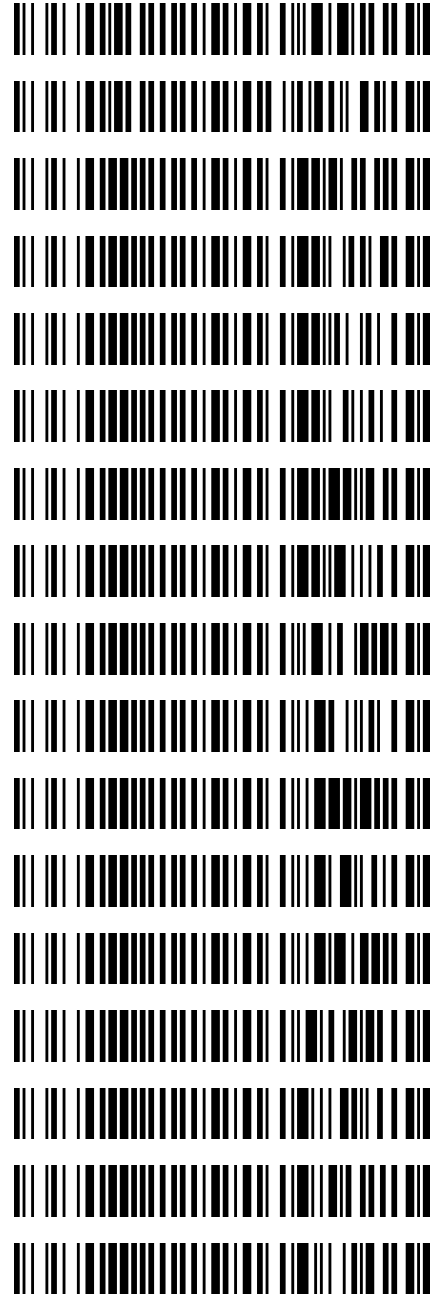


**Worklist: 5904**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-1710	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-1902	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1249	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1250	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1251	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1252	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1253	1	BCK	AM 27 Blood THC Quant by LC-QQQ
<del>P2022-1254</del>	<del>1</del>	<del>BCK</del>	<del>AM 27 Blood THC Quant by LC-QQQ</del>
Not analyzed on AM 27 due to limited sample in tube A. <del>JA</del> 5/24/2022			
P2022-1265	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1278	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1290	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1291	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1293	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1321	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1333	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1334	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1345	2	BCK	AM 27 Blood THC Quant by LC-QQQ



AX

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

Extraction Date: 05/18/2022

Analyst: Amber Gerheart

Plate lot#: 220309

Plate Retest Date: 09/09/2022

**Mobile phase A:** 0.1% Formic Acid in LCMS Water

**Mobile phase B:** 0.1% Formic acid in Acetonitrile

**Blank Blood Lot:** Lampire 22B52016-2

**Column:** UCT Selectra DA 100 x 2.1mm 3um

**LCMS-QQQ ID:** 069901

**Blank Urine Lot:** N/A

### 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: 42**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500µL 0.1% formic acid in water blood sample, 500 µL saturated phosphate buffer in urine** in wells of 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  $\geq 0.98$  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:

Due to poor chromatography, AM 27 column was replaced and the plate was reinjected on 5/20/2022

Curve range: Carboxy-THC 10-1000 ng/mL, OH-THC 3-100 ng/mL

250 AX 6/2/2022

AK

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	P2022-1253-1	P2022-1333-1	IS + Sample	IS + QC_1
B	IS + Cal. 2	Blood Negative	IS + Sample	P2022-1334-1	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2022-1710-2	P2022-1265-1	P2022-1345-2	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	M2022-1902-3	P2022-1278-3	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2022-1249-1	P2022-1290-1	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2022-1250-1	P2022-1291-1	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2022-1251-1	P2022-1293-1	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2022-1252-1	P2022-1321-2	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100  $\mu$ l of residual DMSO

