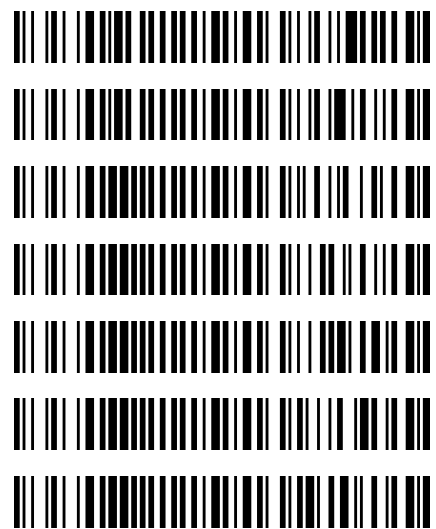


**Worklist: 5662**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-0640	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-0641	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0459	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0468	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0470	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0492	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0594	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: 03/04/2022

Analyst: Tamara Salazar

Plate lot#: IDP-108-3-211018

Plate Re-test Date: 04-18-22

**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

### 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. 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: 750uL
- 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:

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	P2022-0492-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2022-0470-3	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	IS + Sample	P2022-0468-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	IS + Sample	P2022-0459-1*	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	IS + Sample	M2022-0641-1	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	IS + Sample	M2022-0640-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2022-0459-1	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2022-0594-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

\*Sample moved during step 6 of the extraction due to a blood clot.

TS

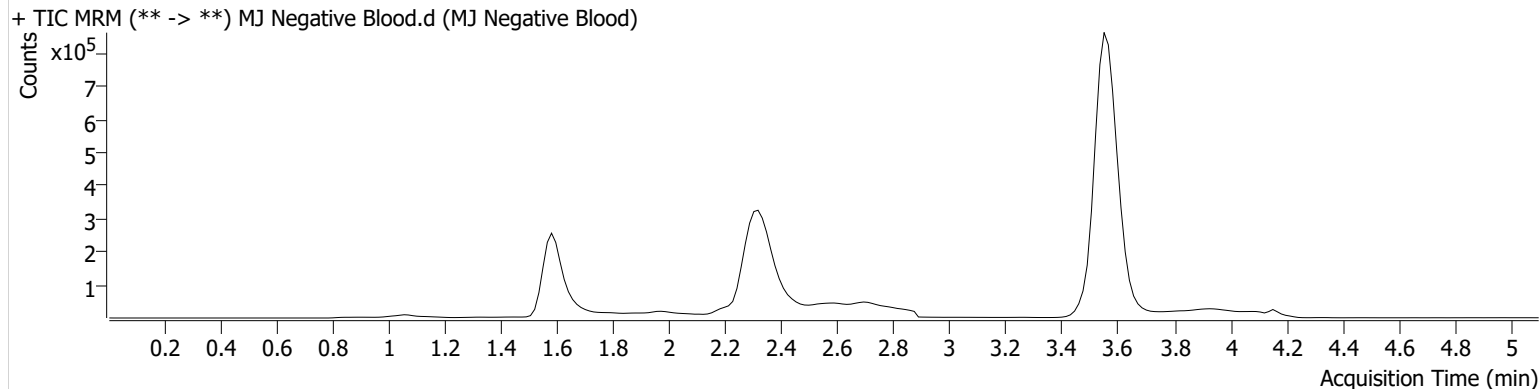


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 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>	Tamara Salazar
<b>Sample Position</b>	P5-G5	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 1:25:08 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



