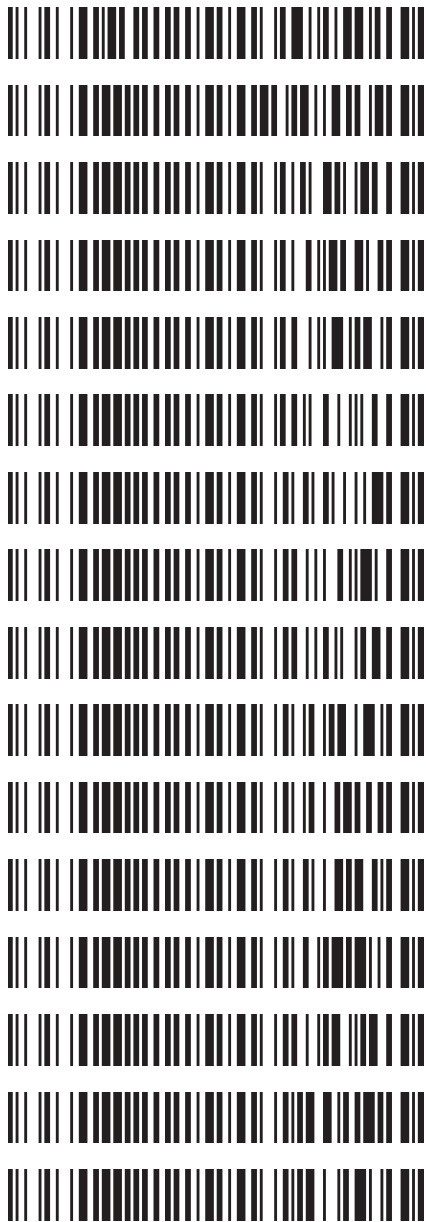


**REVIEWED**  
By Sarah Collins at 11:48 am, Jul 13, 2021

**Worklist: 5083**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-2438	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-3404	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1654	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1700	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1720	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1724	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1752	4	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1984	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1985	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1987	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1988	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2014	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2019	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2029	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2031	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2041	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: 07/07/2021

Analyst: Celena Shrum

Plate lot#: IDP-108-2-210412

Plate Expiration: 10/12/2021

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

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

**Blank Blood Lot:** Lampire 20L20723

**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** of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
- 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 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: Curve Limits: THC-OH not evaluated due to ratios being out of tolerances for some calibrators.

	1	2	3	4	5	6
a	cal 1ng	Blood NC	P2021-1984-1	P2021-2041-1		
b	cal 3 ng	M2021-2438-1	P2021-1985-1			
c	cal 5 ng	P2020-3404-1	P2021-1987-1			
d	cal 10ng	P2021-1654-1	P2021-1988-1			
e	cal 25 ng	P2021-1700-1	P2021-2014-1			
f	cal 50 ng	P2021-1720-1	P2021-2019-1			
g	cal 100 ng	P2021-1724-1	P2021-2029-1			
h	QC 1	P2021-1752-4	P2021-2031-1			

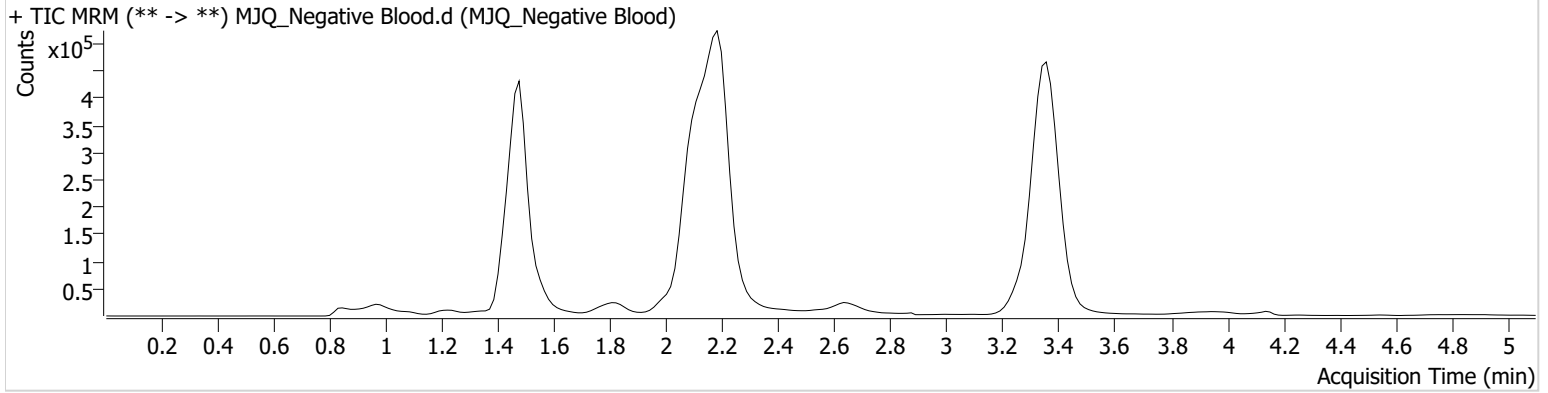


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 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>	Celena Shrum
<b>Sample Position</b>	P1-A2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/8/2021 12:17:20 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



