

REVIEWED

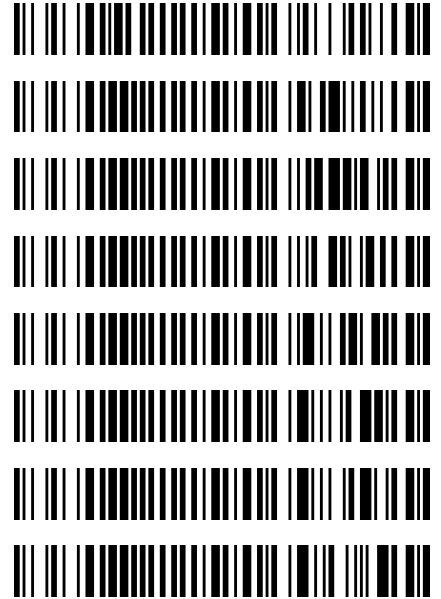
By Anne Nord at 3:39 pm, Nov 10, 2021

TS

11/4/2021

Worklist: 5348

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-4486	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3344	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3374	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3378	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3517	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3530	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3540	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3550	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: 11/09/2021

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: 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: *THC-OH not evaluated due to a possible interfering compound.*

TS

	1	2	3	4	5	6
A	IS + Cal. 1				P2021-3540-1	IS + QC_1
B	IS + Cal. 2				P2021-3530-1	IS + Cal. 7
C	IS + Cal. 3				P2021-3517-1*	IS + Cal. 6
D	IS + Cal. 4				P2021-3378-1	IS + Cal. 5
E	IS + Cal. 5				P2021-3374-1	IS + Cal. 4
F	IS + Cal. 6				P2021-3344-2	IS + Cal. 3
G	IS + Cal. 7			P2021-3517-1	M2021-4486-1	IS + Cal. 2
H	IS + QC_1			P2021-3550-1	Neg	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

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

TS

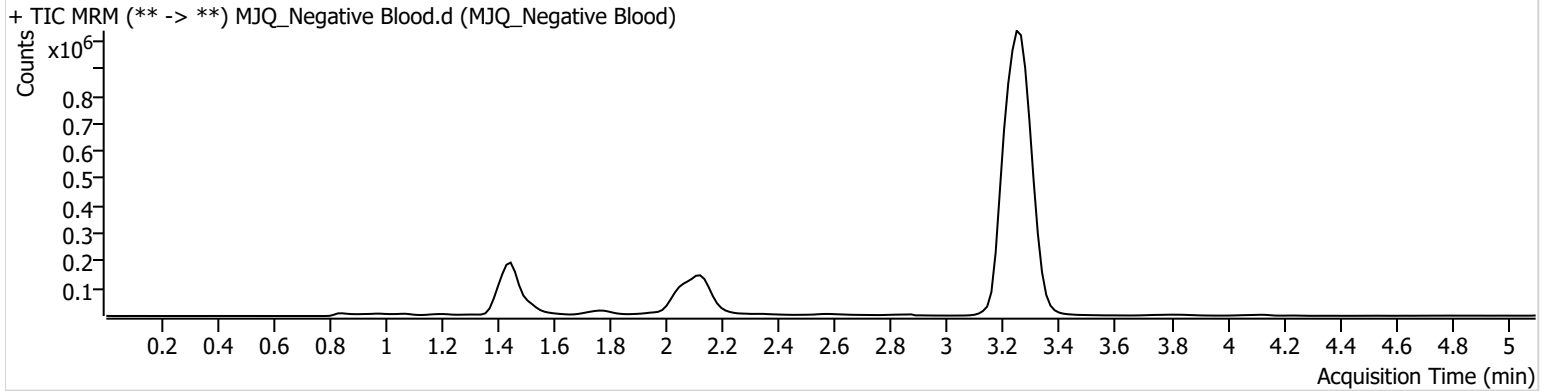


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-H5	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 1:25:38 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.513	166170	∞	3.0 Low	35.41	705948	0.4788 ng/ml Low

