

**REVIEWED**  
By Tamara Salazar at 9:52 am, Dec 21, 2022

**Worklist: 6186**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2021-3542	4	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4184	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4275	4	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4512	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4606	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4840	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4846	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3489	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3490	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3497	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3498	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3581	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3594	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3620	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: 12/09/2022

Analyst: Celena Shrum

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 22B52015-2

**Column:** Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

**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 (if applicable): 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 or 1000µl hydrolyzed urine** into the appropriate wells of the analytical (standards) plate. **Pipette ID: #27**
- 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 or 500µl of saturated phosphate buffer to urine samples** to the appropriate wells of the analytical plate.
- 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 7. Transfer **800µL of blood+acid mixture or urine+acid** to corresponding wells of SLE+ plate.
- 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)** Manifold ID: 067104
- 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. **SPE Dry ID: 067103**
- 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<sup>2</sup> 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 and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/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, 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-COOH curve limit:10-250 (cal 1 removed due to ratio), THC-OH curve limit: 3-100 (cal1 removed due to ratio)

	1	2	3	4	5	6
a				P2022-3620-1	M2022-4840-2	QC 1
b				P2022-3594-1	M2022-4606-1	cal 100 ng
c				P2022-3581-1	M2022-4512-1	cal 50 ng
d				P2022-3498-1	M2022-4275-4	cal 25 ng
e				P2022-3497-1	M2022-4184-1	cal 10ng
f				P2022-3490-1	M2021-3542-4	cal 5 ng
g				P2022-3489-1	NEG Blood	cal 3 ng
h				M2022-4846-2	QC 2	cal 1ng

CS

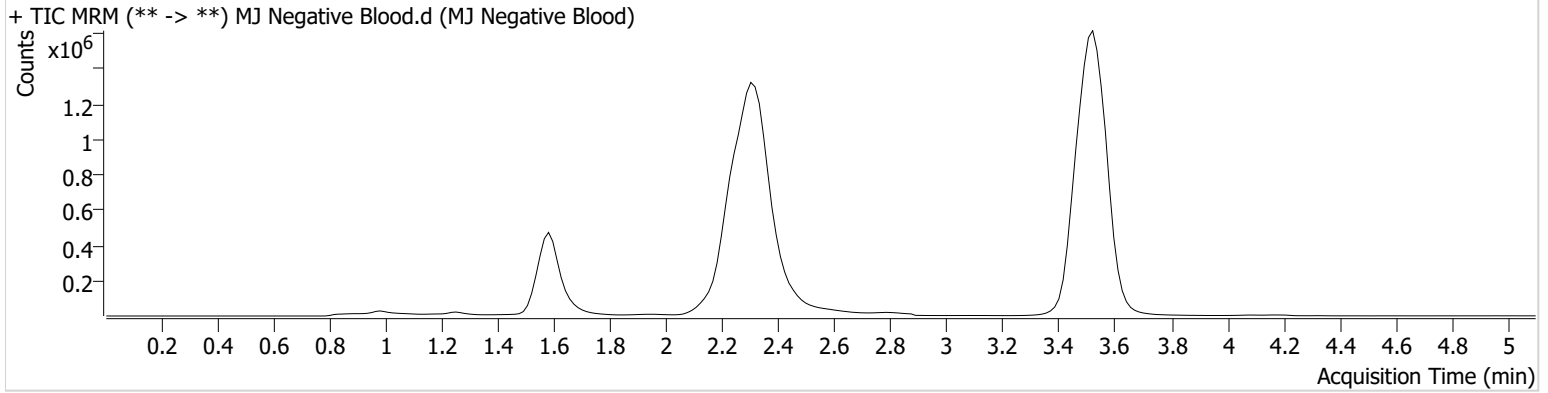


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Negative Blood.d
<b>Type</b>	Sample	<b>Sample</b>	MJ Negative Blood
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Celena Shrum
<b>Sample Position</b>	P1-G5	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	12/9/2022 5:13:32 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



