

WAB

5/13/2024

Worklist: 6817

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2024-0776	9	BLOOD	AM 27 Blood THC Quant by LC-QQQ



NMB

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

Extraction Date: 05/06/24
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: Mikel Buffaloe
Plate Retest Date: 6/12/24
Mobile phase B: 0.1% Formic acid in Acetonitrile
Blank Urine Lot: [Click here to enter text.](#)
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: I41142J** NMB
5/17/2024
- 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. NMB
5/17/2024
- 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: **800 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 r² 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: *Only blood was run.*

MBB

	1	2	3	4	5	6
a	cal 1					
b	cal 2	negative blood				
c	cal 3	Item 9				
d	cal 4					
e	cal 5					
f	cal 6					
g	cal 7					
h	Internal control (blood)					

Plate position 3

c2024-____-__

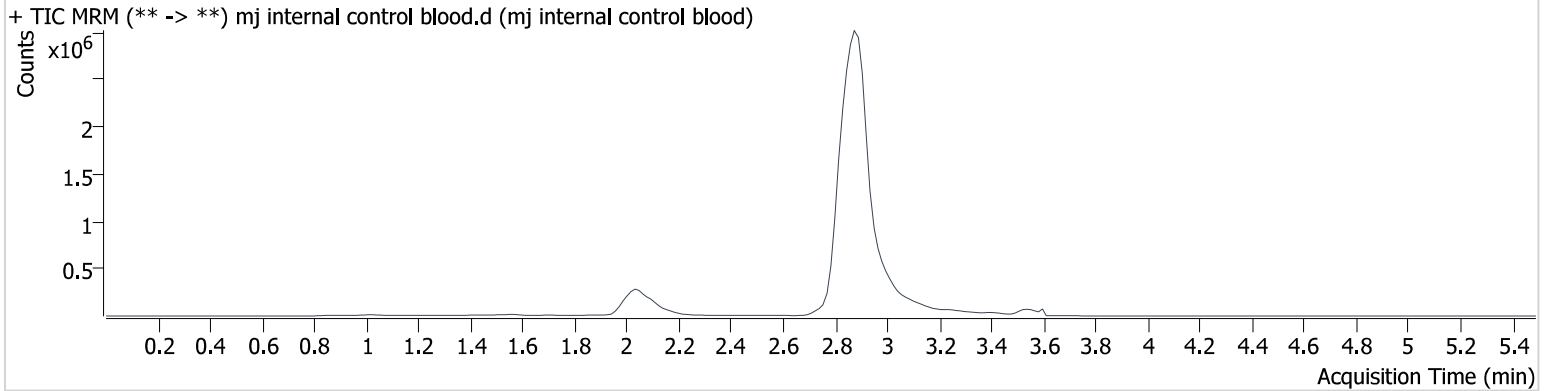
AM #27 Cannabinoids

WAB

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 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	Mikel Buffaloe
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	5/6/2024 5:23:51 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	16272	∞	817.14	∞	1085026	5.013 ng/ml
THC-COOH	2.107	29690	141.9	297.19	574.7	496540	15.258 ng/ml
THC	3.543	24507	∞	23.10	∞	182527	4.821 ng/ml

