

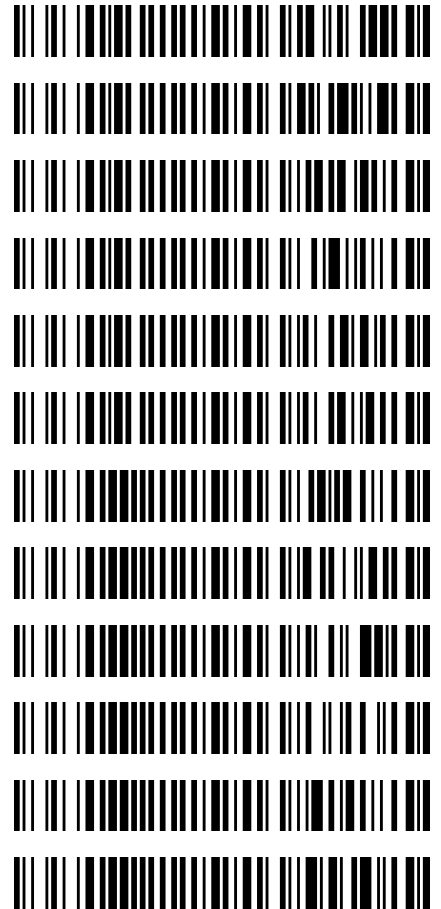
TS

4/4/2022

Worklist: 5748

REVIEWED
By Primary Worklist 1:34 pm, Apr 05, 2022

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-0899	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-0948	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-0961	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-0972	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-0990	3	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-0991	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0547	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0565	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0682	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0687	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0794	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0834	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: 03/31/2022

Analyst: Tamara Salazar

Plate lot#: 211018

Plate Retest Date: 04/18/2022

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 22B52016-2

Blank Urine Lot: N/A

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: 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: 800µL
- 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 3-100--Cal 1 dropped due to poor peak shape

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	M2022-0991-3	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	P2022-0834-1	M2022-0990-3	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	P2022-0834-1*	M2022-0972-2	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	P2022-0794-1	M2022-0961-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	P2022-0687-1	M2022-0948-1	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	P2022-0682-1	M2022-0899-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2022-0565-2	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2022-0547-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Sample moved during step 6 of the extraction procedure due to a blood clot.

TS

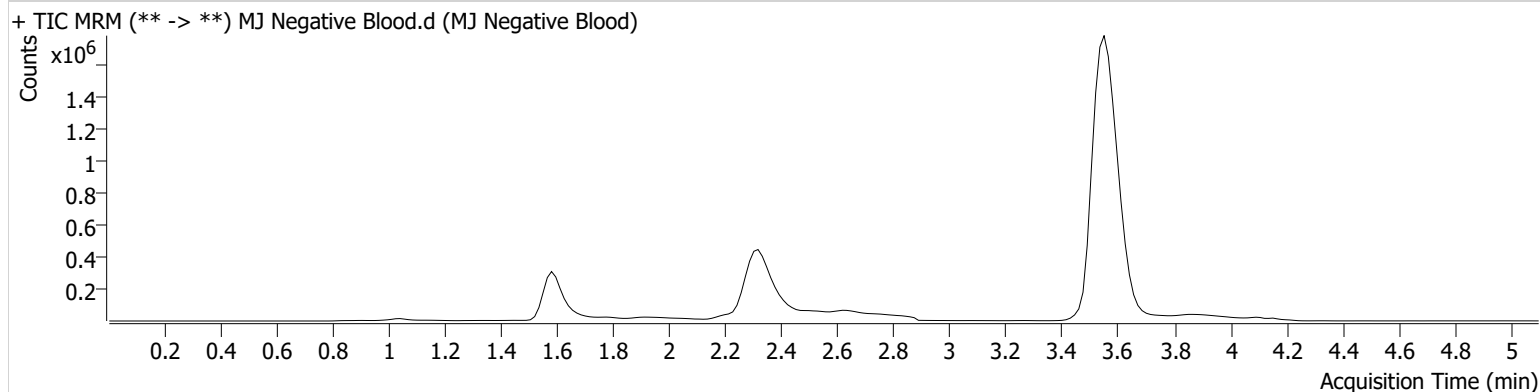


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-G5	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 5:14:21 PM		
Sample Info.			

