

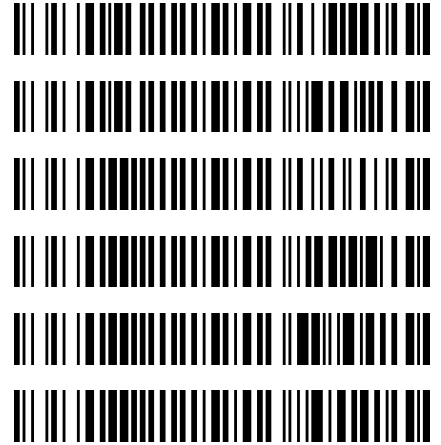
REVIEWED
By Sarah Collins at 1:03 pm, Nov 16, 2022

TS

11/14/2022

Worklist: 6161

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-4186	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-4485	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-3029	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-3223	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-3312	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-3335	1	BCK	AM 27 Blood THC Quant by LC-QQQ



TS

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

Extraction Date: 11-14-2022

Plate lot#: 220802

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 22B52015-2

Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Tamara Salazar

Plate Retest Date: 02/02/2023

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Urine Lot: N/A

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.
- 3. Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: 42**
- 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 5. Add **500µL of 0.1% formic acid in water to blood samples,** and **500µL of saturated phosphate buffer to urine samples** in the wells of the analytical plate.
- 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 7. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate. Amount transferred: 750µL
- 8. 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*)
- 9. Wait 5 minutes.
- 10. Add **2.25mL MTBE.** (*Add in 3 increments of 750uL*)
- 11. Wait 5 minutes.
- 12. Apply positive pressure for approx. 15 seconds. (*10-15 PSI- Selector to the left*).
- 13. Add **2.25mL Hexane.** (*Add in 3 increments of 750uL*)
- 14. Wait 5 minutes.
- 15. Apply positive pressure for approx. 15 seconds. (*10-15 PSI- Selector to the left*).
- 16. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
- 17. 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
THC-OH not evaluated due to possible co-eluting peak.

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	P2022-3335-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2022-3312-1	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	IS + Sample	P2022-3223-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	IS + Sample	P2022-3029-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	IS + Sample	M2022-4485-2	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	IS + Sample	M2022-4186-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	IS + Sample	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

TS

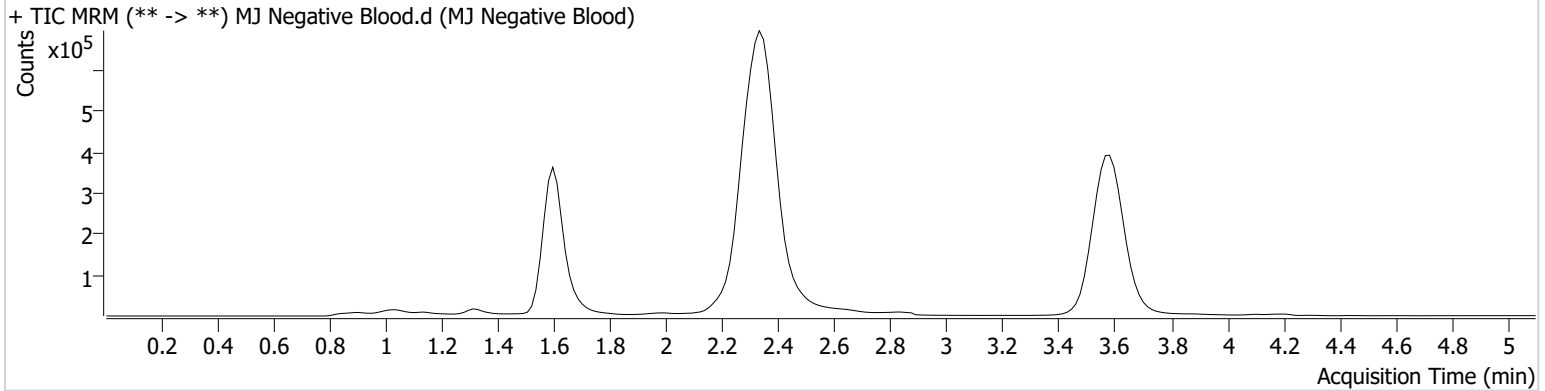


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 12:58:20 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678 High	50276	69.72	2.6 Low	25.44	1543084	0.8413 ng/ml Low