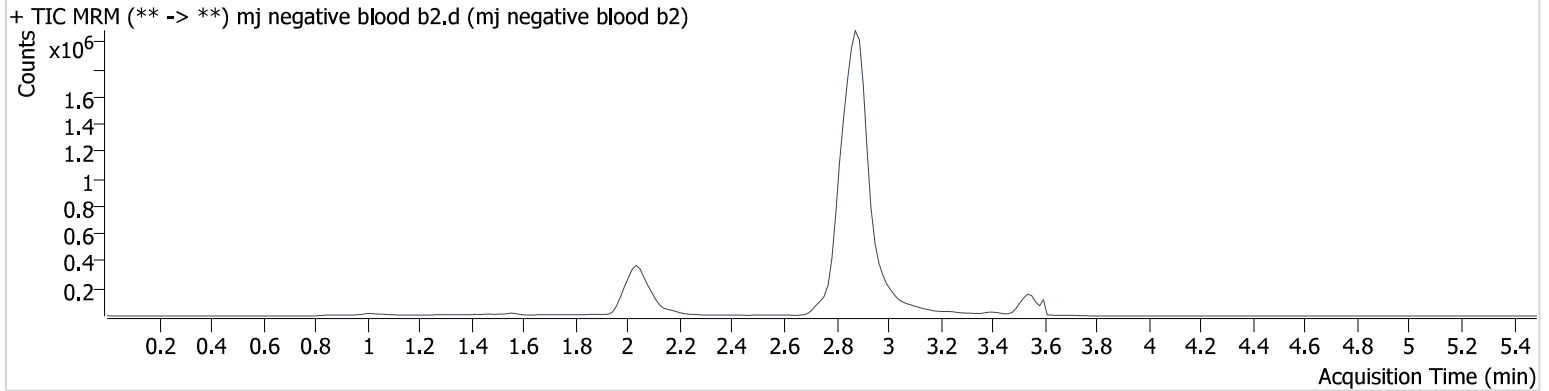
MBB

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument	69679	Data File	mj negative blood b2.d
Type	Sample	Sample	mj negative blood b2
Acq. Method	thc quant 50 50.m	Operator	Mikel Buffaloe
Sample Position	P3-B2	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	5/6/2024 5:30:27 PM		
Sample Info.			

Sample Chromatogram



MKB

AM #27 Cannabinoids

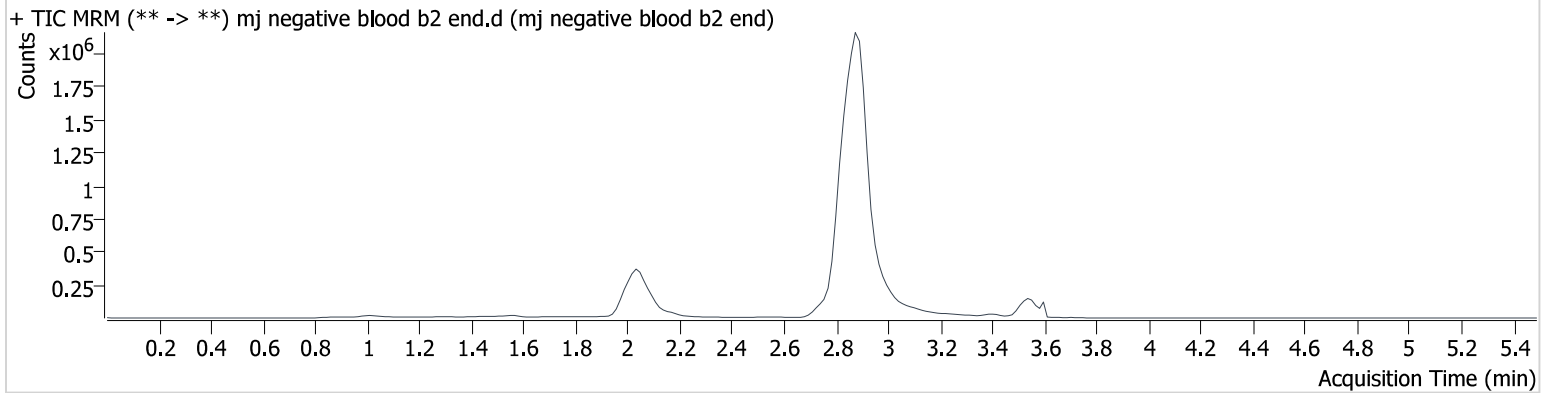
Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679
Type Sample
Acq. Method thc quant 50 50.m
Sample Position P3-B2
Injection Volume 10
Acq. Date-Time 5/6/2024 5:43:40 PM
Sample Info.

Data File mj negative blood b2 end.d
Sample mj negative blood b2 end
Operator Mikel Buffaloe
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



