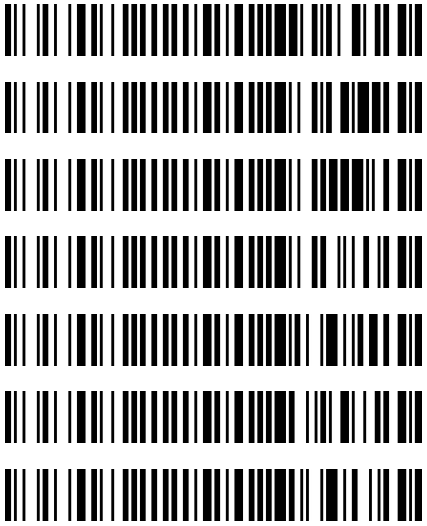
REVIEWED
By Anne Nord at 10:43 am, Apr 09, 2024

4/8/2024

Worklist: 6757

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2024-0564	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0595	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0598	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0603	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0620	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0633	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0647	1	BCK	AM 27 Blood THC Quant by LC-QQQ



BWylie

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

Extraction Date: 4/8/24
Plate lot#: 231212
Mobile phase A: 0.1% Formic Acid in LCMS Water
Blank Blood Lot: 24C52042 (Btl 3)
Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Britany Wylie
Plate Retest Date: 6/12/24
Mobile phase B: 0.1% Formic acid in Acetonitrile
Blank Urine Lot: 1324
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**
☒ 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: **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 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: *4/5/24: Extraction failed for all samples, samples were re-extracted and run on 4/8/24*

	1	2	3	4	5	6
a	cal 1	Internal control urine	0598-1			
b	cal 2	negative blood				
c	cal 3	0595-1				
d	cal 4	0603-1				
e	cal 5	0620-1				
f	cal 6	0633-1				
g	cal 7	0647-1				
h	Internal control (blood)	0564-1				

Plate position 3

c2024-____-_

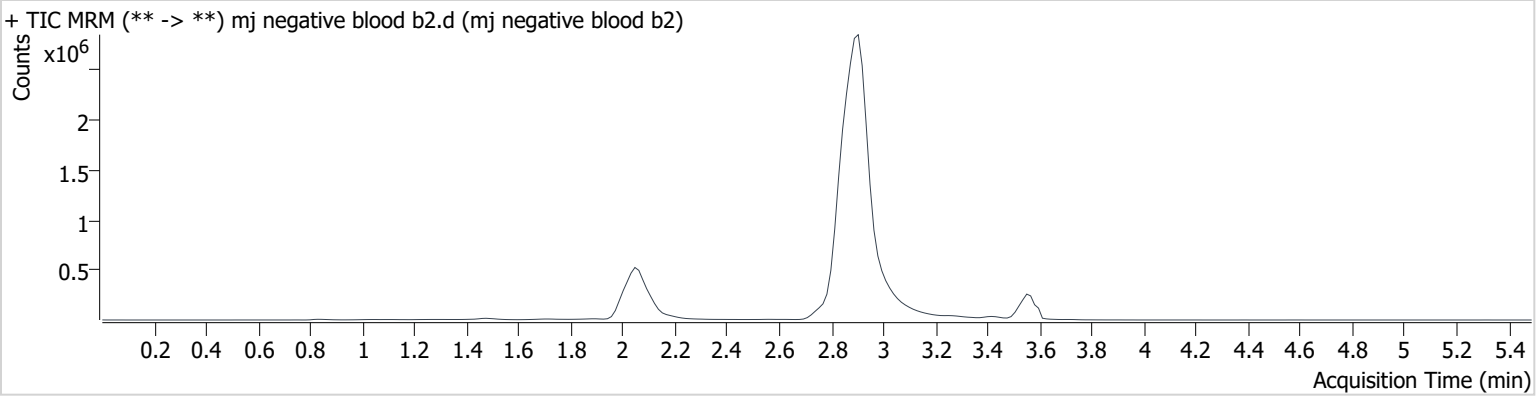
additional samples added to extraction plate upon re-extraction

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update	4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj negative blood b2.d
Type	Sample	**Sample**	mj negative blood b2
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-B2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:55:04 PM		
Sample Info.			