TS

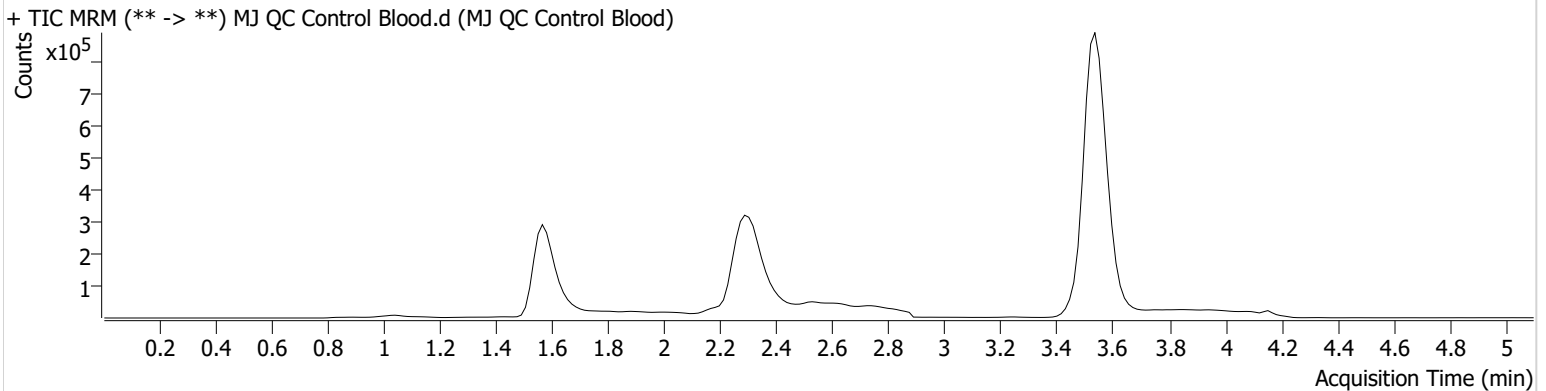


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<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>	Tamara Salazar
<b>Sample Position</b>	P5-A6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 1:09:55 PM		

### Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.573	74713	205.20	15.6	333.84	1089208	4.5257 ng/ml
THC-COOH	1.595	102459	∞	59.0	∞	285839	15.7433 ng/ml
THC	3.540	214804	2782.39	24.2	339.86	4663939	4.9887 ng/ml

TS

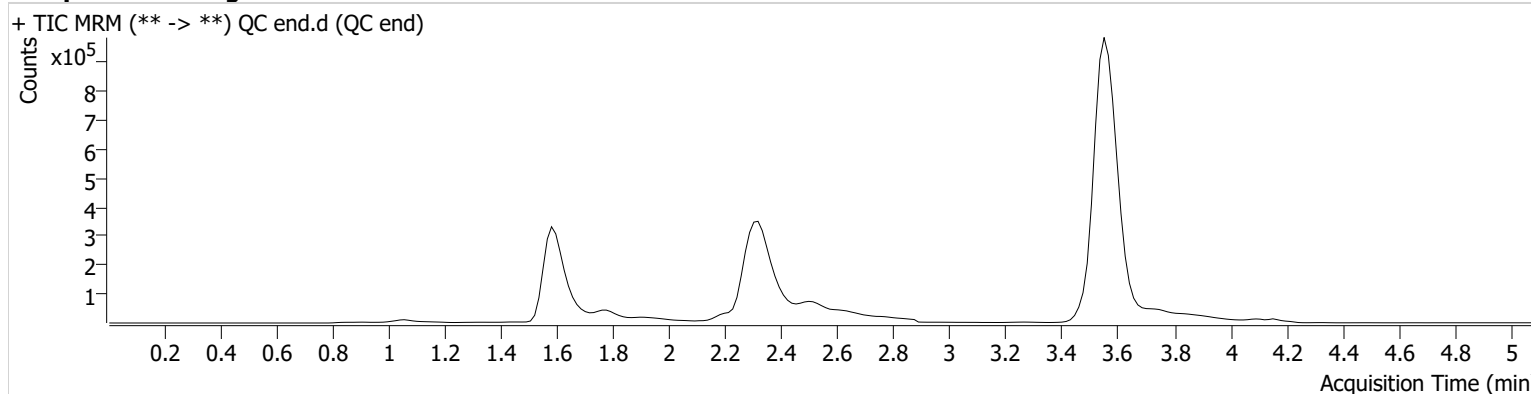


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	QC end.d
<b>Type</b>	QC	<b>Sample</b>	QC end
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-A6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 3:27:01 PM		