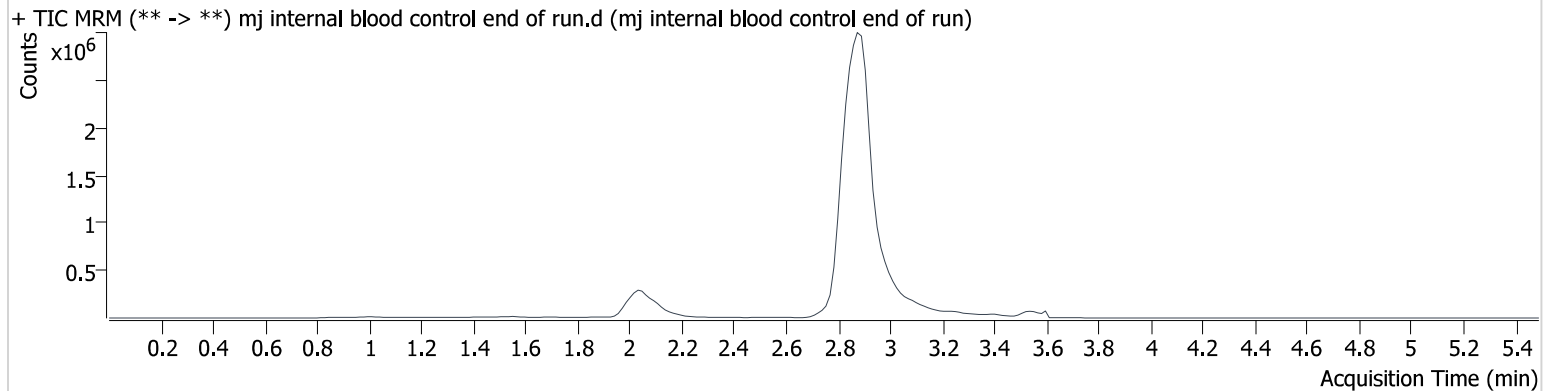
MJB

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679 **Data File** mj internal blood control end of run.d
Type Sample **Sample** mj internal blood control end of run
Acq. Method thc quant 50 50.m **Operator** Mikel Buffaloe
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 5/6/2024 5:50:16 PM
Sample Info.

Sample Chromatogram



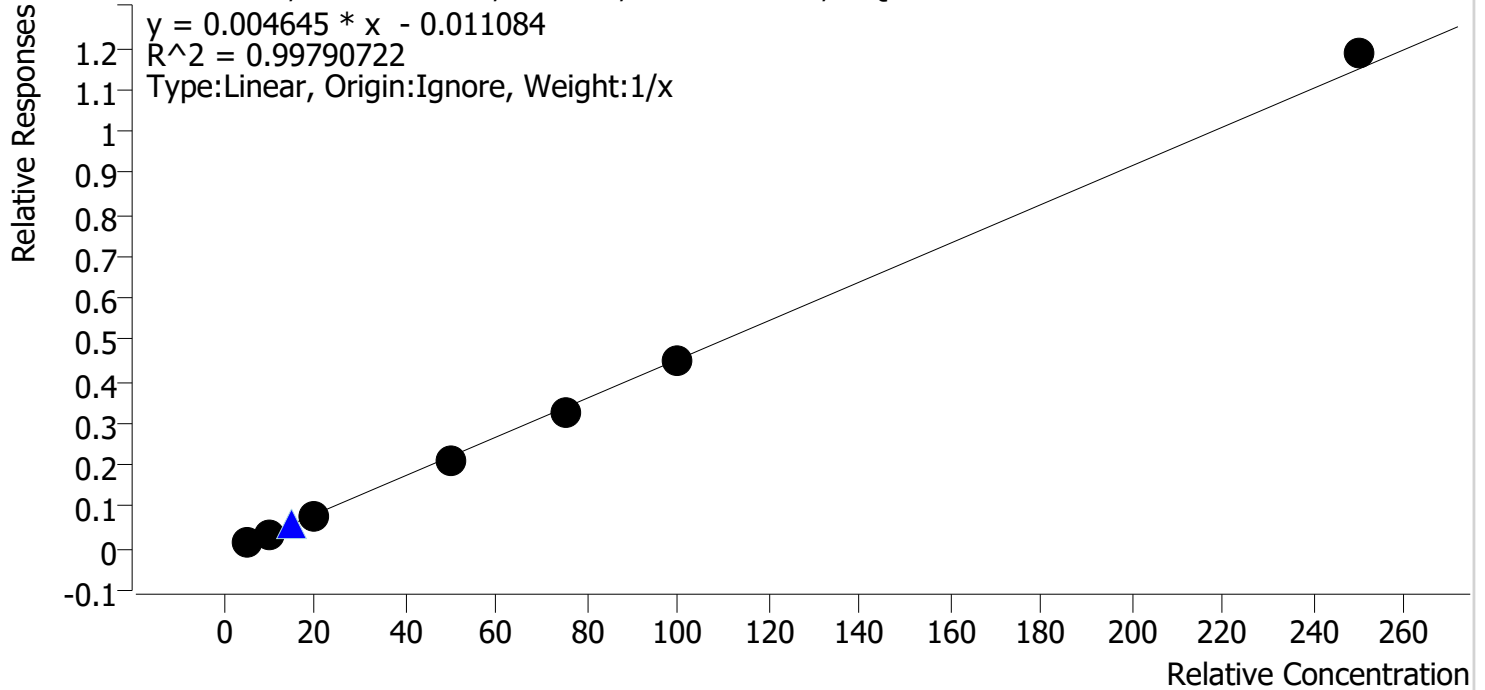
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	17547	∞	778.64	683.2	1113679	5.243 ng/ml
THC-COOH	2.107	31142	51.9	276.36	723.3	520671	15.262 ng/ml
THC	3.543	24751	912.5	20.79	∞	183139	4.851 ng/ml