Sample Chromatogram

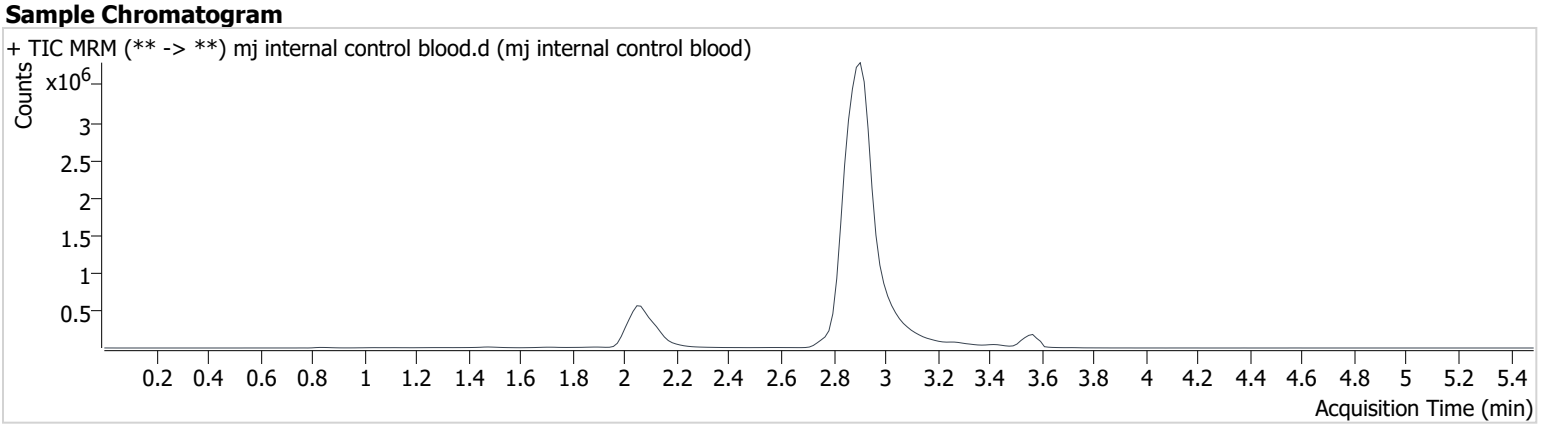


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj internal control blood.d
Type	QC	**Sample**	mj internal control blood
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:48:30 PM		
Sample Info.			

Sample Chromatogram



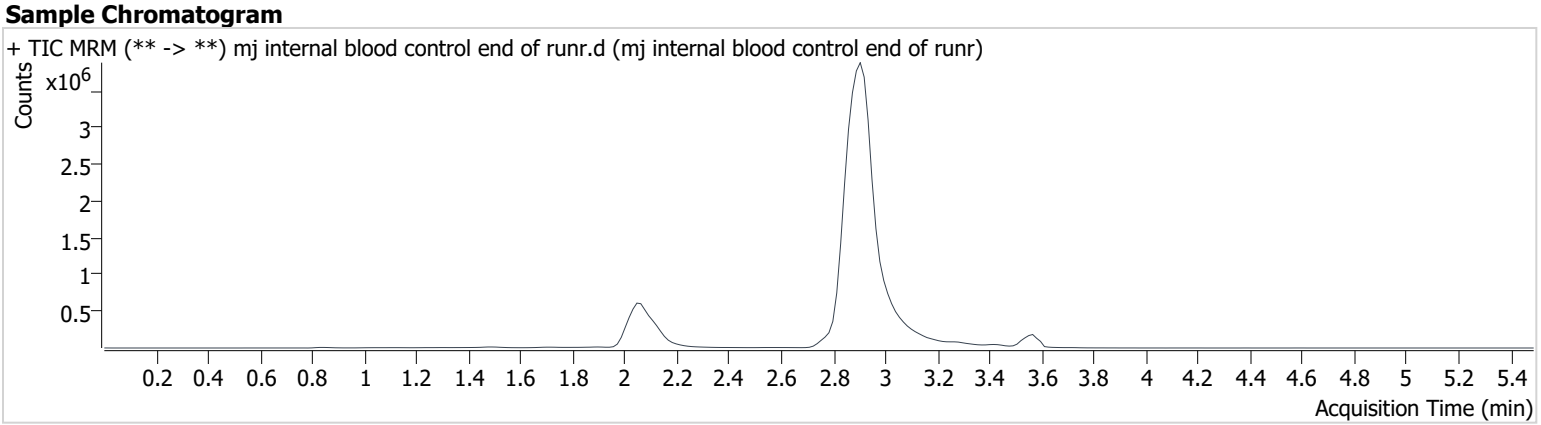
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	33527	604.4	888.21	∞	2333362	4.597	ng/ml
THC-COOH	2.122	60105	38206.7	273.43	372.2	859693	14.075	ng/ml
THC	3.573	66858	∞	23.85	∞	439391	5.108	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj internal blood control end of runr.d
Type	Sample	**Sample**	mj internal blood control end of runr
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 2:34:01 PM		
Sample Info.			