**Sample Chromatogram**



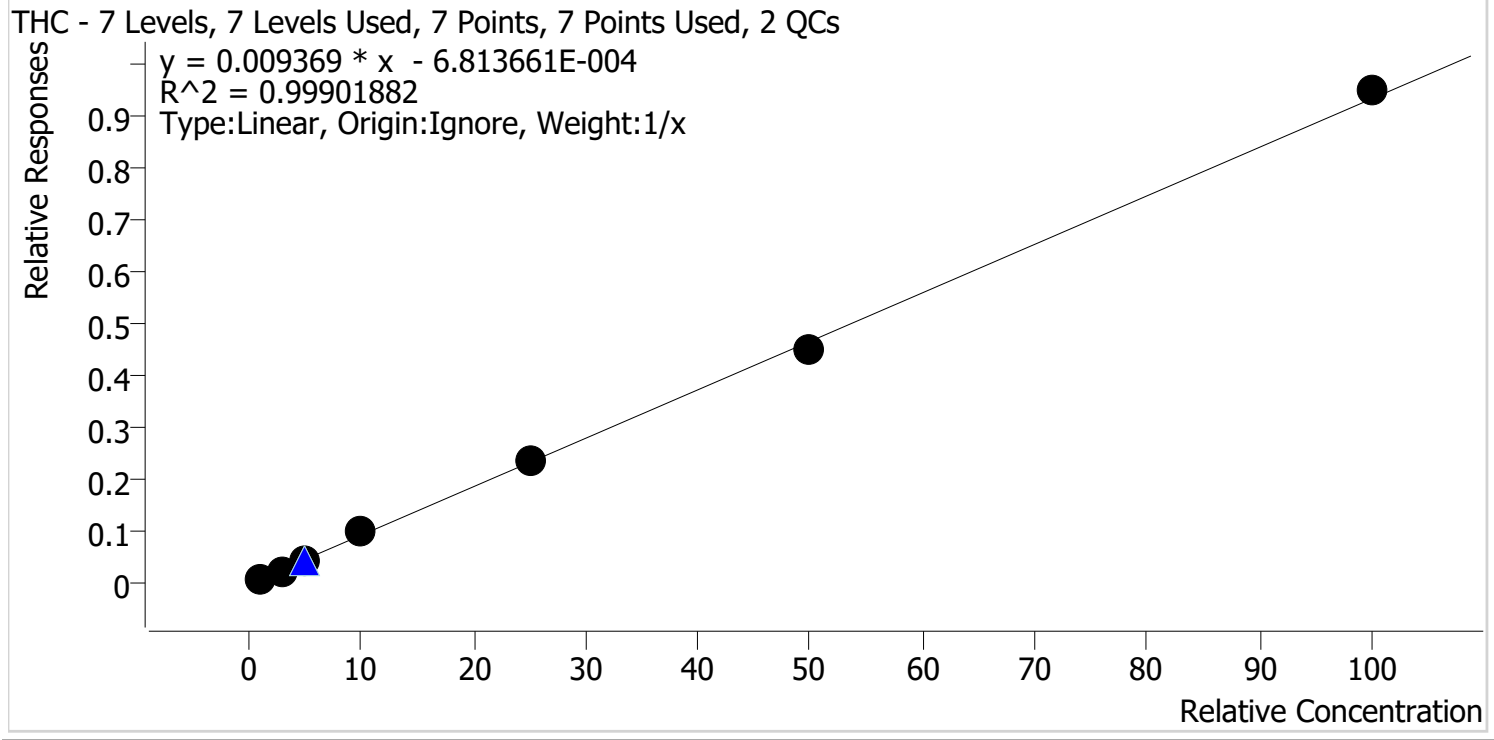
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	88209	157.55	14.6	∞	1147050	5.0866 ng/ml
THC-COOH	1.625	119594	∞	64.3	∞	320370	16.3960 ng/ml
THC	3.555	248506	1580.97	26.4	307.28	6084801	4.4320 ng/ml

TS



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Last Cal. Update** 3/7/2022 11:33 AM  
**Analyst Name** ISP\Datastor  
**Analyte** THC **Internal Standard** THC-D3



