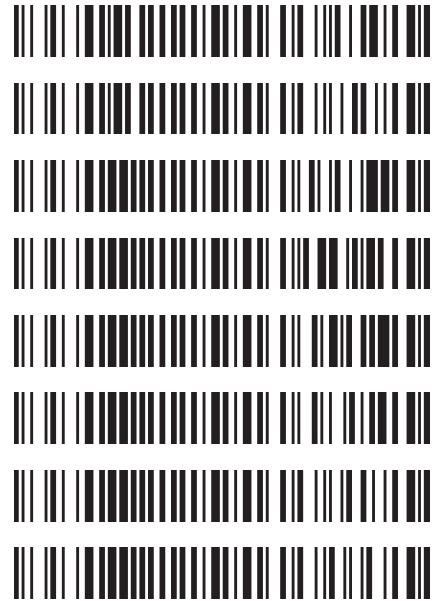


Worklist: 5873

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-1564	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-1661	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1011	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1033	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1215	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1218	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1225	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1227	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: 05/05/2022

Analyst: Tamara Salazar

Plate lot#: 220309

Plate Retest Date: 09/09/2022

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-COOH: 10-250 – Calibrator 1 dropped due to ratio being outside acceptable parameters
THC-OH: 3-100 – Calibrator 1 dropped due to poor peak shape.

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	P2022-1218-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2022-1215-1	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	IS + Sample	P2022-1033-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	IS + Sample	P2022-1011-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	IS + Sample	M2022-1661-2	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	P2022-1225-1	M2022-1564-2	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2022-1227-1	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2022-1225-1**	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

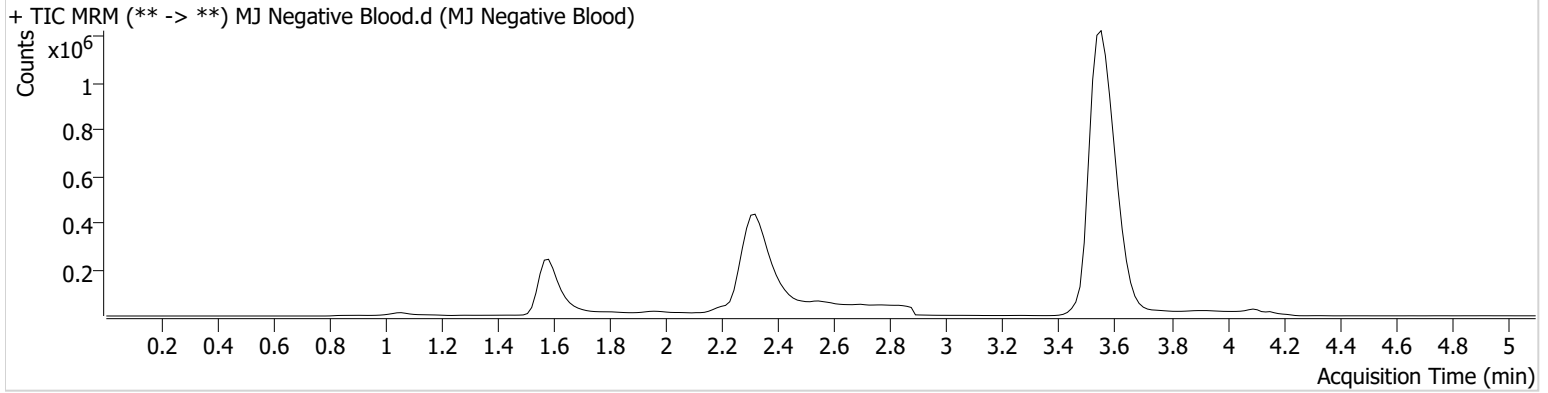


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 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	P1-G5	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 1:30:15 PM		
Sample Info.			

