

**REVIEWED**

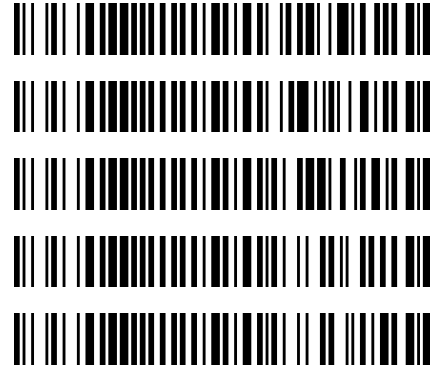
By Celena Shrum at 1:23 pm, Sep 08, 2021

TS

9/4/2021

**Worklist: 5224**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
P2021-1506	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2417	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2722	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2791	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2792	1	BCK	AM 27 Blood THC Quant by LC-QQQ



TS

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

Extraction Date: 09/04/~~221~~ 2021

Plate lot#: IDP-108-2-210609

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 20L20724

LCMS-QQQ ID: 069901

09/08/2021 TS

Analyst: Tamara Salazar

Plate Re-test Date: 12-09-21

Mobile phase B: 0.1% Formic acid in Acetonitrile

Column: UCT Selectra DA 100 x 2.1mm 3um

## 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: 800uL
- 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: *THC-OH not evaluated due to a possible interfering compound.*

TS

	1	2	3	4	5	6
A	IS + Cal. 1					IS + QC_1
B	IS + Cal. 2					IS + Cal. 7
C	IS + Cal. 3				P2021-2792-1	IS + Cal. 6
D	IS + Cal. 4				P2021-2791-1	IS + Cal. 5
E	IS + Cal. 5				P20201-2722-1	IS + Cal. 4
F	IS + Cal. 6				P2021-2417-1	IS + Cal. 3
G	IS + Cal. 7				P2021-1506-1	IS + Cal. 2
H	IS + QC_1				Neg	IS + Cal. 1

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

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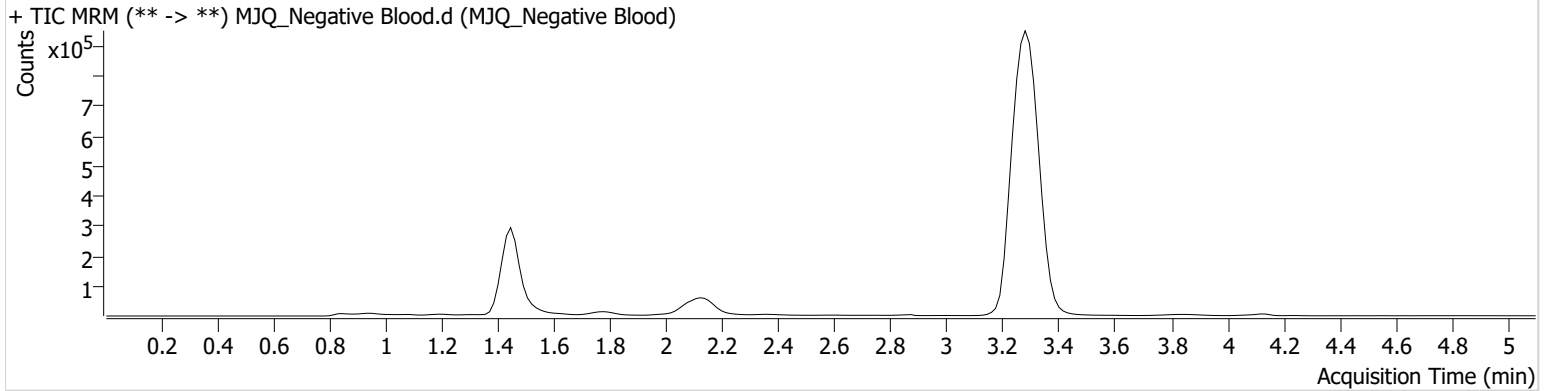


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Negative Blood.d
<b>Type</b>	Sample	<b>Sample</b>	MJQ_Negative Blood
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-H5	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 10:49:06 AM		
<b>Sample Info.</b>			