AK

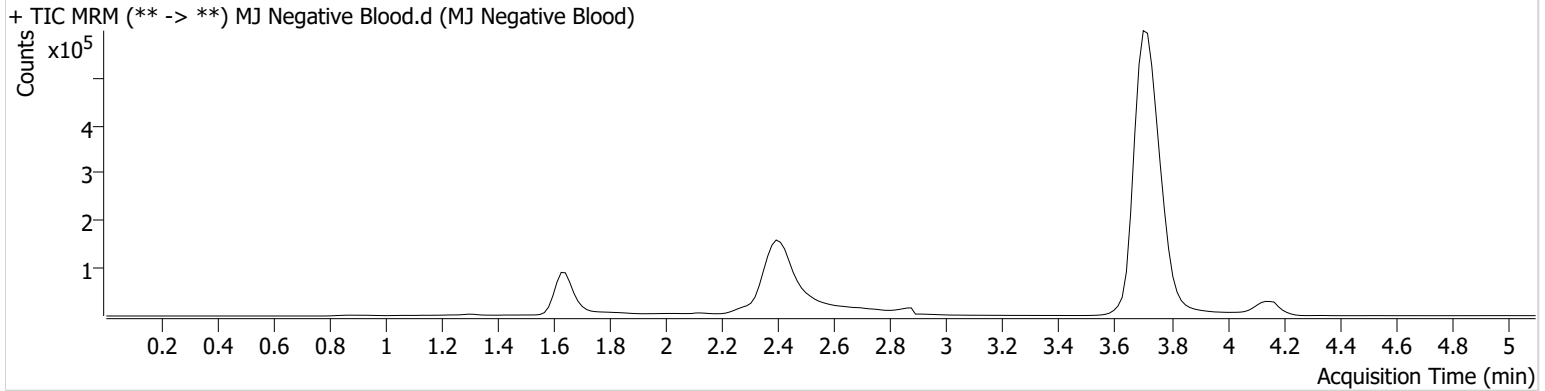


# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<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>	Amber Gerheart
<b>Sample Position</b>	P1-B2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 11:42:08 AM		
<b>Sample Info.</b>			

**Sample Chromatogram**





AX

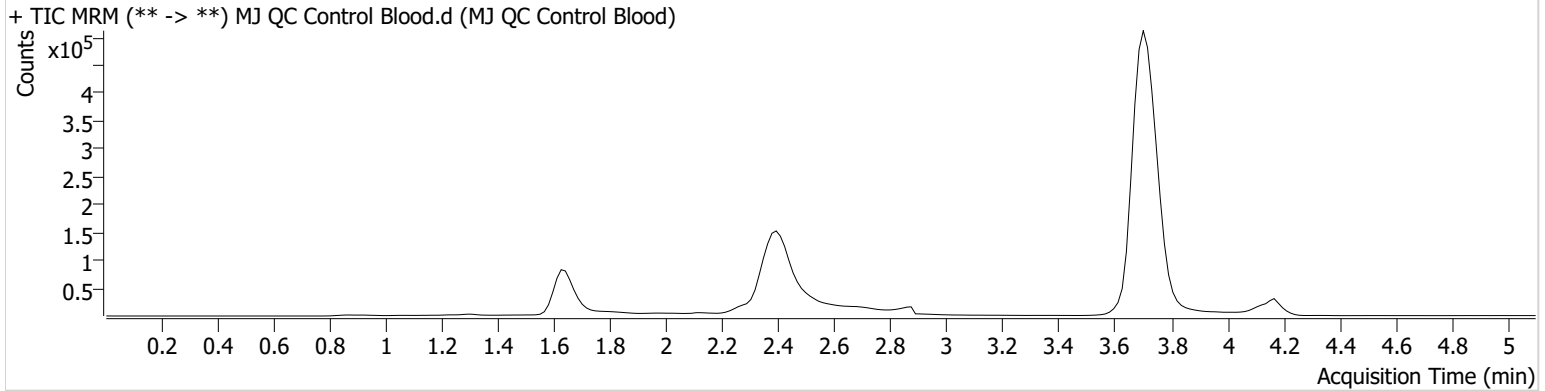


# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ QC Control Blood.d
<b>Type</b>	QC	<b>Sample</b>	MJ QC Control Blood
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 11:26:54 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	18689	94.73	13.2	22.14	284437	4.0996 ng/ml
THC-COOH	1.670	35076	179.39	39.8	∞	78069	15.8946 ng/ml
THC	3.721	121510	∞	29.9	462.73	3034124	4.6770 ng/ml

AX

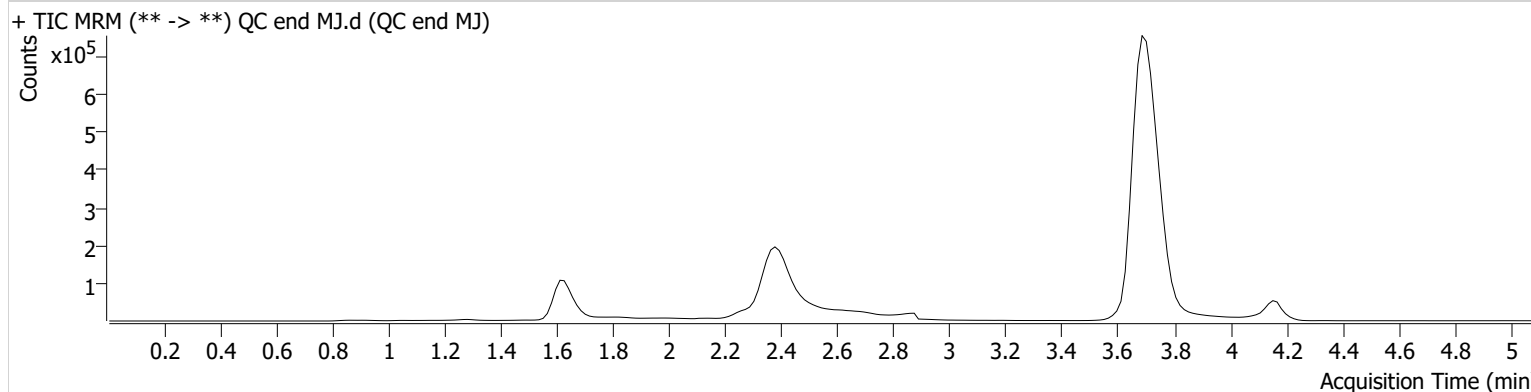


# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	QC end MJ.d
<b>Type</b>	QC	<b>Sample</b>	QC end MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 4:01:04 PM		