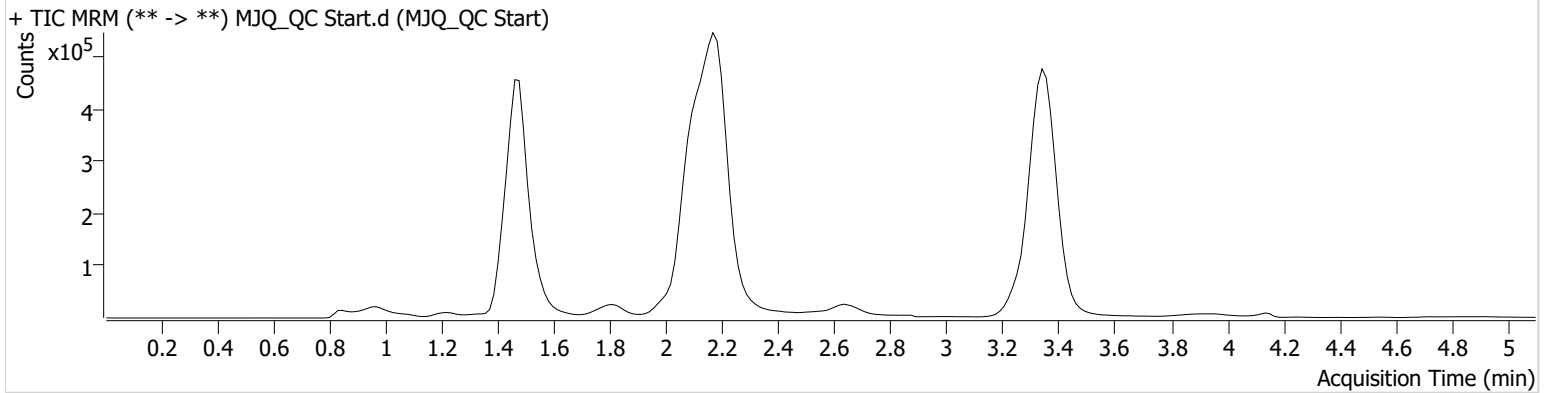
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 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>	Celena Shrum
<b>Sample Position</b>	P1-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/8/2021 12:32:34 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	131626	∞	56.1	361.79	387990	14.2773 ng/ml
THC-OH	1.483	279228	∞	6.2	∞	1682860	4.4447 ng/ml
THC	3.360	151078	∞	30.4	∞	3221620	4.6852 ng/ml

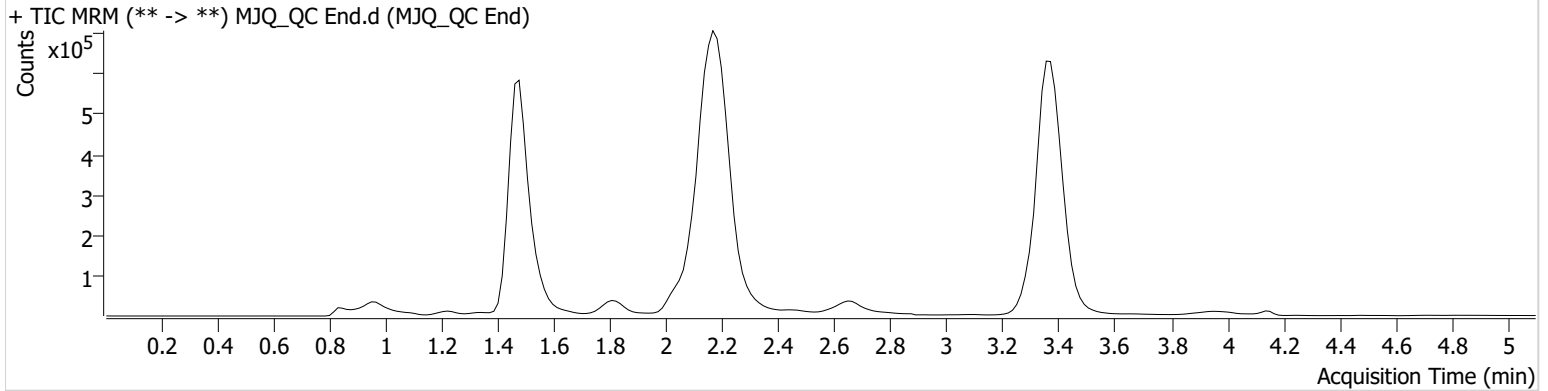


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 PM

**Instrument** Falco (069901) **Data File** MJQ\_QC End.d  
**Type** Sample **Sample** MJQ\_QC End  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-H1 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 7/8/2021 4:51:16 AM  
**Sample Info.**