## Sample Chromatogram





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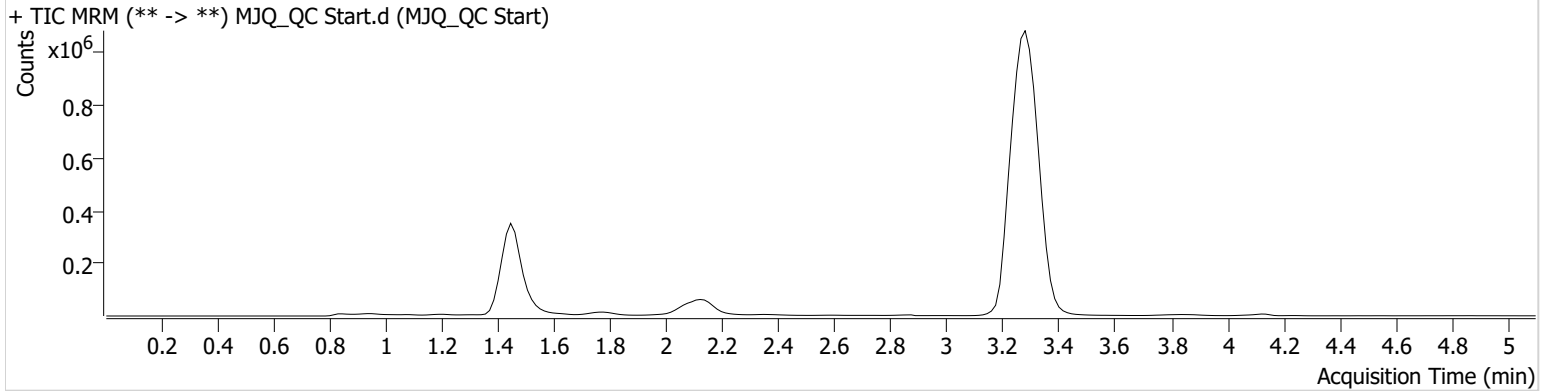


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_QC Start.d
<b>Type</b>	Sample	<b>Sample</b>	MJQ_QC Start
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-A6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 10:33:53 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	103527	∞	60.7	424.69	260887	14.9654 ng/ml
THC-OH	1.468	149758	29.63	8.5	∞	1196339	4.6410 ng/ml
THC	3.300	311618	1775.98	27.1	335.46	7128945	4.7722 ng/ml

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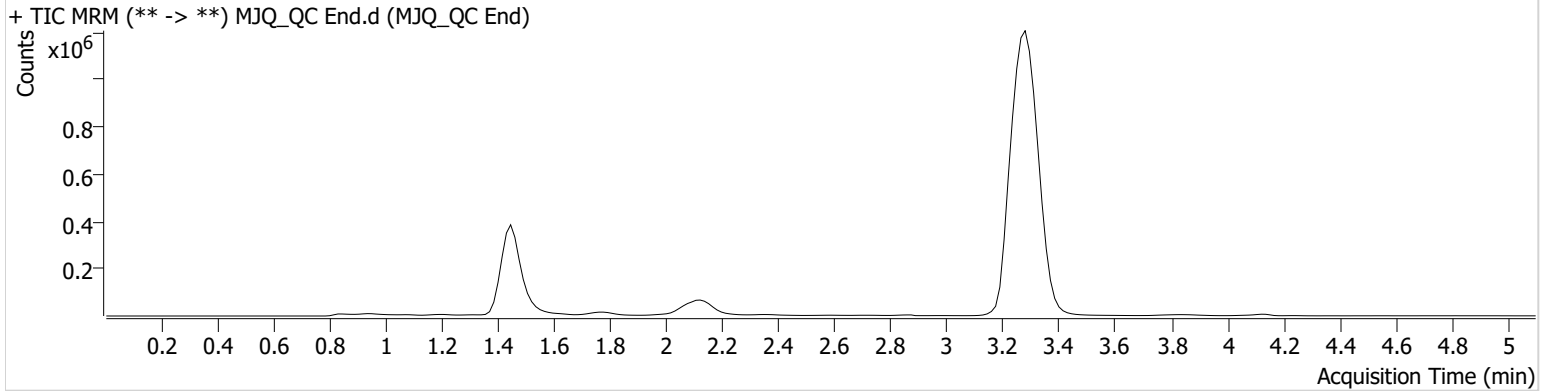


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_QC End.d
<b>Type</b>	Sample	<b>Sample</b>	MJQ_QC End
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-A6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 12:20:19 PM		