TS

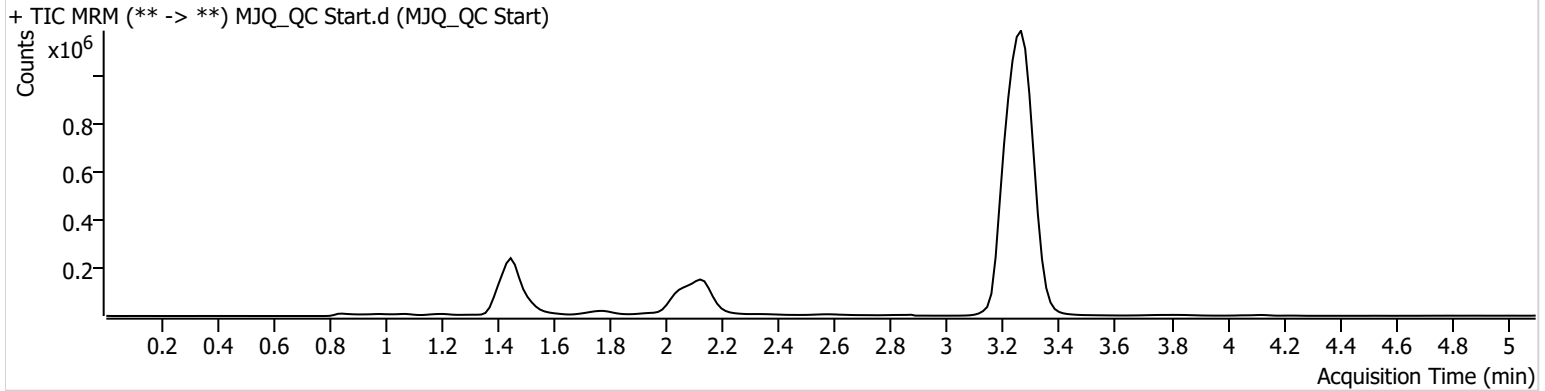


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 1:10:27 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	72763	∞	63.9	940.54	207759	14.8044 ng/ml
THC-OH	1.513	217074	∞	5.9 Low	∞	763052	3.8007 ng/ml
THC	3.285	358971	∞	25.1	277.16	8061798	4.7564 ng/ml