## Sample Chromatogram

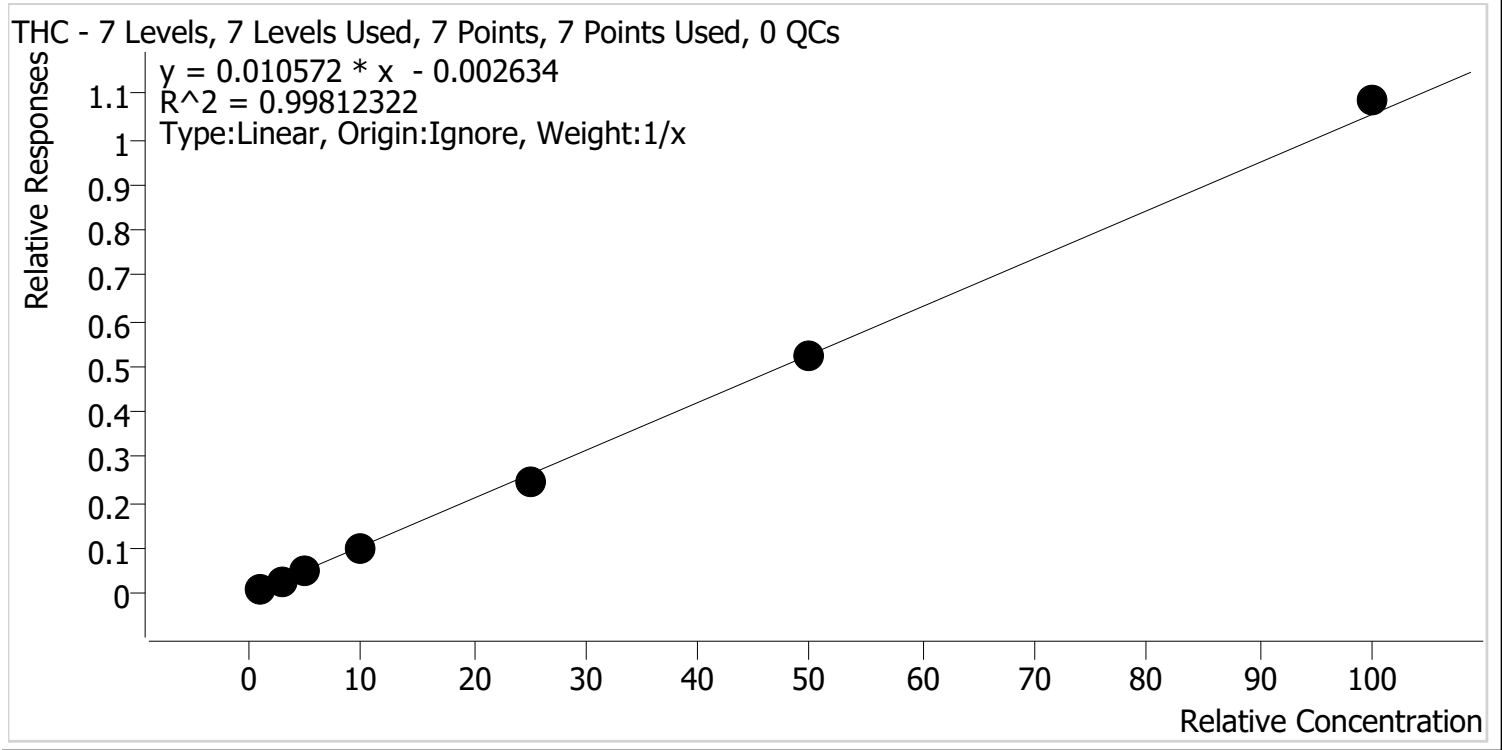


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	151046	331.87	57.6	∞	443564	14.3291 ng/ml
THC-OH	1.543	324640	∞	6.0	∞	1927100	4.6252 ng/ml
THC	3.375	179592	∞	31.2	∞	4001268	4.4949 ng/ml



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 7/12/2021 2:06 PM  
**Analyst Name** ISP\Datastor  
**Analyte** THC **Internal Standard** THC-D3