Sample Chromatogram



TS

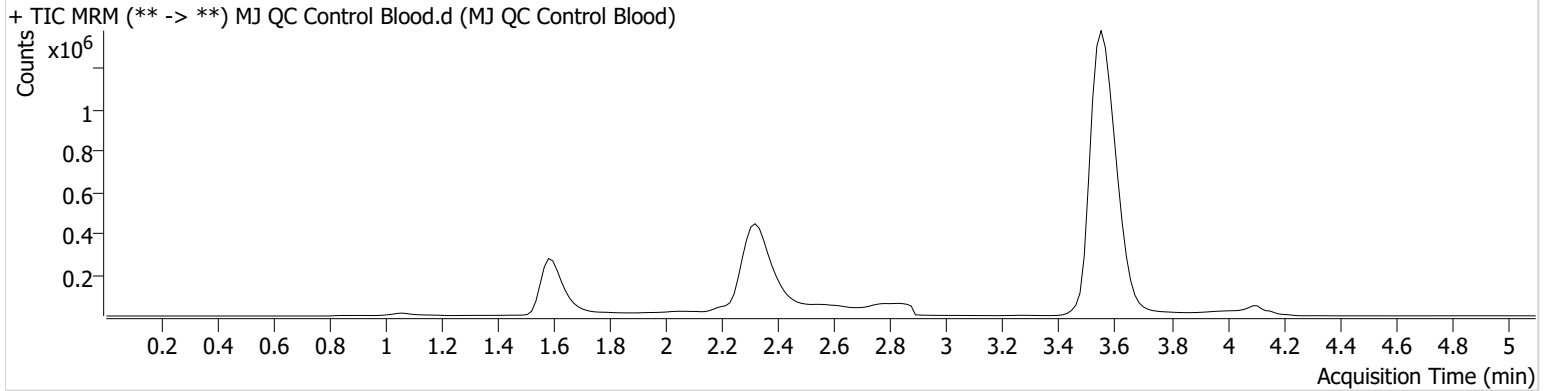
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 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	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 1:15:00 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	85482	∞	12.0	135.73	1052421	4.9293 ng/ml
THC-COOH	1.625	113405	∞	49.5	∞	275829	14.7889 ng/ml
THC	3.570	347369	∞	24.9	135.50	8596027	4.8256 ng/ml

TS

AM #27 Cannabinoid Quant. Results

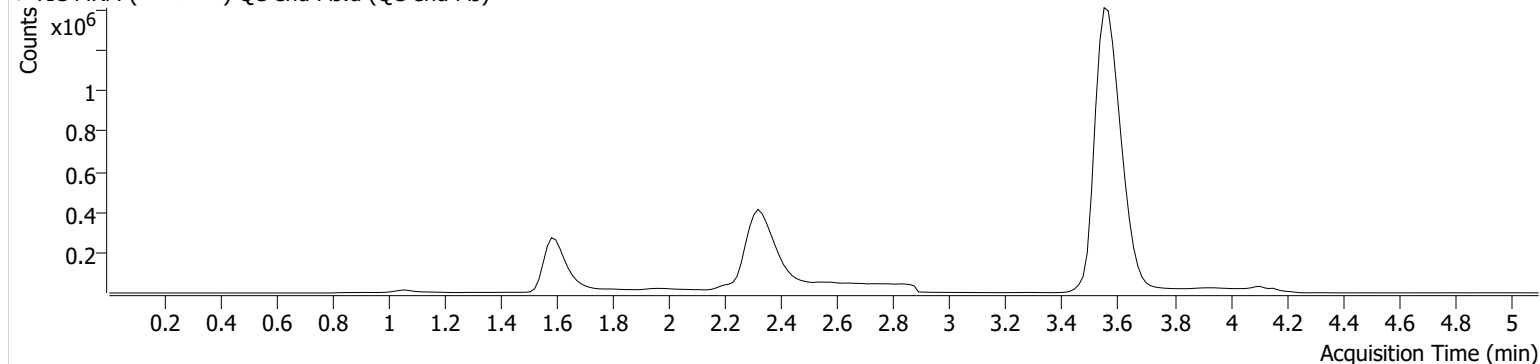


Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 4:02:24 PM		

Sample Chromatogram

+ TIC MRM (** -> **) QC end MJ.d (QC end MJ)



