

REVIEWED

By Amber Gerheart at 3:46 pm, Jun 27, 2022



6/27/2022

Worklist: 6006

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2022-1085	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-1178	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2022-1207	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2022-1283	3	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-1284	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-1287	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-1305	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-1314	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-1318	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2022-1324	1	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 6/16/22
Plate lot#: 220309

Analyst: Anne Nord
Plate re-test: 9/9/2022

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: 22B52016 **Urine Blank:** 21522 **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.

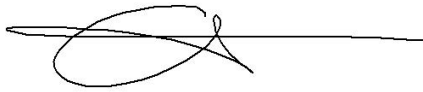
Analytic:

- 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 curve range 3-100*



	1	2	3	4	5	6
a	cal 1	Internal urine	negative urine			
b	cal 2	negative blood	1178-1			
c	cal 3	1085-1	1207-1			
d	cal 4	1283-3	1318-1			
e	Cal 5	1284-1	1324-1			
f	cal 6	1287-1				
g	cal 7	1305-1				
h	Internal control (blood)	1314-2				

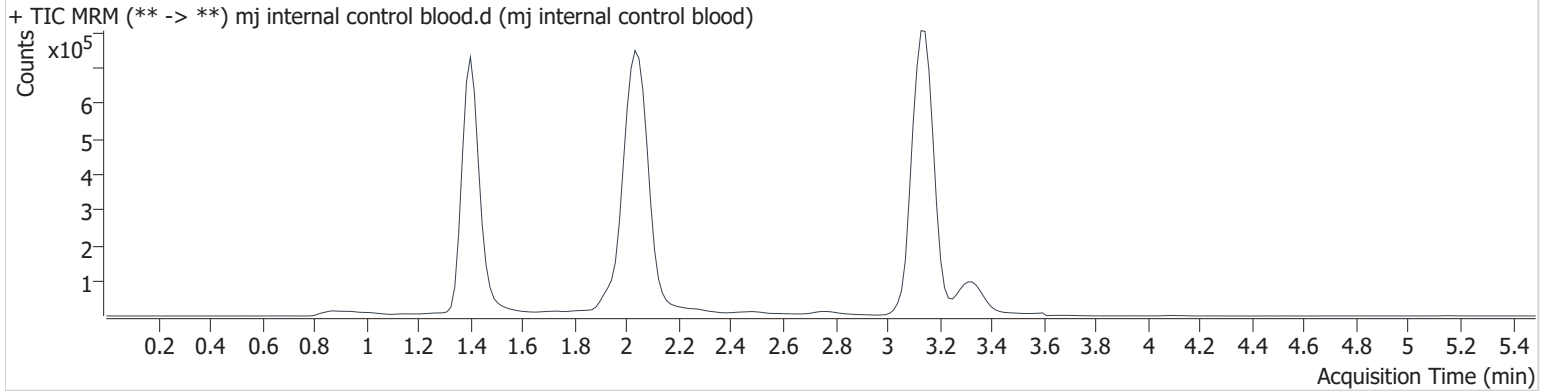
c2022-____-__

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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	6/16/2022 12:16:00 PM		
Sample Info.			

Sample Chromatogram



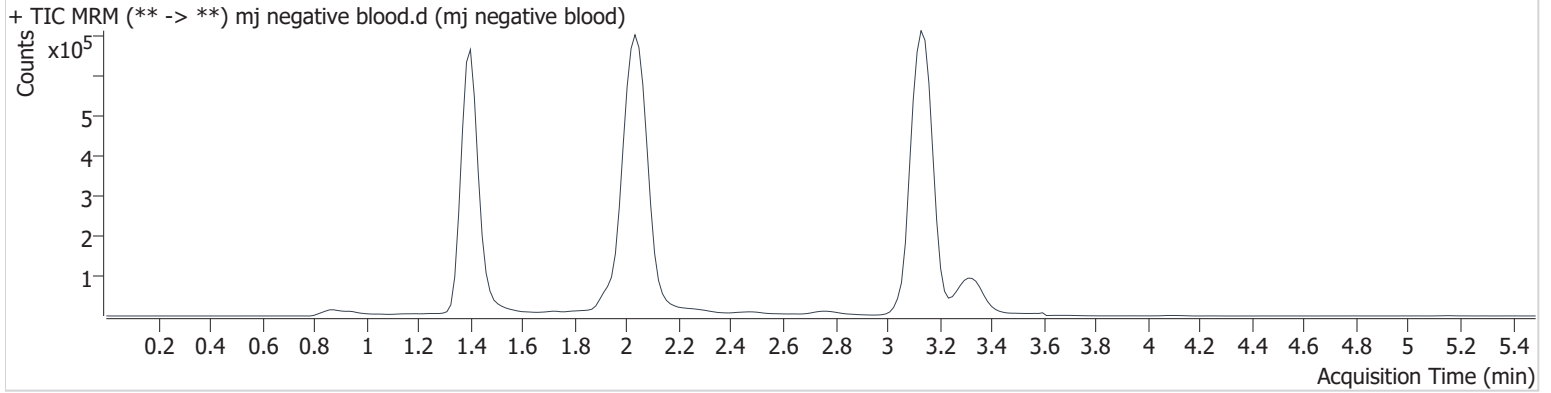
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	297124	∞	11.8	∞	2213166	4.531 ng/ml
THC-COOH	1.433	34793	∞	225.9	∞	594895	14.212 ng/ml
THC	3.152	421976	∞	26.5	∞	3900090	4.745 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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-B2	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2022 12:22:45 PM		
Sample Info.			