**Sample Chromatogram**



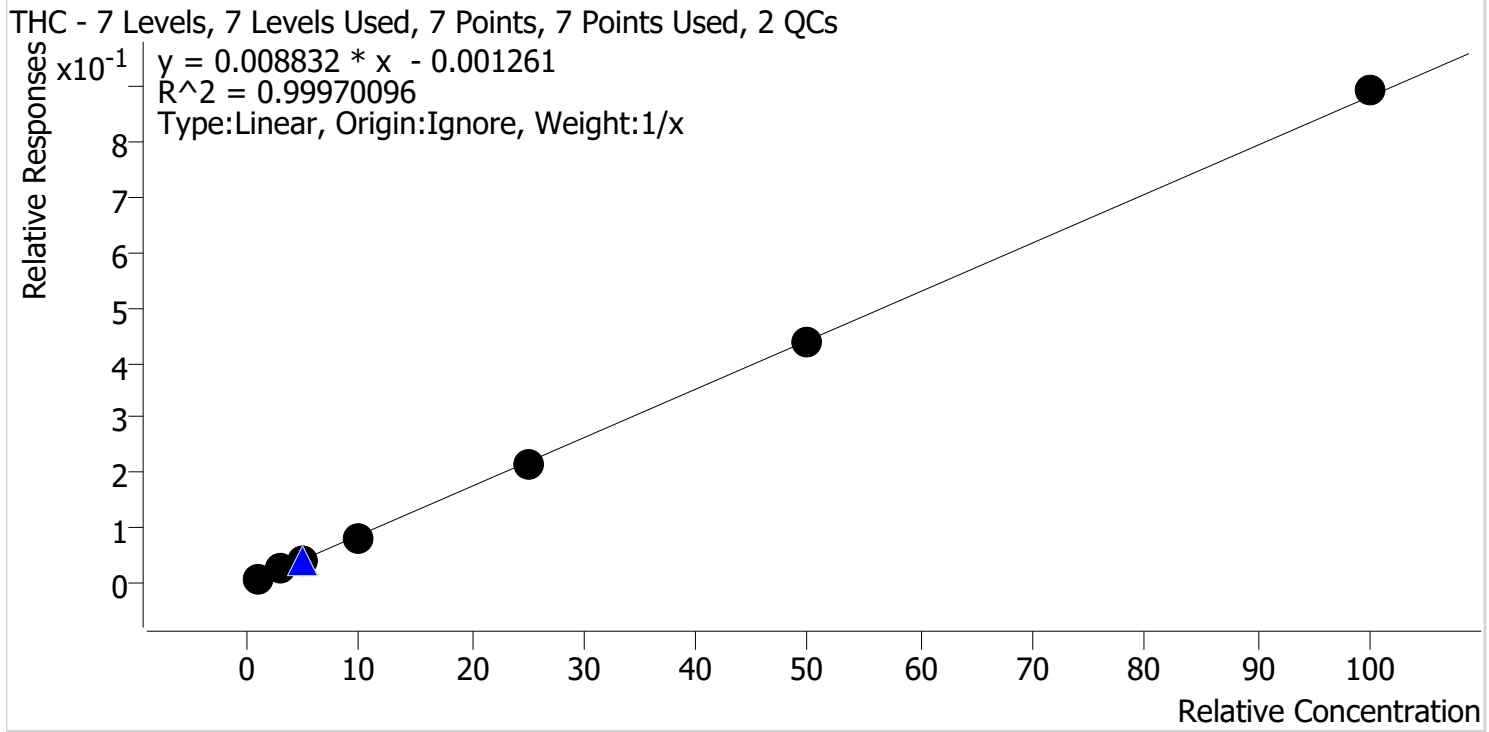
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	23772	205.69	13.2	13.10	368674	4.0291 ng/ml
THC-COOH	1.655	40543	466.88	43.0	380.15	91726	15.5733 ng/ml
THC	3.706	194744	∞	29.2	∞	4726732	4.8075 ng/ml

AX



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** C:\Users\lagerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Last Cal. Update** 5/24/2022 2:51 PM  
**Analyst Name** ISP\lagerheart  
**Analyte** THC **Internal Standard** THC-D3



