

REVIEWED

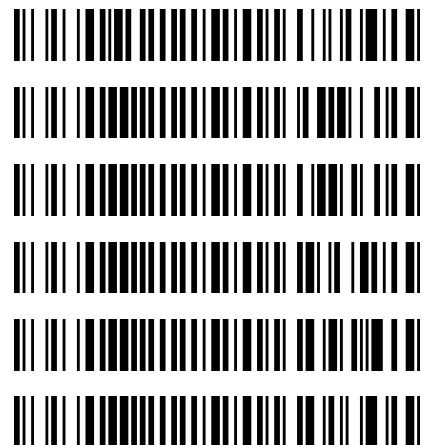
By Celena Shrum at 1:34 pm, Dec 03, 2021

TS

12/3/2021

Worklist: 5421

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-4910	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3742	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3798	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3800	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3803	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3809	1	BCK	AM 27 Blood THC Quant by LC-QQQ



TS

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

Extraction Date: 12/02/2021

Analyst: Tamara Salazar

Plate lot#: IDP-108-2-210609

Plate Re-test Date: 12-09-21

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

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, 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: 750uL
- 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 ≥ 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 samples with calculated concentrations for THC at 1ng/mL or greater and OH-THC at 3ng/mL or greater may be reported quantitatively (blood only). Calculated concentrations for carboxy-THC of 5ng/mL may be reported qualitatively. 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 ratios being out for multiple calibrators.

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	1	2	3	4	5	6
A	IS + Cal. 1				P2021-3742-1	IS + QC_1
B	IS + Cal. 2				P2021-3809-1	IS + Cal. 7
C	IS + Cal. 3				P2021-3803-1	IS + Cal. 6
D	IS + Cal. 4				P2021-3800-1	IS + Cal. 5
E	IS + Cal. 5				P2021-3798-1	IS + Cal. 4
F	IS + Cal. 6				P2021-3742-1*	IS + Cal. 3
G	IS + Cal. 7				M2021-4910-1	IS + Cal. 2
H	IS + QC_1				Neg	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Sample moved during analytical step 6 due to a blood clot.

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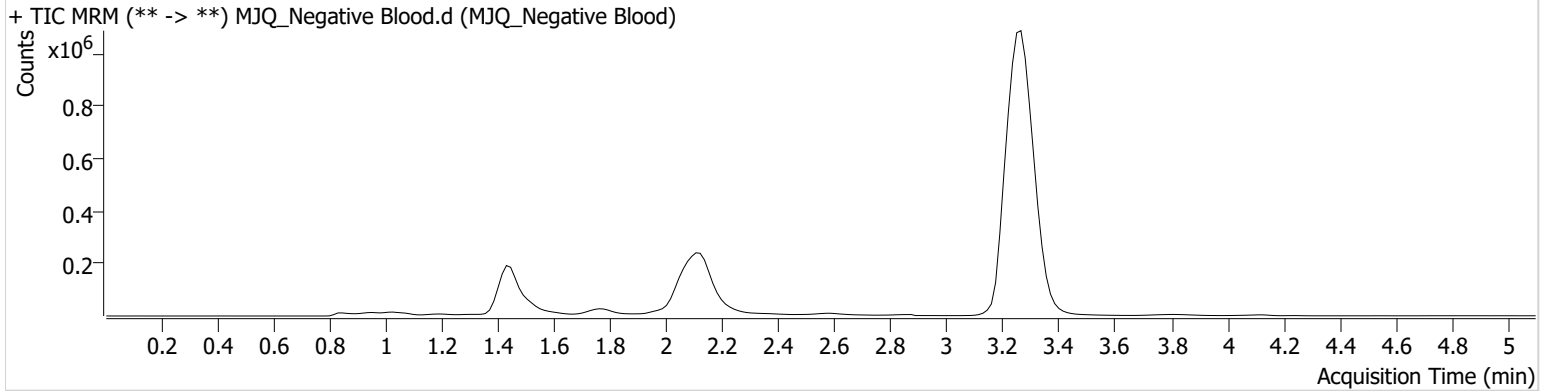


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_Negative Blood.d
Type	Sample	Sample	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 1:22:50 AM		
Sample Info.			

