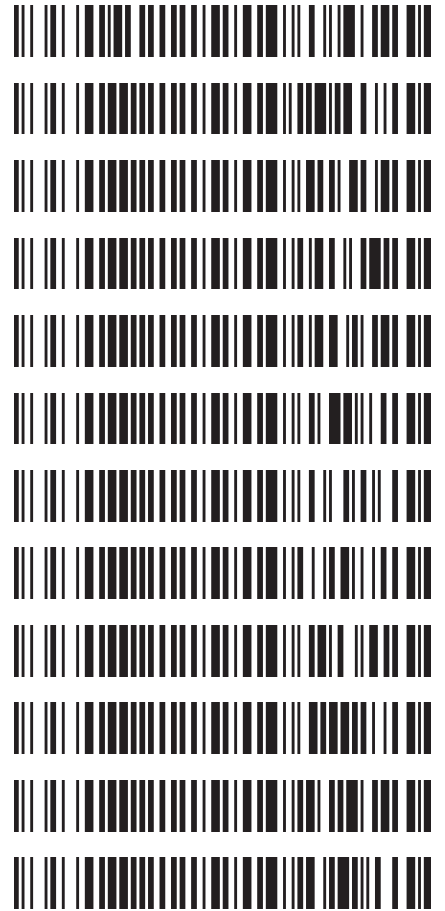


TS

Worklist: 6679

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2024-0282	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0058	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0071	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0083	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0085	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0196	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0205	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0225	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0248	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0279	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0325	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0327	1	BCK	AM 27 Blood THC Quant by LC-QQQ



TS

Worklist: 6683

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
P2023-3861	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0042	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: 02/12/2024

Plate lot#: 231212

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 23E52981

LCMS-QQQ ID: 069901

Analyst: Celena Shrum

Plate Retest Date: 06/12/2024

Mobile phase B: 0.1% Formic acid in Acetonitrile

Column: Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

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 (if applicable): 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 or 1000µl hydrolyzed urine** into the appropriate wells of the 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 or 500µl of saturated phosphate buffer to urine samples** to the appropriate wells of the analytical plate.
- 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 7. Transfer **800µL of blood+acid mixture or urine+acid** to corresponding wells of SLE+ plate.
- 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) Manifold ID: 067104
- 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.
SPE Dry ID: 067103
- 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. THC concentrations of 1-3ng/mL will be reported qualitatively.
- 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: Tamara Salazar had 2 samples included in this run. Tamara pipetted her samples into the plates. Celena Shrum acted as the primary analyst and performed steps 4-16. I, Tamara Salazar approve of all steps utilized in this method. TS

CS
TS

	1	2	3	4	5	6
A	IS + Cal. 1	QC2	P2024-0205-1	P2024-0083-1*		
B	IS + Cal. 2	NEG Blood	P2024-0225-1	P2024-0196-1*		
C	IS + Cal. 3	M2024-0282-2	P2024-0248-1	P2024-0042-1 TS*		
D	IS + Cal. 4	P2024-0058-1	P2024-0279-1			
E	IS + Cal. 5	P2024-0071-2	P2024-0325-1			
F	IS + Cal. 6	P2024-0083-1*	P2024-0327-1			
G	IS + Cal. 7	P2024-0085-1	P2024-3861-1 TS			
H	QC1	P2024-0196-1*	P2024-0042-1 TS*			

