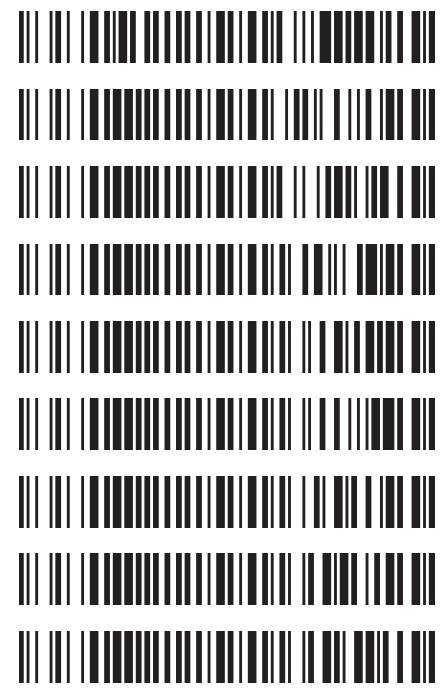


cg

REVIEWED
By Tamara Salazar at 7:08 am, Dec 01, 2021

Worklist: 5416

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-4630	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2312	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3402	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3582	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3646	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3647	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3712	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3739	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3741	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: 11/24/2021

Analyst: Celena Shrum

Plate lot#: 210609

Plate 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 20L20725

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 069901

Blank Urine Lot: POC031319

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 r² values ≥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: THC-OH not evaluated due to ratio issues with the calibrators. THC-COOH curve limit: 10-250.

	1	2	3	4	5	6
a					P2021-3712-1	QC 1
b					P2021-3647-1	cal 100 ng
c					P2021-3646-1	cal 50 ng
d					P2021-3582-1	cal 25 ng
e					P2021-3402-2	cal 10ng
f					P2021-2312-1	cal 5 ng
g				P2021-3741-1	M2021-4630-1	cal 3 ng
h				P2021-3739-1	Blood NC	cal 1ng

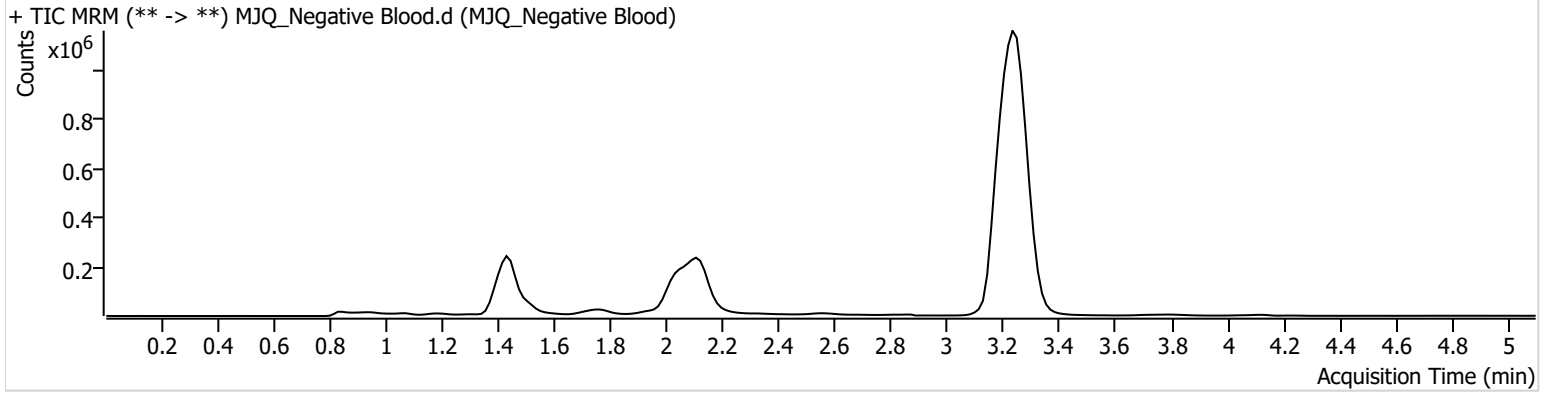
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_Negative Blood.d
Type	Sample	Sample	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 3:46:14 AM		
Sample Info.			