Sample Chromatogram



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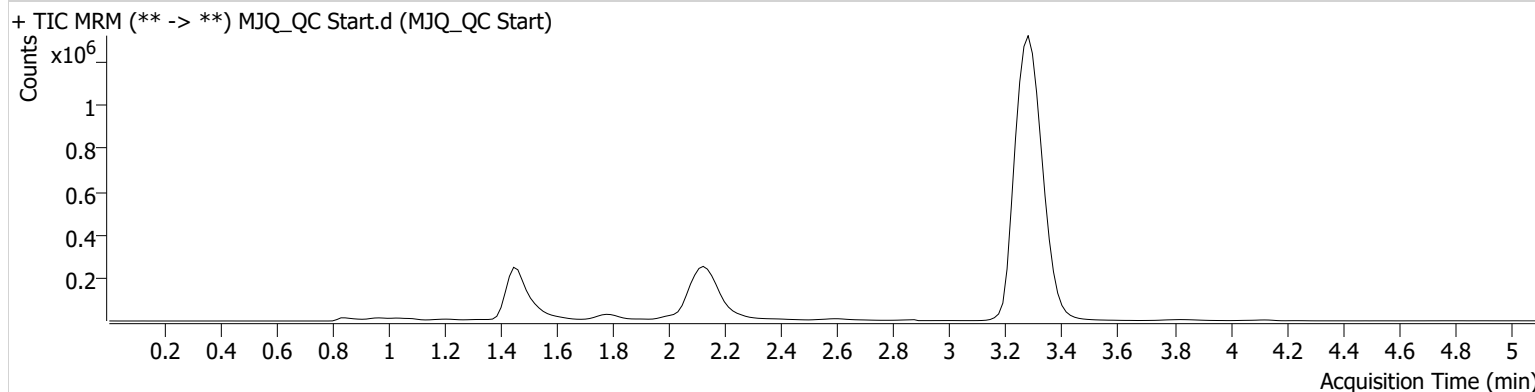


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Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_QC Start.d
Type	Sample	Sample	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 1:07:36 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	59915	∞	70.4	∞	194979	13.8567 ng/ml
THC	3.300	408414	∞	25.4	1299.99	8543872	5.0846 ng/ml

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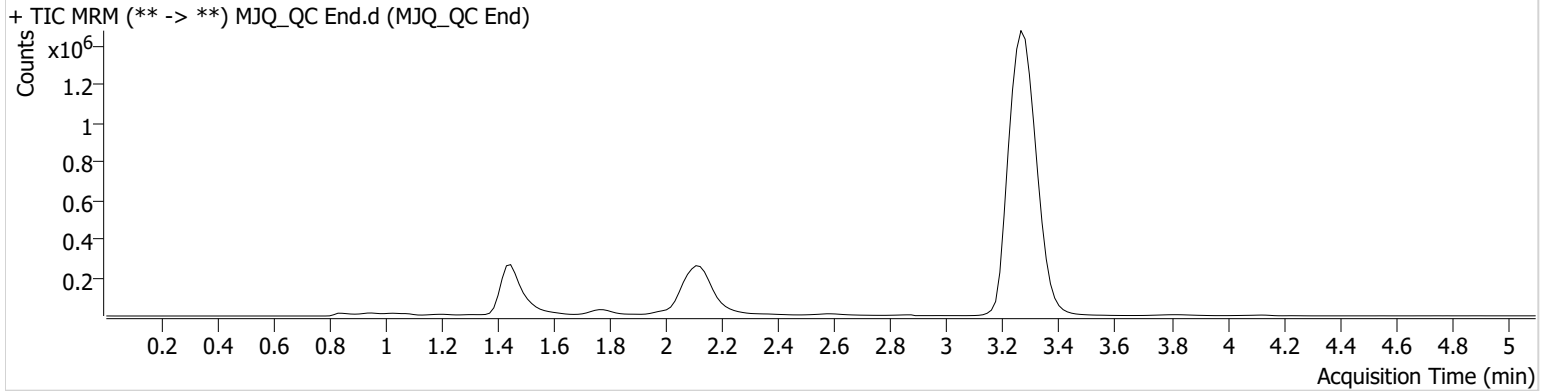


