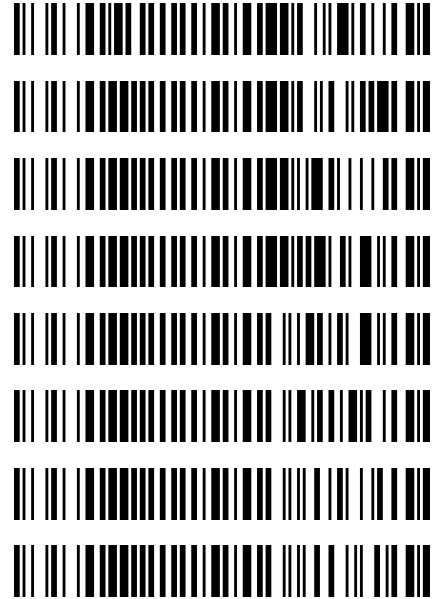


Worklist: 6139

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-3803	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2860	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2883	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2934	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2943	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-3019	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-3044	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-3045	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: 10/20/2022
Plate lot#: IDP-108-3-220802
Mobile phase A: 0.1% Formic Acid in LCMS Water
Blank Blood Lot: Lampire 22B52015-1
Column: UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 069901

Analyst: Sarah Collins
Retest Date: 02/02/2023
Mobile phase B: 0.1% Formic acid in Acetonitrile
Blank Urine Lot: N/A

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: Curve Range: THC-OH 3-100 (calibrator 1 dropped due to ratio and peak shape)

SC

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1 blood end	p2022-3044-1			IS + QC_1 blood start
B	IS + Cal. 2	negative blood	p2022-3045-1			IS + Cal. 7
C	IS + Cal. 3	m2022-3803-1	p2022-2883-1			IS + Cal. 6
D	IS + Cal. 4	p2022-2860-1	p2022-3019-1			IS + Cal. 5
E	IS + Cal. 5	p2022-2883-1*				IS + Cal. 4
F	IS + Cal. 6	p2022-2934-1				IS + Cal. 3
G	IS + Cal. 7	p2022-2943-1				IS + Cal. 2
H	IS + QC_1 blood start	p2022-3019-1*			IS + QC_1 blood end	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Sample moved during analytical step 6 due to blood clot

SC

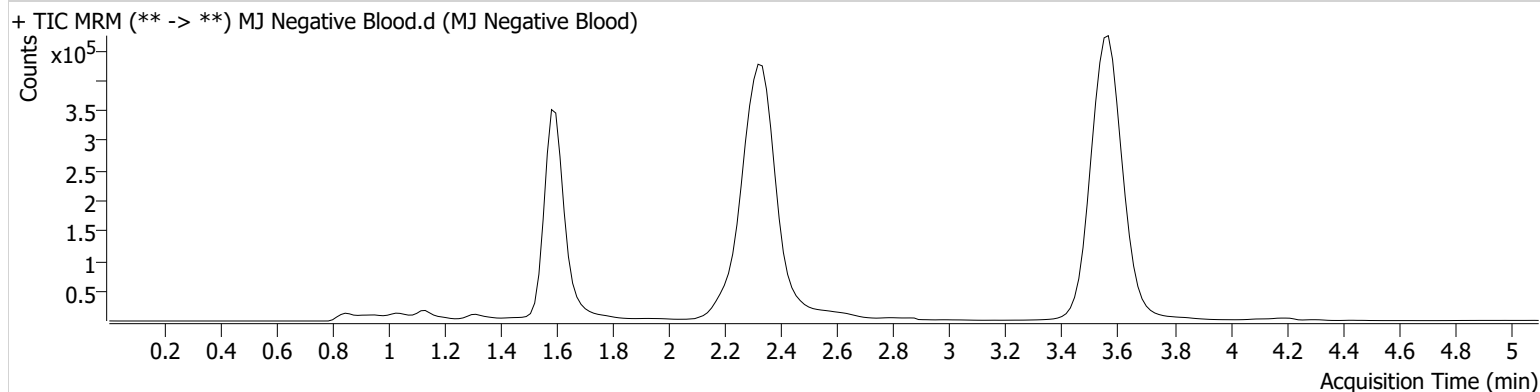


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-B2	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 1:48:43 PM		
Sample Info.			