Sample Chromatogram



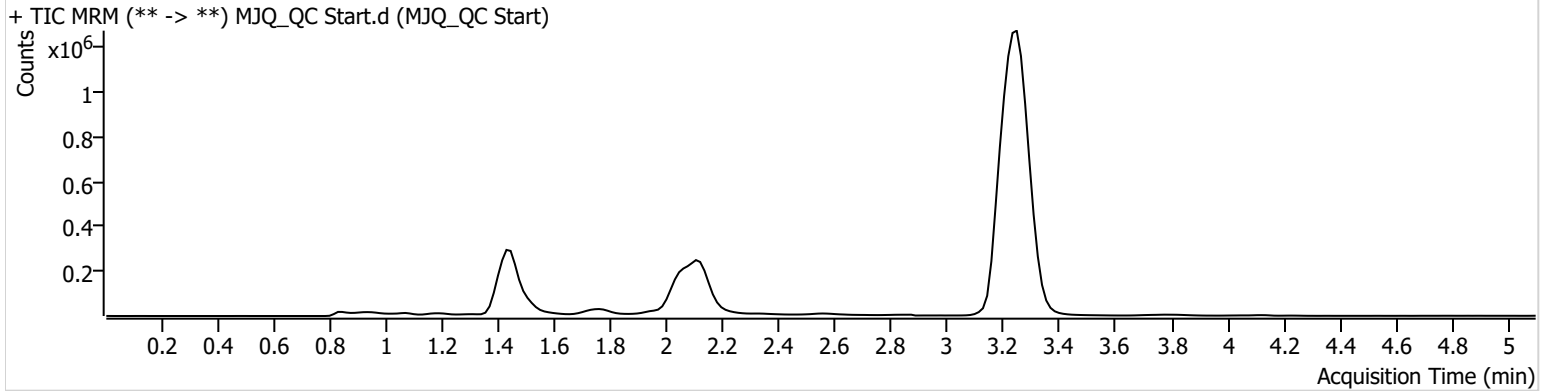
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_QC Start.d
Type	Sample	Sample	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 3:31:02 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	77344	∞	66.8	∞	243864	14.9862 ng/ml
THC	3.254	390624	∞	24.9	∞	8609126	4.8161 ng/ml

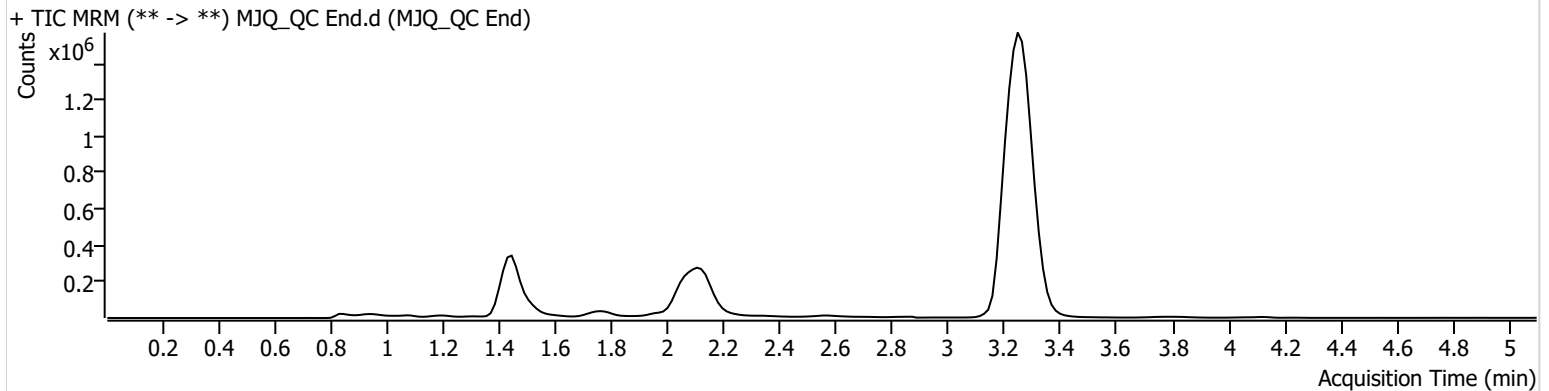
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