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Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_QC End.d
Type	Sample	Sample	MJQ_QC End
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 3:09:29 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	66290	∞	61.6	704.46	200032	14.9304 ng/ml
THC	3.285	446689	∞	26.2	∞	9636243	4.9366 ng/ml

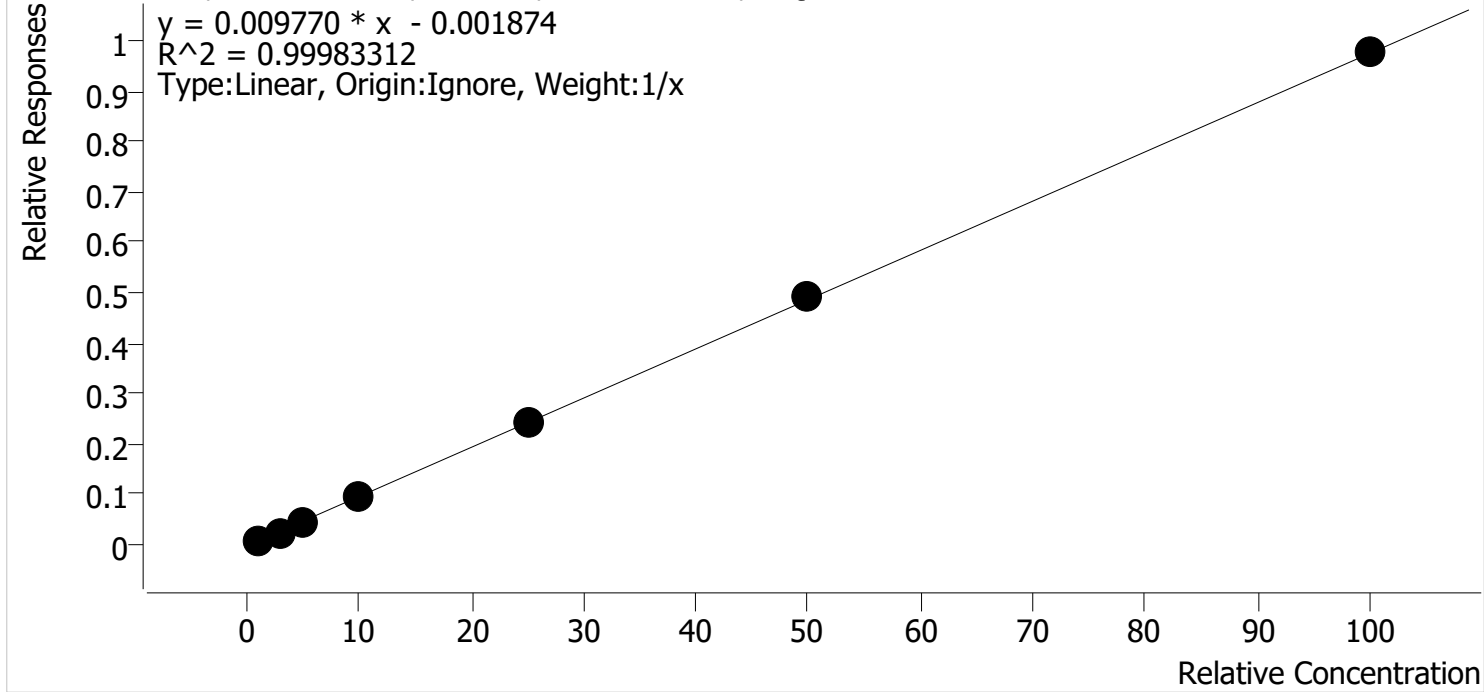
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Last Cal. Update 12/3/2021 12:50 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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