*Moved during the SLE portion of the extraction due to clotting

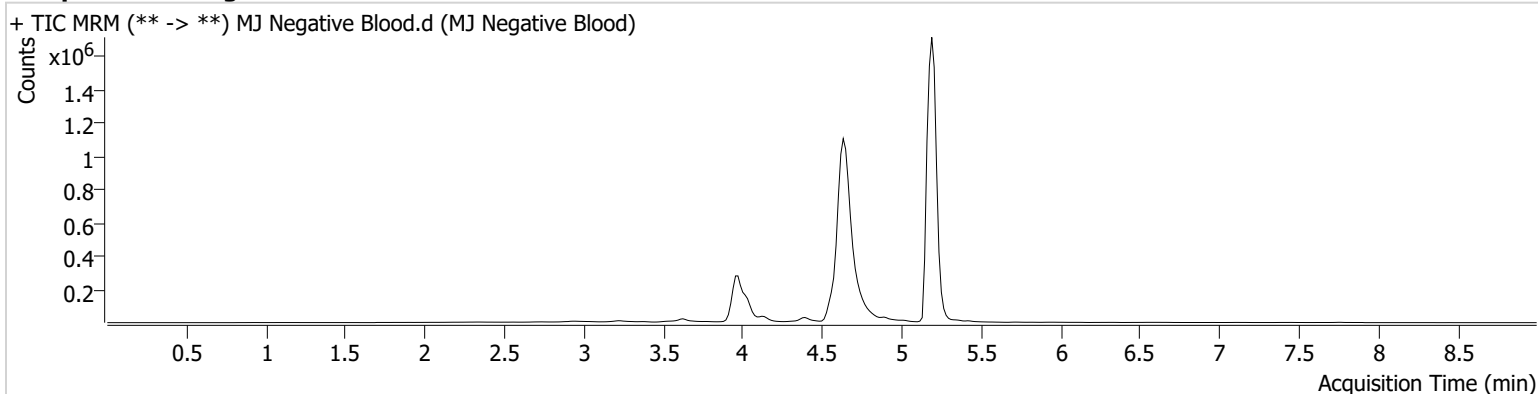


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 Agilent Method.m	Operator	Celena Shrum
Sample Position	P1-B2	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.
Injection Volume	10		
Acq. Date-Time	2/12/2024 5:52:38 PM		
Sample Info.			

Sample Chromatogram





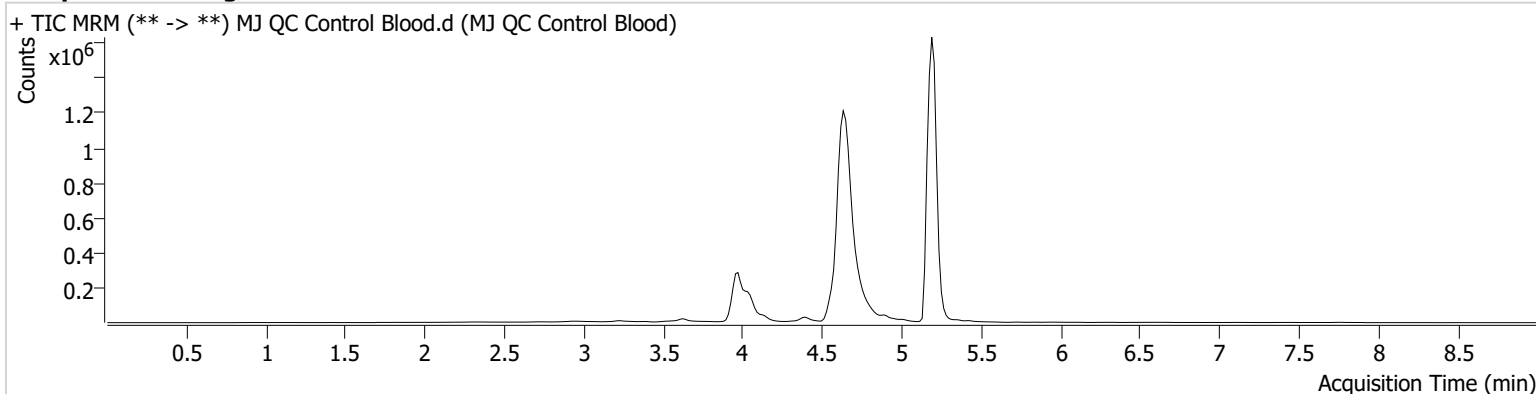
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument Falco (069901) **Data File** MJ QC Control Blood.d
Type QC **Sample** MJ QC Control Blood
Acq. Method AM 27 Agilent Method.m **Operator** Celena Shrum
Sample Position P1-H1 **Comment**
Injection Volume 10
Acq. Date-Time 2/12/2024 5:26:26 PM
Sample Info.

Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.195	277232	∞	24.8	∞	6220138	5.1007 ng/ml
THC-COOH	4.045	35455	∞	219.2	4137.54	380453	14.0815 ng/ml
THC-OH	3.971	97754	∞	12.5	∞	1122840	5.0844 ng/ml

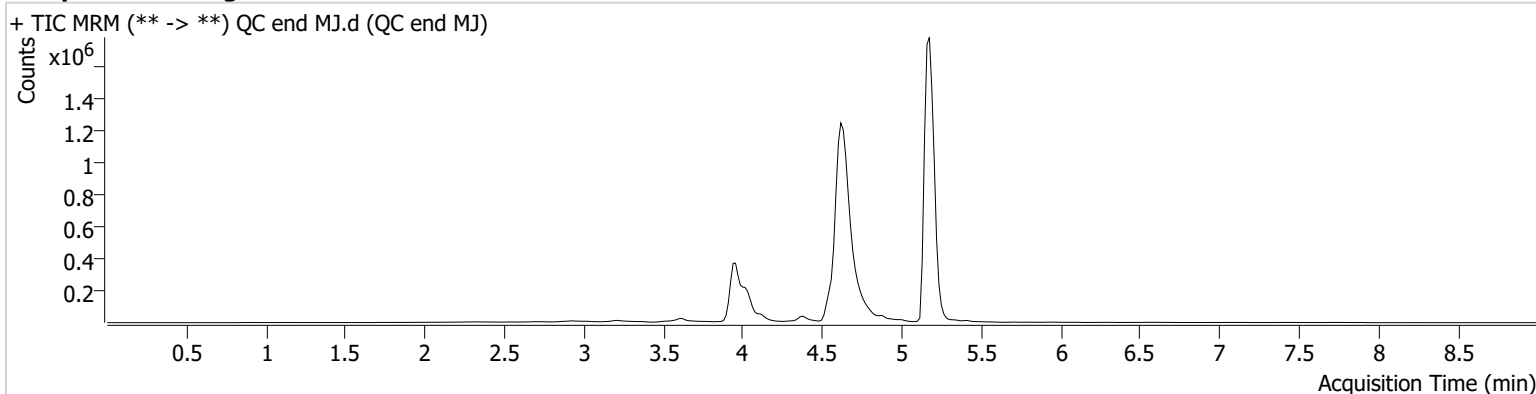


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Celena Shrum
Sample Position	P1-A2	Comment	<p style="color: red;">Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.</p>
Injection Volume	10		
Acq. Date-Time	2/13/2024 12:26:00 AM		
Sample Info.			