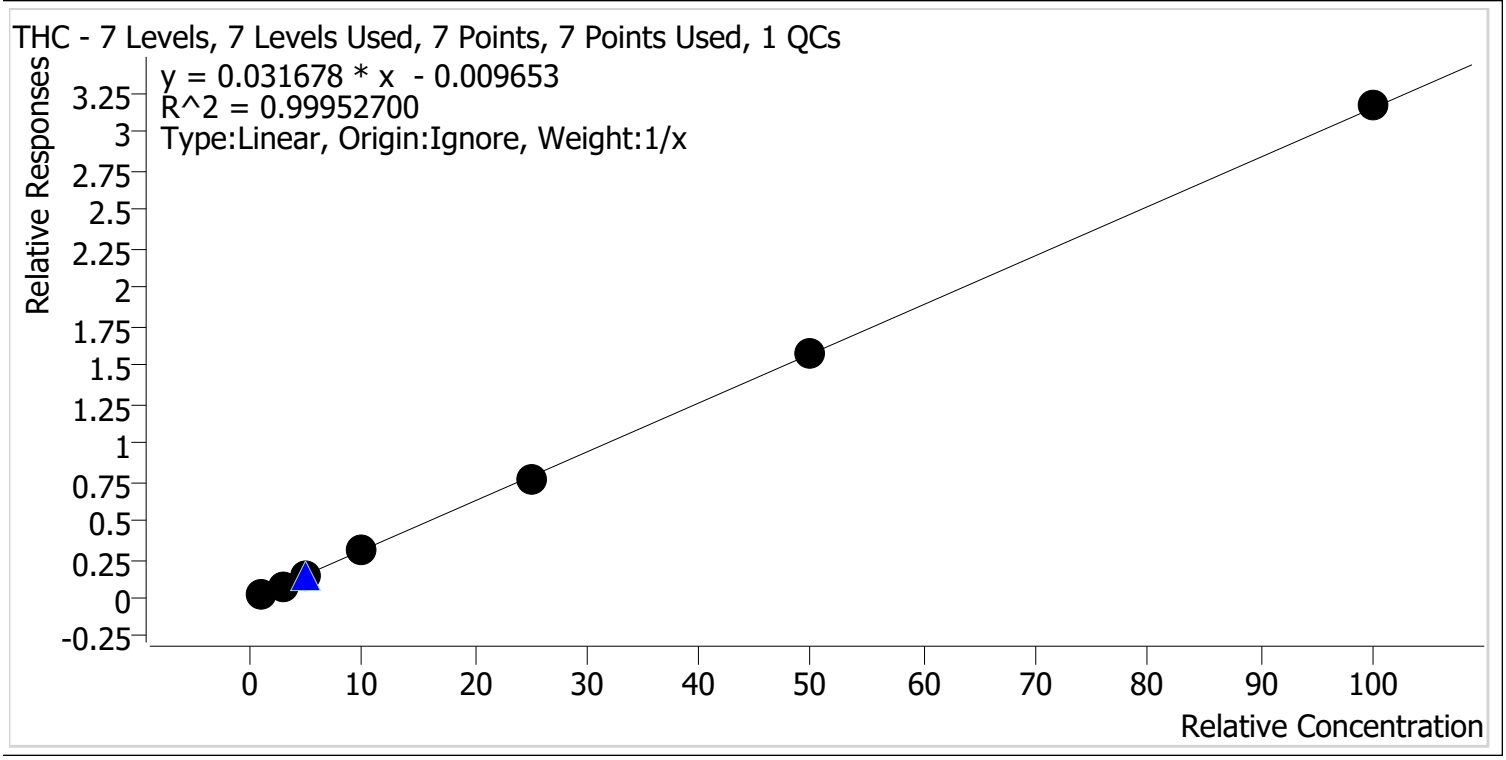
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	36550	217.0	885.00	∞	2525369	4.629	ng/ml
THC-COOH	2.122	63934	1129.3	272.05	280.5	904891	14.211	ng/ml
THC	3.573	63283	18303.9	25.24	85.3	455255	4.693	ng/ml