Compound Calibration Report

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Last Cal. Update 5/7/2024 7:47 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



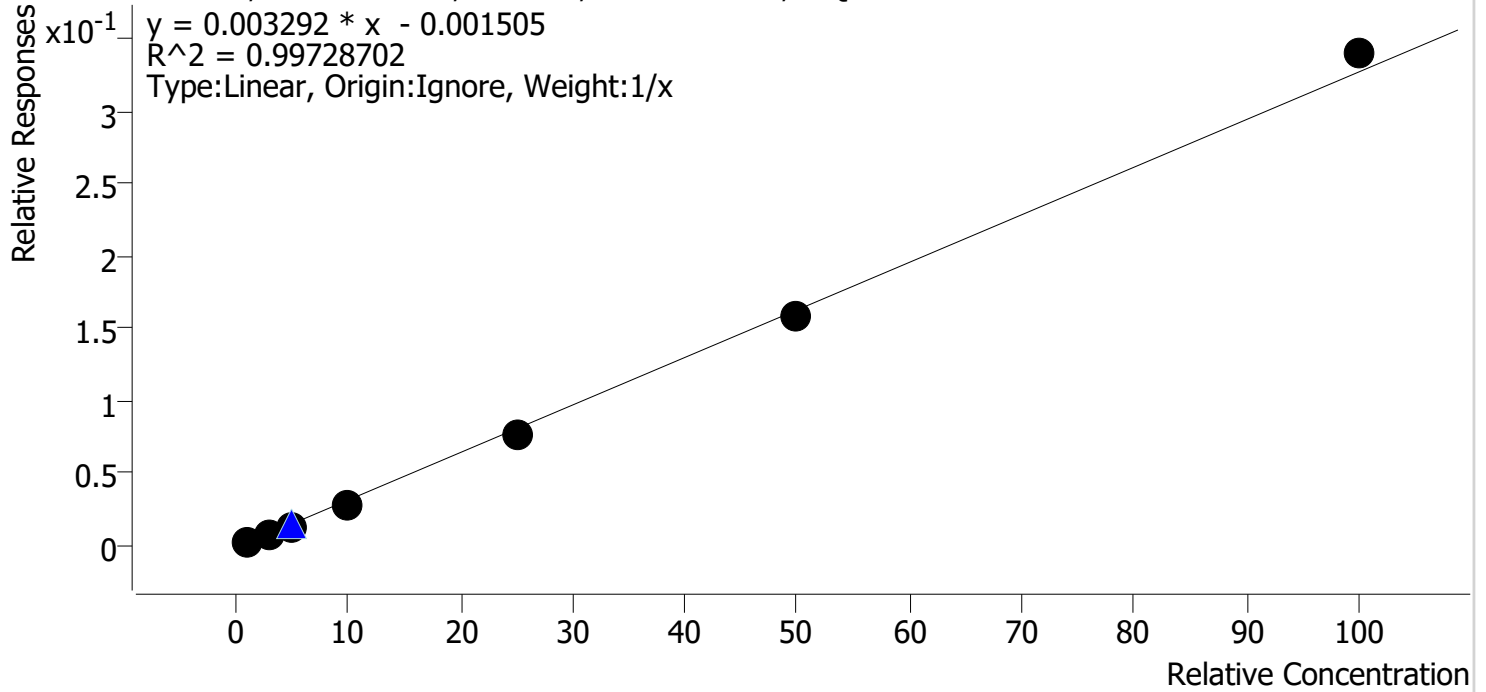
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.8	116.5
mj cal 2	2	✓	10.0	9.9	98.6
mj cal 3	3	✓	20.0	18.6	92.9
mj cal 4	4	✓	50.0	47.1	94.3
mj cal 5	5	✓	75.0	71.6	95.5
mj cal 6	6	✓	100.0	99.1	99.1
mj cal 7	7	✓	250.0	257.9	103.2

Compound Calibration Report

MJB

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Last Cal. Update 5/7/2024 7:47 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, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.3	125.4
mj cal 2	2	✓	3.0	2.9	96.6
mj cal 3	3	✓	5.0	4.6	92.0
mj cal 4	4	✓	10.0	9.0	90.3
mj cal 5	5	✓	25.0	23.6	94.3
mj cal 6	6	✓	50.0	48.8	97.6
mj cal 7	7	✓	100.0	103.8	103.8