Sample Chromatogram



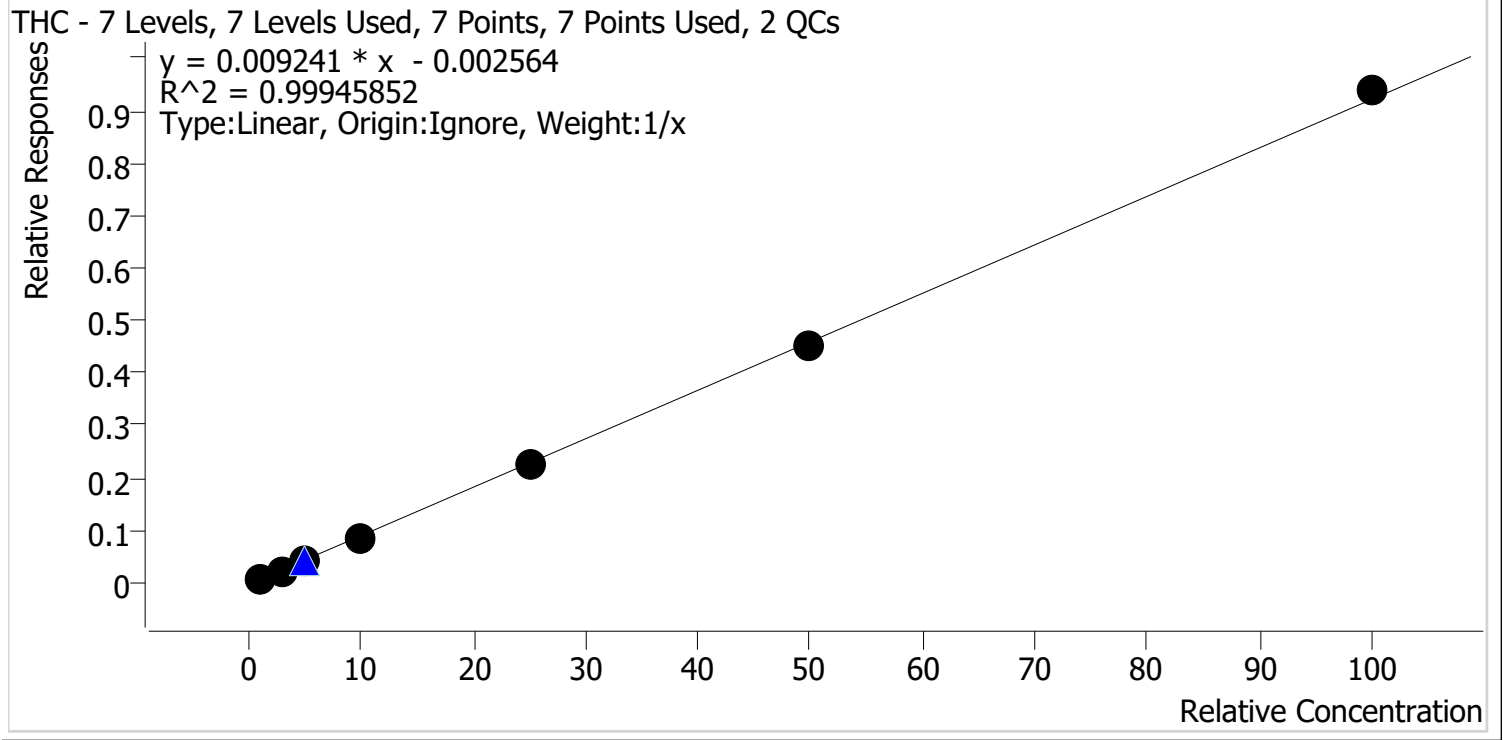
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	317089	2977.90	24.7	∞	7111422	5.1027 ng/ml
THC-COOH	4.030	43660	249.69	230.7	∞	455741	14.4493 ng/ml
THC-OH	3.956	118749	80.99	13.5	∞	1426548	4.8618 ng/ml

TSCS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 2/15/2024 9:31 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	1.1	112.2
Cal 2 MJ	2	✓	3.0	2.9	96.3
Cal 3 MJ	3	✓	5.0	4.8	96.4
Cal 4 MJ	4	✓	10.0	9.7	96.7
Cal 5 MJ	5	✓	25.0	24.5	98.1
Cal 6 MJ	6	✓	50.0	49.2	98.4
Cal 7 MJ	7	✓	100.0	101.8	101.8