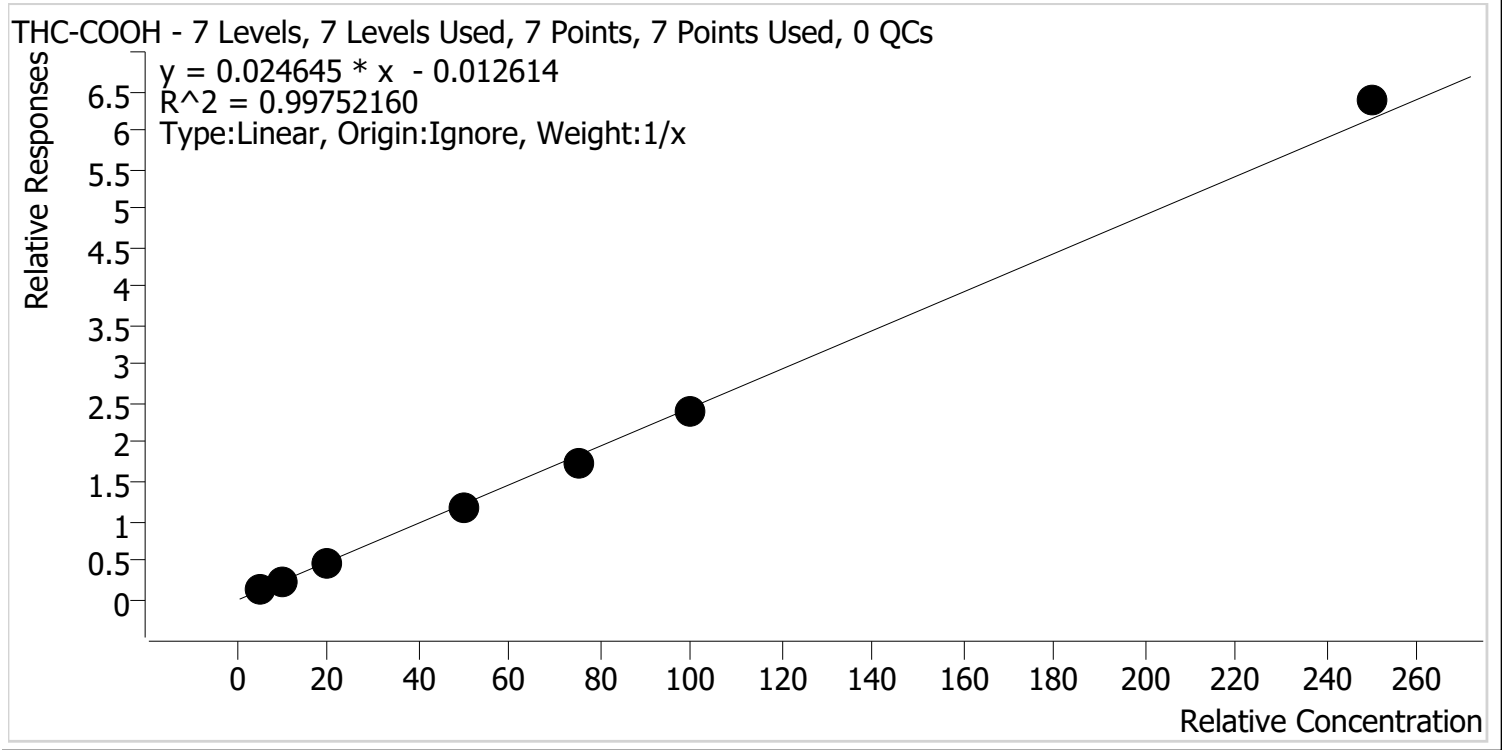


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.2	118.2
MJQ_Cal 2	2	✓	3.0	3.0	98.6
MJQ_Cal 3	3	✓	5.0	4.7	94.5
MJQ_Cal 4	4	✓	10.0	9.4	93.7
MJQ_Cal 5	5	✓	25.0	23.1	92.6
MJQ_Cal 6	6	✓	50.0	49.7	99.5
MJQ_Cal 7	7	✓	100.0	102.9	102.9



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 7/12/2021 2:06 PM  
**Analyst Name** ISP\Datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-D9