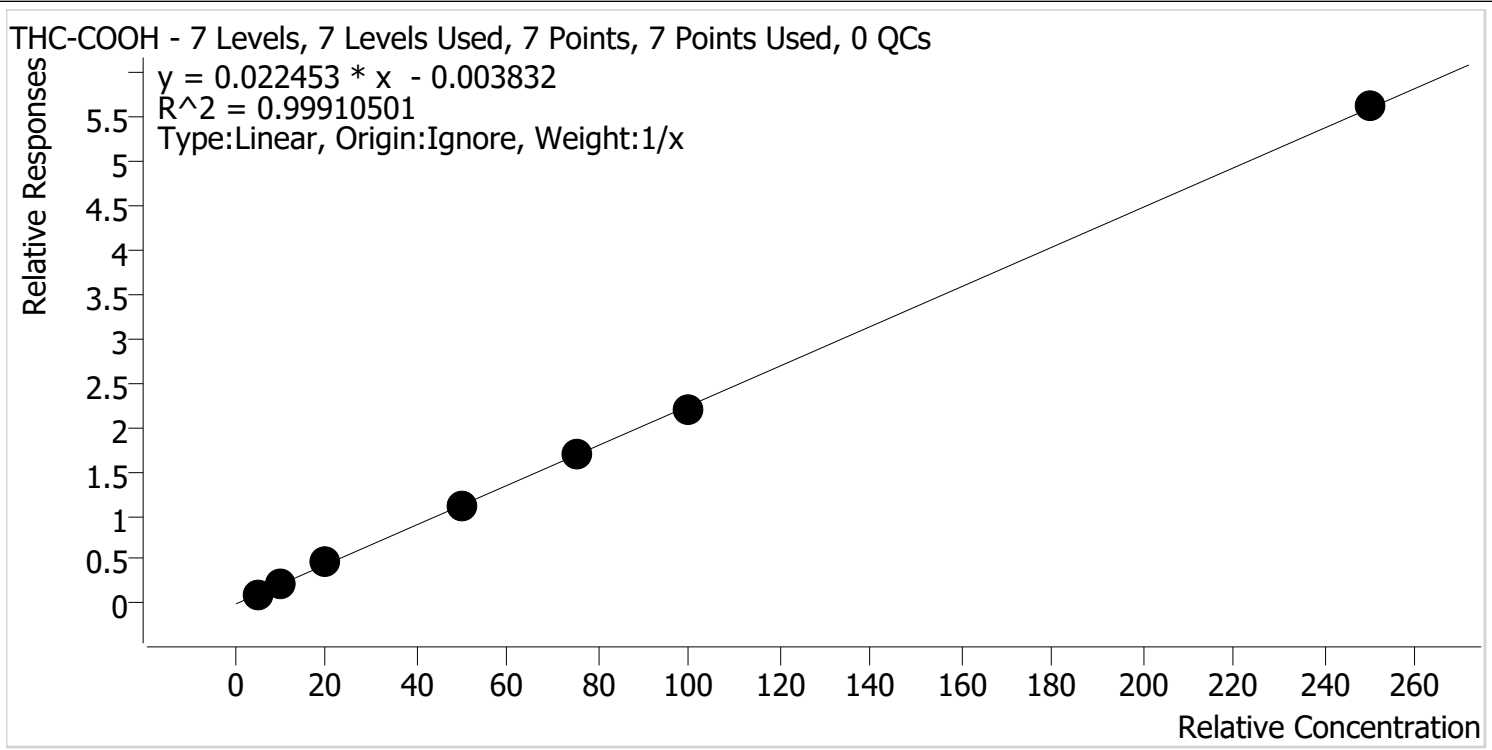
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	109.6
MJQ_Cal 2	2	✓	3.0	2.8	94.8
MJQ_Cal 3	3	✓	5.0	4.8	96.9
MJQ_Cal 4	4	✓	10.0	9.8	98.1
MJQ_Cal 5	5	✓	25.0	24.9	99.8
MJQ_Cal 6	6	✓	50.0	50.3	100.5
MJQ_Cal 7	7	✓	100.0	100.2	100.2

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Last Cal. Update 12/3/2021 12:50 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	4.9	98.6
MJQ_Cal 2	2	✓	10.0	9.3	93.5
MJQ_Cal 3	3	✓	20.0	22.0	109.9
MJQ_Cal 4	4	✓	50.0	49.5	99.0
MJQ_Cal 5	5	✓	75.0	76.2	101.6
MJQ_Cal 6	6	✓	100.0	97.2	97.2
MJQ_Cal 7	7	✓	250.0	250.9	100.4