Sample Chromatogram



TS

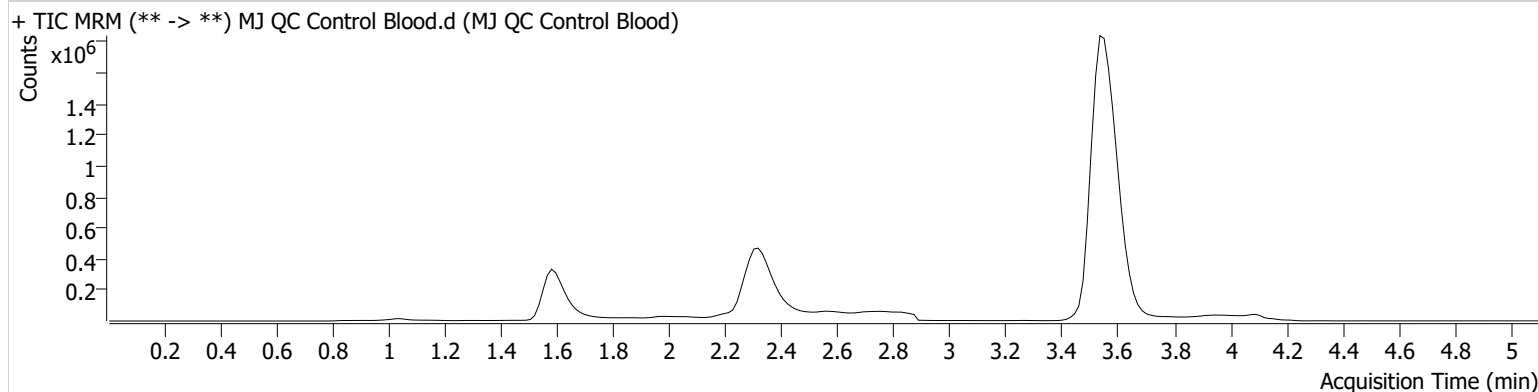


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:59:08 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	92460	71.78	13.6	∞	1213746	5.2725 ng/ml
THC-COOH	1.625	123568	519.61	59.7	535.16	350974	15.3360 ng/ml
THC	3.555	503112	∞	25.9	∞	11552566	4.7248 ng/ml

TS

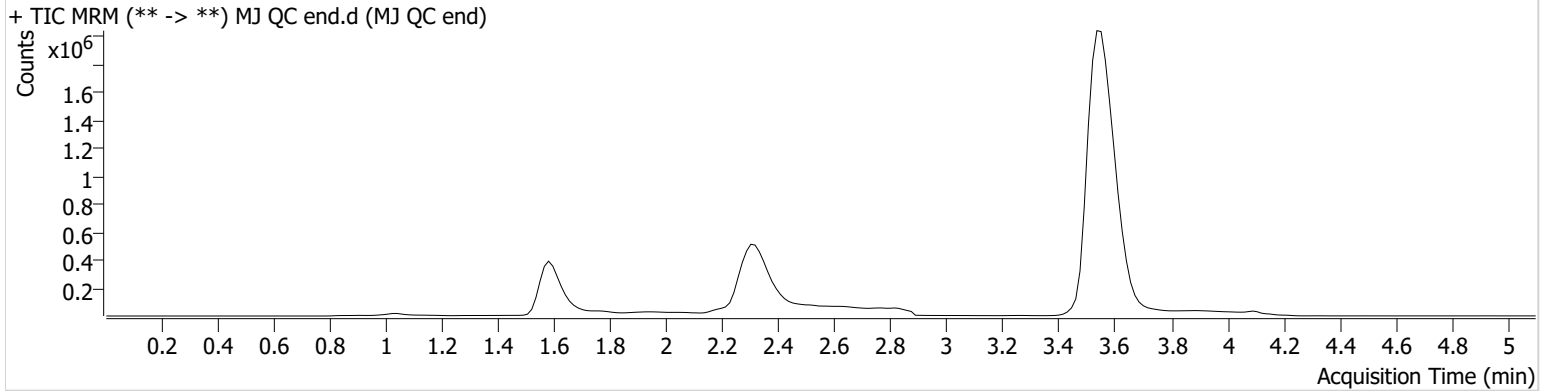


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ QC end.d
Type	QC	Sample	MJ QC end
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 8:32:15 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	110644	192.52	13.0	152.77	1417793	5.4028 ng/ml
THC-COOH	1.625	142146	∞	59.9	497.10	396391	15.6273 ng/ml
THC	3.555	597141	∞	25.2	∞	13459871	4.8093 ng/ml

