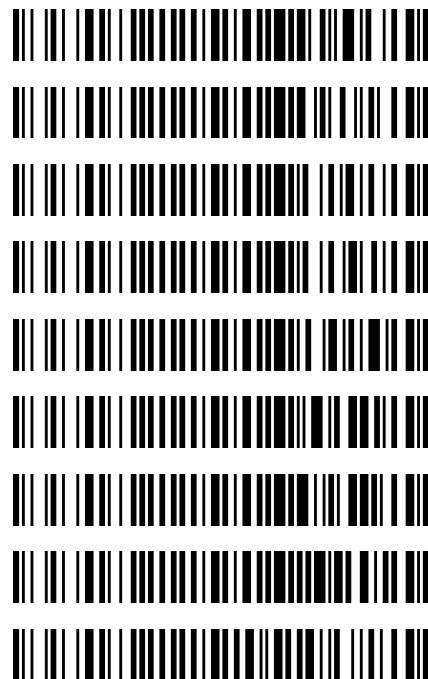


6/6/2024

REVIEWED
By Sarah Collins at 11:47 am, Jun 07, 2024

Worklist: 6837

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2024-086	6 6/7/24	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0877	1	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0919	1	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0924	1	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0934	1	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0985	1	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-1025	1	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-1030	1	1 BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-106	6 6/7/24	1 BLOOD	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: 06/06/24

Plate lot#: 231212

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: 24C52042

Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Anne Nord

Plate Retest Date: 6/12/24

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Urine Lot: Blood only run

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 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes. Using a calibrated pipette, add 1000µl blood and urine (if applicable) (calibrated pipette) into the appropriate wells of analytical (standards) plate. Pipette ID: K52558G
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Add 500µL of 0.1% formic acid in water to blood samples, and 500µL of saturated phosphate buffer to urine samples in the wells of the analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer 700-800µL of blood+acid or urine+acid mixture to corresponding wells of SLE+ plate. Amount transferred: 750 µL
- 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)
- 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.
- 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. Did all QCs pass for each analyte? (if not, describe in comments section)
- 5. Enter QCs into control charting.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: The wrong well position was injected for c2024-1066-1, 6/7/24 the correct well position was injected, and the QC was reconstituted and re-injected.



	1	2	3	4	5	6
a	cal 1	Internal blood control	1025-1			
b	cal 2	negative blood	1030-1			
c	cal 3	0866-1				
d	cal 4	0877-1	1066-1			
e	cal 5	0919-1				
f	cal 6	0924-1				
g	cal 7	0934-1				
h	Internal control (blood)	0985-1				

Plate position 3

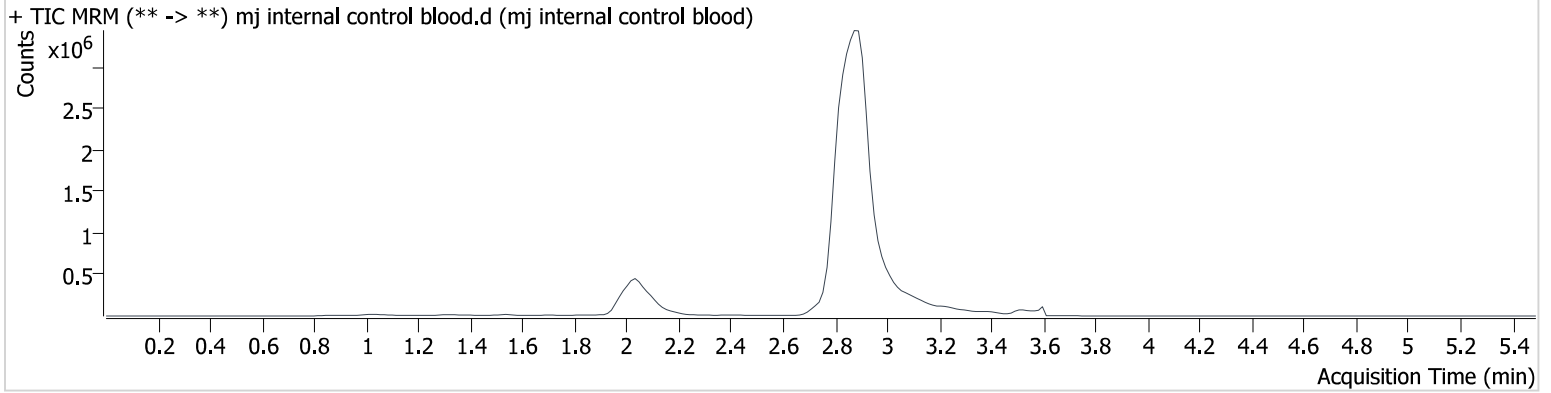
c2024-____-__

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679 **Data File** mj internal control blood.d
Type QC **Sample** mj internal control blood
Acq. Method thc quant 50 50.m **Operator** Anne Nord
Sample Position P3-H1 **Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume 10
Acq. Date-Time 6/6/2024 7:14:38 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	26371	123.8	877.98	∞	1854335	4.813 ng/ml
THC-COOH	2.092	46033	1661.4	280.57	6687.0	808063	12.484 ng/ml
THC	3.558	27576	∞	24.65	139.1	209330	4.910 ng/ml