Compound Calibration Report

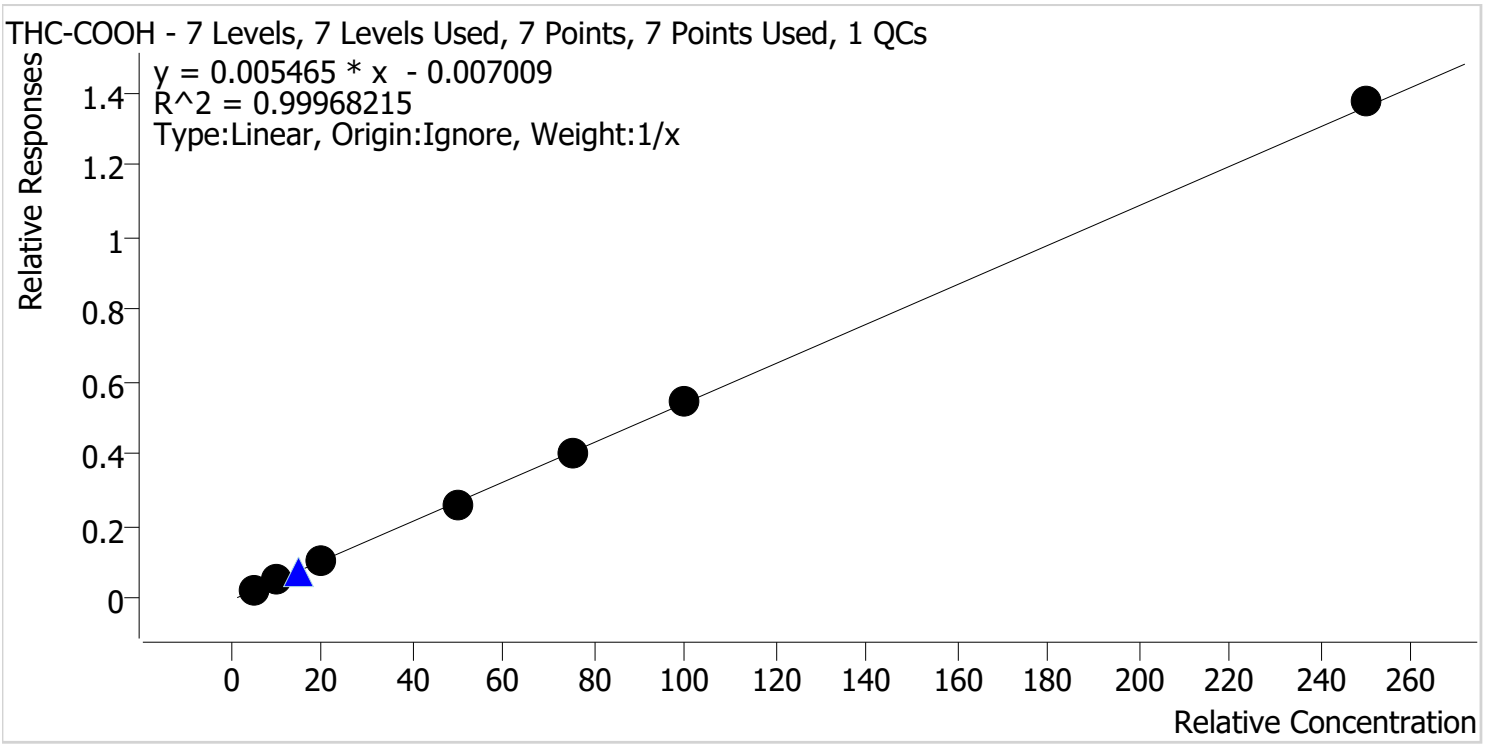
Batch results	D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Last Cal. Update	4/8/2024 3:12 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	115.8
mj cal 2	2	✔	3.0	2.8	93.5
mj cal 3	3	✔	5.0	4.6	92.5
mj cal 4	4	✔	10.0	9.8	97.9
mj cal 5	5	✔	25.0	24.8	99.0
mj cal 6	6	✔	50.0	50.4	100.8
mj cal 7	7	✔	100.0	100.5	100.5

Compound Calibration Report

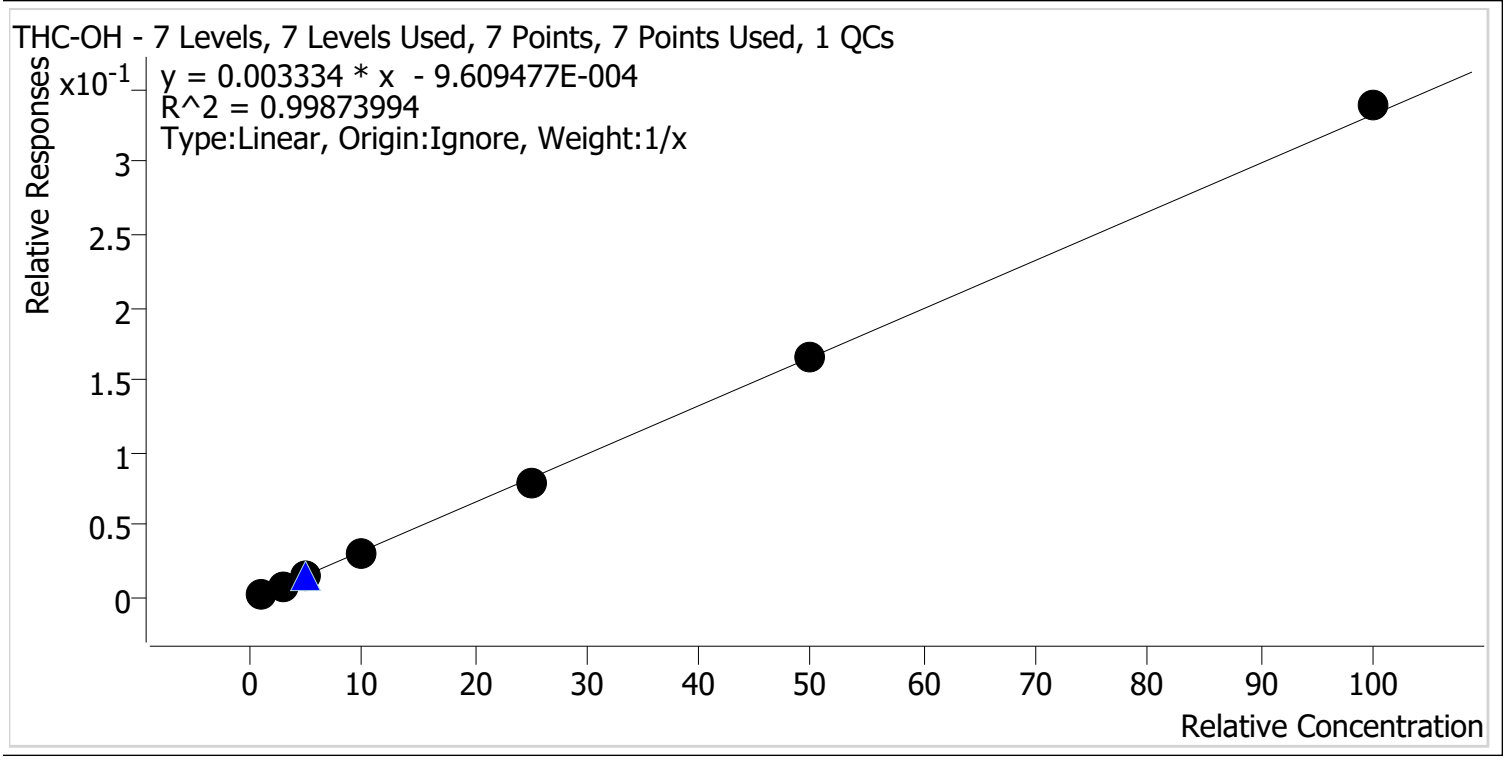
Batch results	D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Last Cal. Update	4/8/2024 3:12 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.3	106.4
mj cal 2	2	✔	10.0	10.0	99.8
mj cal 3	3	✔	20.0	19.4	96.8
mj cal 4	4	✔	50.0	48.7	97.4
mj cal 5	5	✔	75.0	73.8	98.4
mj cal 6	6	✔	100.0	100.1	100.1
mj cal 7	7	✔	250.0	252.7	101.1