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-A6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 6:18:25 AM		

Sample Chromatogram

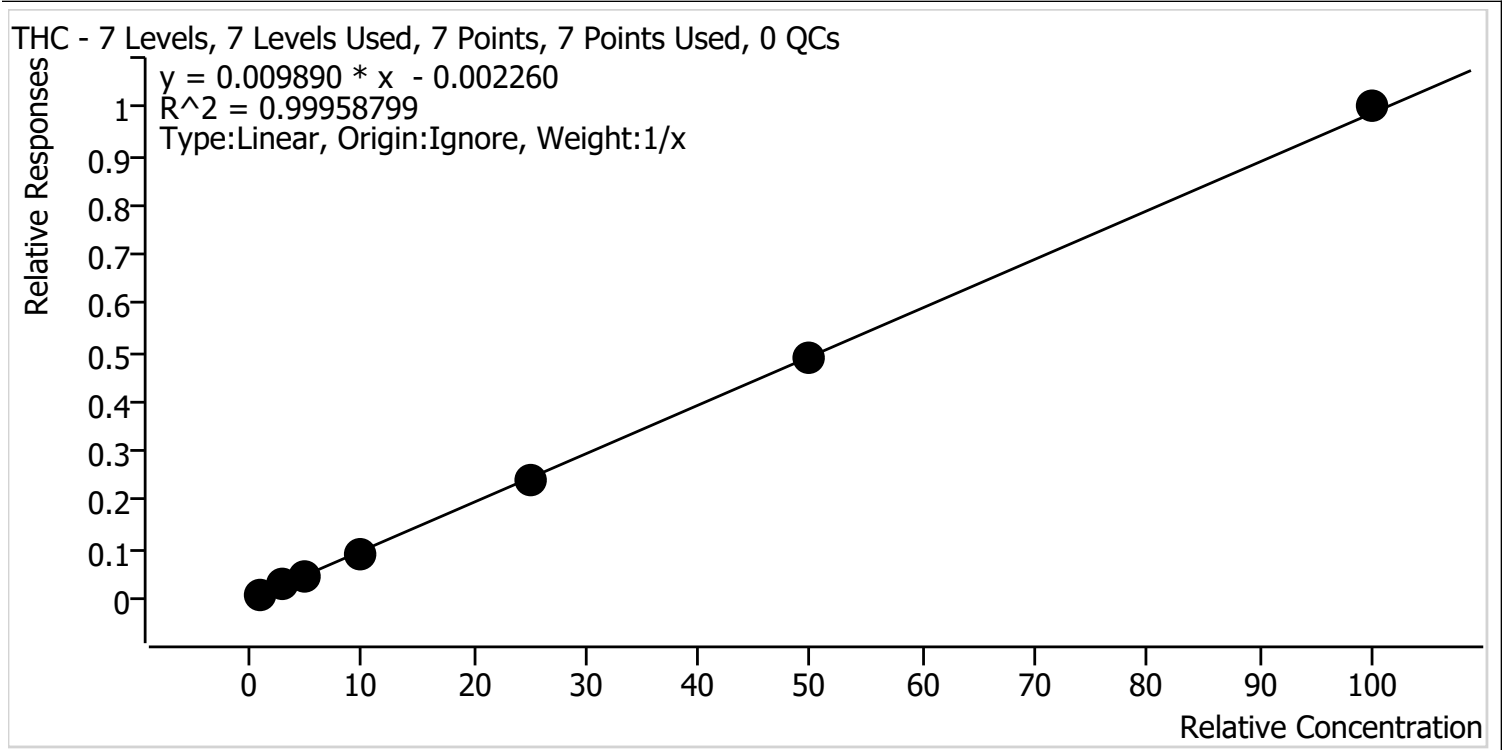


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	80948	∞	71.9	∞	261479	14.6737 ng/ml
THC	3.270	471475	∞	25.6	960.53	10305898	4.8540 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Last Cal. Update 11/30/2021 10:54 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

