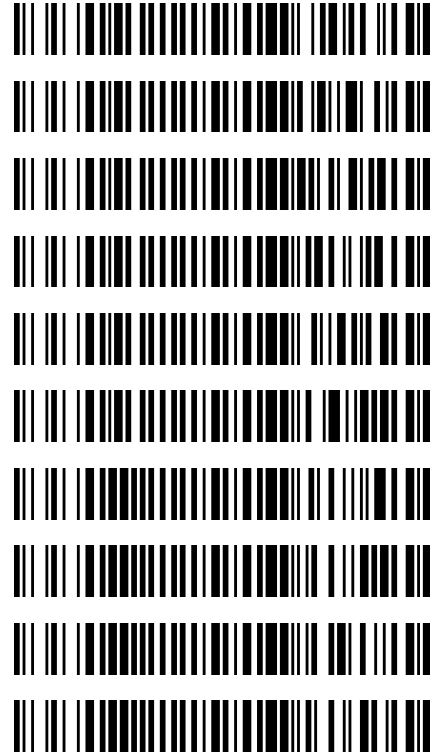


Worklist: 6124

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-3438	3	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3588	3	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3655	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3659	4	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3741	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3742	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2582	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ
P2022-2761	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2765	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2808	1	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: 10/12/2022

Analyst: Tamara Salazar

Plate lot#: 220802

Plate Retest Date: 02/02/2023

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 22B52015-1

Blank Urine Lot: N/A

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 069901

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.
- 3. Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: 42**
- 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 5. 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.
- 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 7. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
Amount transferred: 750µL
- 8. 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)
- 9. Wait 5 minutes.
- 10. Add **2.25mL MTBE. (Add in 3 increments of 750uL)**
- 11. Wait 5 minutes.
- 12. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 13. Add **2.25mL Hexane. (Add in 3 increments of 750uL)**
- 14. Wait 5 minutes.
- 15. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 16. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
- 17. 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 samples with calculated concentrations for THC at 1ng/mL or greater and OH-THC at 3ng/mL or greater may be reported quantitatively (blood only). Calculated concentrations for carboxy-THC of 5ng/mL may be reported qualitatively. 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, 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 – calibrator 1 dropped due to ratio

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	P2022-2582-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	Neg Blood	P2022-2761-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2022-3438-3	P2022-2765-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	M2022-3588-3	P2022-2808-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	M2022-3655-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	M2022-3659-4	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	M2022-3741-2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	M2022-3742-3	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	P2022-2582-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	Neg Blood	P2022-2761-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2022-3438-3*	P2022-2765-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	M2022-3588-3	P2022-2808-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	M2022-3655-1	M2022-3438-3	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	M2022-3659-4	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	M2022-3741-2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	M2022-3742-3	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

*Moved during step 7
of extraction due to
blood clot

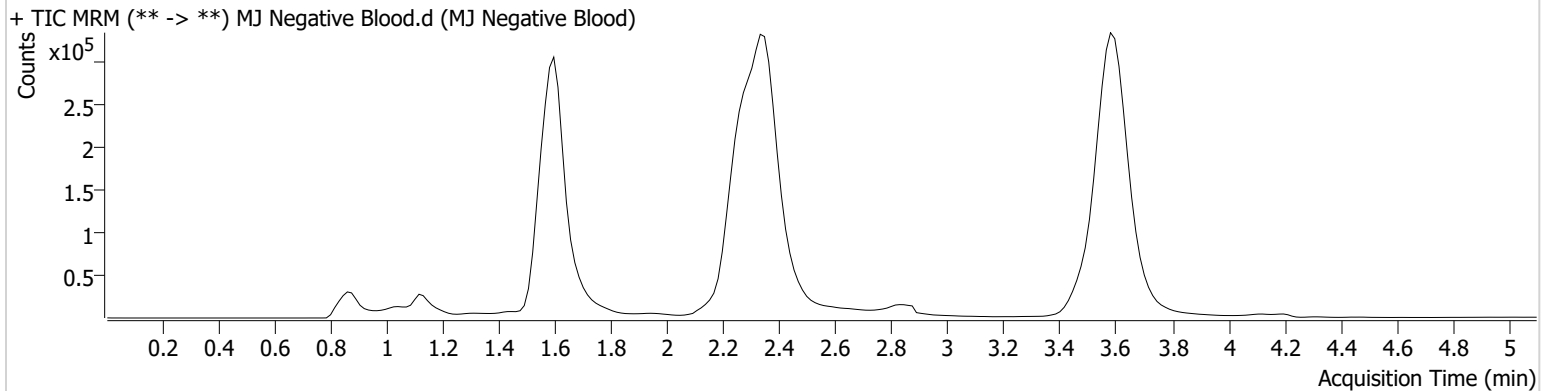
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-B2	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 5:59:19 PM		
Sample Info.			

