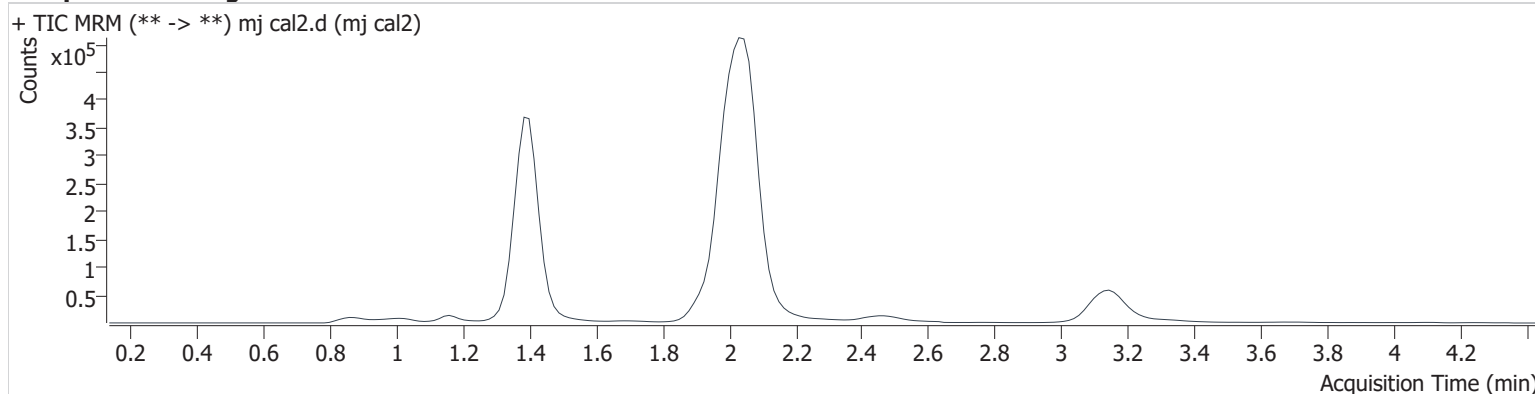
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:08:29 AM		

Sample Info.

Sample Chromatogram



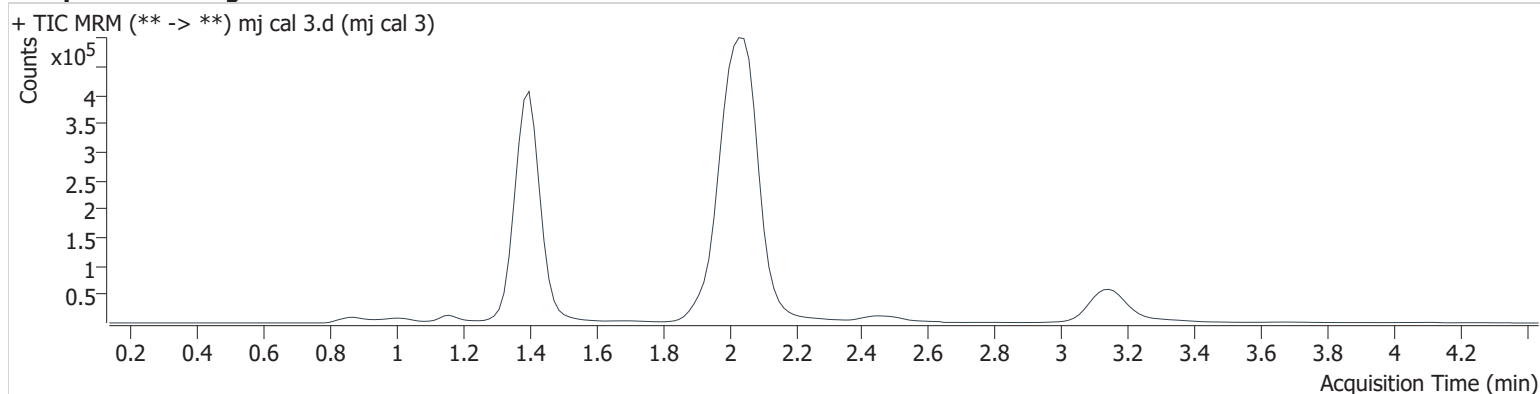
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	60006	∞	10.0	∞	996256	3.352 ng/ml	
THC-COOH	1.416	73560	828.2	35.9	283.1	634247	10.408 ng/ml	
THC	3.164	19492	708.5	26.4	63.9	399221	2.900 ng/ml	Low

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-C1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:16:11 AM		