## Sample Chromatogram



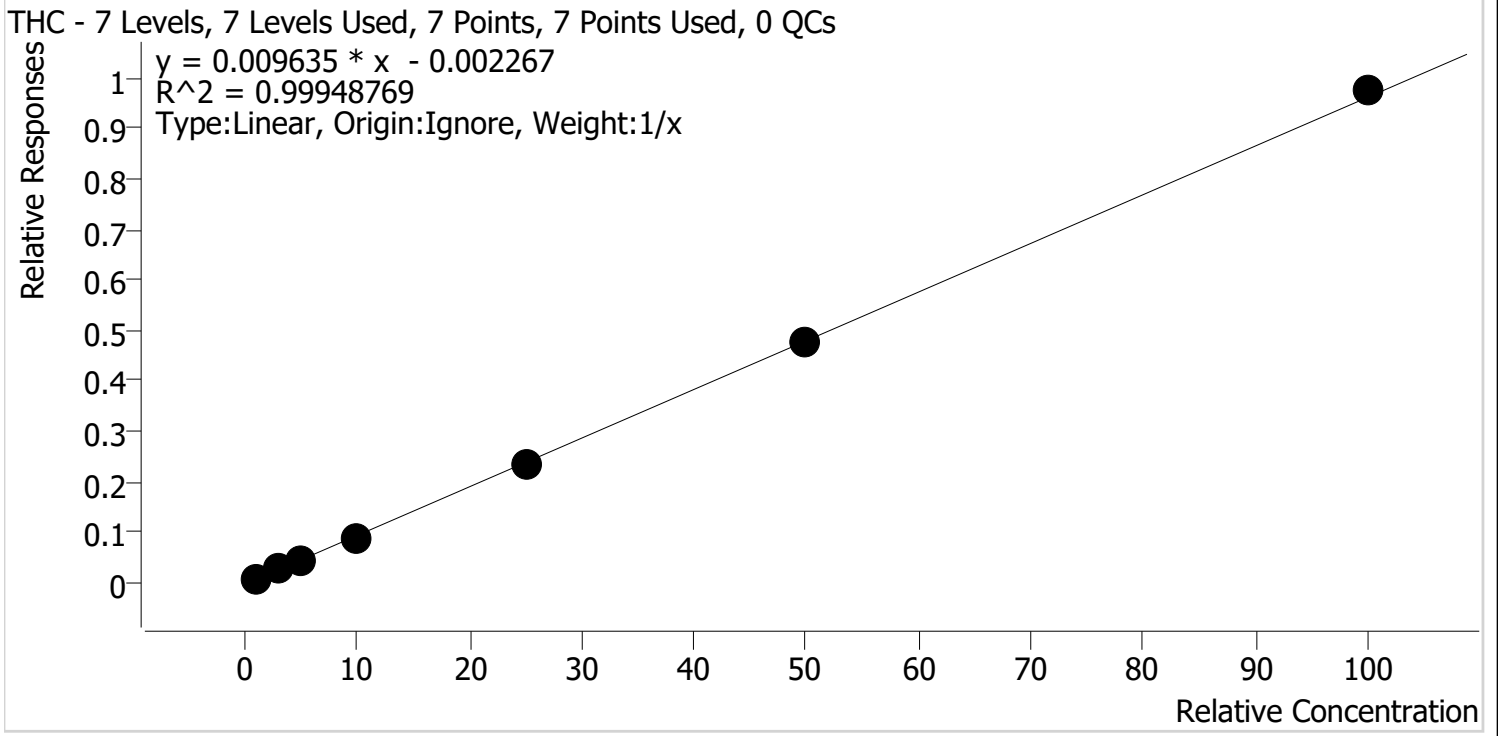
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	107244	242.67	62.2	815.77	273590	14.7730 ng/ml
THC-OH	1.468	159428	29.41	8.0	∞	1281015	4.5798 ng/ml
THC	3.300	345000	2210.17	27.0	∞	7895605	4.7705 ng/ml



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# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Last Cal. Update** 9/4/2021 1:47 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-D3