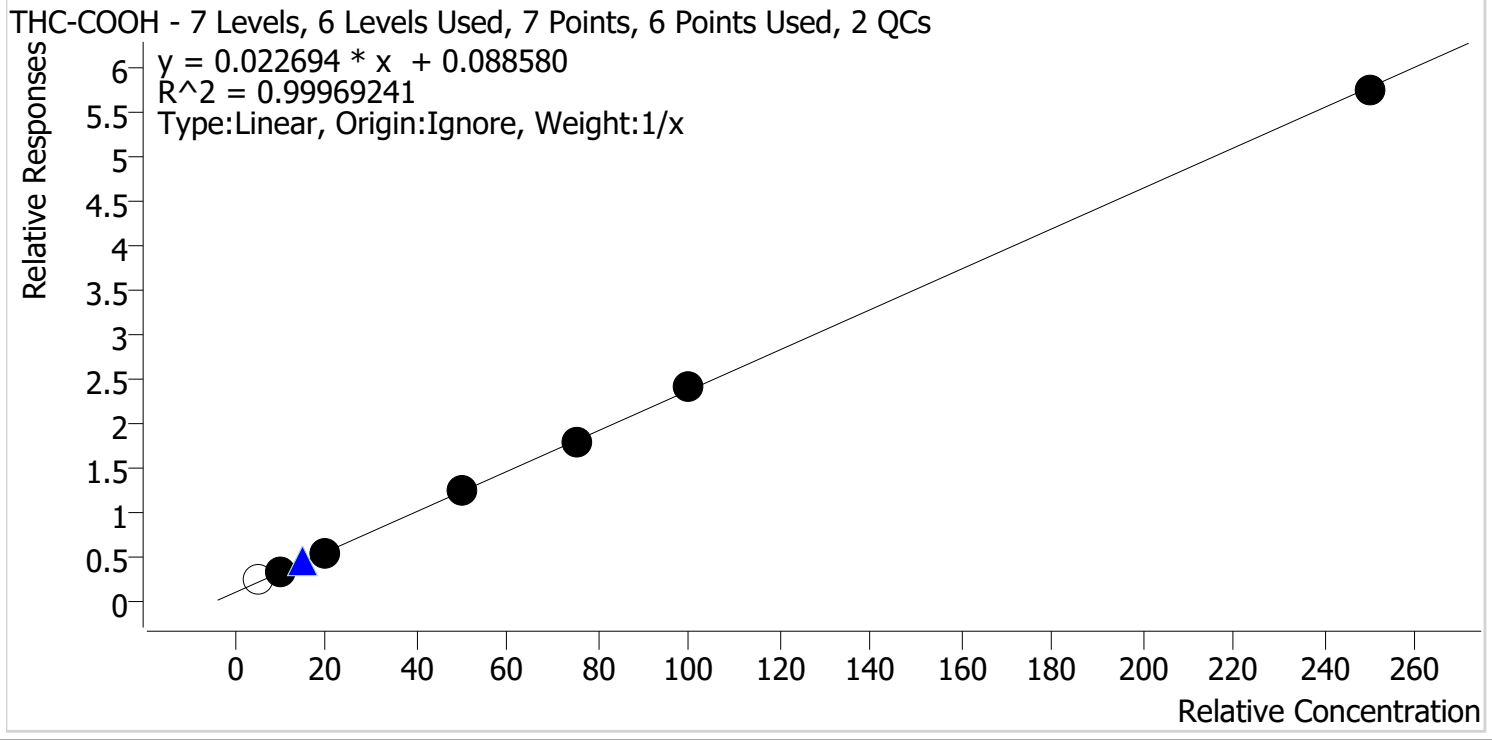
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	108.1
Cal 2 MJ	2	✓	3.0	3.0	99.5
Cal 3 MJ	3	✓	5.0	4.9	97.2
Cal 4 MJ	4	✓	10.0	9.7	96.7
Cal 5 MJ	5	✓	25.0	24.4	97.8
Cal 6 MJ	6	✓	50.0	49.8	99.5
Cal 7 MJ	7	✓	100.0	101.2	101.2

AX



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** C:\Users\lagerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Last Cal. Update** 5/24/2022 2:51 PM  
**Analyst Name** ISP\lagerheart  
**Analyte** THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	5.0	6.8	135.1
Cal 2 MJ	2	✓	10.0	10.1	100.7
Cal 3 MJ	3	✓	20.0	19.4	96.9
Cal 4 MJ	4	✓	50.0	51.0	102.0
Cal 5 MJ	5	✓	75.0	74.3	99.0
Cal 6 MJ	6	✓	100.0	102.0	102.0
Cal 7 MJ	7	✓	250.0	248.3	99.3