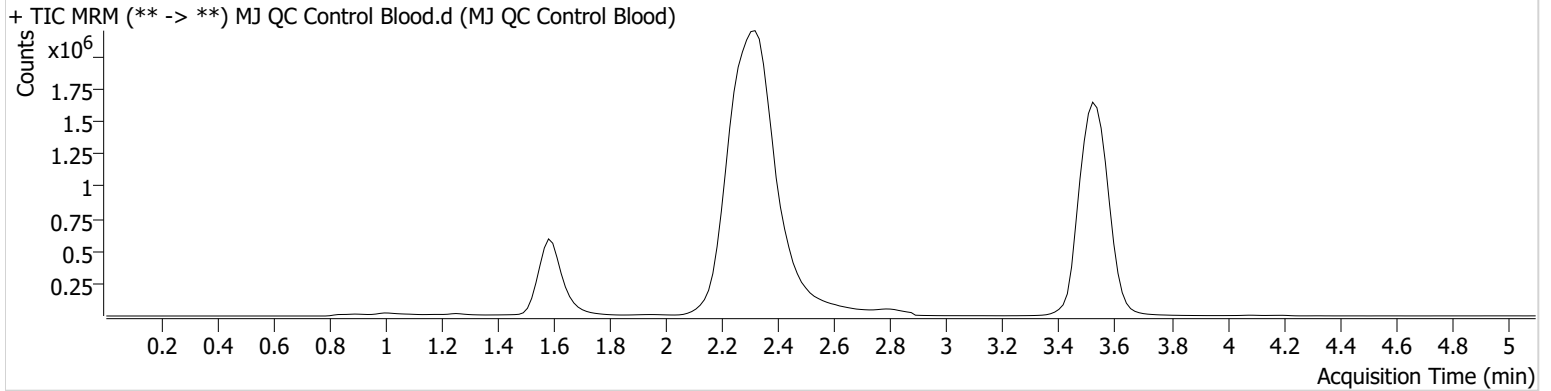


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** MJ QC Control Blood.d  
**Type** QC **Sample** MJ QC Control Blood  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-A6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 4:58:17 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	246741	∞	8.3	541.17	2205208	4.9020 ng/ml
THC-COOH	1.610	297017	232.78	41.7	671.18	602385	16.0871 ng/ml
THC	3.540	535207	2638.51	24.8	∞	11466698	5.0730 ng/ml

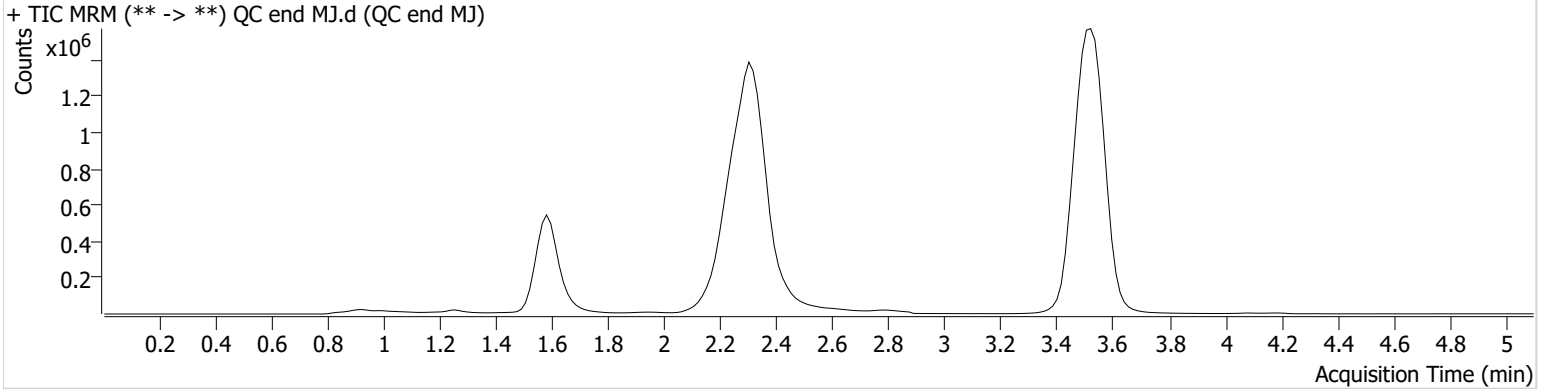


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** QC end MJ.d  
**Type** QC **Sample** QC end MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-H5 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 9:02:09 PM  
**Sample Info.**