AM #27 Cannabinoids

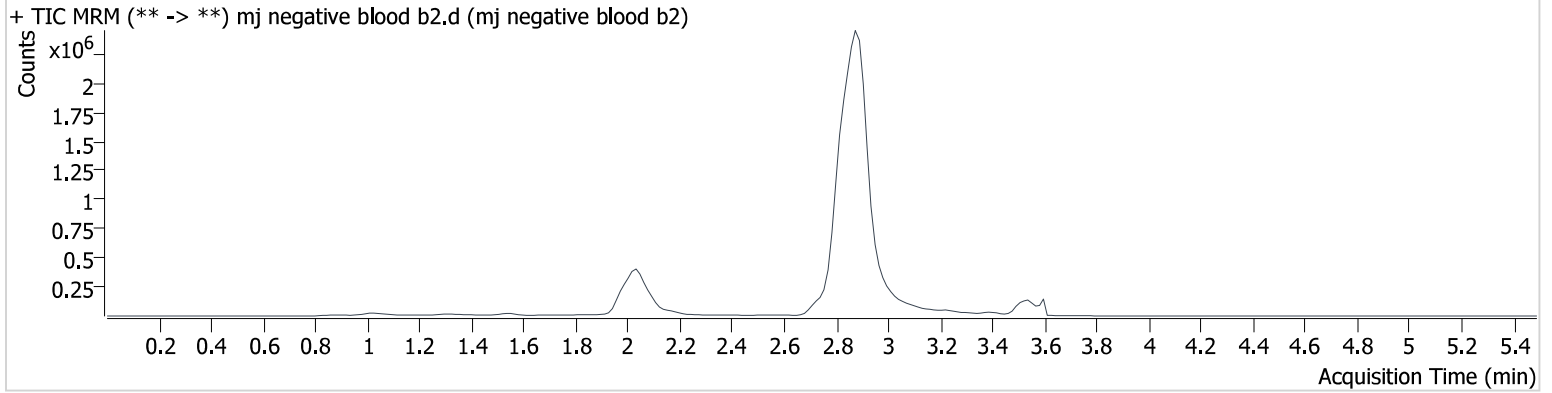
Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Sample
Acq. Method thc quant 50 50.m
Sample Position P3-B2
Injection Volume 10
Acq. Date-Time 6/6/2024 7:21:12 PM
Sample Info.

Data File mj negative blood b2.d
Sample mj negative blood b2
Operator Anne Nord
Comment

Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram

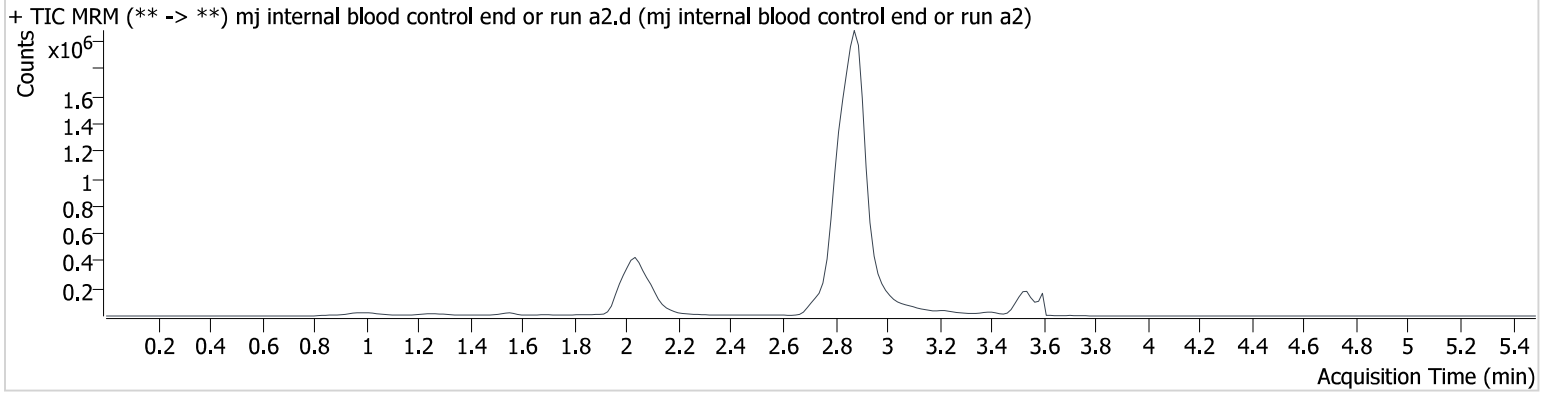


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument	69679	Data File	mj internal blood control end or run a2.d
Type	QC	Sample	mj internal blood control end or run a2
Acq. Method	thc quant 50 50.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	6/6/2024 9:26:28 PM		
Sample Info.			