Sample Chromatogram



SC



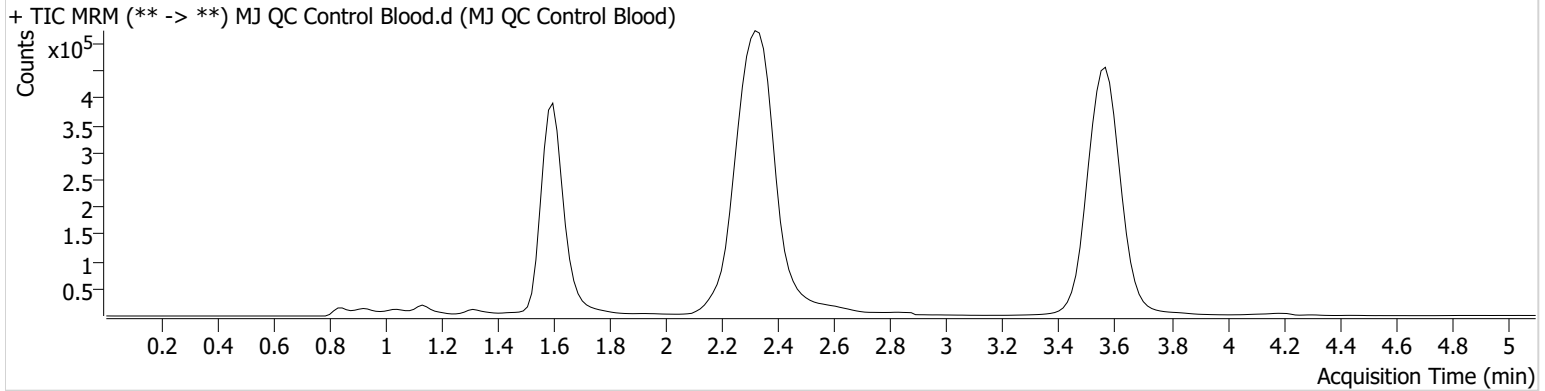
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-H1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 1:33:28 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	133852	∞	13.0	259.78	1513694	4.8551 ng/ml
THC-COOH	1.625	129102	411.03	45.4	550.92	306901	14.1898 ng/ml
THC	3.586	167594	800.67	29.1	∞	3535476	5.1214 ng/ml

SC

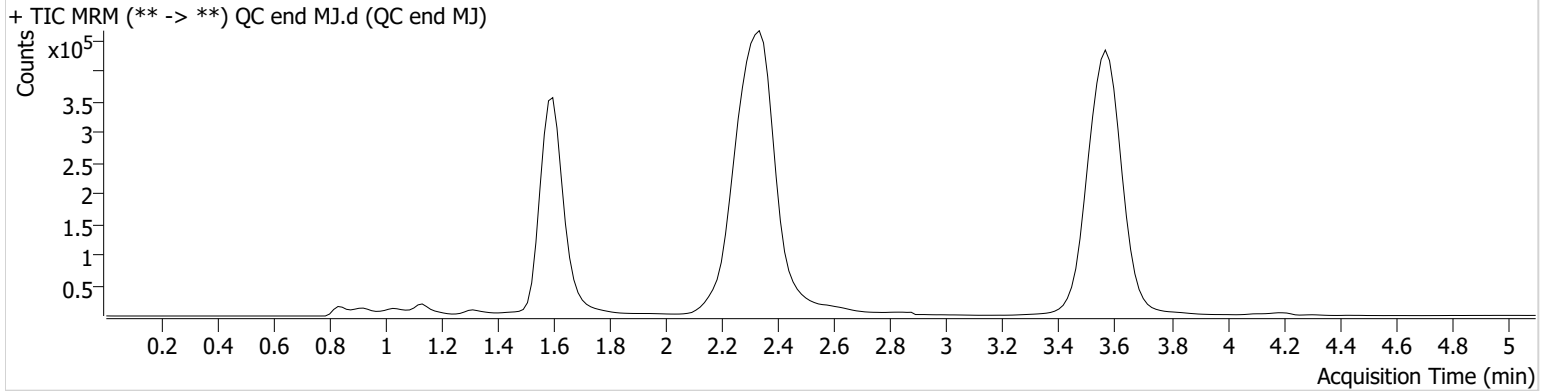


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-A2	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 4:05:53 PM		