MSB

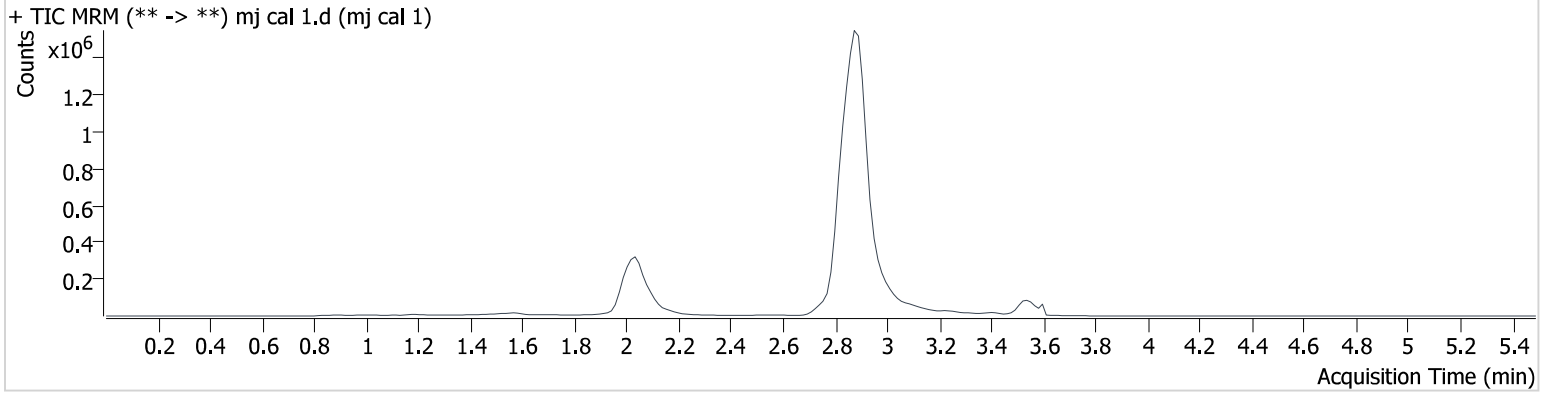
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-A1
Injection Volume 10
Acq. Date-Time 5/6/2024 4:37:31 PM
Sample Info.

Data File mj cal 1.d
Sample mj cal 1
Operator Mikel Buffaloe
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	3824	46.4	803.48	∞	1458824	1.254 ng/ml	Low
THC-COOH	2.092	8351	63.6	259.31	44.2	522702	5.825 ng/ml	
THC	3.543	5749	∞	20.95	158273 049996 4.8	219778	1.160 ng/ml	

MJB

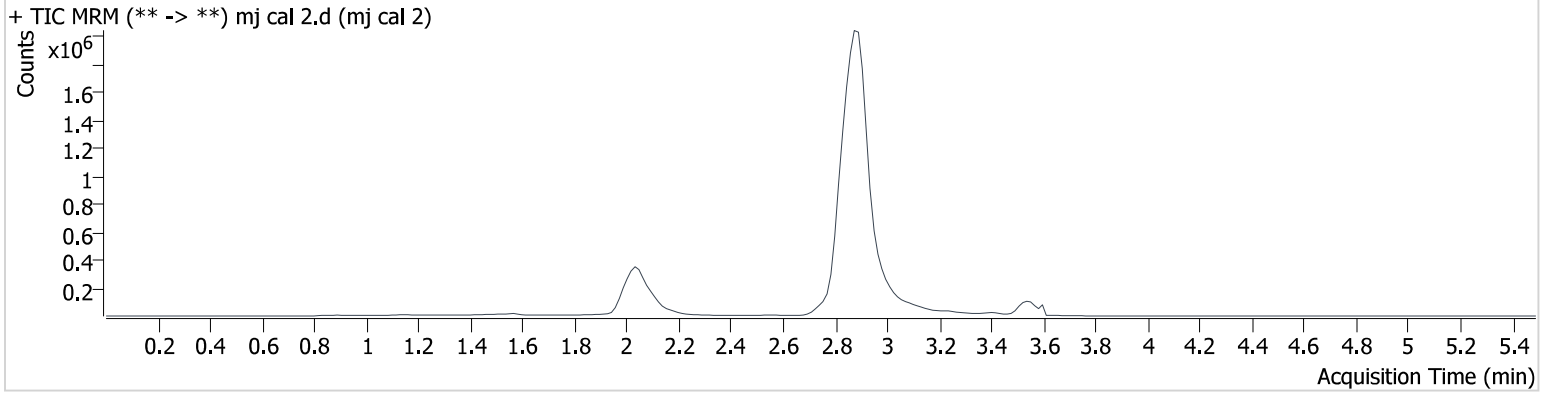
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-B1
Injection Volume 10
Acq. Date-Time 5/6/2024 4:44:15 PM
Sample Info.