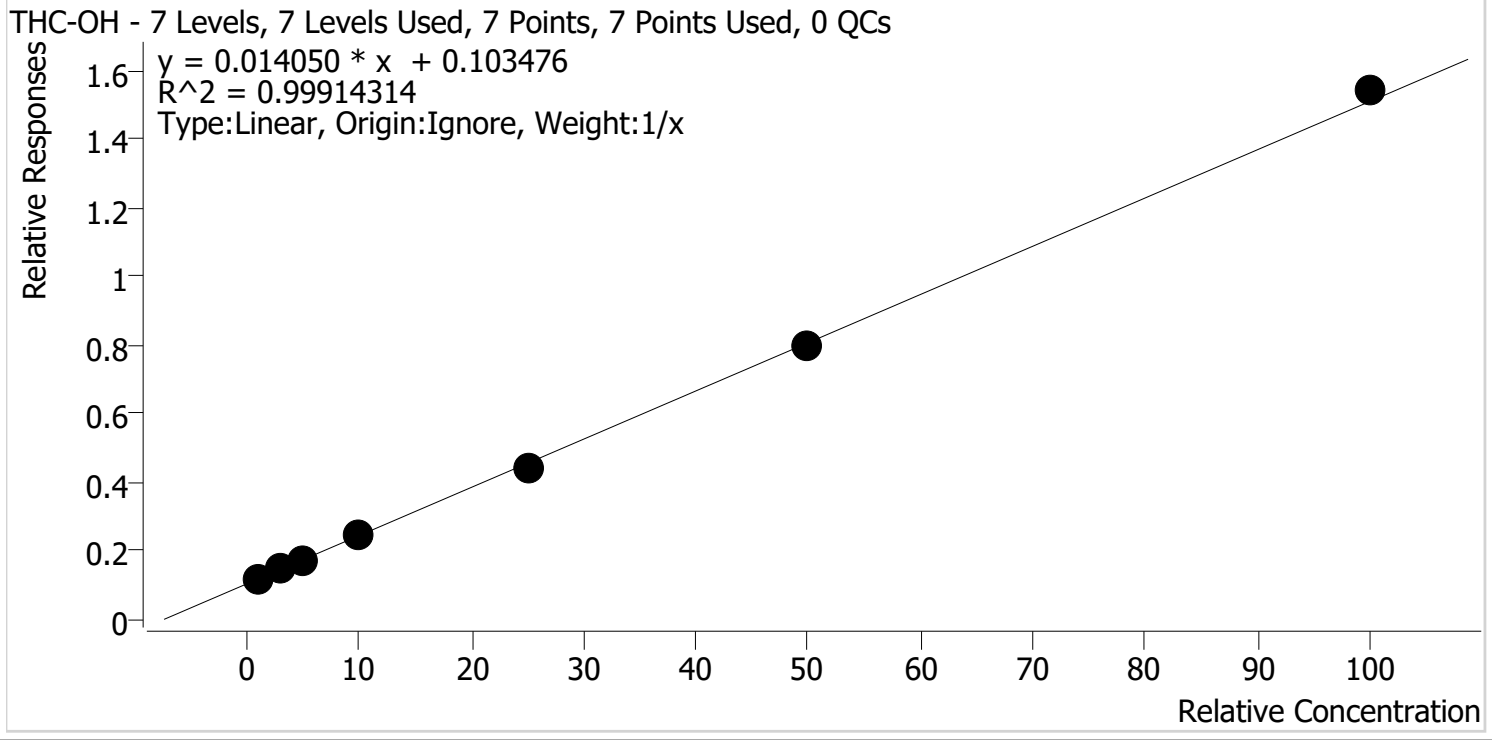
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	5.9	118.7
MJQ_Cal 2	2	✓	10.0	9.5	95.4
MJQ_Cal 3	3	✓	20.0	18.8	93.9
MJQ_Cal 4	4	✓	50.0	48.1	96.2
MJQ_Cal 5	5	✓	75.0	71.2	95.0
MJQ_Cal 6	6	✓	100.0	97.1	97.1
MJQ_Cal 7	7	✓	250.0	259.3	103.7





# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 7/12/2021 2:06 PM  
**Analyst Name** ISP\Datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	106.3
MJQ_Cal 2	2	✓	3.0	3.1	102.5
MJQ_Cal 3	3	✓	5.0	4.9	97.6
MJQ_Cal 4	4	✓	10.0	9.8	98.0
MJQ_Cal 5	5	✓	25.0	23.7	94.8
MJQ_Cal 6	6	✓	50.0	49.3	98.5
MJQ_Cal 7	7	✓	100.0	102.2	102.2

Compound not evaluated due to ratio issues with the calibrators.

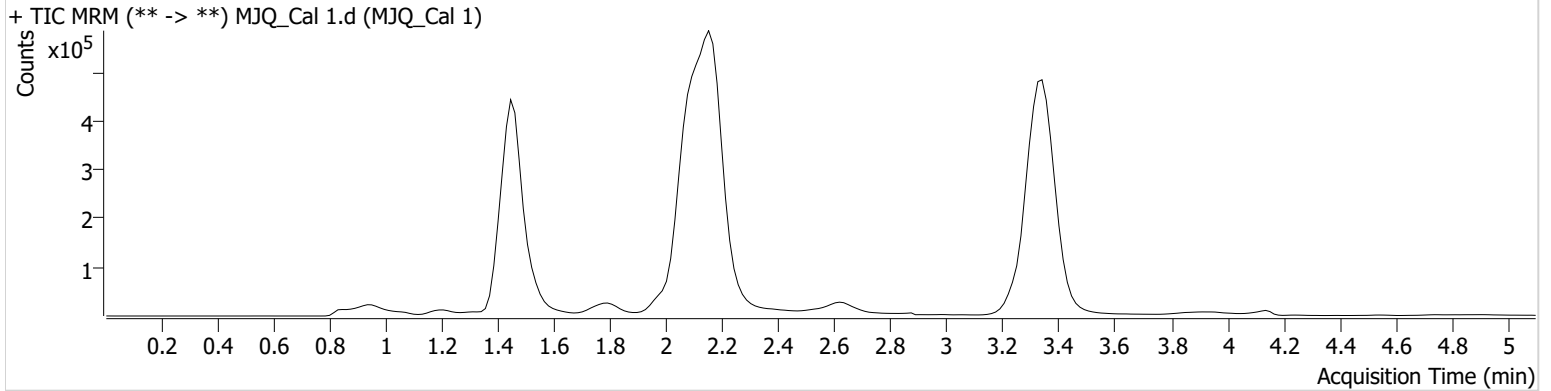


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 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>	Celena Shrum
<b>Sample Position</b>	P1-A1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/7/2021 11:16:26 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	52871	∞	45.2	∞	395559	5.9353 ng/ml
THC-OH	1.528	208526	∞	3.5 <b>Low</b>	37.88	1760947	1.0634 ng/ml <b>Low</b>
THC	3.360	34969	∞	27.4	11.43	3544652	1.1824 ng/ml

