



**Worklist: 5227**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2021-3323	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-3381	5	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-3400	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-3511	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-3690	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2021-3812	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-2861	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-2863	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-2869	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-2917	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-2929	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-2930	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-2947	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

SC

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

Extraction Date: 09/08/21

Analyst: Sarah Collins

Plate lot#: IDP-108-2-210609

Retest Date: 12/09/2021

**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. 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:** 3382167
- 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 uL
- 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: Did not evaluate THC-OH due to interfering peak. Reinject M2021-3690-1 due to low internal standard response in initial injection. Reinject data used.

SC

	1	2	3	4	5	6
A	IS + Cal. 1	negative blood	p2021-2863-1			IS + QC_1
B	IS + Cal. 2	m2021-3323-1	p2021-2869-1			IS + Cal. 7
C	IS + Cal. 3	m2021-3381-5	p2021-2917-1			IS + Cal. 6
D	IS + Cal. 4	m2021-3400-1	p2021-2929-1			IS + Cal. 5
E	IS + Cal. 5	m2021-3511-1	p2021-2930-1			IS + Cal. 4
F	IS + Cal. 6	m2021-3690-1*	p2021-2947-1			IS + Cal. 3
G	IS + Cal. 7	m2021-3812-2	m2021-3690-1			IS + Cal. 2
H	IS + QC_1	p2021-2861-1				IS + Cal. 1

All wells to contain 100 µl of residual DMSO

\*Sample moved during analytical step 6 due to blood clot

SC

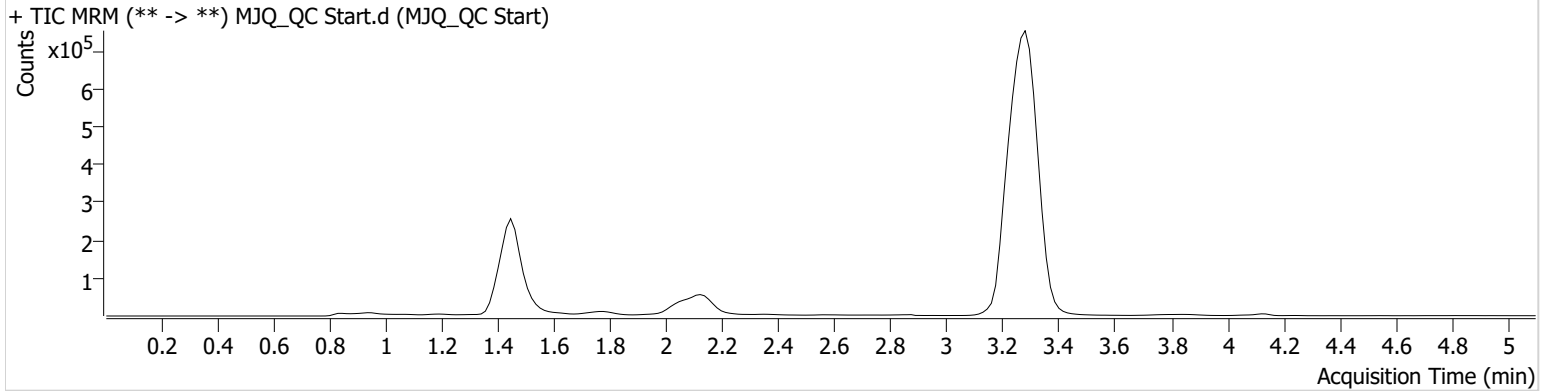


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 11:56:12 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	79500	∞	62.8	∞	211167	15.1471 ng/ml
THC-OH	1.468	125330	∞	8.3	131.50	917198	4.5385 ng/ml
THC	3.300	230622	4137.56	27.5	∞	5274843	4.7746 ng/ml



