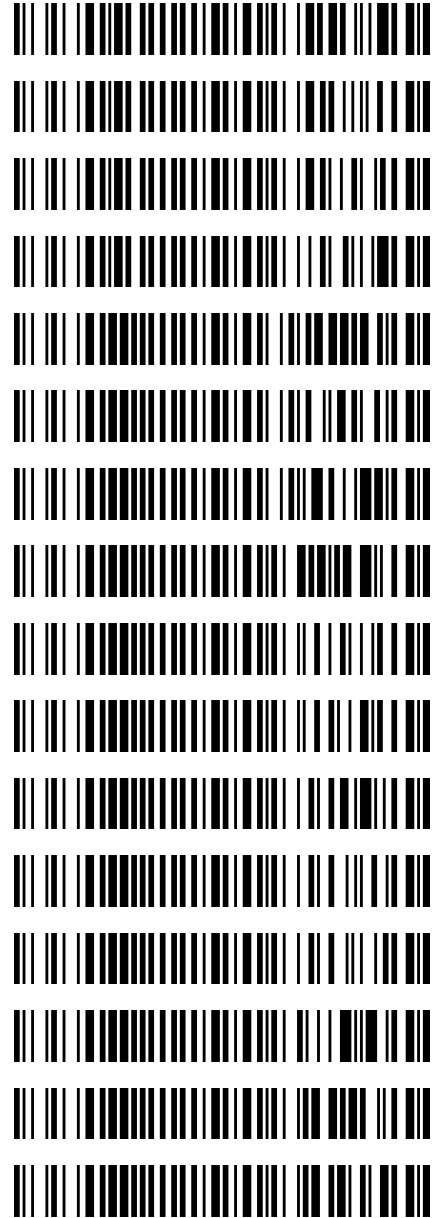


Worklist: 5173

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-3215	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-3226	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-3229	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-3325	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2205	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2282	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2358	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2526	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2546	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2547	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2551	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2562	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2563	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2573	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2587	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2593	1	BCK	AM 27 Blood THC Quant by LC-QQQ



SC

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

Extraction Date: 08/10/21

Analyst: Sarah Collins

Plate lot#: IDP-108-2-210609

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 20L20724

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. 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:** 3382167
- 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: 800 uL
- 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 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: Did not evaluate THC-OH due to interfering peak.

SC

	1	2	3	4	5	6
A				p2021-2587-1	p2021-2358-1	IS + QC_1
B				p2021-2573-1	p2021-2282-1	IS + Cal. 7
C				p2021-2563-1	p2021-2205-1	IS + Cal. 6
D				p2021-2562-1	m2021-3325-3	IS + Cal. 5
E				p2021-2551-1*	m2021-3229-1*	IS + Cal. 4
F			p2021-2551-1	p2021-2547-1	m2021-3226-1	IS + Cal. 3
G			m2021-3229-1	p2021-2546-1	m2021-3215-1	IS + Cal. 2
H			p2021-2593-1	p2021-2526-1	negative blood	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Samples moved during analytical step 6 due to blood clot

SC

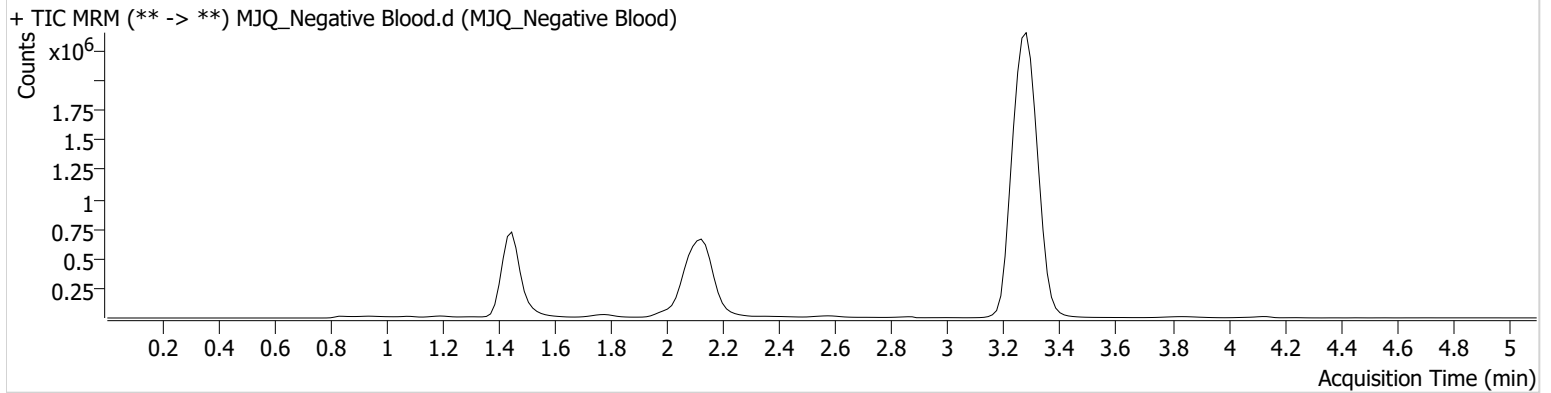


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Negative Blood.d
Type	Sample	Sample	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 5:57:47 PM		
Sample Info.			

