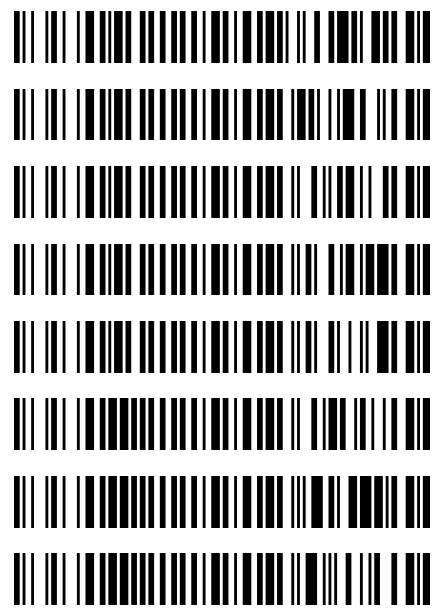


**REVIEWED**  
By Tamara Salazar at 7:34 am, Jan 13, 2021

SL 1/7/2021

**Worklist: 4721**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2020-3888	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-5013	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-5044	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-5103	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-5123	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-3788	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-3834	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-3850	1	BCK	AM 27 Blood THC Quant by LC-QQQ



8C

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

Extraction Date: 01/07/21  
Plate lot#: IDP-108-2-200723

Analyst: Sarah Collins  
Plate Expiration: 01/23/21

**Mobile phase A:** 0.1% Formic Acid in LCMS Water  
MTBE  
**Mobile phase B:** 0.1% Formic acid in Acetonitrile  
Hexane  
**Blank Blood Lot:** Lampire 20L20725  
**LCMS-QQQ ID:** 069901  
**Column:** UCT Selectra DA 100 x 2.1mm 3um  
LCMS Methanol

### 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. Pipette **1000 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: 3382167**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500 µL 0.1% formic acid in LCMS water** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **800 µL of blood+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 85-95 PSI- Selector to the right)*
- 8. Wait 5 minutes.
- 9. Add **2.25 mL MTBE. (Add in 3 increments of 750 µL)**
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 15 seconds. *(12-15 PSI- Selector to the left).*
- 12. Add **2.25 mL Hexane. (Add in 3 increments of 750 µL)**
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 15 seconds. *(12-15 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 **100 µL 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? Y / N
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *Curve range limited: THC 3-100 Reconstituted and reinjected P2020-3788 due to low internal standard response in initial injection. Reinject data used.* THC reported qualitatively.

8C

SL

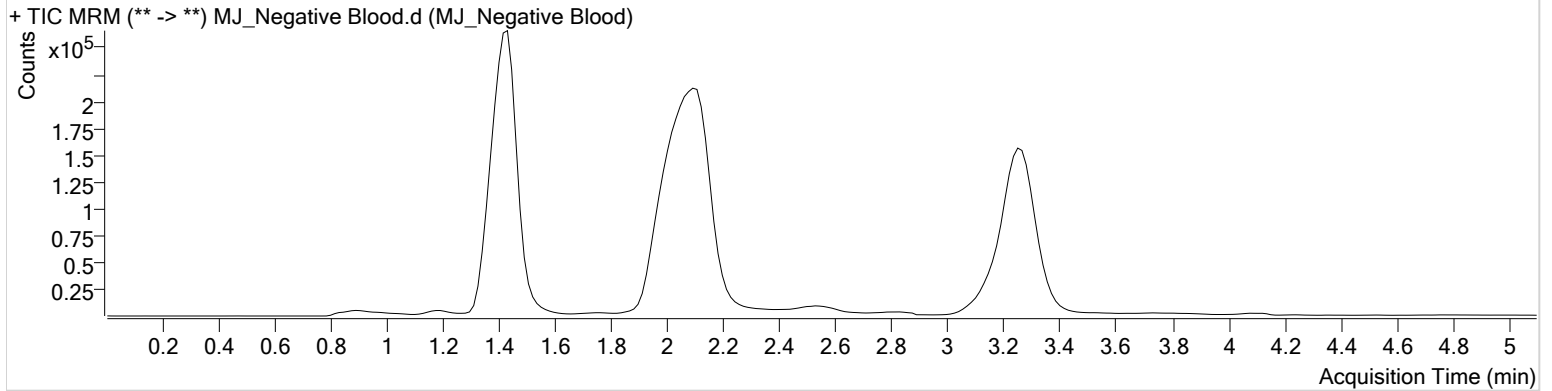


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-H5	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 2:18:48 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