TS

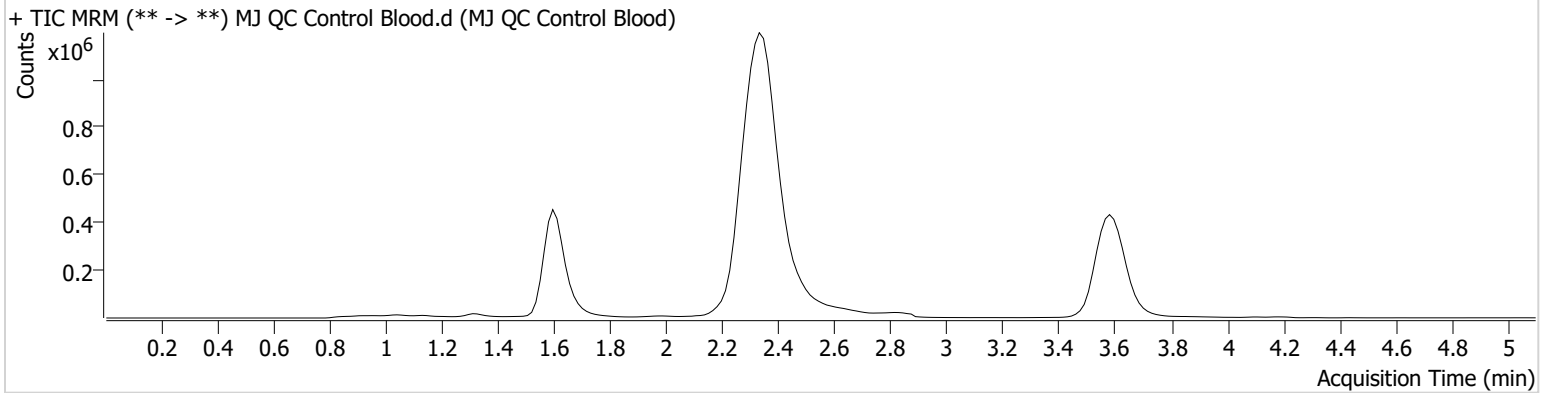


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 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	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	11/14/2022 12:43:06 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	161020	∞	9.6	∞	1702159	4.7711 ng/ml
THC-COOH	1.640	148780	∞	42.8	861.67	321272	17.3429 ng/ml
THC	3.601	147386	477.97	26.2	55.07	3104967	4.9525 ng/ml