Data File mj cal 2.d
Sample mj cal 2
Operator Mikel Buffaloe
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	11911	∞	817.53	∞	1482980	2.897 ng/ml	Low
THC-COOH	2.107	21239	23516.0	284.06	112.9	611774	9.859 ng/ml	
THC	3.558	20437	∞	23.94	∞	272404	2.814 ng/ml	

Web

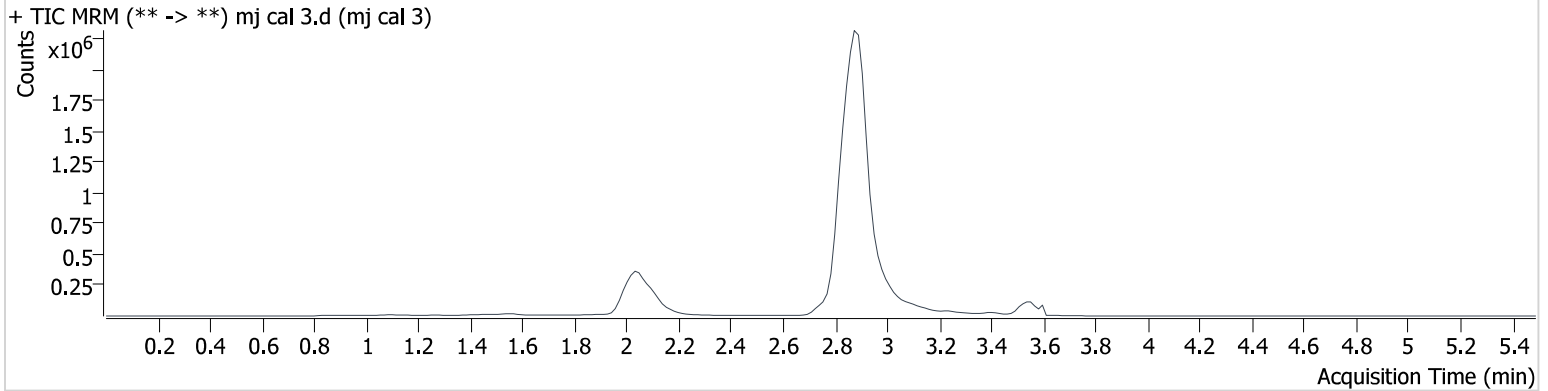
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-C1
Injection Volume 10
Acq. Date-Time 5/6/2024 4:50:51 PM
Sample Info.

Data File mj cal 3.d
Sample mj cal 3
Operator Mikel Buffaloe
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	19520	∞	905.48	∞	1431554	4.599 ng/ml
THC-COOH	2.107	46773	111.3	286.84	∞	621933	18.575 ng/ml
THC	3.558	39278	∞	24.27	243.8	290978	4.845 ng/ml