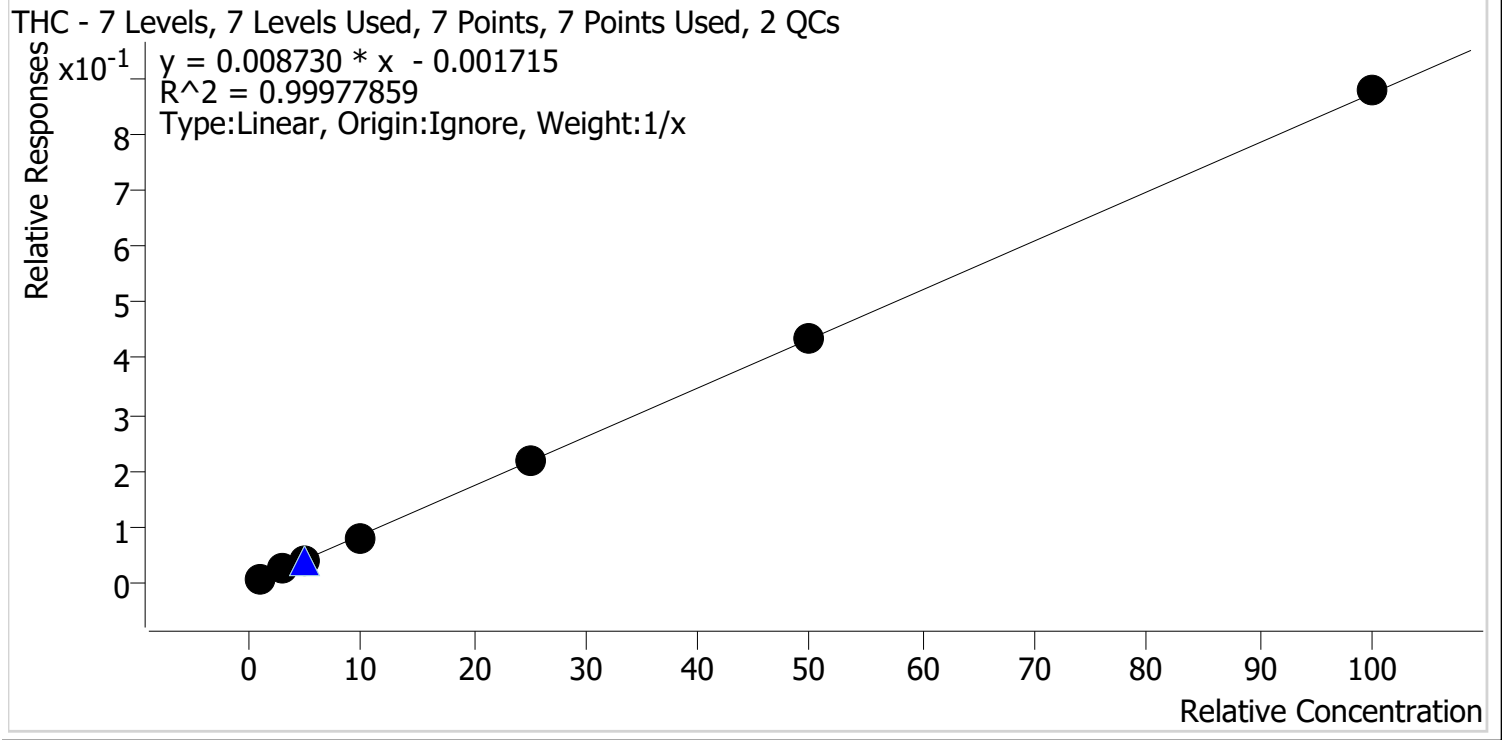
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	79519	127.36	12.2	117.37	1039381	4.6191 ng/ml
THC-COOH	1.625	113984	∞	49.3	∞	275013	14.9254 ng/ml
THC	3.570	352849	∞	25.6	∞	8837862	4.7700 ng/ml

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 5/10/2022 3:35 PM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	109.4
Cal 2 MJ	2	✓	3.0	2.9	97.8
Cal 3 MJ	3	✓	5.0	4.8	96.5
Cal 4 MJ	4	✓	10.0	9.6	95.9
Cal 5 MJ	5	✓	25.0	25.0	99.8
Cal 6 MJ	6	✓	50.0	50.0	100.0
Cal 7 MJ	7	✓	100.0	100.6	100.6