SL

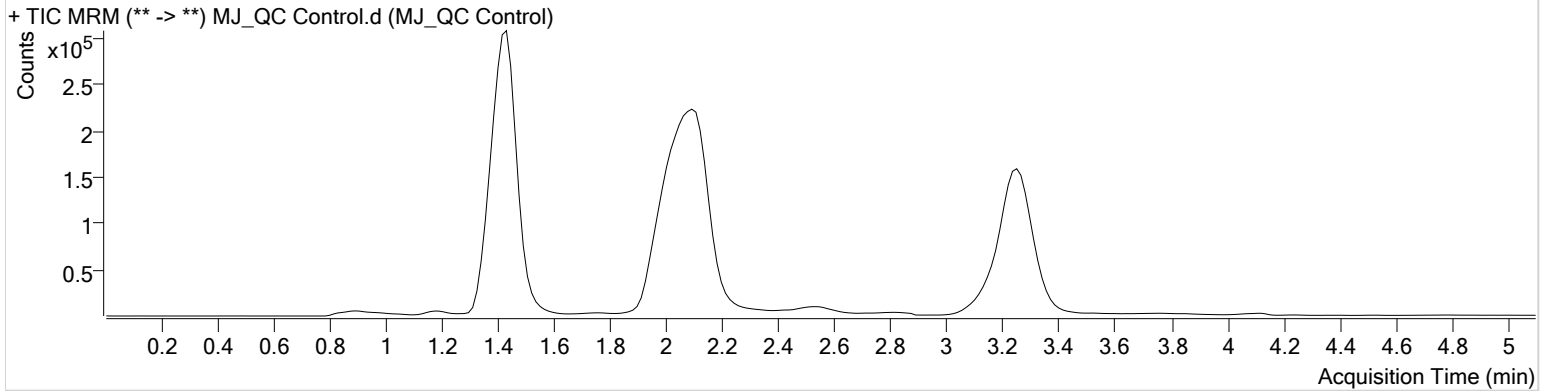


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<b>Data File</b>	MJ_QC Control.d
<b>Type</b>	Sample	<b>Sample</b>	MJ_QC Control
<b>Acq. Method</b>	AM 27 THCQ.m	<b>Operator</b>	Sarah Collins
<b>Sample Position</b>	P1-A6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 2:03:34 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	107771	∞	11.5	∞	1310652	4.6494 ng/ml
THC-COOH	1.459	124056	∞	54.6	∞	338393	15.2771 ng/ml
THC	3.270	59898	478.91	30.8	∞	1356763	4.6970 ng/ml