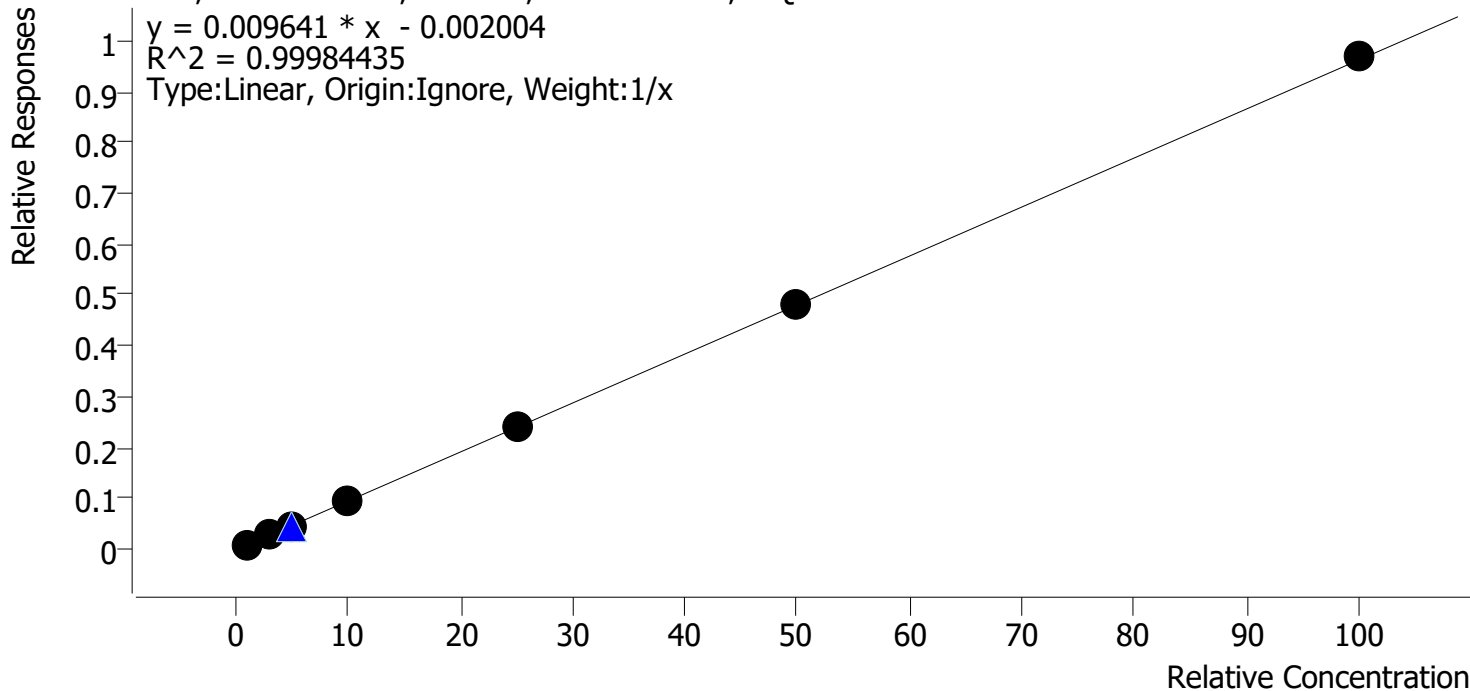
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 4/4/2022 2:18 PM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs



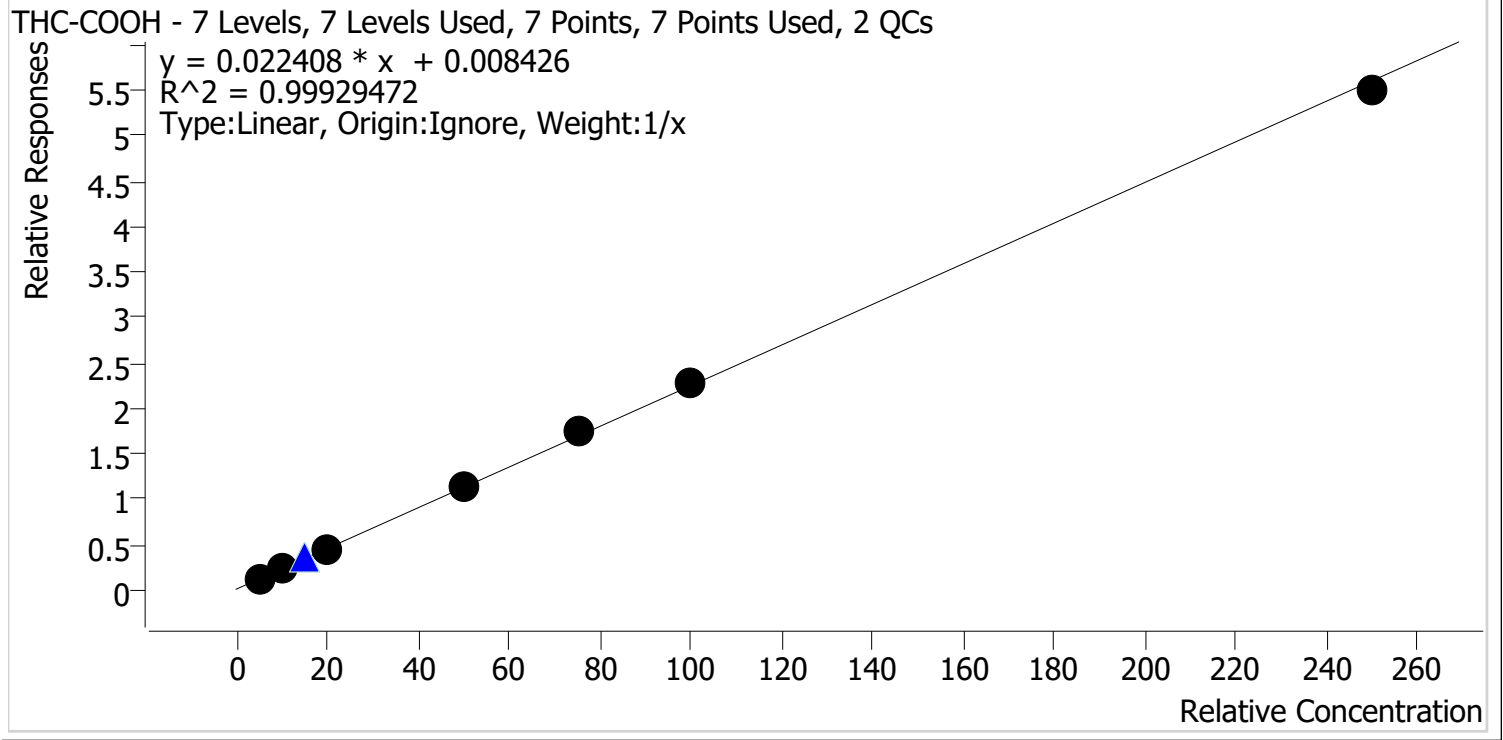
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.1	107.7
MJ Cal 2	2	✓	3.0	3.0	98.5
MJ Cal 3	3	✓	5.0	4.8	96.7
MJ Cal 4	4	✓	10.0	9.7	97.1
MJ Cal 5	5	✓	25.0	24.9	99.5
MJ Cal 6	6	✓	50.0	49.9	99.8
MJ Cal 7	7	✓	100.0	100.7	100.7

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 4/4/2022 2:18 PM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	5.0	4.9	98.2
MJ Cal 2	2	✓	10.0	9.8	98.5
MJ Cal 3	3	✓	20.0	19.6	98.2
MJ Cal 4	4	✓	50.0	50.6	101.1
MJ Cal 5	5	✓	75.0	78.1	104.2
MJ Cal 6	6	✓	100.0	101.7	101.7
MJ Cal 7	7	✓	250.0	245.1	98.1