Sample Chromatogram



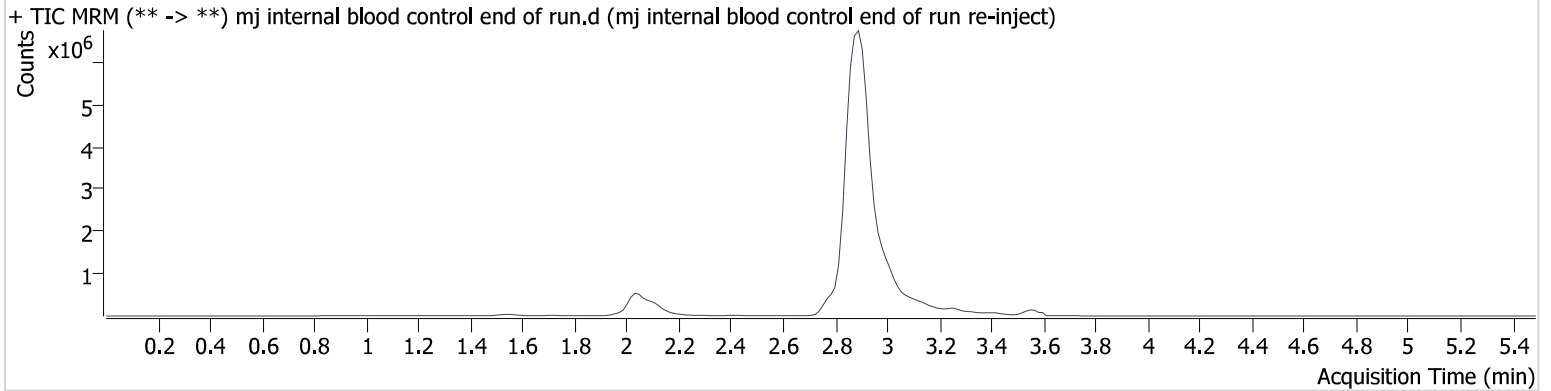
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	23809	514.6	894.08	∞	1718544	4.700 ng/ml
THC-COOH	2.092	47818	66855.0	267.78	215.9	842722	12.439 ng/ml
THC	3.543	56284	13884.6	25.40	601.1	385341	5.416 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument	69679	Data File	mj internal blood control end of run.d
Type	QC	Sample	mj internal blood control end of run re-inject
Acq. Method	thc quant 50 50.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	6/7/2024 9:14:37 AM		
Sample Info.			

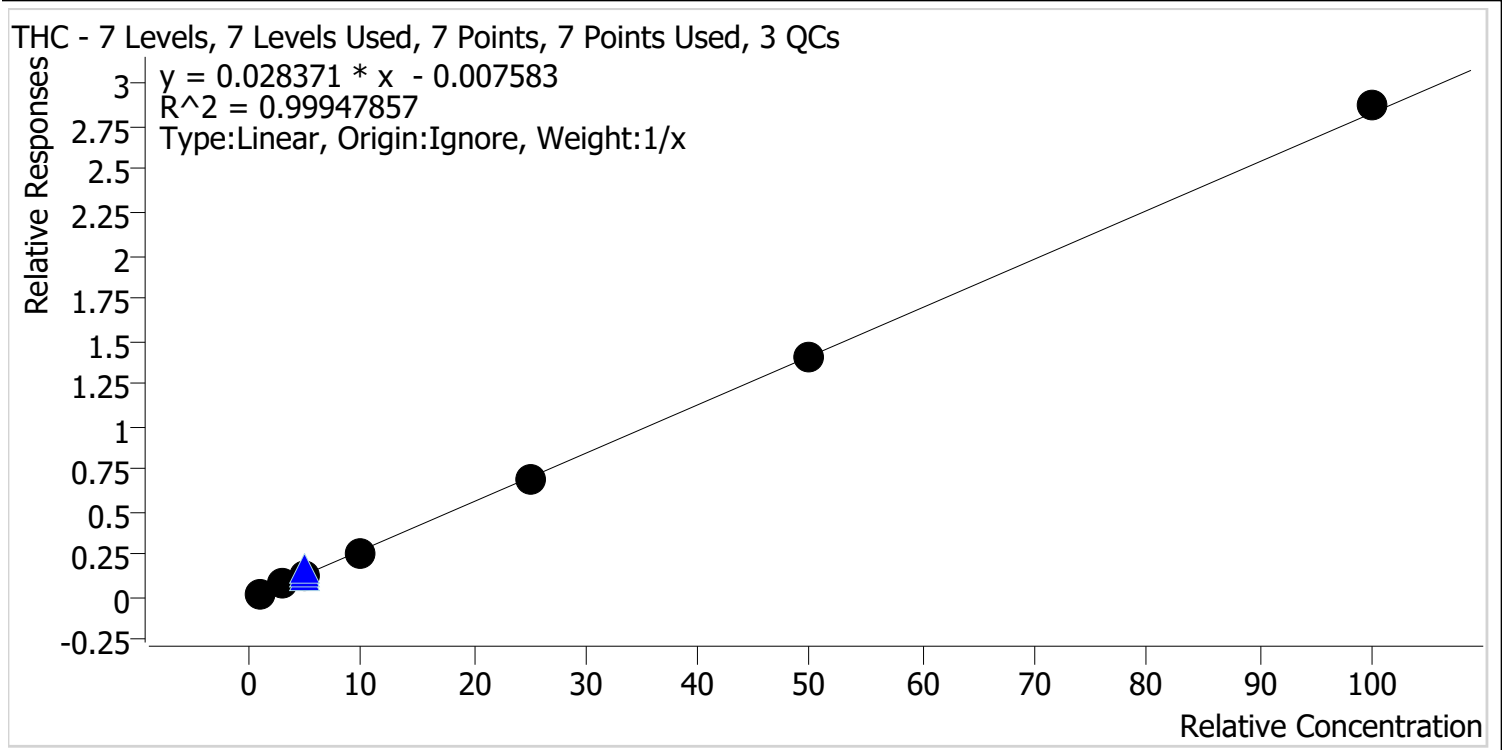
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	27650	∞	810.01	∞	1868579	4.992 ng/ml
THC-COOH	2.122	51158	233.5	279.08	355.1	823230	13.516 ng/ml
THC	3.558	50247	3260.6	28.14	∞	312411	5.936 ng/ml