TS

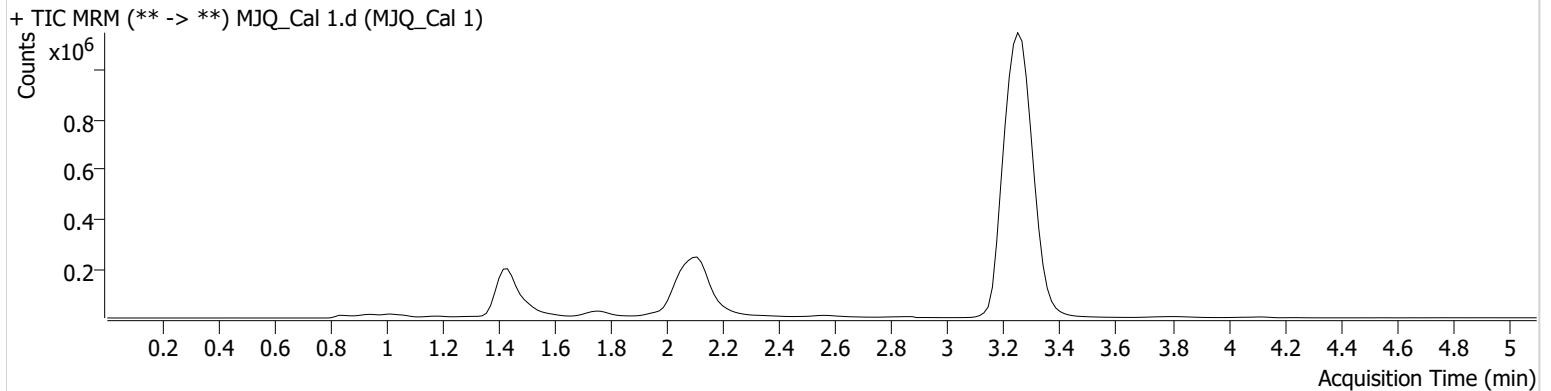


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 1.d
Type	Cal	Sample	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 12:06:37 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	19248	∞	62.9	113.82	180156	4.9291 ng/ml Low
THC	3.270	73348	279.58	29.7	1179.78	8299683	1.0964 ng/ml

TS



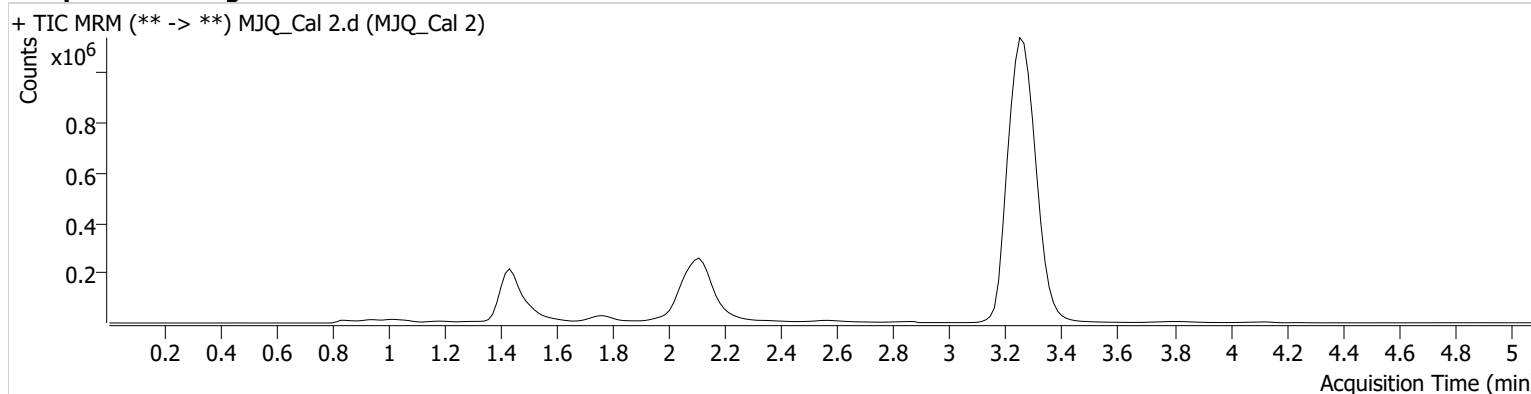
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 2.d
Type	Cal	Sample	MJQ_Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 12:14:23 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	36190	∞	71.3	∞	175685	9.3451 ng/ml
THC	3.270	199892	1559.58	27.2	∞	7711105	2.8452 ng/ml

