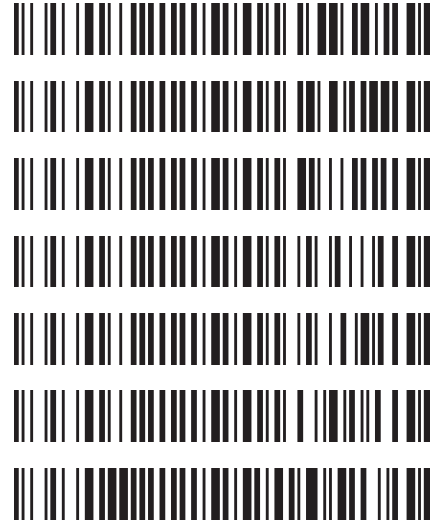


12/7/2021

Worklist: 5428

REVIEWED
By Brittany Wylie at 2:00 pm, Dec 08, 2021

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2021-2508		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2521		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2554		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2599		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2600		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2608	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2012-1953	C1	BCK	AM 27 Blood THC Quant by LC-QQQ





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

Extraction Date 12/07/21
Plate lot#: 210609

Analyst: Anne Nord
Plate Expiration: 12-9-21

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE LCMS Methanol

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 21D52496 **Urine Blank:**

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.

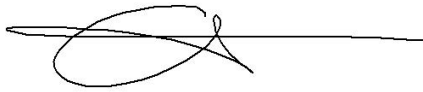
Analytic:

- 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 1ng/ml, 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 3-100*



	1	2	3	4	5	6
a	cal 1	neg blood				
b	cal 2	2508-1				
c	cal 3	2521-1				
d	cal 4	2554-1				
e	Cal 5	2599-1				
f	cal 6	2600-1				
g	cal 7	2608-2				
h	Internal control	p2012-1953-c1				

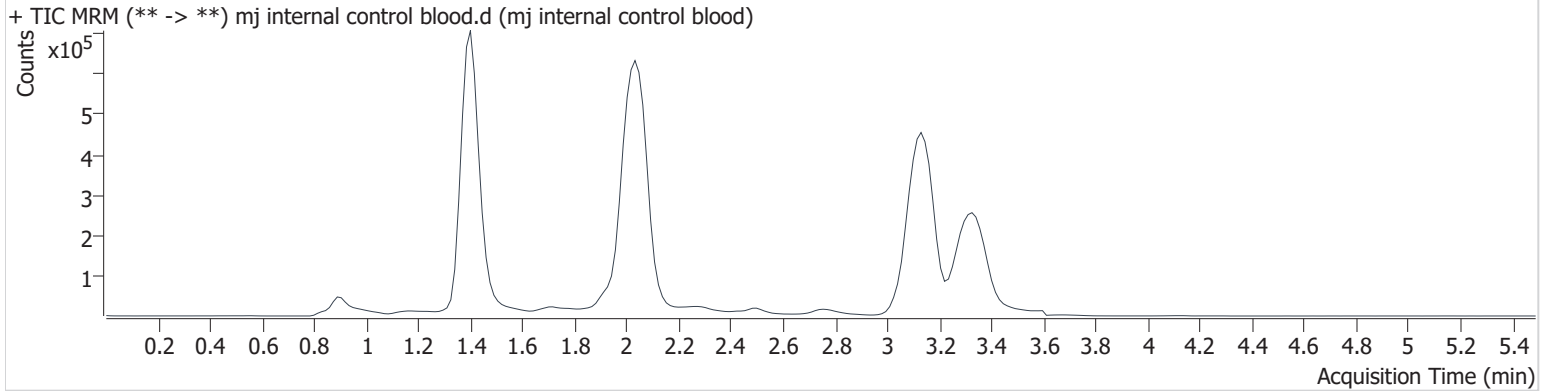
C2021-____-__

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 PM

Instrument	69679	Data File	mj internal control blood.d
Type	QC	Sample	mj internal control blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	12/7/2021 12:29:23 PM		
Sample Info.			

