








REVIEWED

By Celena Shrum at 3:31 pm, May 25, 2021

TS

5/12/2021

Worklist: 4971

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2021-1769	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
M2021-1882	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-1263	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-1410	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-1440	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-1447	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-1518	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	

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

Extraction Date: 05/18/2021

Plate lot#: IDP-108-2-201206

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 20L20723

LCMS-QQQ ID: 069901

Analyst: Tamara Salazar

Plate Expiration: 06/06/2021

Mobile phase B: 0.1% Formic acid in Acetonitrile

Column: UCT Selectra DA 100 x 2.1mm 3um

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. 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: 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: 800uL
- 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: *Only THC-COOH evaluated.*

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	1	2	3	4	5	6
A	IS + Cal. 1	IS + Sample	IS + Sample	IS + Sample	P2021-1440-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2021-1410-1	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	IS + Sample	P2021-1263-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	IS + Sample	M2021-1882-2	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	IS + Sample	M2021-1769-3	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	IS + Sample	Neg Urine	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2021-1518-1	Urine Control	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2021-1447-1	Neg Blood	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO



Idaho State Police Forensic Services

AM #26 Screening of THC and Metabolites and AM #27 Confirmation of THC and Metabolites Blood External Control Prep Sheet

Methanol External Control Solution (Lot: WS03052021)

10 μ L of 1mg/mL THC, 100 μ L of 100 μ g/mL THC-OH, C-THC in 9790 μ L MeOH

Approximate concentration μ g/mL.

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>	<i>Expiration Date</i>
Methanol (LCMS)	Fisher	200921	
THC	Cerilliant	FE01041701	03/31/2022
C-THC	Cerilliant	FE08011801	08/31/2023
THC-OH	Cerilliant	FE07221601	07/31/2021
Prepared:	03/05/2021		
Prepared By:	Tamara Salazar/Amber Gerheart		

Urine External Control Solution (Lot: 04232021)

200 μ L of methanol external control solution was added to 9800 μ L of blood.

Approximately 20ng/mL each

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>
Negative Blood	Lampire	20L20724
Methanol External Control Solution	-	WS03052021
Prepared:	04/23/2021	
Prepared by:	Sarah Collins	

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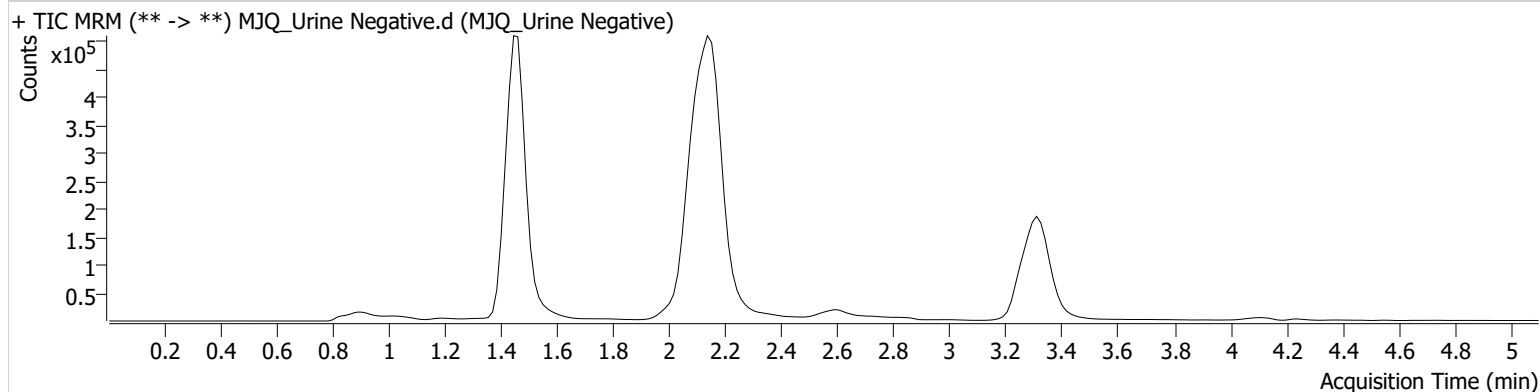


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 PM

Instrument	Falco (069901)	Data File	MJQ_Urine Negative.d
Type	Sample	Sample	MJQ_Urine Negative
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F5	Comment	
Injection Volume	10		
Acq. Date-Time	5/18/2021 2:42:37 PM		
Sample Info.			

