

**Worklist: 4931**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2020-3163	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-1131	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-1512	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-1586	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-1590	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1027	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1085	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1114	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1176	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1177	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1180	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1181	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1209	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-1224	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: 04/22/2021

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 20L20724

**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 Limitations: THC 1-100, c-THC 5-250, THC-OH 3-100.

The run stopped part of the way through due to a clog. The clog was cleared and some of the samples had to be reconstituted and reinjected. There were no issues with the reinjections.

	1	2	3	4	5	6
a	cal 1ng	Blood NC	P2021-1114-1			
b	cal 3 ng	M2020-3163-1	P2021-1176-1			
c	cal 5 ng	M2021-1131-2	P2021-1177-1			
d	cal 10ng	M2021-1512-2	P2021-1180-1			
e	cal 25 ng	M2021-1586-2	P2021-1181-1			
f	cal 50 ng	M2021-1590-2	P2021-1209-1			
g	cal 100 ng	P2021-1027-1	P2021-1224-1			
h	QC 1	P2021-1085-1				

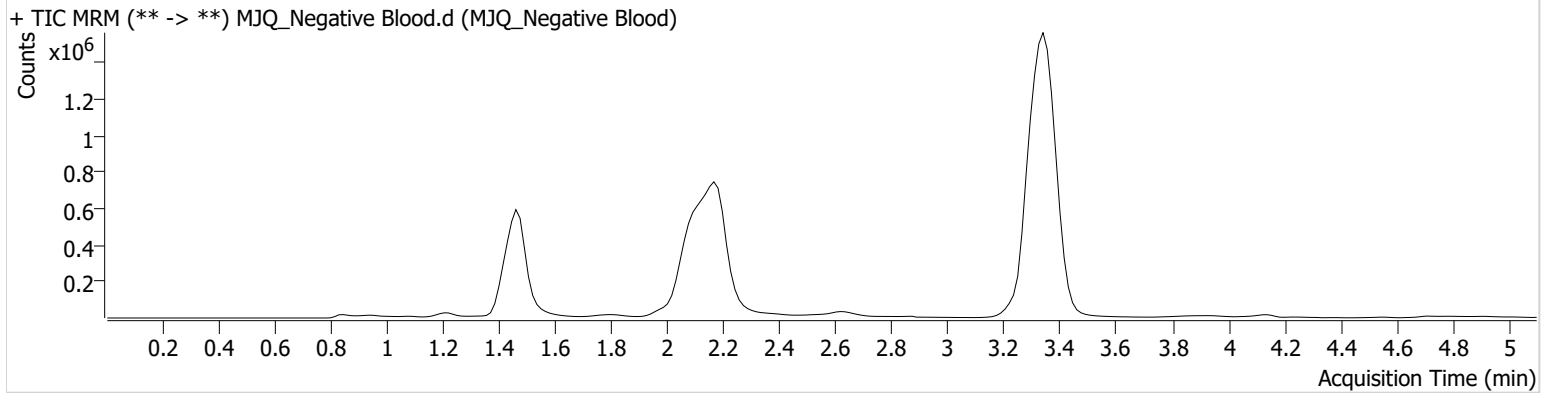
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

<b>Instrument</b>	Instrument 1	<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>	4/22/2021 4:24:47 PM		
<b>Sample Info.</b>			