Sample Chromatogram



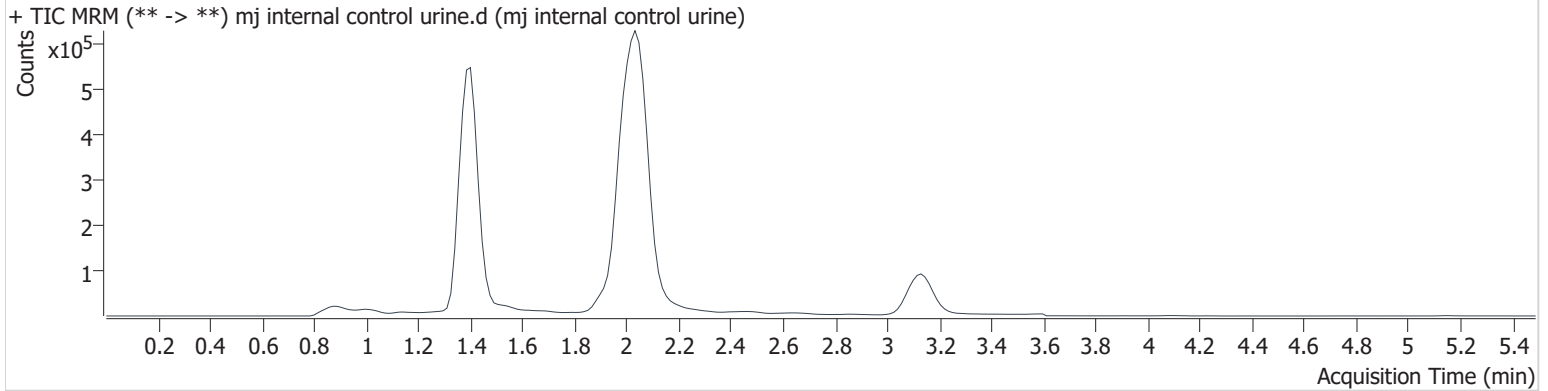
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

Instrument	69679	Data File	mj internal control urine.d
Type	Sample	Sample	mj internal control urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2022 1:49:33 PM		

Sample Info.

Sample Chromatogram



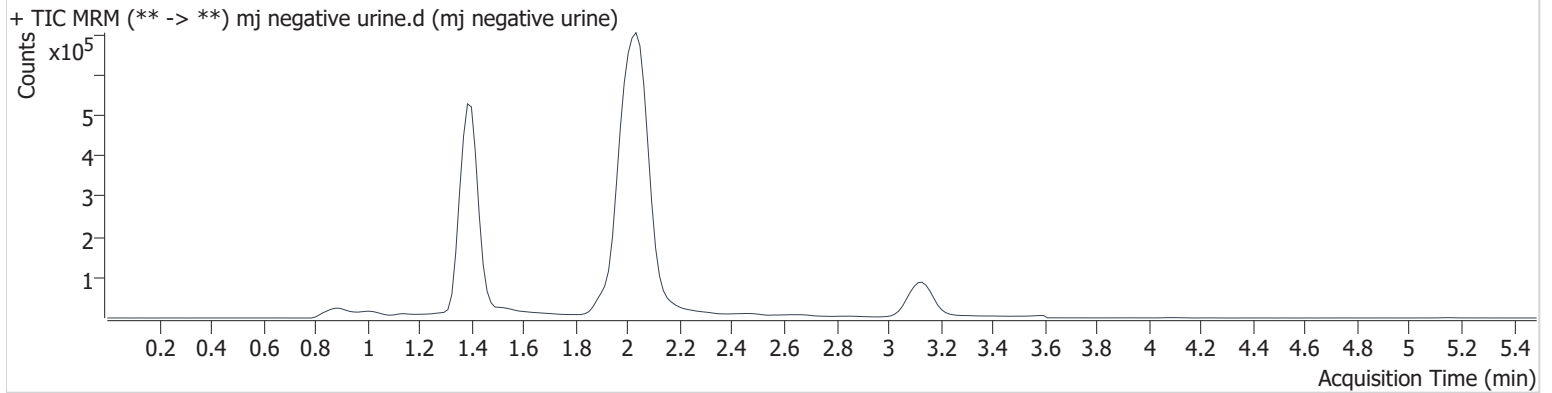
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	210503	∞	13.5	∞	1965401	3.484 ng/ml
THC-COOH	1.433	22011	60.3	186.4	∞	381125	14.056 ng/ml
THC	3.152	58381	∞	31.5	∞	557717	4.602 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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-A3	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2022 1:56:15 PM		
Sample Info.			