Sample Chromatogram



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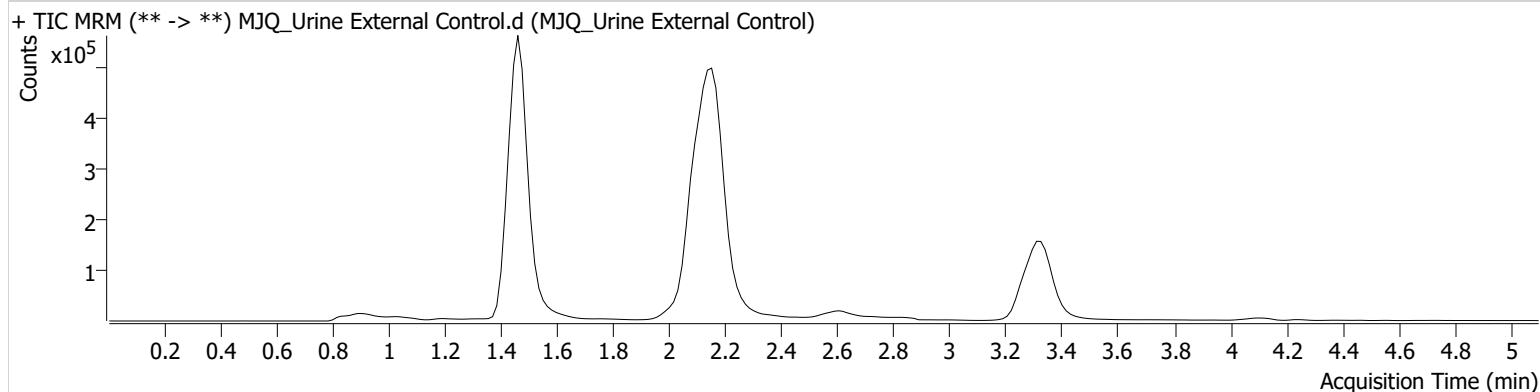


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 PM

Instrument	Falco (069901)	Data File	MJQ_Urine External Control.d
Type	Sample	Sample	MJQ_Urine External Control
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G5	Comment	
Injection Volume	10		
Acq. Date-Time	5/18/2021 2:57:48 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	70722	∞	56.3	∞	405721	7.2702 ng/ml

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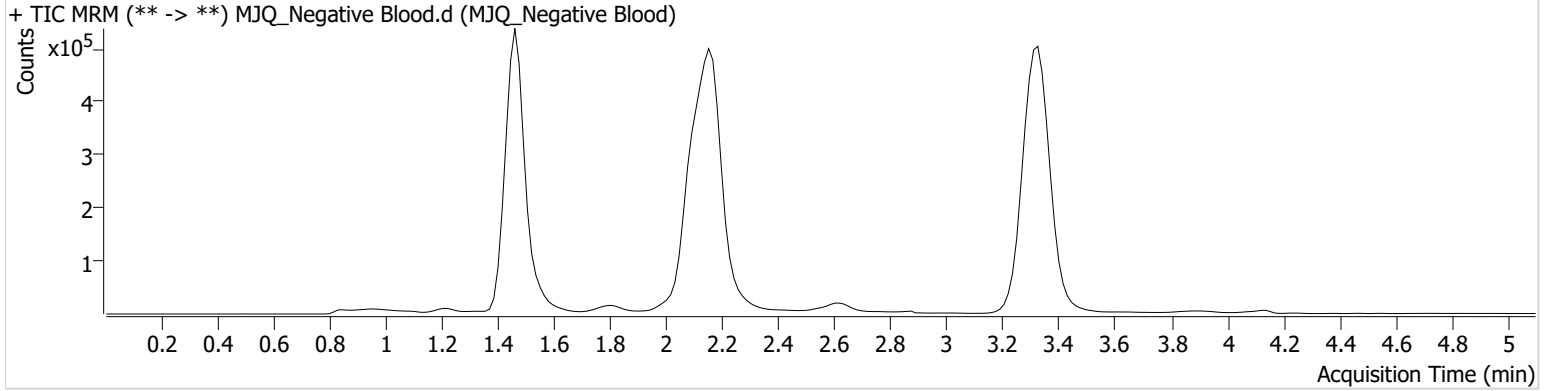