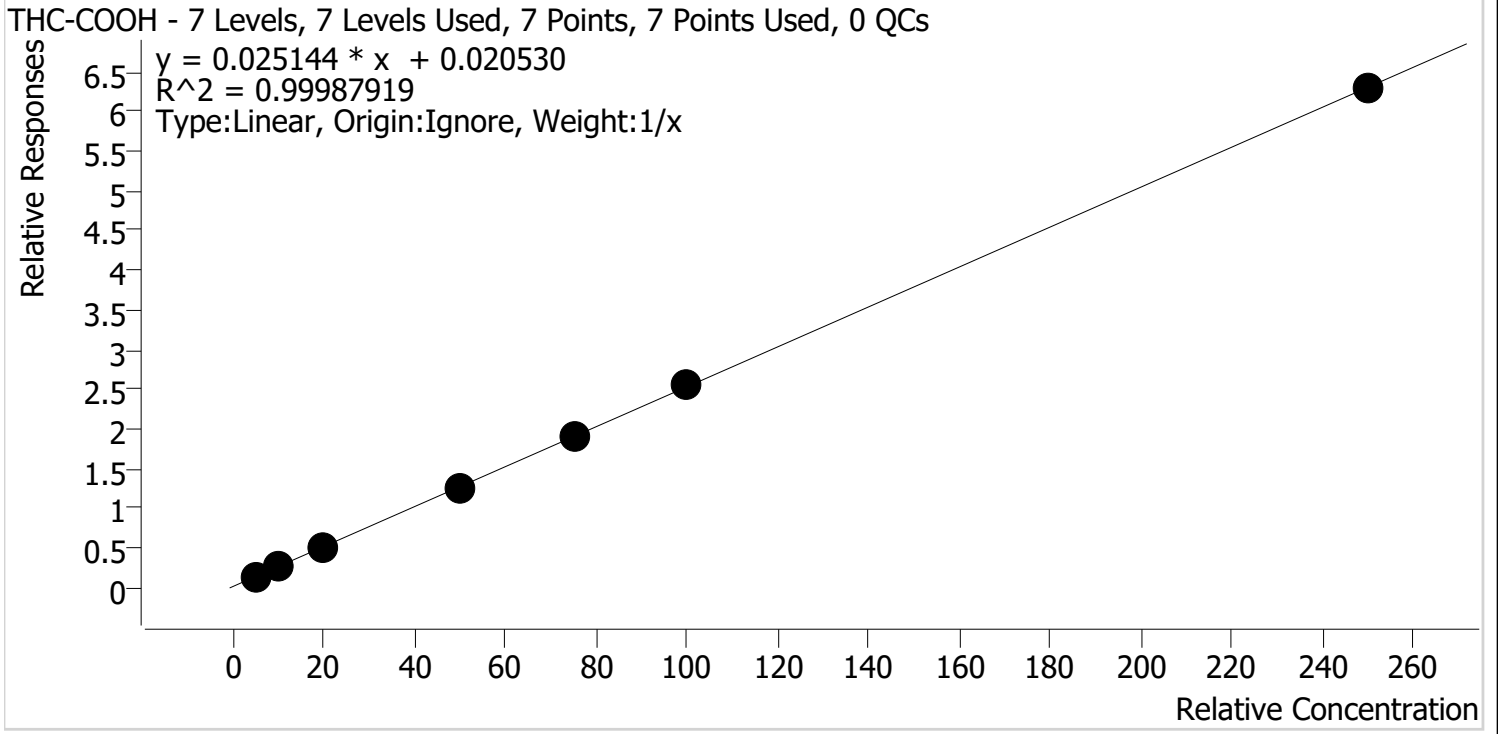
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	113.3
MJQ_Cal 2	2	✓	3.0	2.9	97.1
MJQ_Cal 3	3	✓	5.0	4.8	95.5
MJQ_Cal 4	4	✓	10.0	9.4	94.3
MJQ_Cal 5	5	✓	25.0	24.8	99.1
MJQ_Cal 6	6	✓	50.0	49.6	99.3
MJQ_Cal 7	7	✓	100.0	101.3	101.3



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# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Last Cal. Update** 9/4/2021 1:47 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	4.8	96.3
MJQ_Cal 2	2	✓	10.0	10.4	104.0
MJQ_Cal 3	3	✓	20.0	20.0	100.1
MJQ_Cal 4	4	✓	50.0	49.4	98.7
MJQ_Cal 5	5	✓	75.0	75.0	100.0
MJQ_Cal 6	6	✓	100.0	101.2	101.2
MJQ_Cal 7	7	✓	250.0	249.3	99.7