## Sample Chromatogram





CS

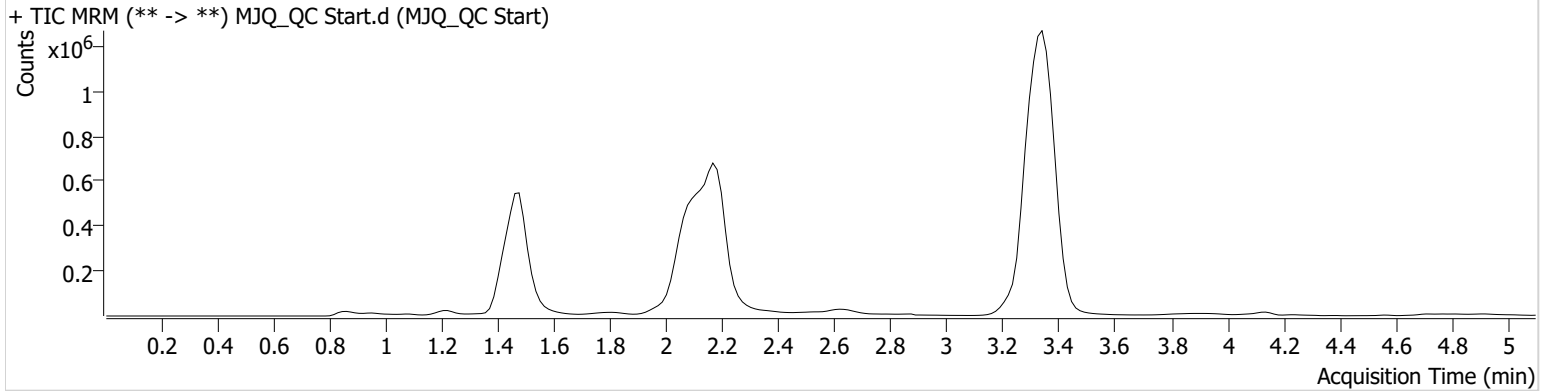


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

<b>Instrument</b>	Instrument 1	<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>	4/22/2021 4:39:59 PM		
<b>Sample Info.</b>			

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	186380	∞	8.7	124.64	2130793	4.8161 ng/ml
THC-COOH	1.504	210399	∞	53.3	371.87	537319	14.3119 ng/ml
THC	3.360	386765	1950.72	26.2	908.32	8802106	4.4493 ng/ml

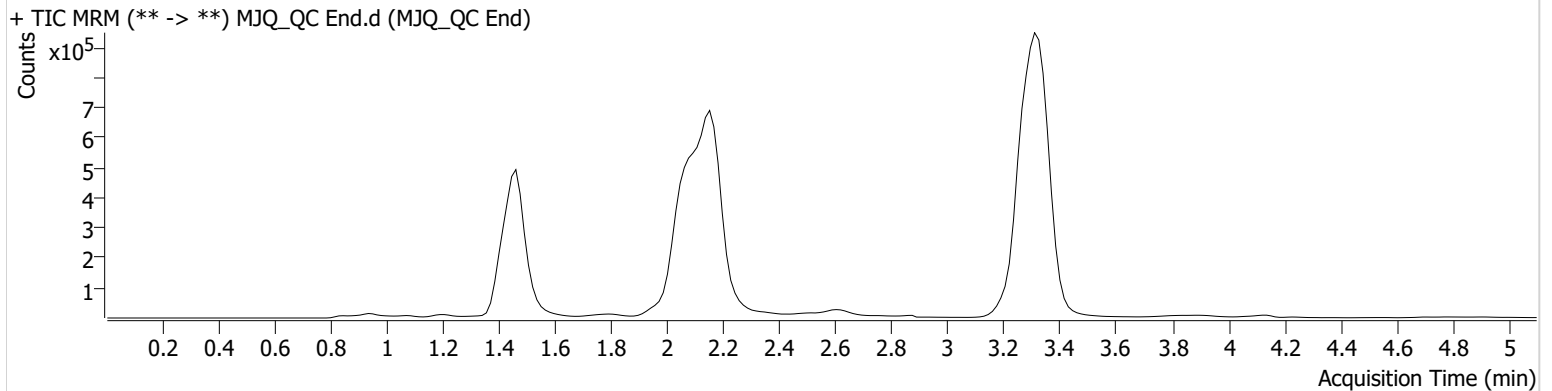
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

<b>Instrument</b>	Instrument 1	<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>	Celena Shrum
<b>Sample Position</b>	P1-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/23/2021 11:13:57 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