Sample Chromatogram



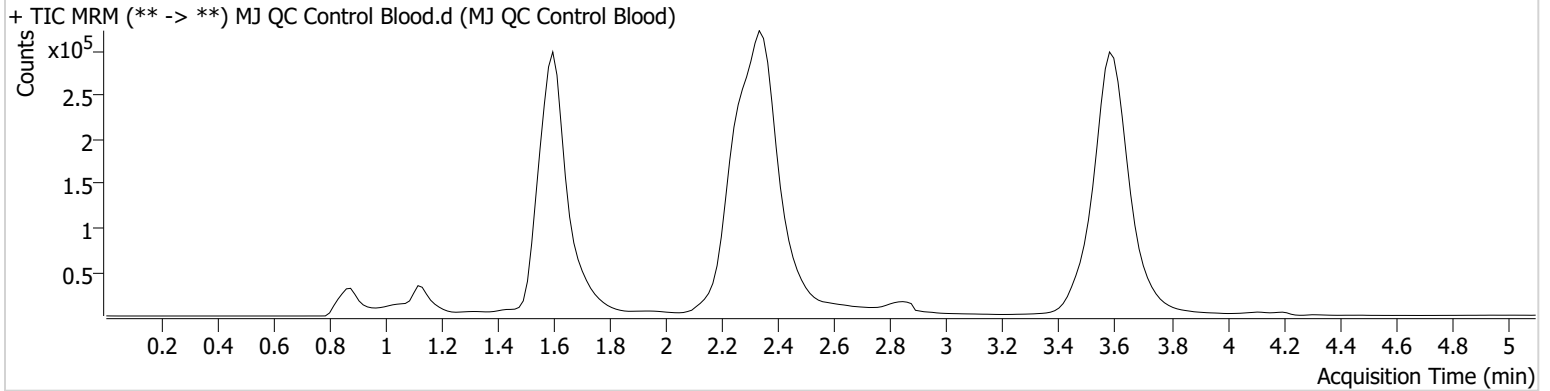


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 5:44:04 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	14068	205.52	921.7	∞	1384708	4.4806 ng/ml
THC-COOH	1.625	104981	∞	56.5	304.18	294260	13.9757 ng/ml
THC	3.601	119227	∞	30.0	188.32	2546174	4.8876 ng/ml

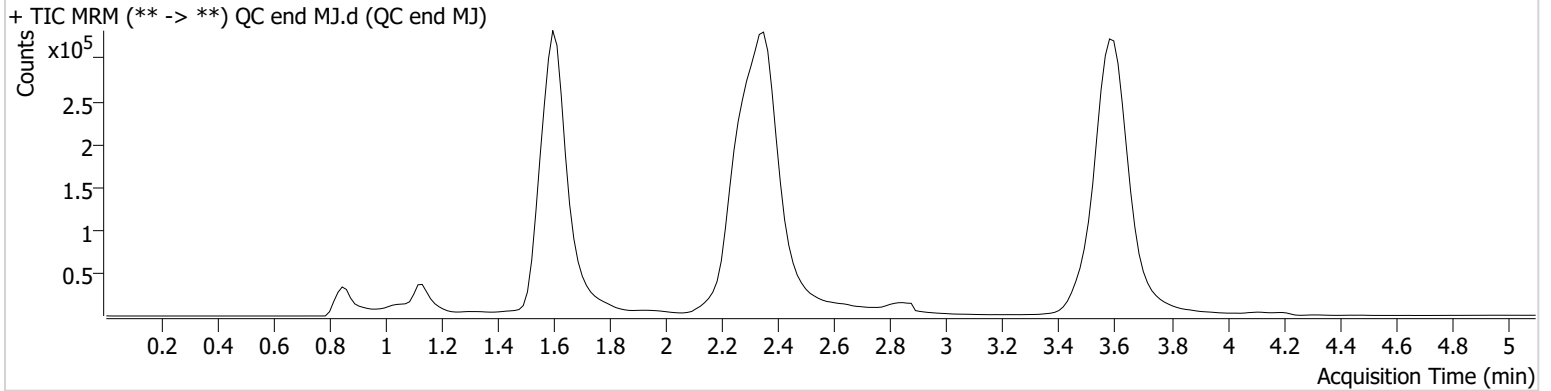


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A2	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 8:46:46 PM		
Sample Info.			