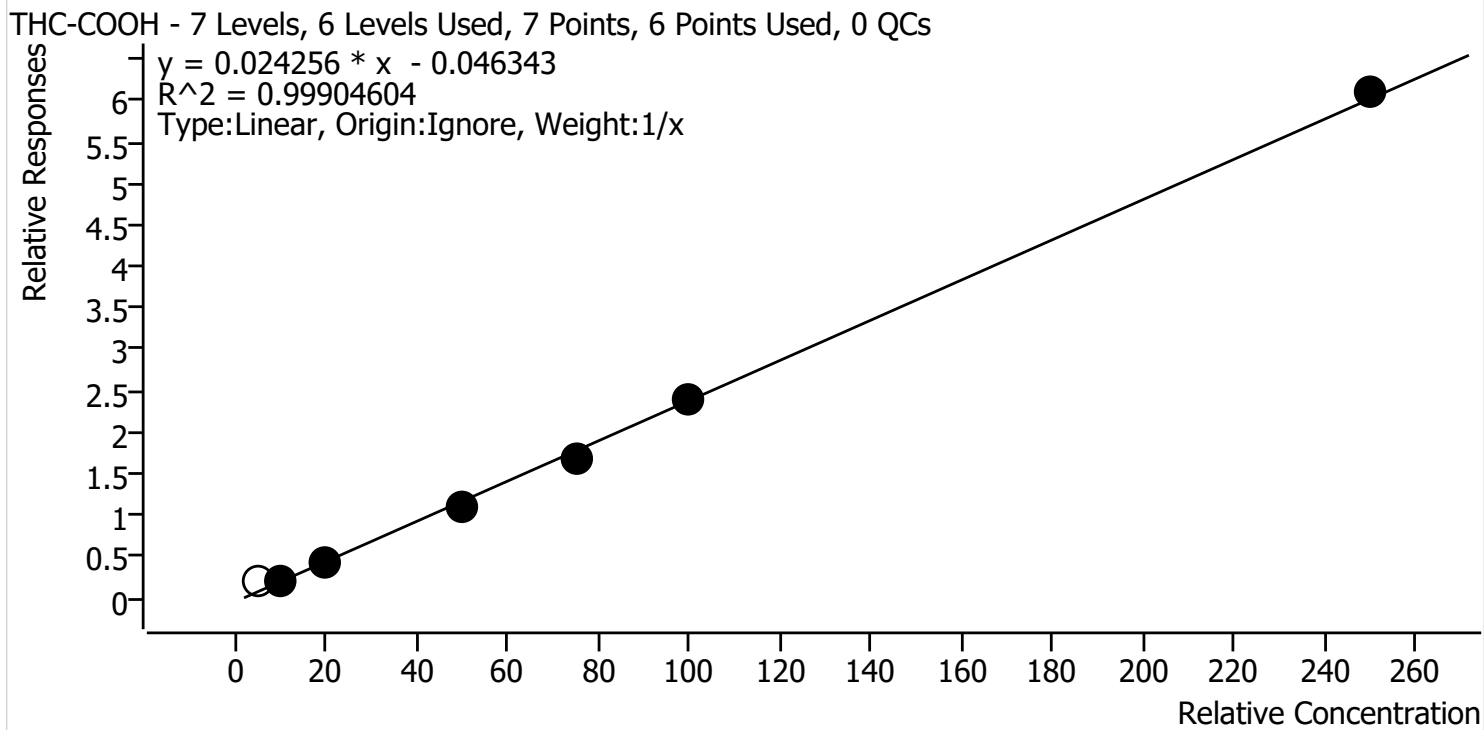


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	110.6
MJQ_Cal 2	2	✓	3.0	2.9	97.9
MJQ_Cal 3	3	✓	5.0	4.9	97.1
MJQ_Cal 4	4	✓	10.0	9.6	95.6
MJQ_Cal 5	5	✓	25.0	24.6	98.5
MJQ_Cal 6	6	✓	50.0	49.5	99.0
MJQ_Cal 7	7	✓	100.0	101.4	101.4



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Last Cal. Update 11/30/2021 10:54 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	x	5.0	10.8	215.5
MJQ_Cal 2	2	✓	10.0	10.6	106.1
MJQ_Cal 3	3	✓	20.0	19.9	99.5
MJQ_Cal 4	4	✓	50.0	47.9	95.7
MJQ_Cal 5	5	✓	75.0	72.0	96.0
MJQ_Cal 6	6	✓	100.0	101.3	101.3
MJQ_Cal 7	7	✓	250.0	253.3	101.3

Calibrator 1 dropped due to not meeting accuracy requirement.