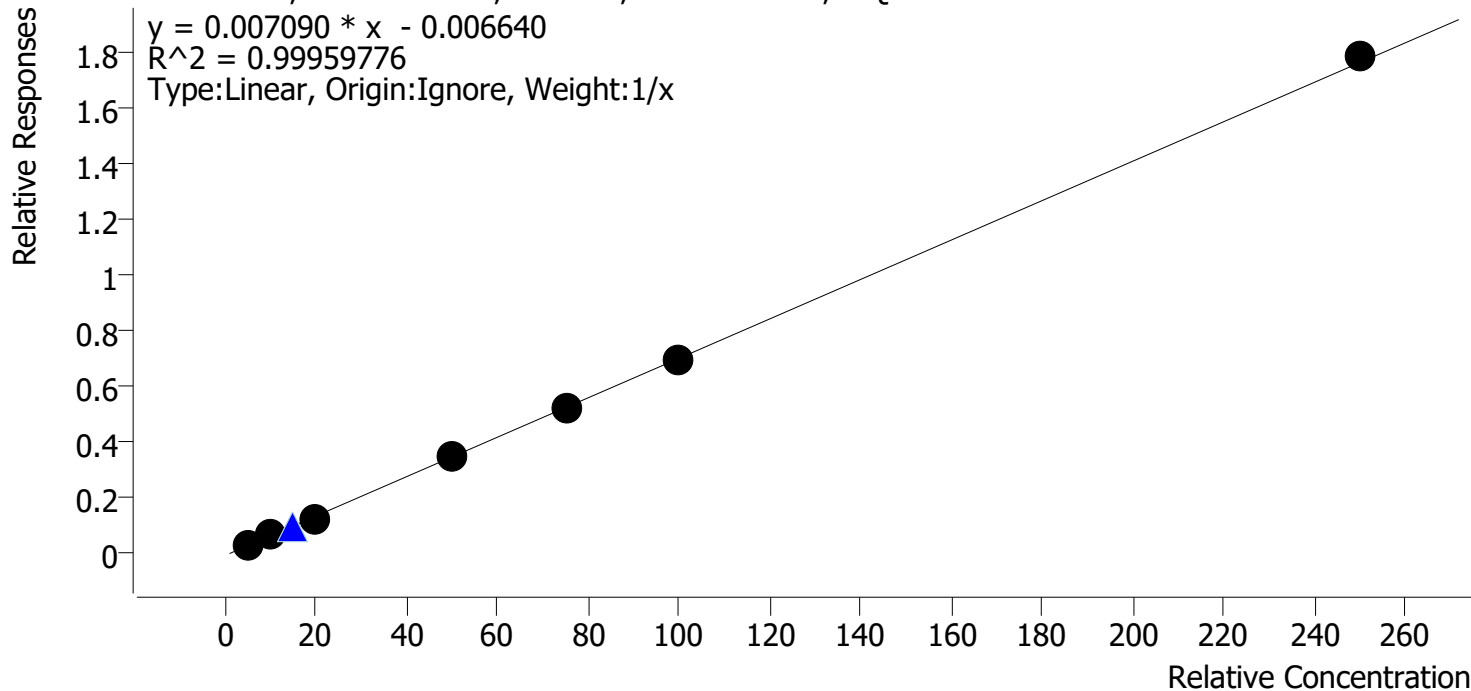
TSQ



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 2/15/2024 9:31 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