TS

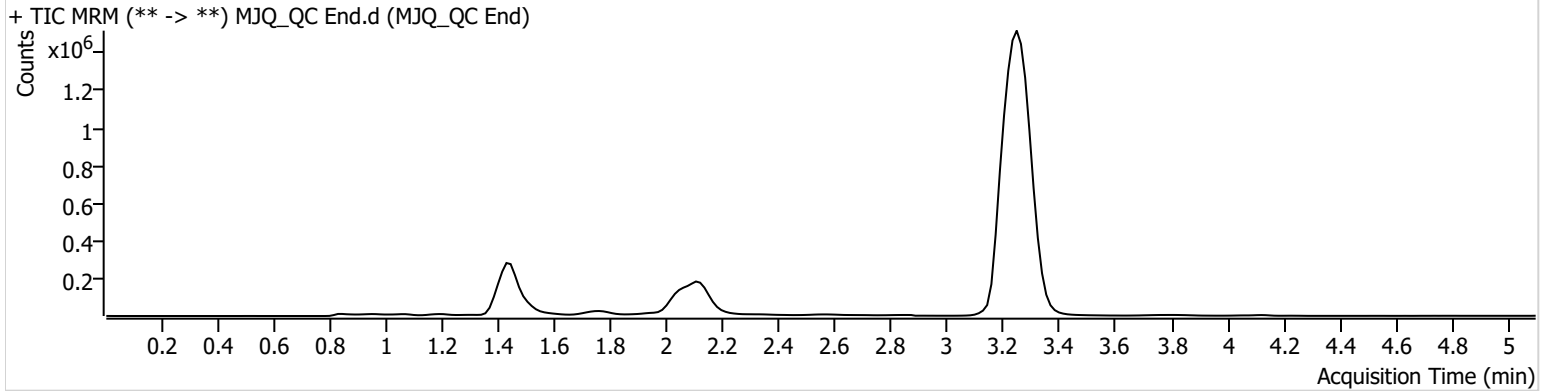


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 3:42:32 PM		

Sample Chromatogram



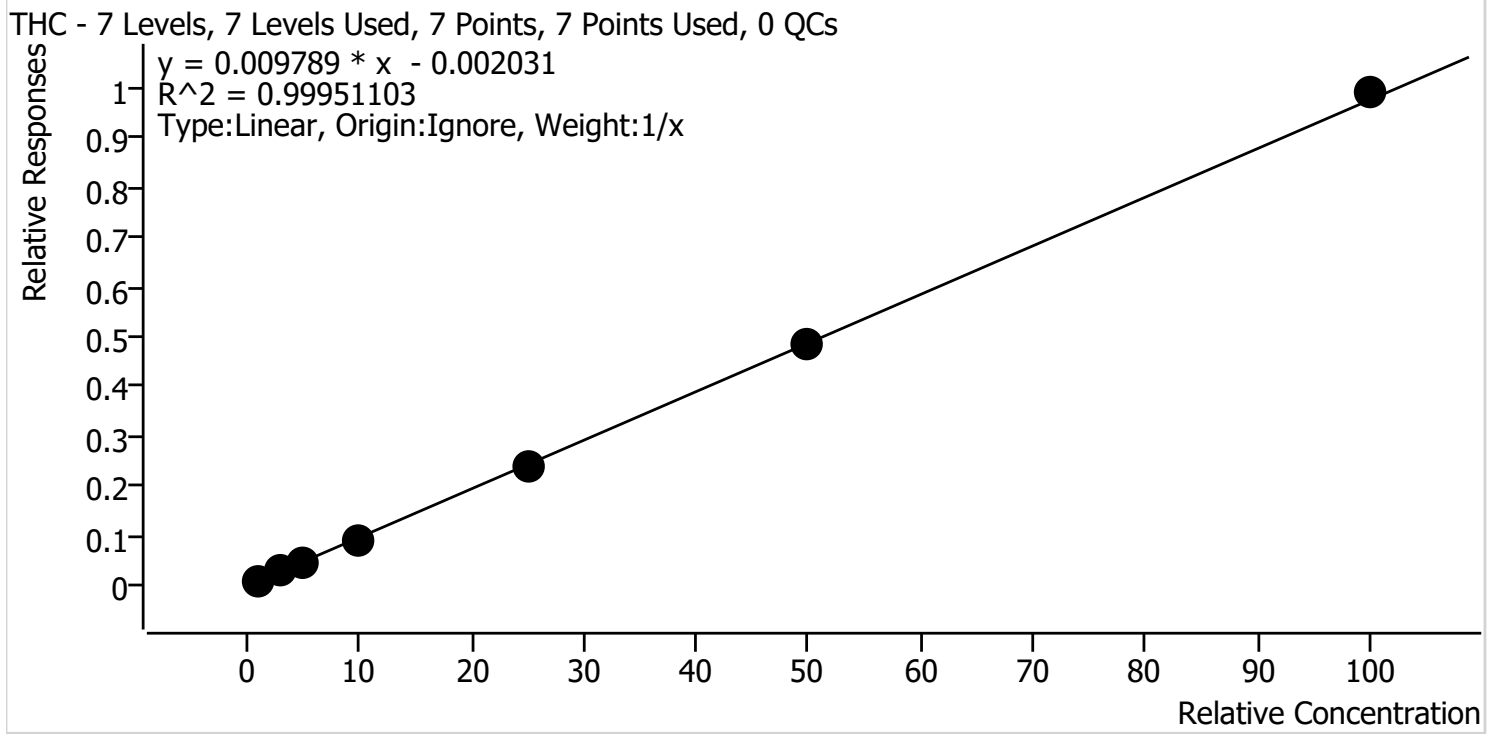
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	76058	∞	68.1	∞	228666	14.0850 ng/ml
THC-OH	1.513	251812	∞	6.0 Low	∞	891422	3.6655 ng/ml
THC	3.270	472883	∞	25.3	749.50	10265595	4.9135 ng/ml



TS

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Last Cal. Update 11/9/2021 3:47 PM
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	111.4
MJQ_Cal 2	2	✓	3.0	2.9	98.3
MJQ_Cal 3	3	✓	5.0	4.8	96.6
MJQ_Cal 4	4	✓	10.0	9.5	94.7
MJQ_Cal 5	5	✓	25.0	24.6	98.4
MJQ_Cal 6	6	✓	50.0	49.5	99.1
MJQ_Cal 7	7	✓	100.0	101.5	101.5