Sample Chromatogram

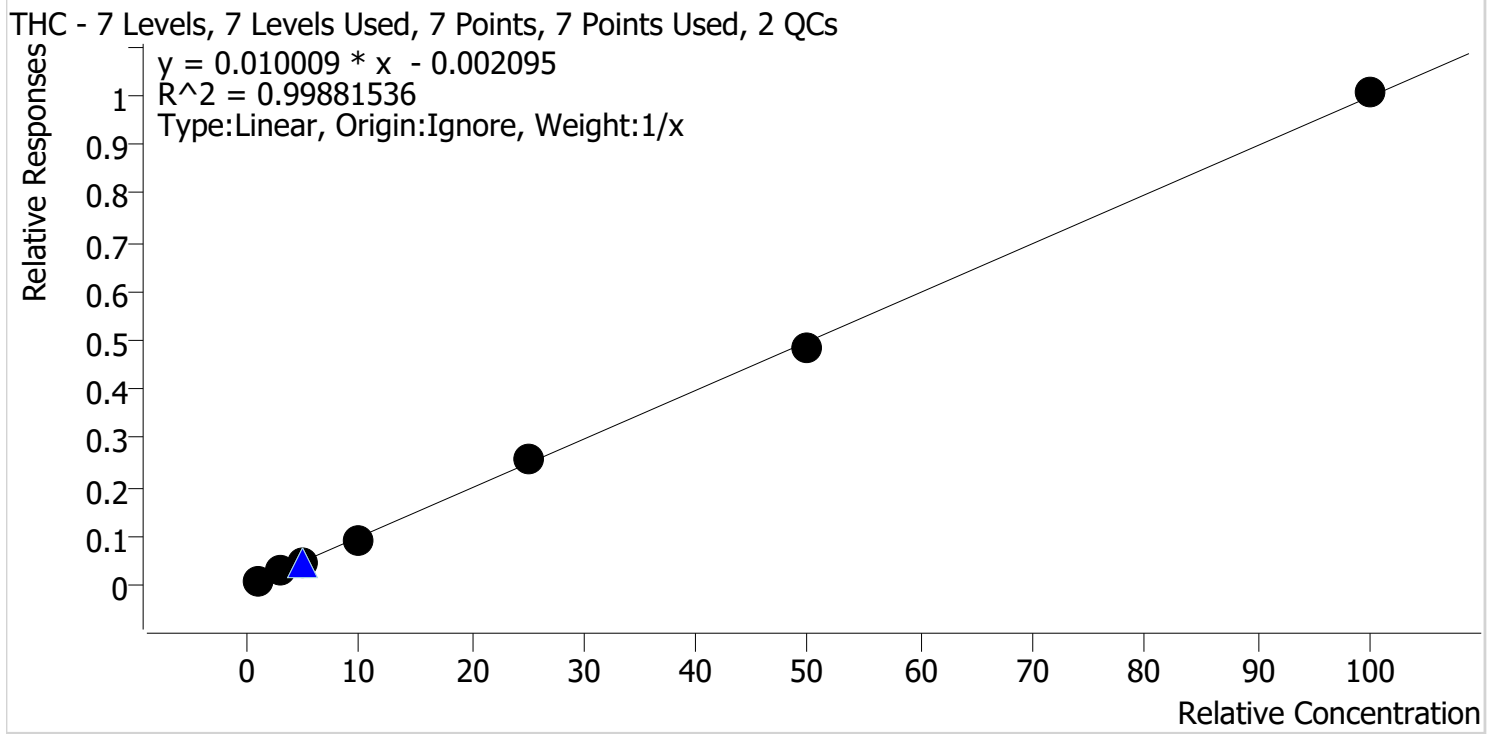


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	16636	∞	799.4	∞	1464901	5.0188 ng/ml
THC-COOH	1.640	110069	377.64	60.3	∞	306093	14.0897 ng/ml
THC	3.601	112222	∞	33.1	∞	2689123	4.3786 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Last Cal. Update 10/13/2022 2:20 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