Sample Chromatogram



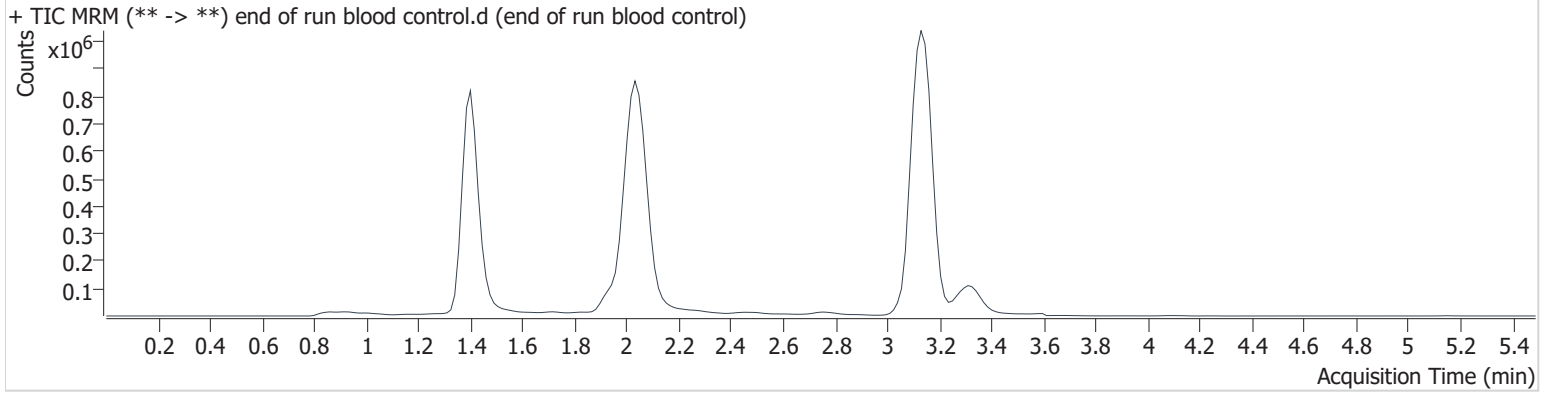
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

Instrument	69679	Data File	end of run blood control.d
Type	Sample	Sample	end of run blood control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2022 3:09:34 PM		

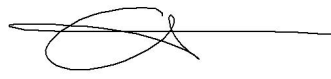
Sample Info.

Sample Chromatogram



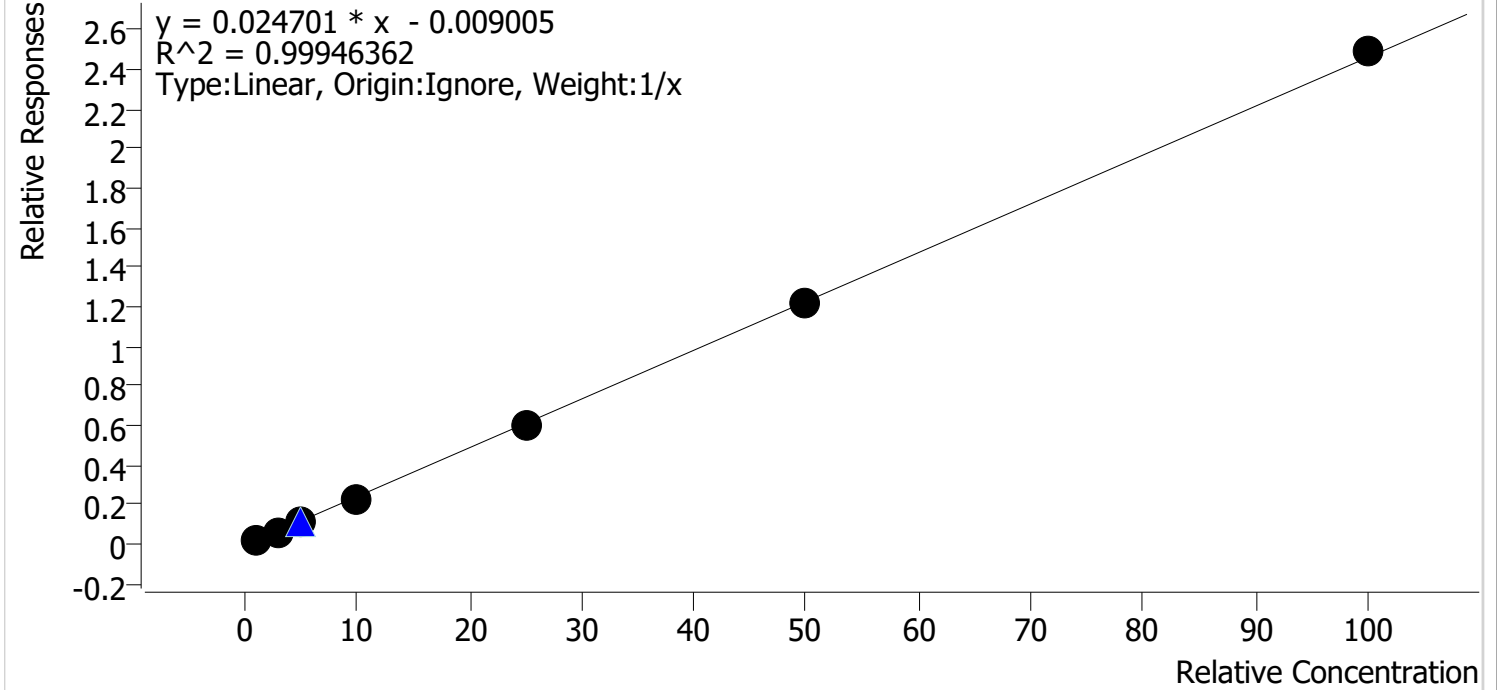
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	325348	∞	11.4	∞	2368209	4.652 ng/ml
THC-COOH	1.433	36916	∞	236.5	1409.8	637450	14.090 ng/ml
THC	3.152	551164	∞	26.1	∞	4910105	4.909 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Last Cal. Update 6/17/2022 11:47 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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