SC

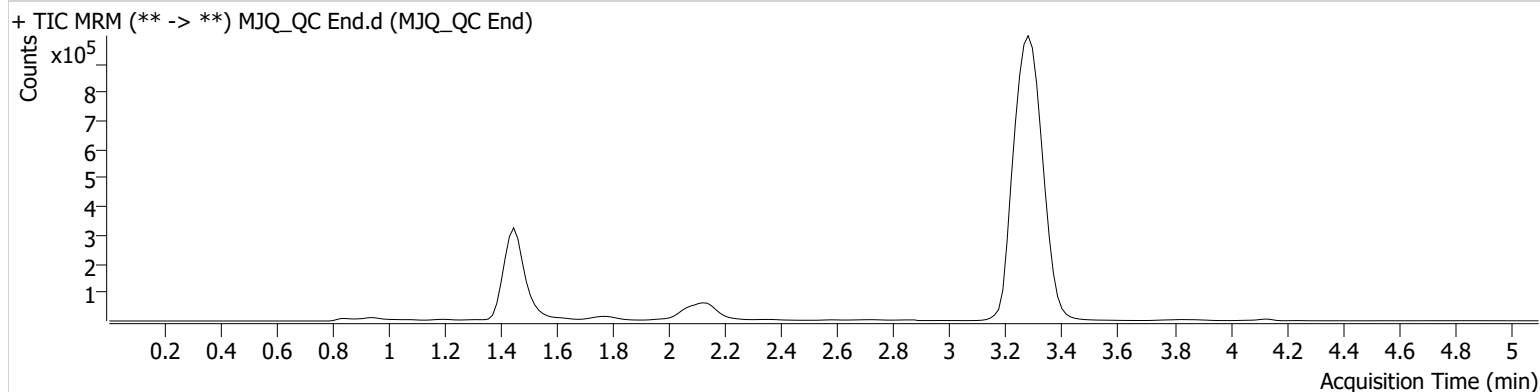


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 3:59:34 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	91745	∞	64.4	350.67	241277	15.2943 ng/ml
THC-OH	1.468	148435	∞	8.4	98.32	1117105	4.2312 ng/ml
THC	3.300	301630	∞	27.5	∞	6864591	4.7976 ng/ml

SC

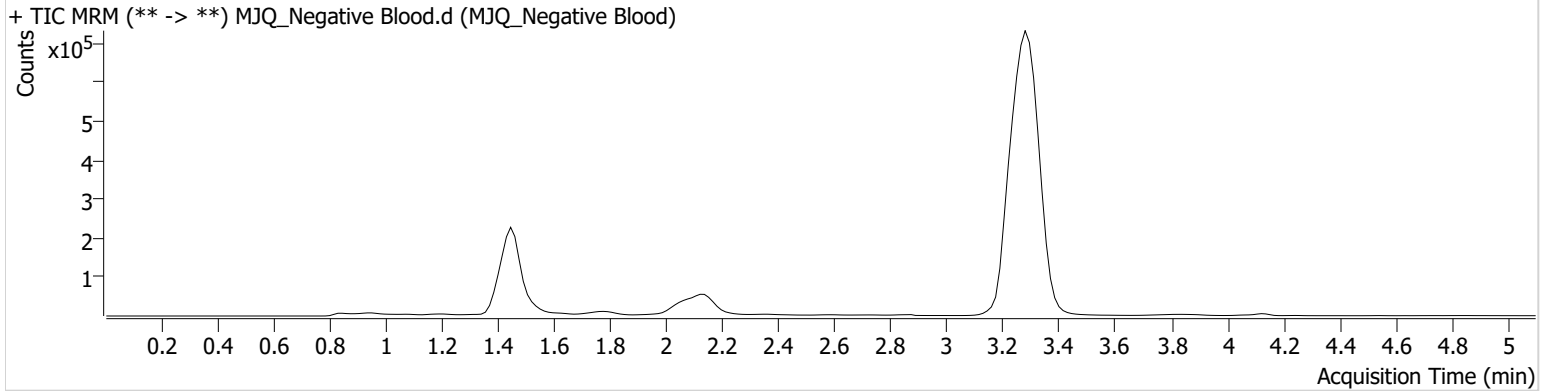


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-A2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 12:11:25 PM		
<b>Sample Info.</b>			