Sample Chromatogram



SC

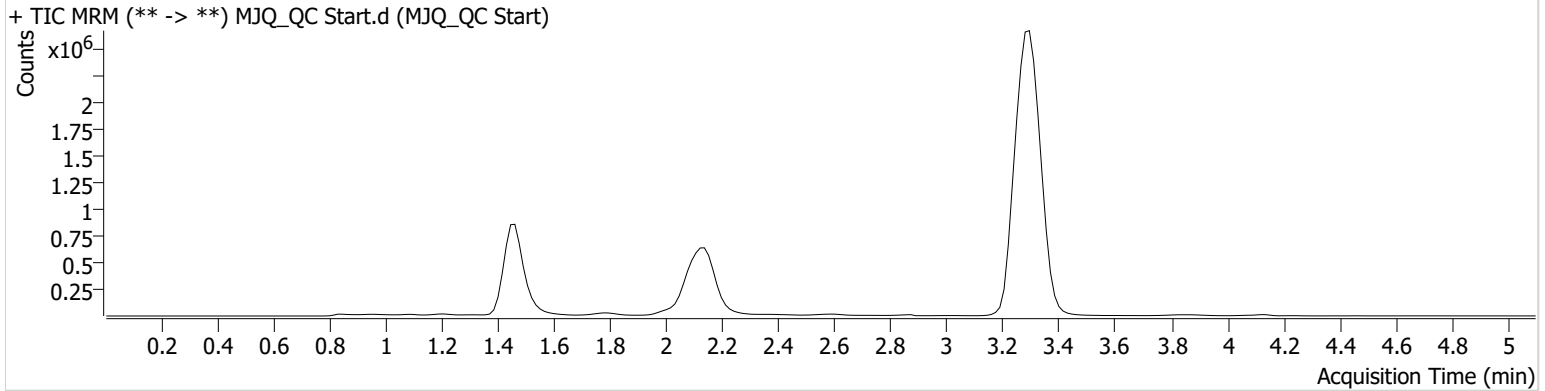


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_QC Start.d
Type	Sample	Sample	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 5:42:31 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	342227	∞	7.0	∞	2733504	4.3994 ng/ml
THC-COOH	1.489	277328	∞	51.3	683.43	738936	14.5327 ng/ml
THC	3.300	726370	935.41	25.7	1049.95	16279265	4.7156 ng/ml

SC

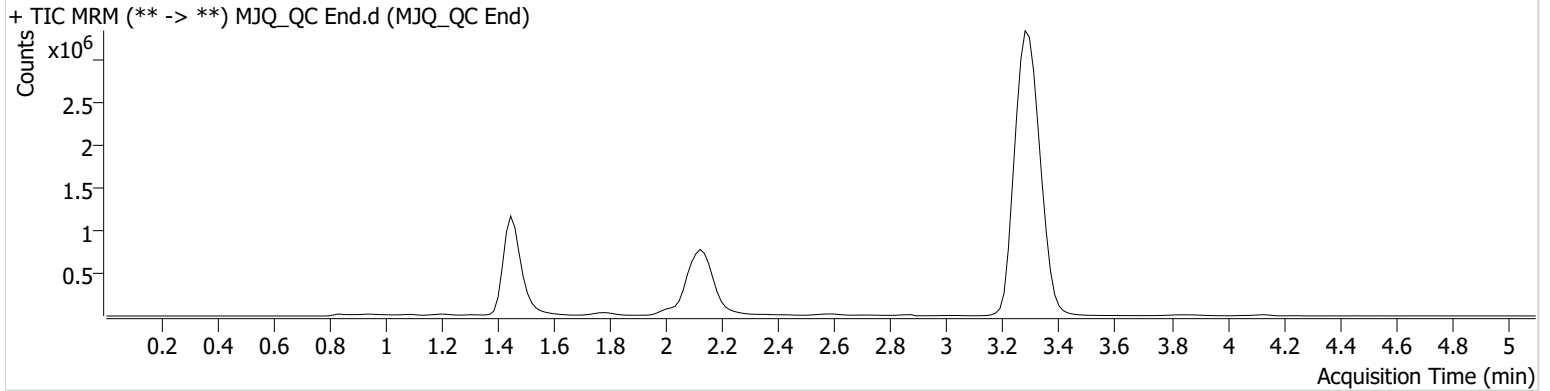


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_QC End.d
Type	Sample	Sample	MJQ_QC End
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 10:16:37 PM		
Sample Info.			