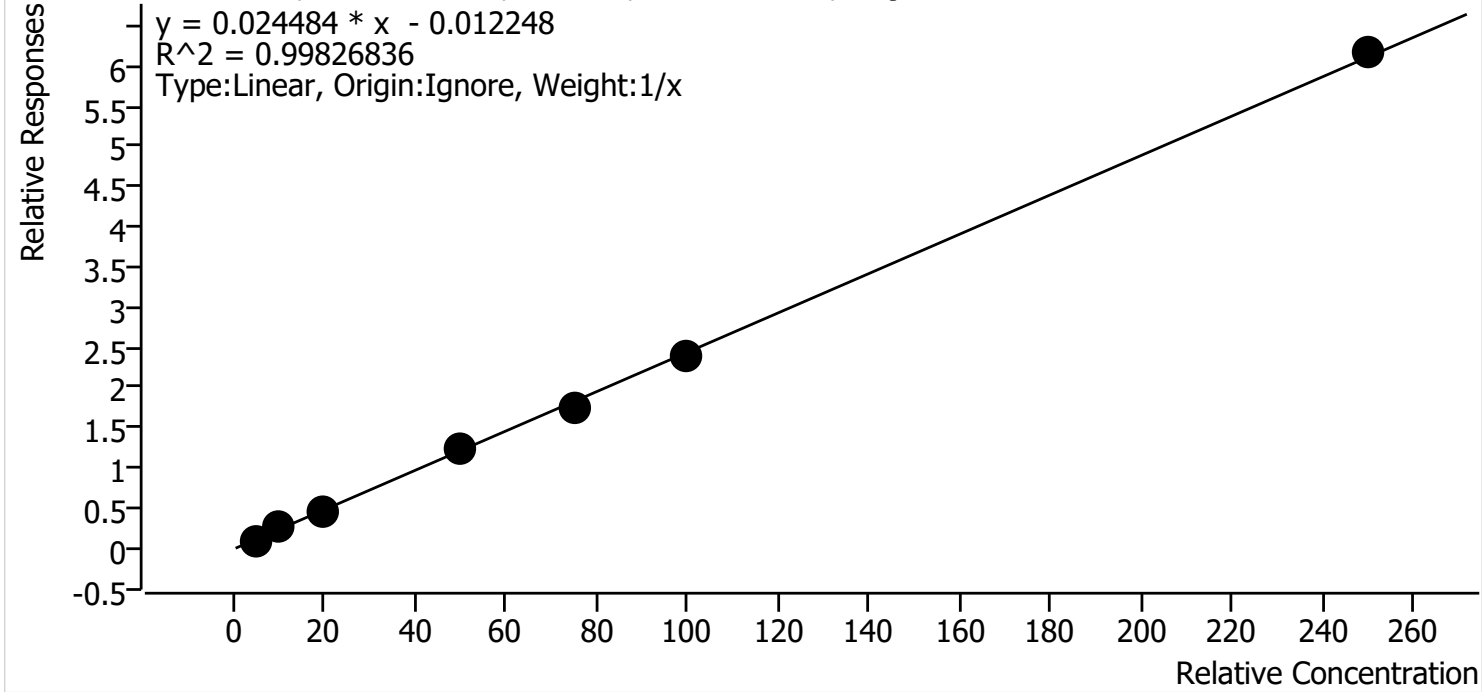
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Last Cal. Update 11/9/2021 3:47 PM
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
MJQ_Cal 1	1	✓	5.0	4.3	86.3
MJQ_Cal 2	2	✓	10.0	11.8	118.5
MJQ_Cal 3	3	✓	20.0	19.2	95.9
MJQ_Cal 4	4	✓	50.0	51.8	103.6
MJQ_Cal 5	5	✓	75.0	72.5	96.7
MJQ_Cal 6	6	✓	100.0	98.1	98.1
MJQ_Cal 7	7	✓	250.0	252.2	100.9