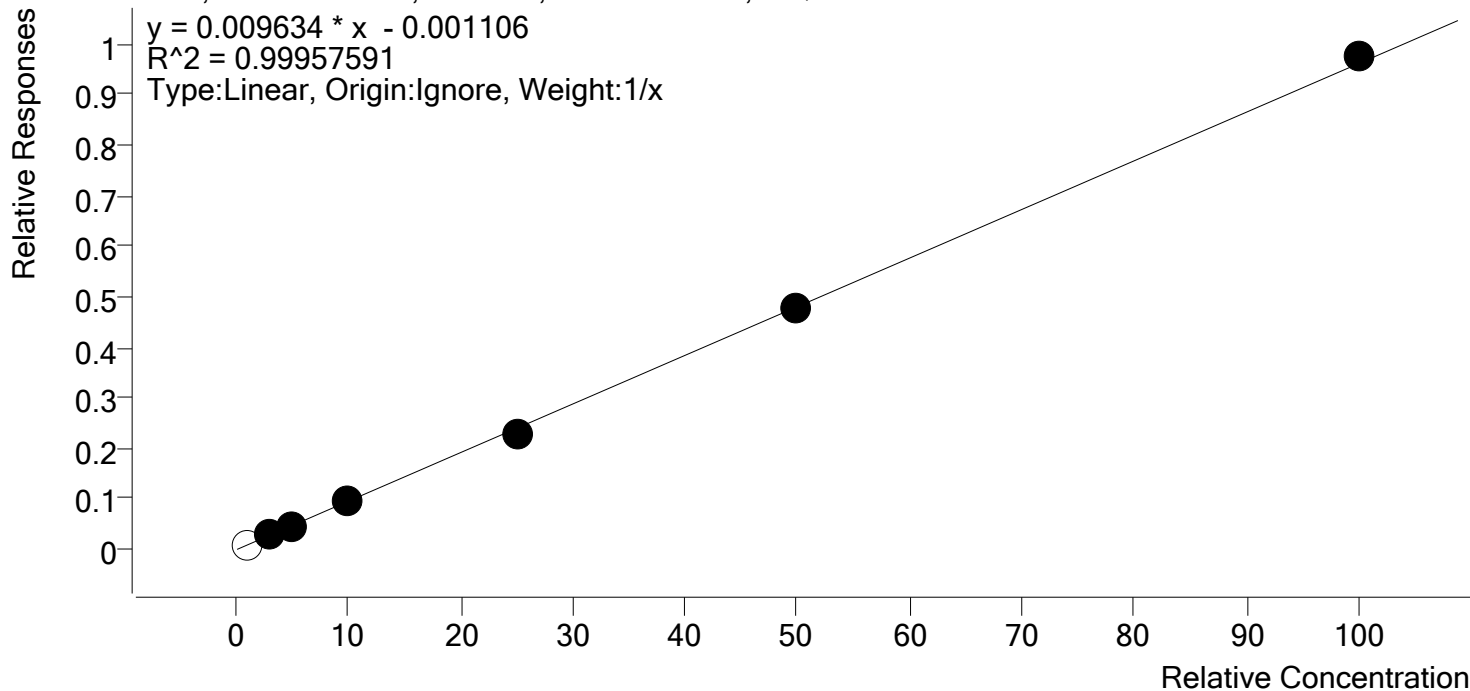
SL



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 1/8/2021 10:11 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-D3

THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	x	1.0	1.1	108.1
MJ_Cal 2	2	✓	3.0	3.1	102.6
MJ_Cal 3	3	✓	5.0	5.1	102.2
MJ_Cal 4	4	✓	10.0	9.8	98.2
MJ_Cal 5	5	✓	25.0	24.2	96.7
MJ_Cal 6	6	✓	50.0	49.4	98.8
MJ_Cal 7	7	✓	100.0	101.4	101.4