TS

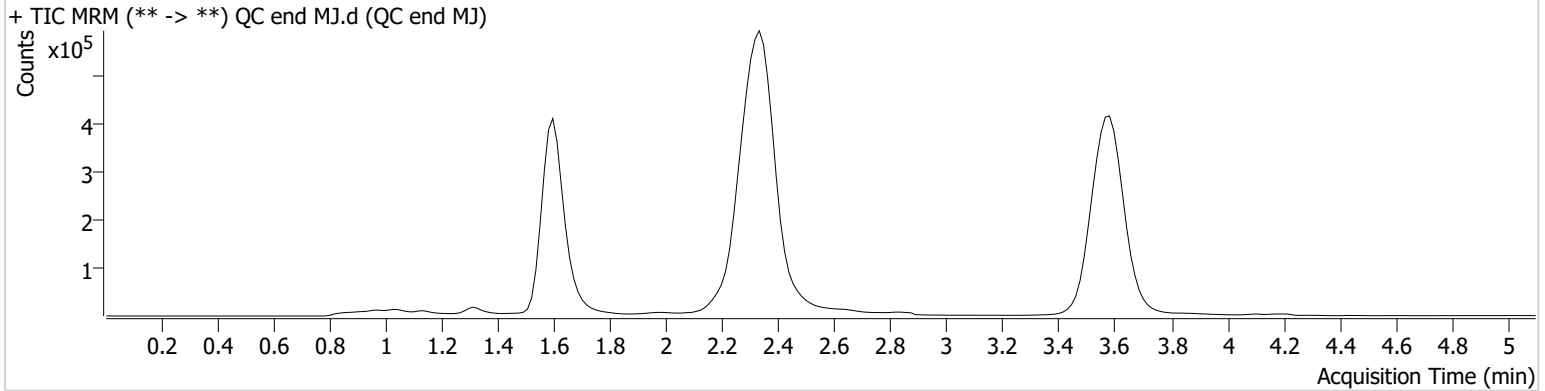


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 2:45:00 PM		

Sample Chromatogram



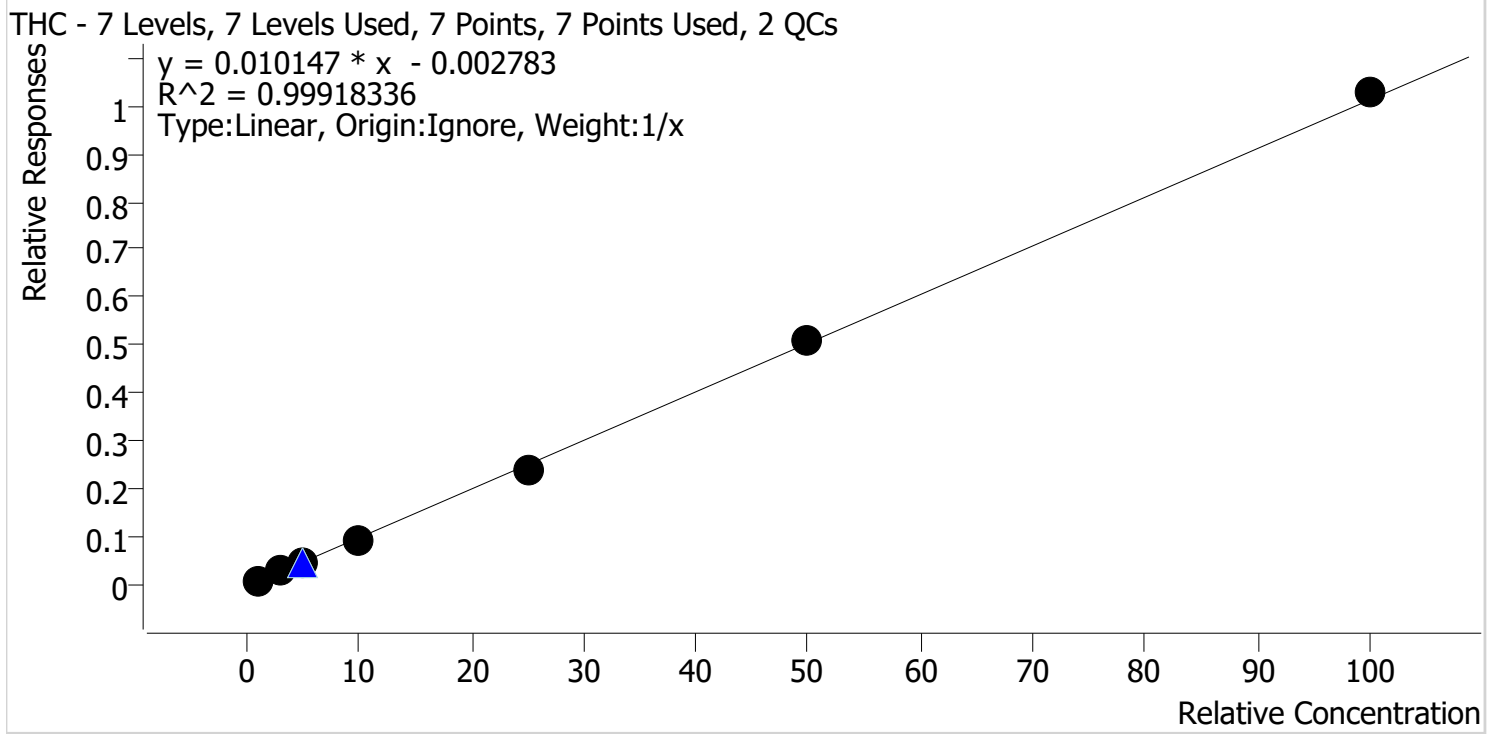
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	154847	∞	9.8	∞	1616141	4.8481 ng/ml
THC-COOH	1.625	114193	∞	52.5	456.10	311225	13.1514 ng/ml
THC	3.601	150916	866.19	25.7	∞	3272623	4.8191 ng/ml

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 11/15/2022 10:10 AM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3