Sample Chromatogram



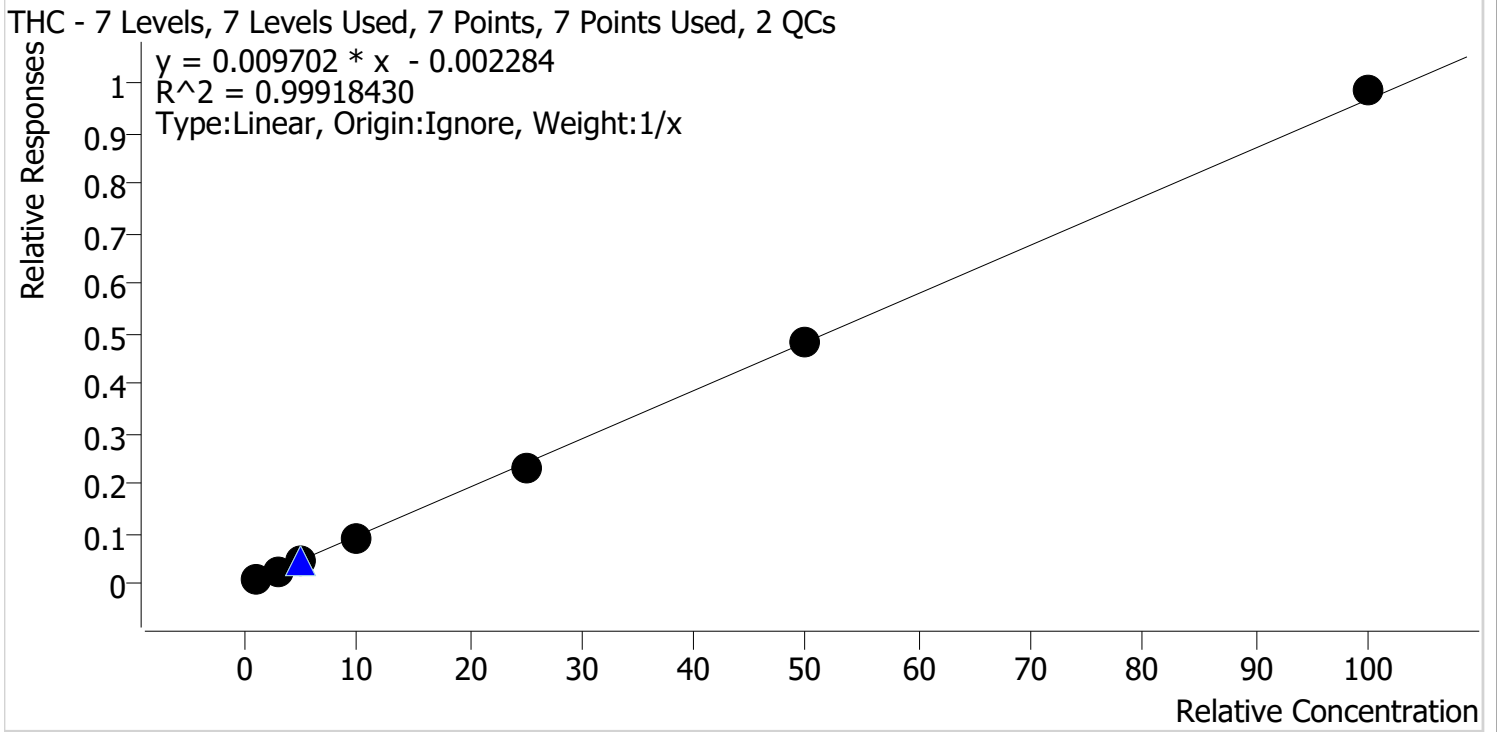
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	130102	203.01	12.0	∞	1423831	5.0193 ng/ml
THC-COOH	1.625	121938	594.96	47.7	∞	302722	13.4675 ng/ml
THC	3.586	155656	732.57	30.6	∞	3503532	4.8148 ng/ml

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 10/21/2022 9:34 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.2	115.9
Cal 2 MJ	2	✓	3.0	2.9	95.5
Cal 3 MJ	3	✓	5.0	4.8	96.5
Cal 4 MJ	4	✓	10.0	9.4	94.0
Cal 5 MJ	5	✓	25.0	24.2	96.7
Cal 6 MJ	6	✓	50.0	49.7	99.5
Cal 7 MJ	7	✓	100.0	101.8	101.8