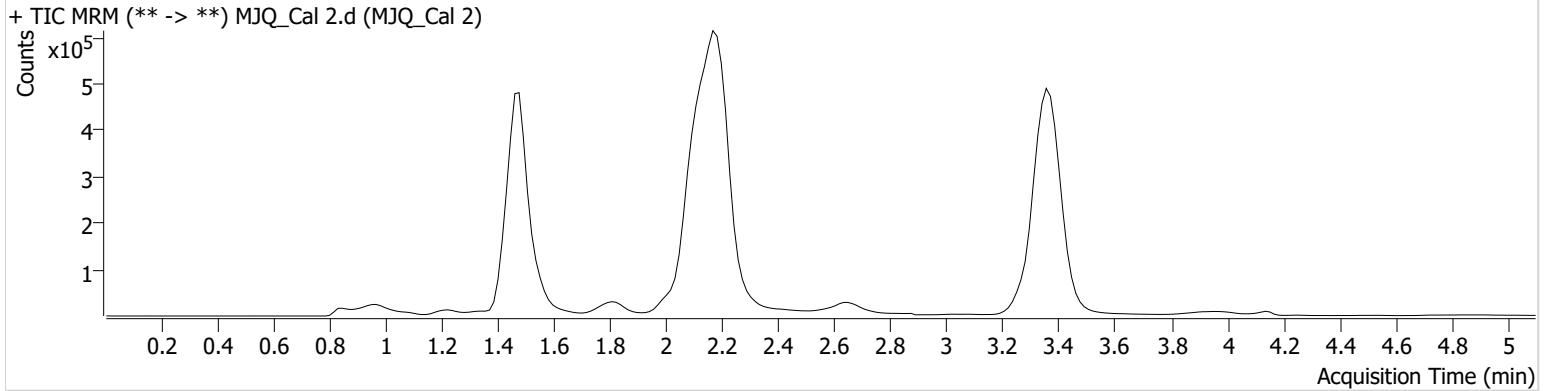


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 PM

**Instrument** Falco (069901) **Data File** MJQ\_Cal 2.d  
**Type** Cal **Sample** MJQ\_Cal 2  
**Acq. Method** AM 27 THCQ.m **Operator** Celena Shrum  
**Sample Position** P1-B1 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 7/7/2021 11:24:12 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	91703	∞	57.1	385.41	412199	9.5389 ng/ml
THC-OH	1.543	261696	∞	4.9 <b>Low</b>	74.69	1783882	3.0765 ng/ml
THC	3.375	96721	∞	27.1	∞	3375819	2.9594 ng/ml

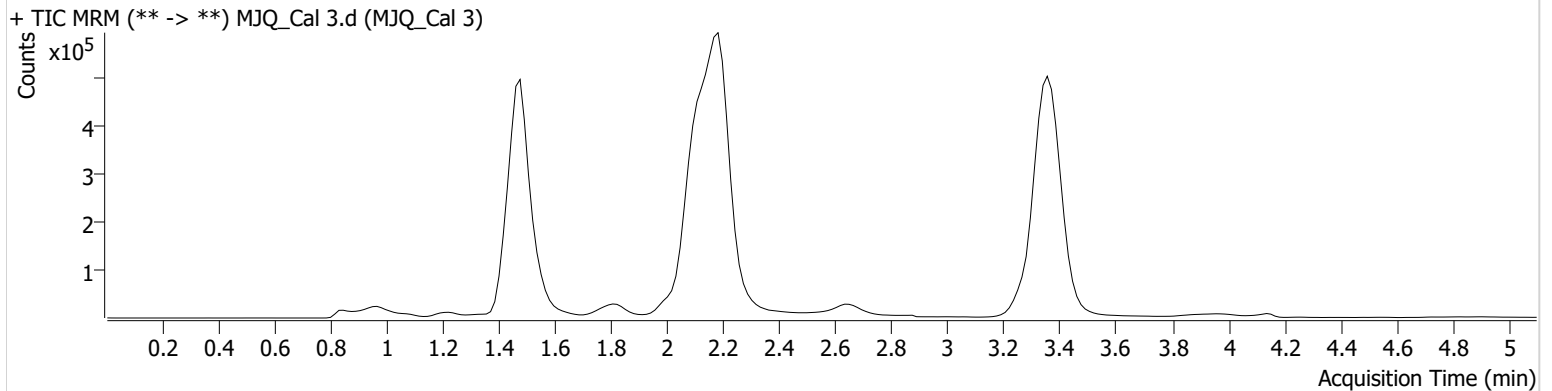
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 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>	Celena Shrum
<b>Sample Position</b>	P1-C1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/7/2021 11:31:47 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	183512	∞	56.9	1175.41	407728	18.7744 ng/ml
THC-OH	1.498	294573	∞	5.5 <b>Low</b>	126.61	1712576	4.8775 ng/ml
THC	3.360	159394	∞	28.4	189.56	3369042	4.7245 ng/ml