## Sample Chromatogram

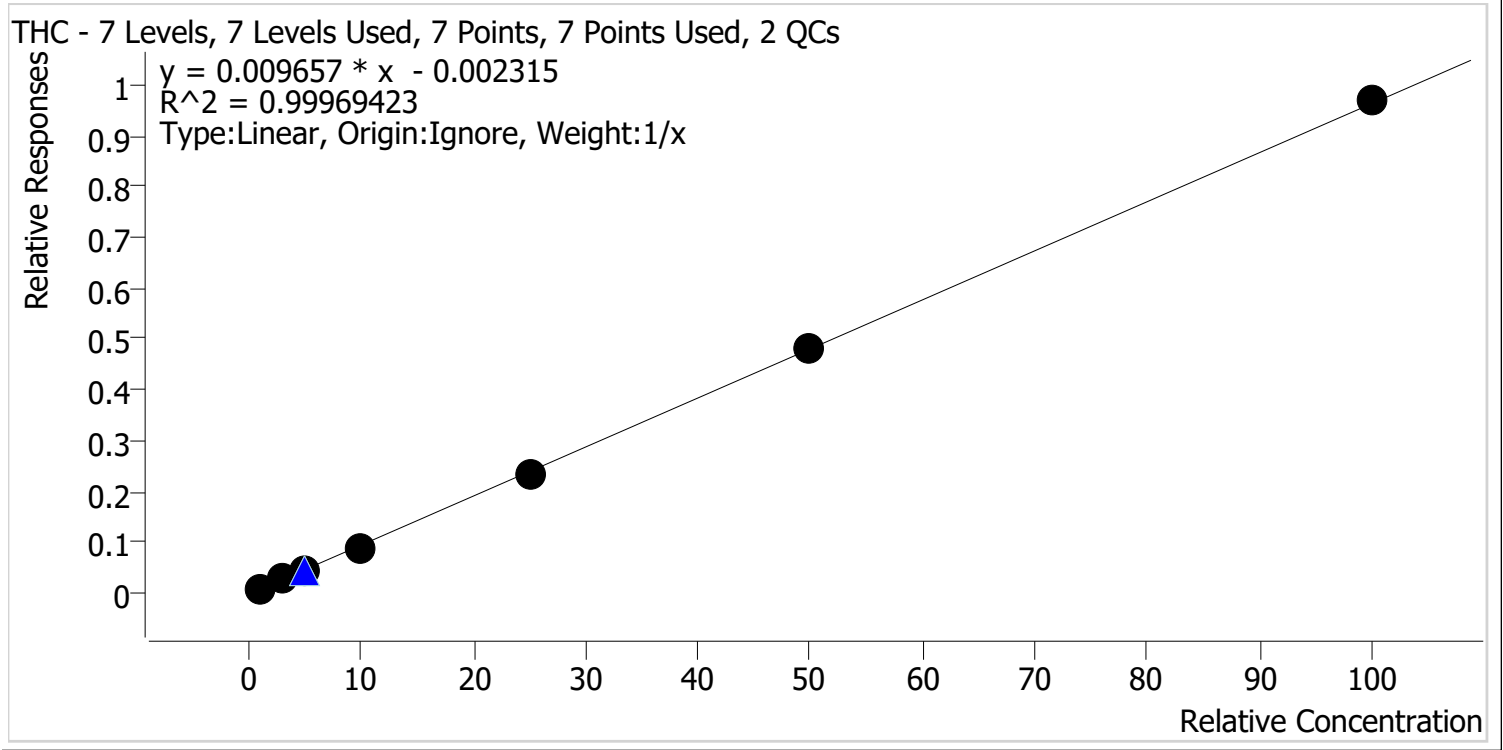


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	222210	∞	8.7	325.57	1991349	4.8833 ng/ml
THC-COOH	1.610	227634	156.77	45.4	∞	543469	13.0507 ng/ml
THC	3.540	508100	2545.24	24.5	∞	11392671	4.8580 ng/ml



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 12/15/2022 8:32 AM  
**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	111.3
Cal 2 MJ	2	✓	3.0	2.9	96.9
Cal 3 MJ	3	✓	5.0	4.8	96.0
Cal 4 MJ	4	✓	10.0	9.6	96.0
Cal 5 MJ	5	✓	25.0	24.7	98.6
Cal 6 MJ	6	✓	50.0	50.4	100.7
Cal 7 MJ	7	✓	100.0	100.6	100.6