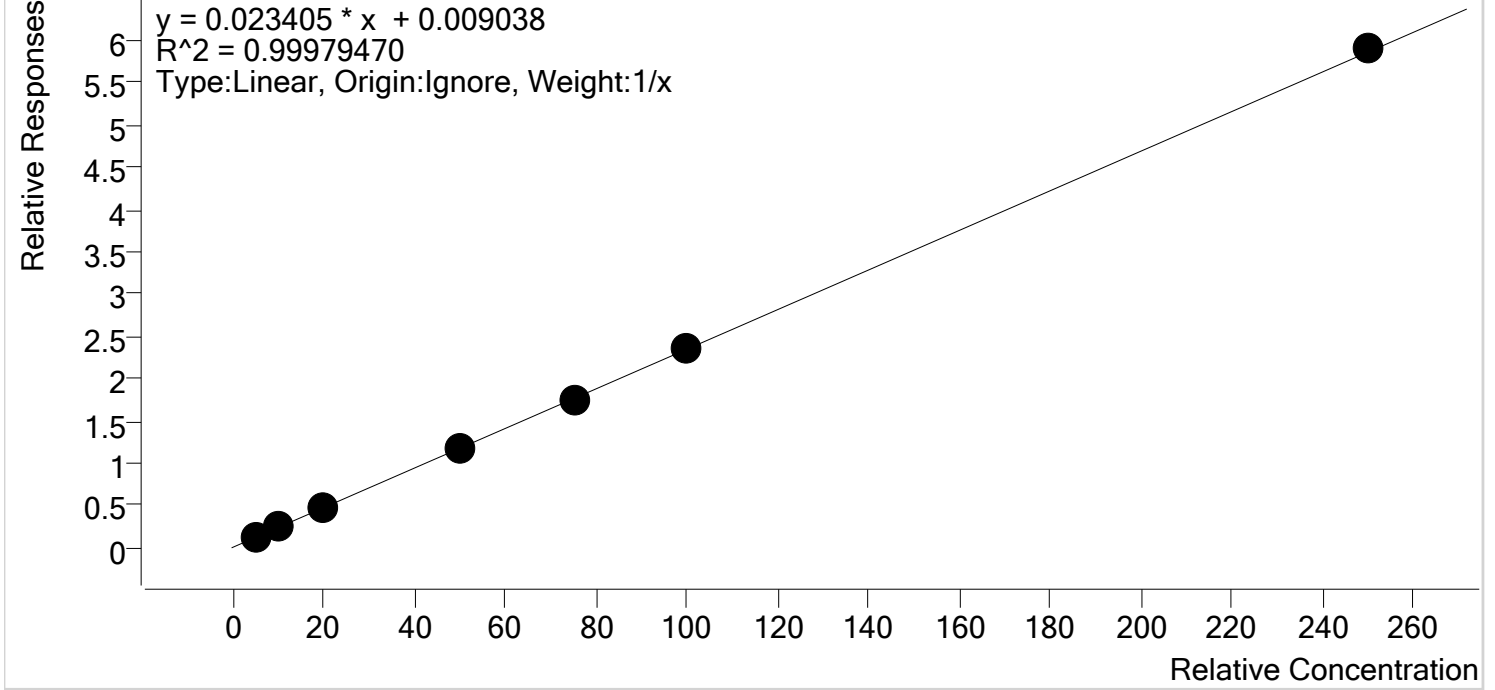
SL



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 1/8/2021 10:11 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-D9

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



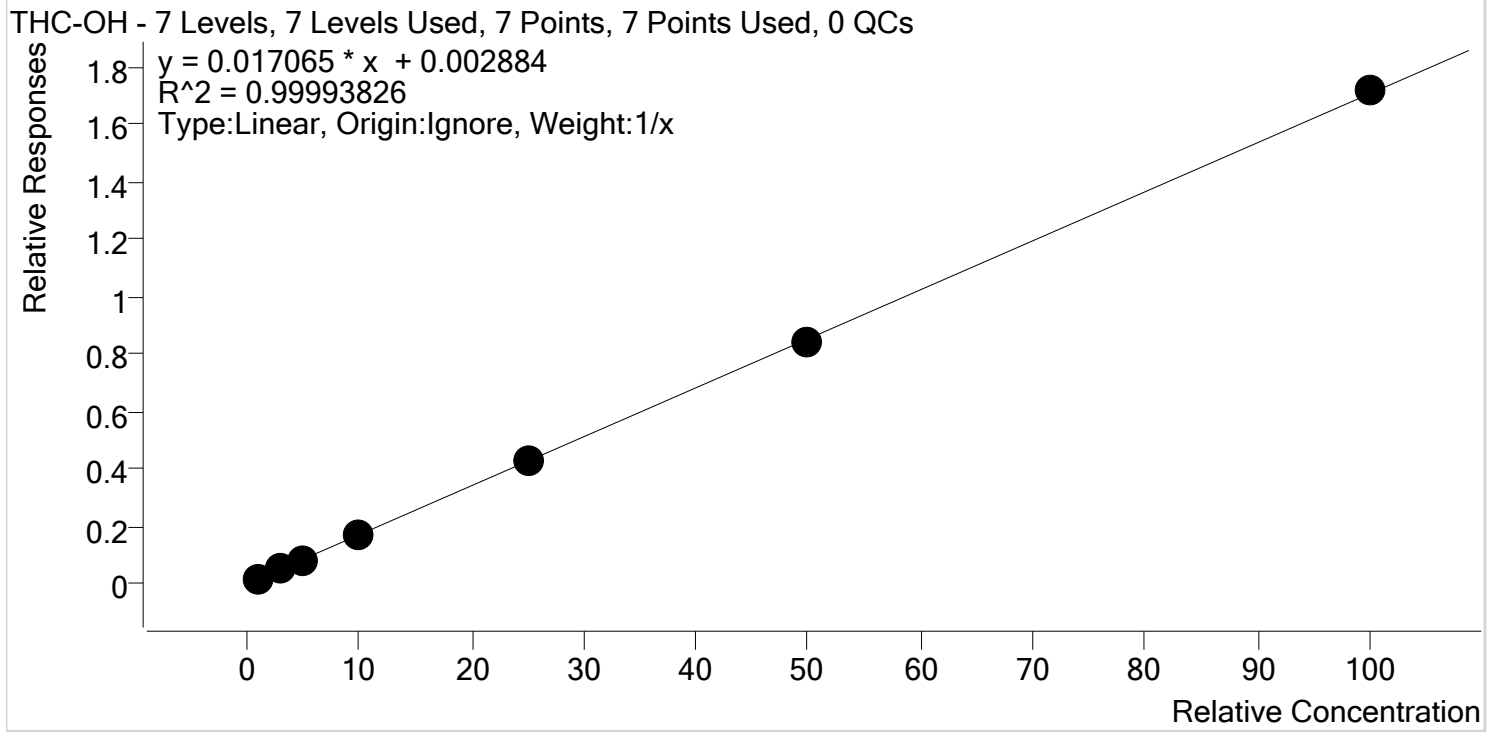
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	✓	5.0	5.2	104.7
MJ_Cal 2	2	✓	10.0	9.8	97.7
MJ_Cal 3	3	✓	20.0	20.0	100.1
MJ_Cal 4	4	✓	50.0	49.0	98.0
MJ_Cal 5	5	✓	75.0	73.6	98.1
MJ_Cal 6	6	✓	100.0	100.6	100.6
MJ_Cal 7	7	✓	250.0	251.7	100.7