Compound Calibration Report

Batch results D:\MassHunter\Data\2024\lam 27-28\060624\QuantResults\lam 27.batch.bin
Last Cal. Update 6/7/2024 9:35 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

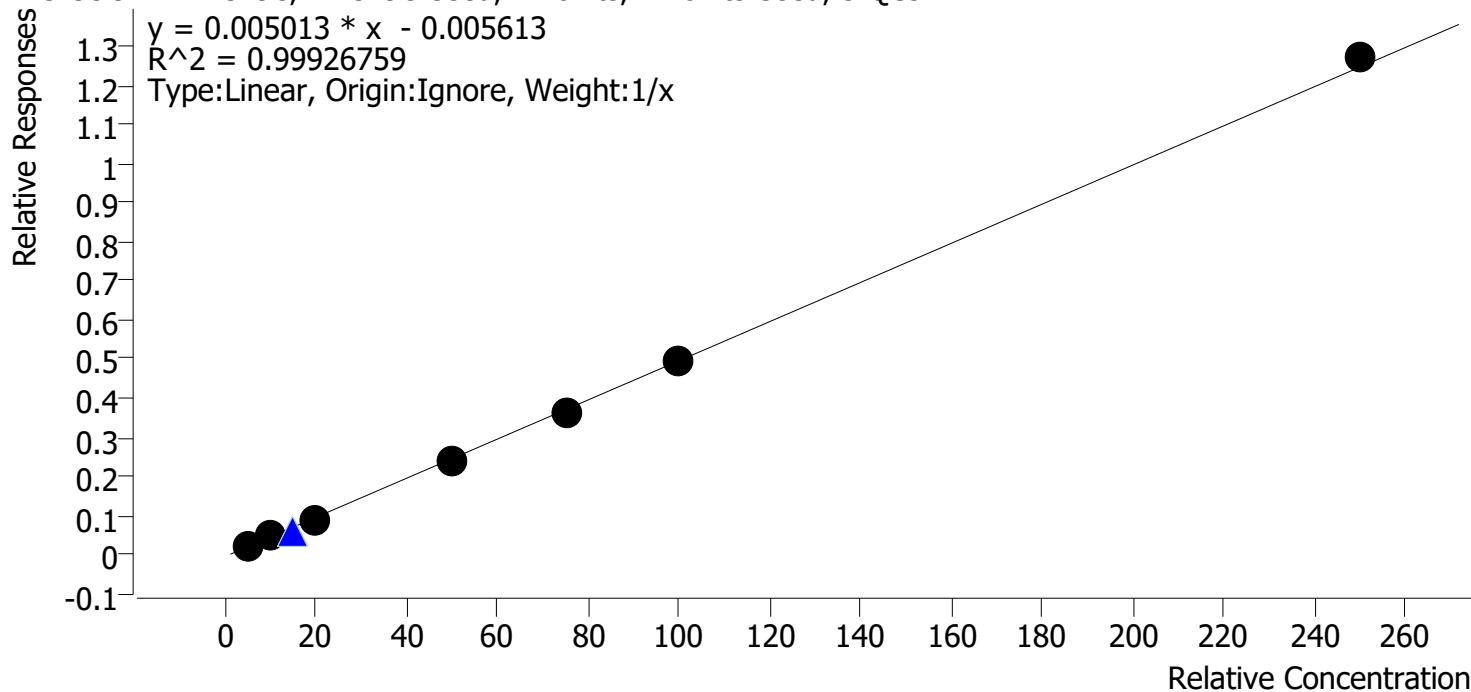


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.1	109.3
mj cal 2	2	✓	3.0	3.0	98.5
mj cal 3	3	✓	5.0	5.0	99.7
mj cal 4	4	✓	10.0	9.5	94.6
mj cal 5	5	✓	25.0	24.2	97.0
mj cal 6	6	✓	50.0	49.7	99.4
mj cal 7	7	✓	100.0	101.5	101.5

Compound Calibration Report

Batch results D:\MassHunter\Data\2024\lam 27-28\060624\QuantResults\lam 27.batch.bin
Last Cal. Update 6/7/2024 9:35 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, 3 QCs