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 2:12:10 PM		
Sample Info.			

Sample Chromatogram



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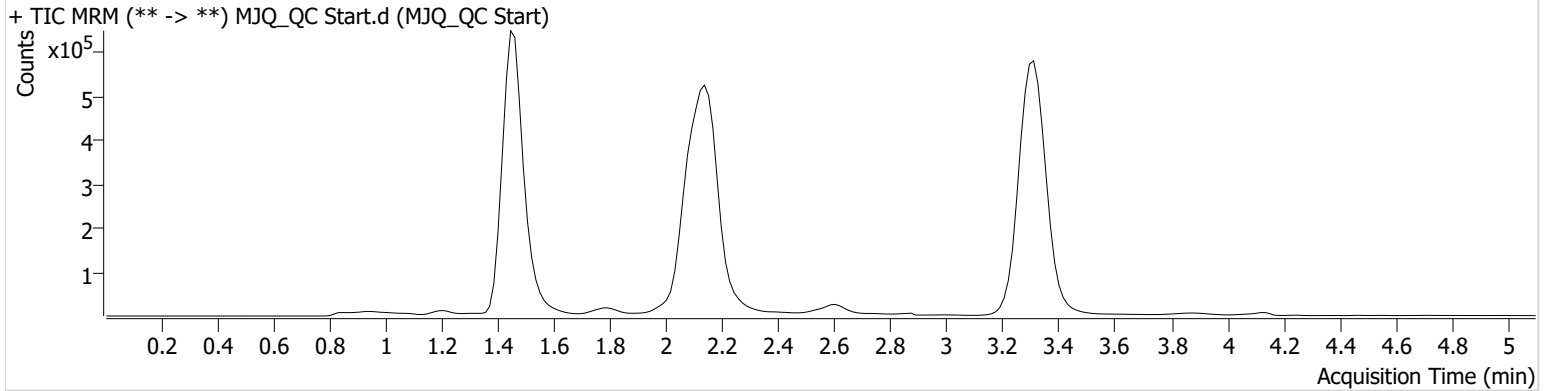


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 2:27:24 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	195706	306.45	53.7	617.18	564473	13.9715 ng/ml

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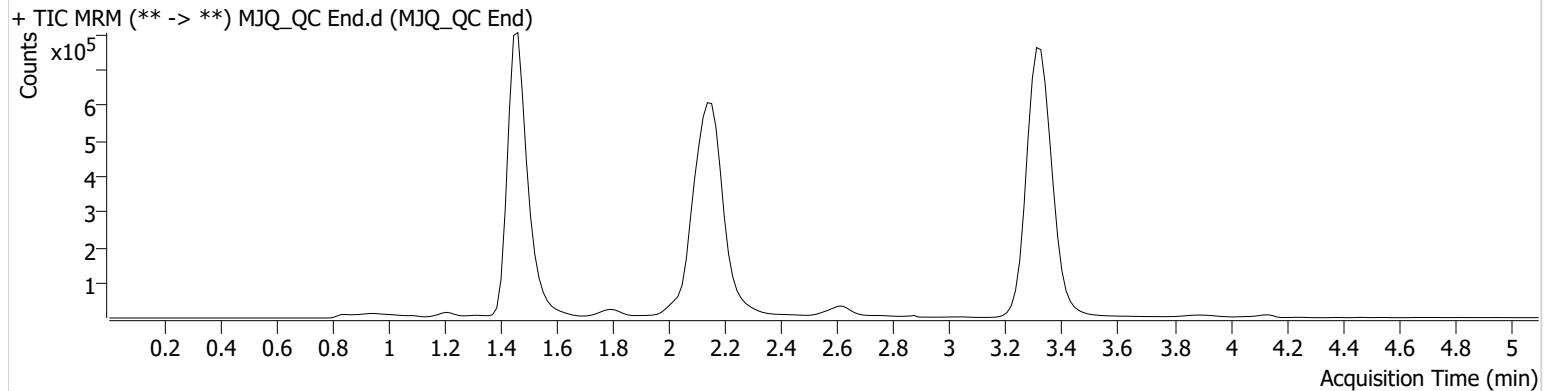