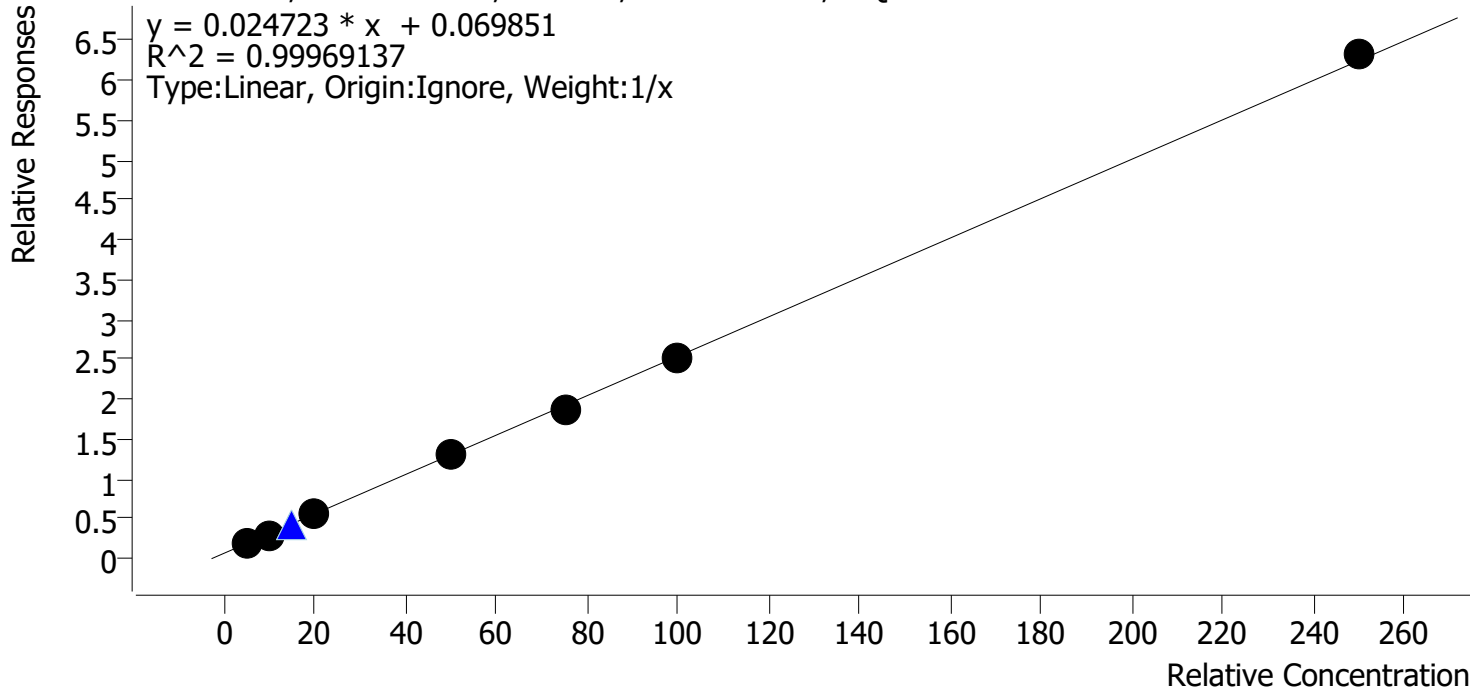
SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 10/21/2022 9:34 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.3	106.1
Cal 2 MJ	2	✓	10.0	9.7	96.7
Cal 3 MJ	3	✓	20.0	20.1	100.5
Cal 4 MJ	4	✓	50.0	49.2	98.5
Cal 5 MJ	5	✓	75.0	73.3	97.8
Cal 6 MJ	6	✓	100.0	99.3	99.3
Cal 7 MJ	7	✓	250.0	253.0	101.2