Compound Calibration Report

Batch results	D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Last Cal. Update	4/8/2024 3:12 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



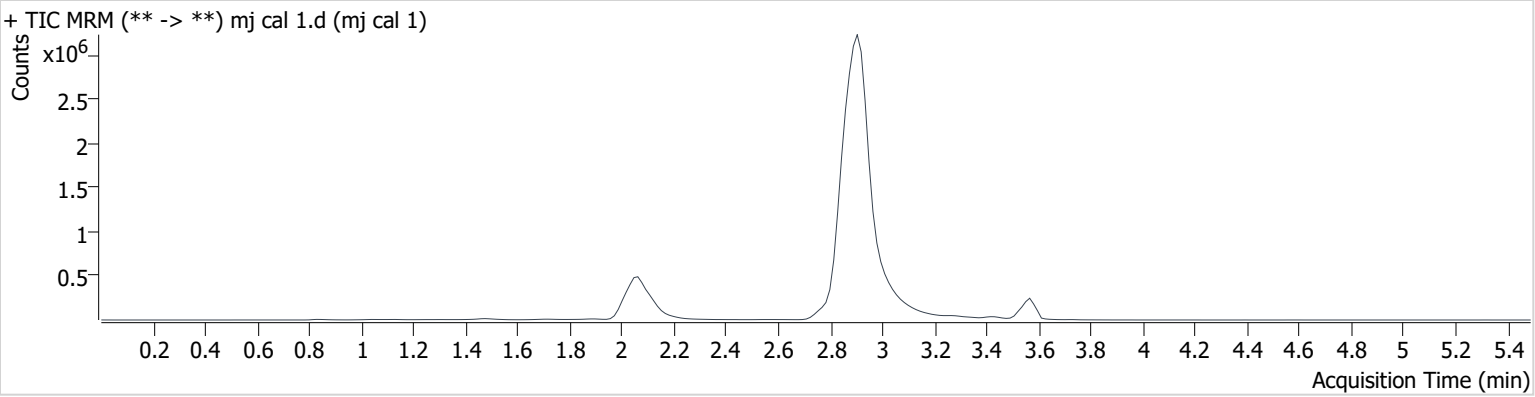
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	119.5
mj cal 2	2	✔	3.0	2.9	97.7
mj cal 3	3	✔	5.0	4.6	92.7
mj cal 4	4	✔	10.0	9.2	91.9
mj cal 5	5	✔	25.0	24.0	96.0
mj cal 6	6	✔	50.0	50.2	100.4
mj cal 7	7	✔	100.0	101.9	101.9

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj cal 1.d
Type	Cal	**Sample**	mj cal 1
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:02:26 PM		
Sample Info.			