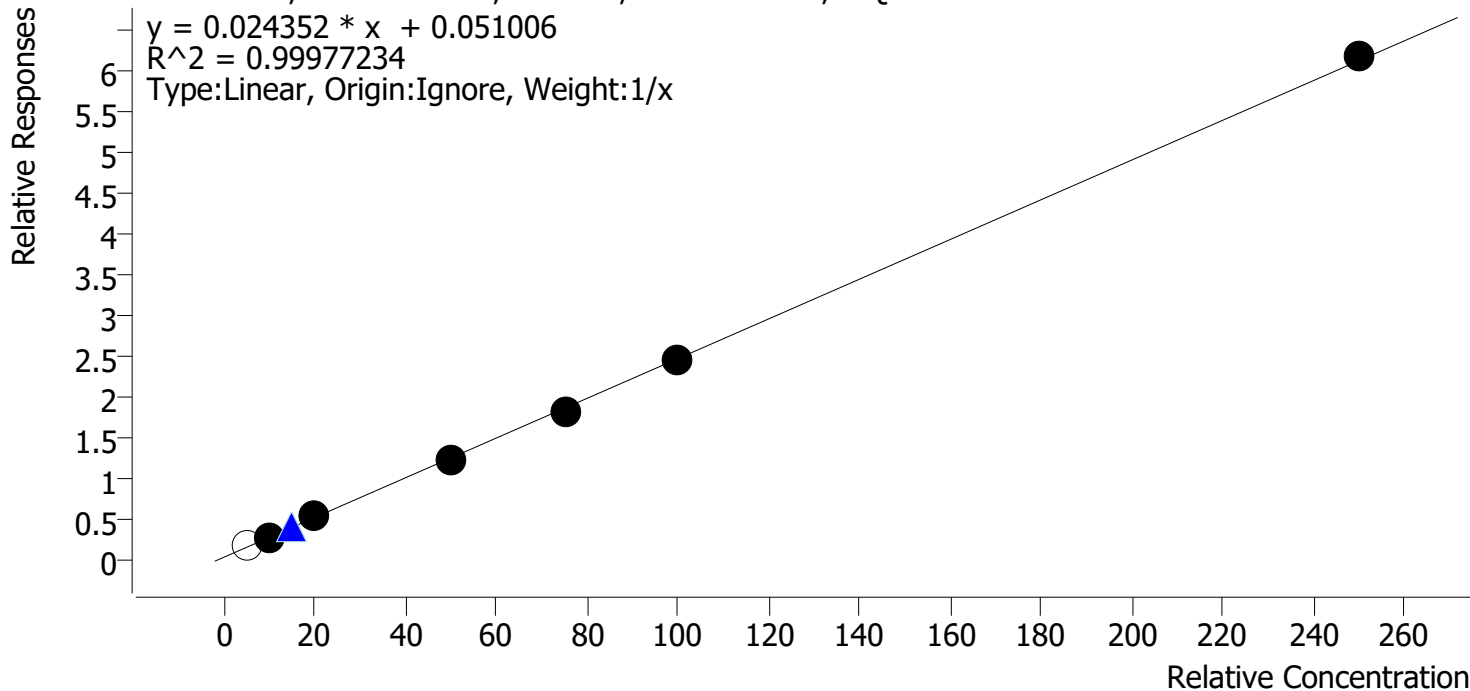
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
 Last Cal. Update 5/10/2022 3:35 PM
 Analyst Name ISP\Datastor
 Analyte THC-COOH Internal Standard THC-COOH-D9

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	5.0	6.2	124.1
Cal 2 MJ	2	✓	10.0	10.0	100.1
Cal 3 MJ	3	✓	20.0	20.6	102.9
Cal 4 MJ	4	✓	50.0	49.4	98.7
Cal 5 MJ	5	✓	75.0	73.5	98.0
Cal 6 MJ	6	✓	100.0	99.6	99.6
Cal 7 MJ	7	✓	250.0	252.0	100.8

Calibrator 1 dropped due to ratio.