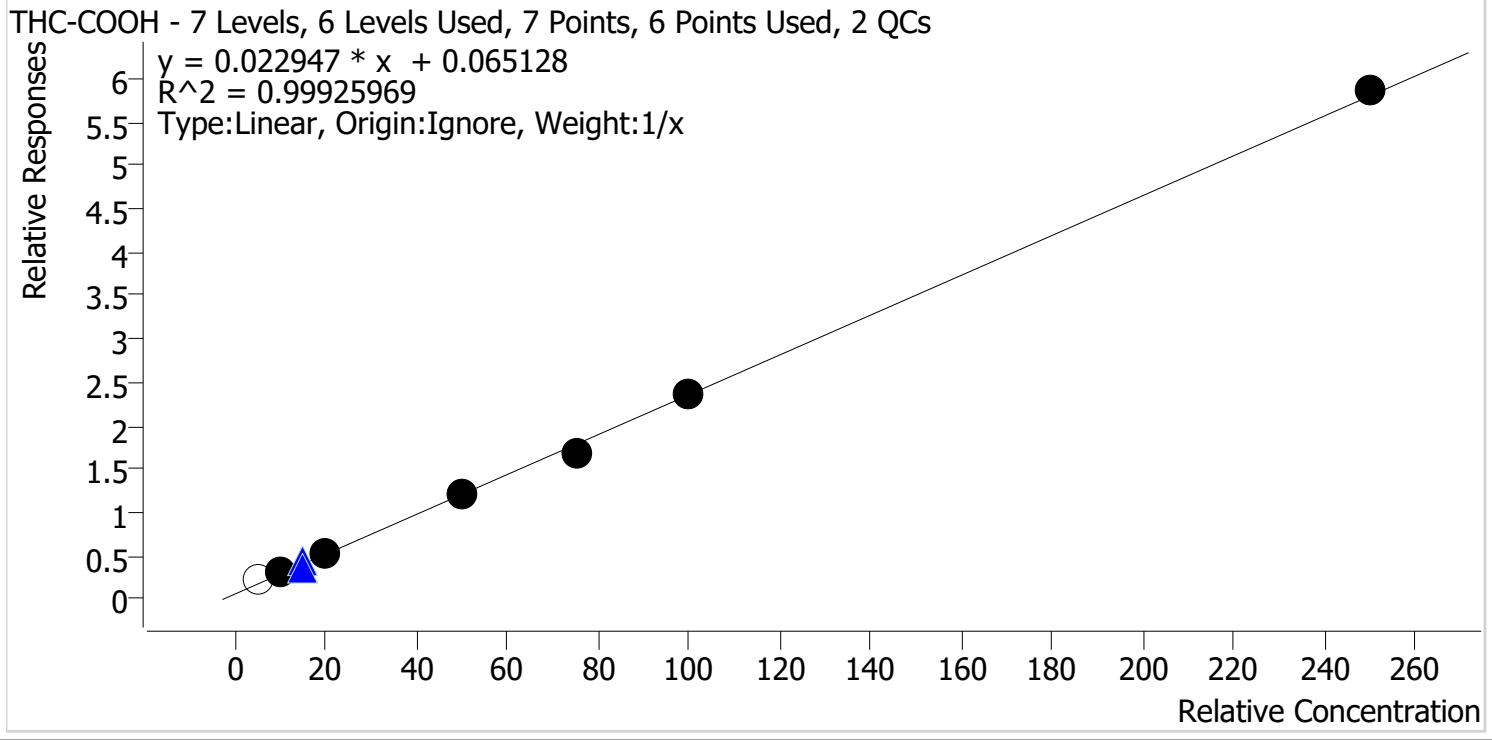
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.2	115.7
Cal 2 MJ	2	✓	3.0	2.9	98.3
Cal 3 MJ	3	✓	5.0	4.7	93.2
Cal 4 MJ	4	✓	10.0	9.4	94.4
Cal 5 MJ	5	✓	25.0	24.1	96.4
Cal 6 MJ	6	✓	50.0	50.2	100.4
Cal 7 MJ	7	✓	100.0	101.5	101.5

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 11/15/2022 10:10 AM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	5.0	7.6	152.3
Cal 2 MJ	2	✓	10.0	10.5	104.8
Cal 3 MJ	3	✓	20.0	19.7	98.4
Cal 4 MJ	4	✓	50.0	49.7	99.5
Cal 5 MJ	5	✓	75.0	71.4	95.2
Cal 6 MJ	6	✓	100.0	101.0	101.0
Cal 7 MJ	7	✓	250.0	252.7	101.1