Sample Chromatogram



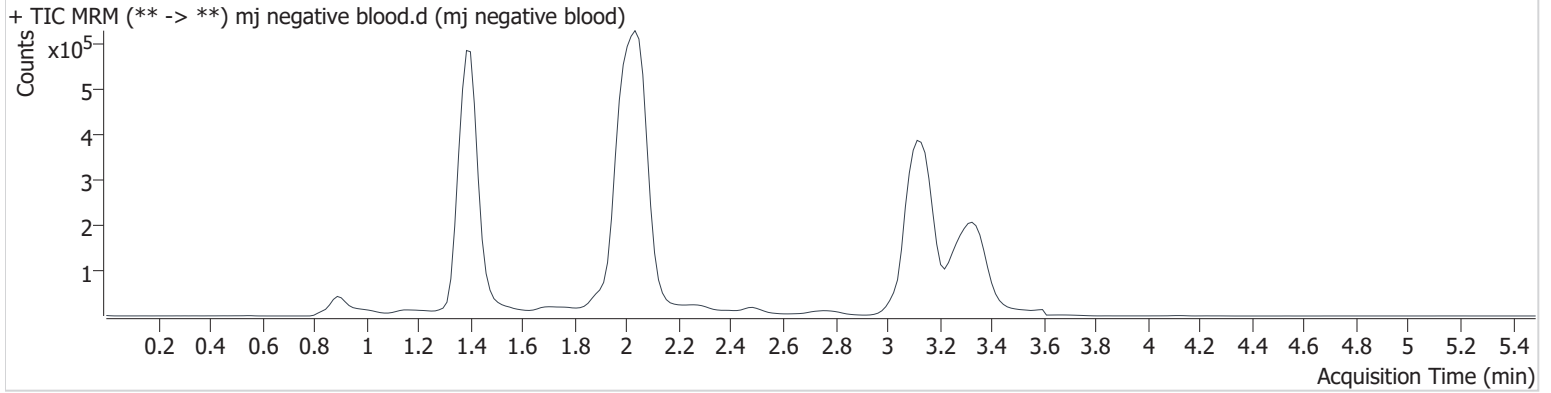
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	31862	97.5	1037.5	155.5	2003863	5.238 ng/ml
THC-COOH	1.431	120180	1034.1	31.6	303.6	583044	16.147 ng/ml
THC	3.156	246999	445.0	25.1	163.7	2085560	4.581 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 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	12/7/2021 12:36:08 PM		
Sample Info.			

Sample Chromatogram

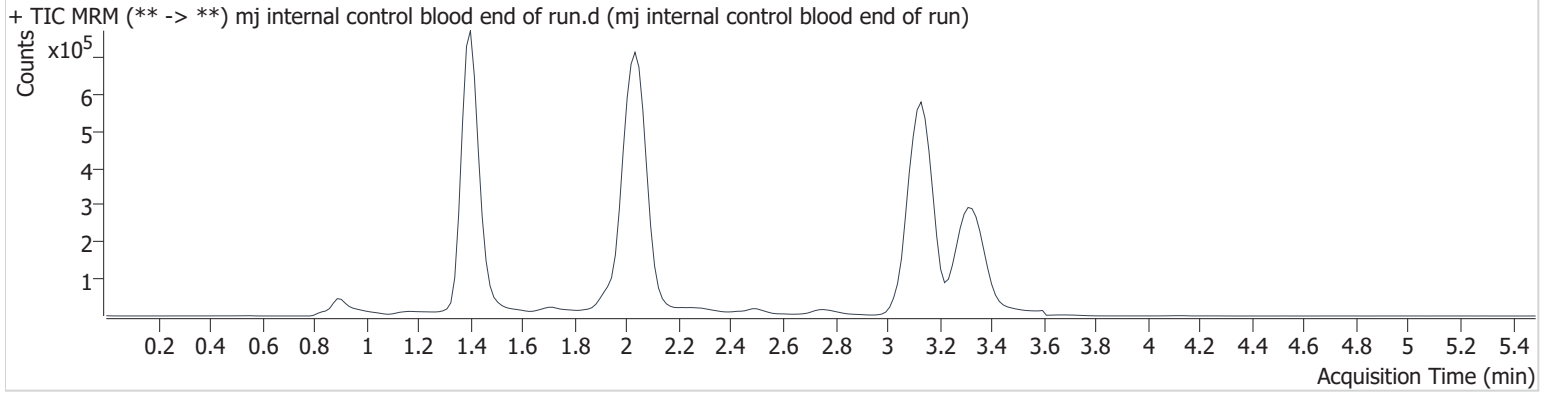


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 PM

Instrument	69679	Data File	mj internal control blood end of run.d
Type	QC	Sample	mj internal control blood end of run
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	12/7/2021 2:16:21 PM		
Sample Info.			