AM #27 Cannabinoids

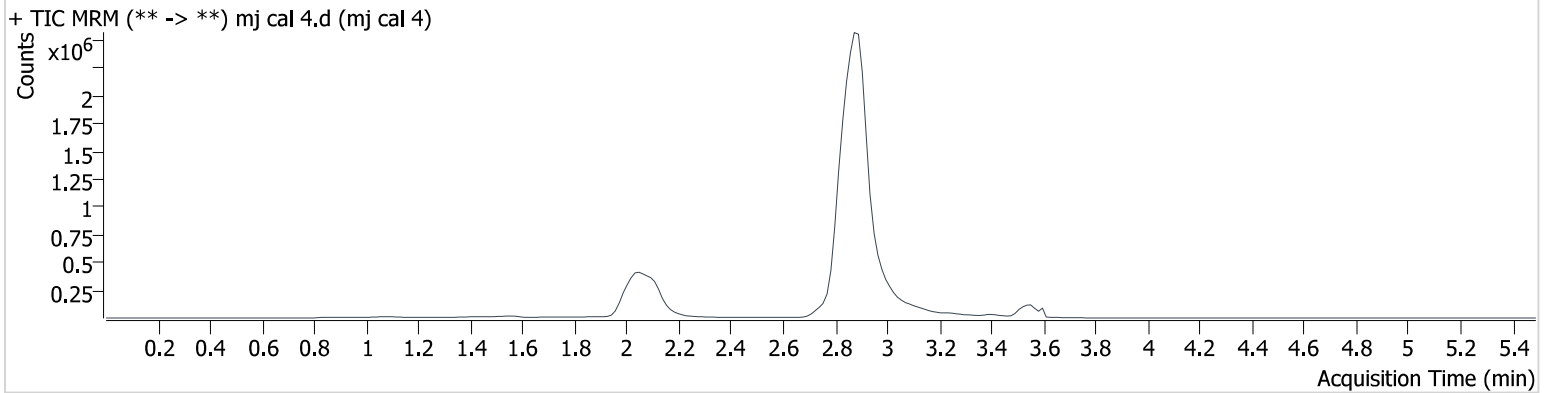
MJB

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-D1
Injection Volume 10
Acq. Date-Time 5/6/2024 4:57:27 PM
Sample Info.

Data File mj cal 4.d
Sample mj cal 4
Operator Mikel Buffaloe
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	40420	1269.3	869.20	∞	1432009	9.031 ng/ml
THC-COOH	2.107	133972	1753.0	262.54	493.9	644567	47.129 ng/ml
THC	3.558	76261	∞	25.24	∞	280691	9.475 ng/ml

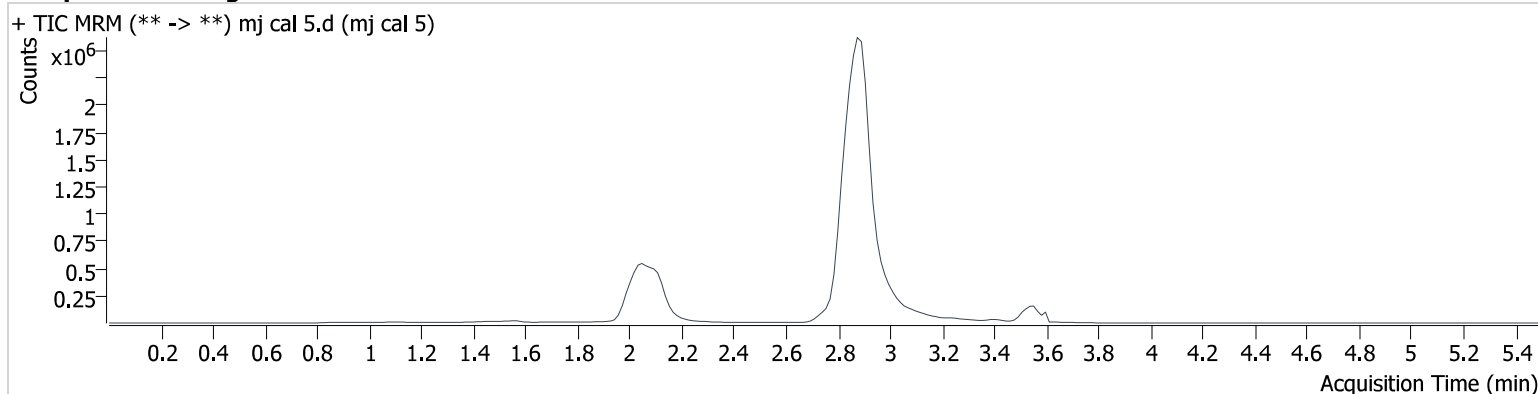
MJB

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	thc quant 50 50.m	Operator	Mikel Buffaloe
Sample Position	P3-E1	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	5/6/2024 5:04:03 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	107205	2694.1	883.85	∞	1408216	23.582 ng/ml
THC-COOH	2.107	207783	835.3	267.71	129.0	645811	71.646 ng/ml
THC	3.558	204280	4116.7	25.08	1262.4	287208	24.361 ng/ml