SL



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 1/8/2021 10:11 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	✓	1.0	1.0	103.6
MJ_Cal 2	2	✓	3.0	2.9	98.1
MJ_Cal 3	3	✓	5.0	5.0	99.9
MJ_Cal 4	4	✓	10.0	9.9	98.9
MJ_Cal 5	5	✓	25.0	24.8	99.4
MJ_Cal 6	6	✓	50.0	49.7	99.4
MJ_Cal 7	7	✓	100.0	100.6	100.6

SL

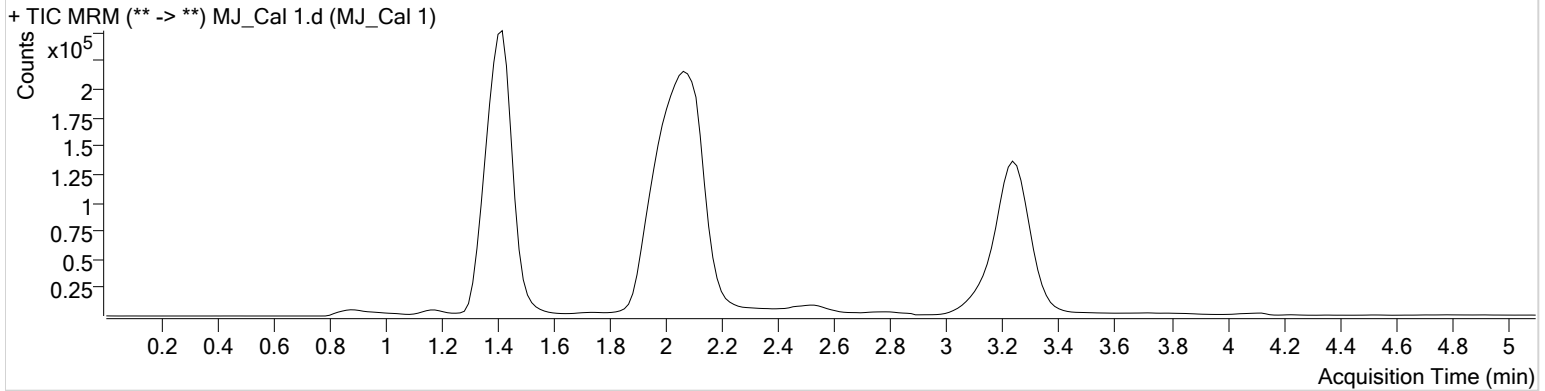


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-H6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 1:02:38 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.423	25603	∞	10.0	34.23	1244567	1.0365 ng/ml <b>Low</b>
THC-COOH	1.444	42282	∞	48.0	∞	321432	5.2340 ng/ml
THC	3.254	11582	52.46	47.8 <b>High</b>	19.52	1243787	1.0812 ng/ml