Cal 1 dropped due to ratio

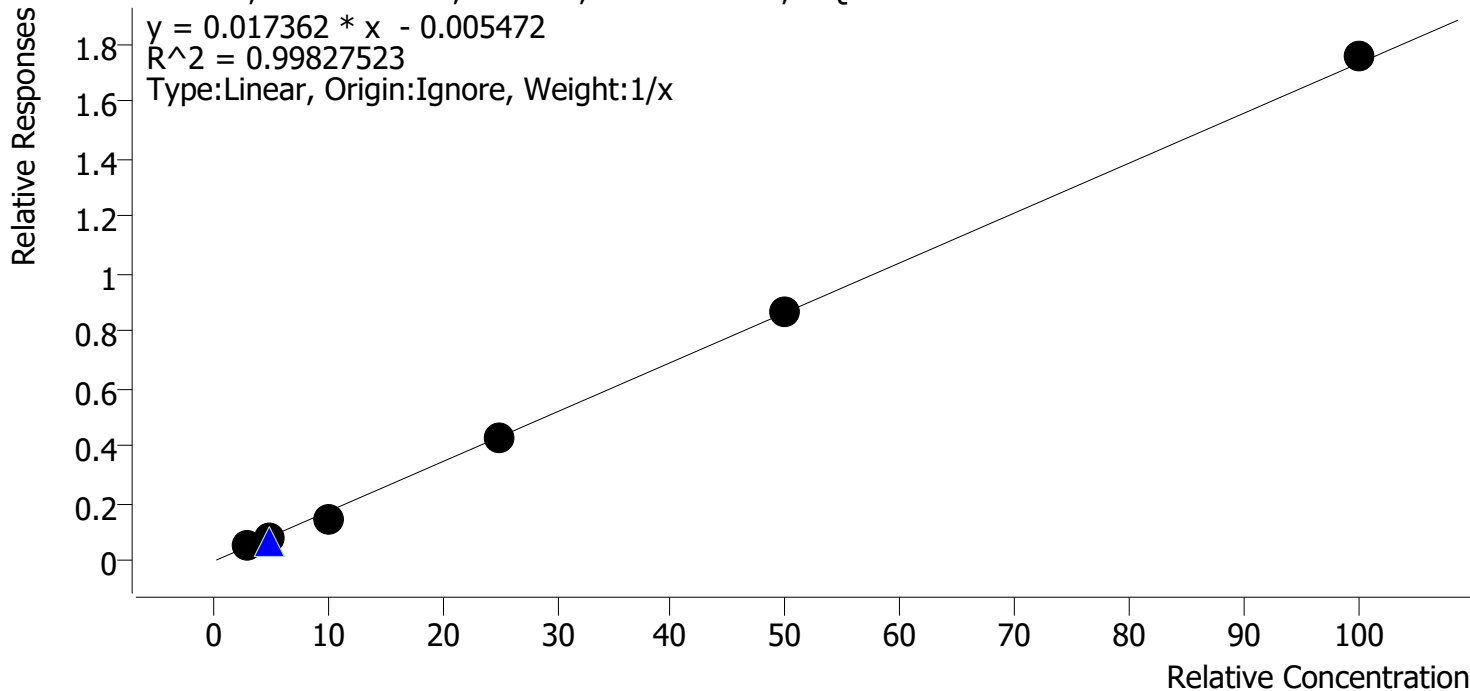
AX



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** C:\Users\lagerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Last Cal. Update** 5/24/2022 2:51 PM  
**Analyst Name** ISP\lagerheart  
**Analyte** THC-OH **Internal Standard** THC-OH-D3

THC-OH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 2 MJ	2	✓	3.0	3.4	113.6
Cal 3 MJ	3	✓	5.0	5.0	99.7
Cal 4 MJ	4	✓	10.0	8.7	87.3
Cal 5 MJ	5	✓	25.0	24.5	98.0
Cal 6 MJ	6	✓	50.0	50.1	100.2
Cal 7 MJ	7	✓	100.0	101.3	101.3

Cal 1 dropped due to ratio and S/N

AX



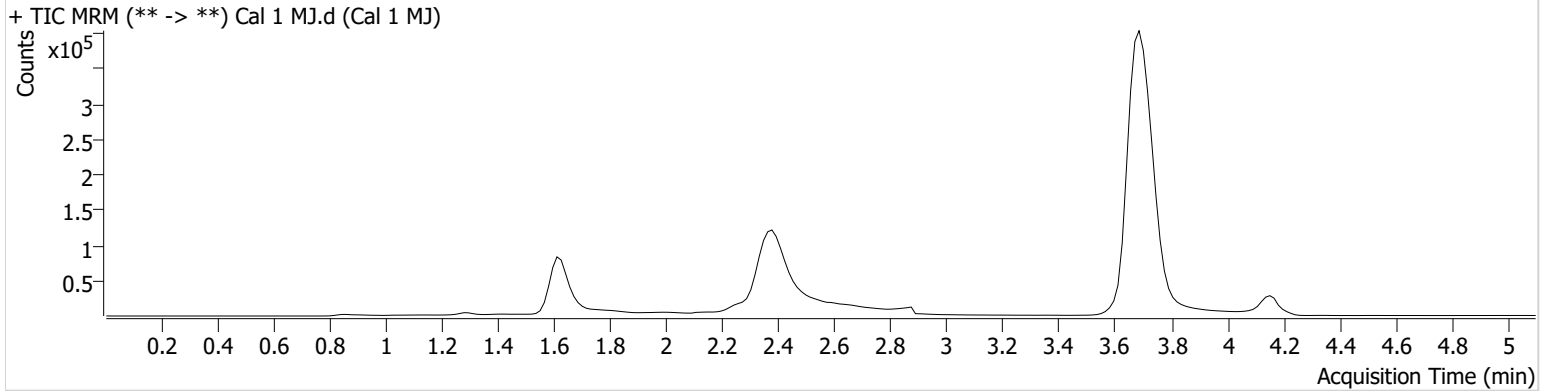
# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 1 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 1 MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-A1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 10:25:54 AM		