TS

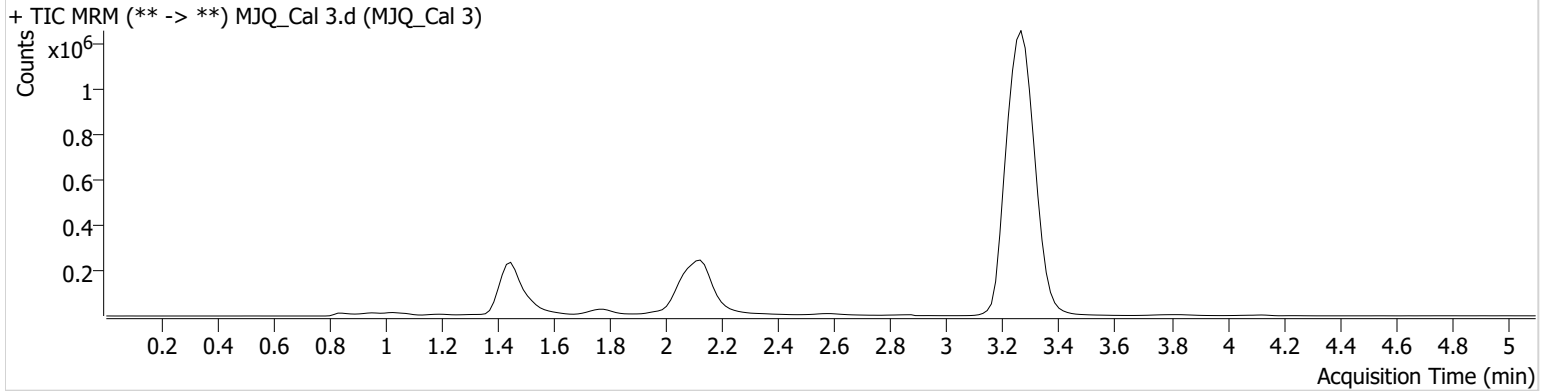


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 3.d
Type	Cal	Sample	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 12:21:59 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	94401	∞	54.0	554.56	192769	21.9815 ng/ml
THC	3.285	381508	1867.51	25.3	584.22	8389958	4.8462 ng/ml

TS



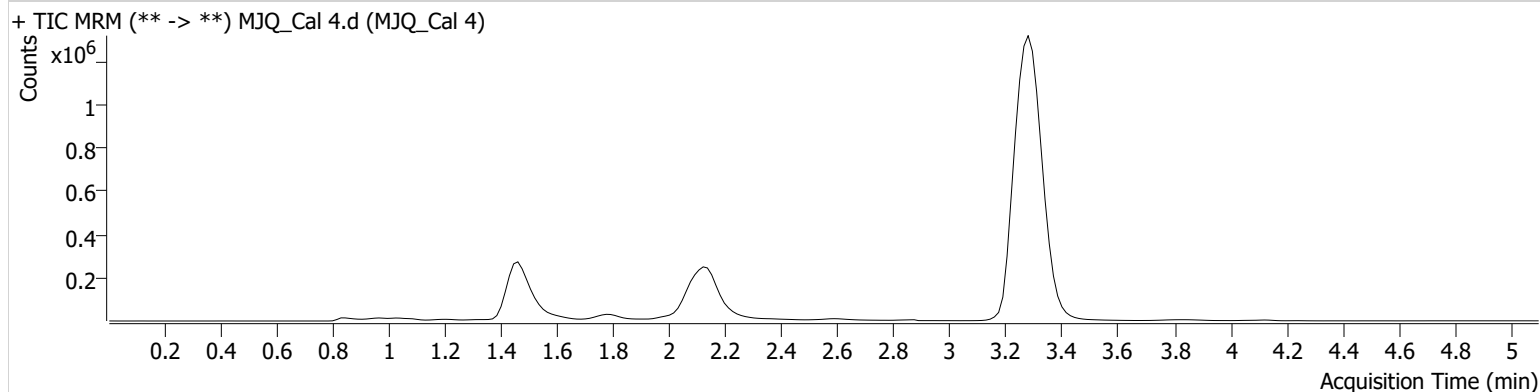
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 4.d
Type	Cal	Sample	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 12:29:35 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	194277	∞	64.7	1314.41	175479	49.4797 ng/ml
THC	3.300	769573	∞	24.4	1029.84	8189936	9.8098 ng/ml