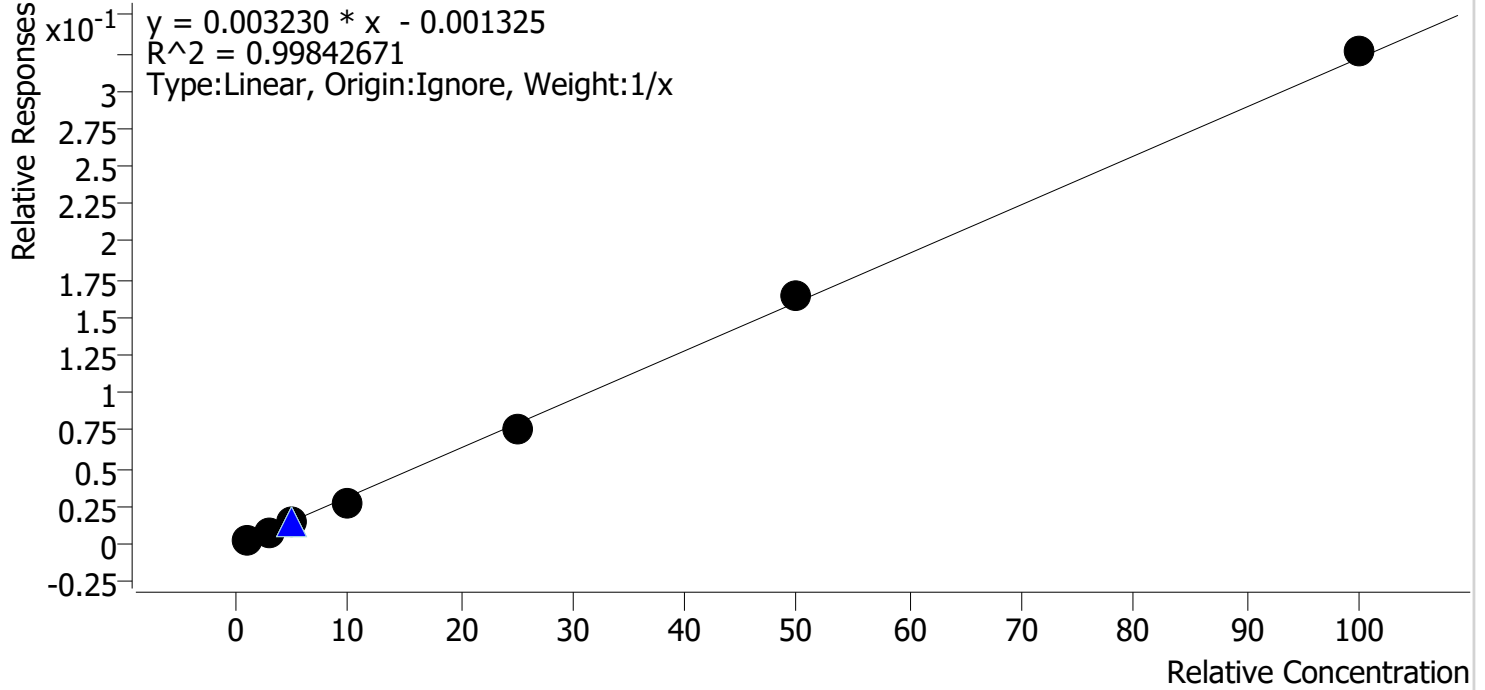


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.5	109.2
mj cal 2	2	✓	10.0	10.0	100.1
mj cal 3	3	✓	20.0	19.0	95.1
mj cal 4	4	✓	50.0	48.7	97.4
mj cal 5	5	✓	75.0	72.9	97.2
mj cal 6	6	✓	100.0	99.1	99.1
mj cal 7	7	✓	250.0	254.8	101.9

Compound Calibration Report

Batch results D:\MassHunter\Data\2024\lam 27-28\060624\QuantResults\lam 27.batch.bin
Last Cal. Update 6/7/2024 9:35 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 3 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	120.8
mj cal 2	2	✓	3.0	2.9	98.2
mj cal 3	3	✓	5.0	4.6	91.8
mj cal 4	4	✓	10.0	9.0	90.3
mj cal 5	5	✓	25.0	23.9	95.5
mj cal 6	6	✓	50.0	51.0	102.0
mj cal 7	7	✓	100.0	101.4	101.4

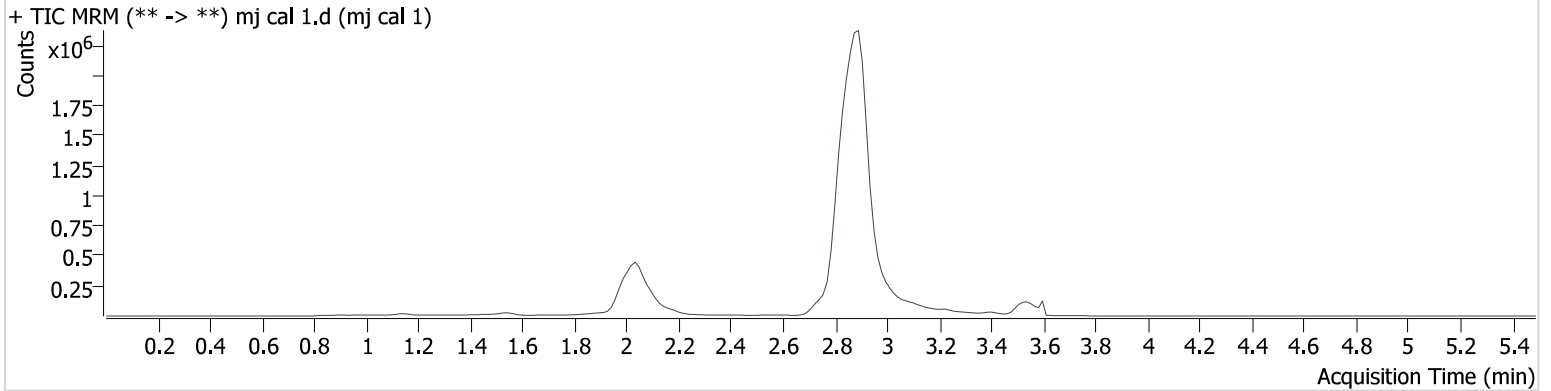
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-A1
Injection Volume 10
Acq. Date-Time 6/6/2024 6:28:22 PM
Sample Info.