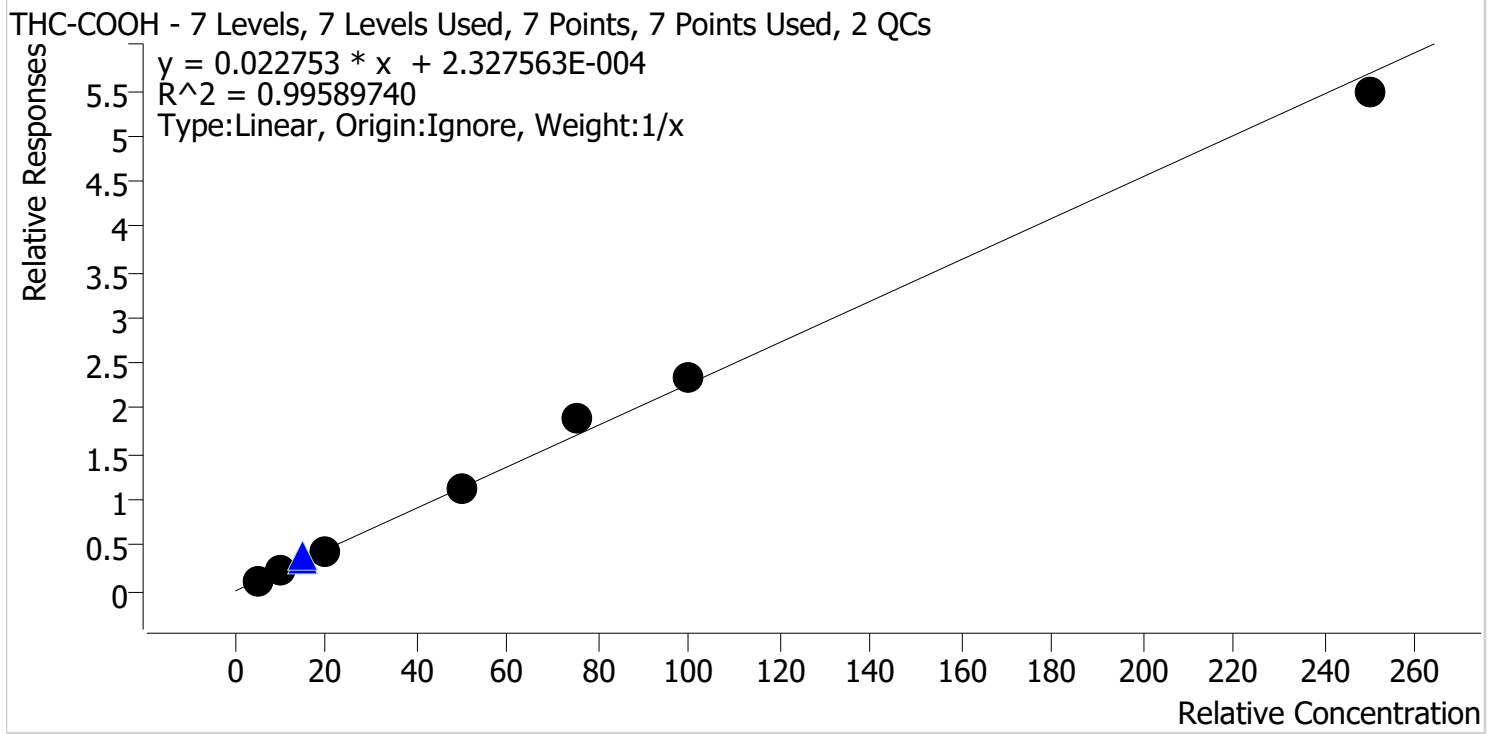
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.0	103.7
MJ Cal 2	2	✓	3.0	2.8	92.0
MJ Cal 3	3	✓	5.0	5.1	101.4
MJ Cal 4	4	✓	10.0	10.5	105.4
MJ Cal 5	5	✓	25.0	25.0	99.9
MJ Cal 6	6	✓	50.0	48.0	95.9
MJ Cal 7	7	✓	100.0	101.7	101.7

TS



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Last Cal. Update** 3/7/2022 11:33 AM  
**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	4.9	98.4
MJ Cal 2	2	✓	10.0	9.8	97.6
MJ Cal 3	3	✓	20.0	19.1	95.5
MJ Cal 4	4	✓	50.0	48.7	97.3
MJ Cal 5	5	✓	75.0	83.9	111.9
MJ Cal 6	6	✓	100.0	103.0	103.0
MJ Cal 7	7	✓	250.0	240.6	96.2