SL

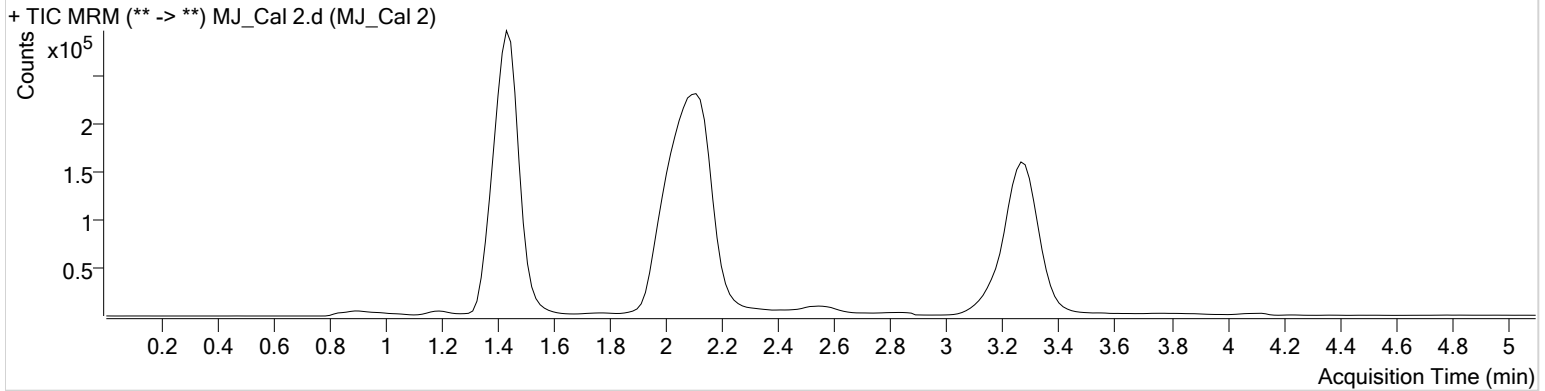


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-G6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 1:10:24 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	70409	∞	12.0	∞	1325276	2.9442 ng/ml <b>Low</b>
THC-COOH	1.474	81745	∞	54.6	531.91	343726	9.7747 ng/ml
THC	3.285	38992	225.11	28.2	36.85	1365958	3.0777 ng/ml

SL

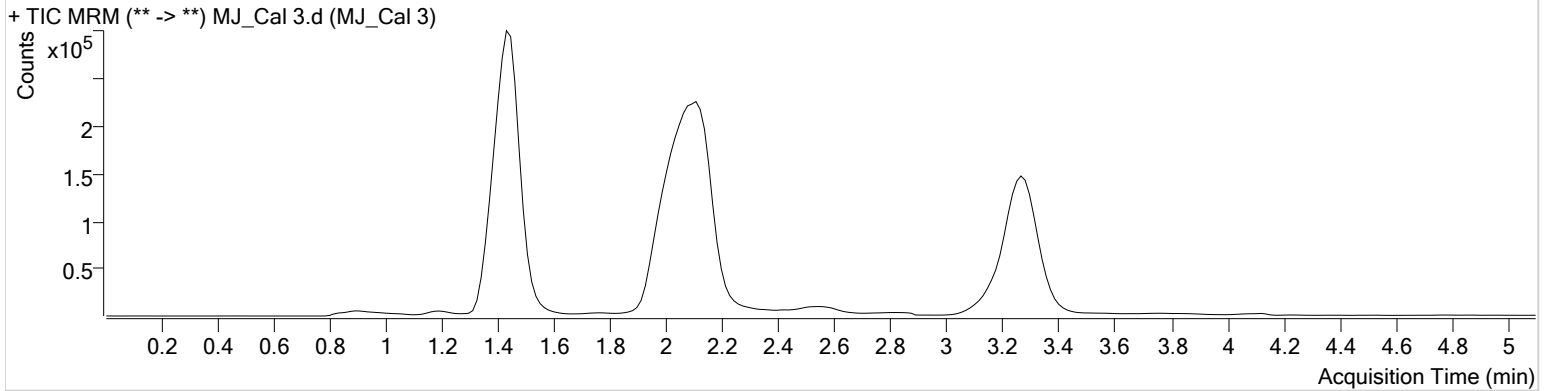


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-F6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 1:17:59 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	109971	∞	11.6	∞	1247379	4.9972 ng/ml
THC-COOH	1.474	154160	∞	56.1	∞	322683	20.0256 ng/ml
THC	3.285	60926	555.94	30.7	56.29	1265520	5.1118 ng/ml