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 4:52:03 PM		

Sample Chromatogram



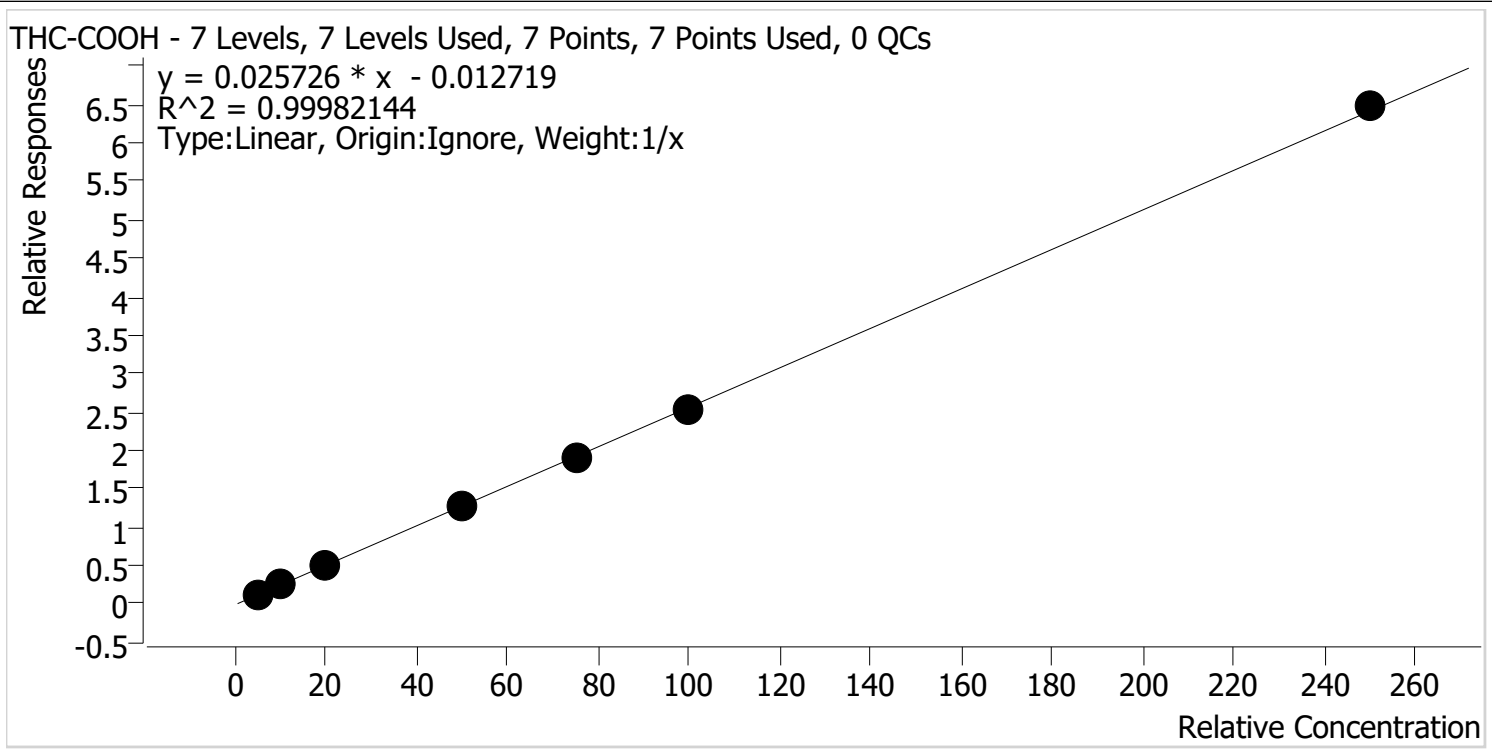
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	226823	∞	53.6	824.45	638963	14.2934 ng/ml