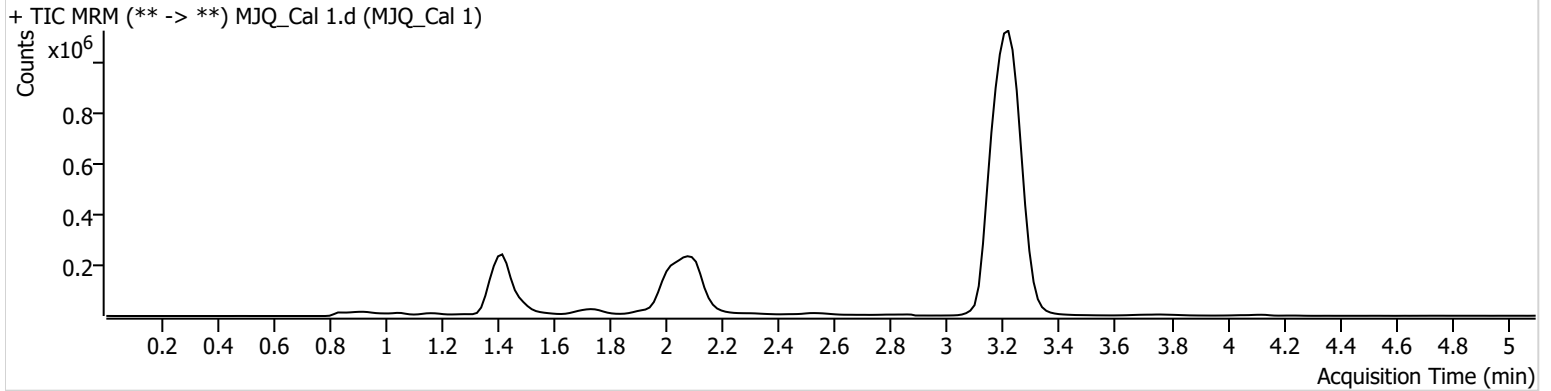
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 1.d
Type	Cal	Sample	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 2:30:03 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.444	48311	∞	33.8 Low	∞	224634	10.7772 ng/ml
THC	3.239	72474	1139.95	29.4	∞	8350240	1.1060 ng/ml

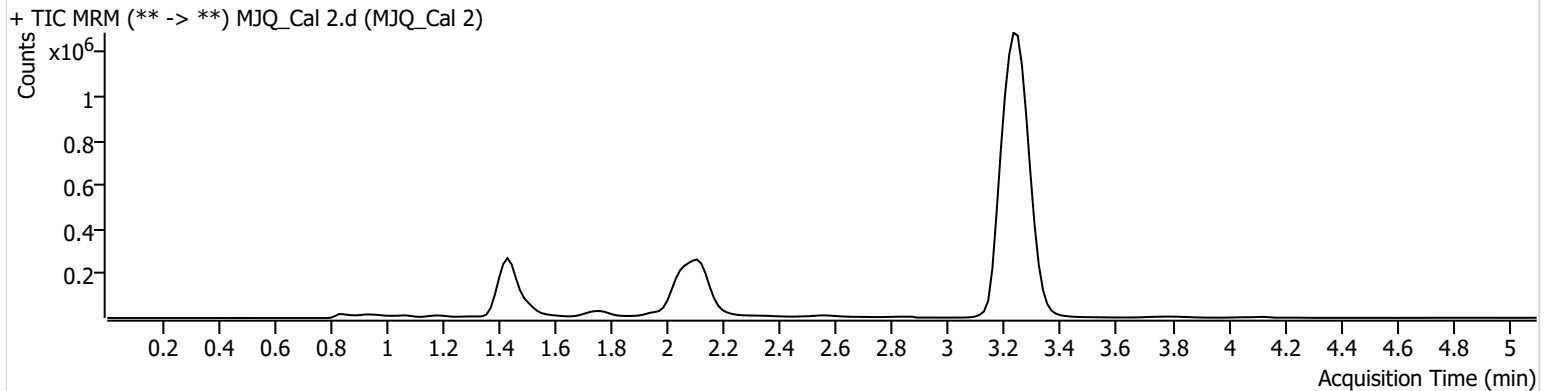
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