SL

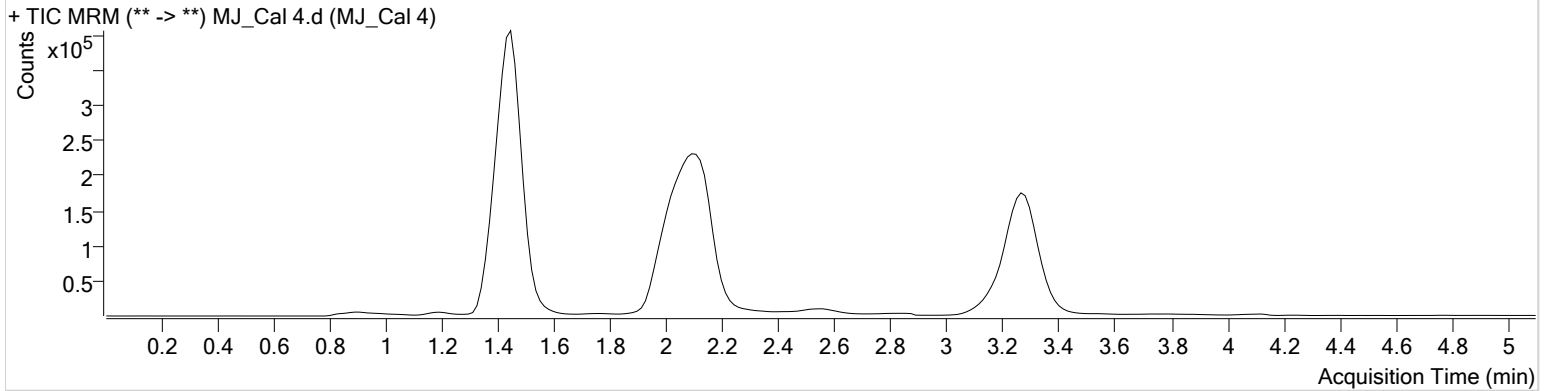


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-E6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 1:25:35 PM		
<b>Sample Info.</b>			

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	228120	∞	11.8	∞	1328499	9.8933 ng/ml
THC-COOH	1.474	402388	∞	58.7	1655.22	348116	49.0000 ng/ml
THC	3.285	129648	759.35	29.2	179.88	1386411	9.8210 ng/ml

SL

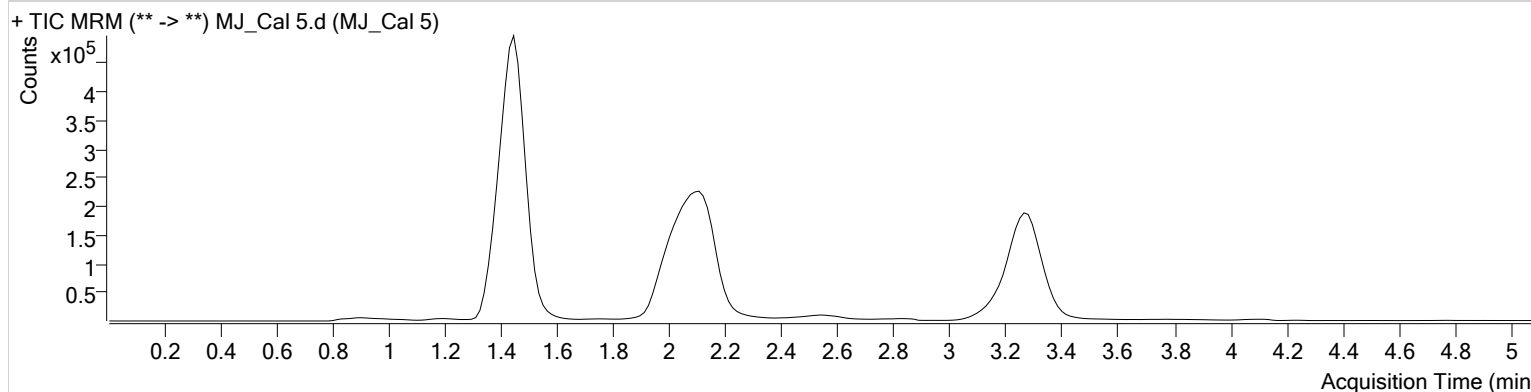


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-D6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 1:33:10 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	545436	∞	11.8	∞	1277997	24.8407 ng/ml
THC-COOH	1.474	584877	∞	59.0	∞	337859	73.5766 ng/ml
THC	3.285	309325	1279.86	29.3	246.76	1333784	24.1864 ng/ml