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Last Cal. Update 5/19/2021 12:56 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	5.2	104.2
MJQ_Cal 2	2	✓	10.0	9.7	97.4
MJQ_Cal 3	3	✓	20.0	20.1	100.6
MJQ_Cal 4	4	✓	50.0	49.6	99.2
MJQ_Cal 5	5	✓	75.0	74.4	99.2
MJQ_Cal 6	6	✓	100.0	98.6	98.6
MJQ_Cal 7	7	✓	250.0	252.4	101.0

TS

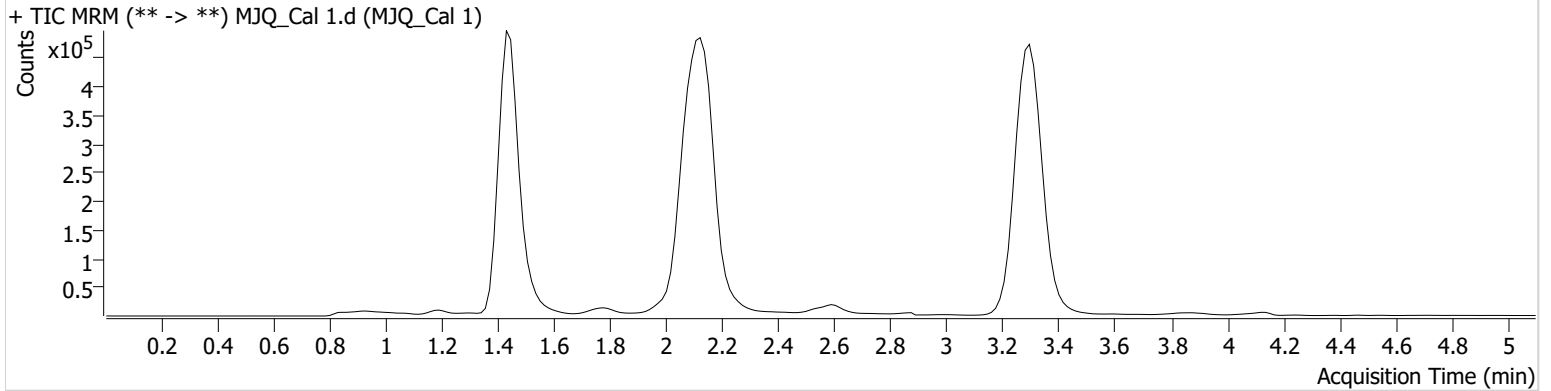


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 1:11:15 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	57464	169.83	50.4	∞	473796	5.2090 ng/ml