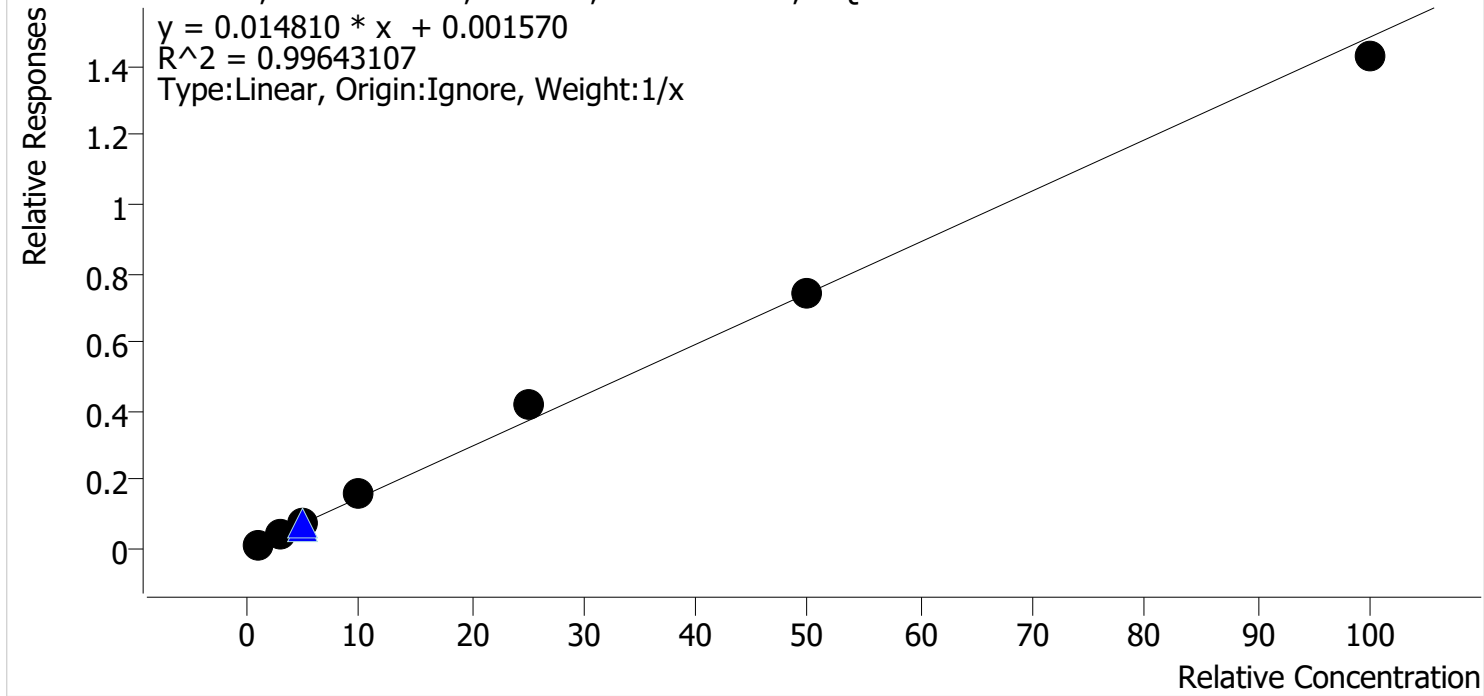
TS



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Last Cal. Update** 3/7/2022 11:33 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, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	0.8	82.3
MJ Cal 2	2	✓	3.0	3.0	99.6
MJ Cal 3	3	✓	5.0	5.1	102.1
MJ Cal 4	4	✓	10.0	10.8	107.5
MJ Cal 5	5	✓	25.0	28.0	111.9
MJ Cal 6	6	✓	50.0	50.2	100.4
MJ Cal 7	7	✓	100.0	96.2	96.2

TS

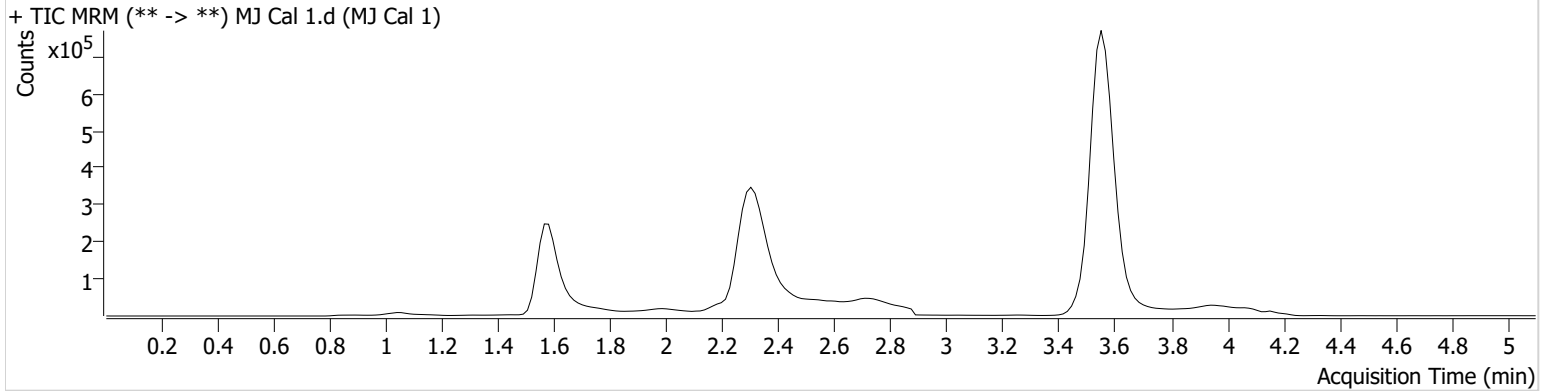


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Cal 1.d
<b>Type</b>	Cal	<b>Sample</b>	MJ Cal 1
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-H6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 12:08:47 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.573	13173	∞	13.9	10.06	957195	0.8232 ng/ml <b>Low</b>
THC-COOH	1.610	32916	164.05	58.3	372.34	293424	4.9199 ng/ml <b>Low</b>
THC	3.555	39670	∞	29.0	11.16	4390289	1.0372 ng/ml