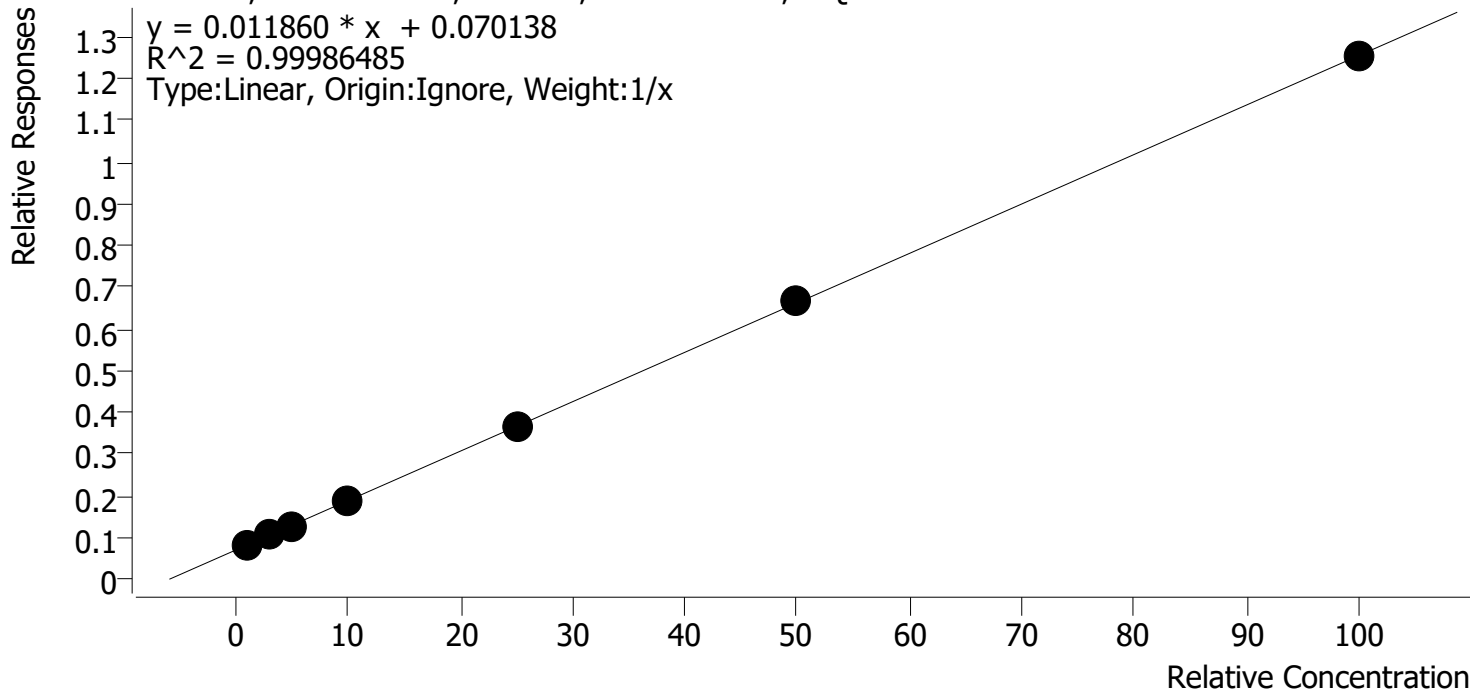
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# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Last Cal. Update** 9/4/2021 1:47 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-D3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	107.2
MJQ_Cal 2	2	✓	3.0	2.9	96.1
MJQ_Cal 3	3	✓	5.0	4.8	97.0
MJQ_Cal 4	4	✓	10.0	9.8	98.4
MJQ_Cal 5	5	✓	25.0	25.2	100.7
MJQ_Cal 6	6	✓	50.0	50.4	100.8
MJQ_Cal 7	7	✓	100.0	99.8	99.8

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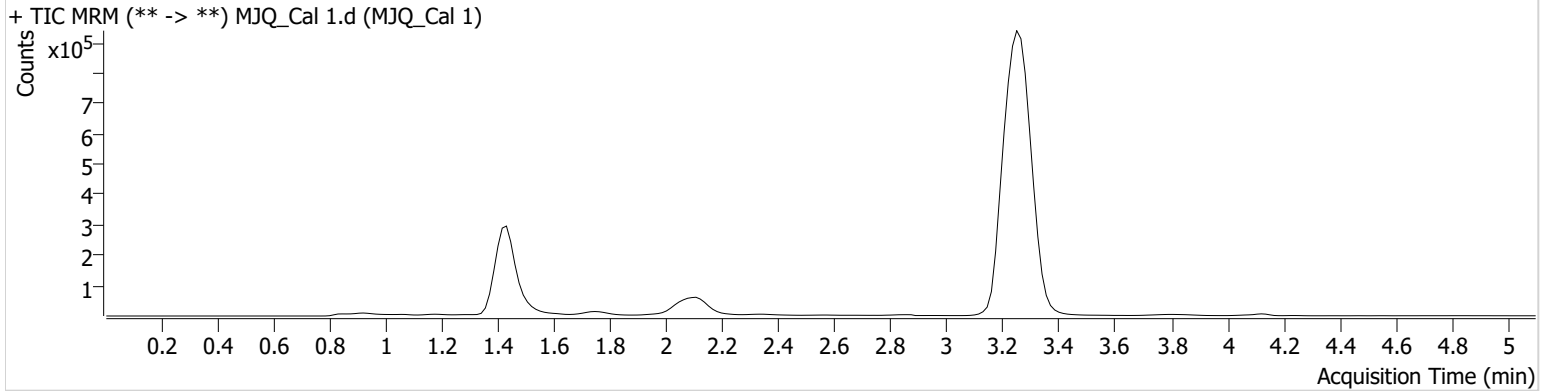


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Cal 1.d
<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 1
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-H6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 9:32:54 AM		
<b>Sample Info.</b>			

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	35256	∞	52.7	240.89	248905	4.8168 ng/ml <b>Low</b>
THC-OH	1.498	95808	∞	3.9 <b>Low</b>	10.62	1156363	1.0721 ng/ml <b>Low</b>
THC	3.270	56063	335.39	32.5	52.84	6481194	1.1331 ng/ml