Sample Chromatogram



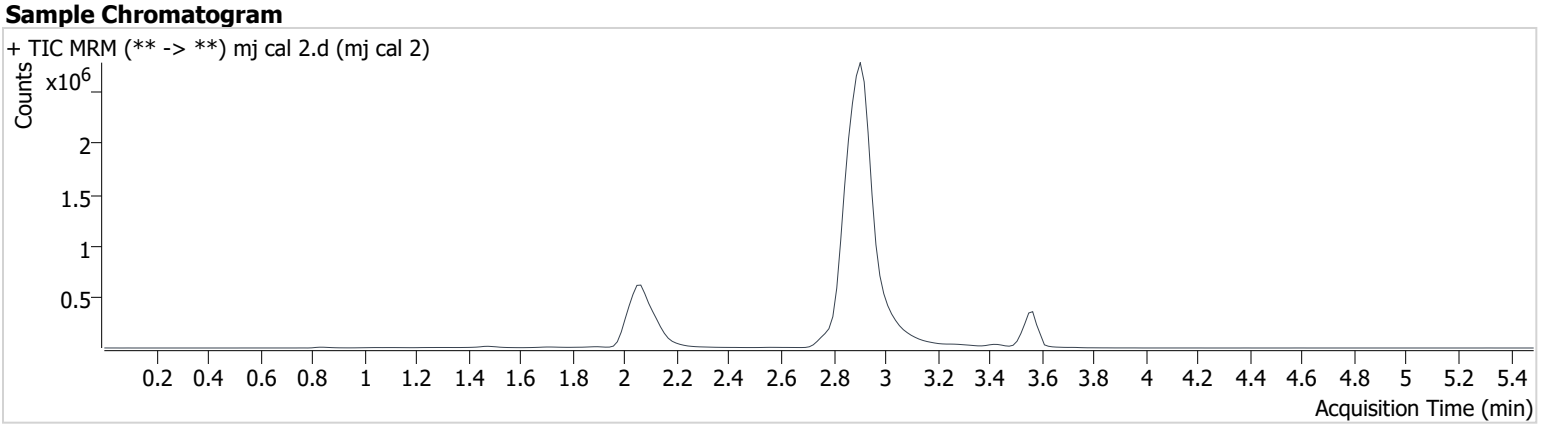
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.066	6785	146.6	716.29	150.2	2243332	1.195	ng/ml Low
THC-COOH	2.137	19046	127.8	279.56	44073.8	863529	5.318	ng/ml
THC	3.573	15977	492.2	24.68	∞	590881	1.158	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj cal 2.d
Type	Cal	**Sample**	mj cal 2
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:09:03 PM		
Sample Info.			

Sample Chromatogram



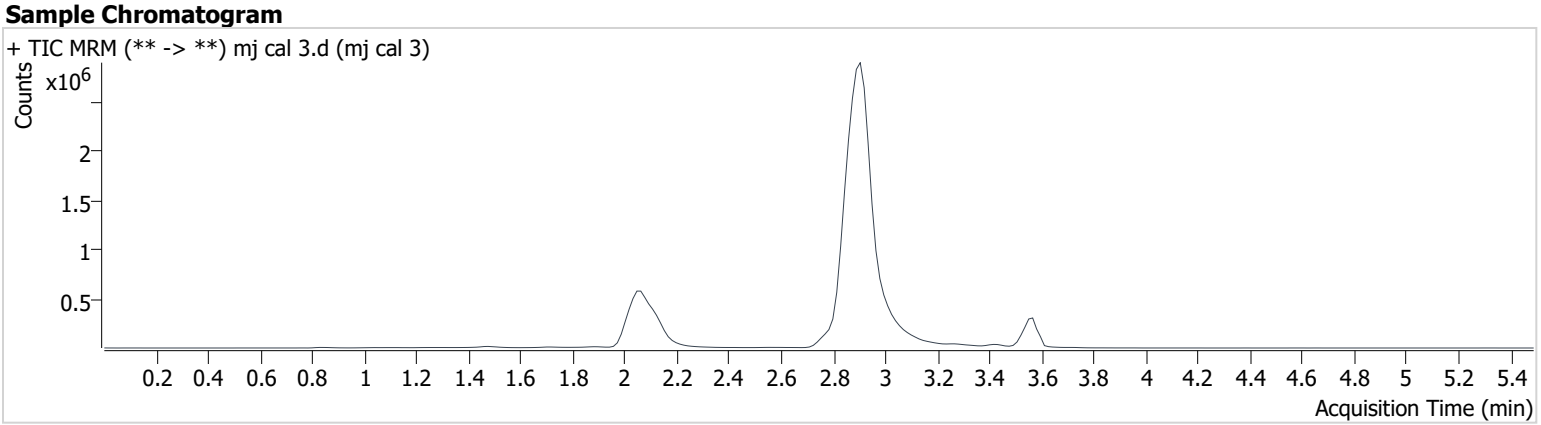
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	24306	∞	816.34	∞	2759058	2.930	ng/ml Low
THC-COOH	2.122	45322	70655.3	268.80	3227.4	953506	9.980	ng/ml
THC	3.573	70385	∞	28.23	∞	888325	2.806	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj cal 3
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:15:37 PM		
Sample Info.			