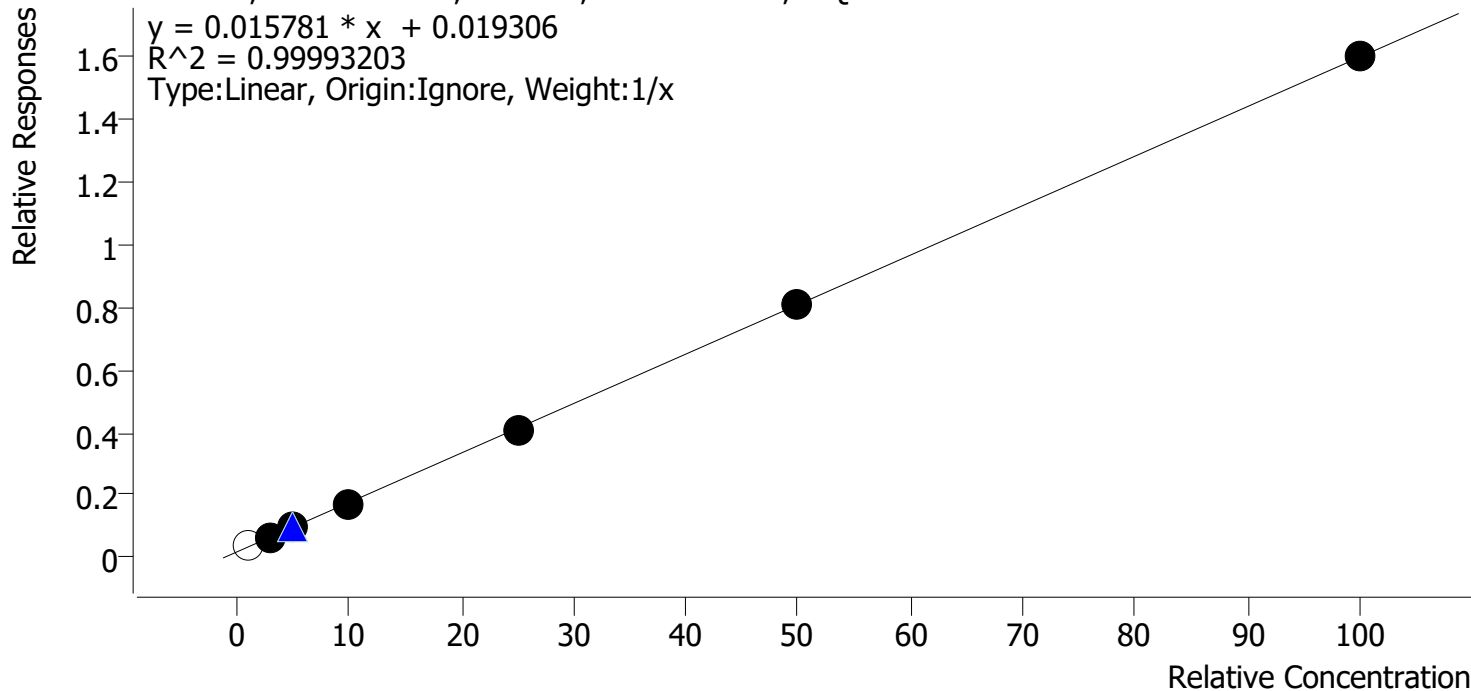
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 11/15/2022 10:10 AM
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
Cal 1 MJ	1	x	1.0	1.5	146.4
Cal 2 MJ	2	✓	3.0	3.1	103.5
Cal 3 MJ	3	✓	5.0	4.9	97.8
Cal 4 MJ	4	✓	10.0	9.9	98.9
Cal 5 MJ	5	✓	25.0	24.8	99.3
Cal 6 MJ	6	✓	50.0	50.2	100.5
Cal 7 MJ	7	✓	100.0	100.1	100.1

Compound not evaluated.

TS

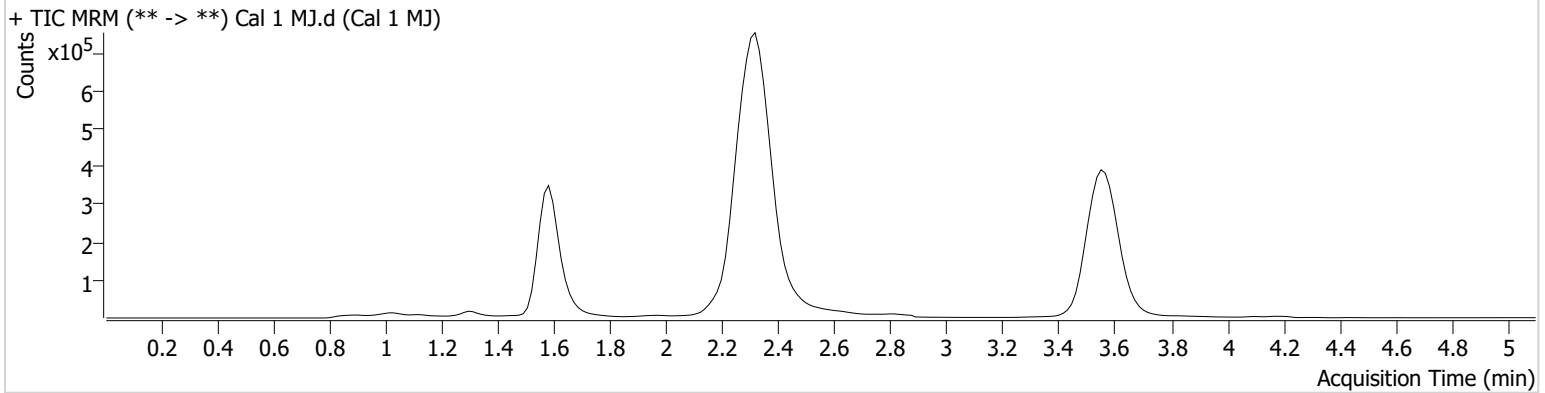


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 11:42:06 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	62179	∞	6.2 Low	19.59	1466006	1.4643 ng/ml Low
THC-COOH	1.625	68416	177.71	27.5 Low	331.88	285243	7.6142 ng/ml
THC	3.570	27775	115.78	29.7	22.45	3099905	1.1573 ng/ml