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-G6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 2:37:50 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	45497	∞	69.7	∞	215602	10.6106 ng/ml
THC	3.254	232675	∞	26.1	∞	8689076	2.9359 ng/ml

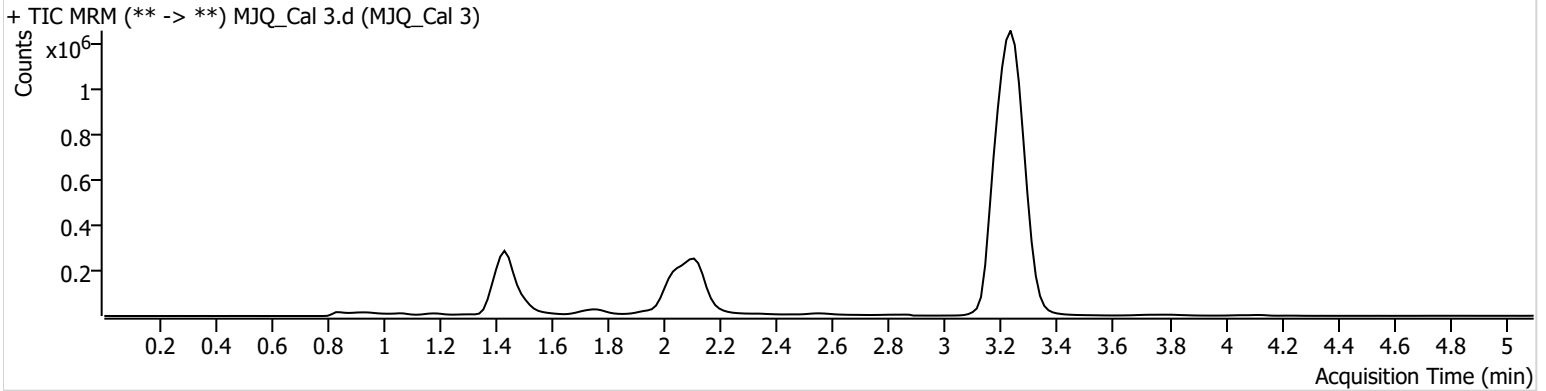
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 3.d
Type	Cal	Sample	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 2:45:27 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	99987	252.21	66.9	∞	229018	19.9100 ng/ml
THC	3.254	396941	∞	25.4	∞	8678291	4.8531 ng/ml