## Sample Chromatogram

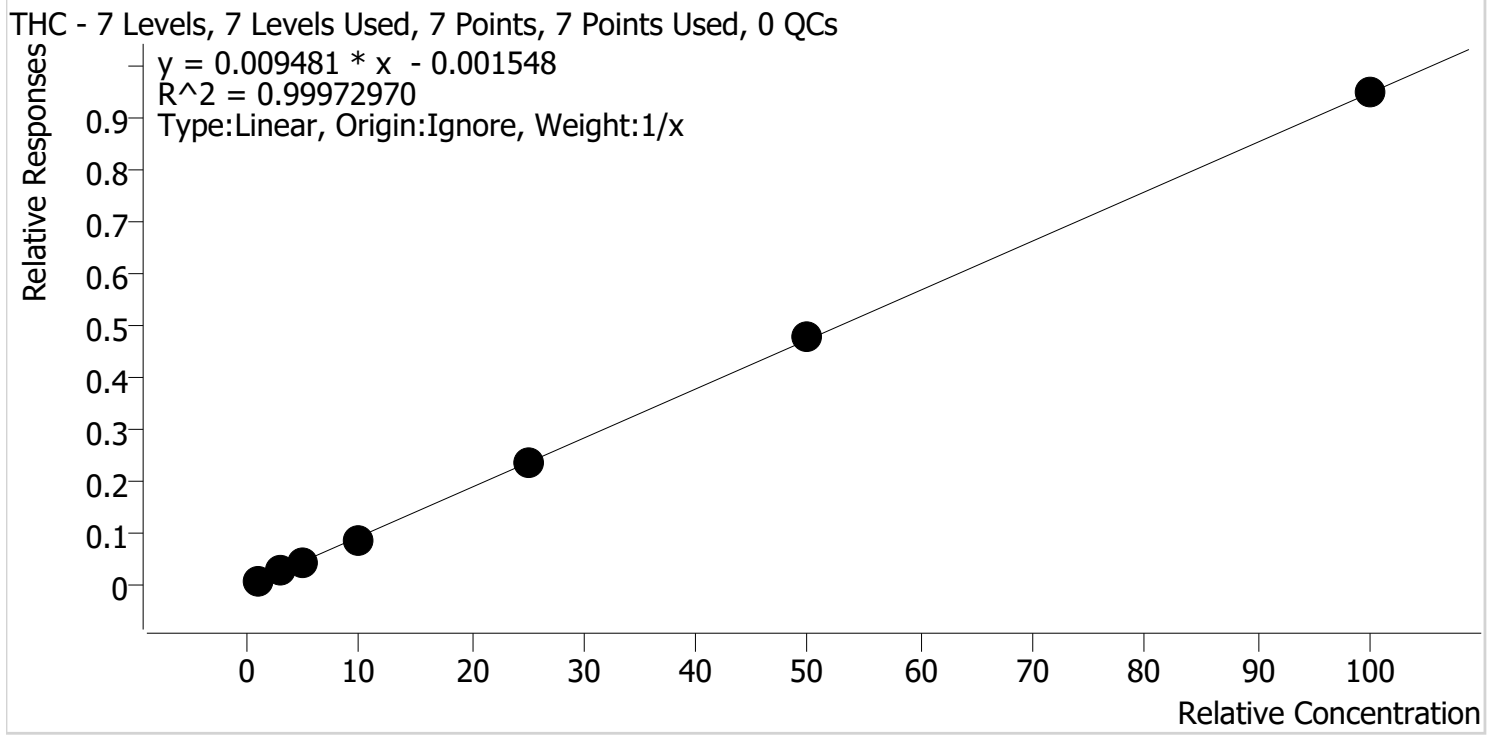


SC



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 9/9/2021 11:59 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-D3