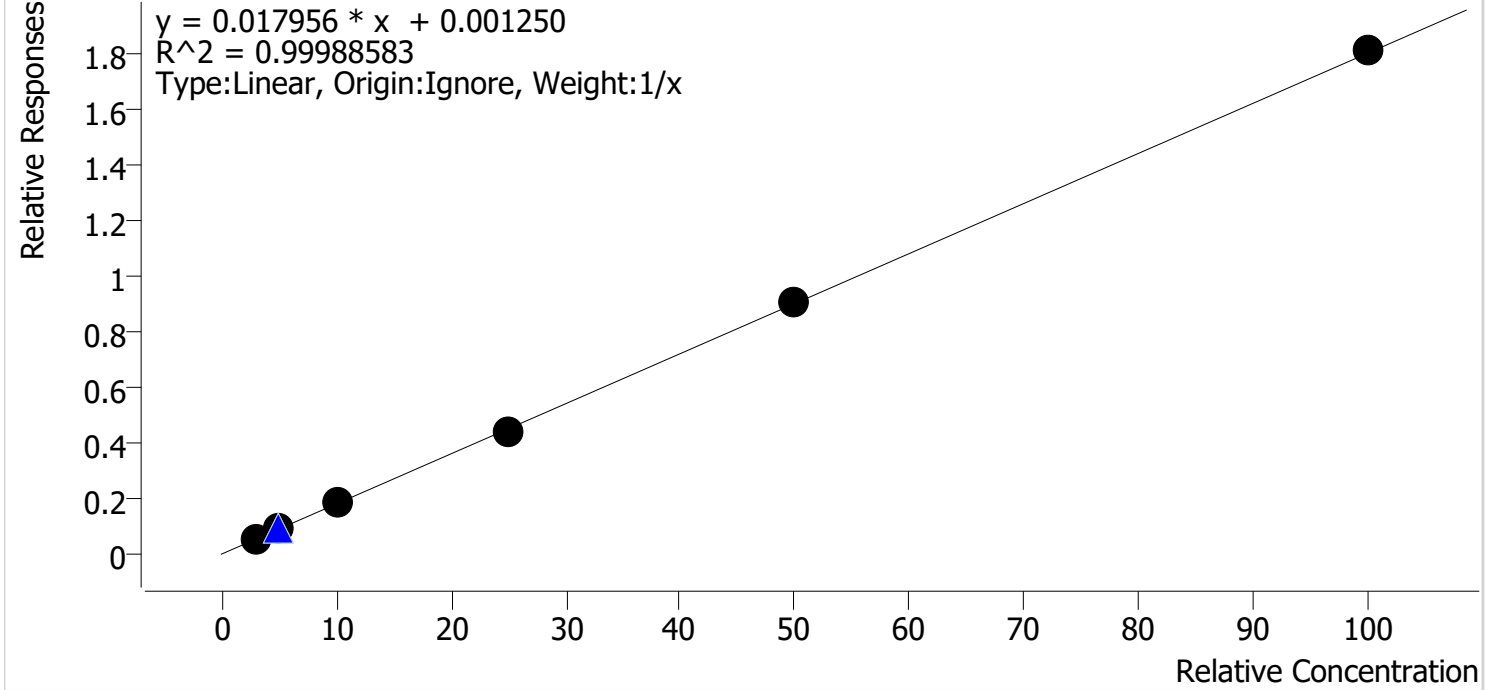
SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 10/21/2022 9:34 AM
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	3.1	102.3
Cal 3 MJ	3	✓	5.0	4.9	98.7
Cal 4 MJ	4	✓	10.0	10.1	100.7
Cal 5 MJ	5	✓	25.0	24.5	97.9
Cal 6 MJ	6	✓	50.0	50.0	99.9
Cal 7 MJ	7	✓	100.0	100.5	100.5

SC

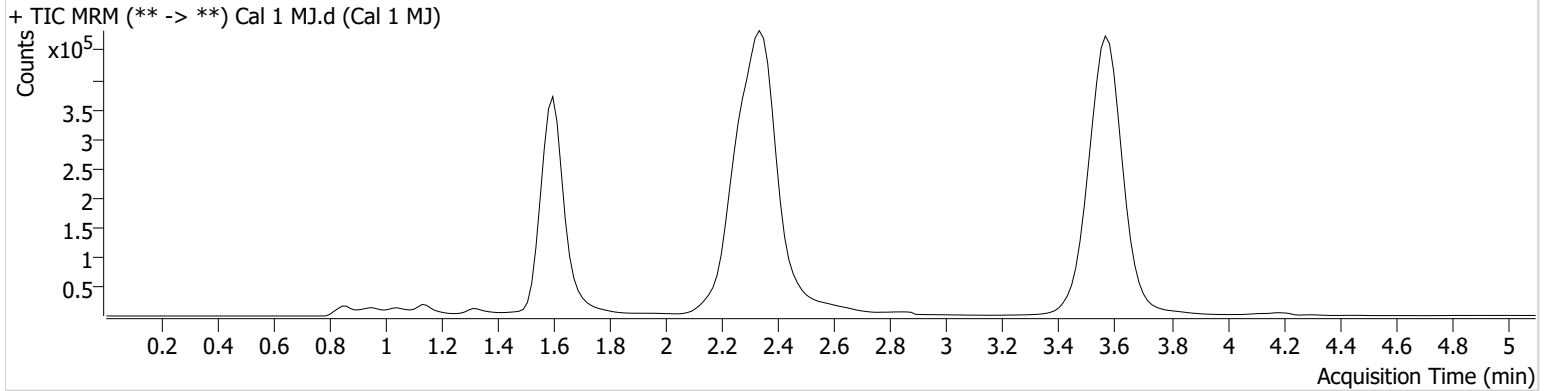


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-A1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 12:32:39 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	69222	∞	39.8	∞	344434	5.3037 ng/ml
THC	3.586	35559	195.24	31.8	17.32	3967187	1.1593 ng/ml