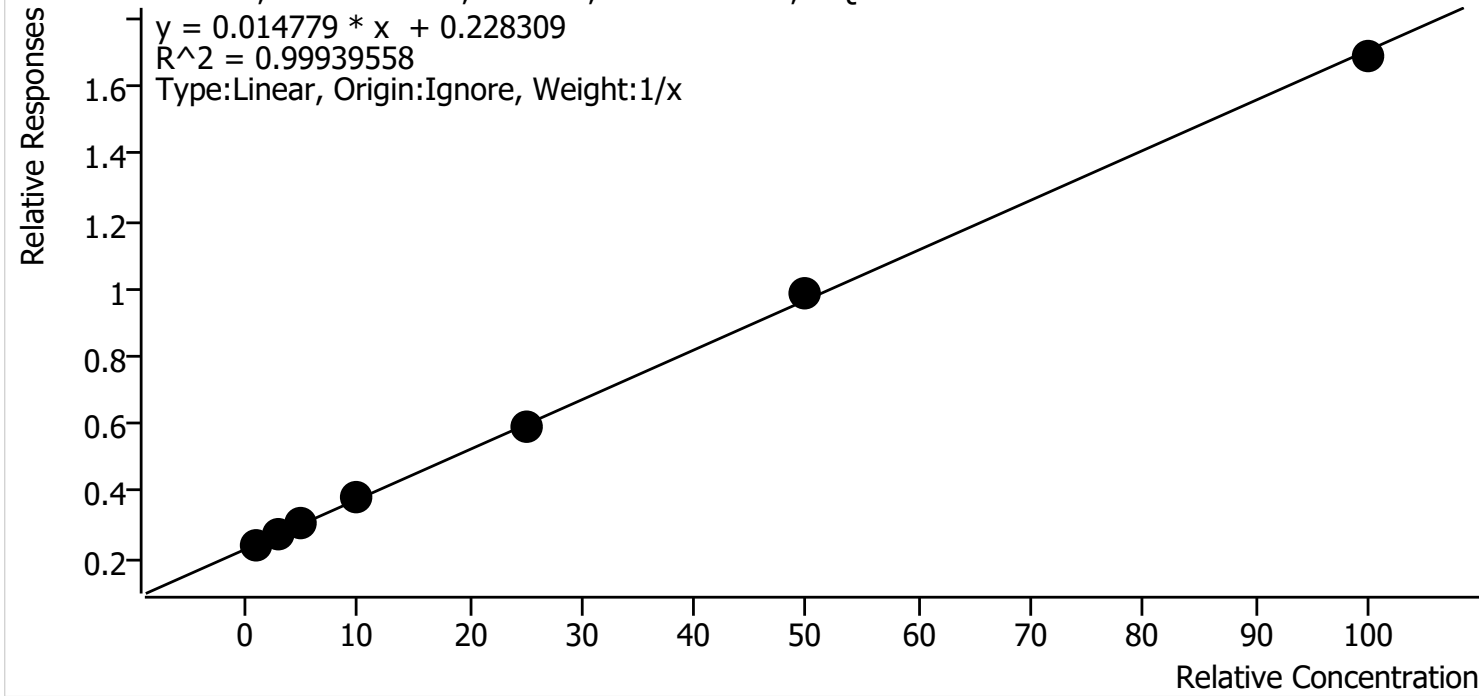
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Last Cal. Update 11/9/2021 3:47 PM
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	1.0	96.5
MJQ_Cal 2	2	✓	3.0	2.9	97.8
MJQ_Cal 3	3	✓	5.0	5.0	100.0
MJQ_Cal 4	4	✓	10.0	10.7	106.5
MJQ_Cal 5	5	✓	25.0	24.5	98.0
MJQ_Cal 6	6	✓	50.0	51.2	102.4
MJQ_Cal 7	7	✓	100.0	98.7	98.7

TS

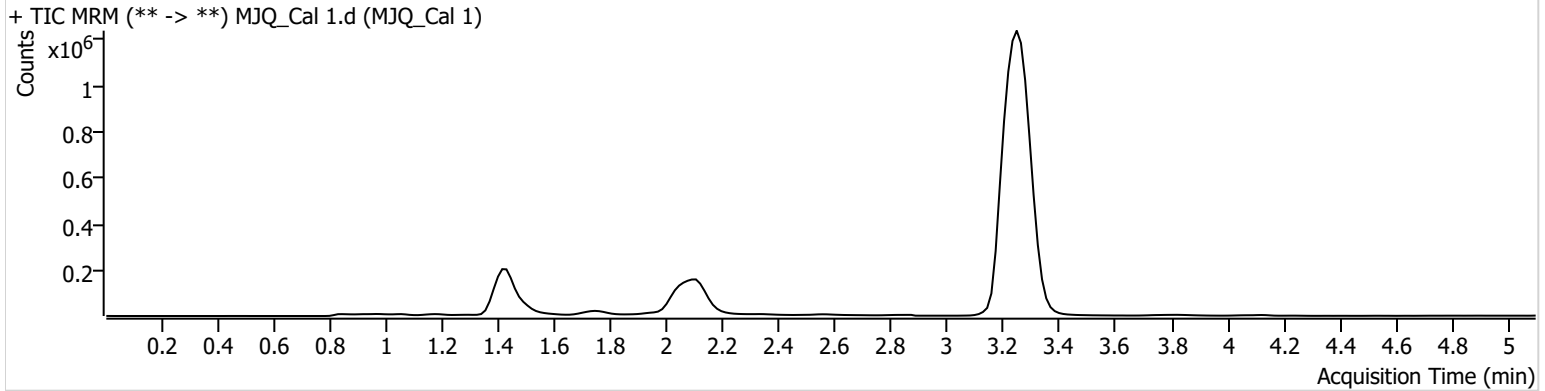


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-H6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 12:09:29 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	18370	∞	73.0	159.57	196589	4.3166 ng/ml Low
THC-OH	1.498	174358	∞	3.7 Low	∞	718779	0.9653 ng/ml Low
THC	3.270	74944	183.71	27.9	∞	8441310	1.1145 ng/ml