Sample Chromatogram



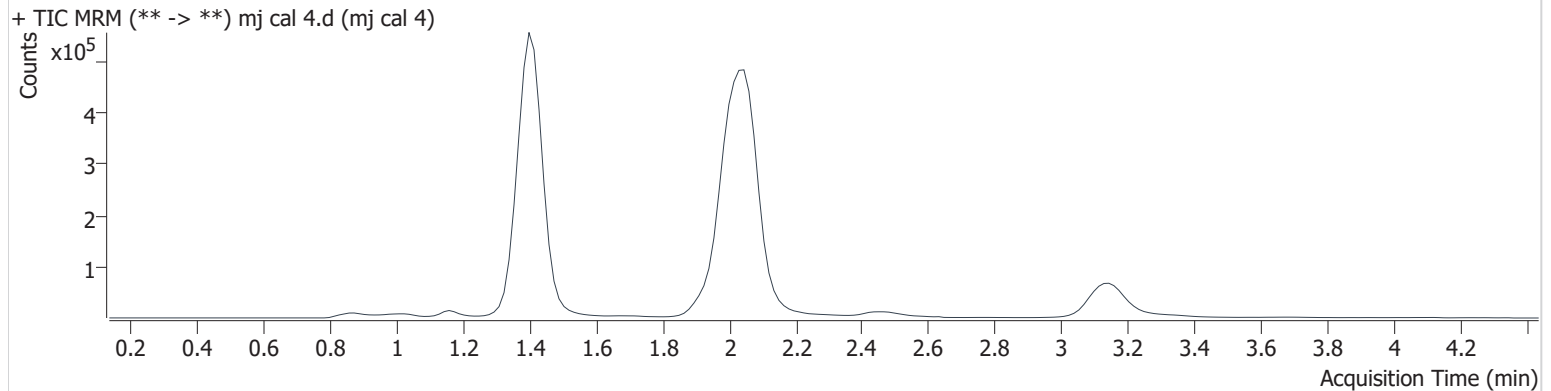
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	92204	∞	9.8	∞	979727	5.229 ng/ml
THC-COOH	1.416	145191	409.6	37.6	888.3	634163	19.451 ng/ml
THC	3.164	32814	∞	25.9	69.2	393641	4.728 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj cal 4.d
Type	Cal	Sample	mj cal 4
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:23:54 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	177322	∞	10.4	∞	968086	10.163 ng/ml
THC-COOH	1.416	378630	850.7	37.8	2931.7	631800	49.094 ng/ml
THC	3.149	69313	∞	24.2	13229. 2	400796	9.471 ng/ml

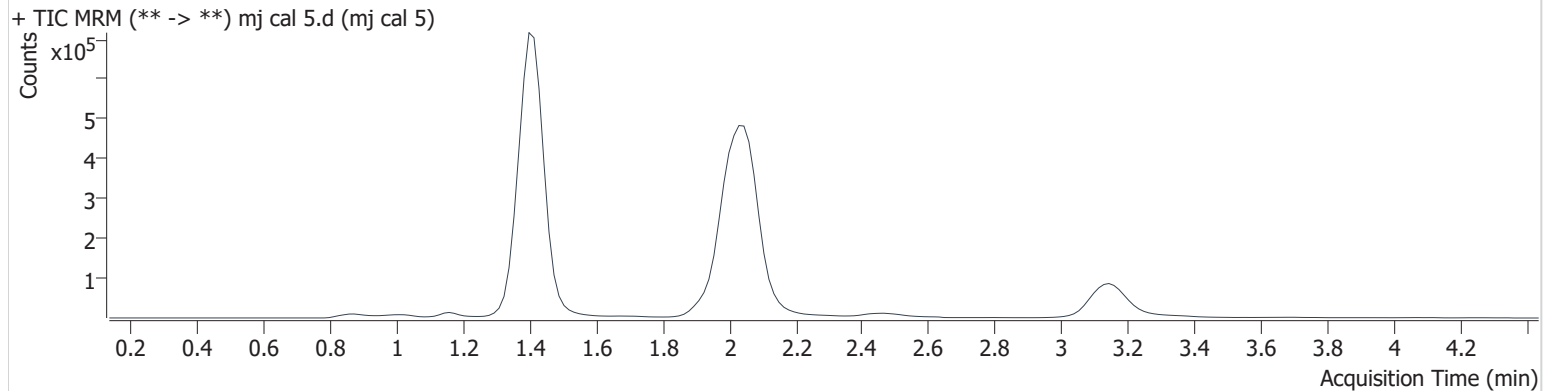
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:31:37 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	423027	∞	10.9	∞	947350	24.755 ng/ml
THC-COOH	1.416	591447	1054.8	37.5	2431.0	642096	74.855 ng/ml
THC	3.164	179686	∞	24.7	11986 16647 15524. 0	410509	23.489 ng/ml