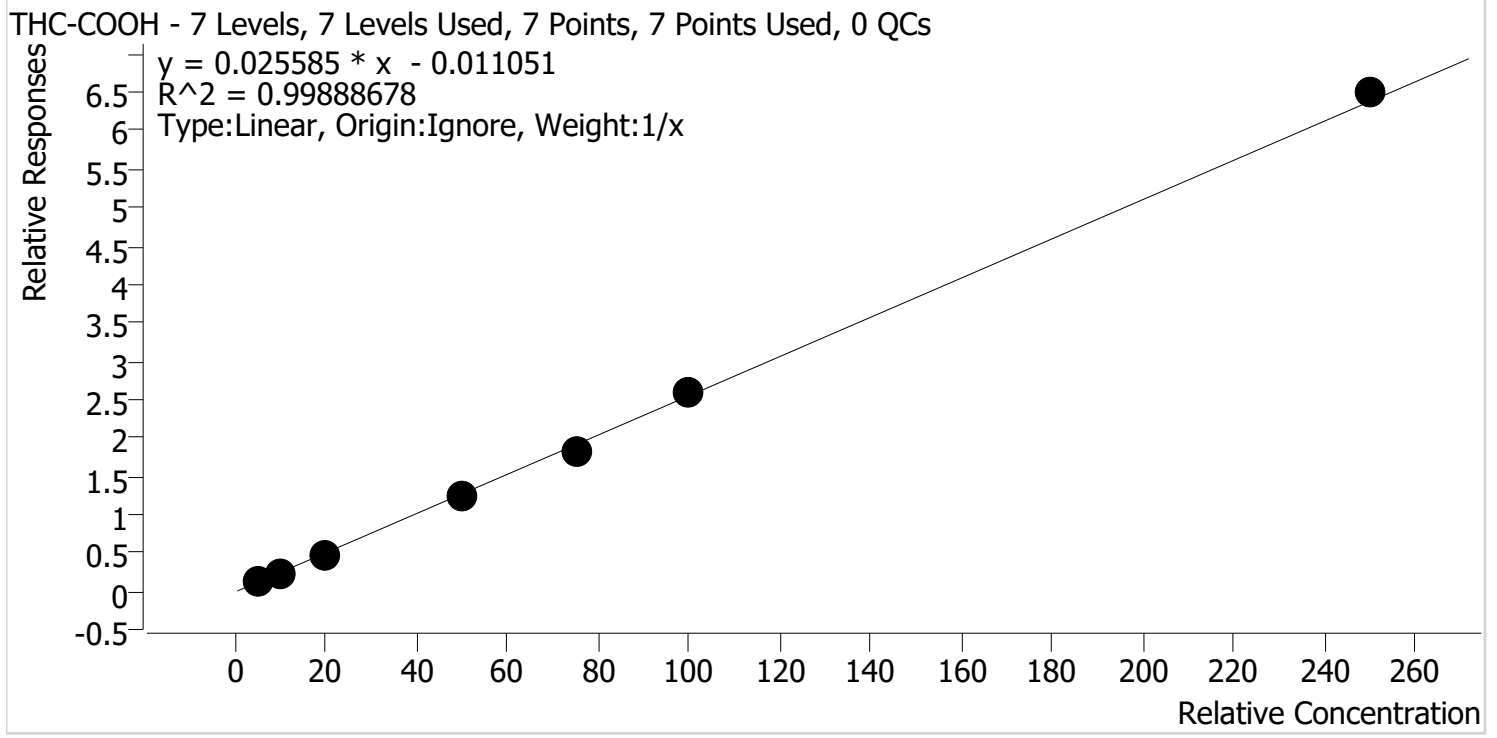
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	108.6
MJQ_Cal 2	2	✓	3.0	2.9	97.5
MJQ_Cal 3	3	✓	5.0	4.9	97.7
MJQ_Cal 4	4	✓	10.0	9.5	94.7
MJQ_Cal 5	5	✓	25.0	25.1	100.5
MJQ_Cal 6	6	✓	50.0	50.5	100.9
MJQ_Cal 7	7	✓	100.0	100.1	100.1

SC



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 9/9/2021 11:59 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	5.7	114.1
MJQ_Cal 2	2	✓	10.0	9.6	96.4
MJQ_Cal 3	3	✓	20.0	18.8	94.2
MJQ_Cal 4	4	✓	50.0	48.0	96.0
MJQ_Cal 5	5	✓	75.0	72.4	96.6
MJQ_Cal 6	6	✓	100.0	100.9	100.9
MJQ_Cal 7	7	✓	250.0	254.5	101.8