TS

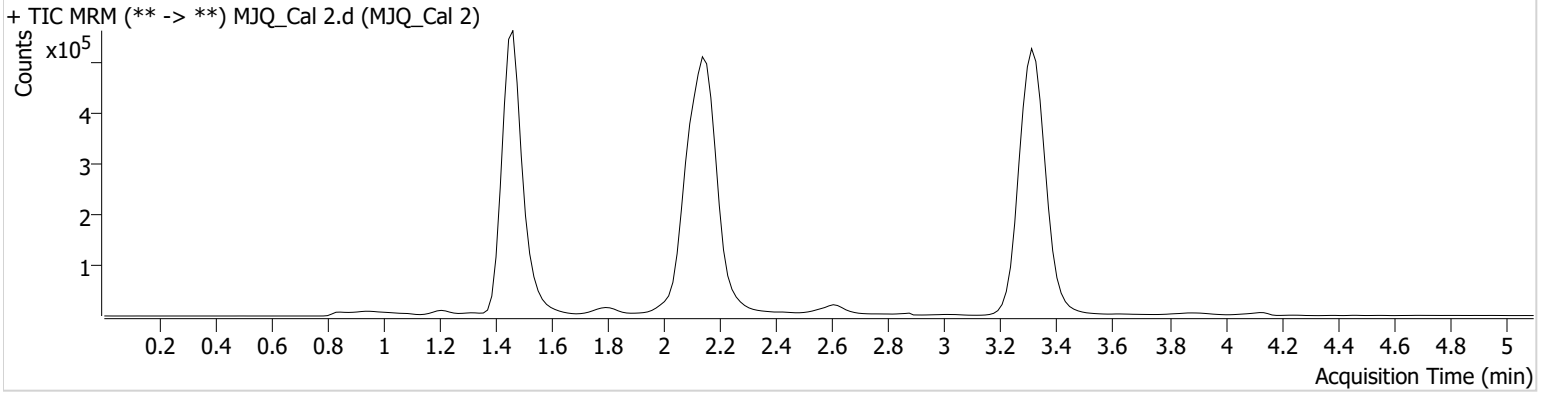


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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 5/18/2021 1:19:01 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	117133	∞	53.2	1476.44	492595	9.7376 ng/ml

TS

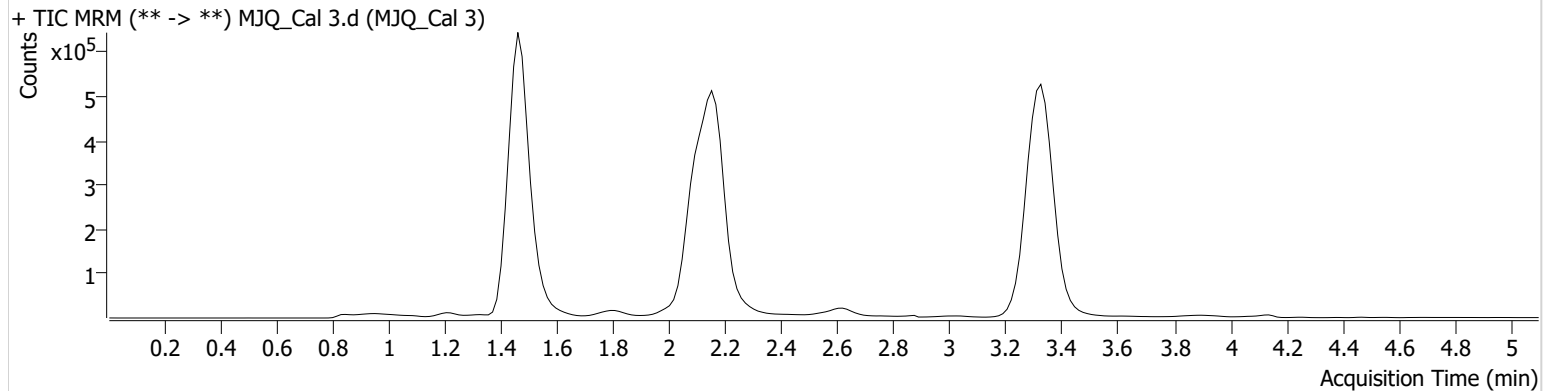


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 1:26:37 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	268829	∞	53.6	796.63	532617	20.1142 ng/ml