Sample Chromatogram



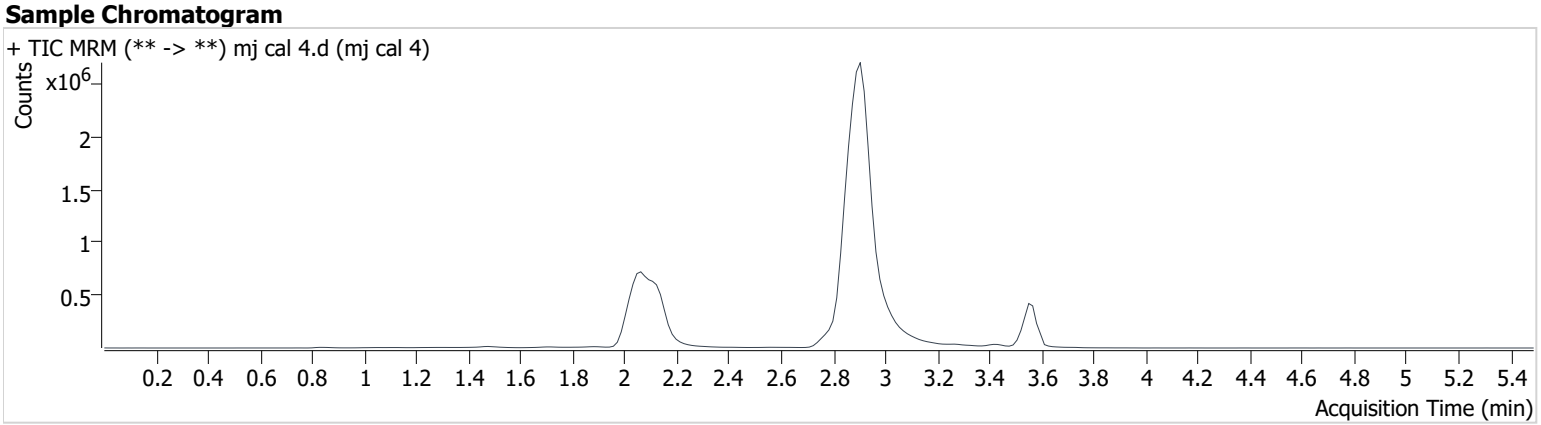
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	34768	∞	873.76	∞	2399562	4.634	ng/ml
THC-COOH	2.122	87616	474.3	268.17	786.4	886563	19.365	ng/ml
THC	3.573	95489	42831.1	28.24	∞	698111	4.623	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj cal 4.d
Type	Cal	**Sample**	mj cal 4
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:22:11 PM		
Sample Info.			

Sample Chromatogram



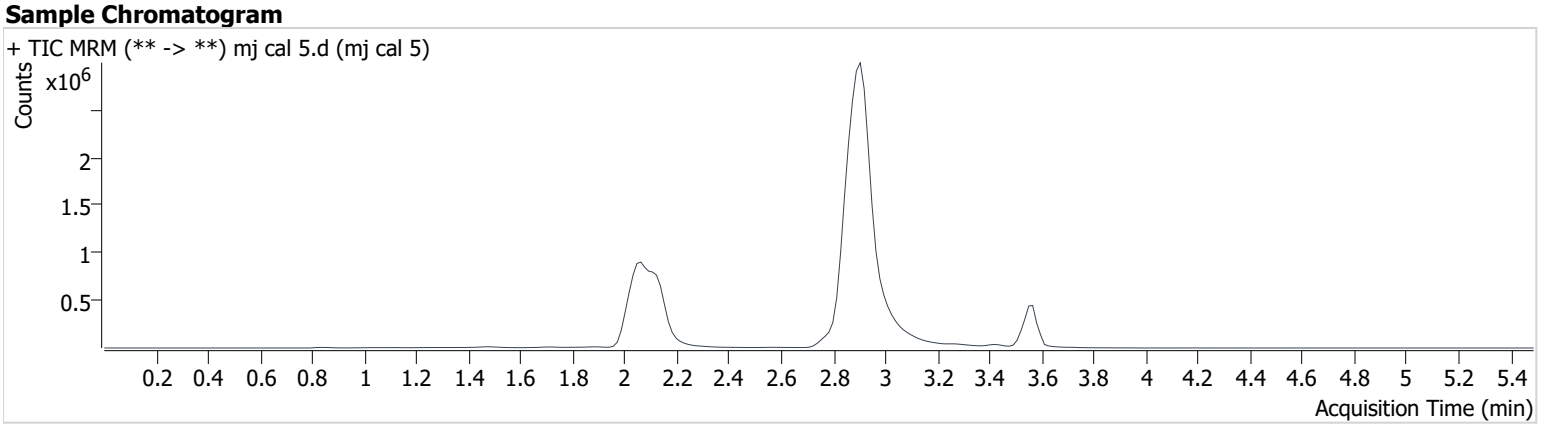
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	76728	∞	886.38	1084.9	2584149	9.193	ng/ml
THC-COOH	2.122	238309	1395.5	269.79	560.7	919193	48.721	ng/ml
THC	3.558	252455	∞	24.29	8290.7	839972	9.793	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj cal 5
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:28:48 PM		
Sample Info.			