Sample Chromatogram



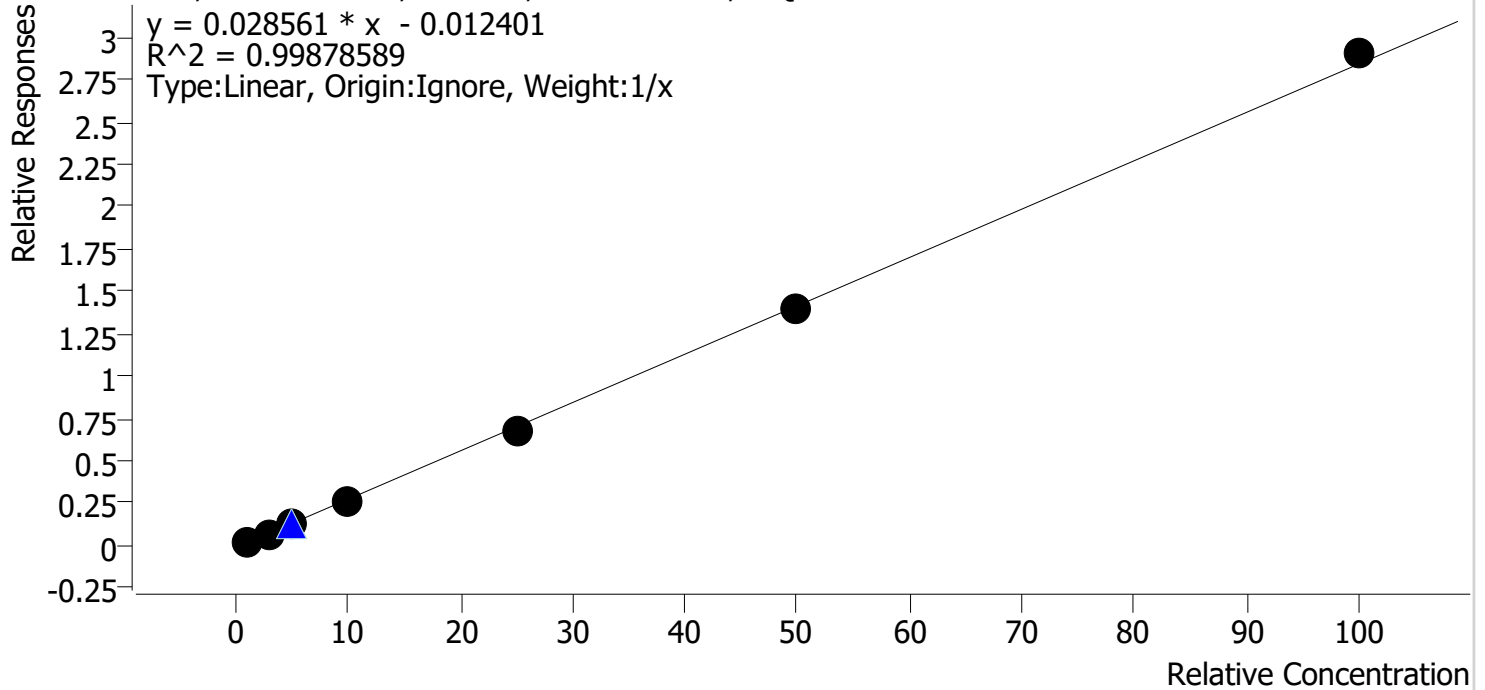
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	32666	239.4	1053.6	153.6	2104272	5.128 ng/ml
THC-COOH	1.431	120436	80.4	35.7	44.3	631634	14.980 ng/ml
THC	3.149	324528	1146.8	24.0	140.9	2616439	4.777 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Last Cal. Update 12/7/2021 2:56 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs



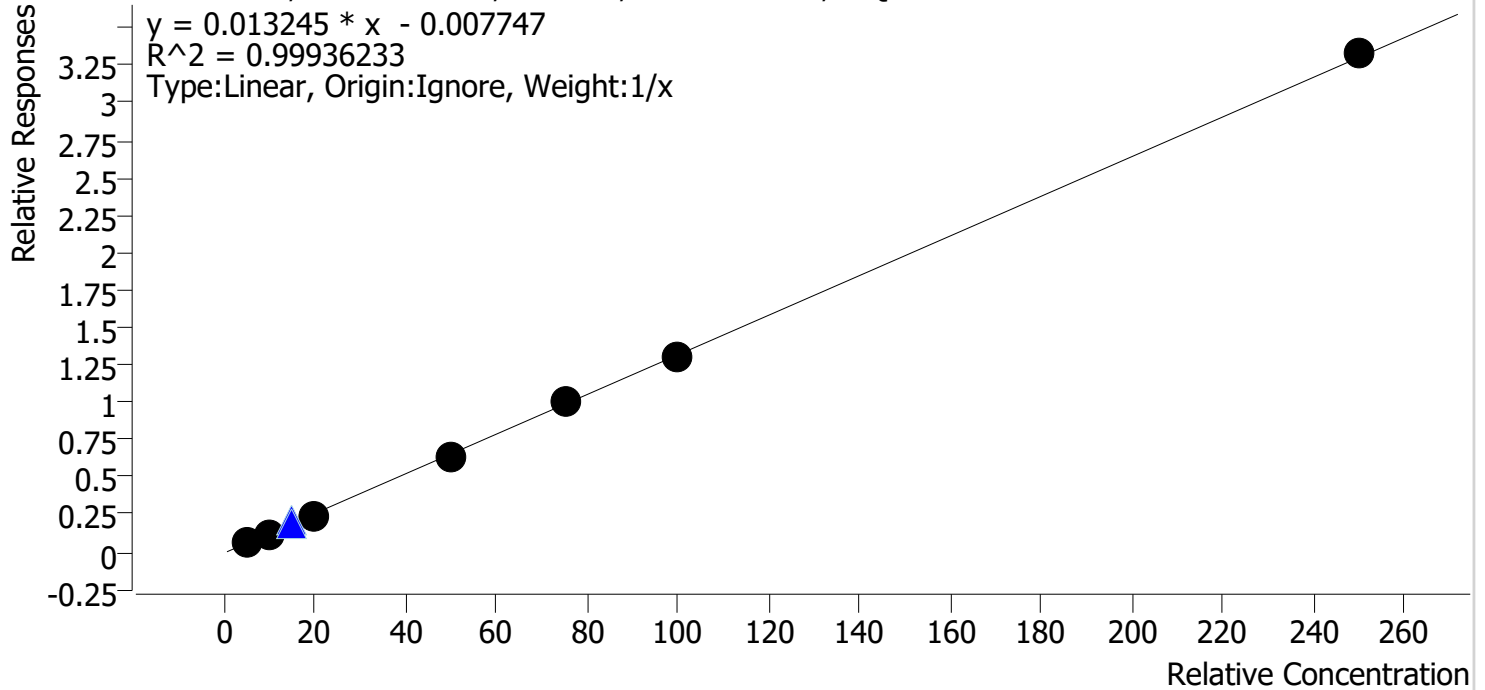
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	121.2
mj cal2	2	✓	3.0	2.8	94.4
mj cal 3	3	✓	5.0	4.7	93.1
mj cal 4	4	✓	10.0	9.3	93.1
mj cal 5	5	✓	25.0	24.1	96.6
mj cal 6	6	✓	50.0	49.7	99.4
mj cal 7	7	✓	100.0	102.1	102.1

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Last Cal. Update 12/7/2021 2:56 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, 2 QCs



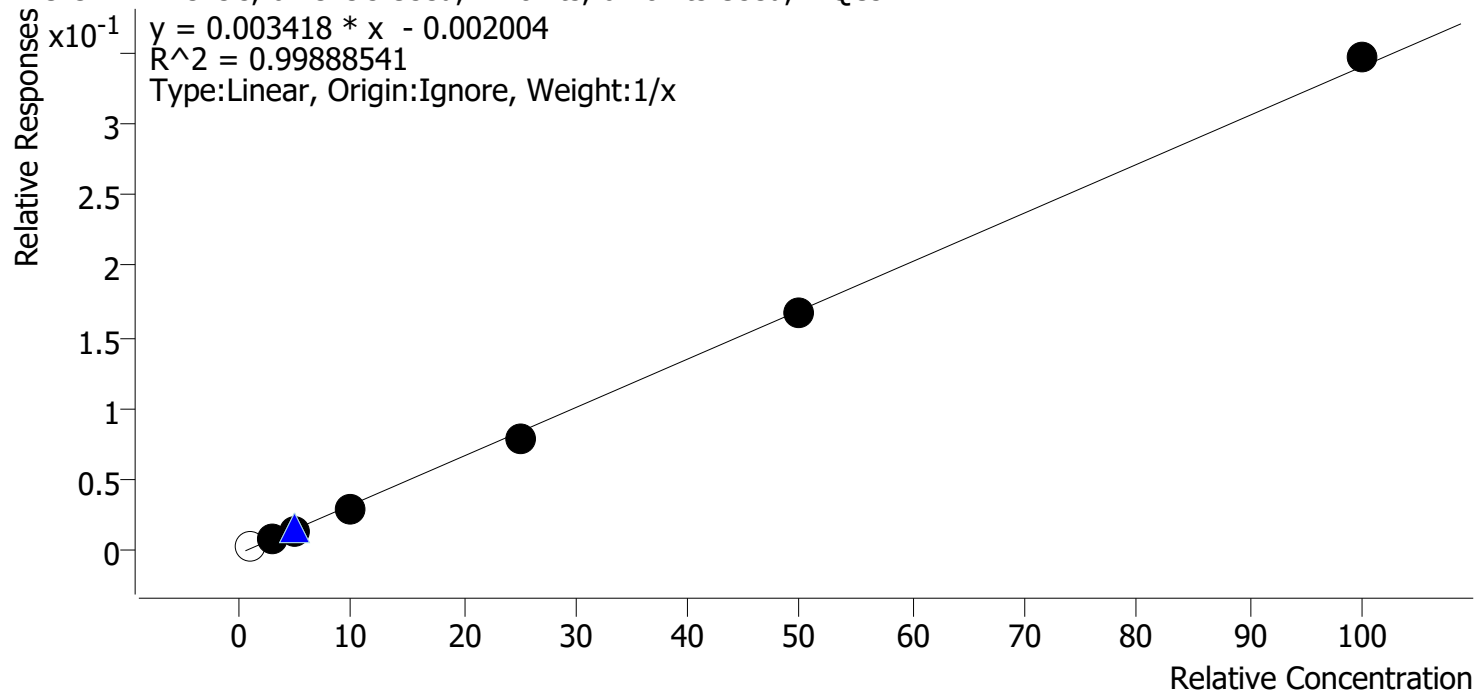
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.7	113.3
mj cal2	2	✓	10.0	9.2	91.7
mj cal 3	3	✓	20.0	19.3	96.3
mj cal 4	4	✓	50.0	48.6	97.2
mj cal 5	5	✓	75.0	75.8	101.0
mj cal 6	6	✓	100.0	99.6	99.6
mj cal 7	7	✓	250.0	251.9	100.8