TS

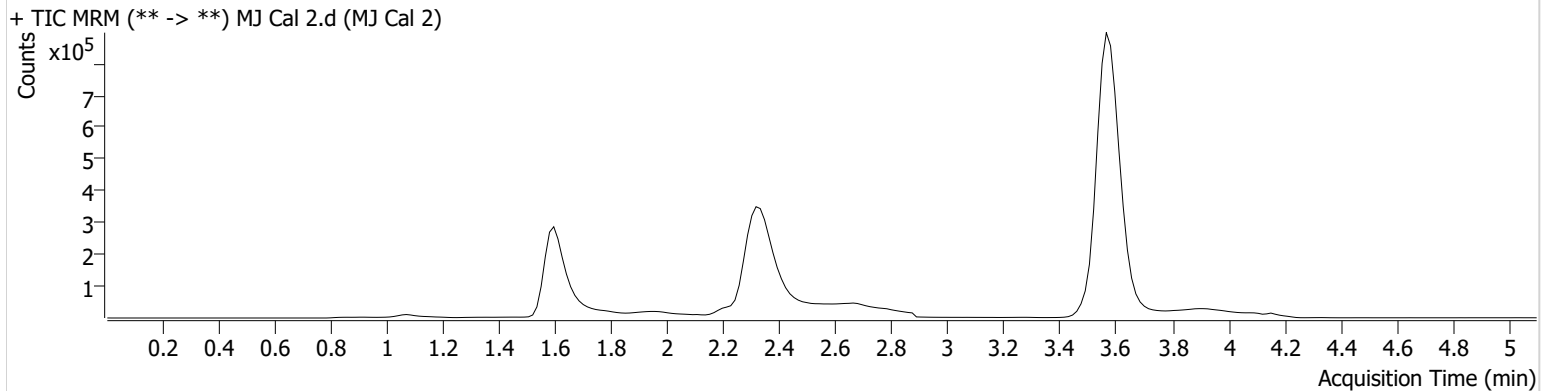
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Cal 2.d
<b>Type</b>	Cal	<b>Sample</b>	MJ Cal 2
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-G6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 12:16:33 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	44324	∞	15.3	121.77	967399	2.9878 ng/ml <b>Low</b>
THC-COOH	1.625	68675	142.02	57.2	662.86	308859	9.7619 ng/ml
THC	3.586	131799	1266.90	28.5	∞	5235102	2.7600 ng/ml

TS

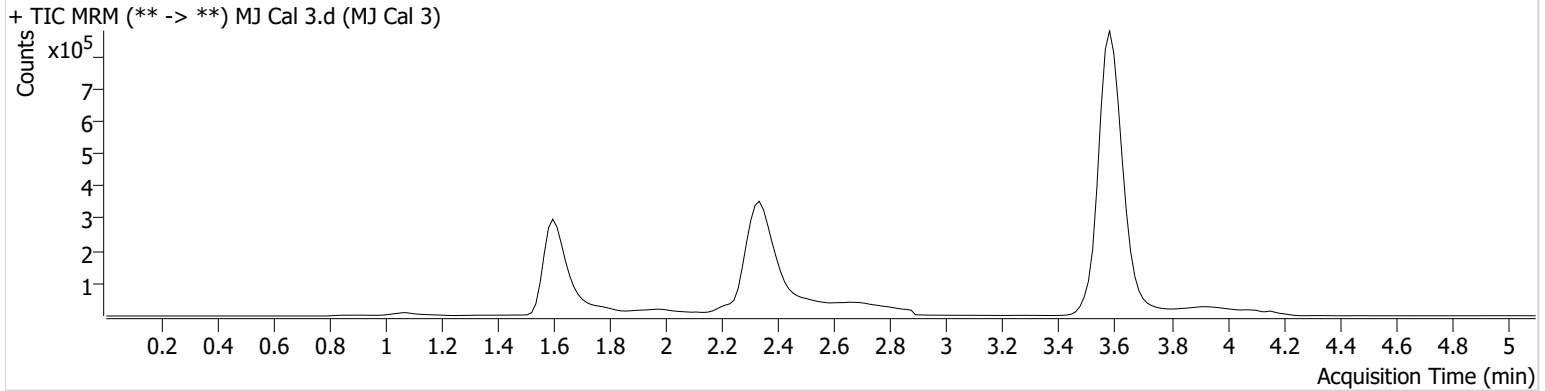


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Cal 3.d
<b>Type</b>	Cal	<b>Sample</b>	MJ Cal 3
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-F6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 12:24:10 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	74601	∞	12.4	140.58	966518	5.1058 ng/ml
THC-COOH	1.625	138452	739.21	59.9	1014.16	318443	19.0980 ng/ml
THC	3.586	222156	1559.90	26.2	401.70	4746646	5.0684 ng/ml