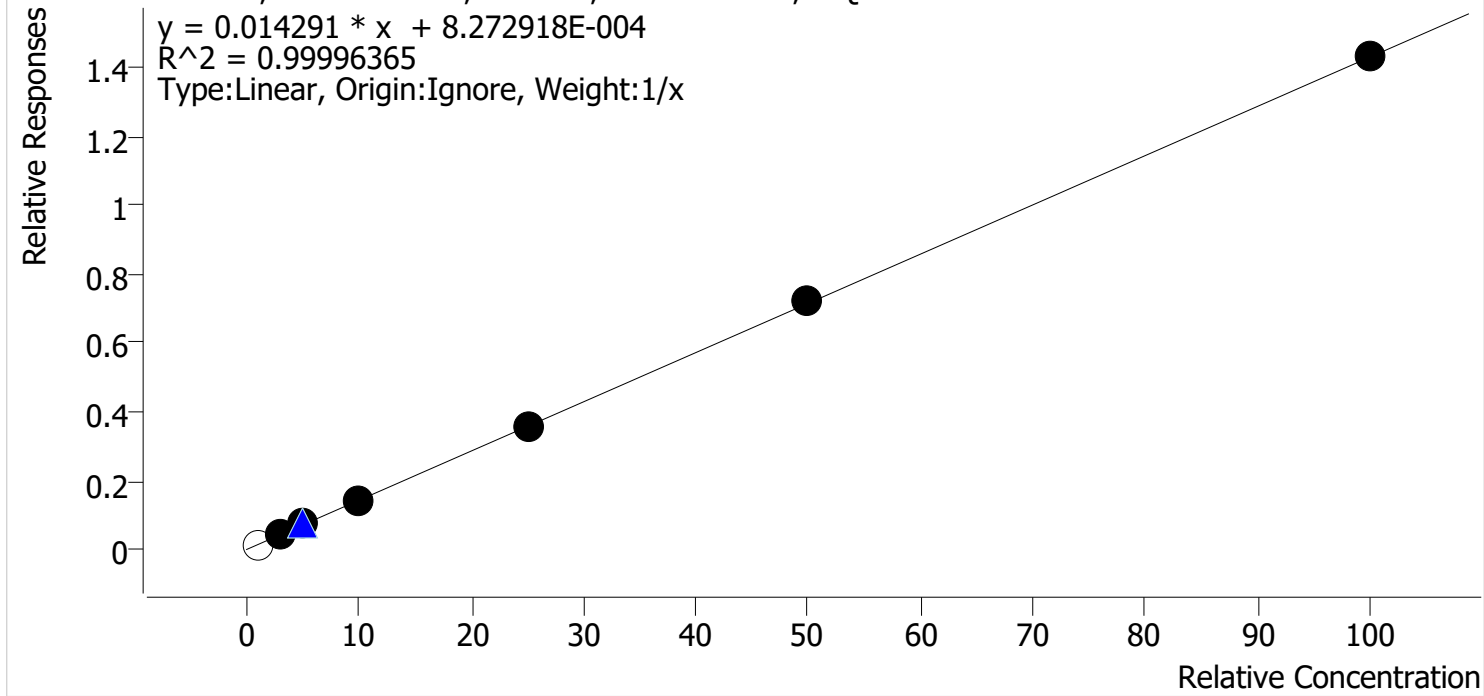
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
 Last Cal. Update 4/4/2022 2:18 PM
 Analyst Name ISP\Datastor
 Analyte THC-OH Internal Standard THC-OH-D3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	x	1.0	1.0	95.8
MJ Cal 2	2	✓	3.0	3.1	101.8
MJ Cal 3	3	✓	5.0	5.0	99.6
MJ Cal 4	4	✓	10.0	9.9	98.9
MJ Cal 5	5	✓	25.0	24.8	99.1
MJ Cal 6	6	✓	50.0	50.2	100.4
MJ Cal 7	7	✓	100.0	100.1	100.1

Calibrator 1 dropped due to poor peak shape.

TS

AM #27 Cannabinoid Quant. Results

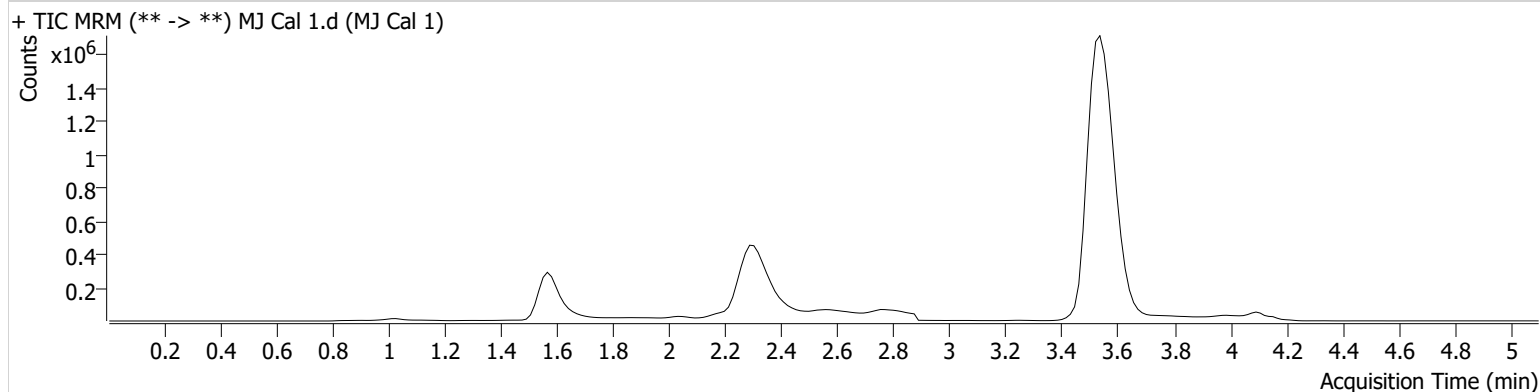


Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Cal 1.d
Type	Cal	Sample	MJ Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-H6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:05:45 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.573	17204	∞	16.4	72.15	1185124	0.9579 ng/ml Low
THC-COOH	1.610	40545	181.21	57.0	183.27	342380	4.9087 ng/ml Low
THC	3.540	96101	424.45	29.6	190.99	11462556	1.0775 ng/ml