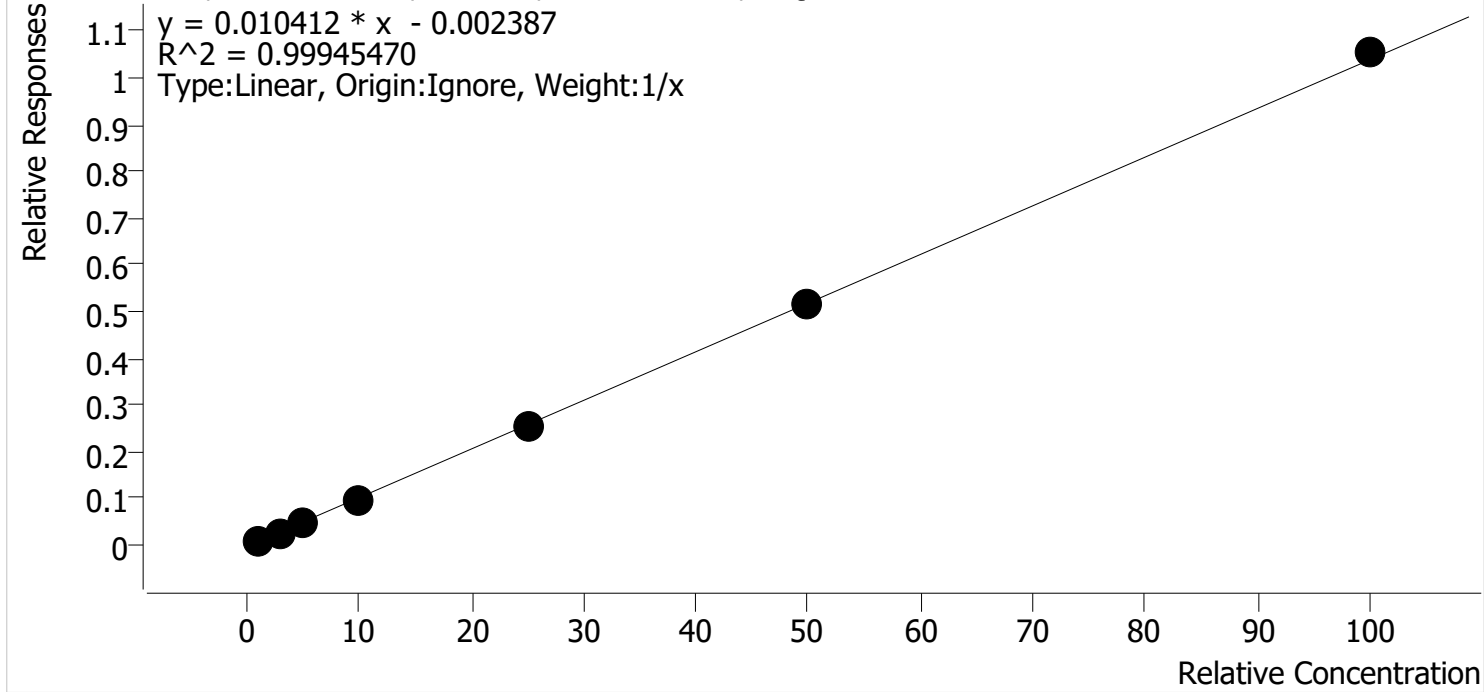
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	163081	∞	8.7	98.15	1869820	4.7977 ng/ml
THC-COOH	1.489	173161	∞	54.0	509.96	472815	13.3231 ng/ml
THC	3.330	290959	∞	26.2	56.21	6783954	4.3484 ng/ml



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 4/26/2021 6:48 AM  
**Analyst Name** ISP\Datastor  
**Analyte** THC **Internal Standard** THC-D3