TS



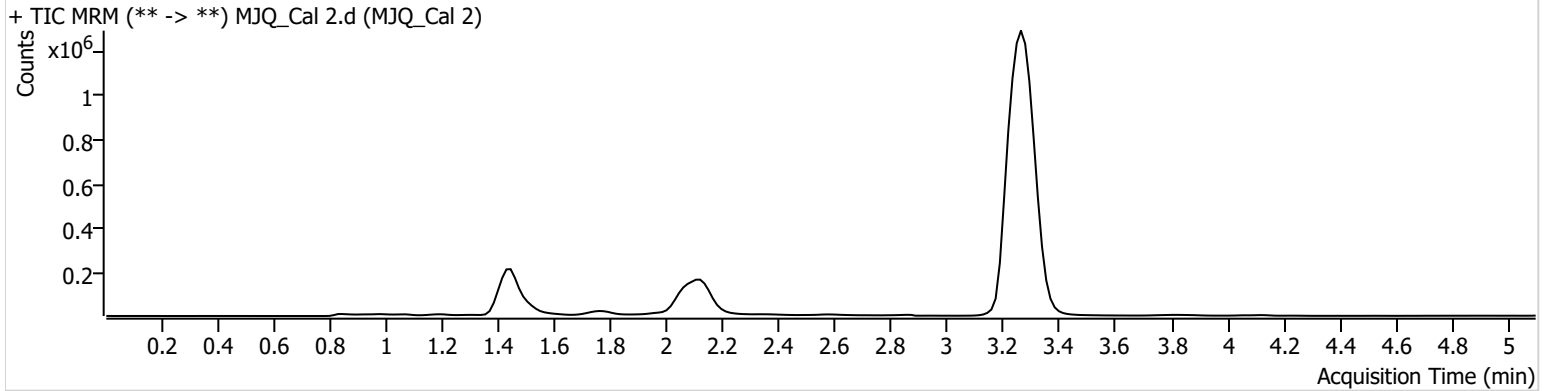
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-G6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 12:17:16 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	53018	∞	53.8	∞	190821	11.8480 ng/ml
THC-OH	1.513	189204	∞	4.8 Low	45.58	696459	2.9336 ng/ml Low
THC	3.285	225506	∞	25.3	275.94	8405899	2.9481 ng/ml

TS

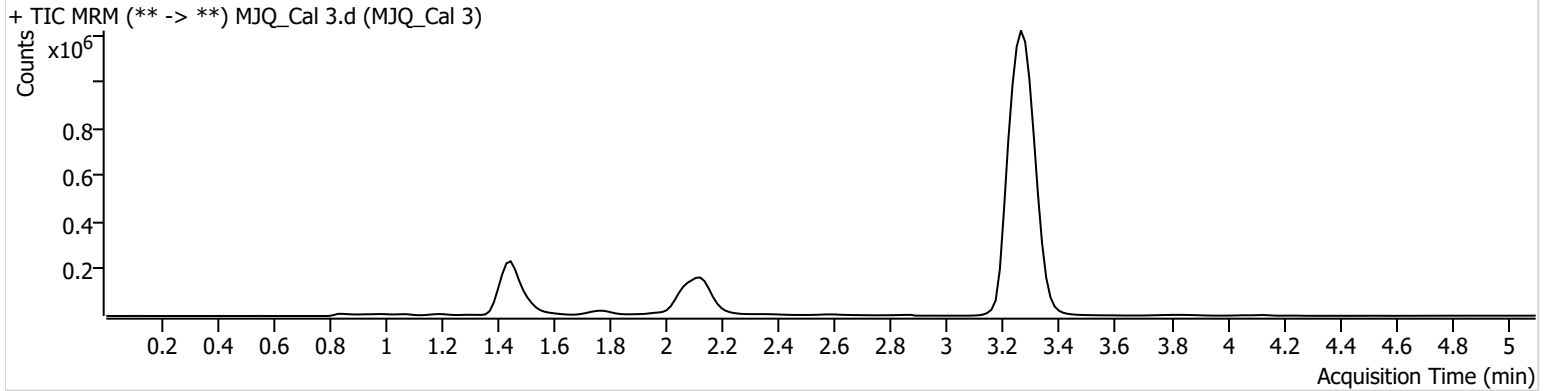


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-F6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 12:24:53 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	88151	∞	63.4	325.88	192723	19.1814 ng/ml
THC-OH	1.513	212268	∞	5.8 Low	∞	702412	4.9995 ng/ml
THC	3.285	346401	4171.04	24.9	383.66	7653486	4.8313 ng/ml