TS



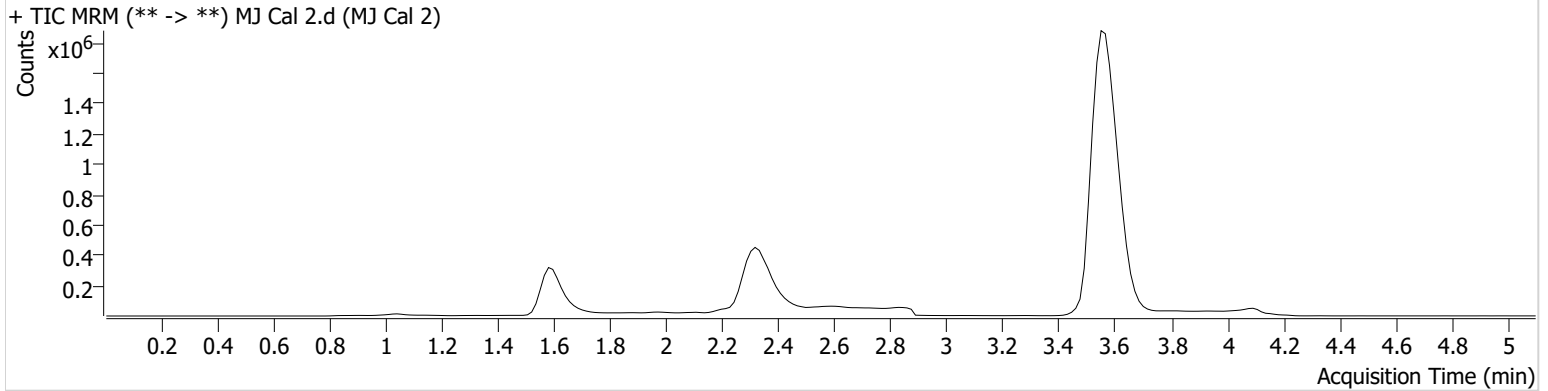
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Cal 2.d
Type	Cal	Sample	MJ Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-G6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:13:31 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	54038	∞	13.3	101.22	1214770	3.0548 ng/ml
THC-COOH	1.625	81559	∞	58.4	318.76	355978	9.8486 ng/ml
THC	3.570	320196	∞	25.7	∞	12089177	2.9550 ng/ml

TS

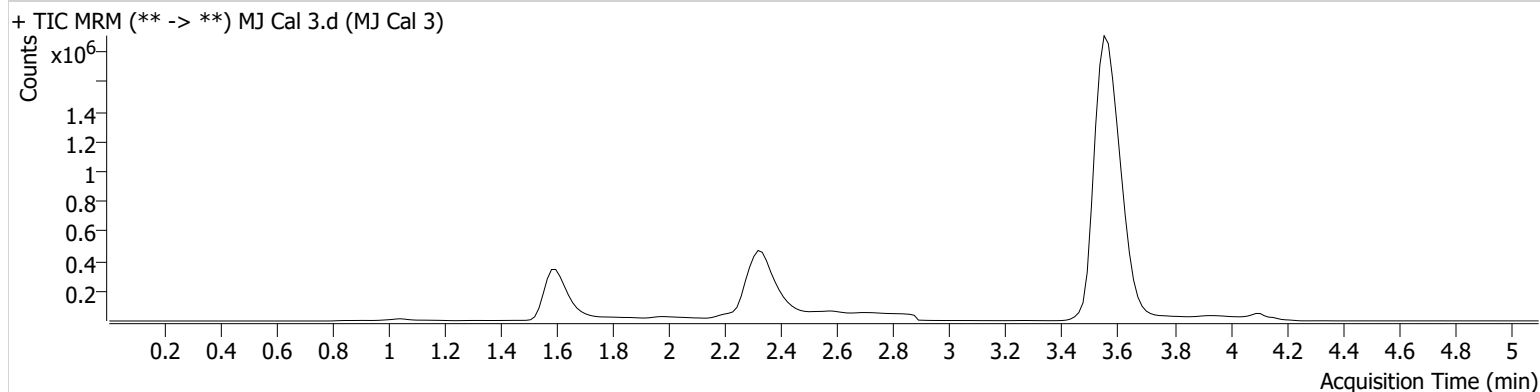


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Cal 3.d
Type	Cal	Sample	MJ Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-F6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:21:07 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	91317	∞	13.5	266.15	1267872	4.9818 ng/ml
THC-COOH	1.625	166178	271.78	59.8	∞	370482	19.6413 ng/ml
THC	3.570	530483	∞	25.3	∞	11889139	4.8357 ng/ml