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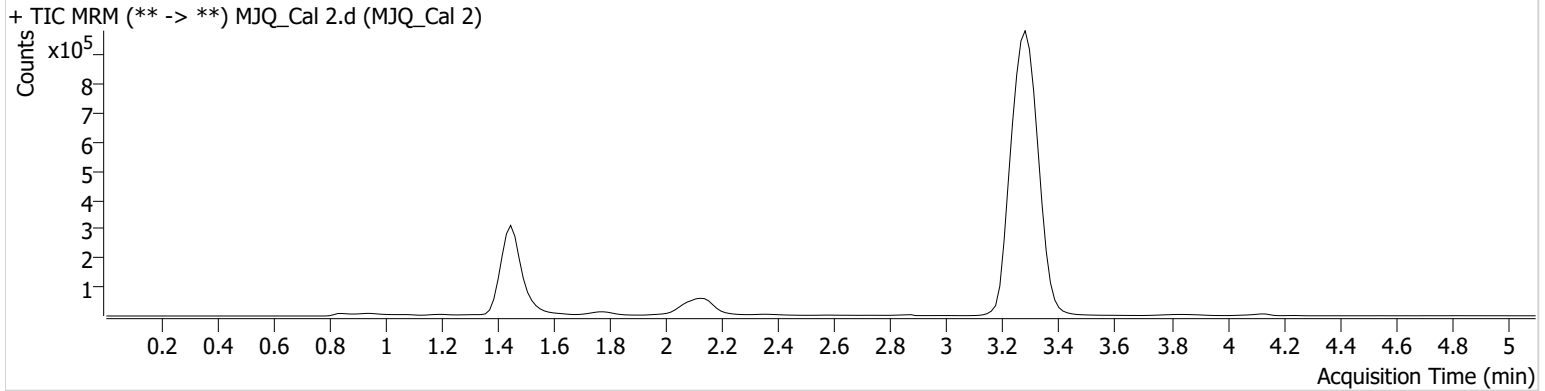


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Cal 2.d
<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 2
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-G6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 9:40:40 AM		
<b>Sample Info.</b>			

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	68996	∞	55.4	261.00	244559	10.4037 ng/ml
THC-OH	1.513	117387	∞	6.7 <b>Low</b>	∞	1125050	2.8838 ng/ml <b>Low</b>
THC	3.300	168145	∞	29.4	∞	6516086	2.9136 ng/ml

TS

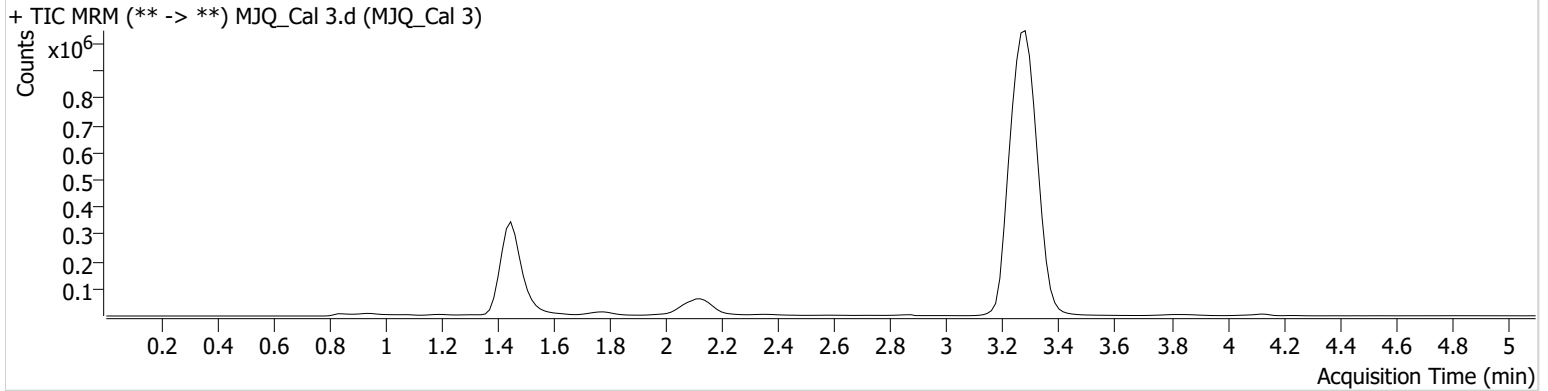


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Cal 3.d
<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 3
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-F6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 9:48:17 AM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	131107	∞	61.5	∞	250305	20.0148 ng/ml
THC-OH	1.483	148288	∞	7.9	∞	1161678	4.8493 ng/ml
THC	3.285	298307	1736.88	27.0	383.61	6818975	4.7759 ng/ml