TS

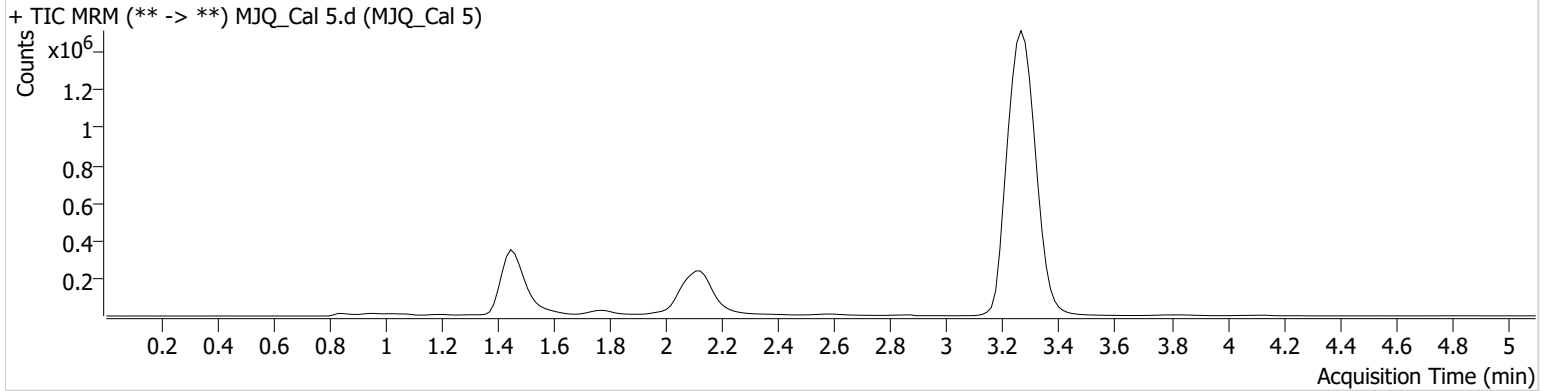


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 5.d
Type	Cal	Sample	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 12:37:11 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	303198	∞	64.7	1339.68	177701	76.1625 ng/ml
THC	3.285	1990812	14180.20	24.4	∞	8232759	24.9432 ng/ml