TS



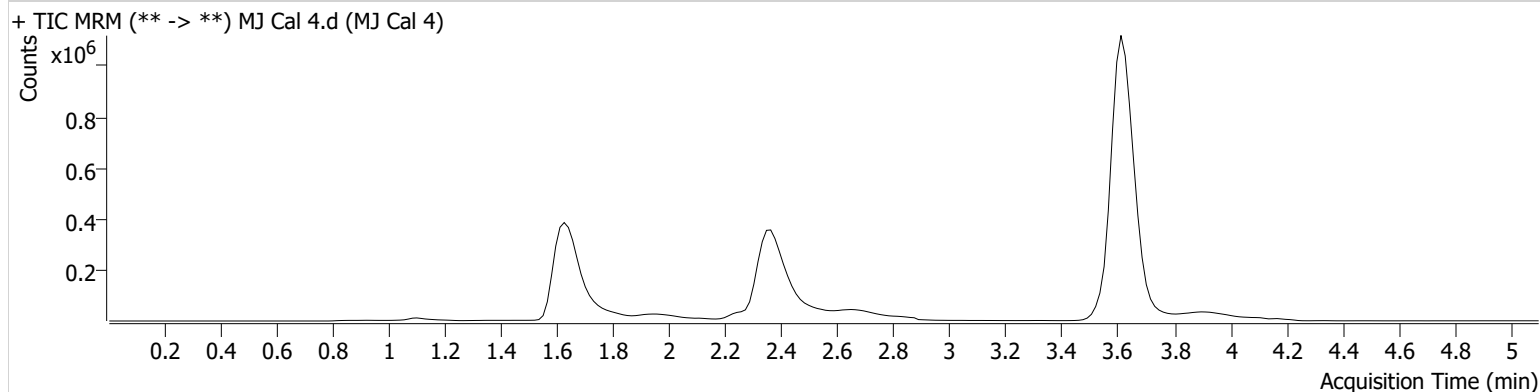
# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Cal 4.d
<b>Type</b>	Cal	<b>Sample</b>	MJ Cal 4
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-E6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 12:31:46 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	174284	3167.83	13.8	602.44	1083796	10.7524 ng/ml
THC-COOH	1.655	380176	5664.48	70.5	∞	343329	48.6558 ng/ml
THC	3.616	538298	5494.34	25.3	∞	5488548	10.5412 ng/ml

TS

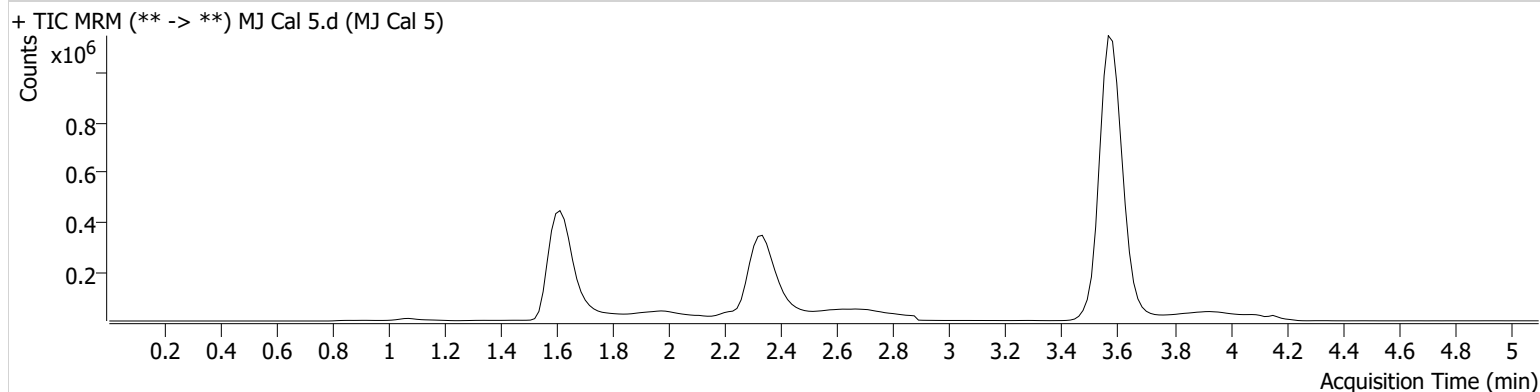


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Cal 5.d
<b>Type</b>	Cal	<b>Sample</b>	MJ Cal 5
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-D6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 12:39:22 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	382981	∞	14.1	1058.47	920827	27.9779 ng/ml
THC-COOH	1.625	510981	1370.48	61.1	4555.55	267523	83.9350 ng/ml
THC	3.586	1227823	7120.40	25.0	836.34	5260467	24.9860 ng/ml