NAB

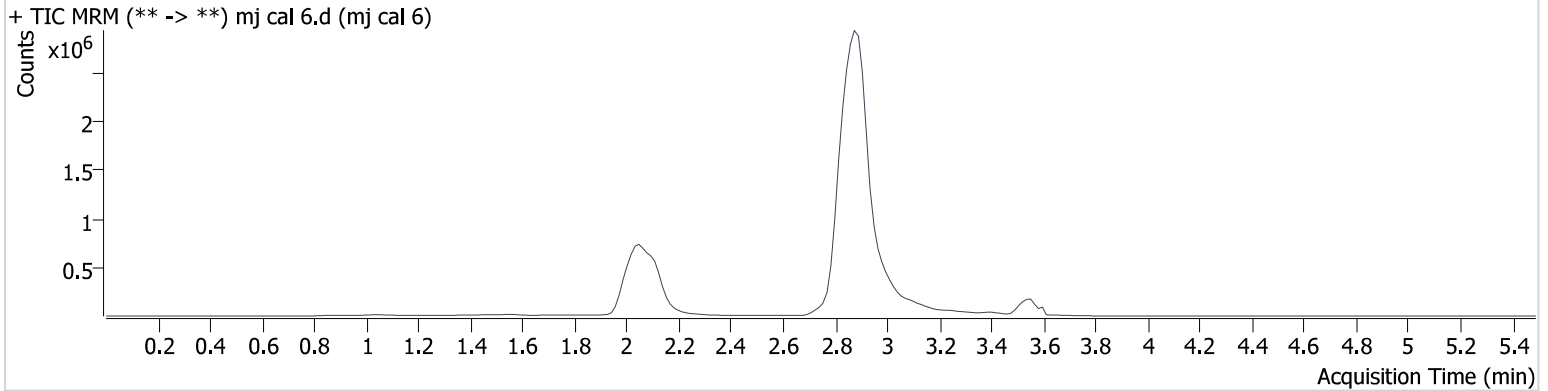
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-F1
Injection Volume 10
Acq. Date-Time 5/6/2024 5:10:40 PM
Sample Info.

Data File mj cal 6.d
Sample mj cal 6
Operator Mikel Buffaloe
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	210784	7341.1	847.93	∞	1324307	48.806 ng/ml
THC-COOH	2.107	272077	134447.2	270.01	1308.2	605664	99.088 ng/ml
THC	3.558	348796	∞	23.40	∞	238564	49.787 ng/ml

MJB

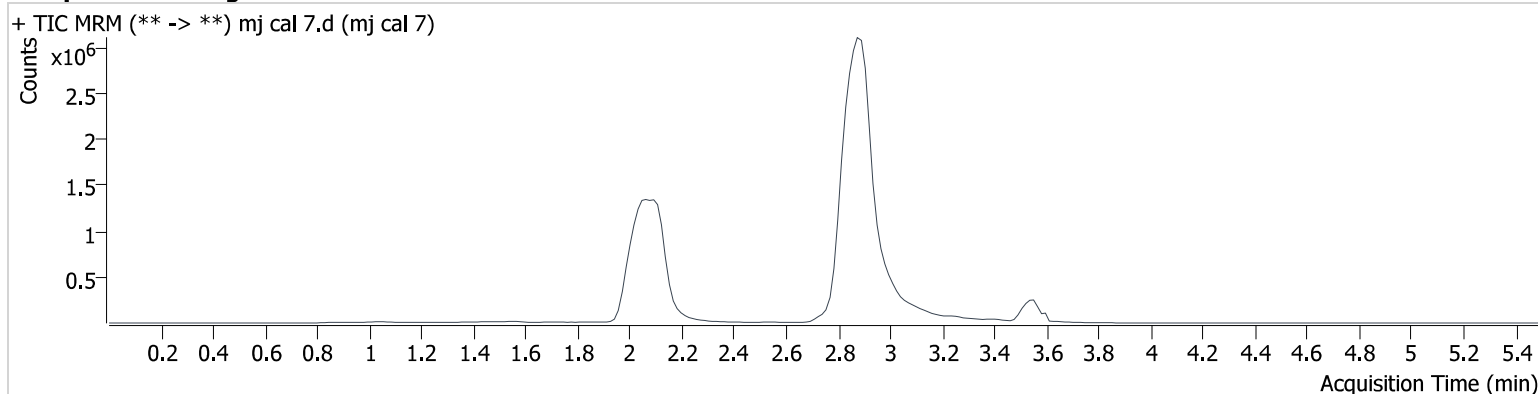
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\050624\QuantResults\thc.batch.bin
Calibration Last Update 5/7/2024 7:47:05 AM

Instrument 69679
Type Cal
Acq. Method thc quant 50 50.m
Sample Position P3-G1
Injection Volume 10
Acq. Date-Time 5/6/2024 5:17:16 PM
Sample Info.

Data File mj cal 7.d
Sample mj cal 7
Operator Mikel Buffaloe
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	481584	10523.3	812.74	∞	1415135	103.831 ng/ml
THC-COOH	2.107	713889	1443.2	264.19	1479.1	601497	257.876 ng/ml
THC	3.558	681139	∞	24.90	2821.2	227742	101.559 ng/ml