TS

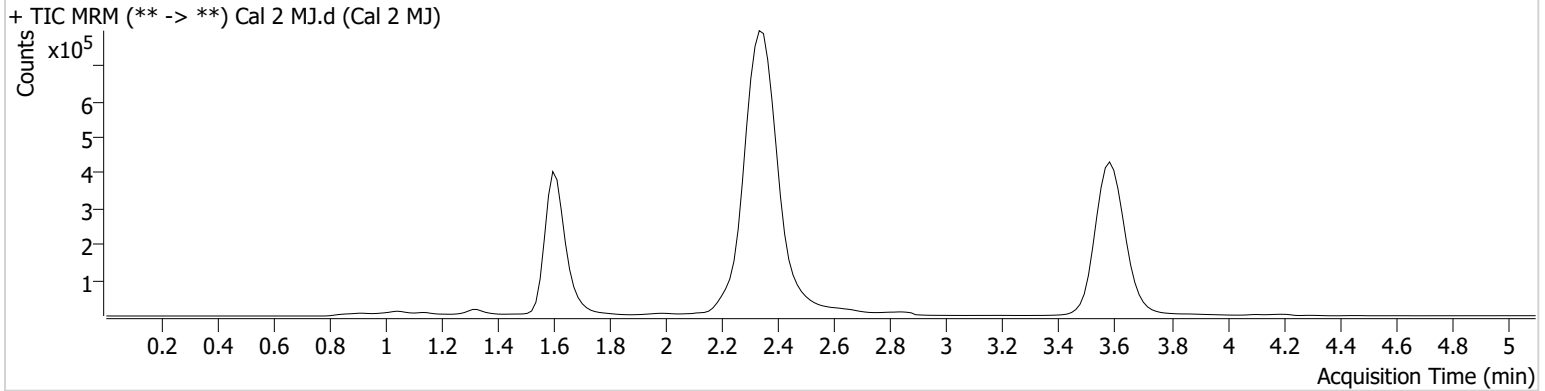


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 11:49:52 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	103687	∞	9.6	265.19	1518227	3.1043 ng/ml
THC-COOH	1.640	89853	286.56	42.9	322.07	293935	10.4834 ng/ml
THC	3.601	85039	190.24	25.9	78.12	3132291	2.9500 ng/ml

TS



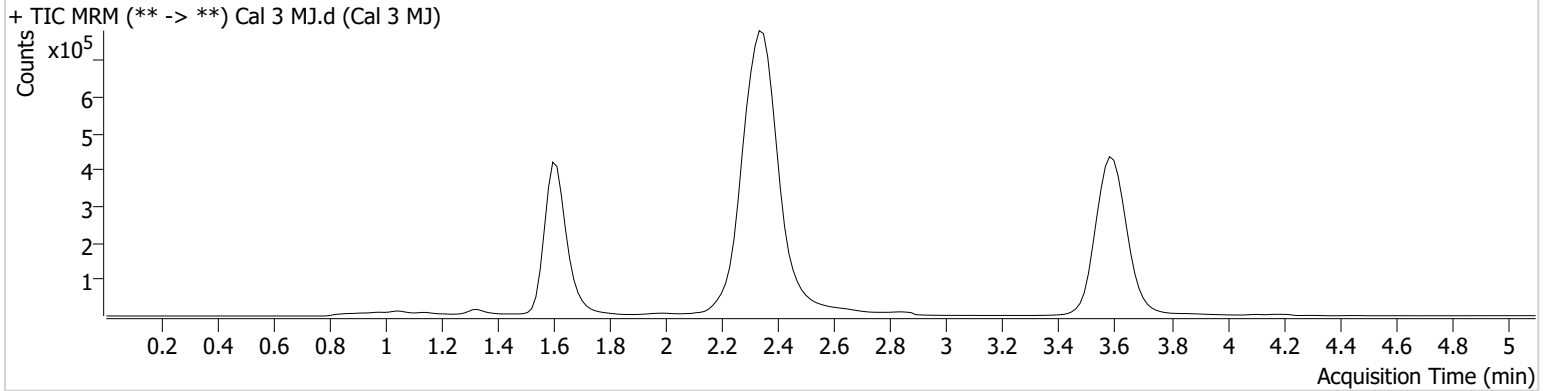
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 11:57:28 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	151251	∞	10.4	∞	1567428	4.8914 ng/ml
THC-COOH	1.640	156689	∞	50.5	913.13	303323	19.6736 ng/ml
THC	3.601	142670	260.64	27.4	77.99	3205963	4.6601 ng/ml