TS

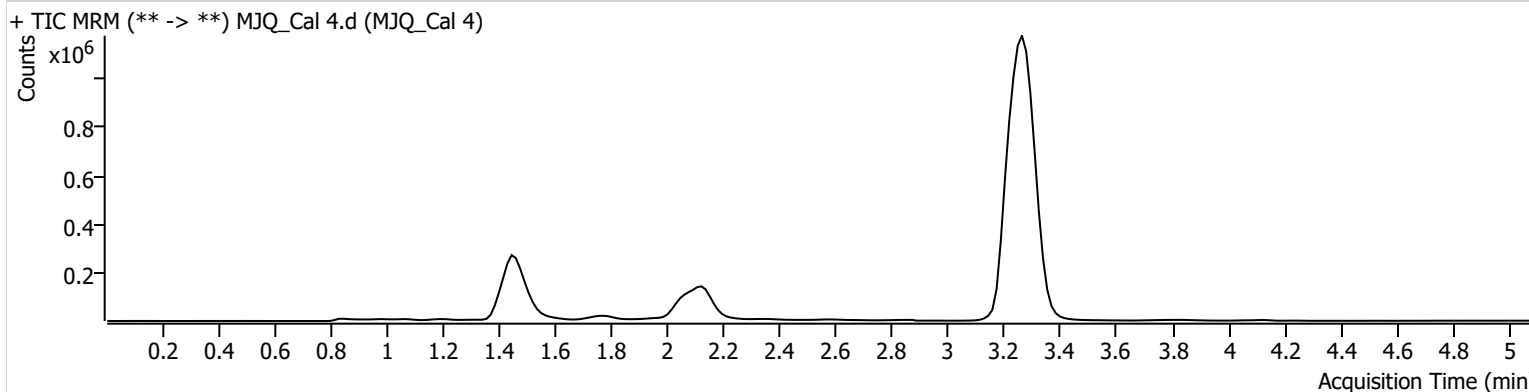


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-E6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 12:32:28 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	219436	181.31	68.0	∞	174722	51.7947 ng/ml
THC-OH	1.513	264254	∞	7.3	∞	685009	10.6539 ng/ml
THC	3.285	659532	∞	25.1	∞	7276991	9.4665 ng/ml

TS

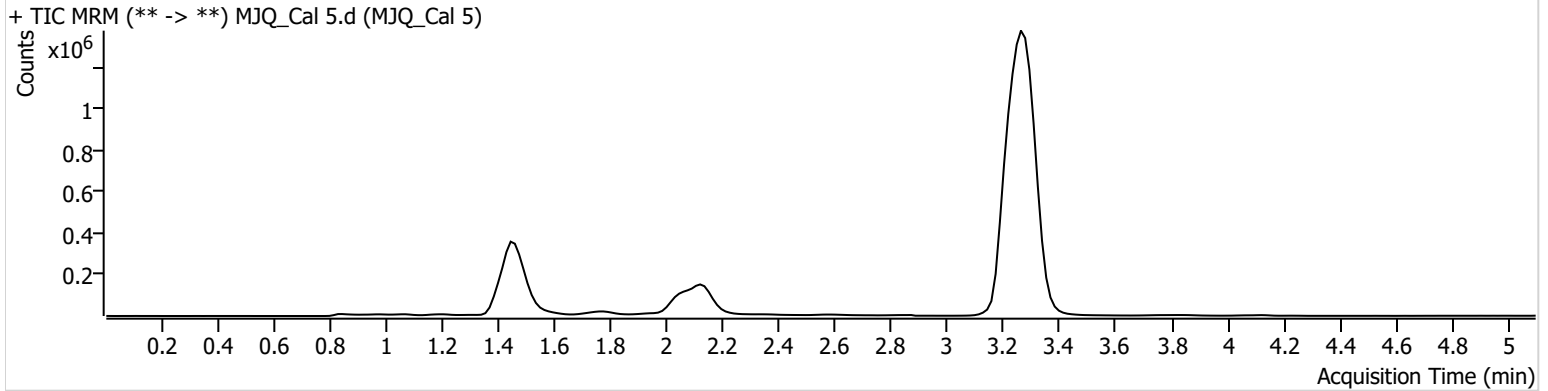


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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 P5-D6 **Comment**
Injection Volume 10
Acq. Date-Time 11/8/2021 12:40:03 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	357637	383.43	66.1	∞	202804	72.5245 ng/ml
THC-OH	1.453 Low	432266	∞	9.8 High	∞	732115	24.5021 ng/ml
THC	3.285	1848514	∞	24.4	∞	7741838	24.6002 ng/ml