**Sample Info.**

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	17315	∞	27.4 <b>Low</b>	∞	71597	6.7531 ng/ml
THC	3.706	21419	∞	35.3	76.23	2585183	1.0808 ng/ml

AX



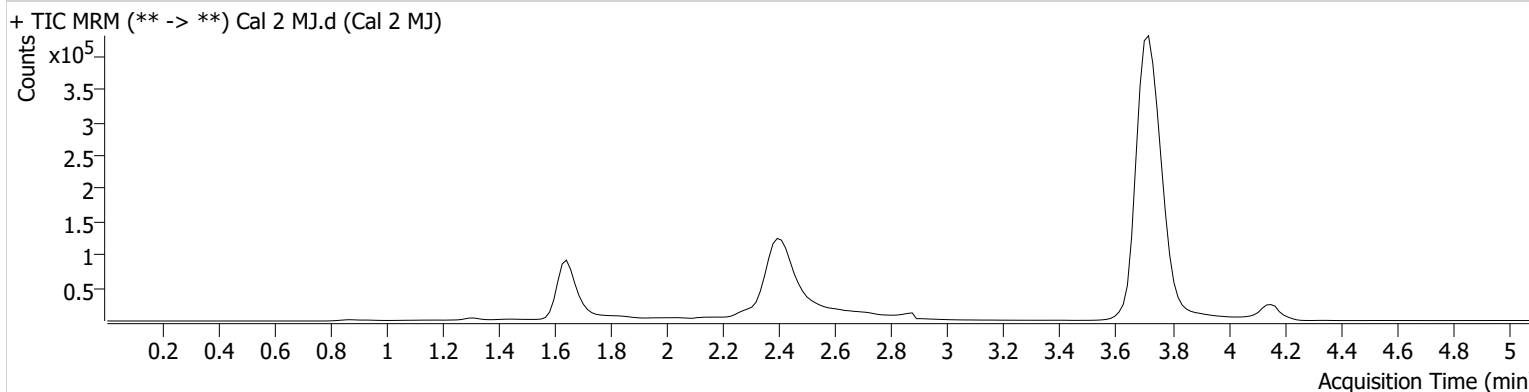
# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 2 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 2 MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-B1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 10:33:40 AM		

**Sample Info.**

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	13656	52.34	12.2	15.50	254260	3.4087 ng/ml
THC-COOH	1.670	26249	∞	42.4	132.72	82761	10.0727 ng/ml
THC	3.721	66885	∞	30.8	∞	2664069	2.9853 ng/ml

AX



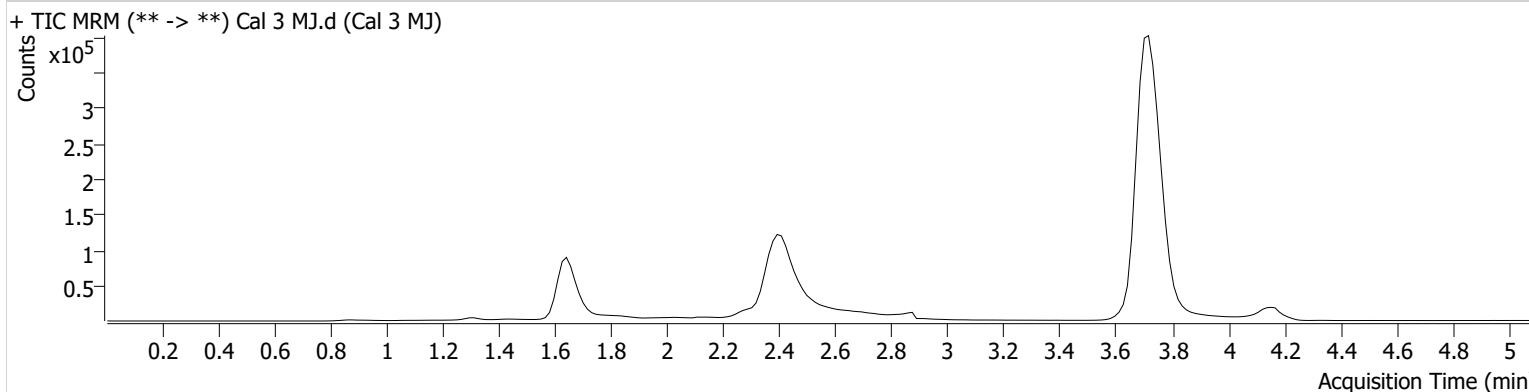
# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 3 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 3 MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-C1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 10:41:16 AM		