Sample Chromatogram



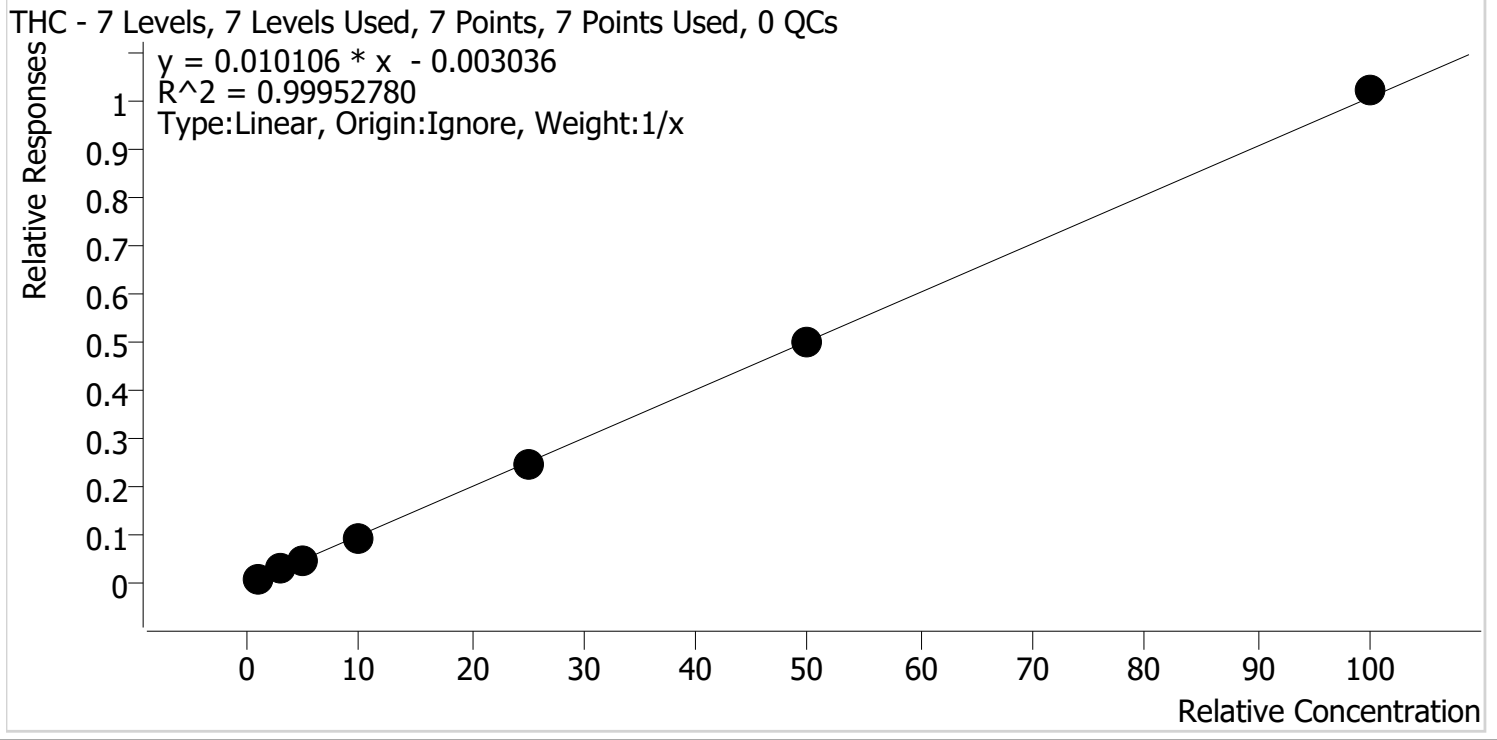
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	409604	∞	7.1	122.91	3410739	4.0411 ng/ml
THC-COOH	1.474	303572	309.70	54.3	∞	855493	13.6741 ng/ml
THC	3.300	936869	1160.24	26.9	∞	20012643	4.9327 ng/ml

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 8/11/2021 12:48 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