TS



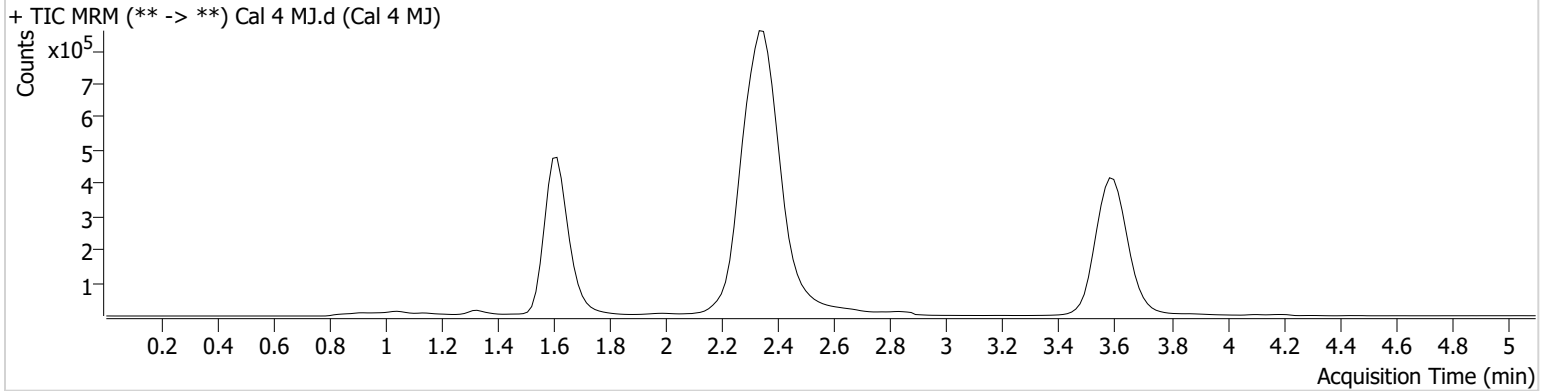
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 12:05:04 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	279536	∞	11.0	1396.31	1593851	9.8903 ng/ml
THC-COOH	1.640	369681	∞	55.0	∞	306422	49.7373 ng/ml
THC	3.601	283919	4356.25	25.6	∞	3051851	9.4430 ng/ml

TS

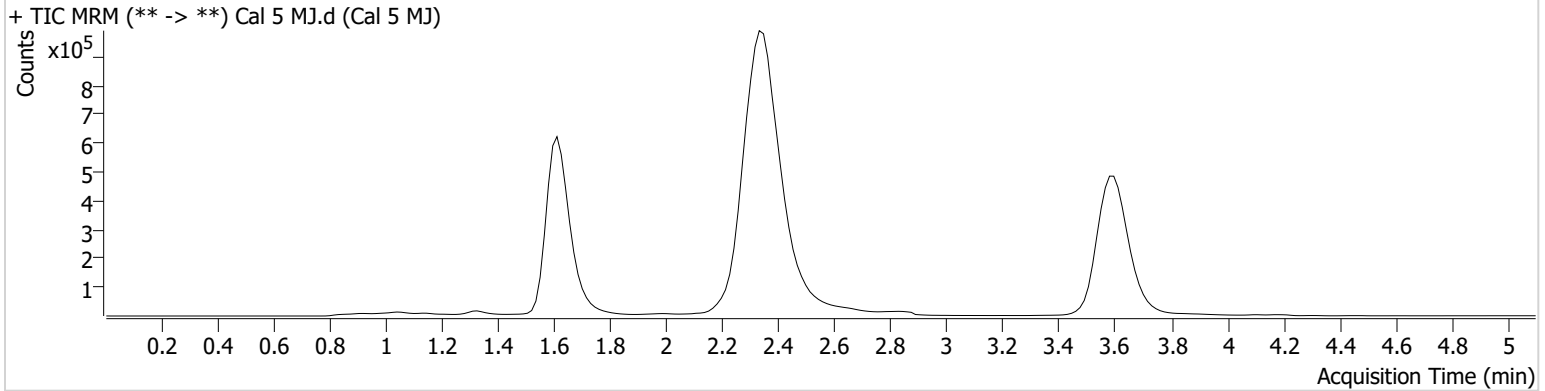


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 12:12:40 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	666420	∞	11.8	∞	1621729	24.8163 ng/ml
THC-COOH	1.640	539340	∞	56.4	∞	316485	71.4269 ng/ml
THC	3.601	726843	6523.22	24.1	1004.92	3007098	24.0957 ng/ml