TS

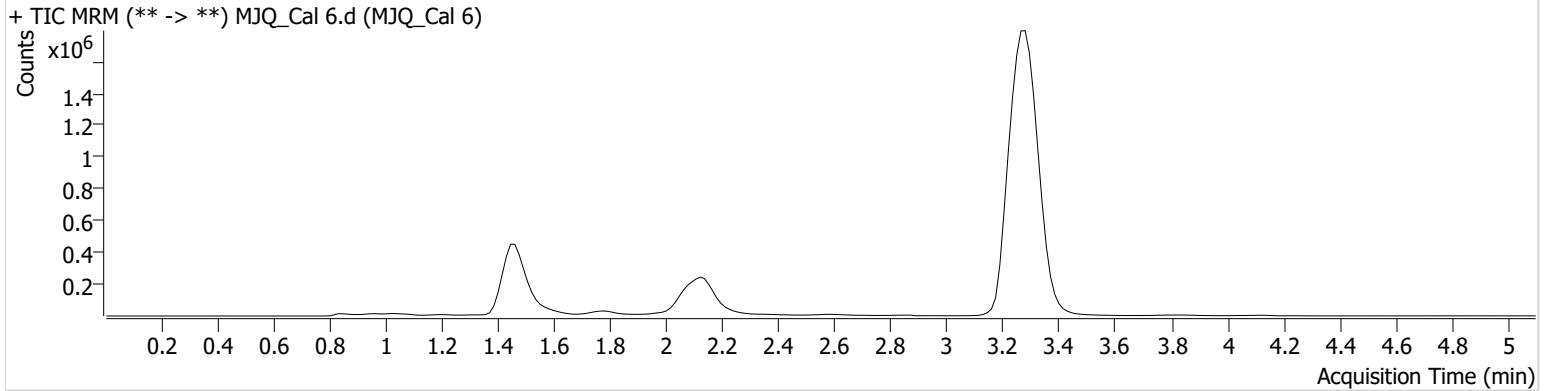


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 6.d
Type	Cal	Sample	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	12/3/2021 12:44:47 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	420736	∞	65.7	5171.50	193158	97.1830 ng/ml
THC	3.285	3996565	∞	24.8	∞	8168698	50.2701 ng/ml

TS

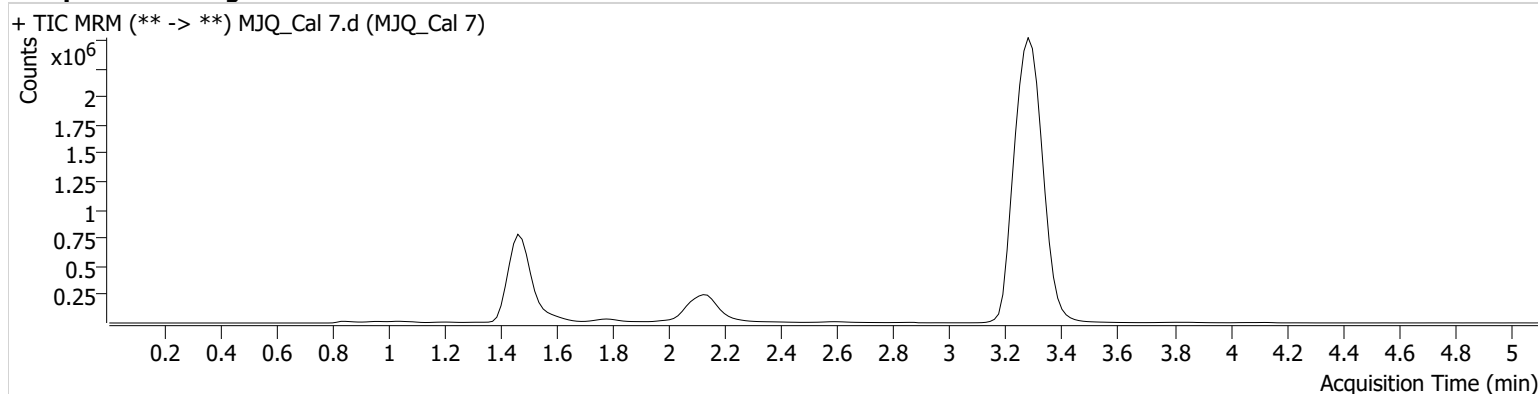


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\12022021 AM 27 28 TS\QuantResults\AM 27_Hydroxy removed.batch.bin
Calibration Last Update 12/3/2021 12:50:14 PM

Instrument Falco (069901) **Data File** MJQ_Cal 7.d
Type Cal **Sample** MJQ_Cal 7
Acq. Method AM 27 THCQ.m **Operator** Tamara Salazar
Sample Position P1-B6 **Comment**
Injection Volume 10
Acq. Date-Time 12/3/2021 12:52:23 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	1022965	∞	64.8	6038.16	181699	250.9190 ng/ml
THC	3.300	7791282	∞	24.5	6860.24	7975099	100.1891 ng/ml