Data File mj cal 1.d
Sample mj cal 1
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.036	5352	∞	681.62	∞	2076464	1.208 ng/ml	Low
THC-COOH	2.092	15414	154.2	284.55	28546.0	708180	5.462 ng/ml	
THC	3.543	8034	∞	27.15	∞	343092	1.093 ng/ml	

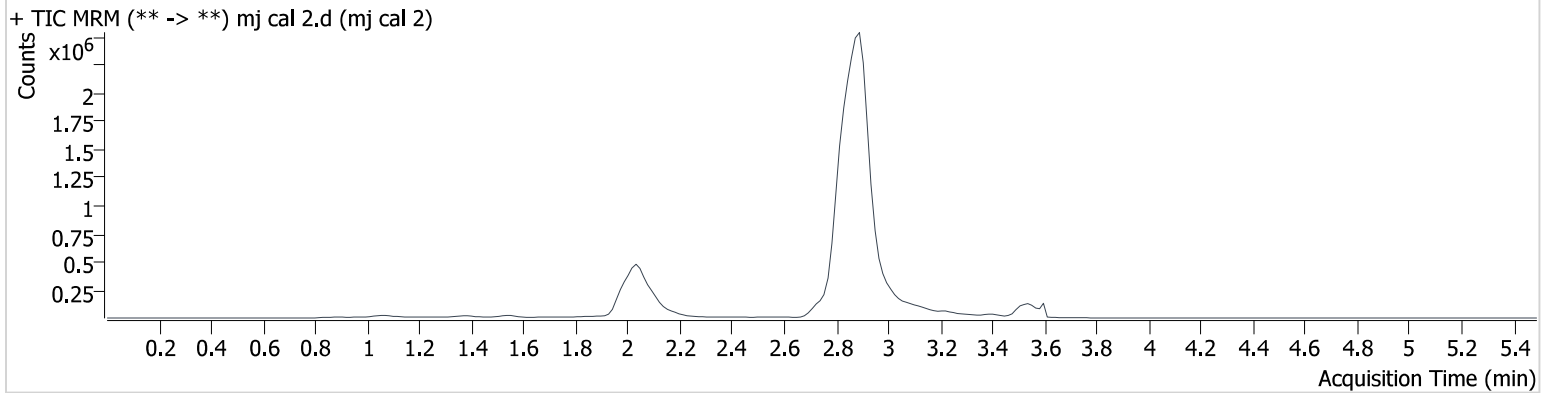
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-B1
Injection Volume 10
Acq. Date-Time 6/6/2024 6:35:06 PM
Sample Info.

Data File mj cal 2.d
Sample mj cal 2
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.036	17029	∞	864.33	∞	2079832	2.945 ng/ml	Low
THC-COOH	2.107	32705	226.7	283.27	77.5	734218	10.006 ng/ml	
THC	3.543	27649	1155.9	25.45	∞	362657	2.954 ng/ml	

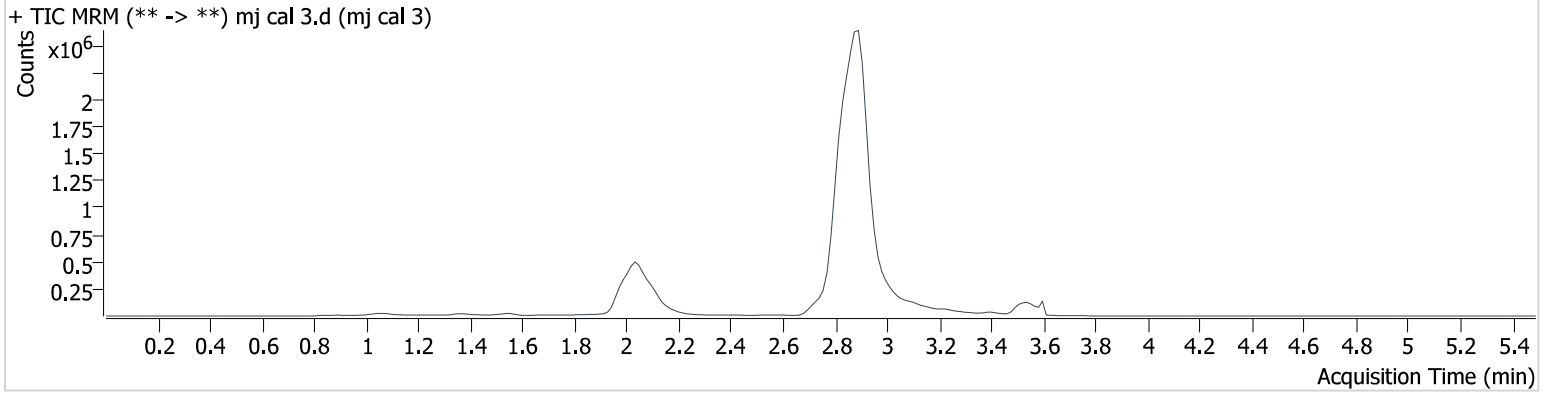
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-C1
Injection Volume 10
Acq. Date-Time 6/6/2024 6:41:40 PM
Sample Info.