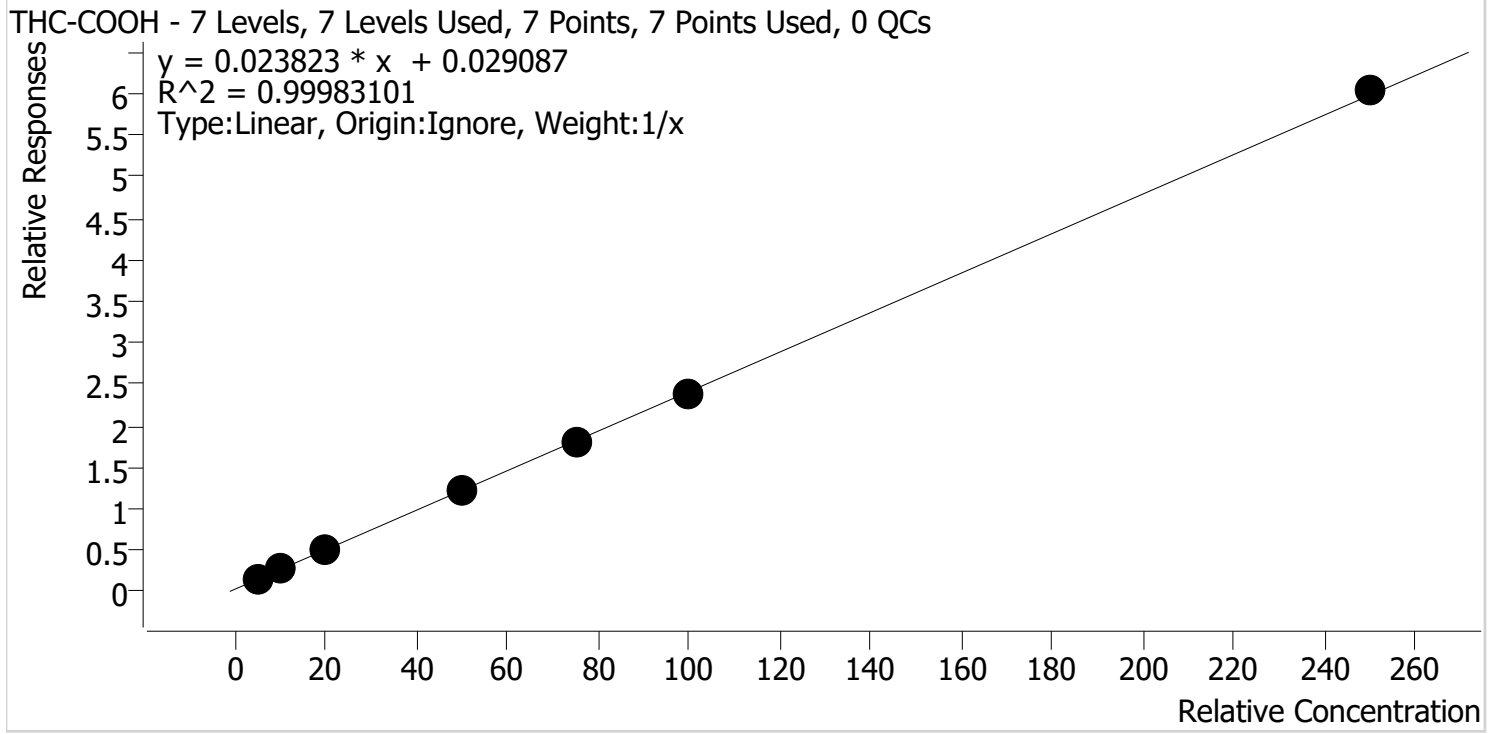
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	111.8
MJQ_Cal 2	2	✓	3.0	2.9	98.0
MJQ_Cal 3	3	✓	5.0	4.8	96.0
MJQ_Cal 4	4	✓	10.0	9.6	95.9
MJQ_Cal 5	5	✓	25.0	24.3	97.4
MJQ_Cal 6	6	✓	50.0	49.8	99.6
MJQ_Cal 7	7	✓	100.0	101.4	101.4

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 8/11/2021 12:48 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	104.7
MJQ_Cal 2	2	✓	10.0	10.0	99.8
MJQ_Cal 3	3	✓	20.0	19.5	97.6
MJQ_Cal 4	4	✓	50.0	49.3	98.5
MJQ_Cal 5	5	✓	75.0	74.3	99.1
MJQ_Cal 6	6	✓	100.0	99.4	99.4
MJQ_Cal 7	7	✓	250.0	252.3	100.9