Sample Chromatogram



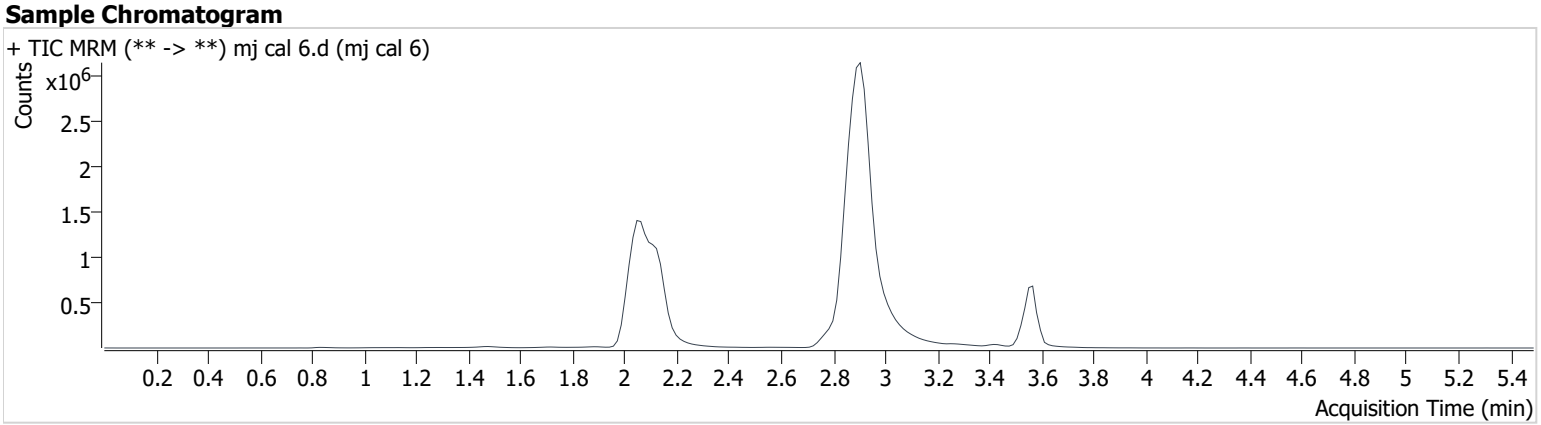
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	190947	3355.4	868.13	∞	2416197	23.989	ng/ml
THC-COOH	2.122	338650	28469.8	267.20	1735.7	854682	73.783	ng/ml
THC	3.573	521621	25634.2	25.54	2651.0	673587	24.751	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj cal 6
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:35:22 PM		
Sample Info.			

Sample Chromatogram



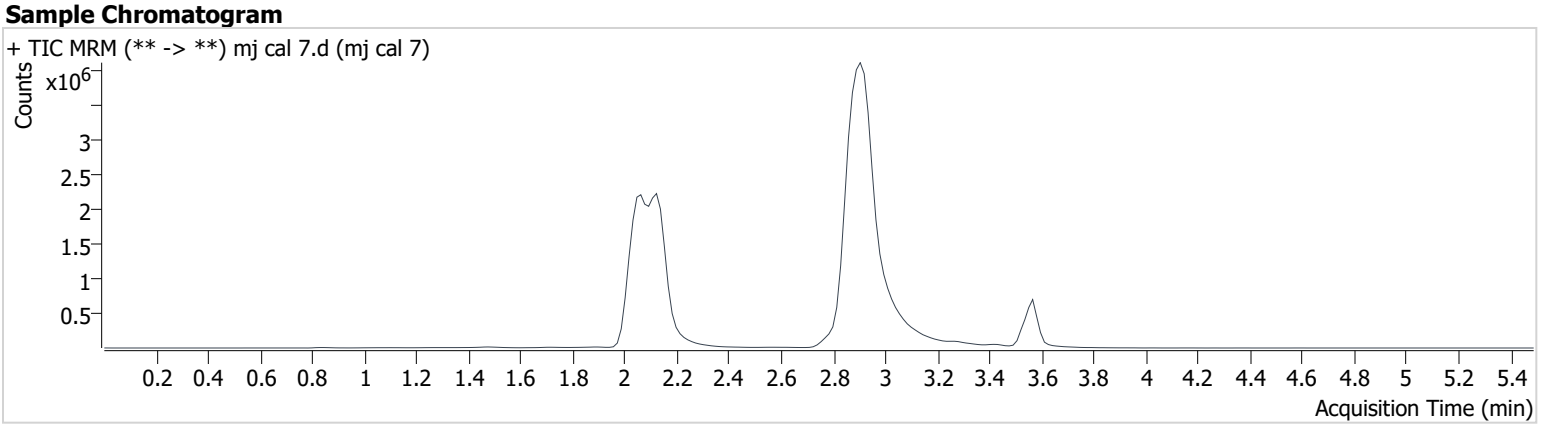
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	446307	∞	834.83	∞	2682449	50.186	ng/ml
THC-COOH	2.122	493108	572702.5	271.37	483205.8	913118	100.095	ng/ml
THC	3.573	1126680	764372.2	26.36	∞	710200	50.385	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2024\am 27-28\040824\QuantResults\am 27 4-8-24.batch.bin
Calibration Last Update 4/8/2024 3:12:16 PM

Instrument	69679	**Data File**	mj cal 7.d
Type	Cal	**Sample**	mj cal 7
Acq. Method	thc quant 50 50.m	**Operator**	Britany Wylie
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/8/2024 12:41:56 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.051	864904	36265.7	834.40	∞	2553369	101.874	ng/ml
THC-COOH	2.122	1140854	1287.8	268.09	∞	830168	252.737	ng/ml
THC	3.573	1431052	79687.3	25.61	18591.3	450943	100.485	ng/ml