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

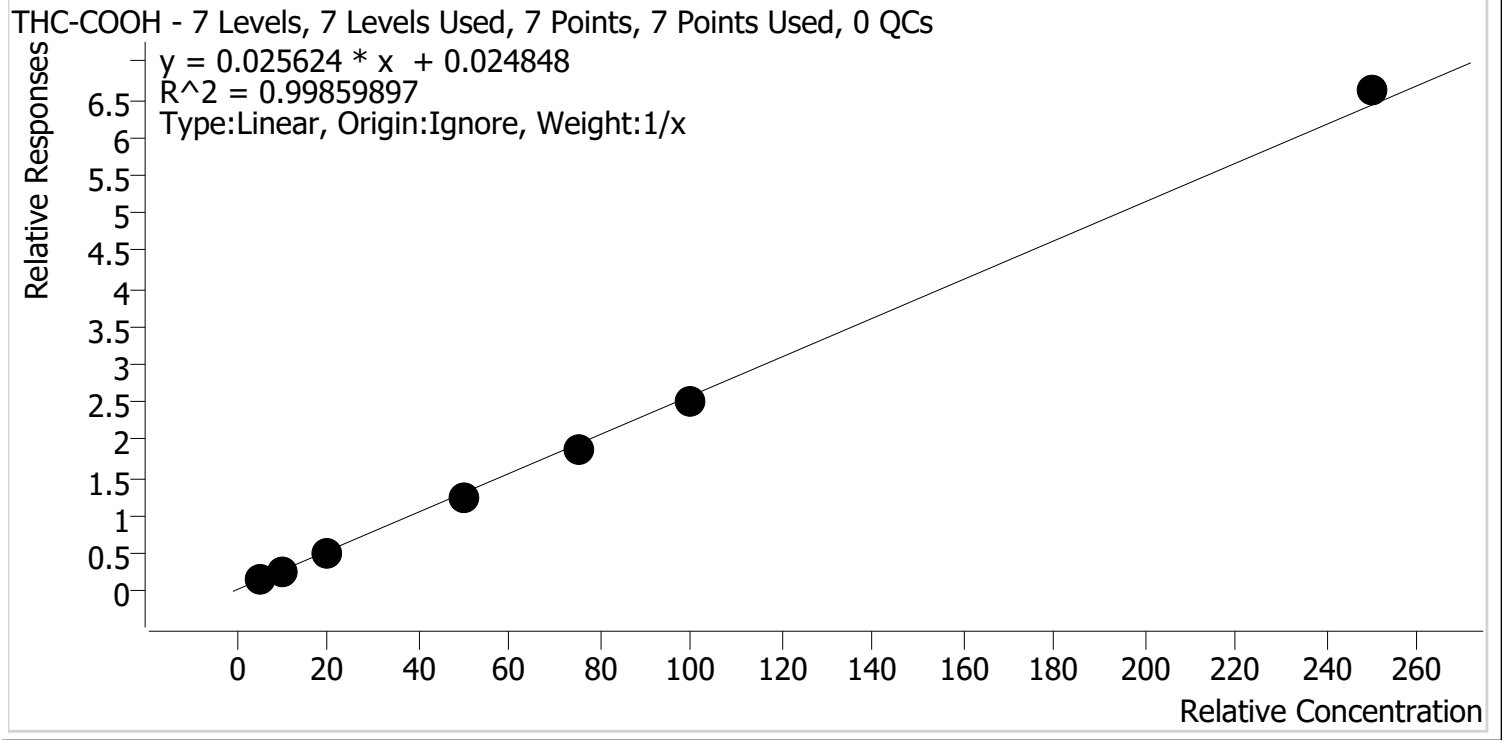


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	114.6
MJQ_Cal 2	2	✓	3.0	2.9	95.6
MJQ_Cal 3	3	✓	5.0	4.8	95.6
MJQ_Cal 4	4	✓	10.0	9.5	95.2
MJQ_Cal 5	5	✓	25.0	24.6	98.3
MJQ_Cal 6	6	✓	50.0	49.7	99.3
MJQ_Cal 7	7	✓	100.0	101.4	101.4



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 4/26/2021 6:48 AM  
**Analyst Name** ISP\Datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-D9



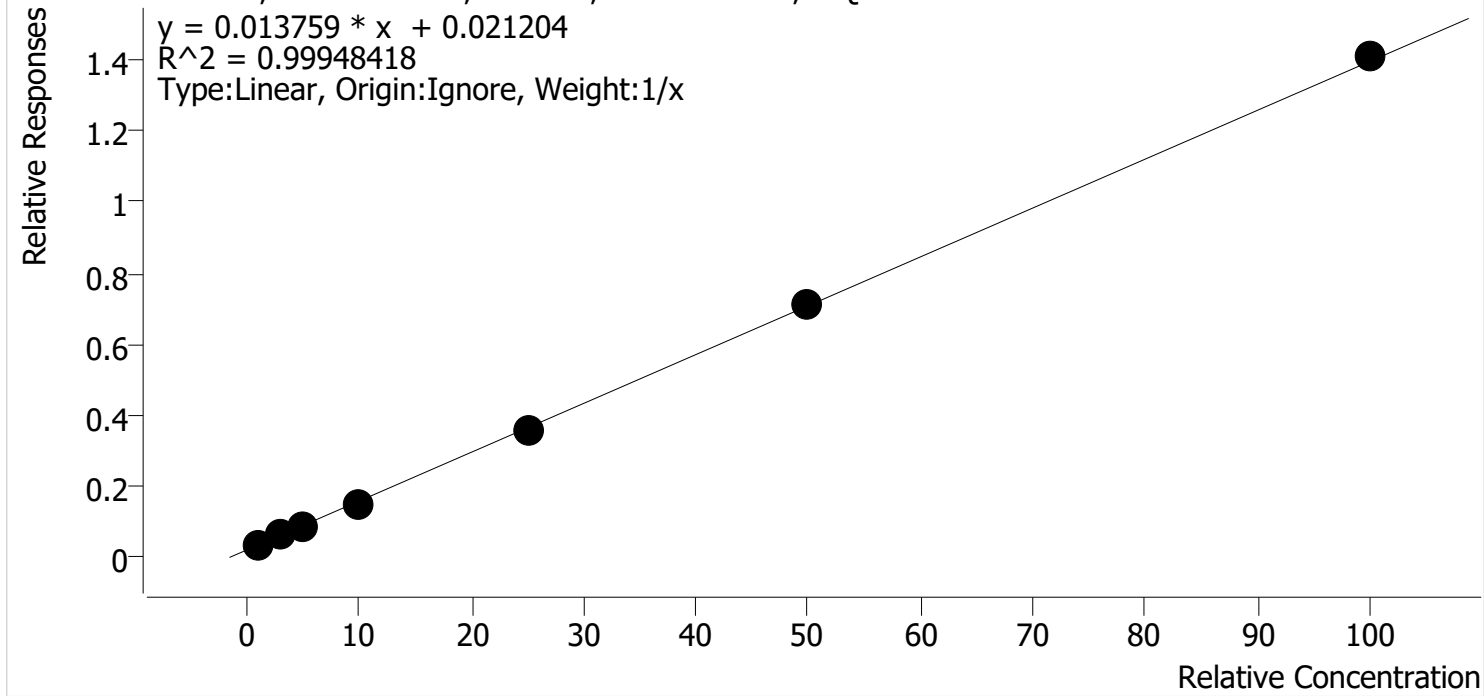
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	5.6	111.3
MJQ_Cal 2	2	✓	10.0	10.0	99.5
MJQ_Cal 3	3	✓	20.0	19.2	96.1
MJQ_Cal 4	4	✓	50.0	48.0	96.0
MJQ_Cal 5	5	✓	75.0	72.8	97.0
MJQ_Cal 6	6	✓	100.0	97.1	97.1
MJQ_Cal 7	7	✓	250.0	257.4	103.0