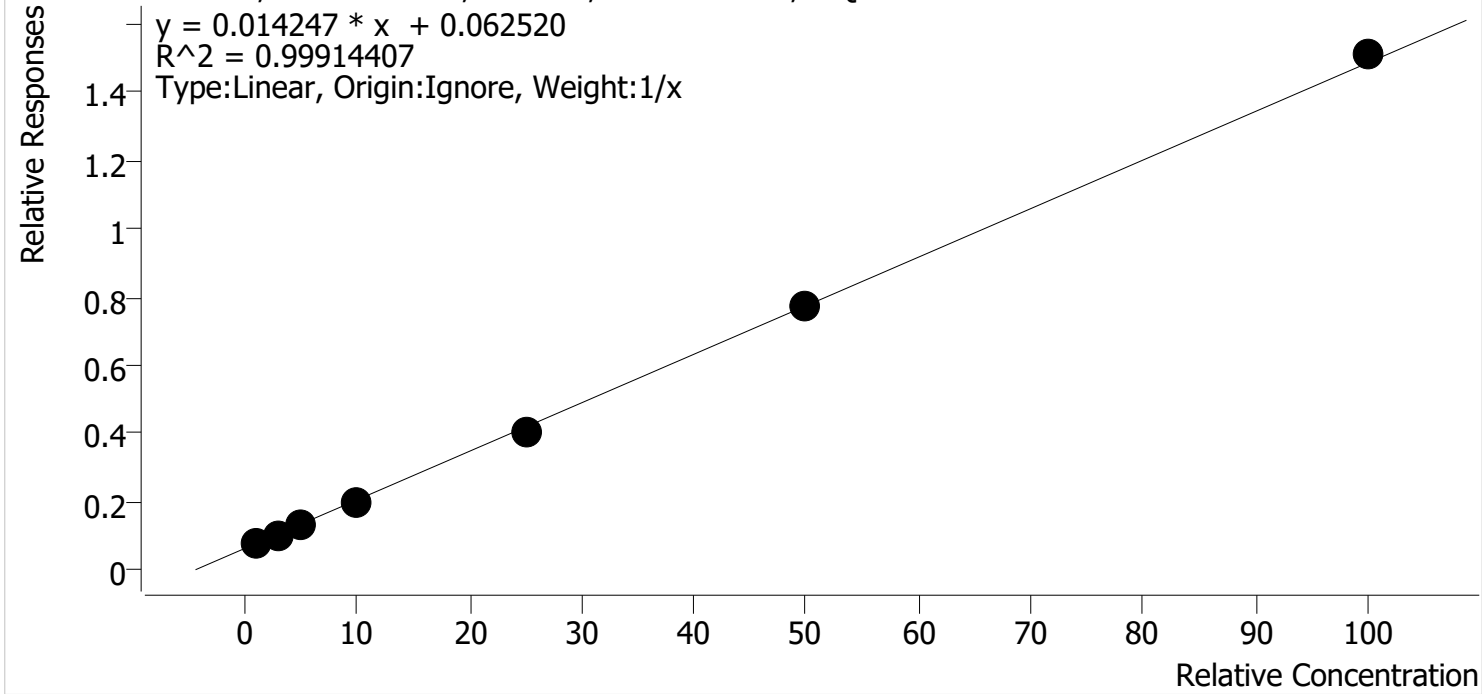
SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 8/11/2021 12:48 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	1.2	117.8
MJQ_Cal 2	2	✓	3.0	2.9	96.0
MJQ_Cal 3	3	✓	5.0	4.6	92.7
MJQ_Cal 4	4	✓	10.0	9.5	95.2
MJQ_Cal 5	5	✓	25.0	24.2	96.7
MJQ_Cal 6	6	✓	50.0	49.9	99.9
MJQ_Cal 7	7	✓	100.0	101.7	101.7

Did not evaluate due to interfering peak

SC

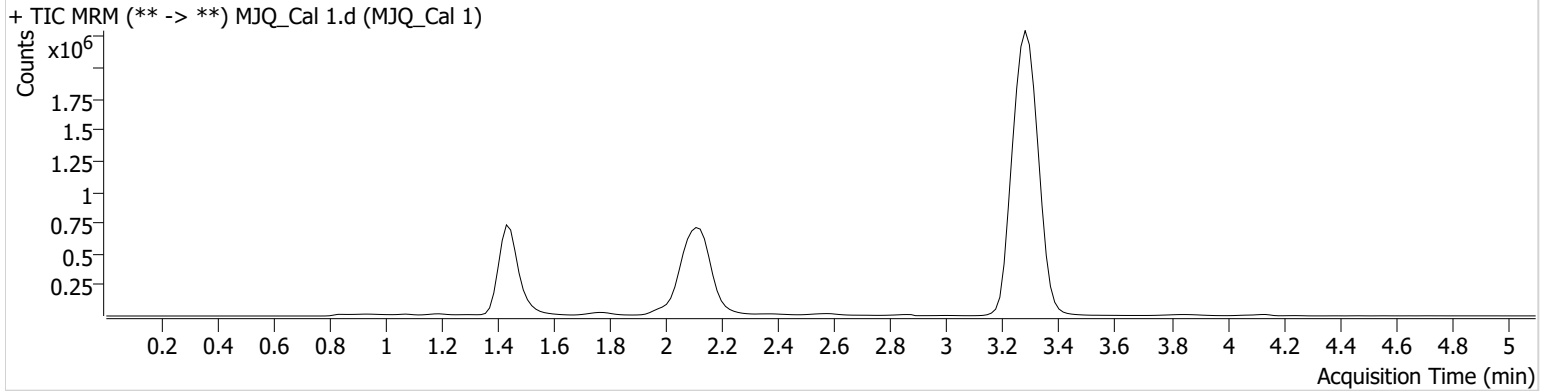


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 1.d
Type	Cal	Sample	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 4:41:35 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.498 High	203639	∞	4.1 Low	∞	2567713	1.1783 ng/ml Low
THC-COOH	1.474	106222	∞	43.2	243.30	690712	5.2343 ng/ml
THC	3.300	121465	233.21	27.9	∞	14708525	1.1176 ng/ml