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Last Cal. Update 12/7/2021 2:56 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	x	1.0	1.6	163.3
mj cal2	2	✓	3.0	3.3	110.6
mj cal 3	3	✓	5.0	4.9	98.5
mj cal 4	4	✓	10.0	9.5	94.5
mj cal 5	5	✓	25.0	23.8	95.2
mj cal 6	6	✓	50.0	49.6	99.3
mj cal 7	7	✓	100.0	101.9	101.9

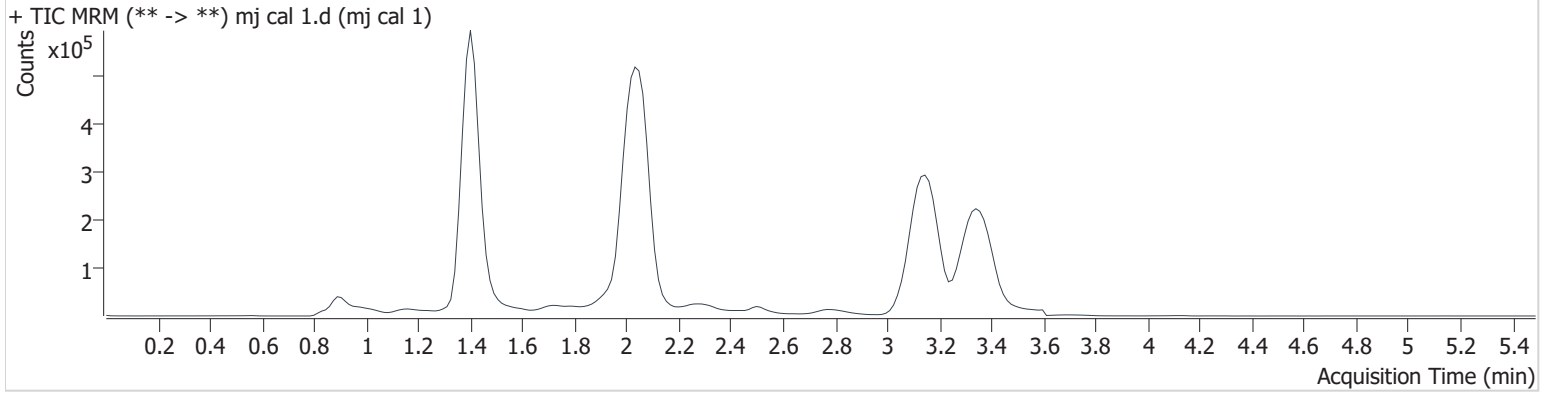
dropped cal 1 ratio out of range.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 PM

Instrument	69679	Data File	mj cal 1.d
Type	Cal	Sample	mj cal 1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	12/7/2021 11:35:50 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.409	7149	14.8	1576.6 High	43.4	1997748	1.633 ng/ml	Low
THC-COOH	1.431	33234	75.0	31.8	66.0	493717	5.667 ng/ml	
THC	3.168	32141	90.9	24.1	14.8	1446883	1.212 ng/ml	