Data File mj cal 3.d
Sample mj cal 3
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	27669	505.5	894.10	∞	2048884	4.592 ng/ml
THC-COOH	2.107	67404	235.0	278.64	114.8	751485	19.013 ng/ml
THC	3.543	46795	∞	26.07	276.2	349750	4.983 ng/ml

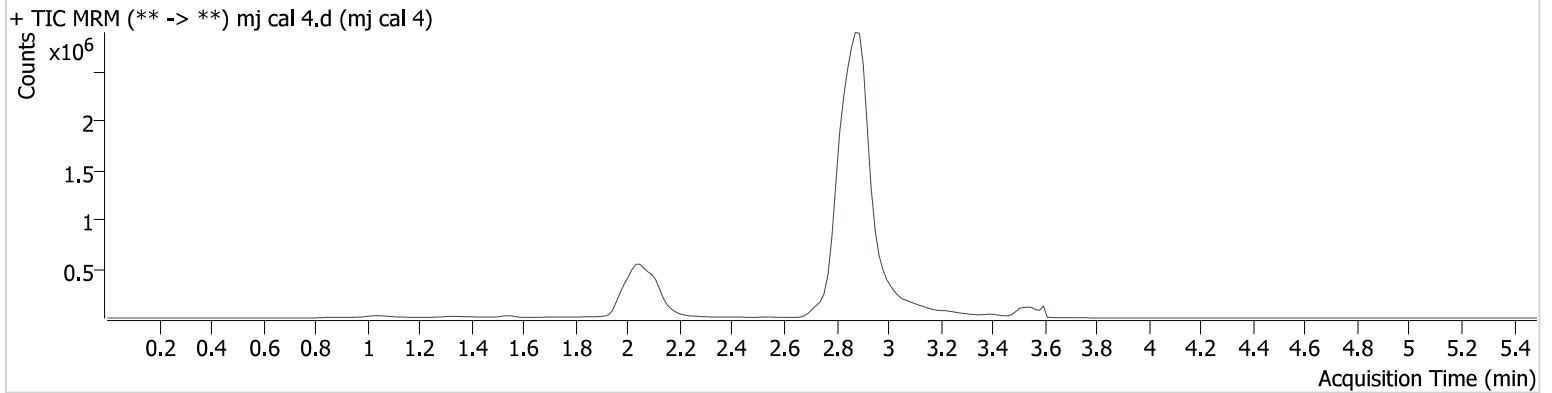
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-D1
Injection Volume 10
Acq. Date-Time 6/6/2024 6:48:14 PM
Sample Info.

Data File mj cal 4.d
Sample mj cal 4
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	53278	1384.6	926.73	∞	1913329	9.032 ng/ml
THC-COOH	2.092	171510	1788.8	274.25	444324	719086	48.700 ng/ml
THC	3.558	79577	∞	25.44	1352.5	305038	9.462 ng/ml

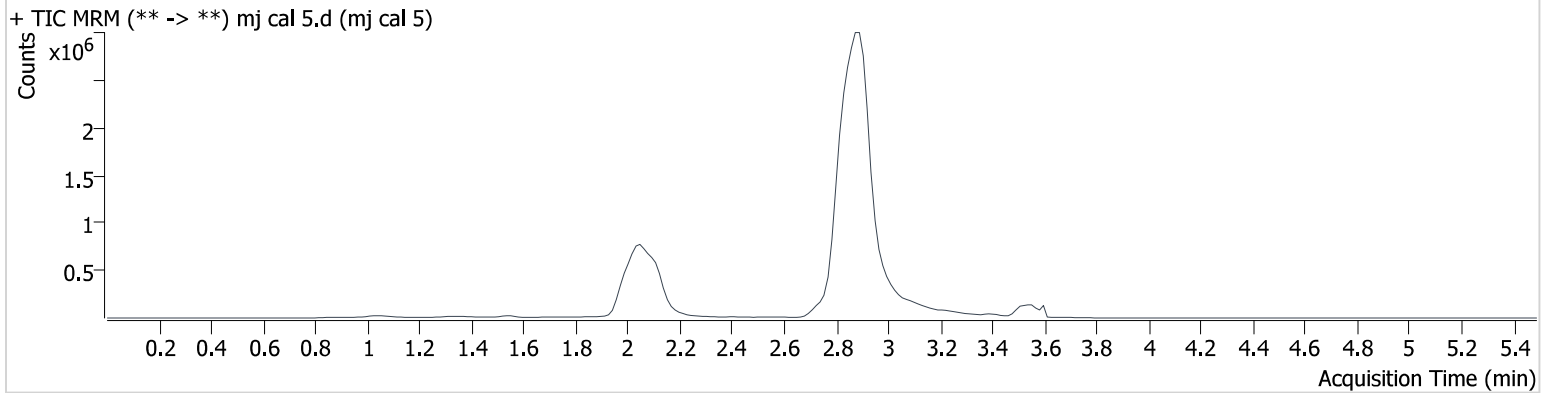
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-E1
Injection Volume 10
Acq. Date-Time 6/6/2024 6:54:50 PM
Sample Info.