TS

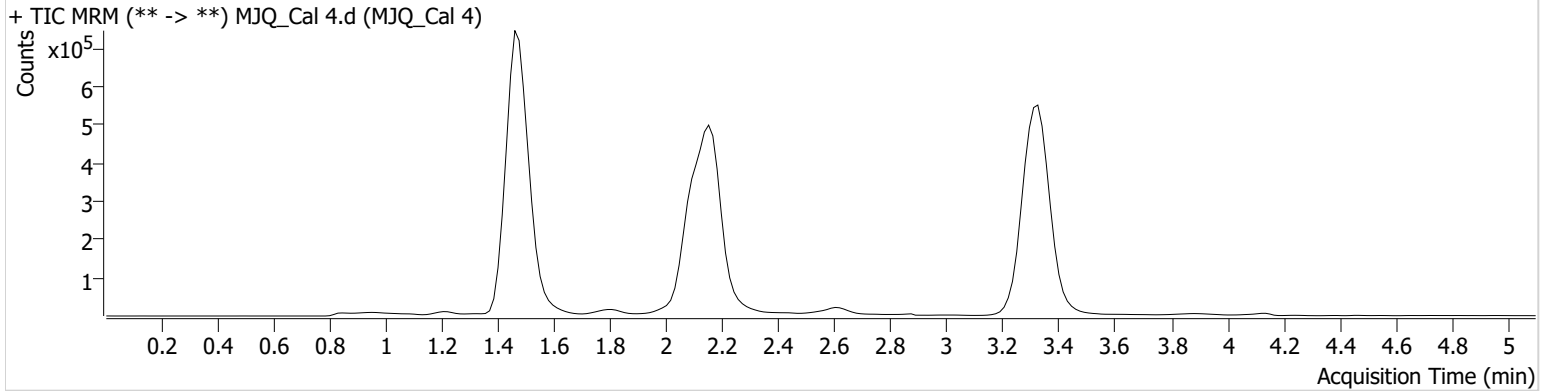


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 1:34:12 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	651097	∞	53.6	∞	515499	49.5911 ng/ml

TS

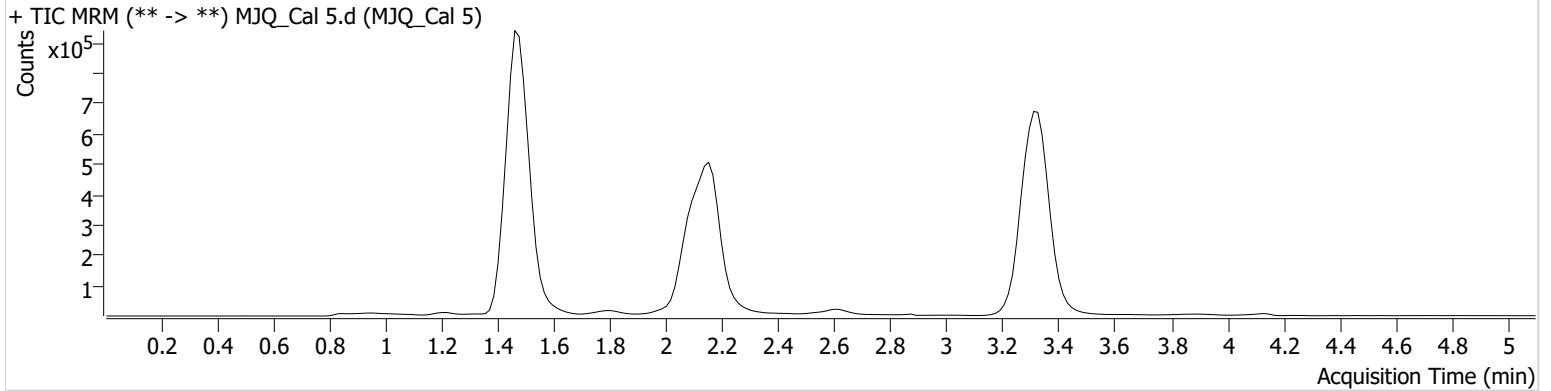


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 1:41:48 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	1001839	1911.64	54.1	3506.98	527088	74.3781 ng/ml