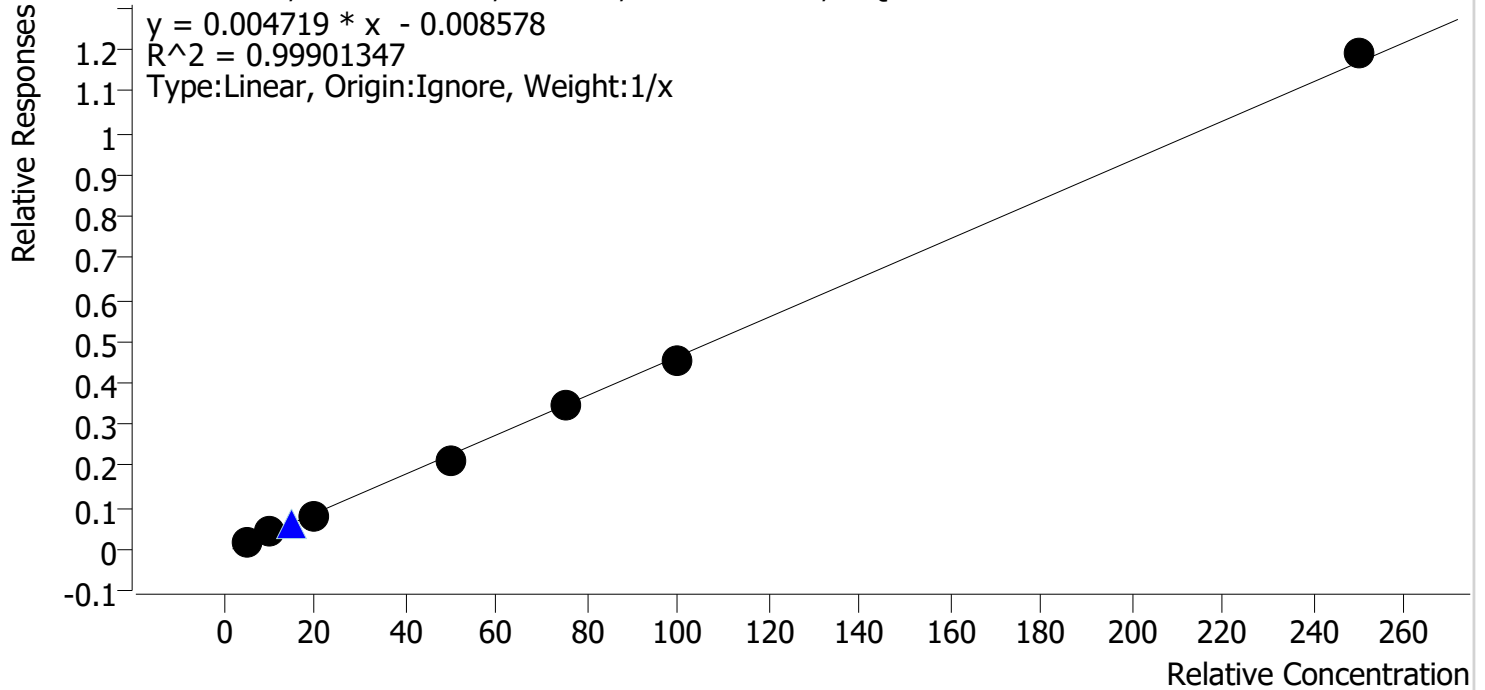
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	115.8
mj cal 2	2	✓	3.0	2.8	94.9
mj cal 3	3	✓	5.0	4.7	93.7
mj cal 4	4	✓	10.0	9.5	95.4
mj cal 5	5	✓	25.0	24.9	99.6
mj cal 6	6	✓	50.0	49.7	99.4
mj cal 7	7	✓	100.0	101.2	101.2

Compound Calibration Report



Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Last Cal. Update 6/17/2022 11:47 AM
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