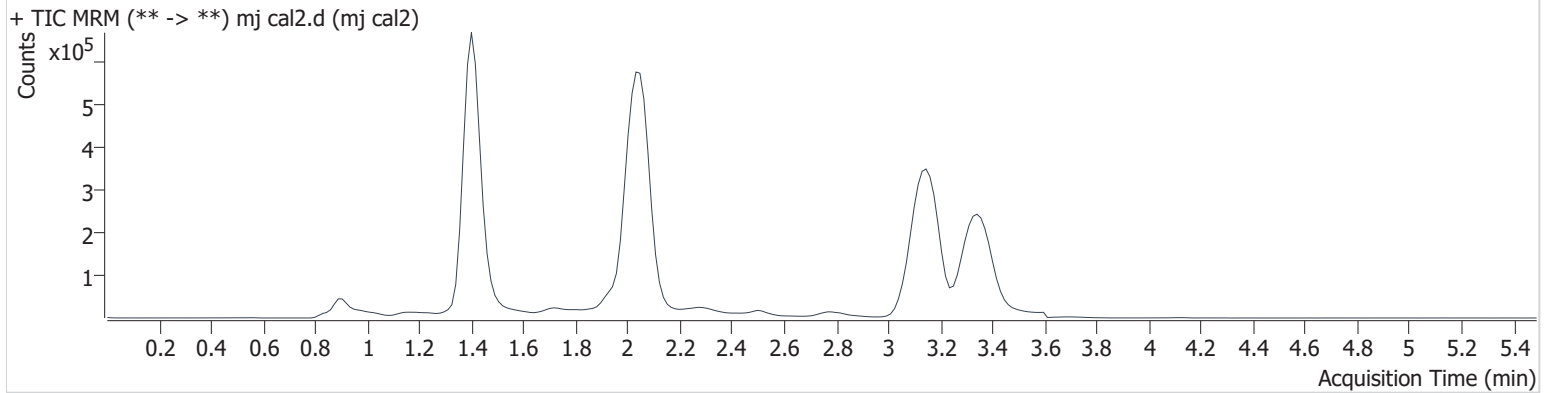
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 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	12/7/2021 11:42:34 AM		

Sample Info.

Sample Chromatogram



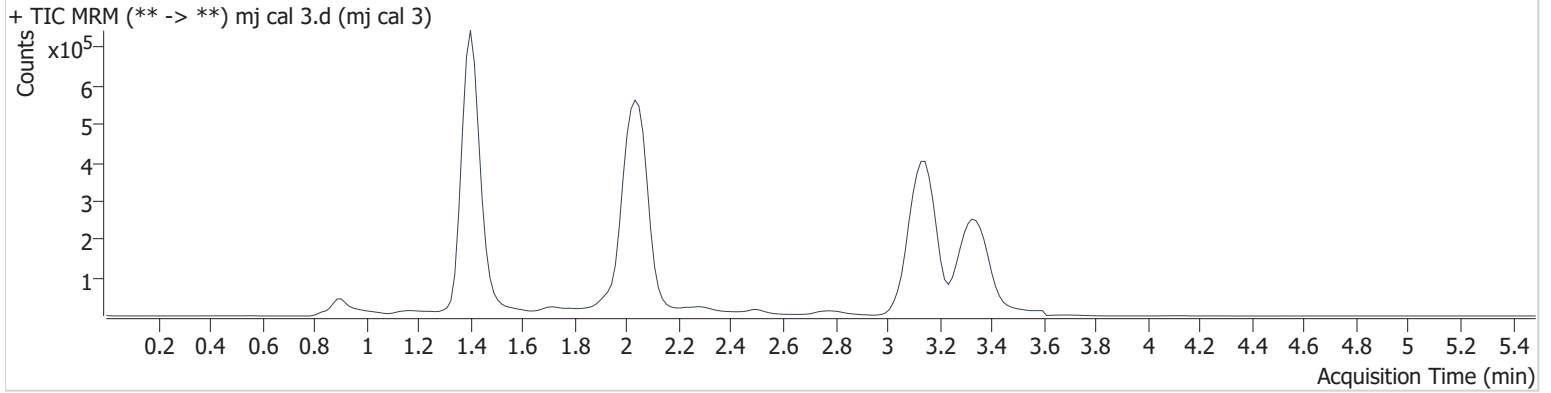
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	18917	98.7	1163.8	67.5	2026880	3.317 ng/ml
THC-COOH	1.431	60268	35.1	38.7	21695.8	529913	9.171 ng/ml
THC	3.174	111531	370.1	25.2	104.7	1628732	2.832 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 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	12/7/2021 11:49:16 AM		
Sample Info.			