TS

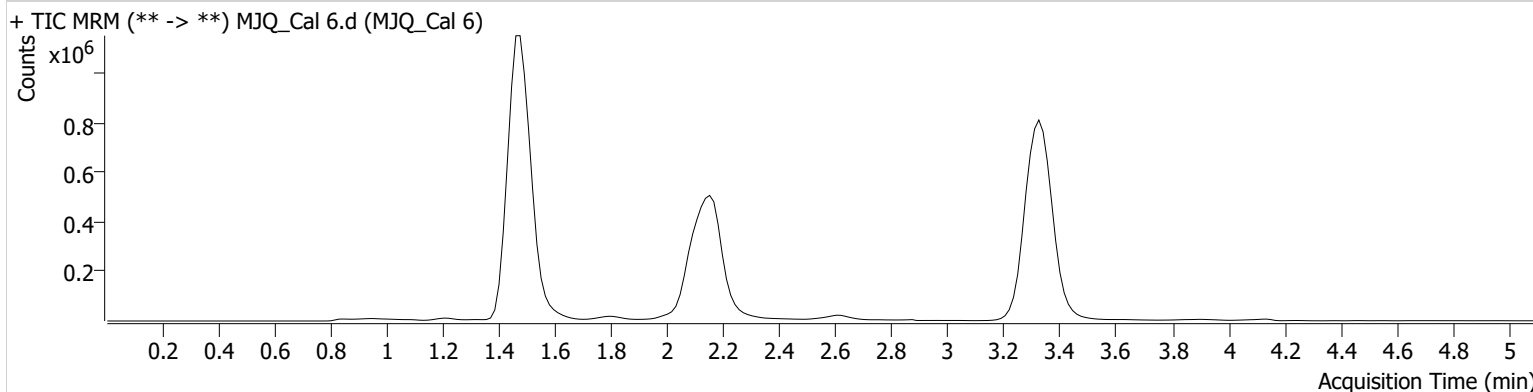


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 1:49:23 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.504	1272409	∞	55.8	∞	504398	98.5533 ng/ml

TS

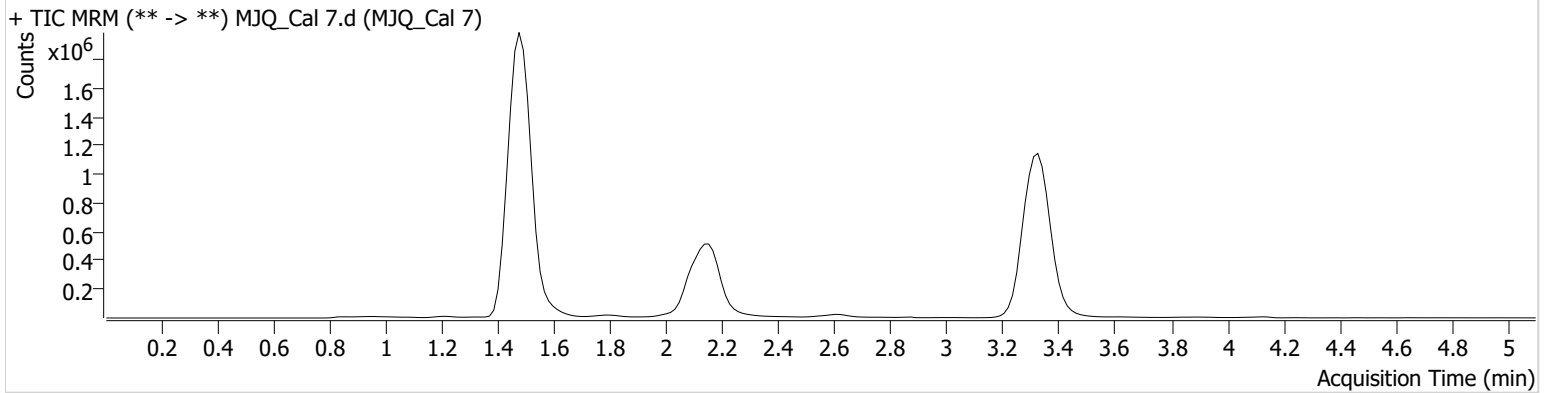


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\051821 AM 27 28 TS\QuantResults\AM 27_evaluated only.batch.bin
Calibration Last Update 5/19/2021 12:56:37 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	5/18/2021 1:56:59 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	3103171	∞	54.1	∞	478821	252.4166 ng/ml