**Sample Info.**

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	20193	1769.42	13.2	43.07	249178	4.9830 ng/ml
THC-COOH	1.670	38806	∞	45.6	432.02	73420	19.3868 ng/ml
THC	3.721	99906	671.89	30.8	219.07	2397033	4.8617 ng/ml



AX



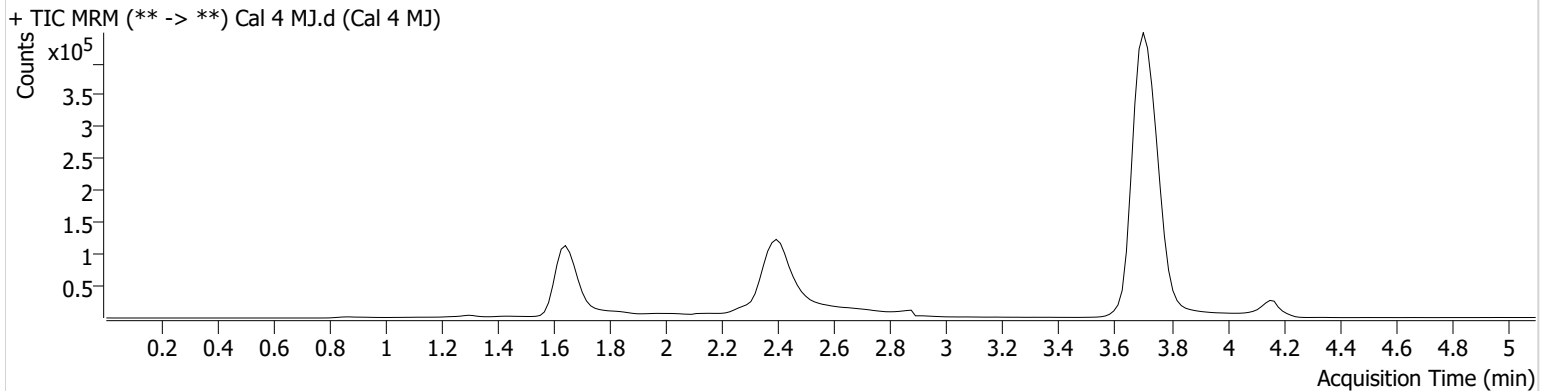
# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 4 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 4 MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-D1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 10:48:54 AM		

**Sample Info.**

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	48445	∞	12.6	184.01	331653	8.7287 ng/ml
THC-COOH	1.670	103733	∞	52.0	1467.05	83245	51.0067 ng/ml
THC	3.721	219440	∞	28.7	164.41	2608339	9.6680 ng/ml

AX

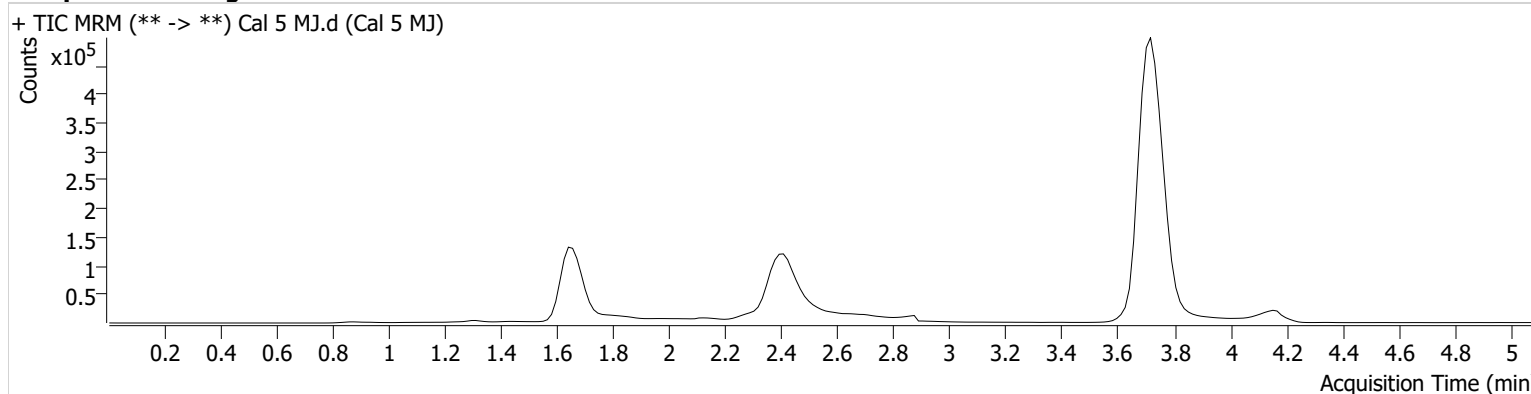


# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 5 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 5 MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-E1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 10:56:29 AM		
<b>Sample Info.</b>			

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	105658	668.38	13.3	503.43	251762	24.4878 ng/ml
THC-COOH	1.670	134308	∞	52.9	873.68	75718	74.2577 ng/ml
THC	3.721	526831	∞	28.4	1417.79	2454200	24.4473 ng/ml