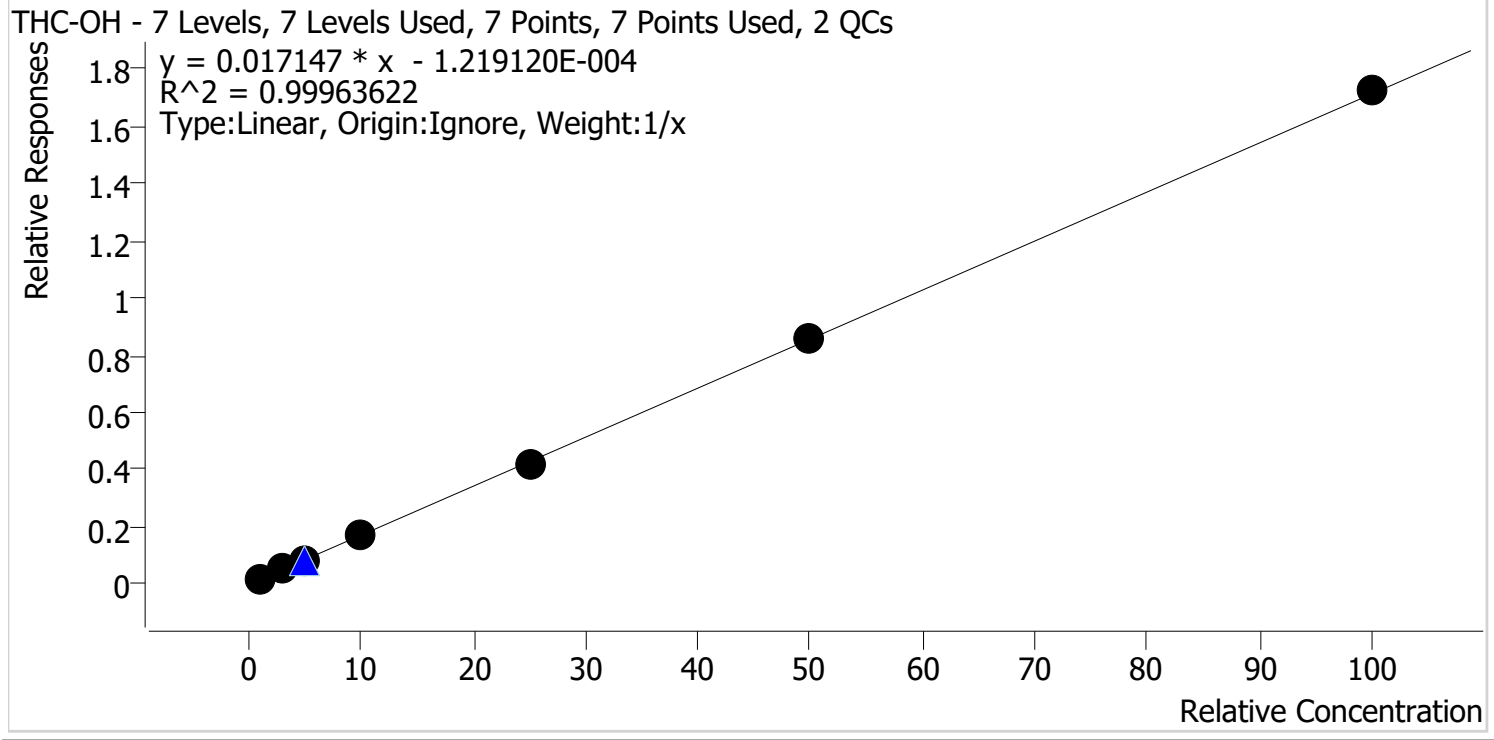
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.4	108.5
Cal 2 MJ	2	✓	10.0	9.9	98.9
Cal 3 MJ	3	✓	20.0	18.9	94.5
Cal 4 MJ	4	✓	50.0	49.3	98.6
Cal 5 MJ	5	✓	75.0	74.5	99.3
Cal 6 MJ	6	✓	100.0	99.0	99.0
Cal 7 MJ	7	✓	250.0	253.0	101.2

TSCS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 2/15/2024 9:31 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	112.0
Cal 2 MJ	2	✓	3.0	2.9	96.3
Cal 3 MJ	3	✓	5.0	4.7	94.2
Cal 4 MJ	4	✓	10.0	9.9	98.6
Cal 5 MJ	5	✓	25.0	24.4	97.5
Cal 6 MJ	6	✓	50.0	50.4	100.8
Cal 7 MJ	7	✓	100.0	100.6	100.6