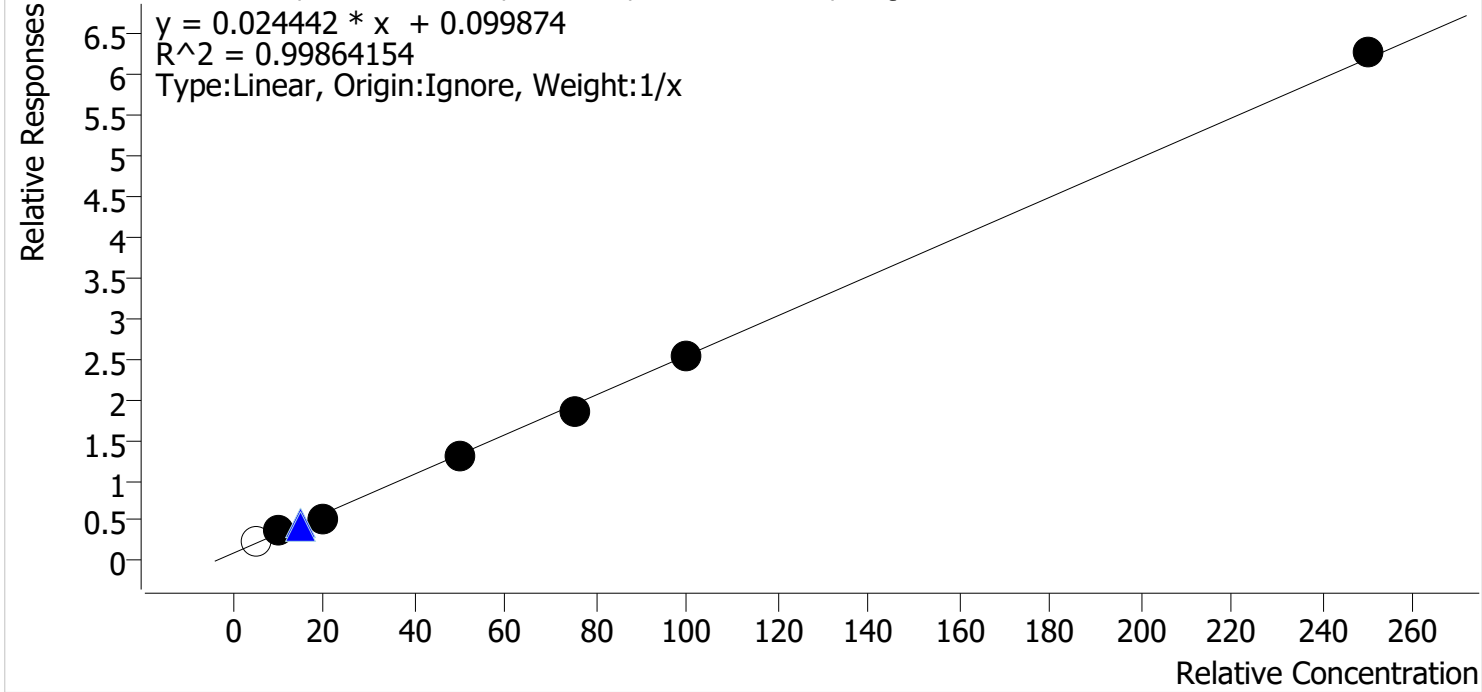
CS



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 12/15/2022 8:32 AM  
**Analyst Name** ISP\Datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-D9

THC-COOH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 2 QCs



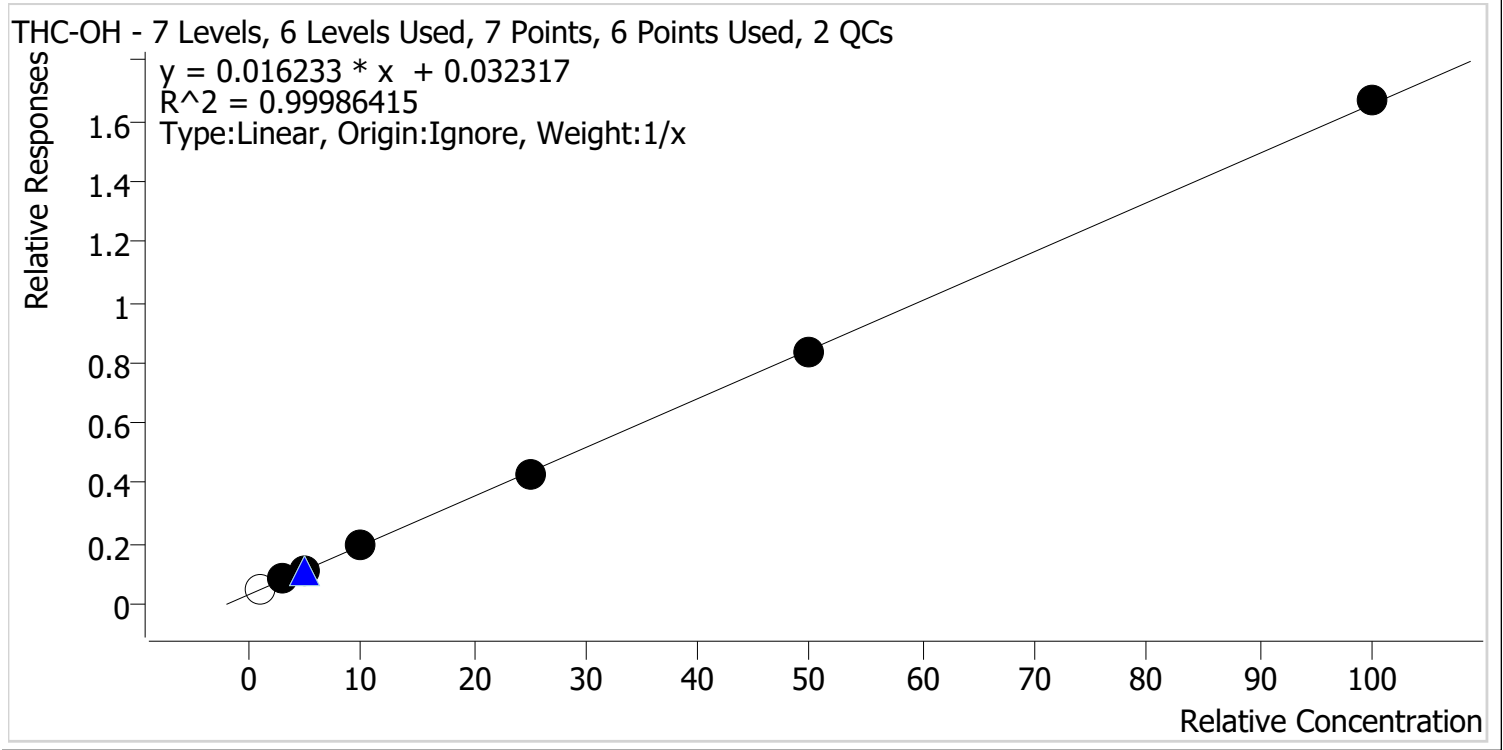
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	5.0	6.3	125.8
Cal 2 MJ	2	✓	10.0	11.1	111.5
Cal 3 MJ	3	✓	20.0	18.1	90.5
Cal 4 MJ	4	✓	50.0	49.9	99.8
Cal 5 MJ	5	✓	75.0	72.4	96.6
Cal 6 MJ	6	✓	100.0	100.5	100.5
Cal 7 MJ	7	✓	250.0	253.0	101.2





# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 12/15/2022 8:32 AM  
**Analyst Name** ISP\Datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	1.2	120.3
Cal 2 MJ	2	✓	3.0	3.1	103.8
Cal 3 MJ	3	✓	5.0	4.9	98.1
Cal 4 MJ	4	✓	10.0	10.0	99.5
Cal 5 MJ	5	✓	25.0	24.7	98.7
Cal 6 MJ	6	✓	50.0	49.6	99.2
Cal 7 MJ	7	✓	100.0	100.7	100.7

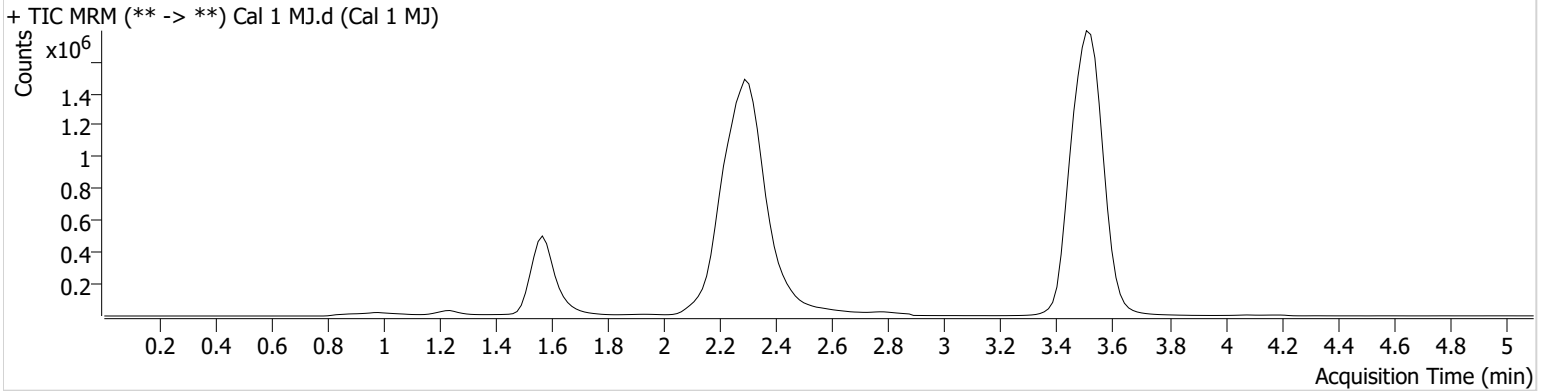


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** Cal 1 MJ.d  
**Type** Cal **Sample** Cal 1 MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-H6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 3:57:17 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	110469	∞	5.1 <b>Low</b>	17.55	2130837	1.2029 ng/ml <b>Low</b>
THC-COOH	1.610	153196	∞	25.5 <b>Low</b>	∞	604074	6.2897 ng/ml
THC	3.525	120865	290.76	26.6	∞	14339630	1.1125 ng/ml