Sample Chromatogram



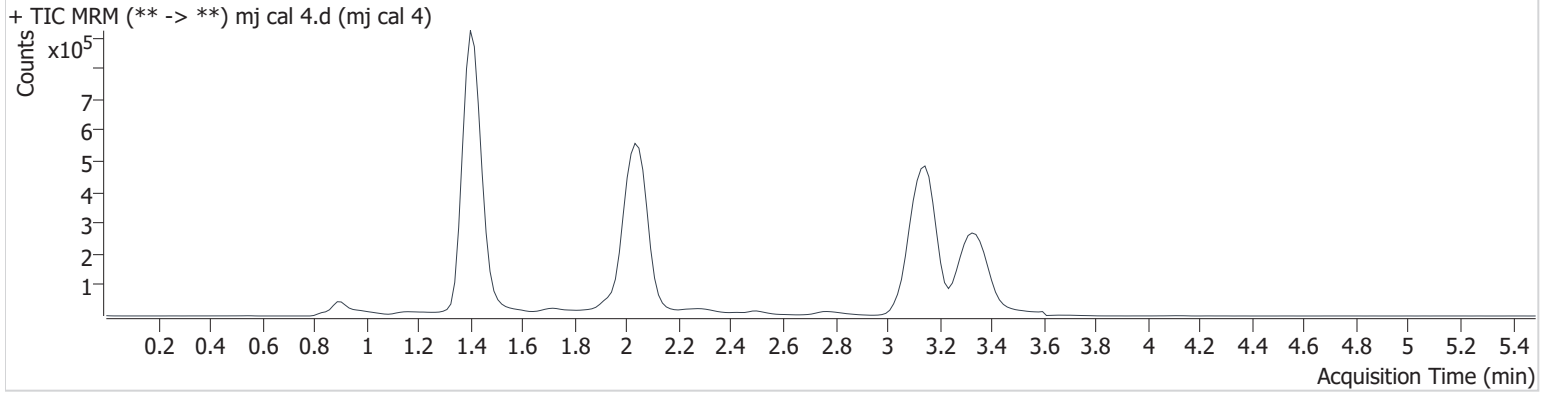
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	31573	88.7	1121.2	134.8	2128284	4.927 ng/ml
THC-COOH	1.431	138932	96.9	36.0	70788.6	561551	19.264 ng/ml
THC	3.163	224917	1175.5	24.2	115.5	1865081	4.656 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 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	12/7/2021 11:55:59 AM		
Sample Info.			

Sample Chromatogram



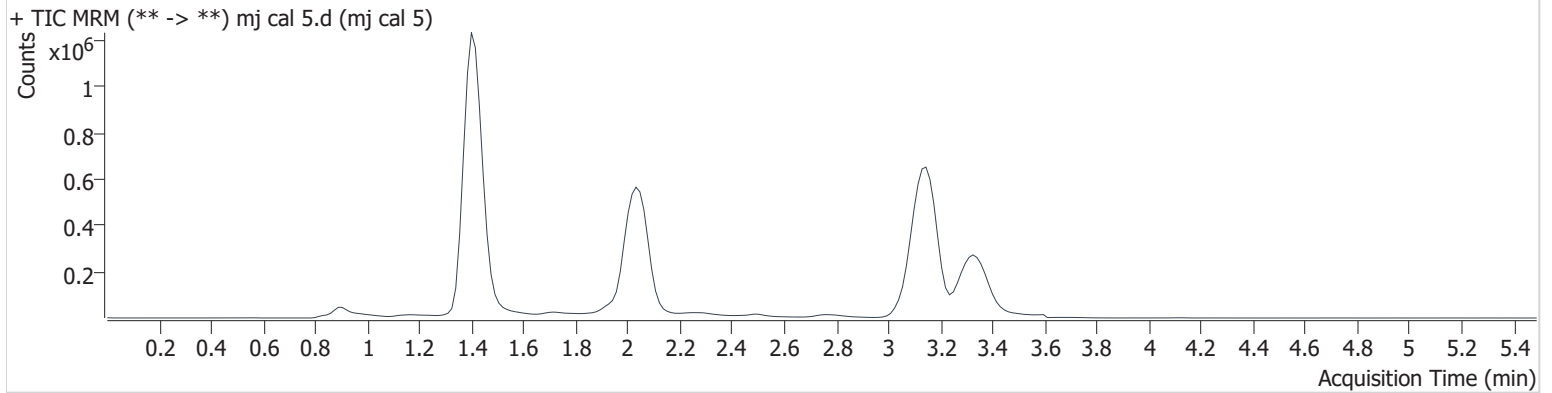
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	64711	249.6	1000.0	325.4	2135188	9.453 ng/ml
THC-COOH	1.431	356277	1000.3	37.1	12839.7	560001	48.617 ng/ml
THC	3.165	506740	7017.5	23.5	332.2	1997870	9.315 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 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	12/7/2021 12:02:41 PM		
Sample Info.			