SC

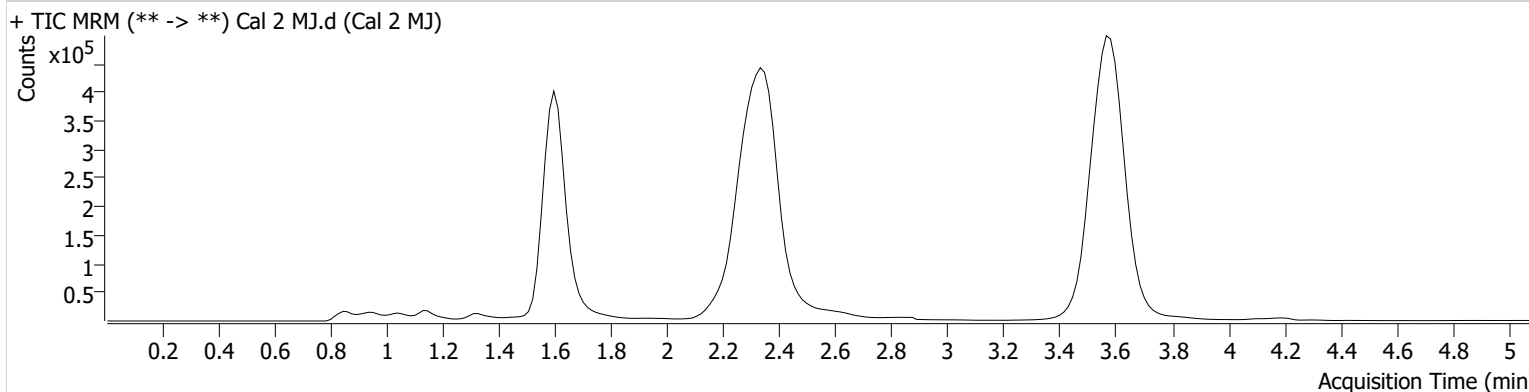


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-B1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 12:40:15 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	93298	∞	12.5	387.93	1656232	3.0676 ng/ml
THC-COOH	1.640	103536	∞	42.6	80.37	335282	9.6653 ng/ml
THC	3.586	105193	362.98	30.5	∞	4122293	2.8656 ng/ml

SC

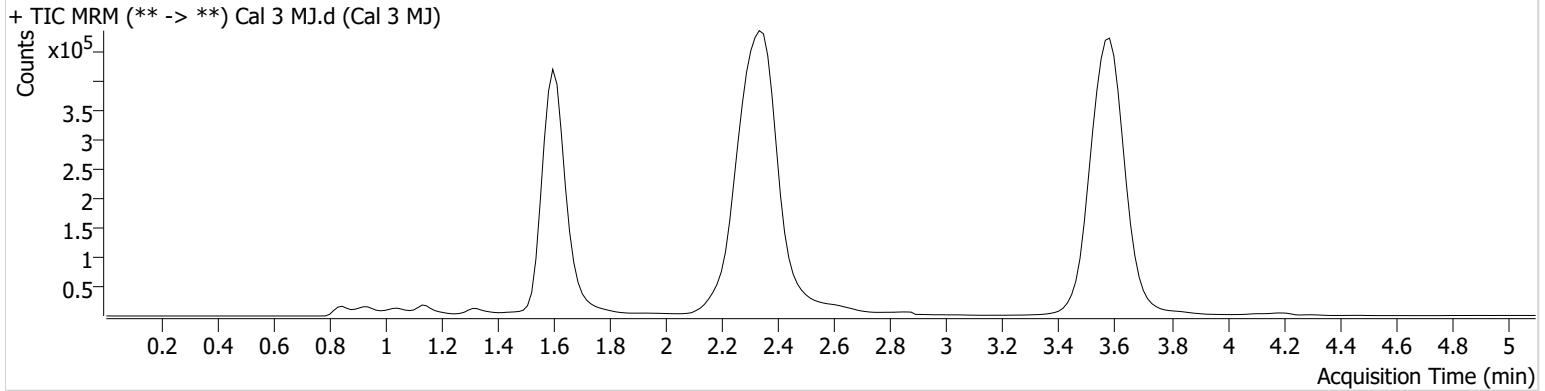


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-C1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 12:47:50 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	146970	∞	12.5	243.73	1635834	4.9341 ng/ml
THC-COOH	1.640	183689	∞	47.6	486.05	324200	20.0924 ng/ml
THC	3.601	170585	910.31	28.3	∞	3831468	4.8244 ng/ml