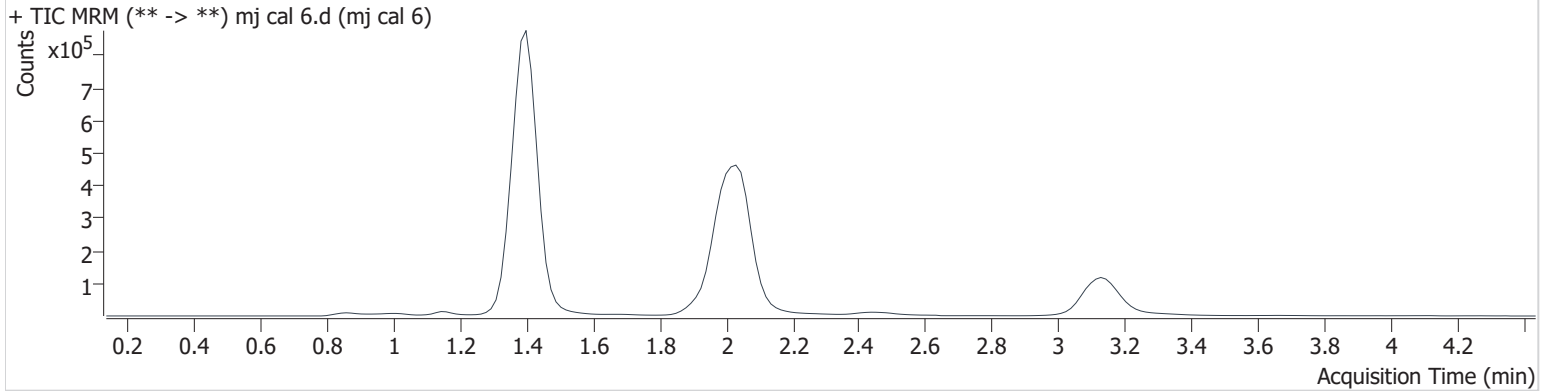
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj cal 6.d
Type	Cal	Sample	mj cal 6
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-F1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:39:21 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	826640	∞	11.7	∞	937489	48.868 ng/ml
THC-COOH	1.416	733373	1558.2	38.0	2630.5	613126	96.867 ng/ml
THC	3.134	377014	∞	23.9	∞	410950	48.886 ng/ml

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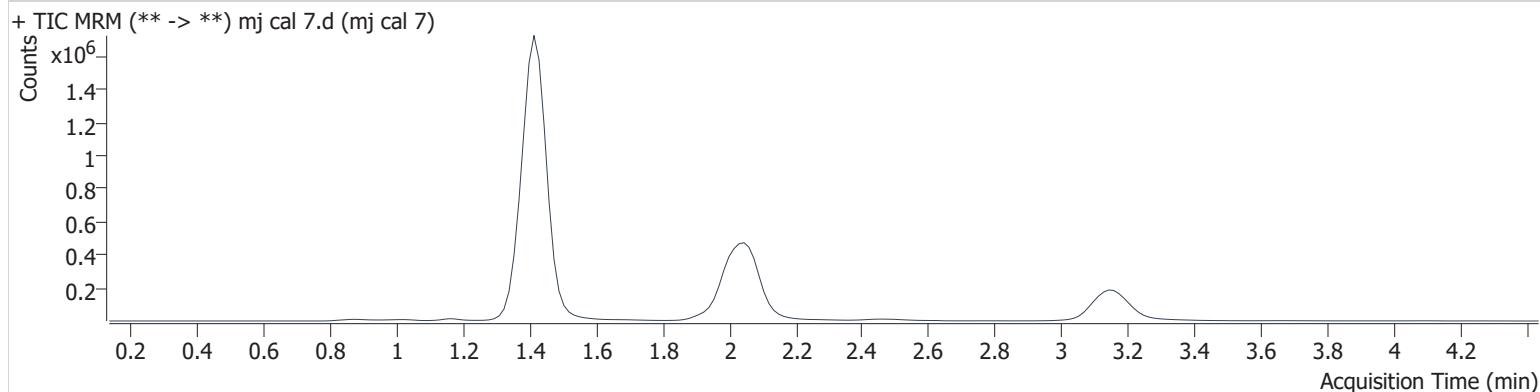
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 082720\QuantResults\cann.batch.bin
Calibration Last Update 8/27/2020 12:50:25 PM

Instrument	69679	Data File	mj cal 7.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2020 10:47:04 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.408	1669675	∞	11.9	∞	917958	100.789 ng/ml
THC-COOH	1.431	1881366	56322.4	38.2	12372.3	595004	254.219 ng/ml
THC	3.149	818991	∞	23.6	∞	420879	103.337 ng/ml