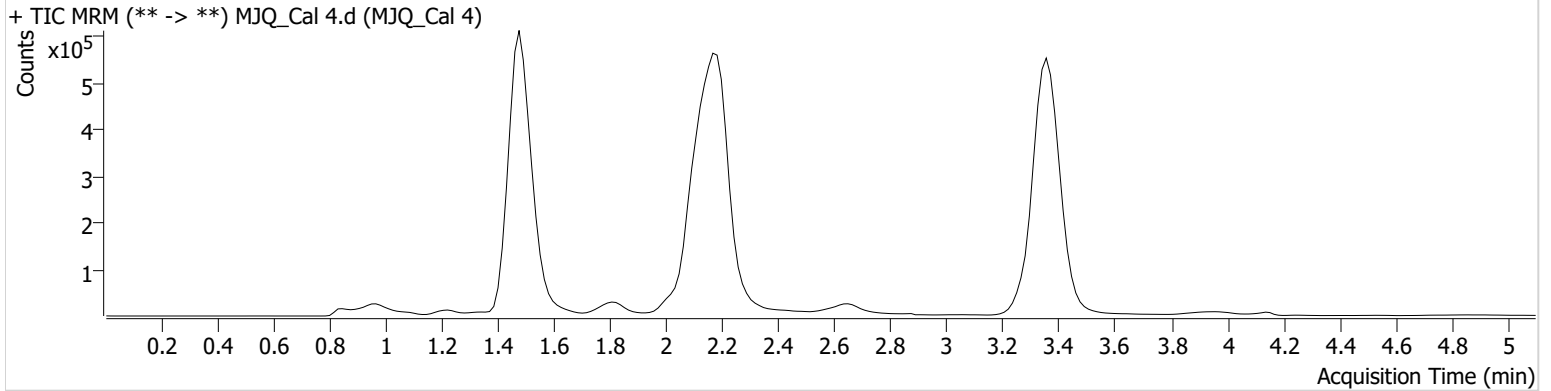


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 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>	Celena Shrum
<b>Sample Position</b>	P1-D1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/7/2021 11:39:22 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	465047	∞	59.0	∞	396398	48.1148 ng/ml
THC-OH	1.483	415937	∞	7.1	246.53	1724220	9.8046 ng/ml
THC	3.375	328379	∞	28.1	∞	3406247	9.3685 ng/ml

CS

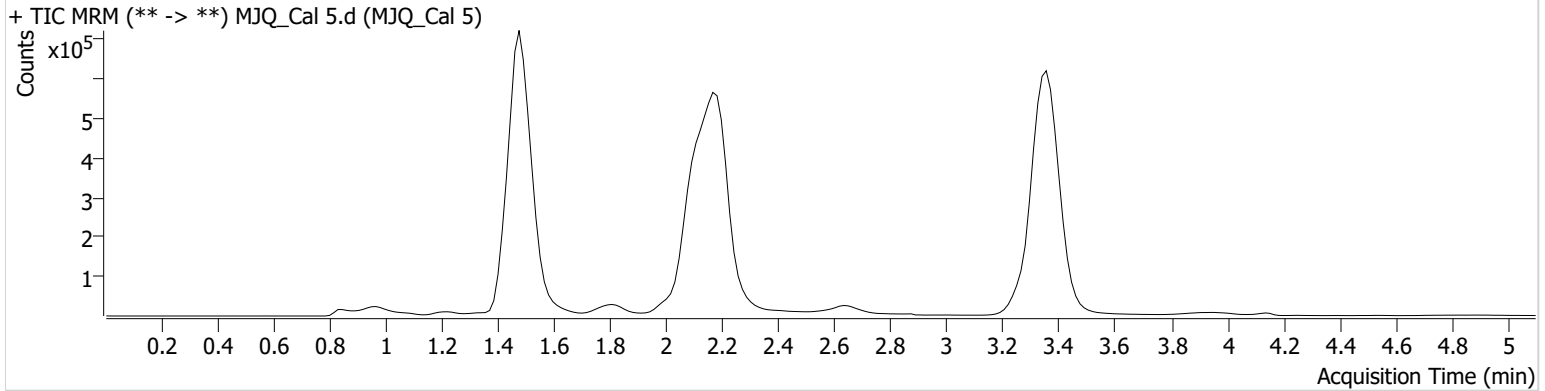


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 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>	Celena Shrum
<b>Sample Position</b>	P1-E1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/7/2021 11:46:57 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	672828	871.22	59.4	∞	386037	71.2320 ng/ml
THC-OH	1.483	735412	∞	9.4 <b>High</b>	244.61	1685583	23.6879 ng/ml
THC	3.360	818216	∞	27.3	1199.58	3380346	23.1457 ng/ml