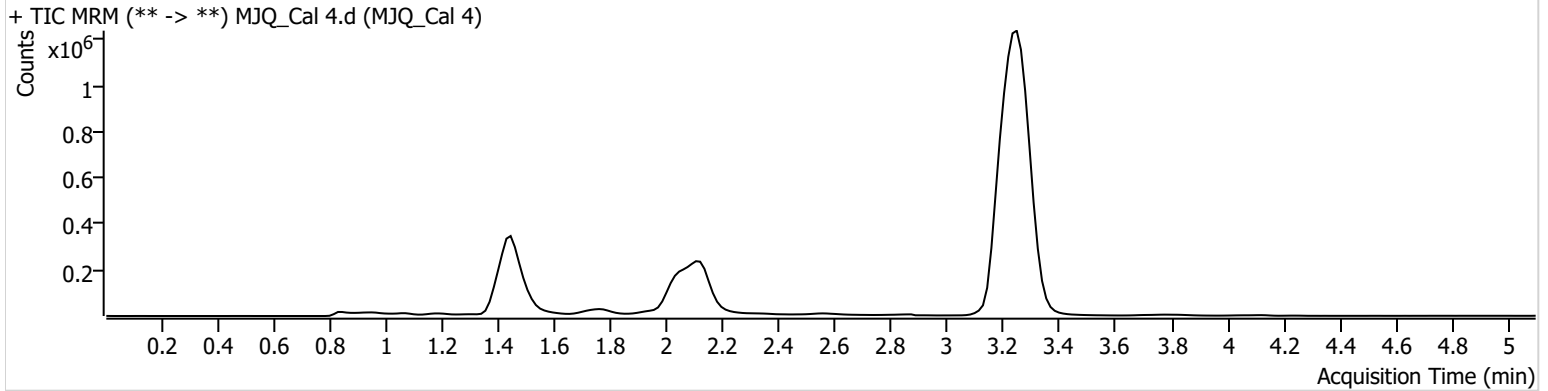
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 4.d
Type	Cal	Sample	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 2:53:02 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	257286	196.41	66.4	690.14	230845	47.8601 ng/ml
THC	3.270	766803	6260.48	24.5	∞	8309852	9.5583 ng/ml

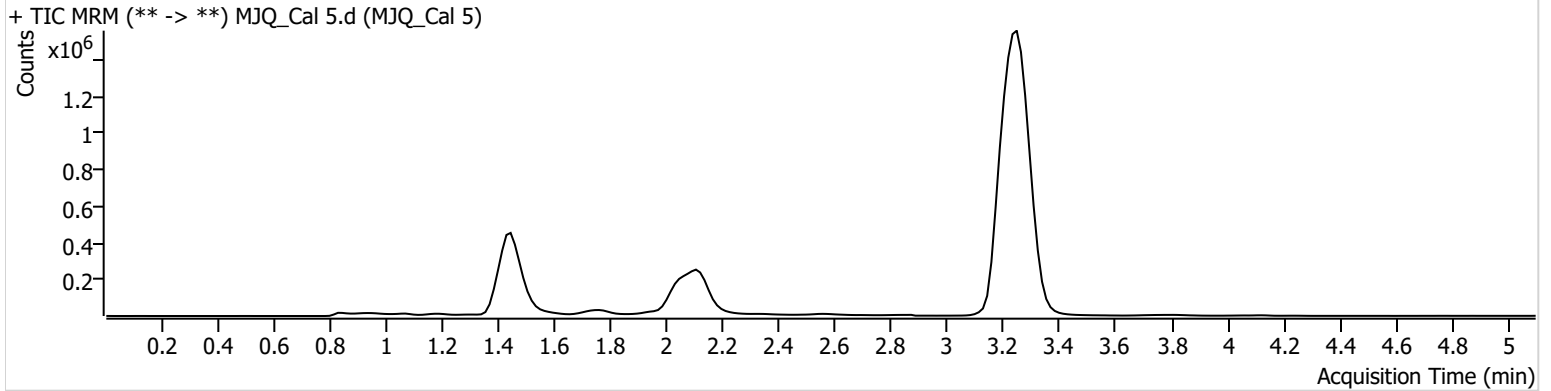
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 5.d
Type	Cal	Sample	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 3:00:38 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	397122	∞	70.2	∞	233520	72.0214 ng/ml
THC	3.254	2101602	4515.92	24.4	∞	8713242	24.6152 ng/ml