Sample Chromatogram



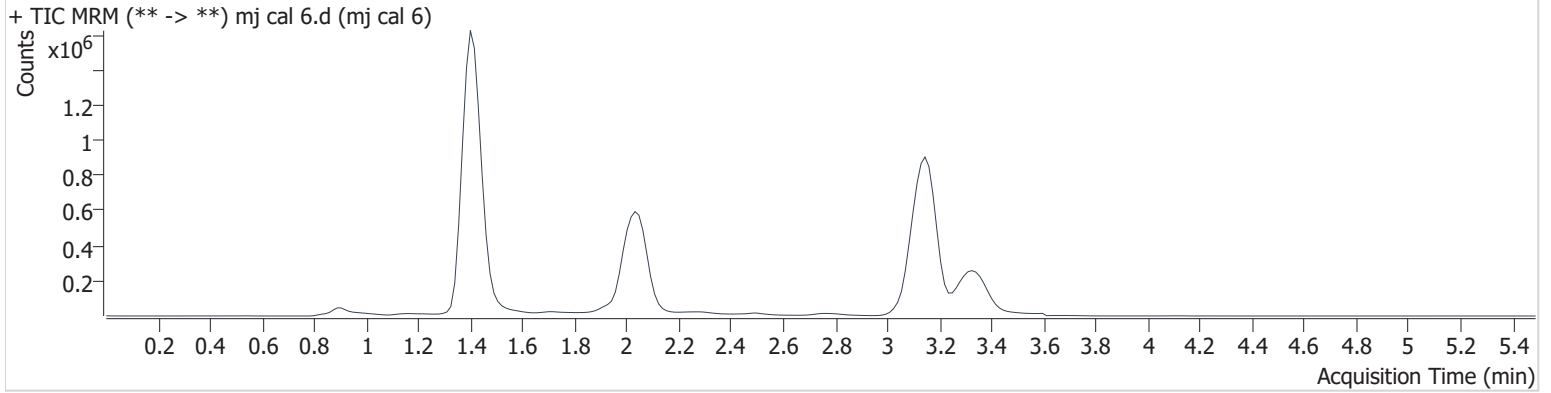
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	166184	541.8	934.0	639.2	2093713	23.808 ng/ml
THC-COOH	1.431	545911	501.2	37.3	20897	548222	75.765 ng/ml
THC	3.161	1342239	8245.2	23.4	1224.2	1982048	24.144 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 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	12/7/2021 12:09:23 PM		
Sample Info.			

Sample Chromatogram



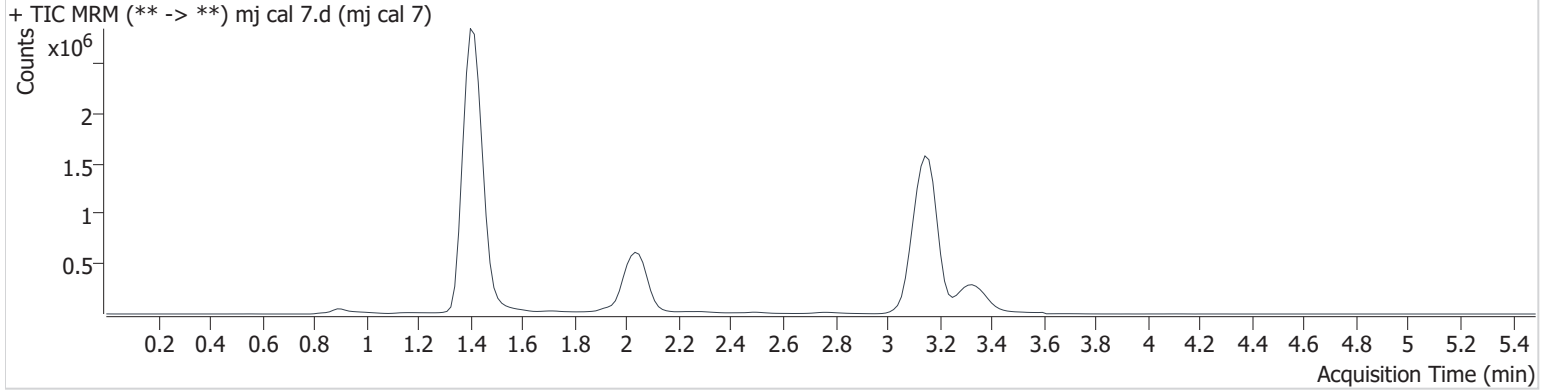
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	337494	1922.8	878.4	917.4	2012882	49.640 ng/ml
THC-COOH	1.431	704924	666.6	37.5	2072.5	537426	99.613 ng/ml
THC	3.158	2676030	∞	23.9	2476.2	1901108	49.718 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\120721\QuantResults\cann.batch.bin
Calibration Last Update 12/7/2021 2:56:43 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	12/7/2021 12:16:05 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	699062	864.6	858.4	1052.2	2019617	101.854 ng/ml
THC-COOH	1.431	1779445	1733.7	37.5	1437.1	534560	251.903 ng/ml
THC	3.161	5991306	9427.5	24.2	6783.3	2062862	102.123 ng/ml