TS

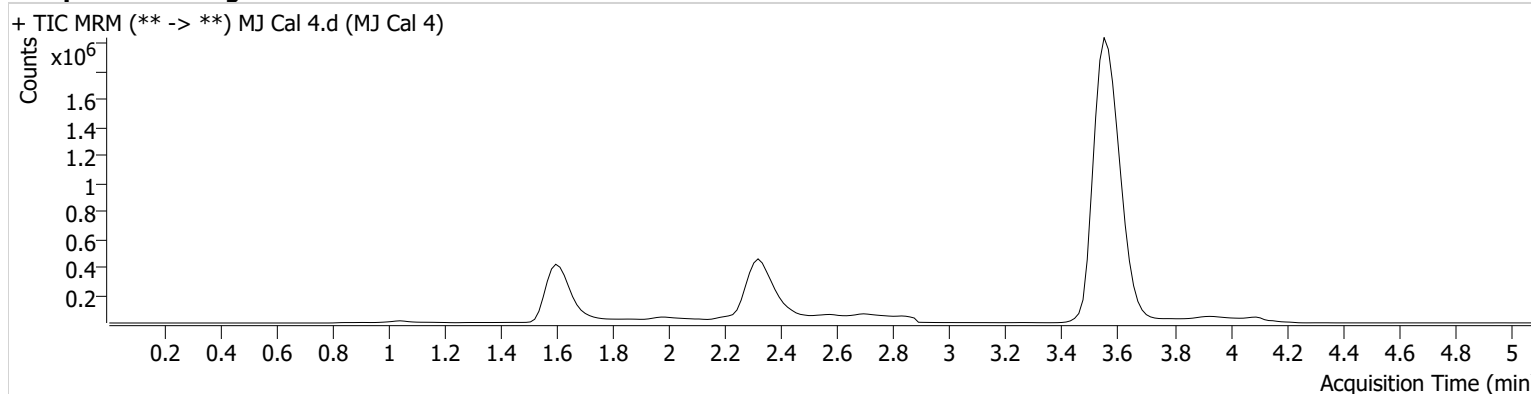


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Cal 4.d
Type	Cal	Sample	MJ Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-E6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:28:43 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	177706	∞	13.5	560.64	1249729	9.8920 ng/ml
THC-COOH	1.625	410352	∞	61.4	∞	359431	50.5736 ng/ml
THC	3.570	1113064	∞	24.7	∞	12149191	9.7102 ng/ml

TS

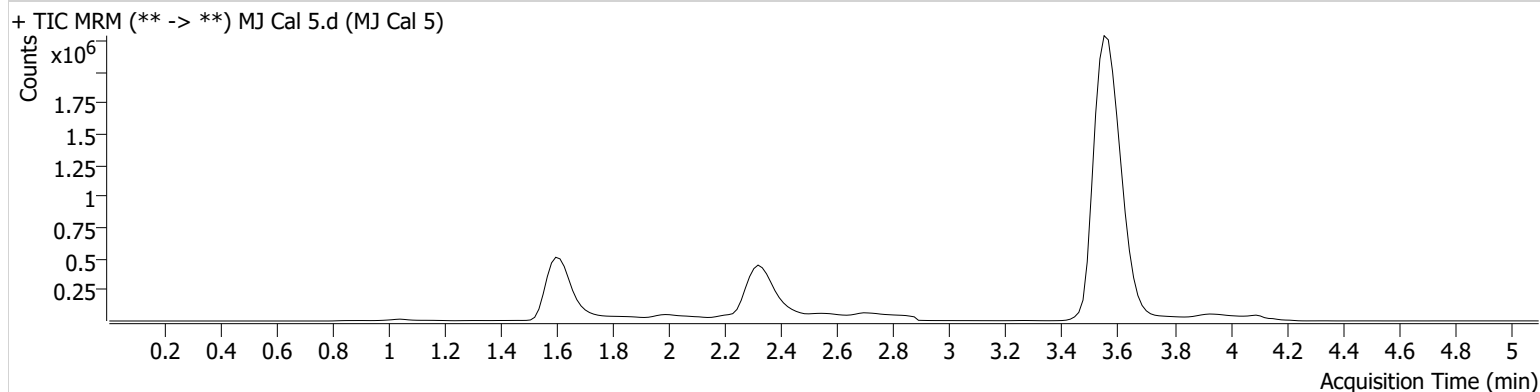


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Cal 5.d
Type	Cal	Sample	MJ Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-D6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:36:19 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	432768	∞	13.9	683.84	1219272	24.7783 ng/ml
THC-COOH	1.625	602439	∞	62.0	2784.81	342436	78.1356 ng/ml
THC	3.570	2866951	∞	24.7	3434.95	12053832	24.8770 ng/ml