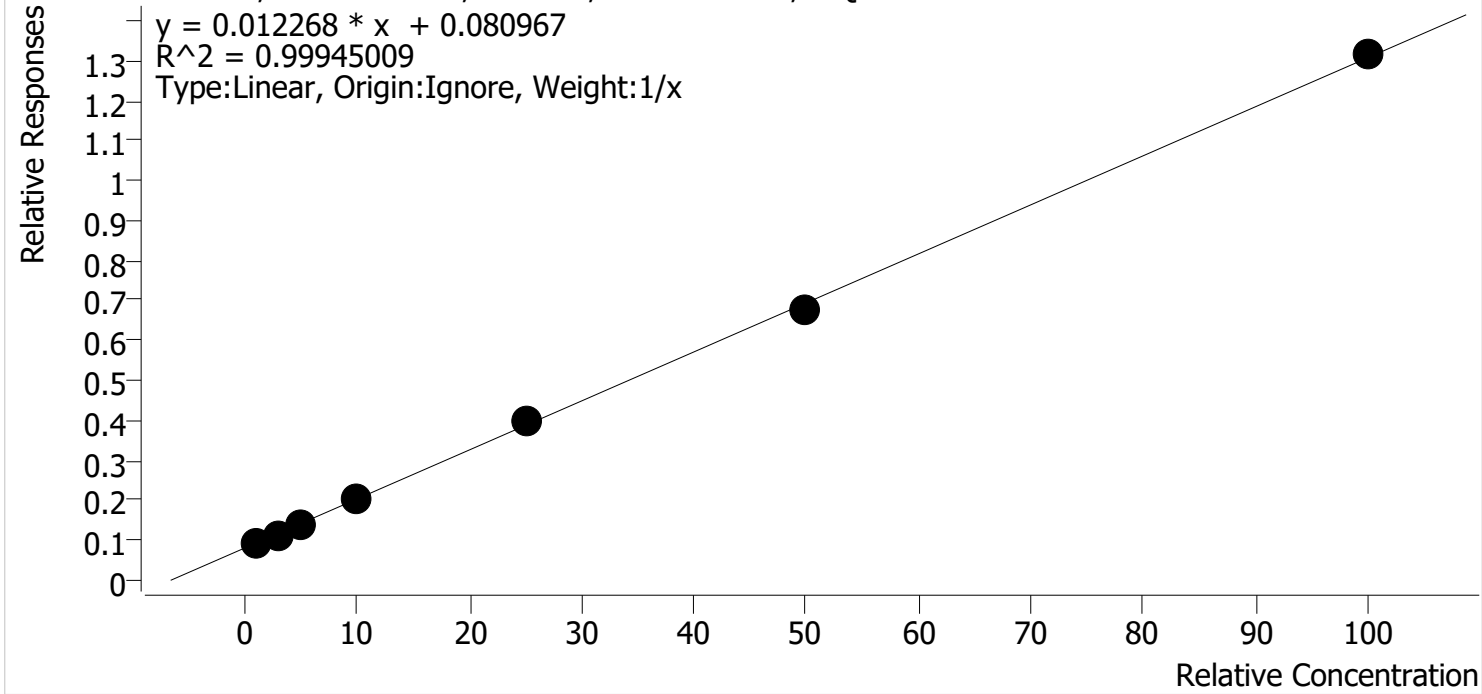
SC



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 9/9/2021 11:59 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	107.3
MJQ_Cal 2	2	✓	3.0	2.7	90.7
MJQ_Cal 3	3	✓	5.0	5.0	100.4
MJQ_Cal 4	4	✓	10.0	10.0	100.3
MJQ_Cal 5	5	✓	25.0	25.8	103.2
MJQ_Cal 6	6	✓	50.0	48.8	97.5
MJQ_Cal 7	7	✓	100.0	100.6	100.6

SC



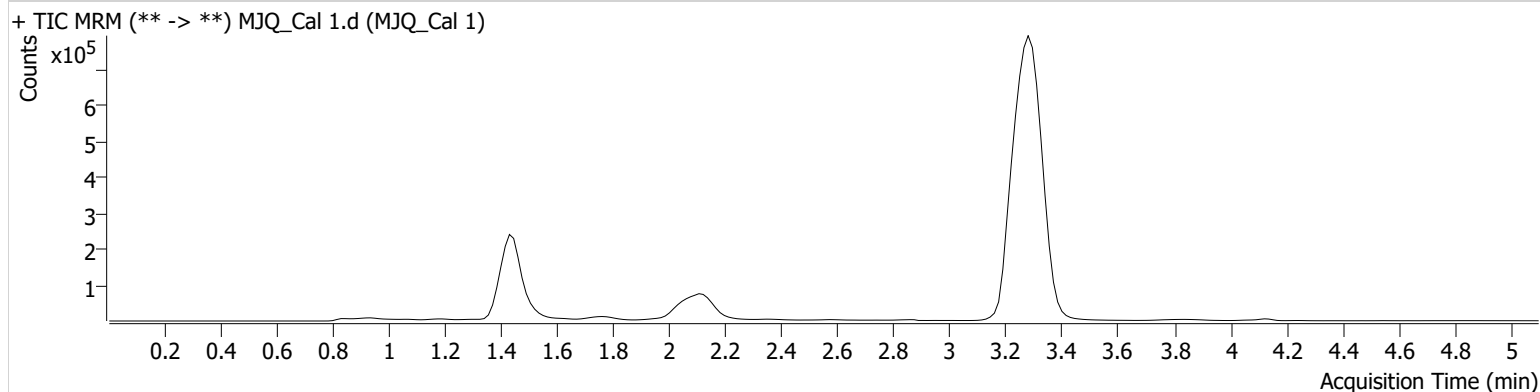
# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-A1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 10:55:16 AM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	29675	61.82	52.5	366.68	220067	5.7025 ng/ml
THC-OH	1.498	88771	∞	4.8 <b>Low</b>	20.57	943110	1.0726 ng/ml <b>Low</b>
THC	3.300	50736	646.88	33.0	∞	5798458	1.0862 ng/ml