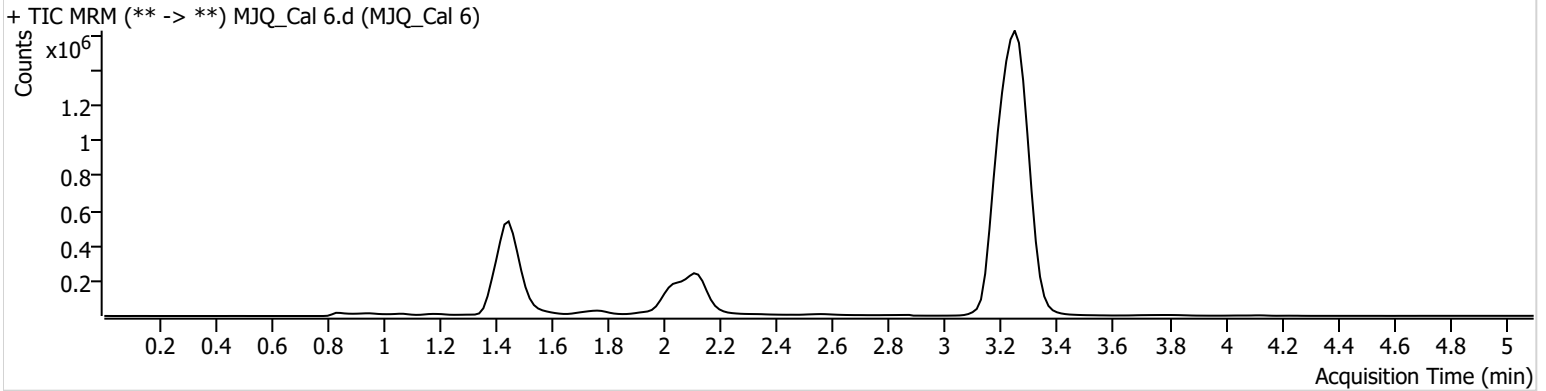
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 6.d
Type	Cal	Sample	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 3:08:14 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	549290	∞	63.0	∞	227941	101.2600 ng/ml
THC	3.270	3856975	∞	24.6	∞	7915526	49.4948 ng/ml

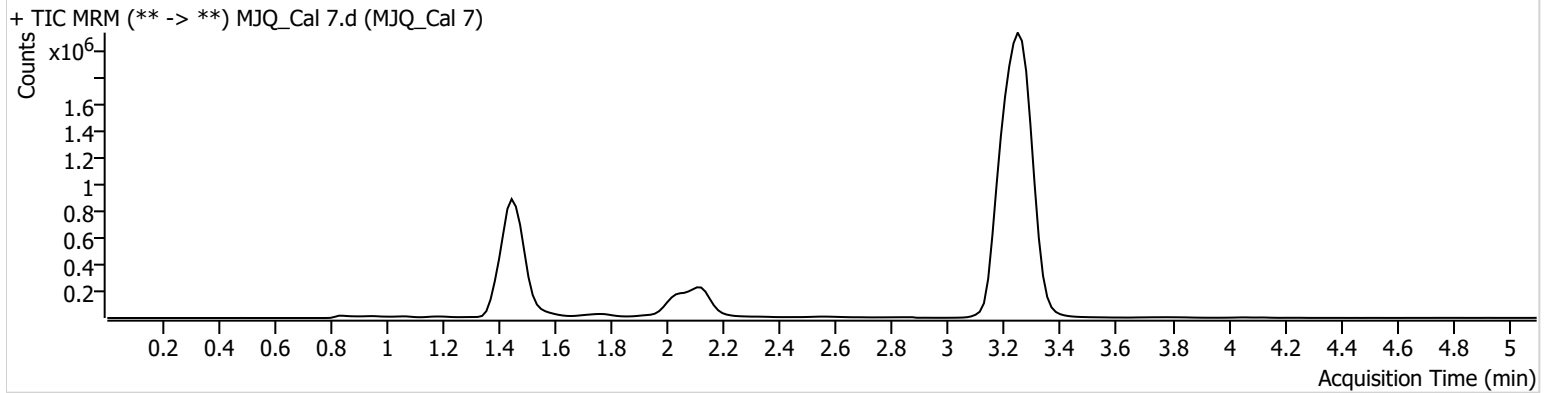
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\112421 AM 27 28 CS\QuantResults\AM 27 THC-OH removed.batch.bin
Calibration Last Update 11/30/2021 10:54:03 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 7.d
Type	Cal	Sample	MJQ_Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-B6	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2021 3:15:50 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	1332204	∞	62.6	3145.60	218446	253.3379 ng/ml
THC	3.270	7573585	∞	24.7	∞	7566036	101.4367 ng/ml