AX



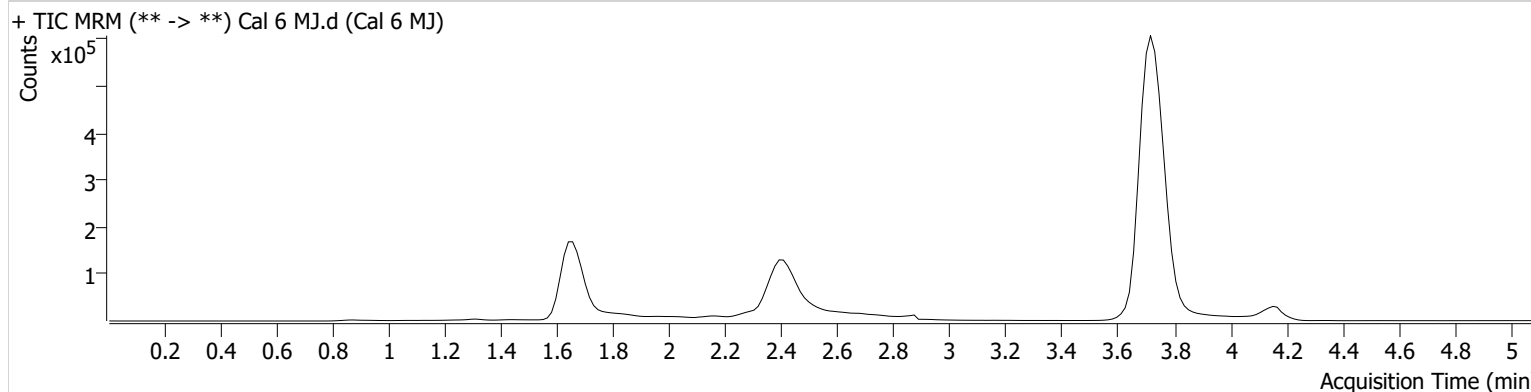
# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 6 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 6 MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-F1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 11:04:04 AM		

**Sample Info.**

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	216796	∞	13.5	793.92	250888	50.0869 ng/ml
THC-COOH	1.670	182001	∞	53.6	826.88	75722	102.0077 ng/ml
THC	3.721	1068101	∞	28.7	∞	2437683	49.7517 ng/ml

AX

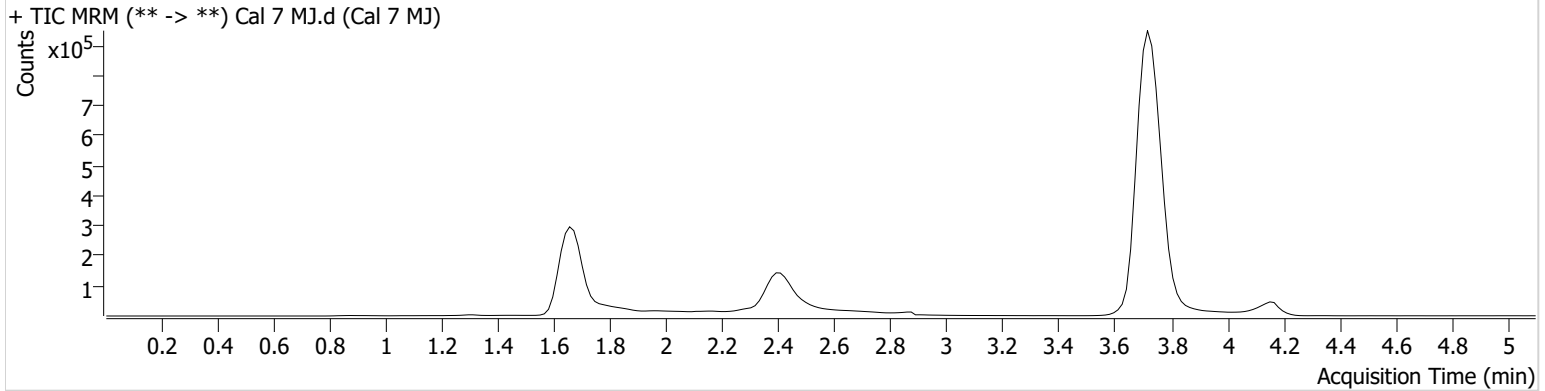


# AM #27 Cannabinoid Quant. Results

**Batch results** C:\Users\agerheart\Desktop\052022 AM 27 AG\QuantResults\AM 27 Final.batch.bin  
**Calibration Last Update** 5/24/2022 2:51:20 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 7 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 7 MJ
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P1-G1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/20/2022 11:11:40 AM		
<b>Sample Info.</b>			

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	442013	1724.38	12.7	1511.68	252098	101.3049 ng/ml
THC-COOH	1.670	449419	∞	56.2	∞	78531	248.2684 ng/ml
THC	3.721	2450430	∞	28.2	∞	2745223	101.2052 ng/ml