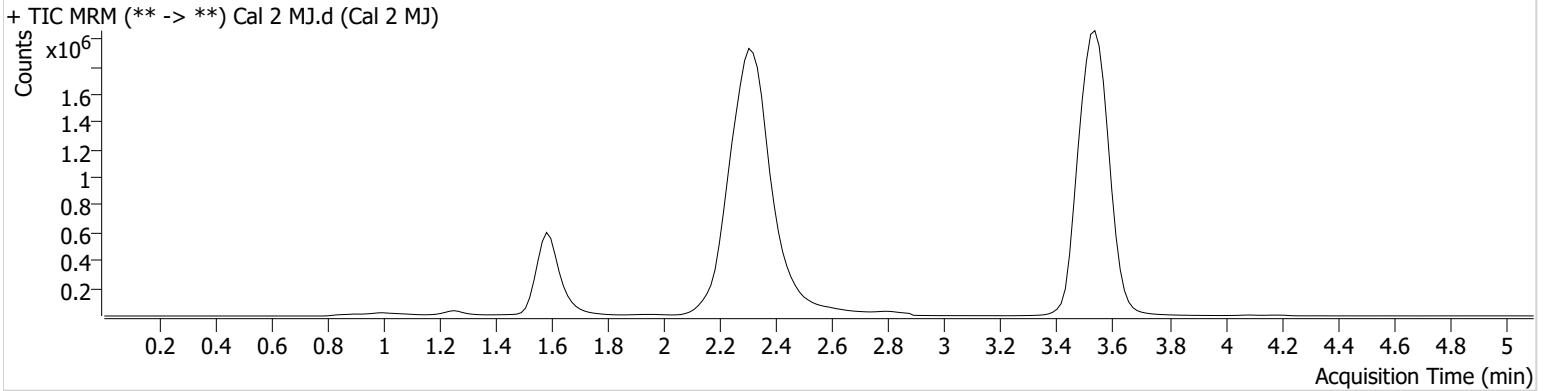


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** Cal 2 MJ.d  
**Type** Cal **Sample** Cal 2 MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-G6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 4:05:04 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	192592	∞	8.2	148.24	2324784	3.1126 ng/ml
THC-COOH	1.625	239594	∞	43.9	383.33	643396	11.1496 ng/ml
THC	3.555	395767	∞	25.8	387.70	15368318	2.9064 ng/ml

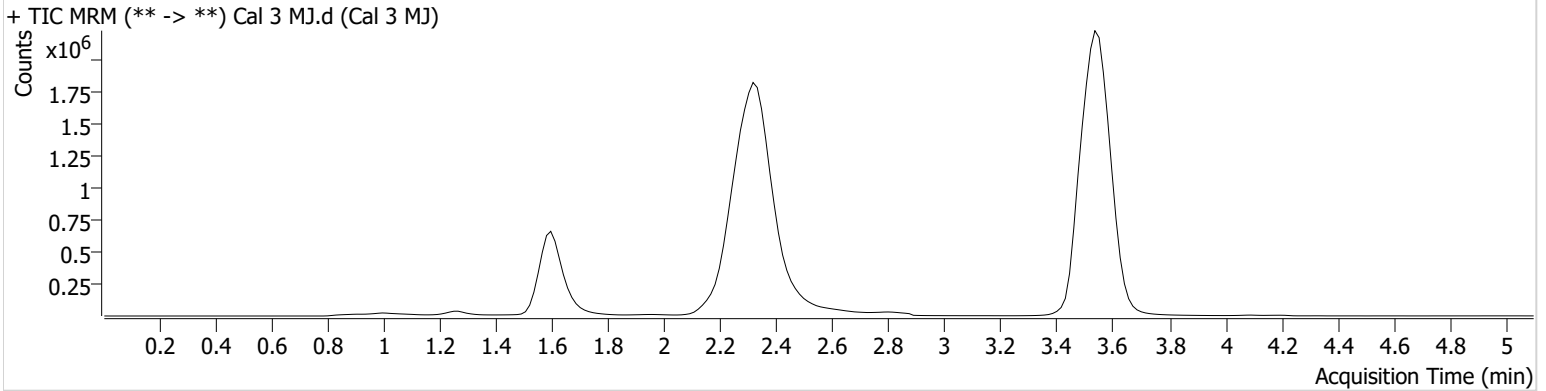


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** Cal 3 MJ.d  
**Type** Cal **Sample** Cal 3 MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-F6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 4:12:40 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	263916	∞	8.4	238.84	2358180	4.9035 ng/ml
THC-COOH	1.625	354727	∞	48.7	467.07	654231	18.0974 ng/ml
THC	3.555	700169	1231.89	24.4	514.03	15900938	4.7994 ng/ml