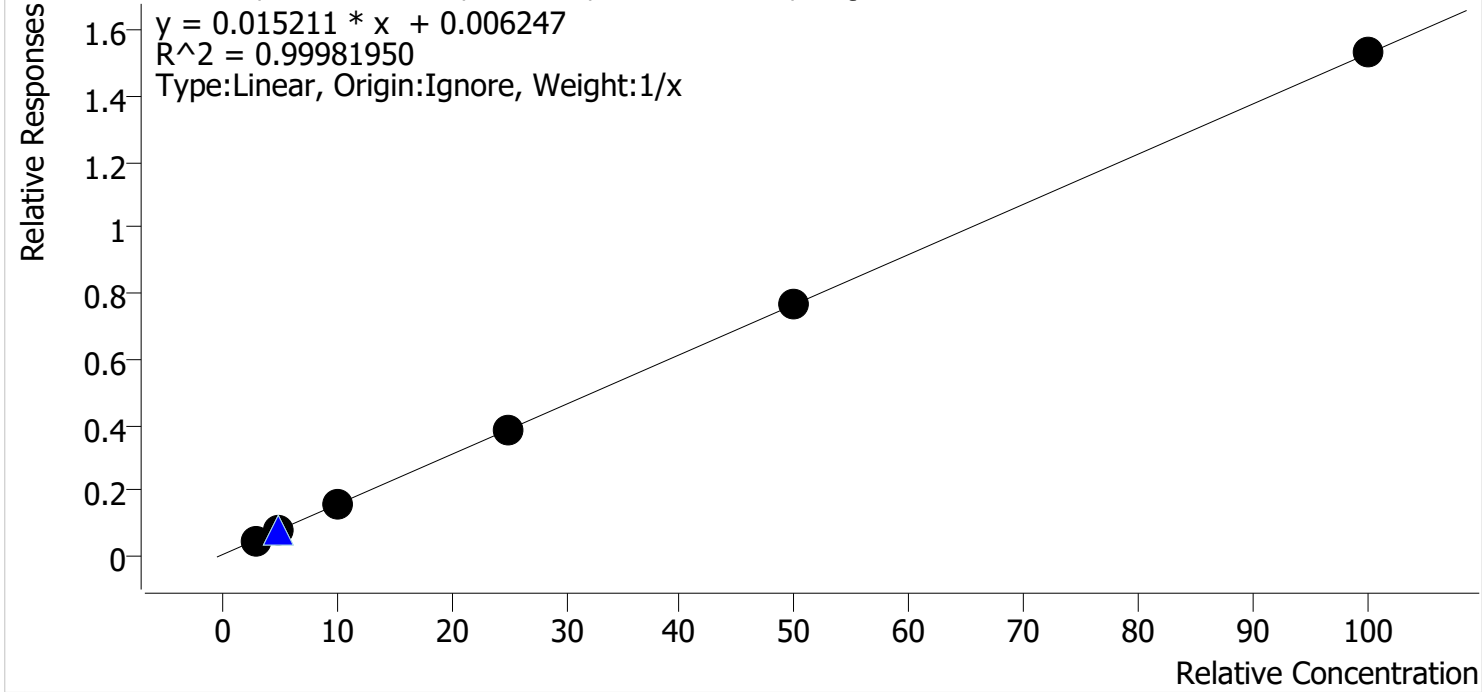
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 5/10/2022 3:35 PM
Analyst Name ISP\Datastor
Analyte THC-OH **Internal Standard** THC-OH-D3

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 2 MJ	2	✓	3.0	2.8	95.0
Cal 3 MJ	3	✓	5.0	5.2	104.5
Cal 4 MJ	4	✓	10.0	10.2	101.8
Cal 5 MJ	5	✓	25.0	24.7	98.8
Cal 6 MJ	6	✓	50.0	49.9	99.7
Cal 7 MJ	7	✓	100.0	100.2	100.2

Calibrator 1 dropped due to poor peak shape.

TS

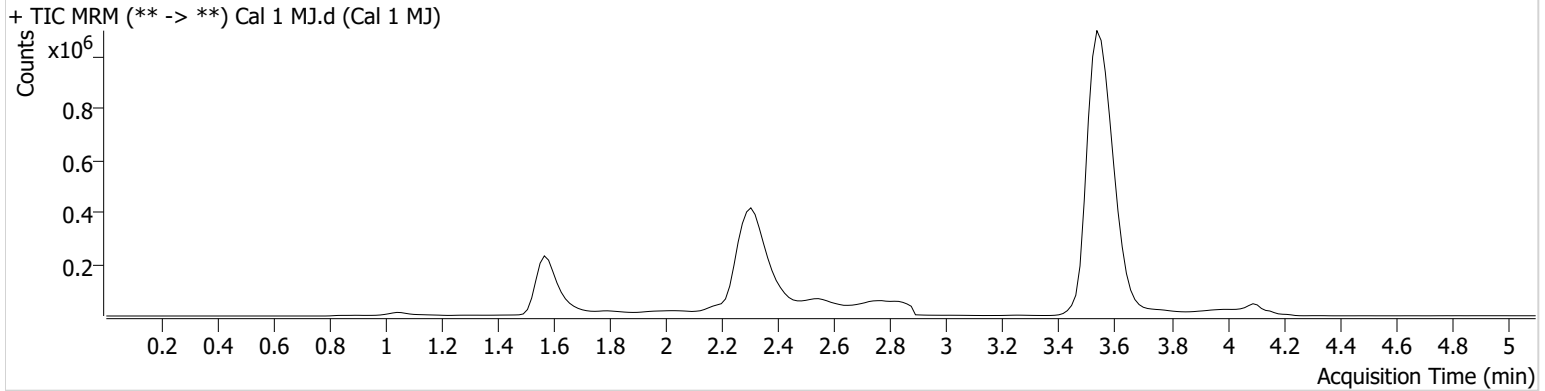


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 12:13:56 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.610	49623	∞	32.1 Low	∞	245473	6.2067 ng/ml
THC	3.555	56560	∞	28.1	∞	7220853	1.0938 ng/ml