SL

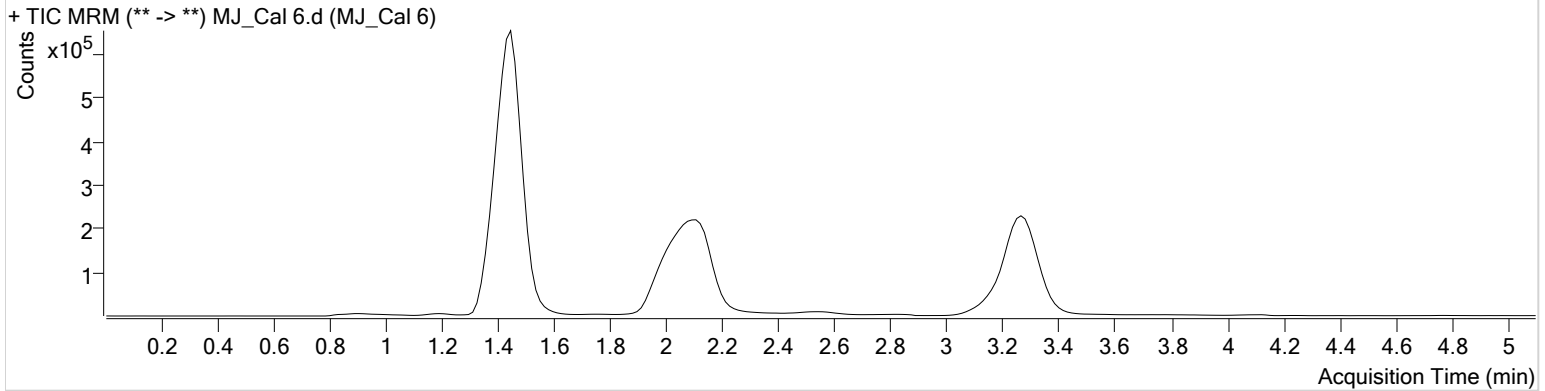


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-C6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 1:40:45 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	1139844	∞	12.0	∞	1339859	49.6827 ng/ml
THC-COOH	1.459	797470	∞	57.3	∞	337245	100.6447 ng/ml
THC	3.285	629204	∞	25.5	386.57	1324634	49.4177 ng/ml

SL

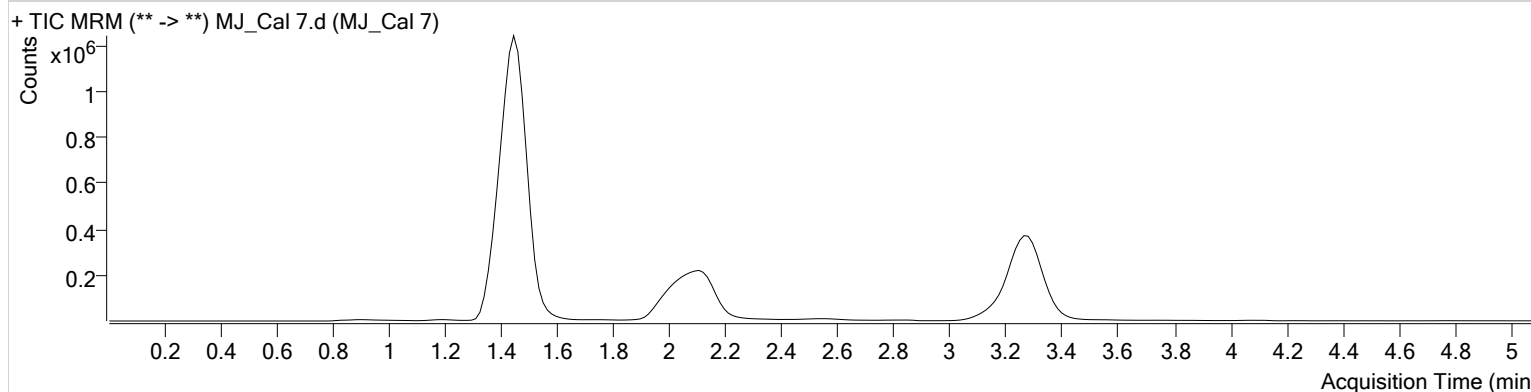


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\010721 AM 27 28 SC\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 1/8/2021 10:11:52 AM

<b>Instrument</b>	Instrument 1	<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>	Sarah Collins
<b>Sample Position</b>	P1-B6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/7/2021 1:48:21 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	2597627	∞	12.1	∞	1510501	100.6054 ng/ml
THC-COOH	1.474	2041810	∞	59.1	∞	345999	251.7444 ng/ml
THC	3.285	1497975	∞	24.7	1923.64	1535314	101.3855 ng/ml