TS

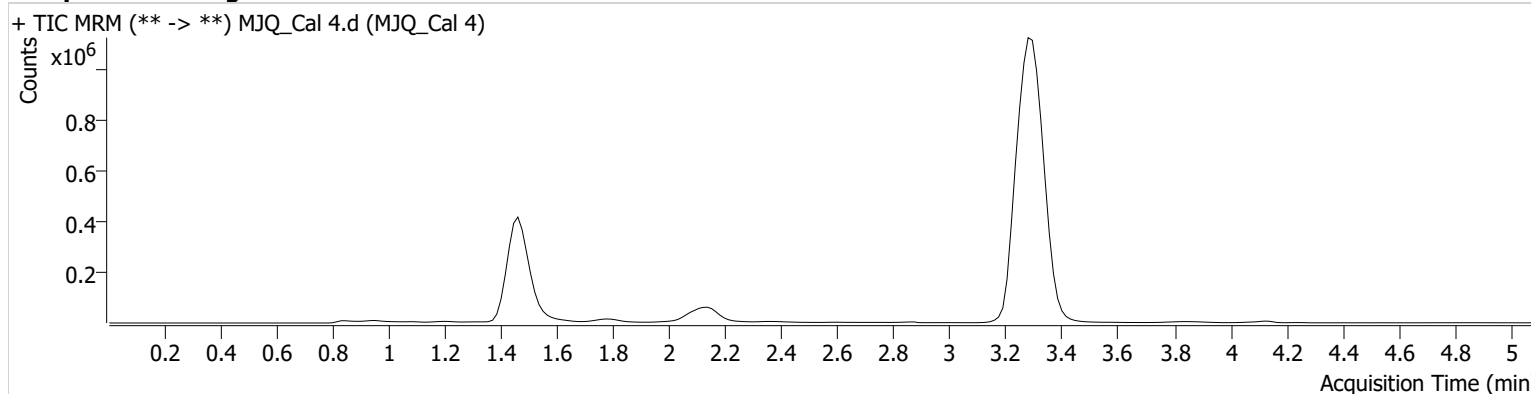


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Cal 4.d
<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 4
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-E6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 9:55:52 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	321372	1357.18	68.7	3354.11	254727	49.3589 ng/ml
THC-OH	1.468	221201	∞	9.3	320.48	1184242	9.8355 ng/ml
THC	3.300	614534	6571.14	26.9	∞	6934236	9.4337 ng/ml

TS

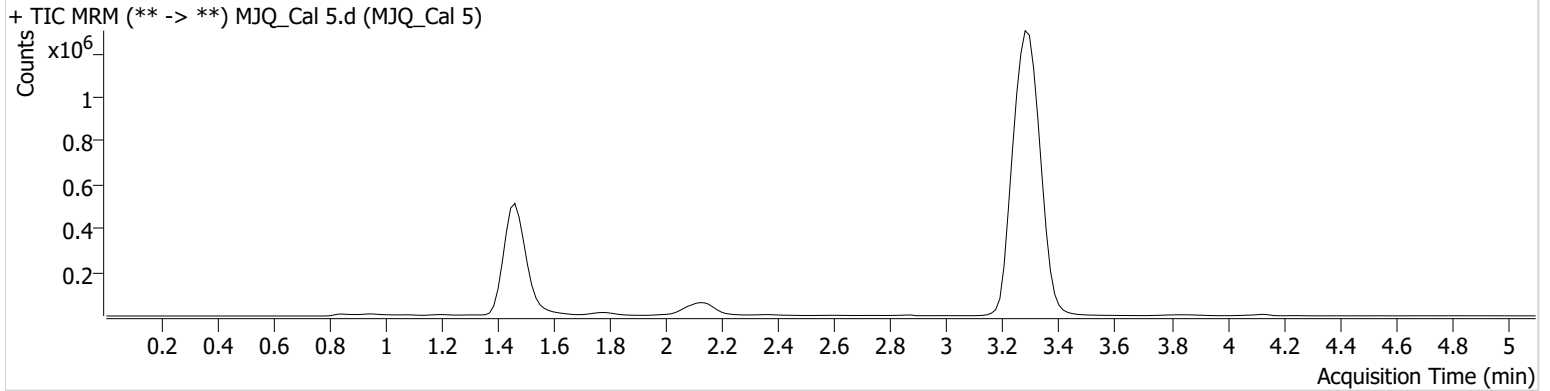


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Cal 5.d
<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 5
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-D6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 10:03:28 AM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	476540	∞	65.0	2362.07	250047	74.9777 ng/ml
THC-OH	1.453	434918	∞	11.4 <b>High</b>	∞	1179233	25.1835 ng/ml
THC	3.300	1627673	15936.98	25.8	1395.42	6883416	24.7782 ng/ml