TS

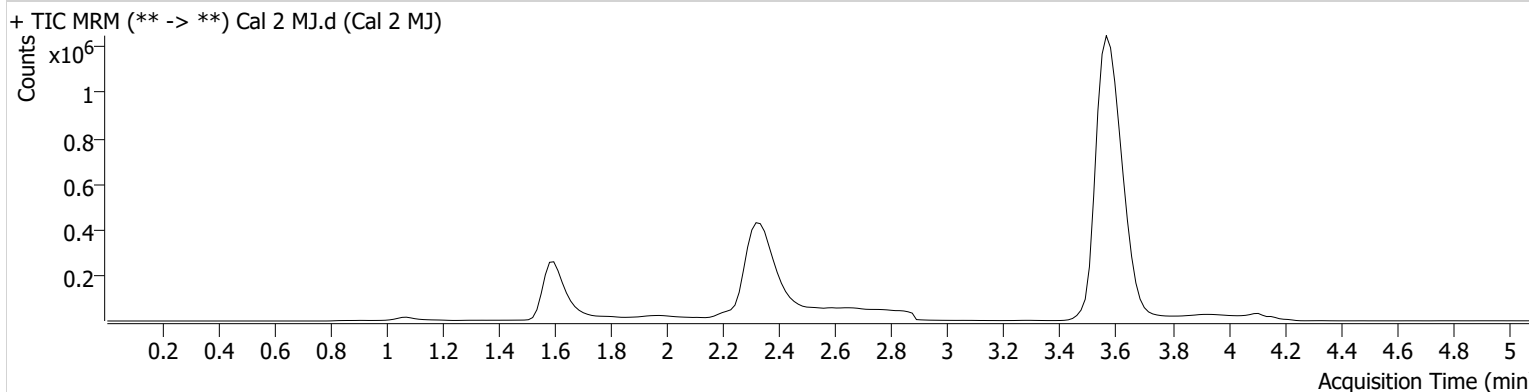


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 12:21:42 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	52022	24.19	13.2	80.34	1049028	2.8496 ng/ml Low
THC-COOH	1.625	78093	∞	46.6	∞	265012	10.0063 ng/ml
THC	3.586	191106	5647.45	25.5	156.67	7999480	2.9331 ng/ml

TS

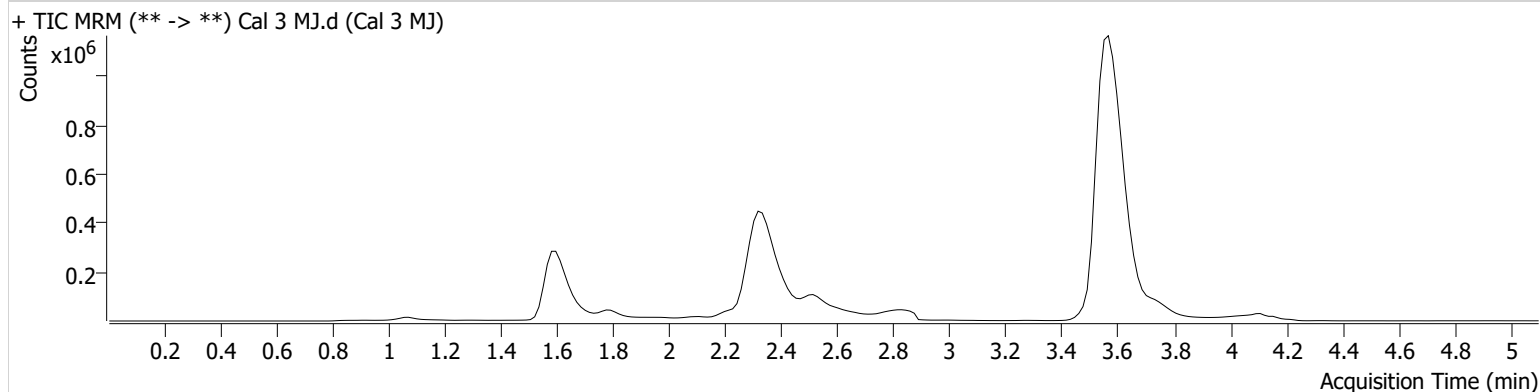


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 12:29:18 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	91988	∞	12.0	280.59	1072953	5.2257 ng/ml
THC-COOH	1.625	152155	∞	48.8	∞	275630	20.5742 ng/ml
THC	3.586	315124	∞	24.7	495.77	7801216	4.8237 ng/ml