TS



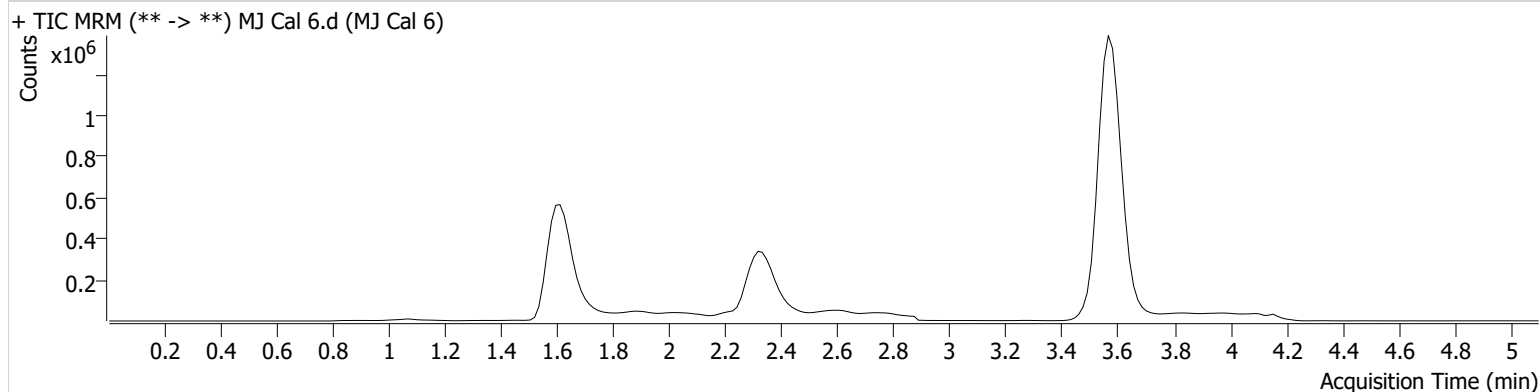
# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Cal 6.d
<b>Type</b>	Cal	<b>Sample</b>	MJ Cal 6
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-C6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 12:46:57 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	706489	∞	16.1	3216.72	948712	50.1780 ng/ml
THC-COOH	1.625	683161	∞	58.5	∞	291398	103.0256 ng/ml
THC	3.586	2333079	20657.89	26.6	∞	5201344	47.9505 ng/ml

TS

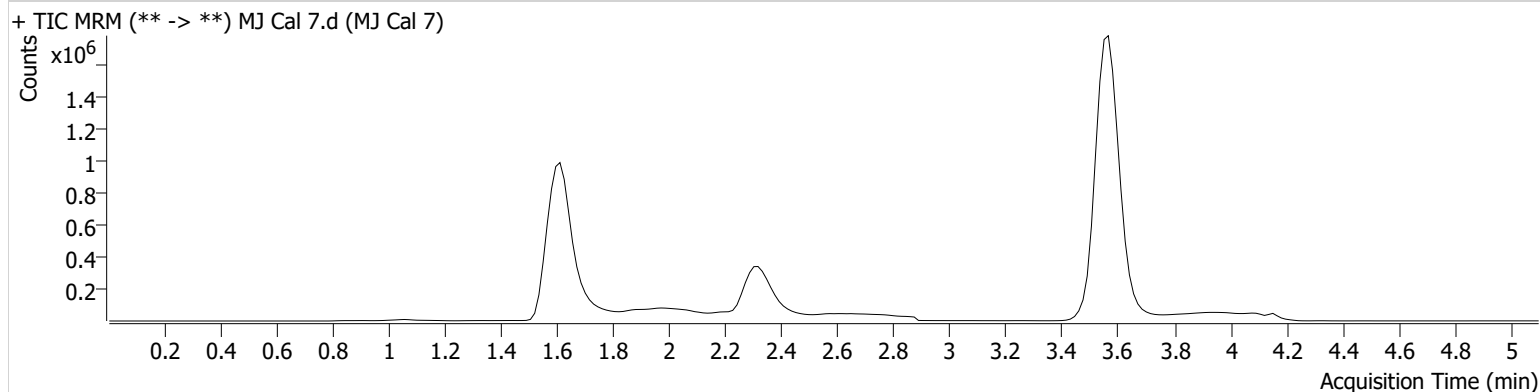


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2022\AM 27-28\030422 AM 27 28 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 3/7/2022 11:33:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Cal 7.d
<b>Type</b>	Cal	<b>Sample</b>	MJ Cal 7
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P5-B6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	3/4/2022 12:54:35 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	1320453	8935.78	15.0	3501.50	926066	96.1749 ng/ml
THC-COOH	1.625	1537068	∞	64.6	9685.37	280753	240.6037 ng/ml
THC	3.570	4721949	22807.68	25.5	∞	4961533	101.6567 ng/ml