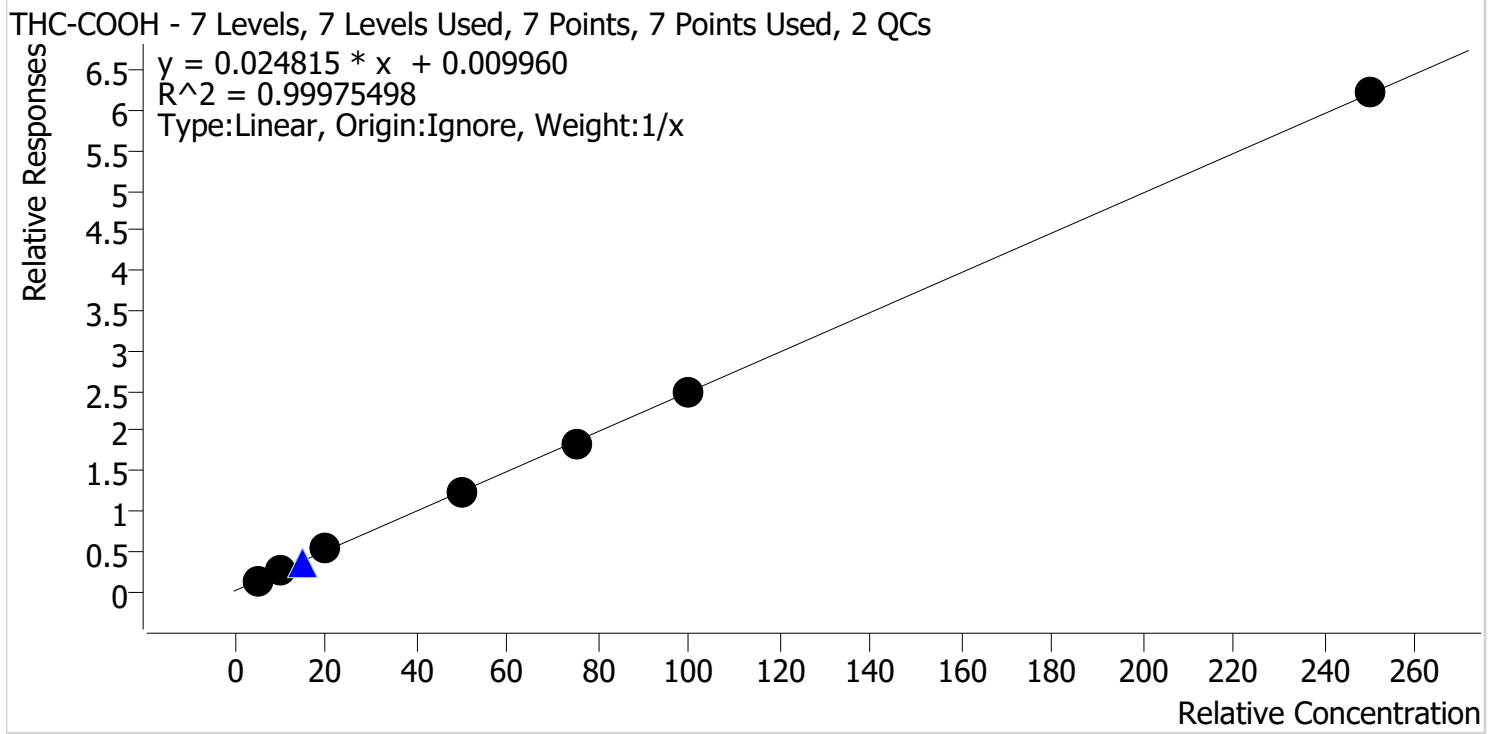


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	106.0
Cal 2 MJ	2	✓	3.0	3.2	105.1
Cal 3 MJ	3	✓	5.0	4.7	93.9
Cal 4 MJ	4	✓	10.0	9.1	91.5
Cal 5 MJ	5	✓	25.0	26.2	105.0
Cal 6 MJ	6	✓	50.0	48.9	97.8
Cal 7 MJ	7	✓	100.0	100.8	100.8



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Last Cal. Update 10/13/2022 2:20 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