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 4/26/2021 6:48 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, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	107.0
MJQ_Cal 2	2	✓	3.0	3.2	106.0
MJQ_Cal 3	3	✓	5.0	4.6	92.0
MJQ_Cal 4	4	✓	10.0	9.6	95.9
MJQ_Cal 5	5	✓	25.0	24.5	97.8
MJQ_Cal 6	6	✓	50.0	50.1	100.3
MJQ_Cal 7	7	✓	100.0	101.0	101.0



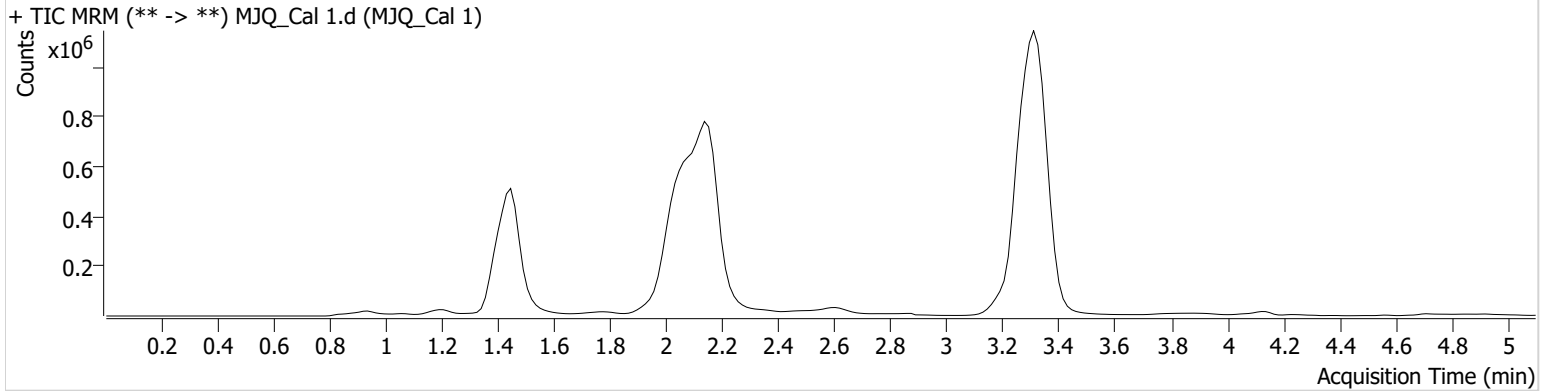
# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

**Instrument** Instrument 1  
**Type** Cal  
**Acq. Method** AM 27 THCQ.m  
**Sample Position** P1-A1  
**Injection Volume** 10  
**Acq. Date-Time** 4/22/2021 3:23:49 PM  
**Sample Info.**

**Data File** MJQ\_Cal 1.d  
**Sample** MJQ\_Cal 1  
**Operator** Celena Shrum  
**Comment**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	80994	∞	8.0	23.48	2254802	1.0696 ng/ml <b>Low</b>
THC-COOH	1.474	92055	79.36	43.1	192.36	549699	5.5658 ng/ml
THC	3.330	80771	633.20	28.8	∞	8464285	1.1457 ng/ml