SC

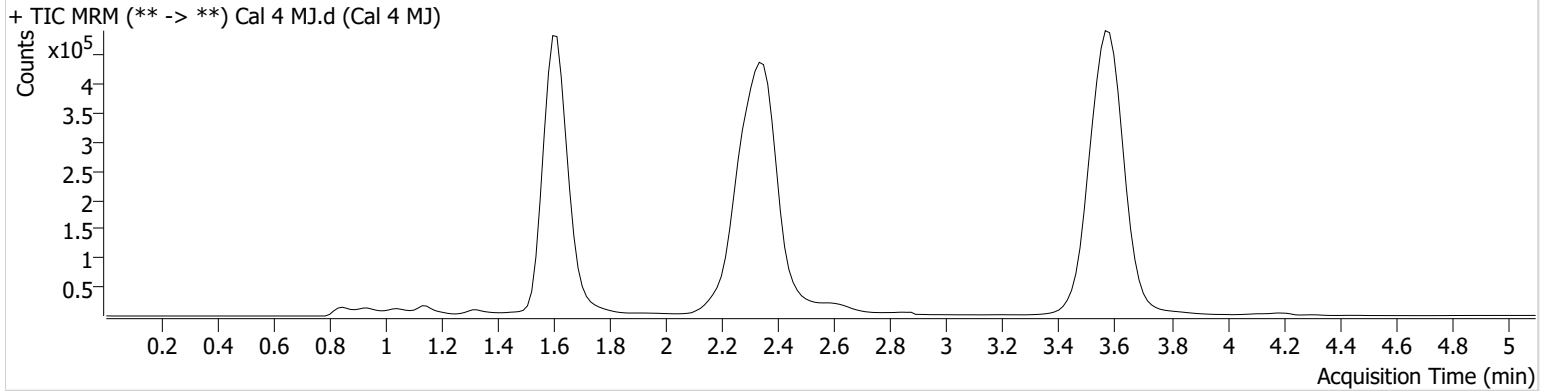


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-D1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 12:55:26 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	288830	∞	12.7	320.06	1585795	10.0740 ng/ml
THC-COOH	1.640	414257	1483.16	52.1	2218.83	321825	49.2404 ng/ml
THC	3.586	339474	453.37	28.1	∞	3816754	9.4030 ng/ml

SC

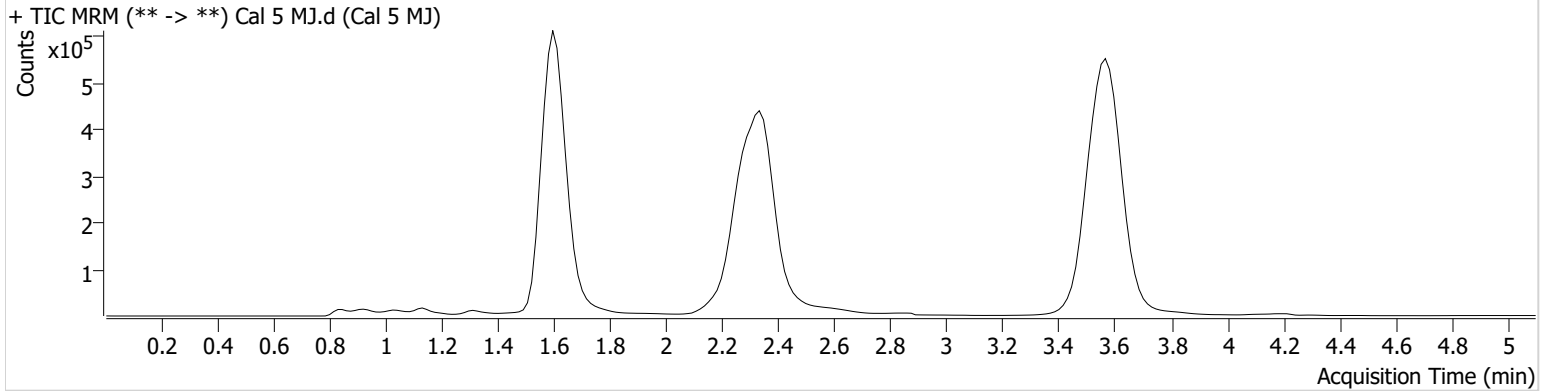


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-E1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 1:03:03 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	698600	∞	13.6	∞	1585015	24.4771 ng/ml
THC-COOH	1.625	582081	∞	54.1	∞	309132	73.3373 ng/ml
THC	3.586	855320	4374.60	27.7	∞	3680814	24.1866 ng/ml