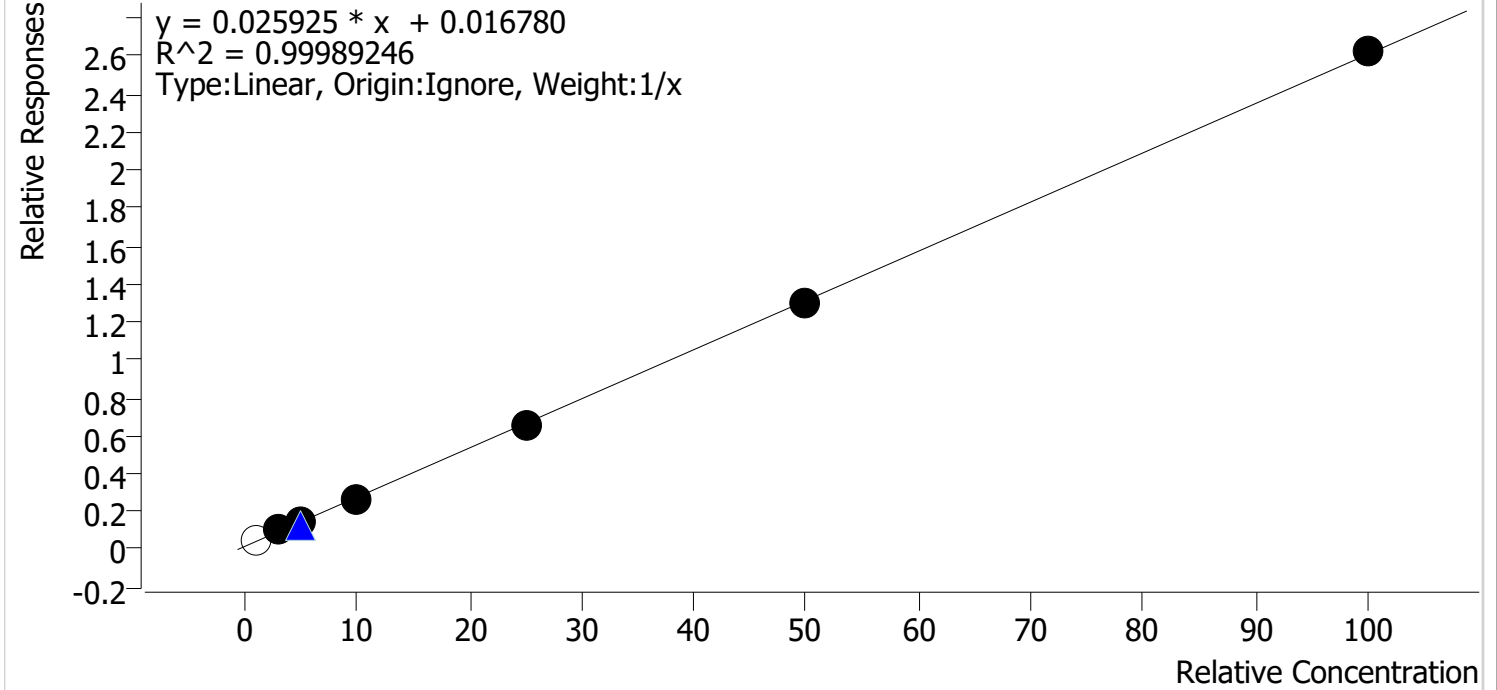
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.5	109.9
mj cal 2	2	✓	10.0	10.1	101.5
mj cal 3	3	✓	20.0	18.5	92.6
mj cal 4	4	✓	50.0	47.6	95.2
mj cal 5	5	✓	75.0	75.5	100.7
mj cal 6	6	✓	100.0	98.4	98.4
mj cal 7	7	✓	250.0	254.3	101.7

Compound Calibration Report



Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Last Cal. Update 6/17/2022 11:47 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	x	1.0	1.4	135.9
mj cal 2	2	✓	3.0	3.1	104.2
mj cal 3	3	✓	5.0	4.9	98.1
mj cal 4	4	✓	10.0	9.8	98.1
mj cal 5	5	✓	25.0	24.9	99.7
mj cal 6	6	✓	50.0	49.7	99.4
mj cal 7	7	✓	100.0	100.5	100.5

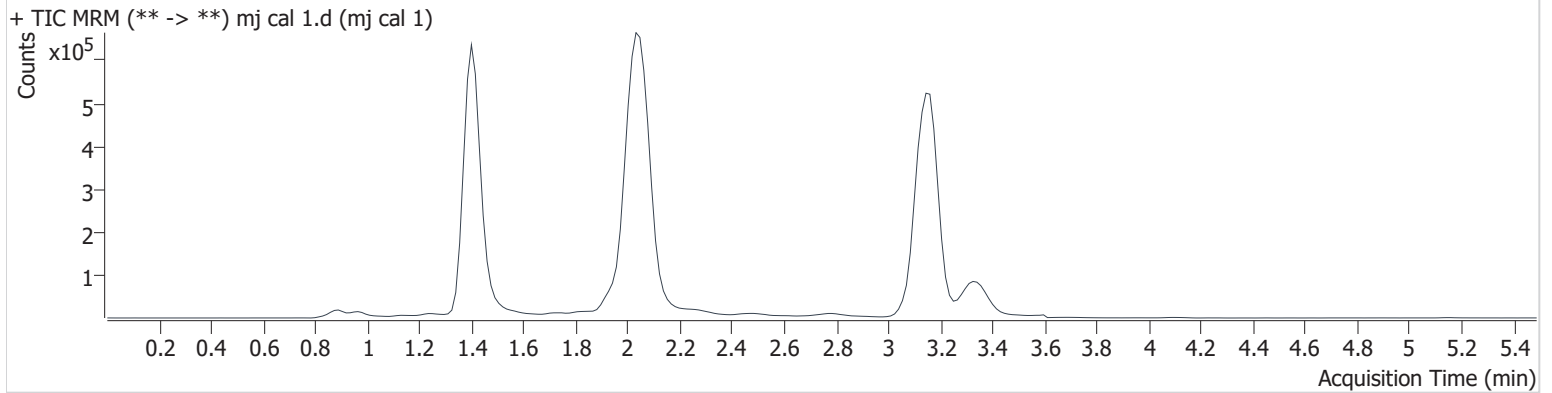
dropped cal 1 ratio out of range.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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	6/16/2022 11:22:24 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	113494	2220856944 6985.1	7.5 Low	∞	2182503	1.359 ng/ml Low
THC-COOH	1.433	9322	∞	229.3	∞	537116	5.496 ng/ml
THC	3.167	55854	4620425885 5318.5	28.0	144.9	2850724	1.158 ng/ml