Data File mj cal 5.d
Sample mj cal 5
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	157058	1346.5	878.03	∞	2072486	23.874 ng/ml
THC-COOH	2.107	281209	347494.3	274.02	1092.6	781333	72.917 ng/ml
THC	3.558	212244	7236.6	24.94	267.8	311982	24.246 ng/ml

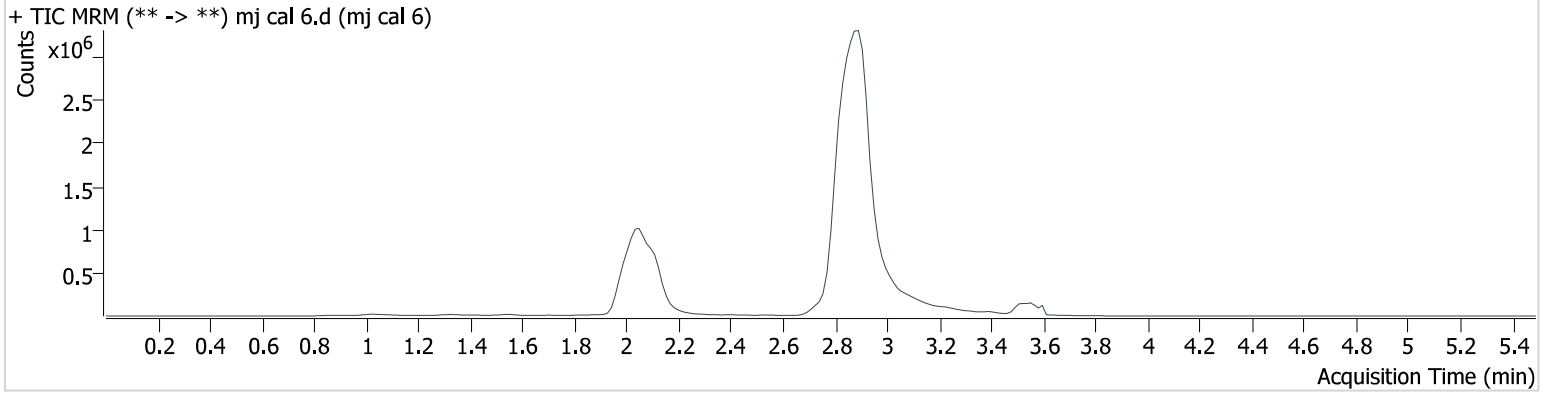
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-F1
Injection Volume 10
Acq. Date-Time 6/6/2024 7:01:26 PM
Sample Info.

Data File mj cal 6.d
Sample mj cal 6
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	317663	2551.4	844.24	∞	1944483	50.991 ng/ml
THC-COOH	2.107	356302	1899.3	272.61	4361.0	725434	99.100 ng/ml
THC	3.558	359276	7960.0	25.11	252.8	256060	49.722 ng/ml

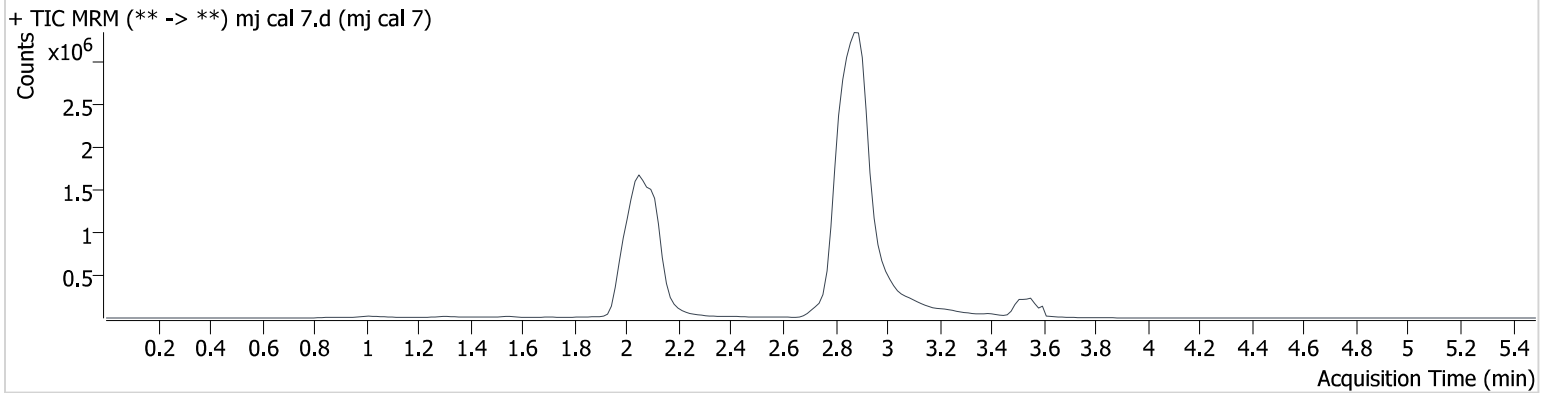
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\060624\QuantResults\am 27.batch.bin
Calibration Last Update 6/7/2024 9:35:11 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-G1
Injection Volume 10
Acq. Date-Time 6/6/2024 7:08:03 PM
Sample Info.

Data File mj cal 7.d
Sample mj cal 7
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	597609	20296.1	863.68	∞	1832909	101.358 ng/ml
THC-COOH	2.092	841054	1501027.2	262.33	4339.5	661376	254.803 ng/ml
THC	3.558	716494	28438.9	26.15	∞	249370	101.539 ng/ml