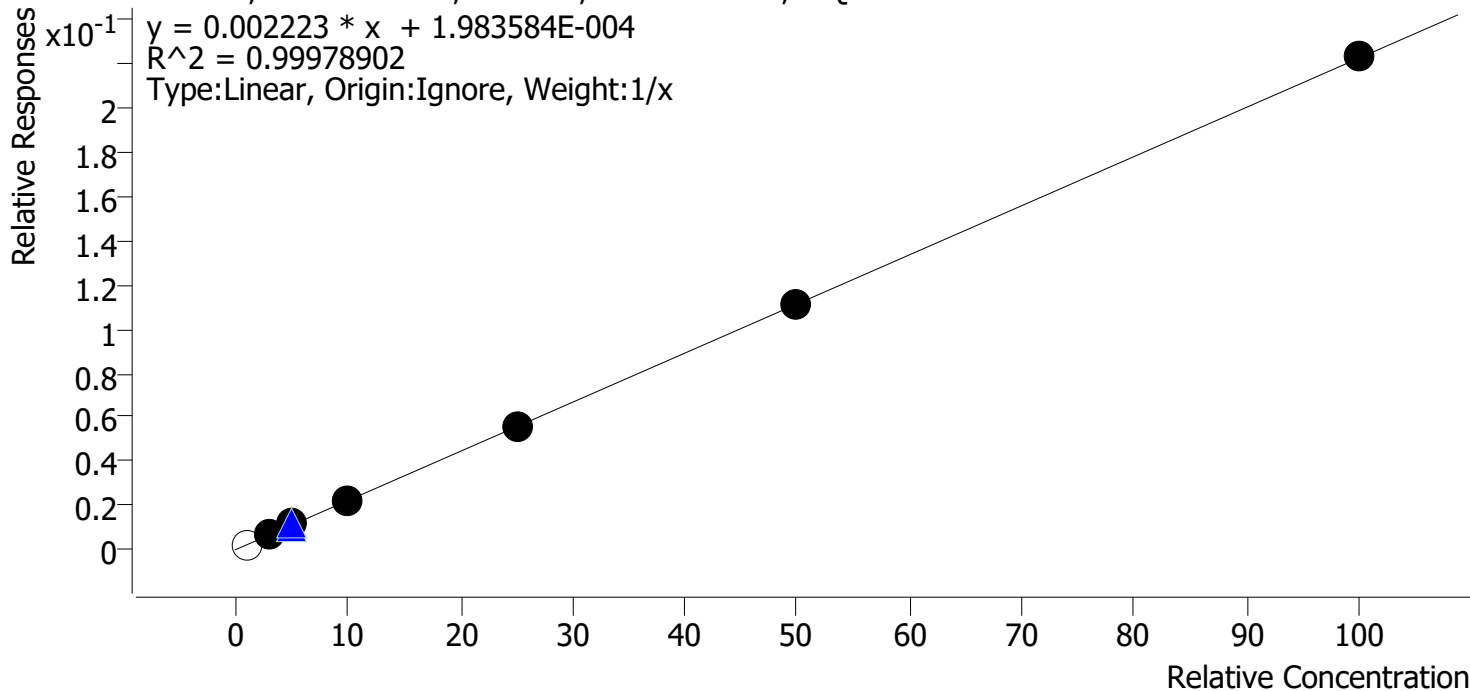
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	4.9	98.0
Cal 2 MJ	2	✓	10.0	9.9	98.6
Cal 3 MJ	3	✓	20.0	21.1	105.6
Cal 4 MJ	4	✓	50.0	49.5	98.9
Cal 5 MJ	5	✓	75.0	73.8	98.4
Cal 6 MJ	6	✓	100.0	100.4	100.4
Cal 7 MJ	7	✓	250.0	250.5	100.2



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Last Cal. Update 10/13/2022 2:20 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
Cal 1 MJ	1	x	1.0	1.0	99.6
Cal 2 MJ	2	✓	3.0	3.0	100.7
Cal 3 MJ	3	✓	5.0	5.1	102.9
Cal 4 MJ	4	✓	10.0	9.6	95.5
Cal 5 MJ	5	✓	25.0	25.3	101.1
Cal 6 MJ	6	✓	50.0	49.7	99.4
Cal 7 MJ	7	✓	100.0	100.3	100.3



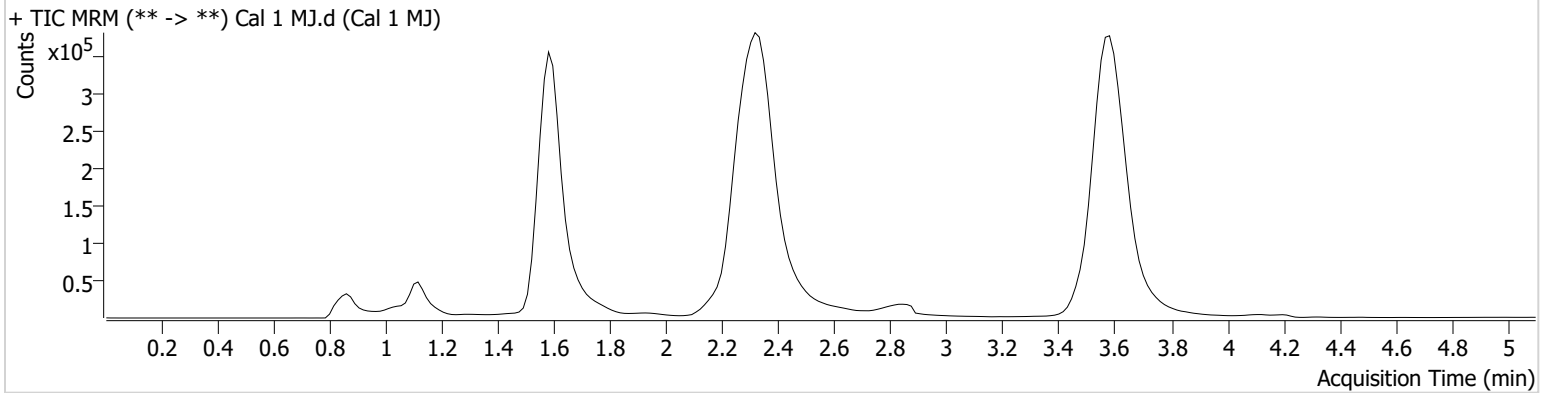
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 4:43:03 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.590	3983	18.85	1435.0 High	9.32 Low	1650356	0.9963 ng/ml Low
THC-COOH	1.625	43063	90.08	51.9	111.66	327339	4.9001 ng/ml Low
THC	3.601	27882	13.54	37.1	34.95	3274145	1.0601 ng/ml