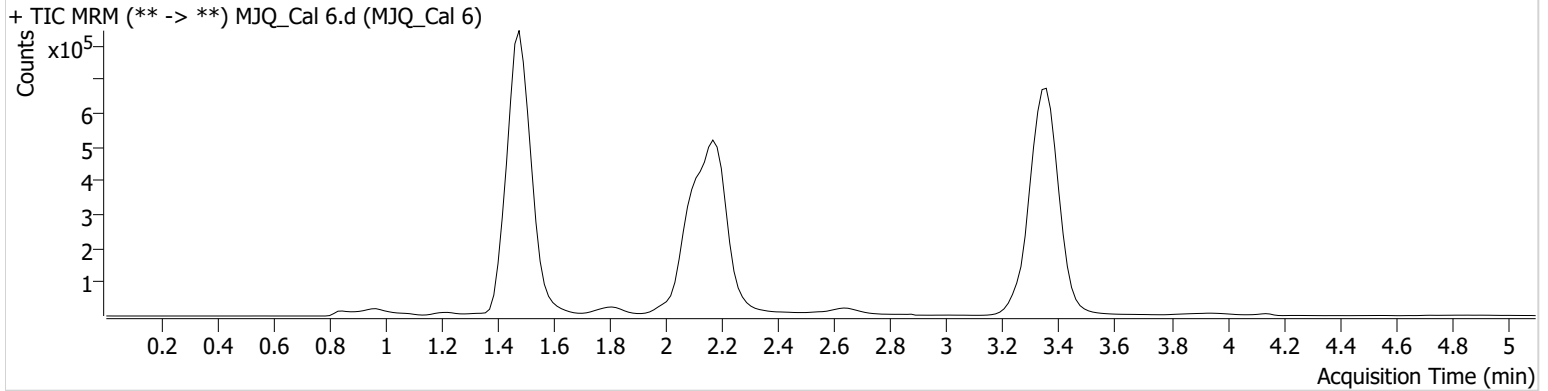
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 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>	Celena Shrum
<b>Sample Position</b>	P1-F1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/7/2021 11:54:33 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	856801	∞	59.3	2992.77	359896	97.1105 ng/ml
THC-OH	1.468	1264303	∞	10.6 <b>High</b>	∞	1588896	49.2687 ng/ml
THC	3.360	1571203	∞	25.4	∞	3003116	49.7398 ng/ml

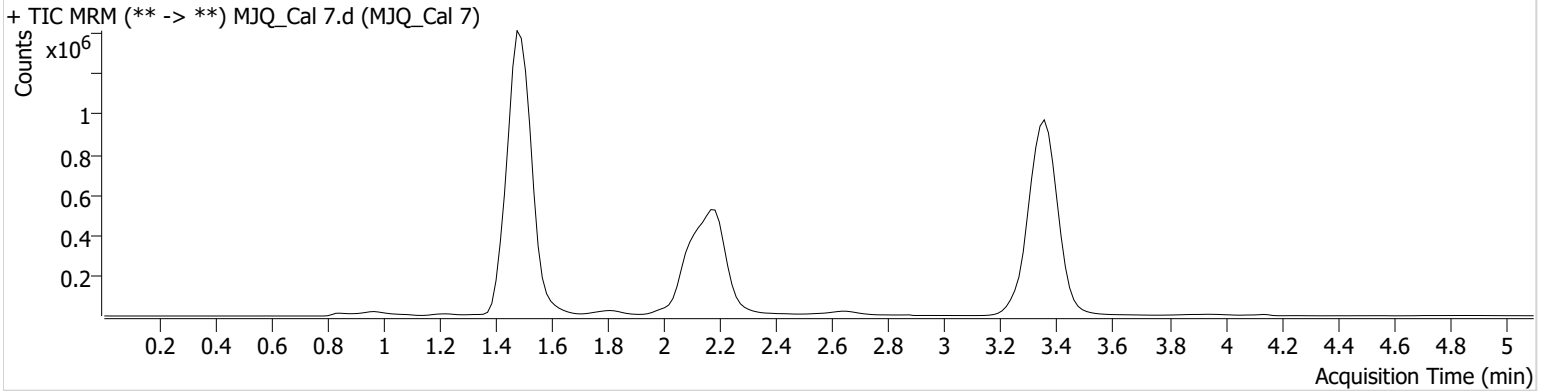
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\070721 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 7/12/2021 2:06:51 PM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJQ_Cal 7.d
<b>Type</b>	Cal	<b>Sample</b>	MJQ_Cal 7
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Celena Shrum
<b>Sample Position</b>	P1-G1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	7/8/2021 12:02:09 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	2218987	∞	56.9	∞	347927	259.2942 ng/ml
THC-OH	1.468	2376299	∞	11.2 <b>High</b>	∞	1543344	102.2214 ng/ml
THC	3.360	3296259	∞	25.6	4194.55	3038134	102.8798 ng/ml