SC

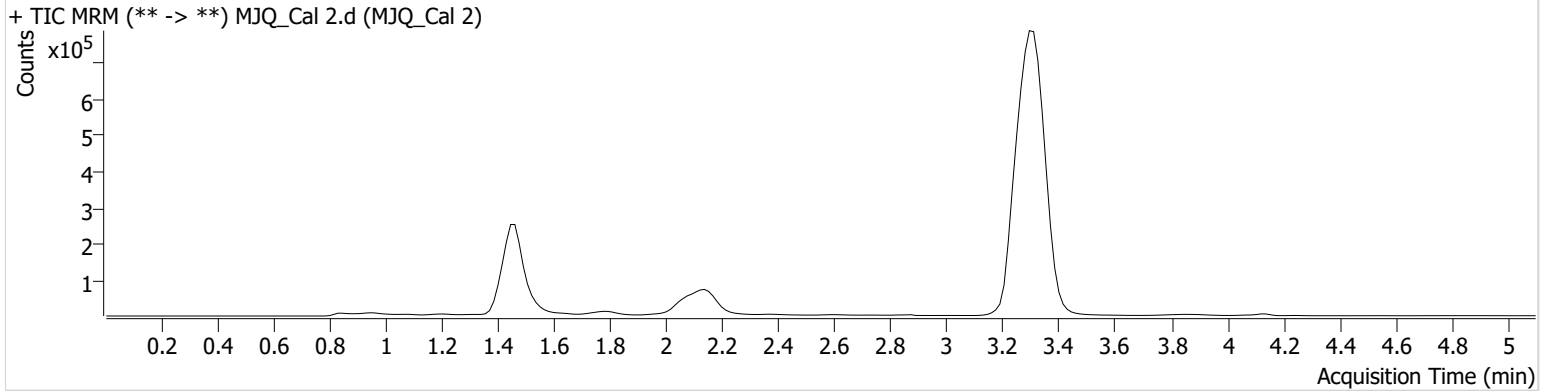


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-B1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 11:03:01 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	51487	∞	64.0	215.75	218545	9.6402 ng/ml
THC-OH	1.528 <b>High</b>	107783	∞	6.7 <b>Low</b>	44.26	942477	2.7221 ng/ml <b>Low</b>
THC	3.330	145688	1082.33	28.5	283.27	5565281	2.9243 ng/ml

SC

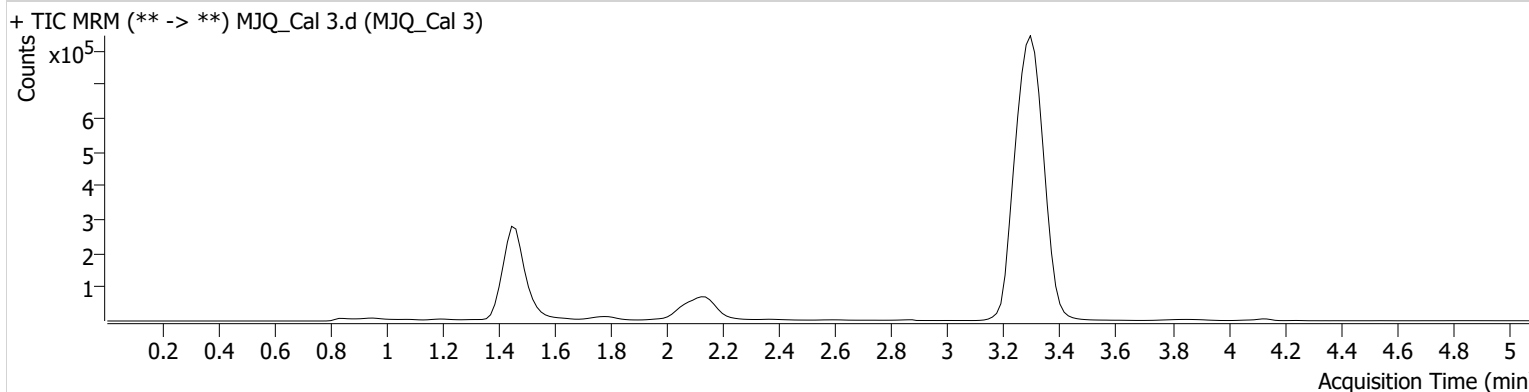


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-C1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 11:10:37 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	103953	∞	66.2	∞	220614	18.8492 ng/ml
THC-OH	1.468	136545	∞	8.1	76.45	957797	5.0209 ng/ml
THC	3.315	255521	1984.50	27.1	1027.32	5705401	4.8868 ng/ml