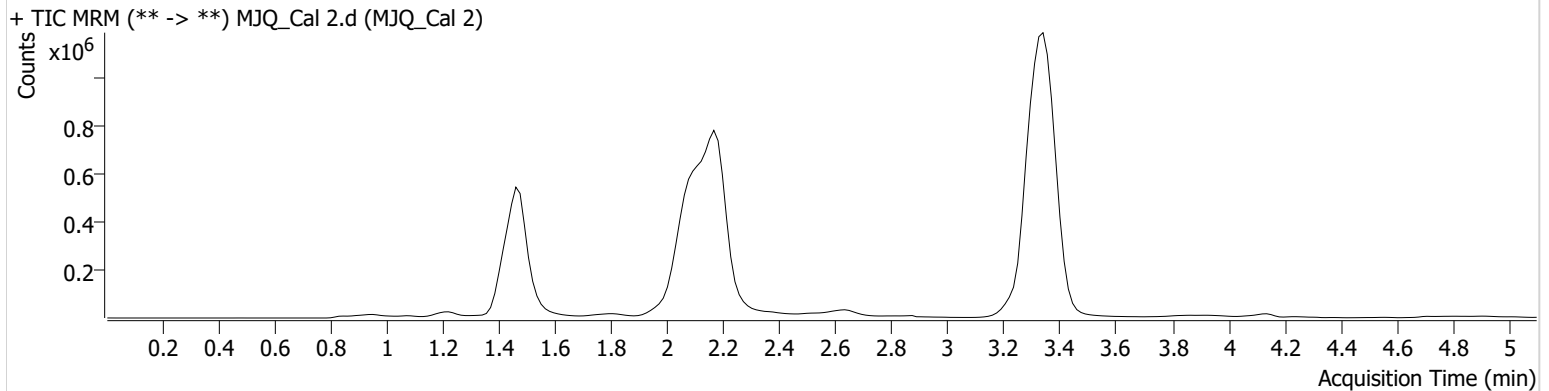
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

<b>Instrument</b>	Instrument 1	<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>	Celena Shrum
<b>Sample Position</b>	P1-B1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/22/2021 3:31:34 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	141475	∞	8.4	204.18	2177806	3.1802 ng/ml
THC-COOH	1.504	152831	∞	48.6	402.80	546094	9.9523 ng/ml
THC	3.360	228549	∞	28.7	61.72	8321867	2.8669 ng/ml

CS

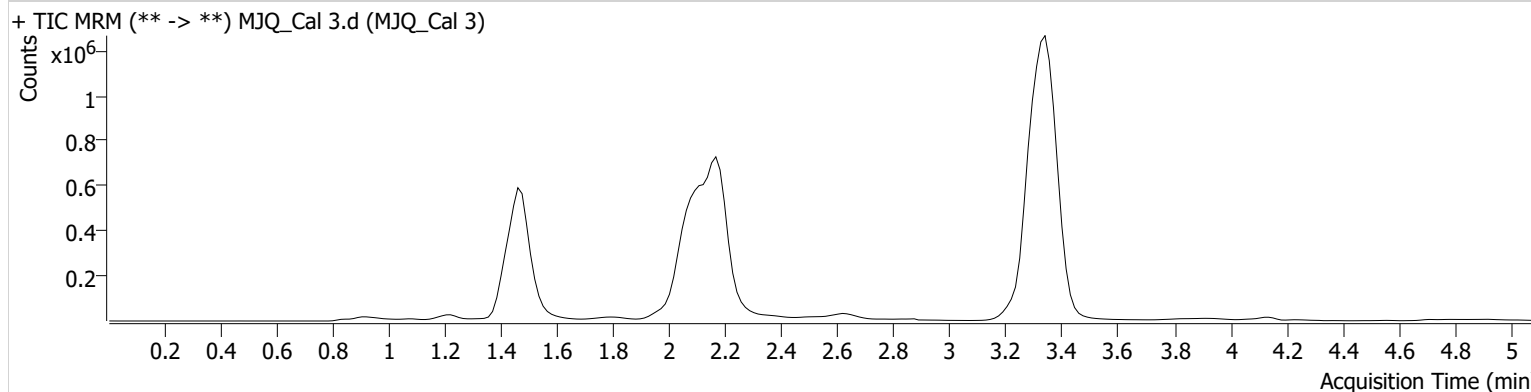


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

<b>Instrument</b>	Instrument 1	<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>	4/22/2021 3:39:10 PM		
<b>Sample Info.</b>			

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	185297	∞	9.5	161.85	2192087	4.6024 ng/ml
THC-COOH	1.504	280370	∞	53.7	∞	542189	19.2111 ng/ml
THC	3.360	411627	567.76	25.7	∞	8691391	4.7778 ng/ml