SC

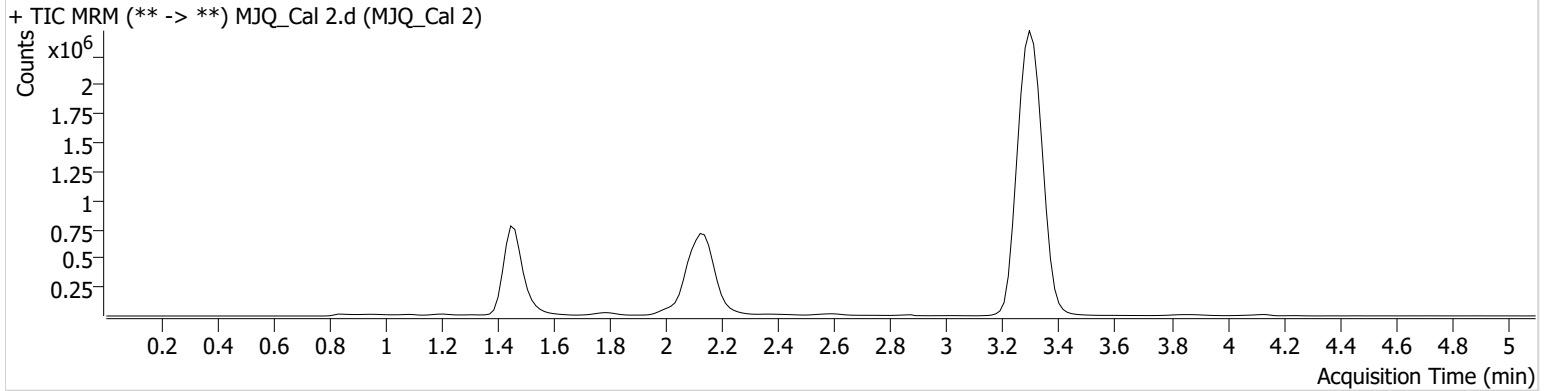


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 2.d
Type	Cal	Sample	MJQ_Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 4:49:21 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.513 High	261395	31.39	5.6 Low	95.09	2524015	2.8808 ng/ml Low
THC-COOH	1.489	181751	∞	46.0	∞	680813	9.9849 ng/ml
THC	3.315	397024	∞	28.0	742.61	14883645	2.9400 ng/ml

SC

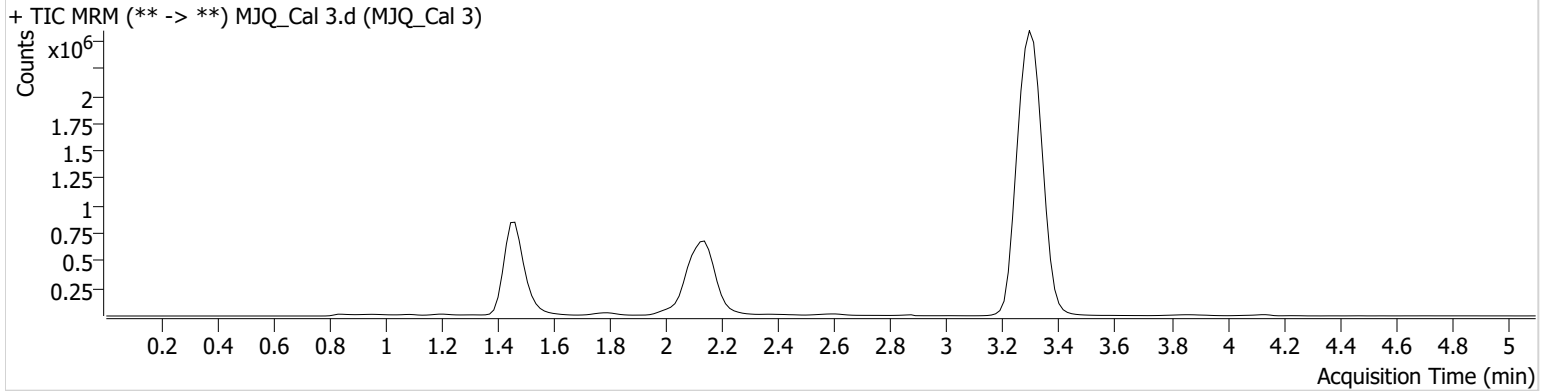


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 3.d
Type	Cal	Sample	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 4:56:57 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	342139	∞	6.8	∞	2661085	4.6362 ng/ml
THC-COOH	1.489	351215	∞	51.7	407.43	711130	19.5100 ng/ml
THC	3.315	701834	3647.36	26.9	2287.42	15436949	4.7992 ng/ml