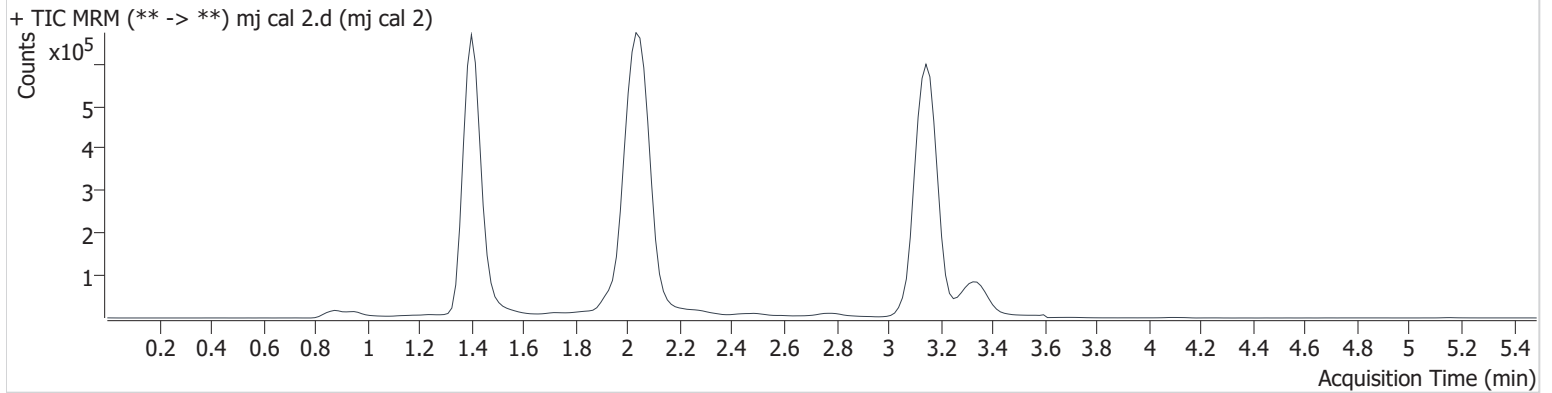
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

Instrument	69679	Data File	mj cal 2.d
Type	Cal	Sample	mj cal 2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2022 11:29:08 AM		

Sample Info.

Sample Chromatogram



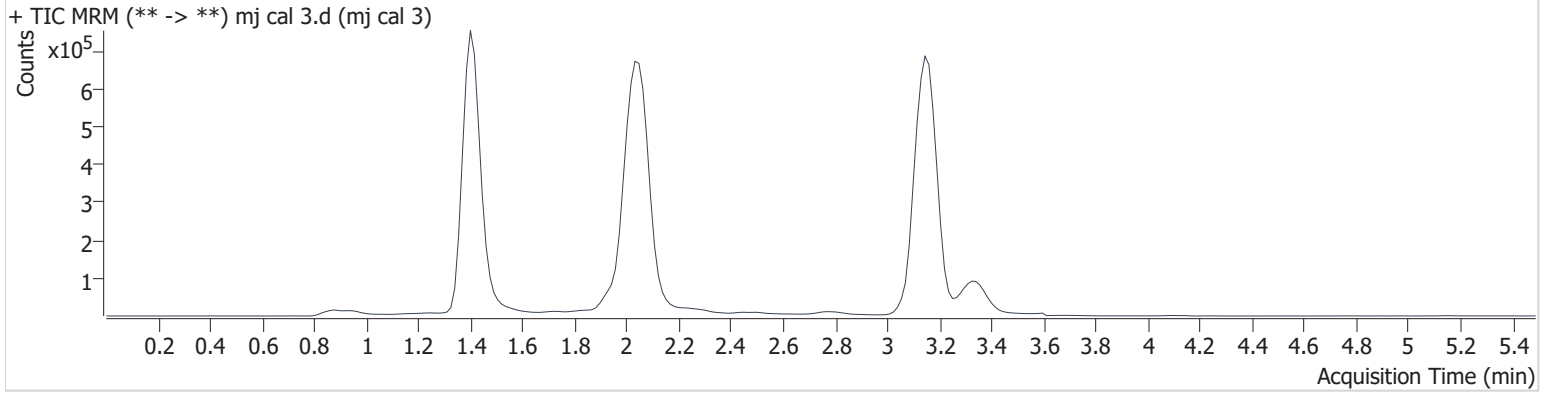
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	217497	∞	10.1	∞	2224199	3.125 ng/ml
THC-COOH	1.433	22186	118.6	220.2	∞	564377	10.148 ng/ml
THC	3.167	190267	∞	26.8	763.7	3104620	2.846 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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	6/16/2022 11:35:51 AM		
Sample Info.			