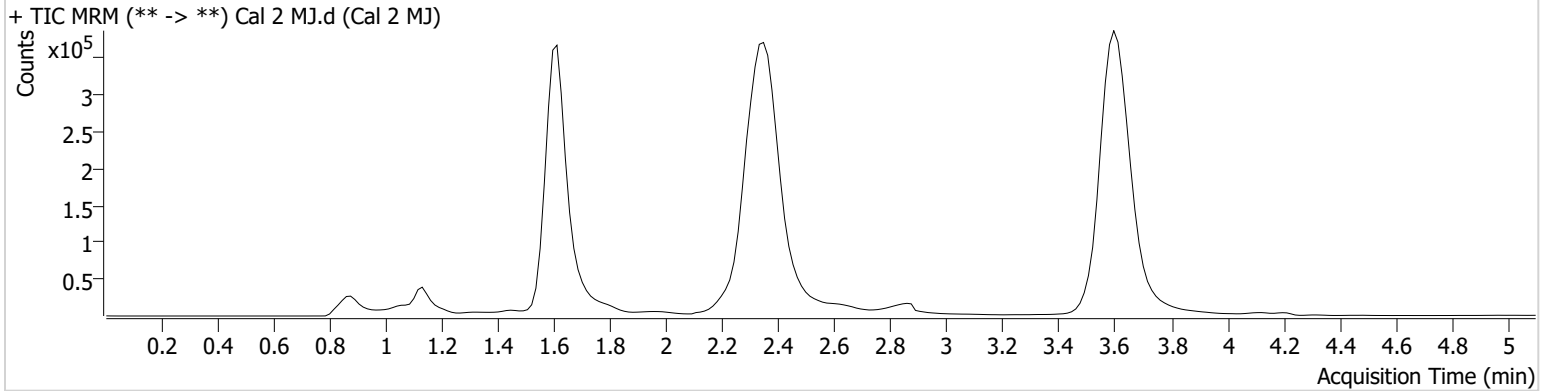


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-B1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 4:50:49 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	9965	163.31	957.9	∞	1440689	3.0219 ng/ml
THC-COOH	1.640	76273	106.79	53.6	∞	299677	9.8553 ng/ml
THC	3.616	79558	∞	33.9	73.16	2700082	3.1531 ng/ml

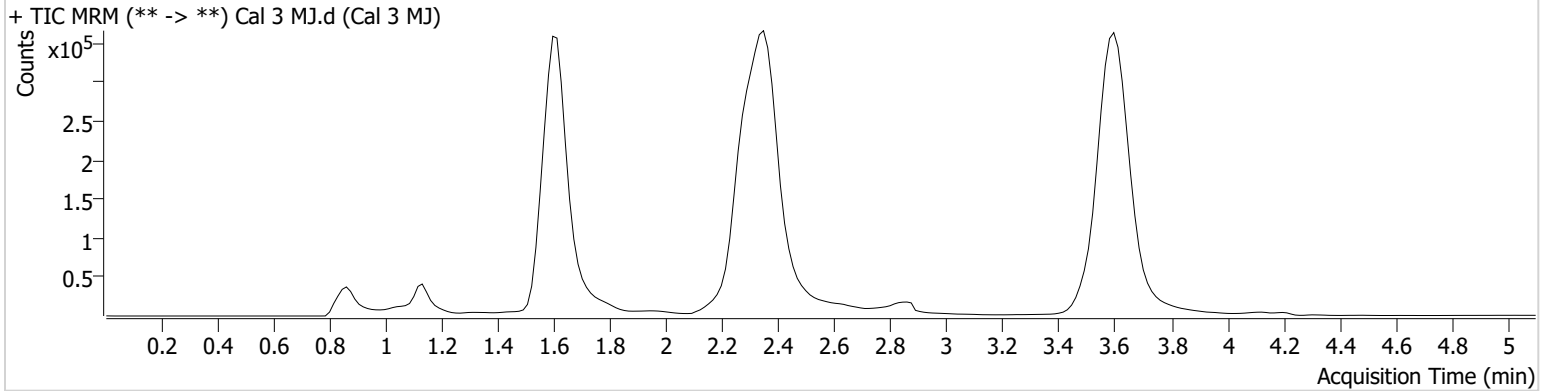


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 4:58:25 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	17016	274.12	813.1	∞	1462073	5.1455 ng/ml
THC-COOH	1.640	157476	∞	54.9	466.03	294940	21.1151 ng/ml
THC	3.616	118228	565.72	33.6	64.78	2634267	4.6932 ng/ml