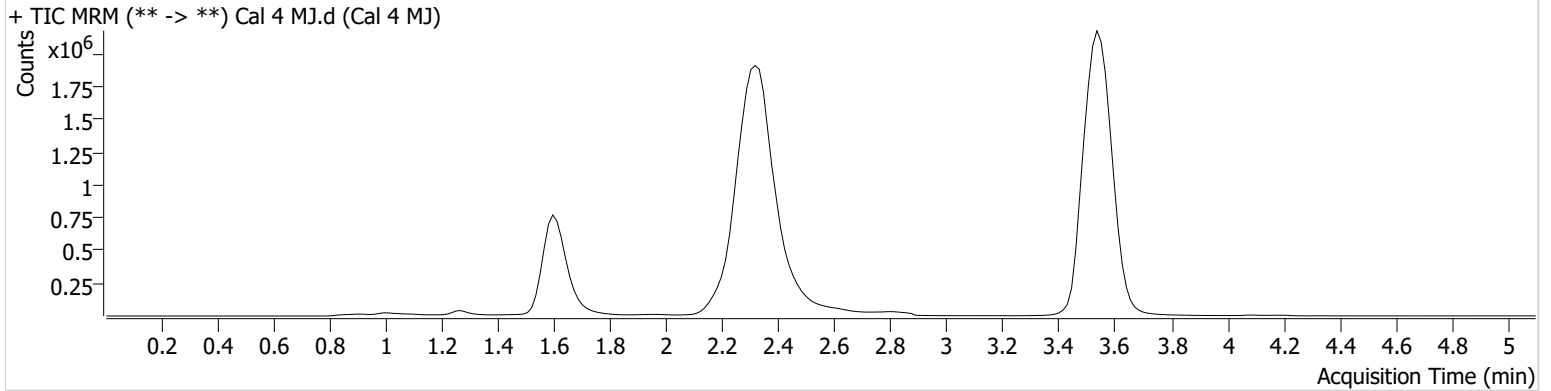


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** Cal 4 MJ.d  
**Type** Cal **Sample** Cal 4 MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-E6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 4:20:16 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	424831	∞	9.8	282.24	2191231	9.9526 ng/ml
THC-COOH	1.625	791308	∞	51.2	10778.7	599722	49.8978 ng/ml
THC	3.555	1264034	5731.43	24.3	∞	13986756	9.5981 ng/ml

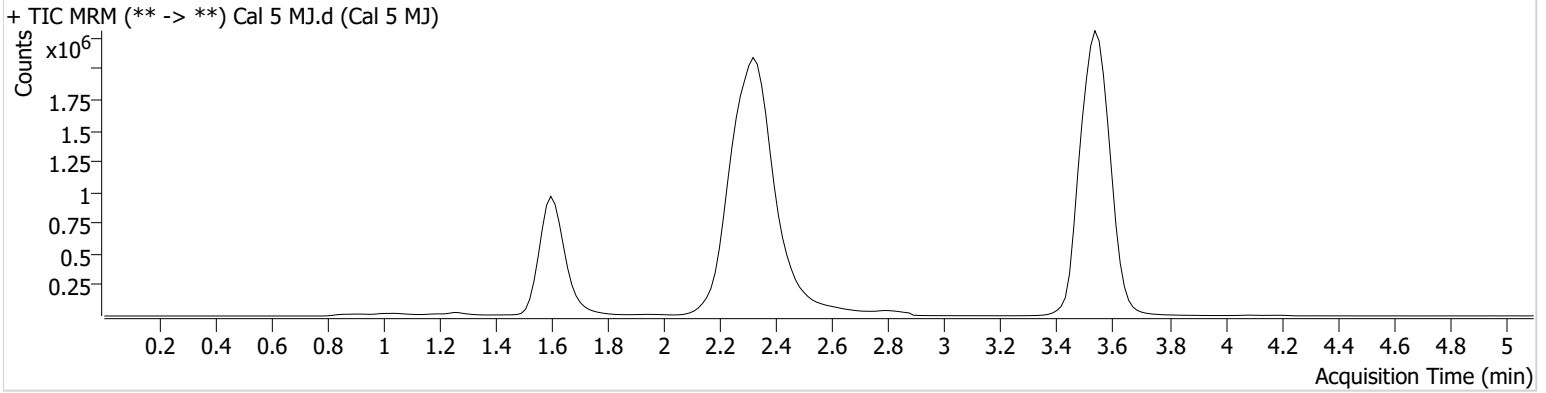


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** Cal 5 MJ.d  
**Type** Cal **Sample** Cal 5 MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-D6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 4:27:51 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	996051	∞	10.9	348.63	2301613	24.6686 ng/ml
THC-COOH	1.625	1159243	∞	54.0	1988.00	619868	72.4284 ng/ml
THC	3.555	3157798	20908.06	24.4	1734.50	13395481	24.6506 ng/ml