SC

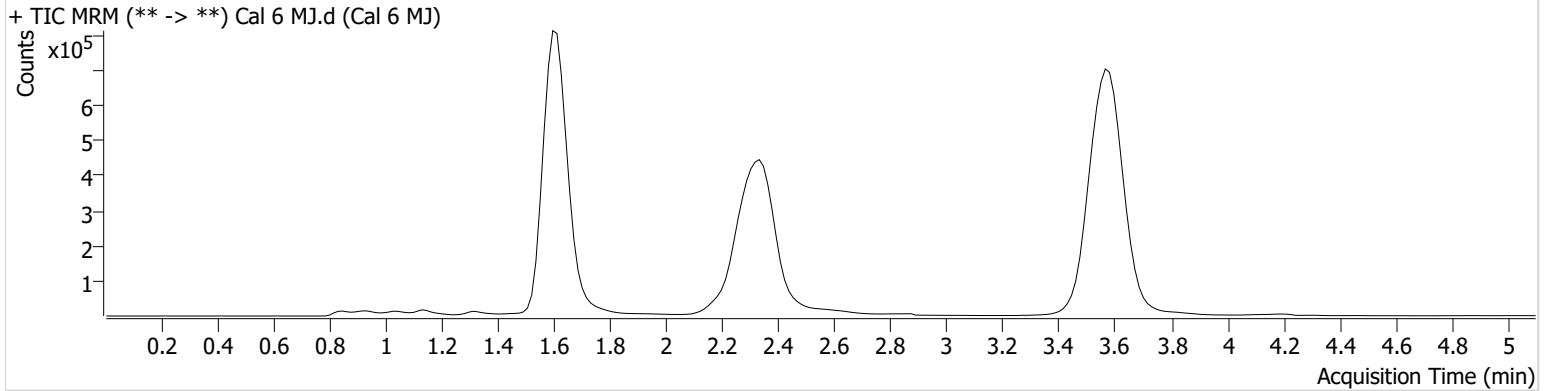


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-F1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 1:10:39 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	1427470	∞	13.6	2382.38	1588782	49.9684 ng/ml
THC-COOH	1.625	778858	∞	54.2	3655.91	308363	99.3389 ng/ml
THC	3.586	1792579	24909.82	27.4	1402.33	3733409	49.7251 ng/ml

SC

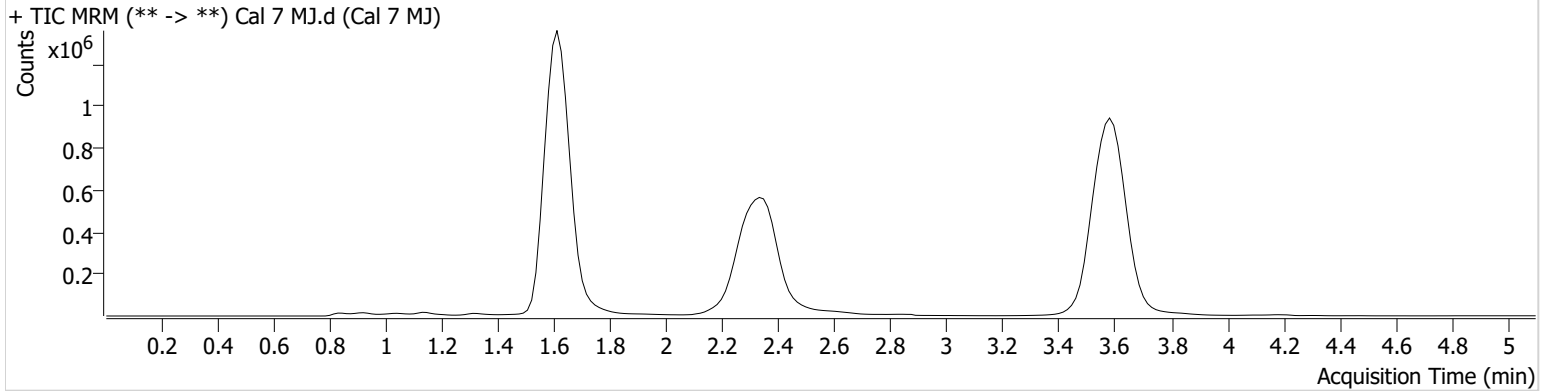


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\102022 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/21/2022 9:34:54 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-G1	Comment	
Injection Volume	10		
Acq. Date-Time	10/20/2022 1:18:14 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	2844977	∞	13.8	4869.50	1575799	100.4788 ng/ml
THC-COOH	1.640	1875211	5043.36	55.9	5643.13	296464	253.0221 ng/ml
THC	3.601	3473600	18566.54	27.8	2003.50	3523910	101.8361 ng/ml