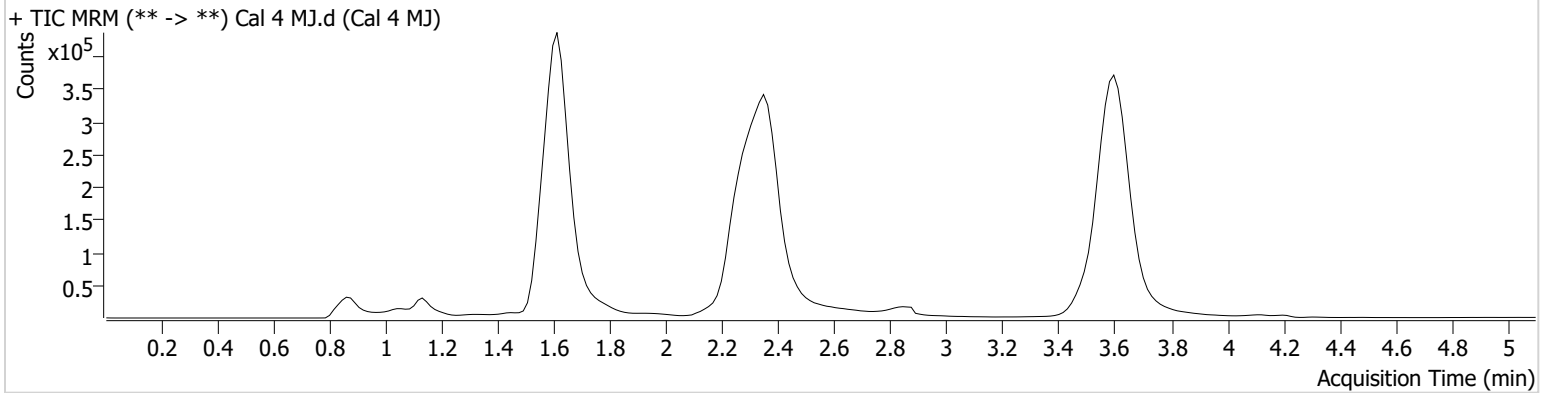


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 5:06:01 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	32387	408.48	810.5	∞	1510602	9.5542 ng/ml
THC-COOH	1.640	384581	∞	58.2	∞	310794	49.4646 ng/ml
THC	3.616	232498	∞	31.5	∞	2598840	9.1473 ng/ml

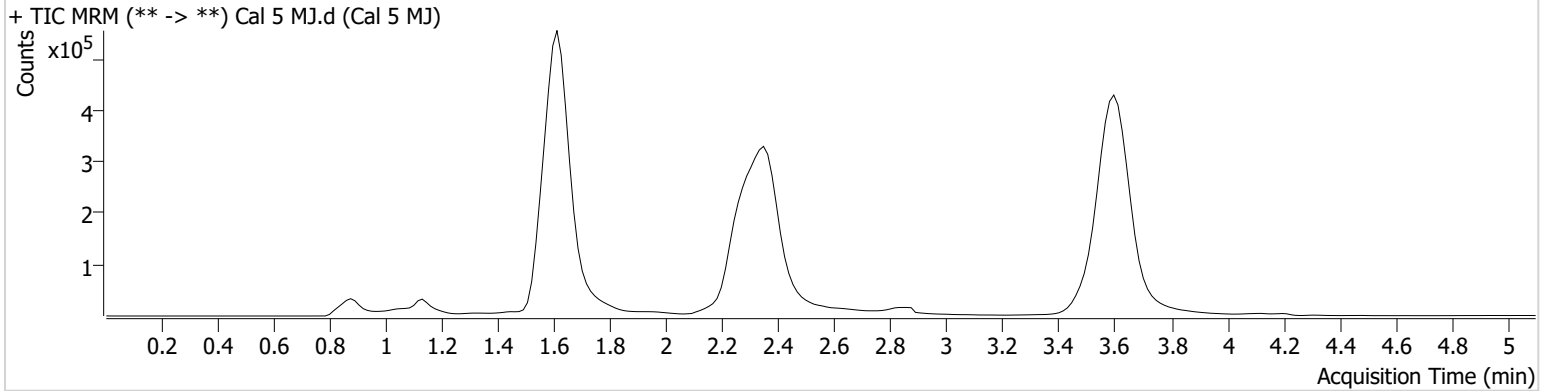


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 5:13:37 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	85428	1002.69	734.5	∞	1514788	25.2775 ng/ml
THC-COOH	1.640	568488	∞	59.0	1756.86	308882	73.7669 ng/ml
THC	3.616	676211	2681.64	27.1	∞	2595410	26.2393 ng/ml