Sample Chromatogram



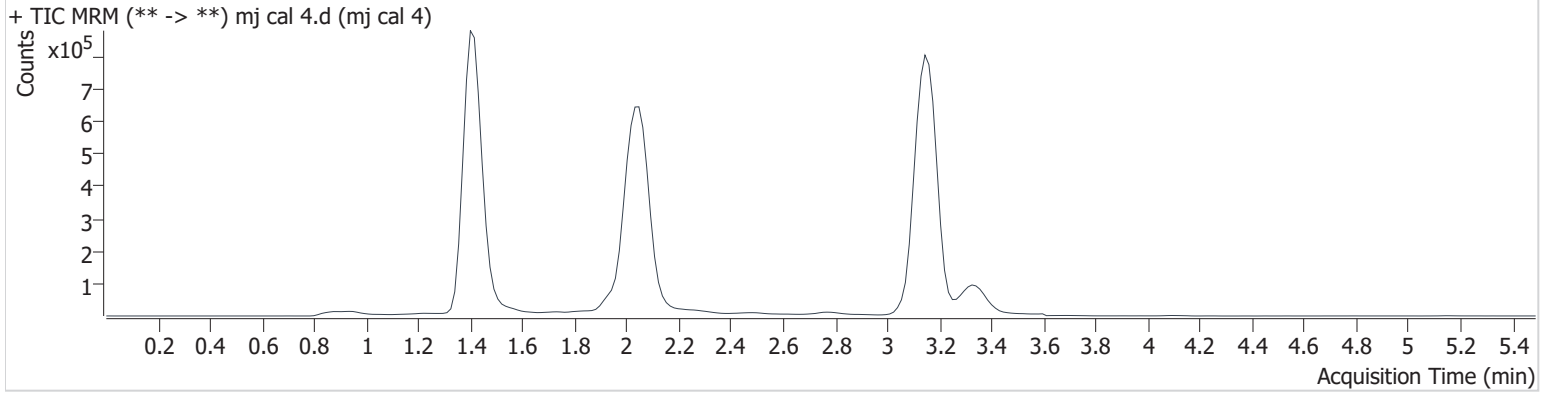
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	332158	∞	11.6	∞	2307838	4.904 ng/ml
THC-COOH	1.433	45901	2353.9	238.6	191.5	582328	18.521 ng/ml
THC	3.167	358136	∞	26.6	∞	3354493	4.687 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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	6/16/2022 11:42:33 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	615334	∞	12.1	10391 79852 33557 0.0	2269473	9.811 ng/ml
THC-COOH	1.433	124916	760.1	229.6	841.0	578453	47.580 ng/ml
THC	3.167	796376	∞	26.3	19770 75852 1053.2	3512026	9.544 ng/ml

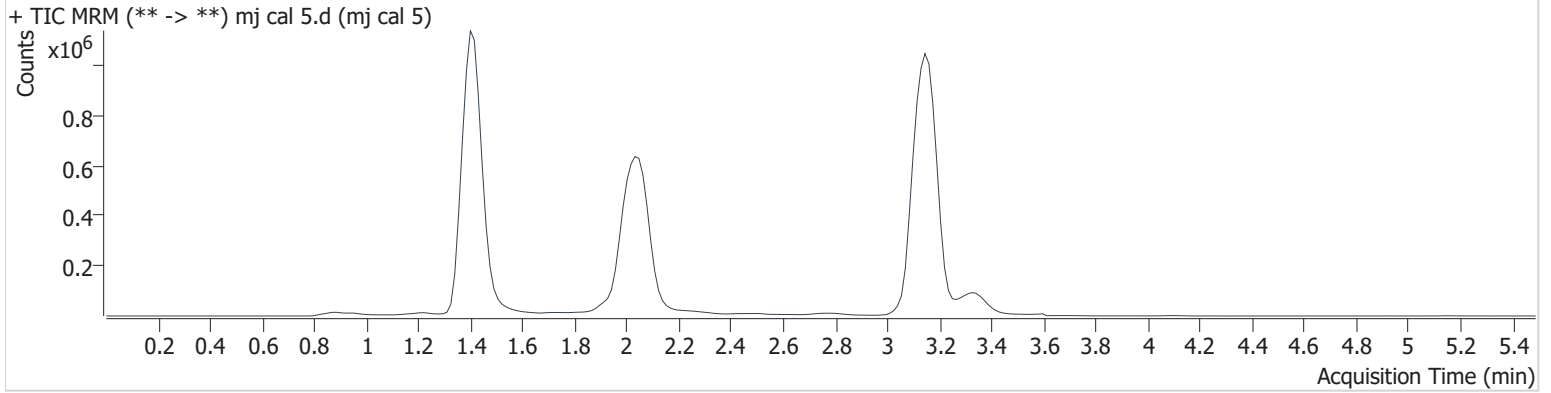
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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	6/16/2022 11:49:15 AM		

Sample Info.