TS

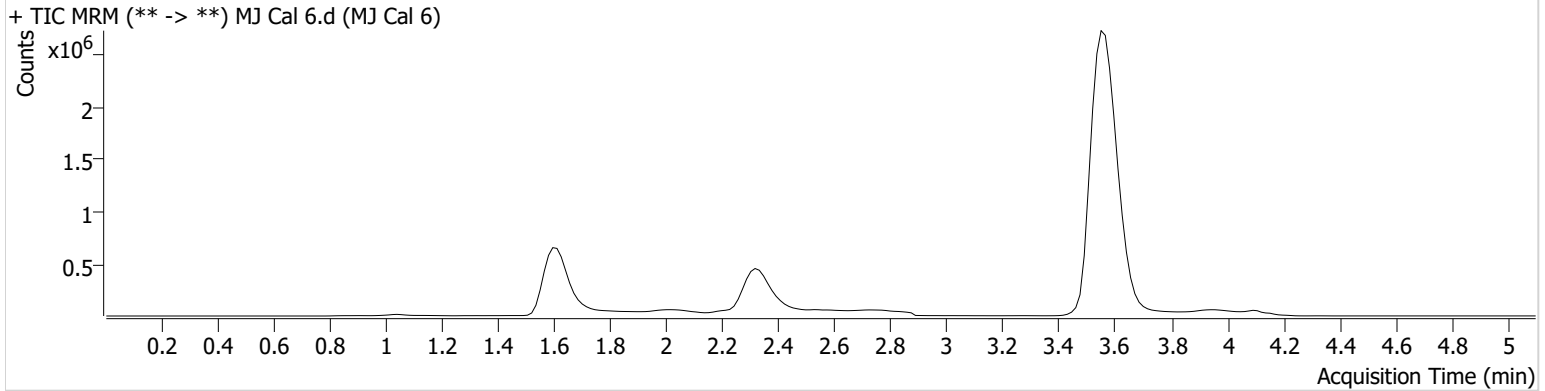


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Cal 6.d
Type	Cal	Sample	MJ Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-C6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:43:55 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	873867	∞	14.0	3396.56	1216412	50.2106 ng/ml
THC-COOH	1.625	783978	∞	59.1	3348.86	342592	101.7476 ng/ml
THC	3.570	5499454	∞	25.4	∞	11482532	49.8831 ng/ml

TS

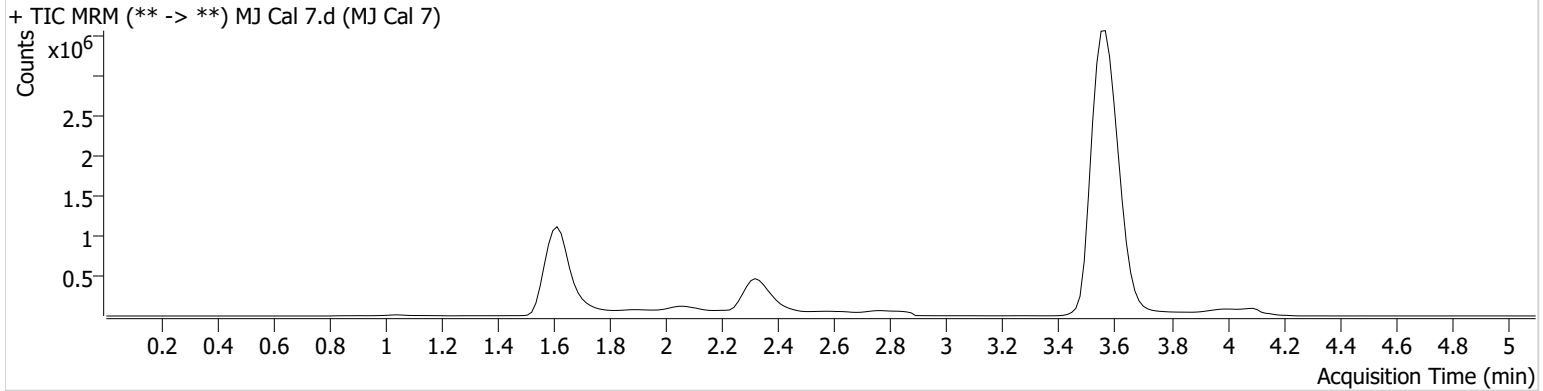


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\033122 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2022 2:18:20 PM

Instrument	Falco (069901)	Data File	MJ Cal 7.d
Type	Cal	Sample	MJ Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P5-B6	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2022 4:51:31 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	1680656	∞	14.2	1410.39	1174358	100.0825 ng/ml
THC-COOH	1.625	1756626	∞	62.1	6369.91	319294	245.1446 ng/ml
THC	3.570	10666248	∞	25.4	∞	11012952	100.6615 ng/ml