TS

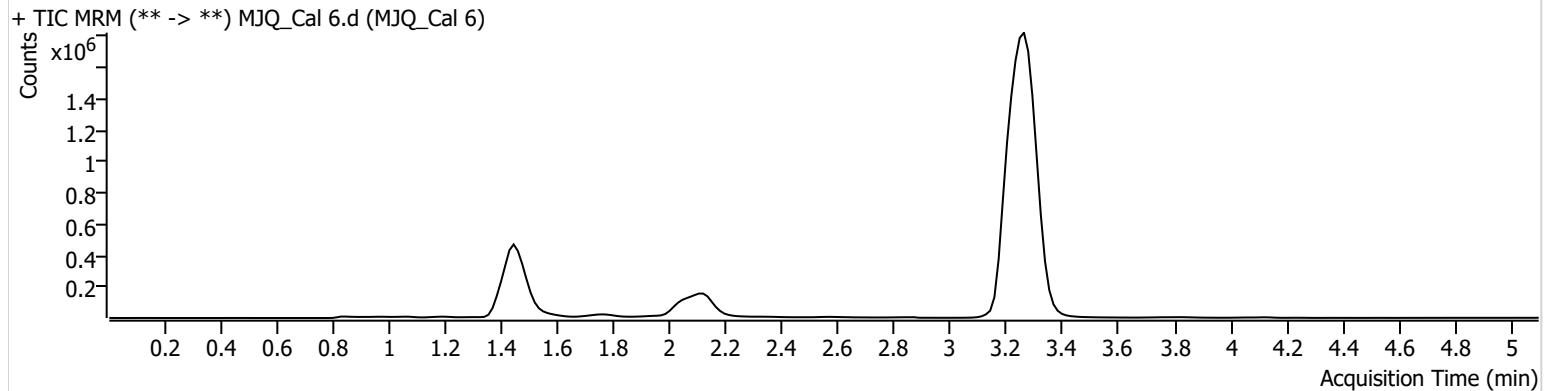


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-C6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 12:47:40 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	480464	∞	65.5	2378.74	201064	98.0975 ng/ml
THC-OH	1.453 Low	734222	∞	11.4 High	∞	745386	51.2006 ng/ml
THC	3.270	3979306	∞	24.5	∞	8239043	49.5489 ng/ml

TS

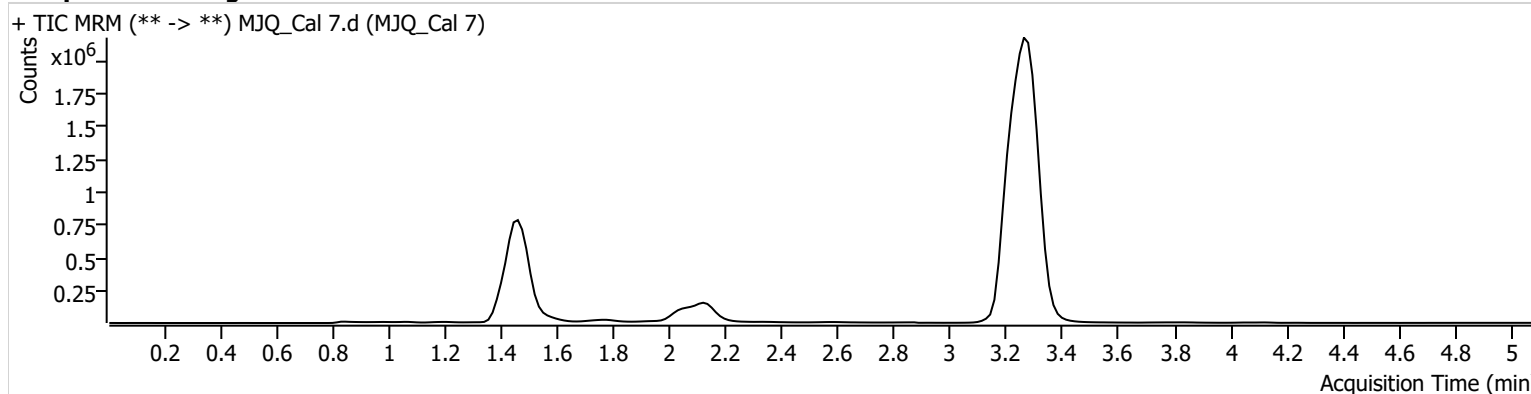


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\110821 AM 27 28 TS CS\QuantResults\AM 27_TS.batch.bin
Calibration Last Update 11/9/2021 3:47:12 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	P5-B6	Comment	
Injection Volume	10		
Acq. Date-Time	11/8/2021 12:55:15 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	1194340	∞	65.6	∞	193773	252.2374 ng/ml
THC-OH	1.453 Low	1226323	∞	13.3 High	521.70	726624	98.7450 ng/ml
THC	3.285	7287725	∞	24.9	∞	7350830	101.4905 ng/ml