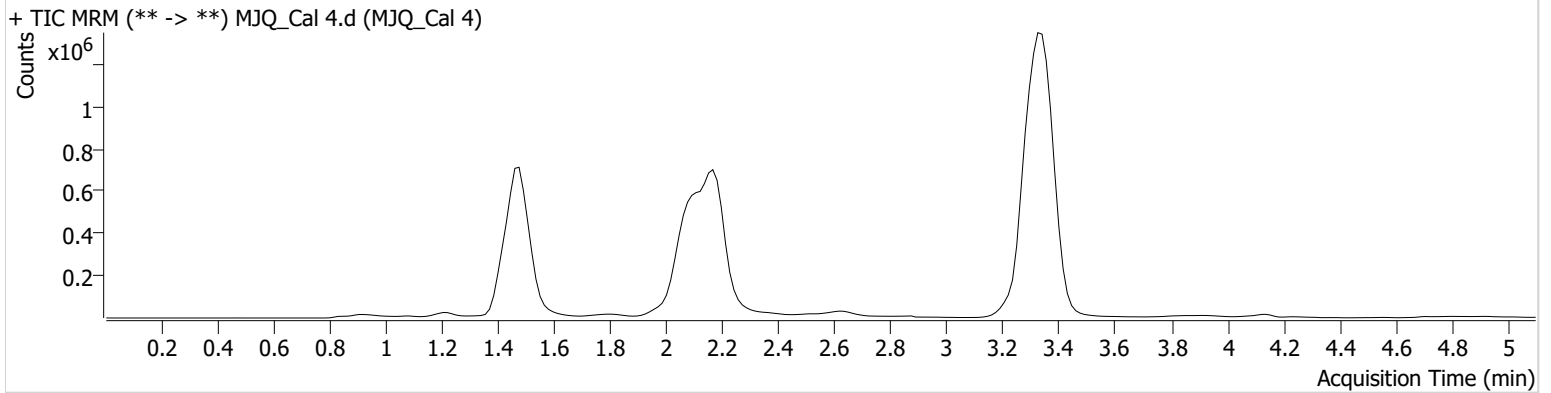
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

<b>Instrument</b>	Instrument 1	<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>	4/22/2021 3:46:45 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	339551	∞	10.2	542.01	2216704	9.5916 ng/ml
THC-COOH	1.504	683028	∞	56.4	6487.21	544243	48.0086 ng/ml
THC	3.345	859058	2656.20	25.5	257.01	8878433	9.5220 ng/ml



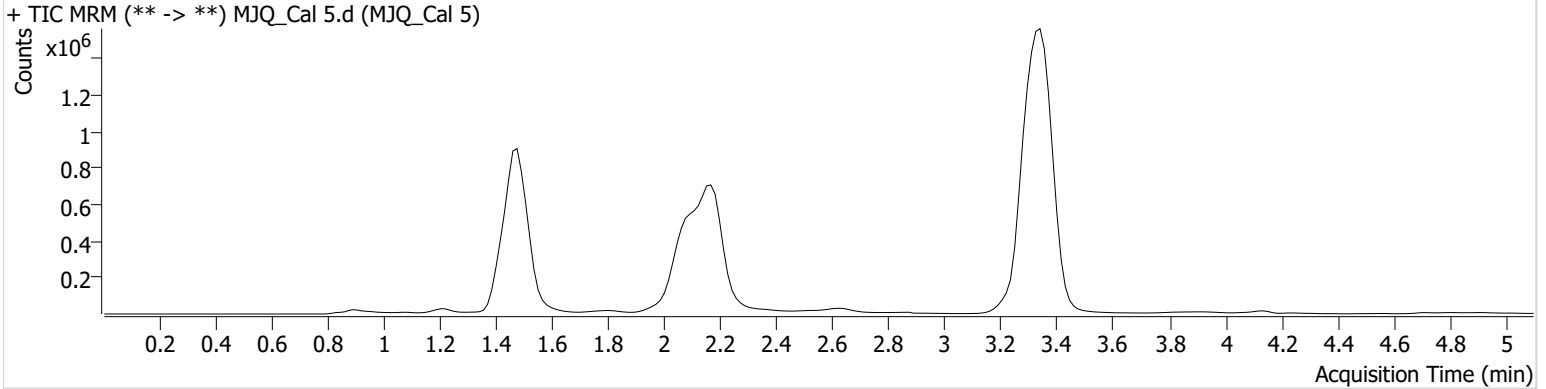
# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

**Instrument** Instrument 1  
**Type** Cal  
**Acq. Method** AM 27 THCQ.m  
**Sample Position** P1-E1  
**Injection Volume** 10  
**Acq. Date-Time** 4/22/2021 3:54:20 PM  
**Sample Info.**

**Data File** MJQ\_Cal 5.d  
**Sample** MJQ\_Cal 5  
**Operator** Celena Shrum  
**Comment**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	805266	∞	11.1	1910.90	2251017	24.4583 ng/ml
THC-COOH	1.504	1034126	∞	56.9	5865.25	547346	72.7647 ng/ml
THC	3.360	2246059	∞	25.1	∞	8858988	24.5789 ng/ml