TS



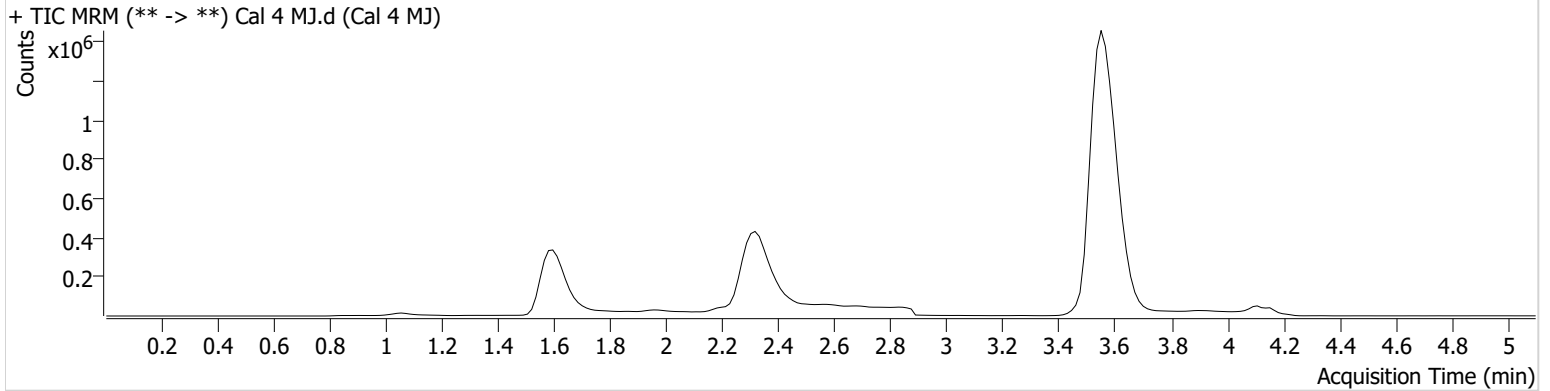
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 12:36:56 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	175885	∞	12.1	189.50	1091625	10.1821 ng/ml
THC-COOH	1.625	347195	∞	57.5	∞	277097	49.3585 ng/ml
THC	3.570	720016	∞	24.5	∞	8777507	9.5932 ng/ml

TS

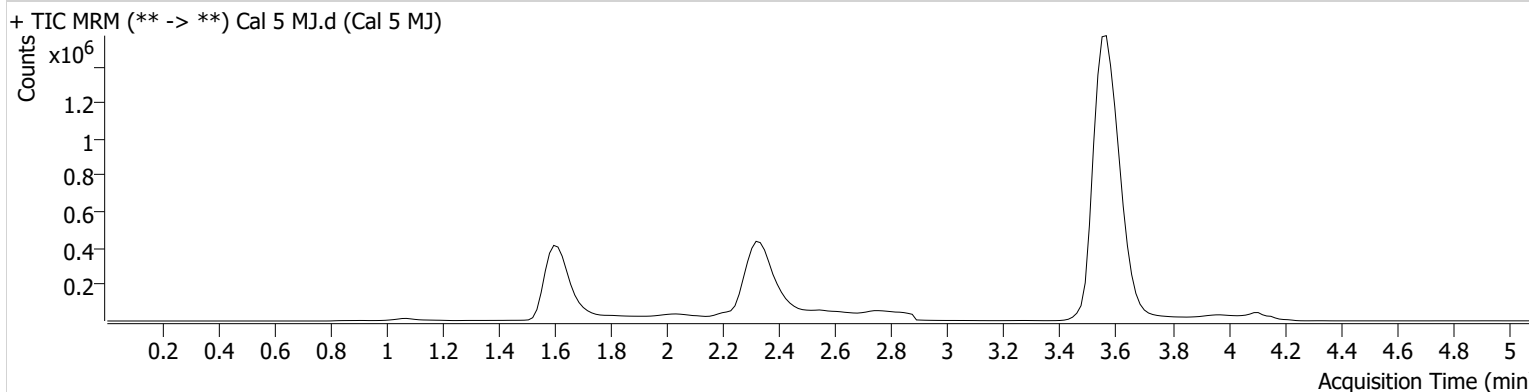


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 12:44:32 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	404238	1216.42	13.1	1433.48	1058717	24.6916 ng/ml
THC-COOH	1.625	488461	∞	58.7	∞	265407	73.4819 ng/ml
THC	3.570	1770207	∞	24.3	∞	8188114	24.9619 ng/ml