TS

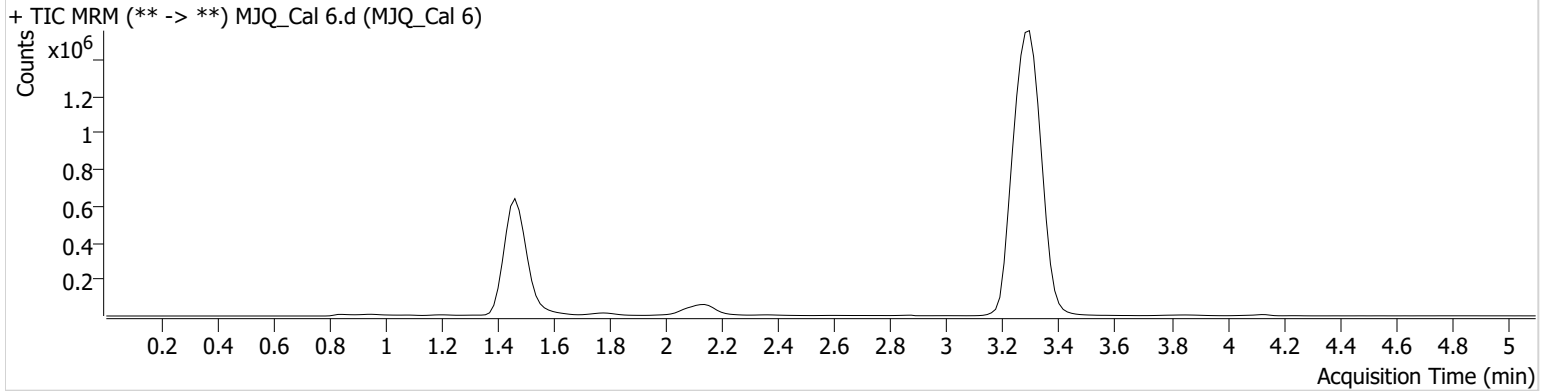


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Cal 6.d
<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 6
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-C6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 10:11:03 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	637374	∞	68.1	5295.72	248583	101.1558 ng/ml
THC-OH	1.453	795715	∞	12.8 <b>High</b>	1147.76	1191063	50.4159 ng/ml
THC	3.300	3268484	30670.43	26.3	∞	6867393	49.6343 ng/ml

TS

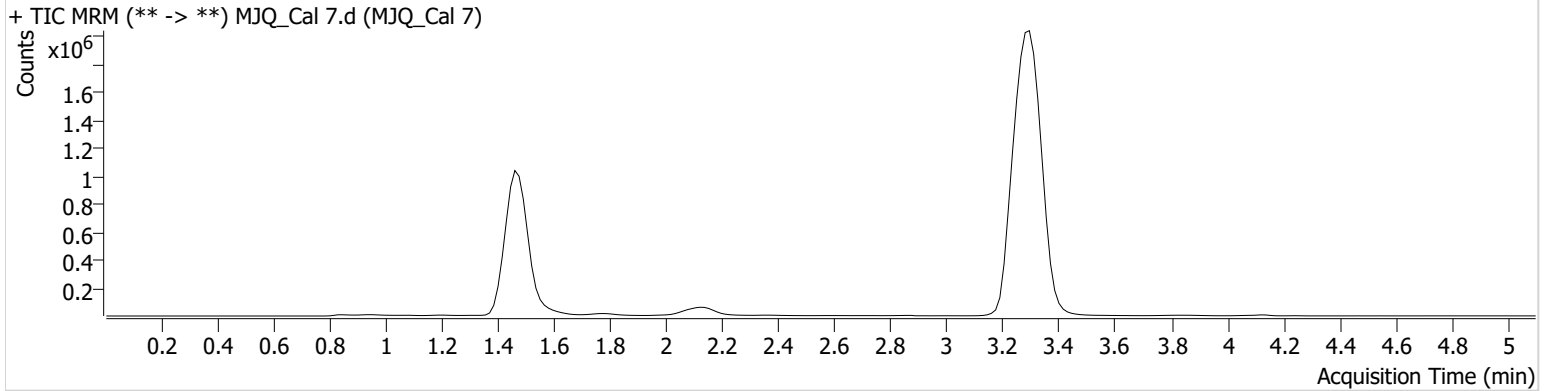


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090421 AM 27 TS\QuantResults\AM 27 TS.batch.bin  
**Calibration Last Update** 9/4/2021 1:47:26 PM

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<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 7
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Tamara Salazar
<b>Sample Position</b>	P1-B6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/4/2021 10:18:38 AM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	1443628	∞	69.0	∞	229572	249.2724 ng/ml
THC-OH	1.453	1436961	∞	13.8 <b>High</b>	3469.30	1146549	99.7600 ng/ml
THC	3.300	6327775	∞	26.3	∞	6496519	101.3312 ng/ml