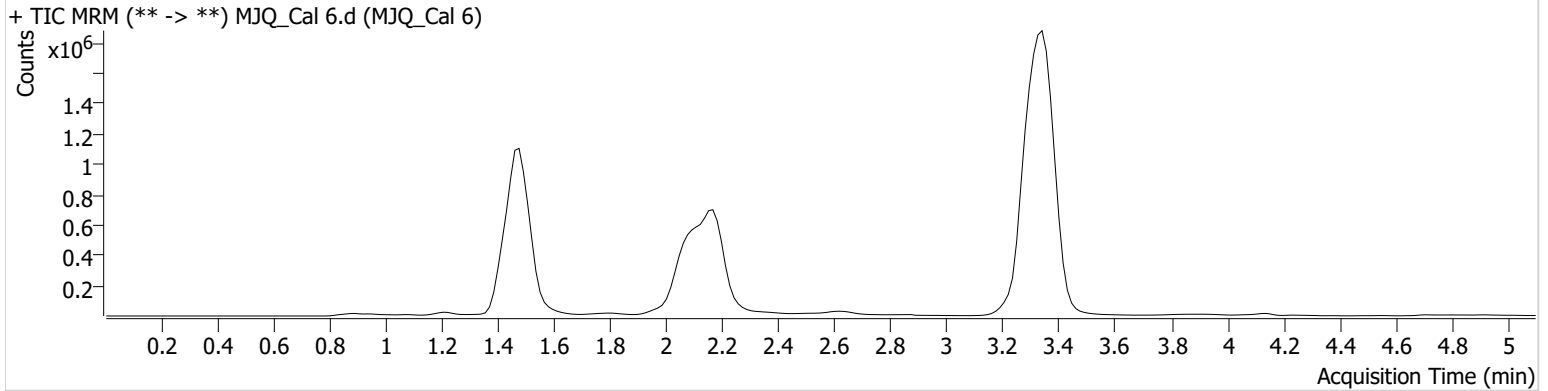
# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

**Instrument** Instrument 1  
**Type** Cal  
**Acq. Method** AM 27 THCQ.m  
**Sample Position** P1-F1  
**Injection Volume** 10  
**Acq. Date-Time** 4/22/2021 4:01:56 PM  
**Sample Info.**

**Data File** MJQ\_Cal 6.d  
**Sample** MJQ\_Cal 6  
**Operator** Celena Shrum  
**Comment**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	1547074	∞	11.4	1838.24	2175627	50.1397 ng/ml
THC-COOH	1.504	1317799	∞	57.5	1924.00	524330	97.1153 ng/ml
THC	3.345	4417643	∞	25.8	2558.05	8582735	49.6627 ng/ml

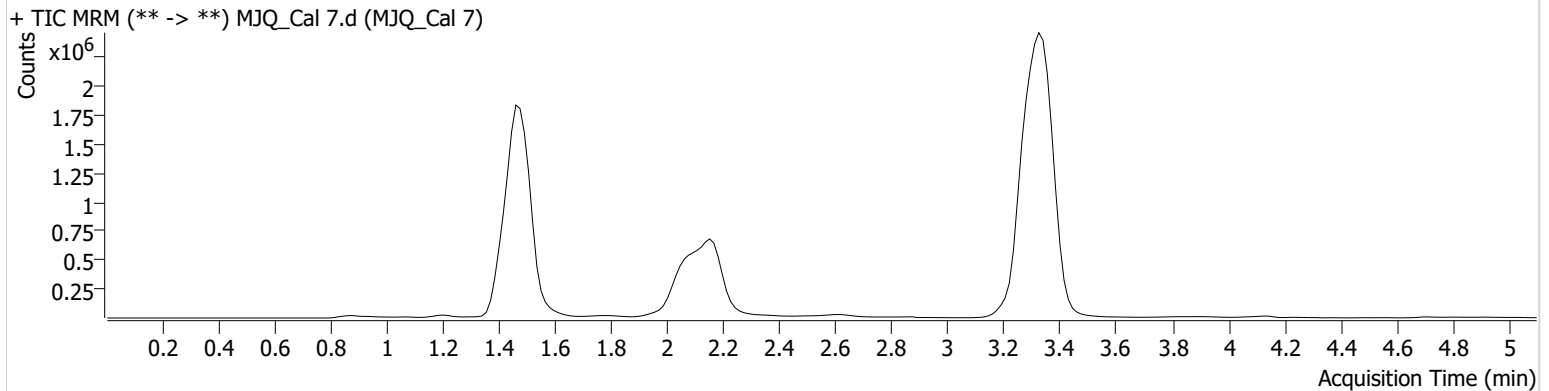
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2021\AM 27-28\042221 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 4/26/2021 6:48:53 AM

<b>Instrument</b>	Instrument 1	<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>	4/22/2021 4:09:33 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	3013441	∞	11.2	15470.3	2136703	100.9582 ng/ml
THC-COOH	1.489	3149724	4225.43	57.3	∞	475795	257.3821 ng/ml
THC	3.345	8676870	∞	25.4	6942.40	8233160	101.4460 ng/ml