TS

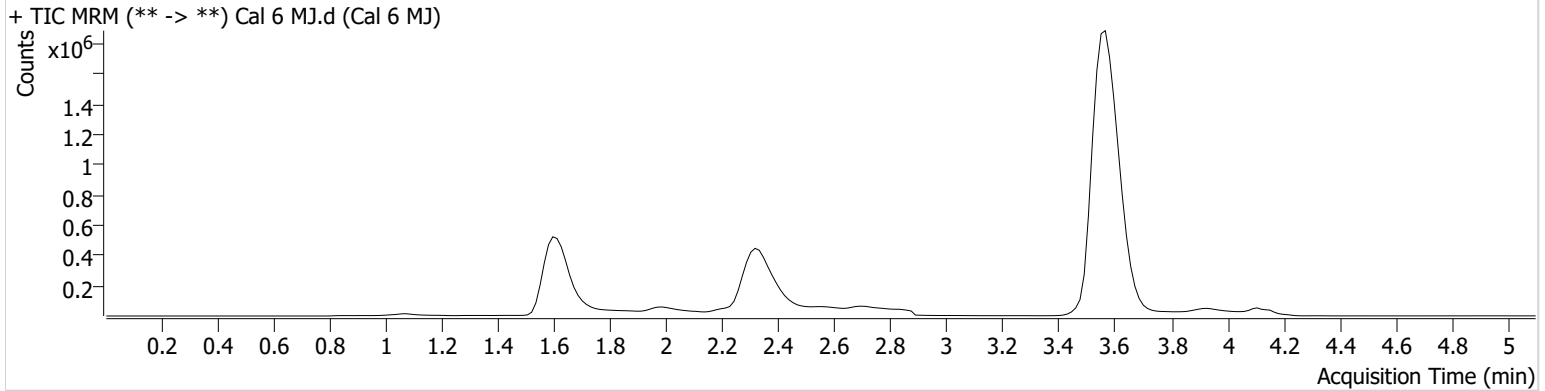


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 12:52:08 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	832181	∞	13.2	∞	1088302	49.8611 ng/ml
THC-COOH	1.625	667618	∞	58.3	∞	269668	99.5695 ng/ml
THC	3.570	3598024	∞	24.7	∞	8275230	50.0034 ng/ml

TS

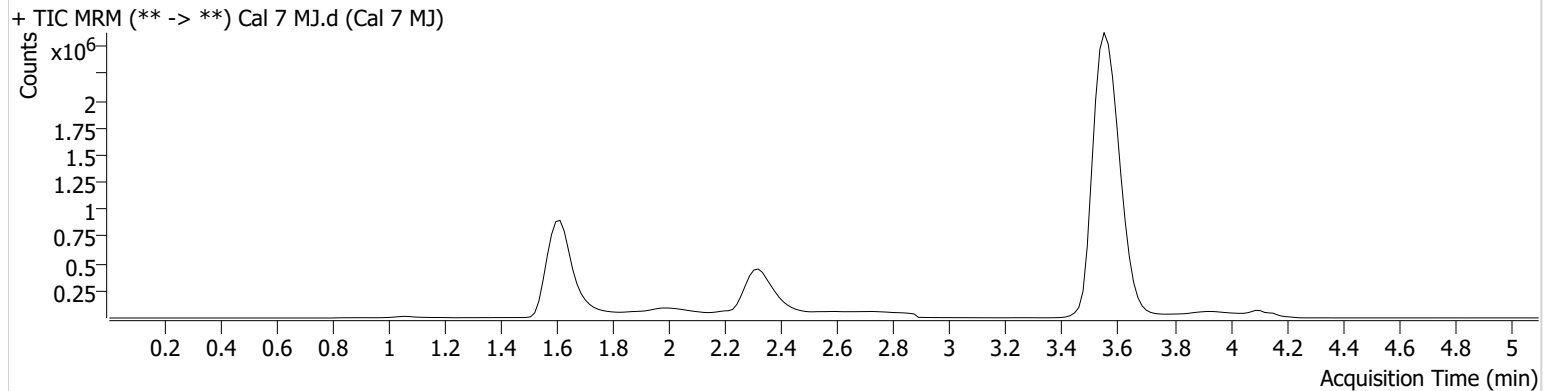


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\050522 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/10/2022 3:35:37 PM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-B6	Comment	
Injection Volume	10		
Acq. Date-Time	5/5/2022 12:59:45 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	1599392	∞	13.4	∞	1045226	100.1899 ng/ml
THC-COOH	1.625	1553049	∞	60.2	∞	250982	252.0096 ng/ml
THC	3.555	7395169	∞	24.8	∞	8438084	100.5909 ng/ml