SC

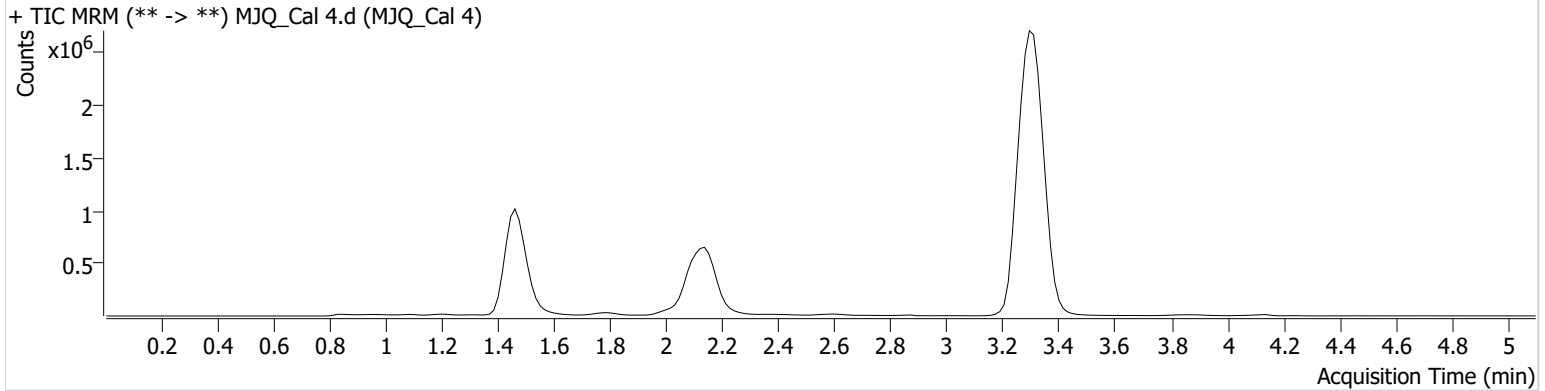


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 4.d
Type	Cal	Sample	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 5:04:33 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	523011	∞	8.4	∞	2640360	9.5153 ng/ml
THC-COOH	1.489	844835	∞	53.6	∞	702505	49.2589 ng/ml
THC	3.315	1449394	12340.35	25.3	1768.73	15444440	9.5866 ng/ml

SC



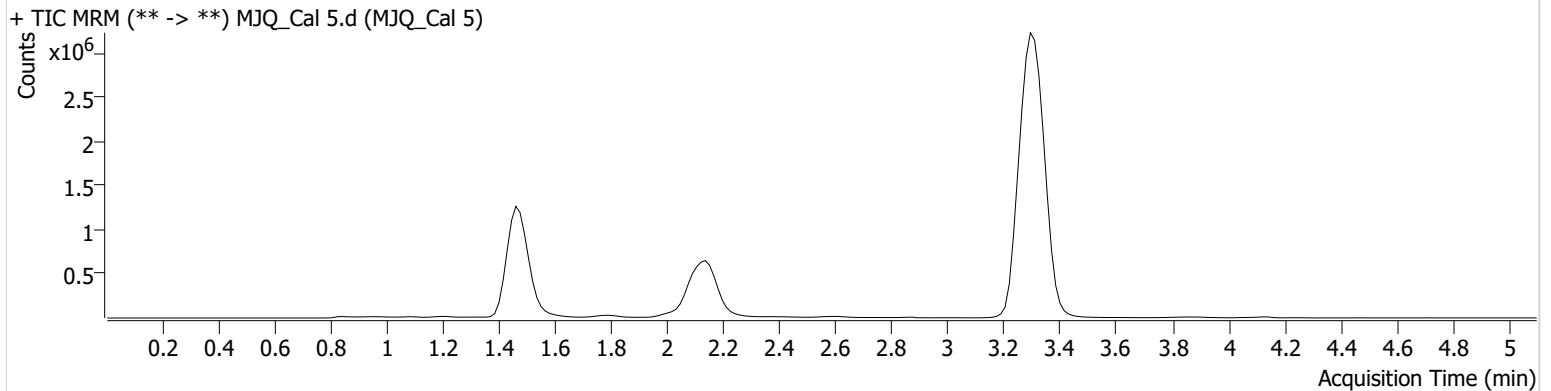
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 5.d
Type	Cal	Sample	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 5:12:09 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	1073808	∞	10.1 High	∞	2638361	24.1794 ng/ml
THC-COOH	1.489	1250769	∞	56.3	∞	695036	74.3170 ng/ml
THC	3.315	3791306	21615.07	25.4	5589.48	15604858	24.3412 ng/ml