Sample Chromatogram



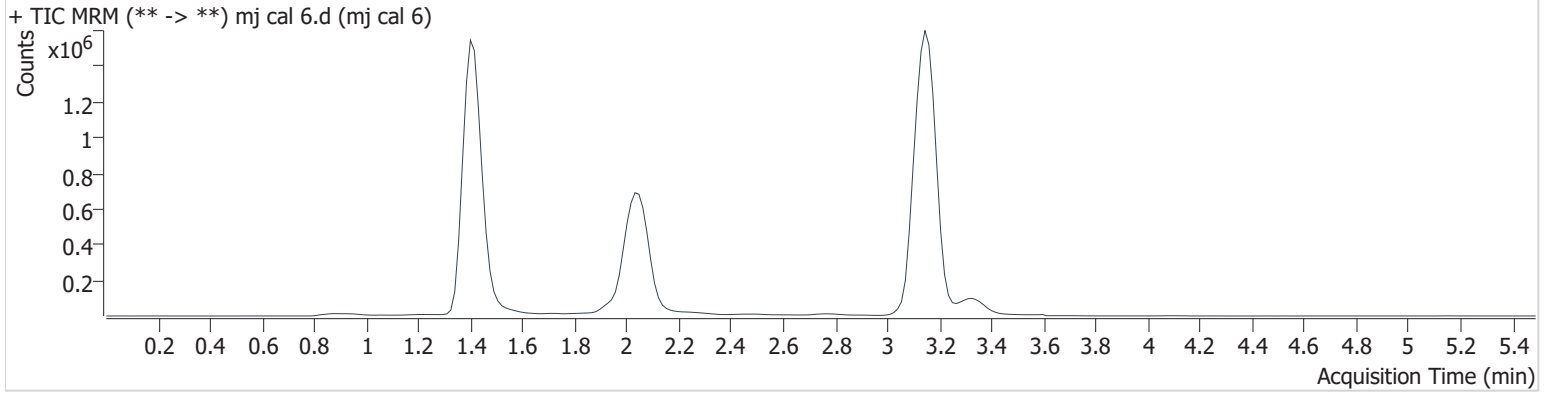
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1555533	∞	13.1	∞	2346459	24.924 ng/ml
THC-COOH	1.433	196310	∞	226.5	∞	564400	75.526 ng/ml
THC	3.167	2201259	∞	26.2	∞	3630909	24.908 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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	6/16/2022 11:55:58 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	2925687	∞	13.9	∞	2241076	49.710 ng/ml
THC-COOH	1.433	249427	1426.0	227.6	∞	547263	98.402 ng/ml
THC	3.167	4372746	∞	27.0	∞	3588013	49.702 ng/ml

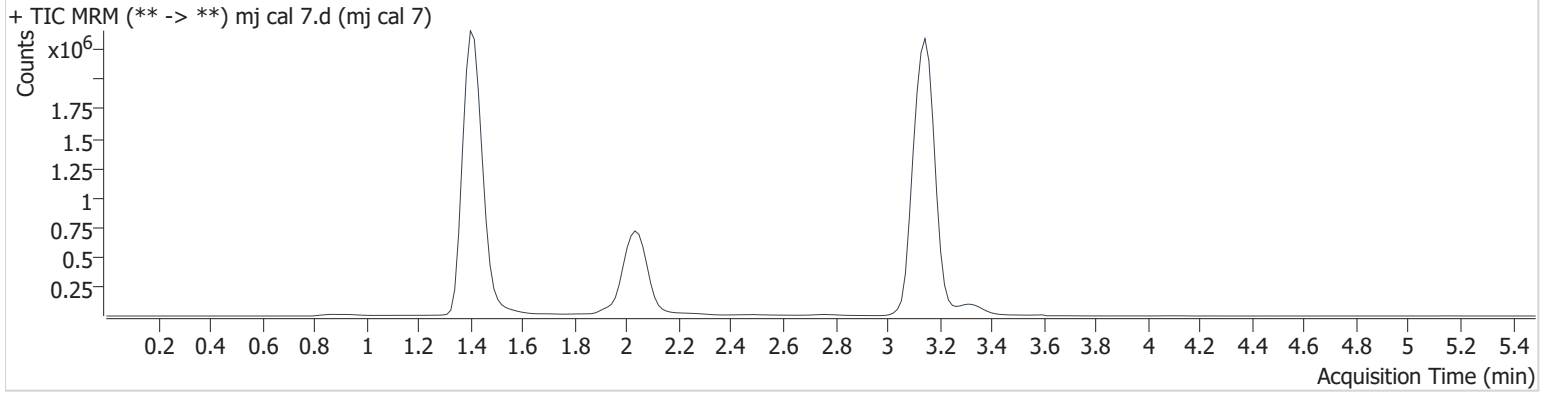
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\061622\QuantResults\cann.batch.bin
Calibration Last Update 6/17/2022 11:47:36 AM

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	6/16/2022 12:02:40 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	5439887	∞	14.3	∞	2074011	100.526 ng/ml
THC-COOH	1.433	571248	∞	226.4	∞	479410	254.326 ng/ml
THC	3.152	7987221	∞	26.9	∞	3208136	101.155 ng/ml