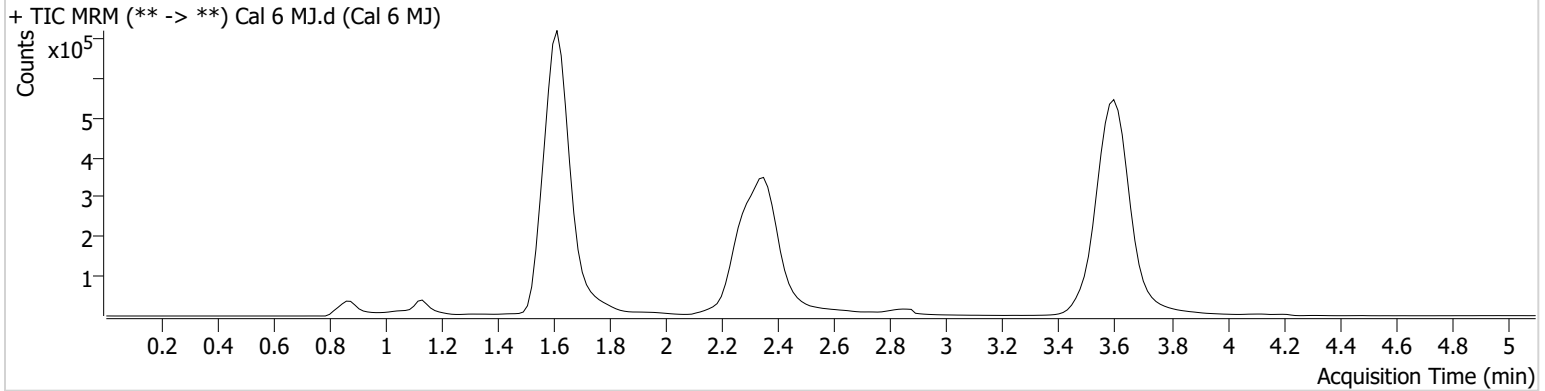


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 5:21:13 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	166882	3678.92	748.9	∞	1507395	49.7069 ng/ml
THC-COOH	1.640	756928	1643.29	58.8	∞	302682	100.3747 ng/ml
THC	3.601	1298934	∞	29.2	503.16	2666040	48.8857 ng/ml

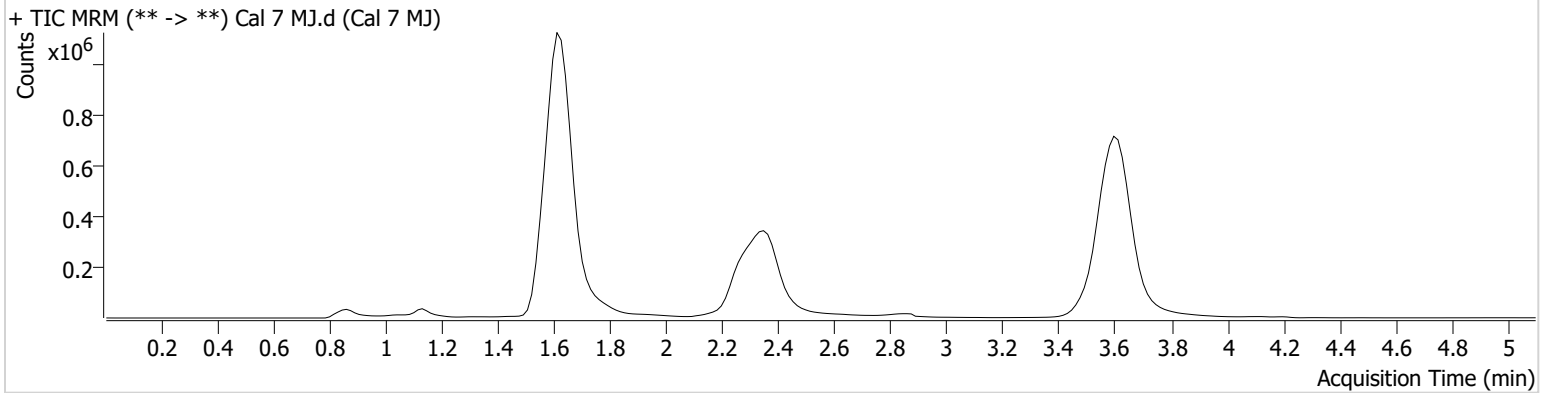


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\101222 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 10/13/2022 2:20:56 PM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	10/12/2022 5:28:48 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.605	312003	6382.02	736.2	∞	1398011	100.2940 ng/ml
THC-COOH	1.640	1734318	∞	59.8	∞	278532	250.5233 ng/ml
THC	3.616	2514210	∞	27.1	1239.35	2496606	100.8213 ng/ml