SC

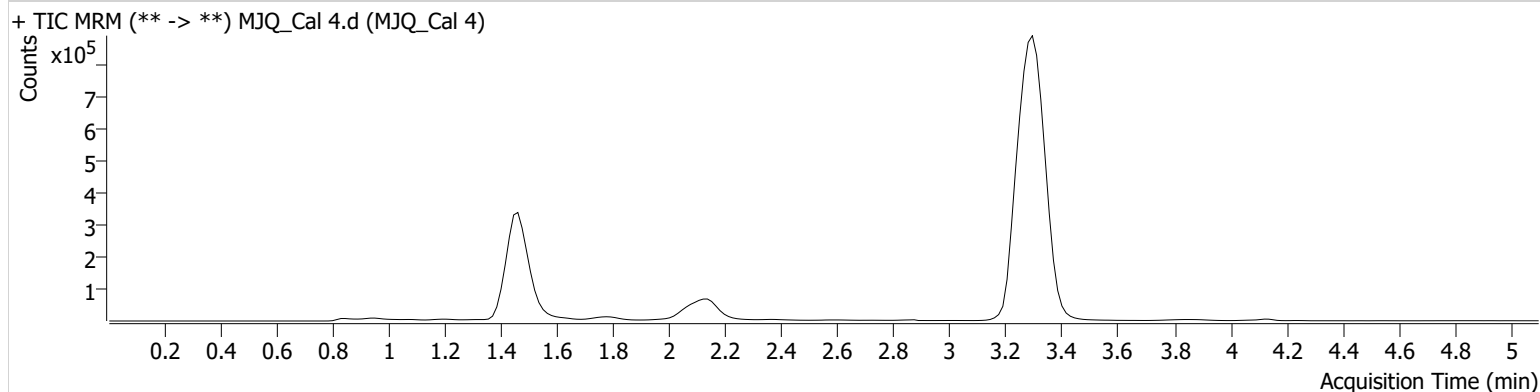


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-D1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 11:18:13 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	265247	835.39	65.8	∞	217836	48.0249 ng/ml
THC-OH	1.468	195648	∞	8.9	∞	959121	10.0280 ng/ml
THC	3.315	495214	7019.05	27.3	571.19	5611777	9.4705 ng/ml

SC

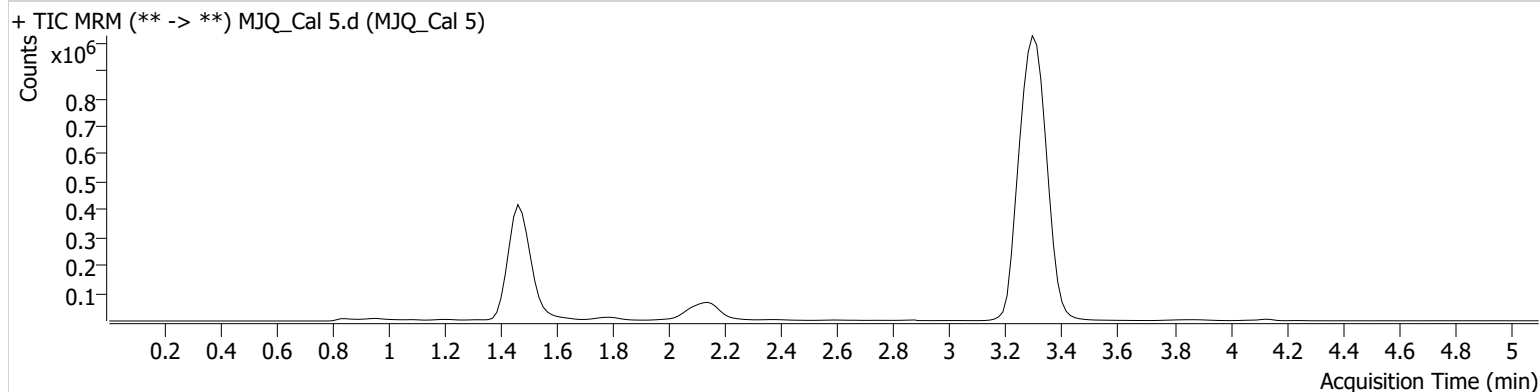


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-E1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 11:25:48 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	384045	∞	67.0	2127.92	208469	72.4370 ng/ml
THC-OH	1.468	373576	∞	11.4	336.17	940004	25.7957 ng/ml
THC	3.315	1292806	20868.99	26.0	884.43	5461813	25.1279 ng/ml