TS

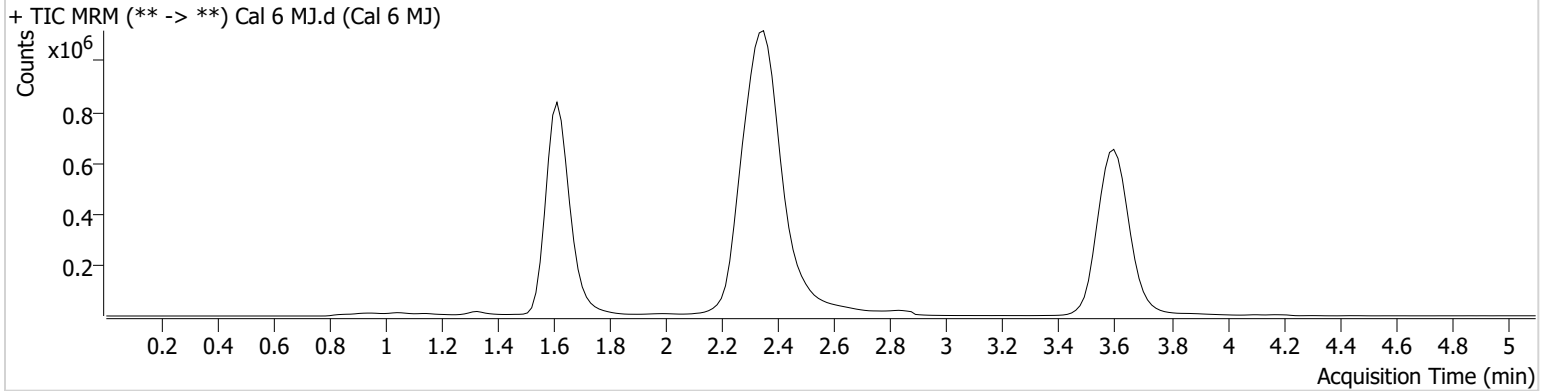


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 12:20:16 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	1393862	∞	12.2	2497.44	1716637	50.2293 ng/ml
THC-COOH	1.640	756962	∞	56.2	1017.14	317599	101.0272 ng/ml
THC	3.601	1625825	11964.72	24.1	756.83	3207852	50.2241 ng/ml

TS

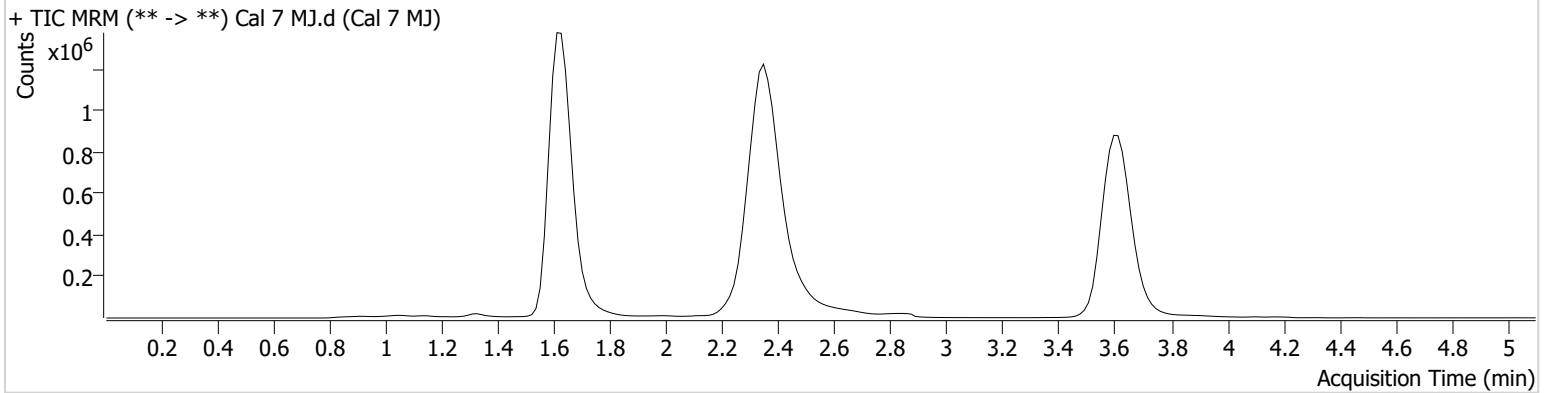


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\111422 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 11/15/2022 10:10:43 AM

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	11/14/2022 12:27:52 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	2633041	∞	12.3	15002.1 0	1647212	100.0684 ng/ml
THC-COOH	1.640	1713969	∞	57.9	∞	292351	252.6517 ng/ml
THC	3.616	2971923	23251.09	25.4	∞	2894344	101.4698 ng/ml