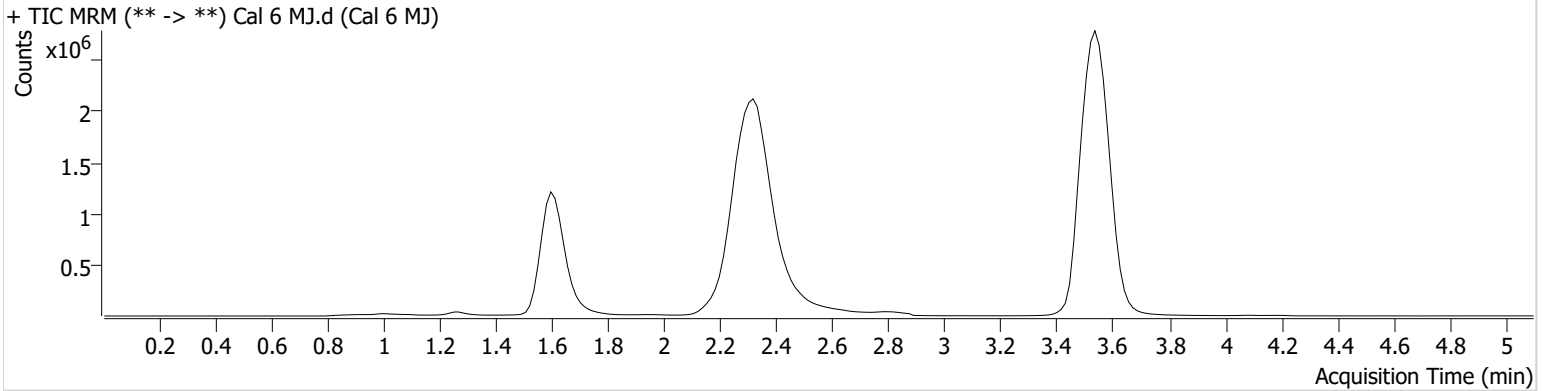


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** Cal 6 MJ.d  
**Type** Cal **Sample** Cal 6 MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-C6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 4:35:27 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	1807464	∞	11.3	∞	2157622	49.6147 ng/ml
THC-COOH	1.625	1458767	∞	54.1	30902.8	570854	100.4653 ng/ml
THC	3.540	6066942	44159.42	24.5	4127.89	12534774	50.3597 ng/ml

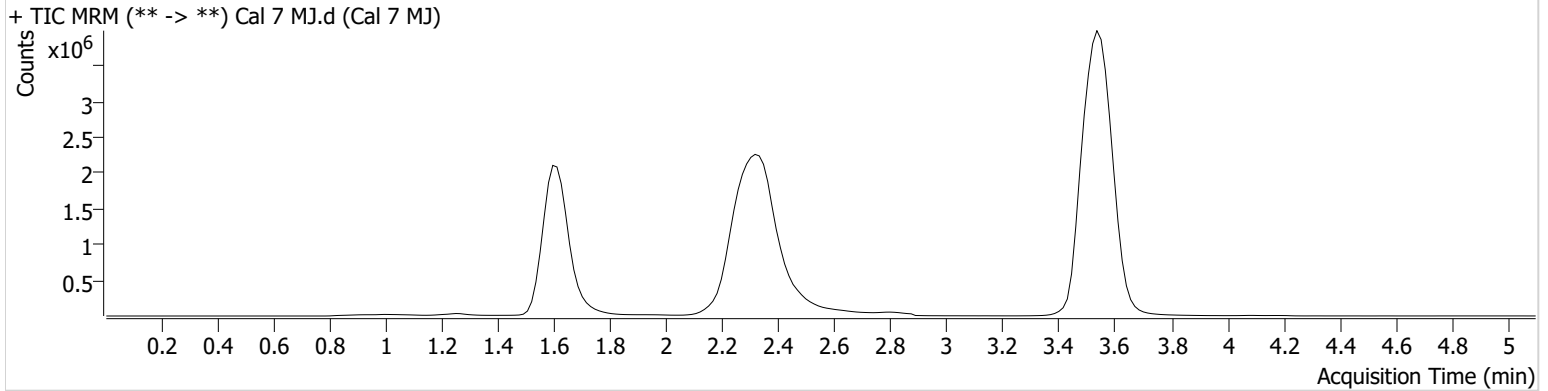


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\120922 AM 27 28 P1 P2 CS TS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 12/15/2022 8:32:12 AM

**Instrument** Falco (069901) **Data File** Cal 7 MJ.d  
**Type** Cal **Sample** Cal 7 MJ  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-B6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 12/9/2022 4:43:03 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	3862935	∞	11.3	∞	2316243	100.7480 ng/ml
THC-COOH	1.625	3622912	∞	55.5	∞	576652	252.9615 ng/ml
THC	3.555	13286023	58513.62	25.0	7957.99	13712200	100.5731 ng/ml