SC

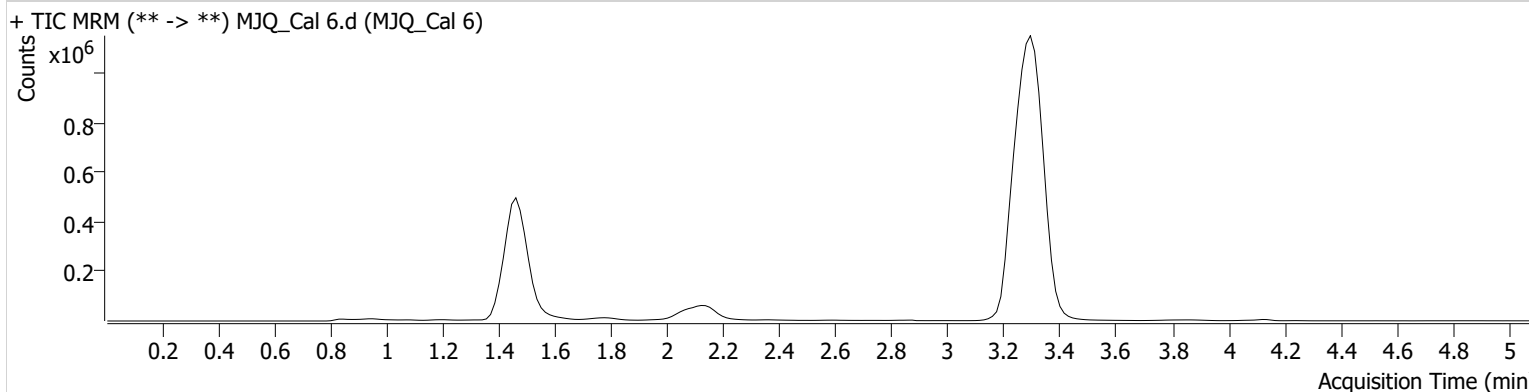


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-F1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 11:33:24 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	522553	∞	68.1	∞	203327	100.8836 ng/ml
THC-OH	1.453	632047	∞	13.1 <b>High</b>	1967.65	930671	48.7592 ng/ml
THC	3.300	2499837	∞	26.4	∞	5243002	50.4507 ng/ml

SC

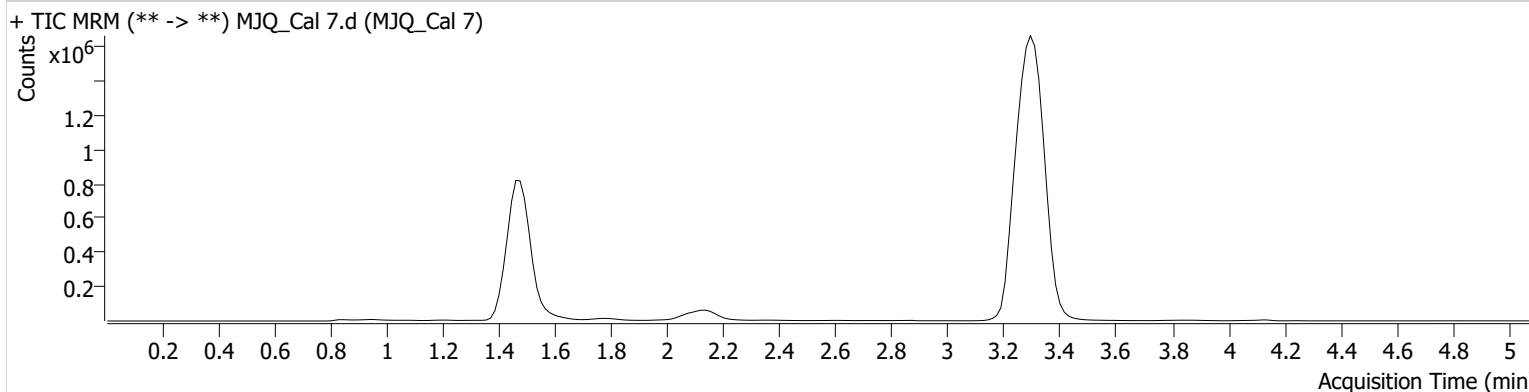


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\090821 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 9/9/2021 11:59:57 AM

<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>	Sarah Collins
<b>Sample Position</b>	P1-G1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	9/8/2021 11:40:58 AM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	1223461	∞	68.5	∞	188246	254.4625 ng/ml
THC-OH	1.453	1178962	∞	13.8 <b>High</b>	1385.81	896471	100.6015 ng/ml
THC	3.315	5081333	∞	27.1	3707.42	5365155	100.0536 ng/ml