SC

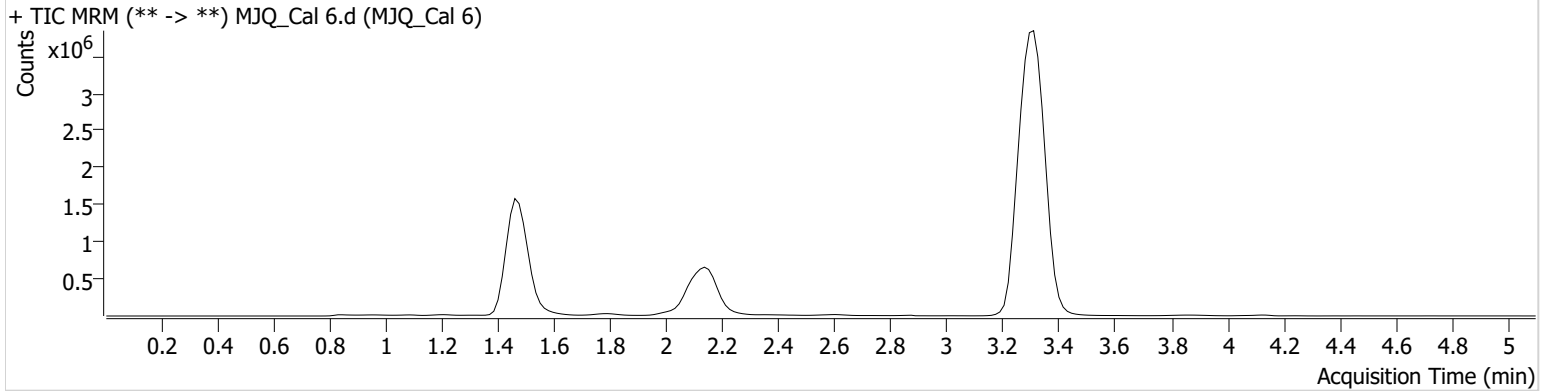


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 6.d
Type	Cal	Sample	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 5:19:44 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	2046678	∞	10.7 High	∞	2644500	49.9354 ng/ml
THC-COOH	1.489	1675492	∞	57.0	∞	699065	99.3842 ng/ml
THC	3.315	7727627	∞	25.8	∞	15444934	49.8089 ng/ml

SC

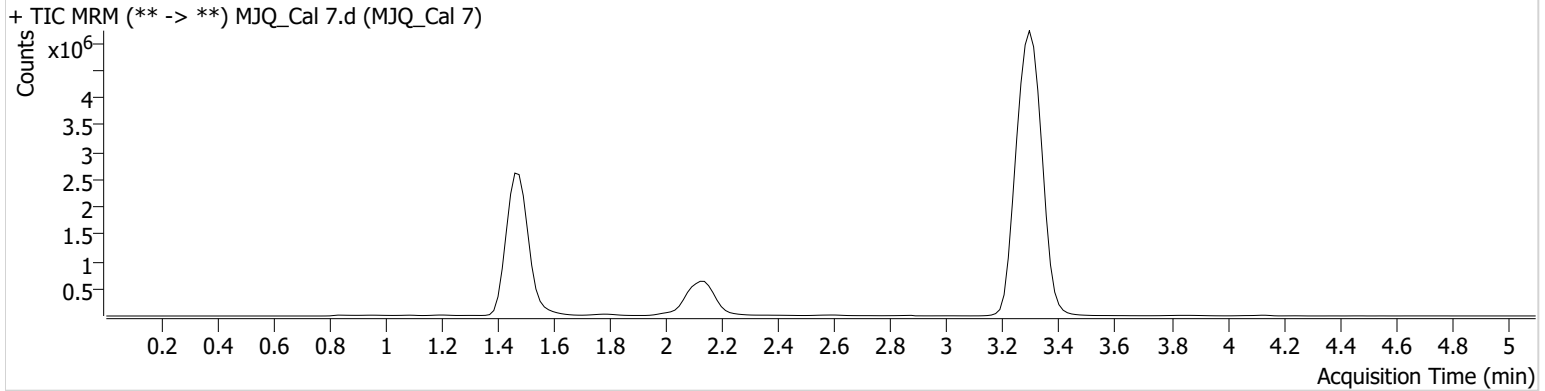


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\081021 AM 27 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/11/2021 12:48:08 PM

Instrument	Falco (069901)	Data File	MJQ_Cal 7.d
Type	Cal	Sample	MJQ_Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-B6	Comment	
Injection Volume	10		
Acq. Date-Time	8/10/2021 5:27:20 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	3909318	∞	11.2 High	∞	2587148	101.6745 ng/ml
THC-COOH	1.489	3831797	∞	55.9	6667.05	634404	252.3107 ng/ml
THC	3.300	14853438	∞	26.1	∞	14536816	101.4065 ng/ml