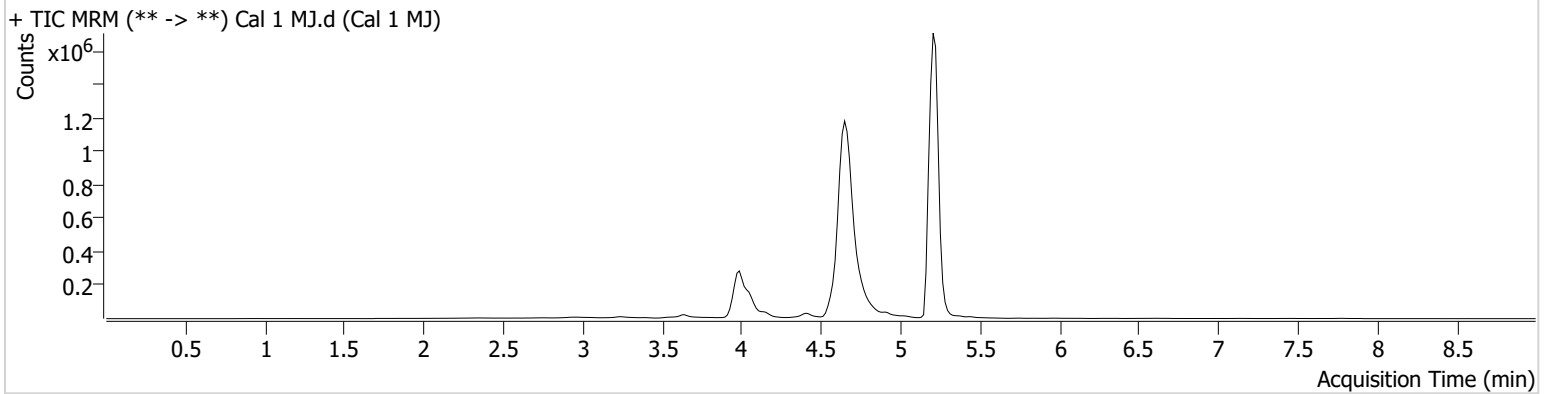


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Celena Shrum
Sample Position	P1-A1	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.
Injection Volume	10		
Acq. Date-Time	2/12/2024 3:41:23 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.225	53827	883.94	29.0	∞	6894959	1.1223 ng/ml
THC-COOH	4.075	12920	∞	224.5	∞	406157	5.4236 ng/ml
THC-OH	3.986	23289	∞	11.1	14.57	1220418	1.1200 ng/ml

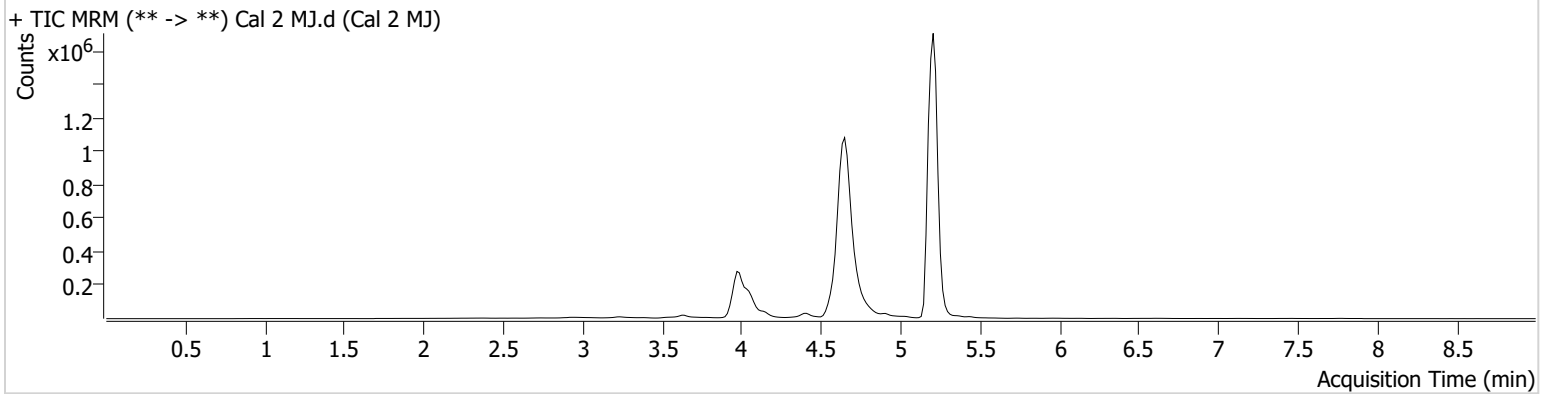


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Celena Shrum
Sample Position	P1-B1	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.
Injection Volume	10		
Acq. Date-Time	2/12/2024 3:54:39 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.210	165700	2385.67	25.6	∞	6863284	2.8901 ng/ml
THC-COOH	4.060	25188	∞	212.7	∞	396879	9.8886 ng/ml
THC-OH	3.986	58569	73.48	13.2	∞	1185672	2.8880 ng/ml



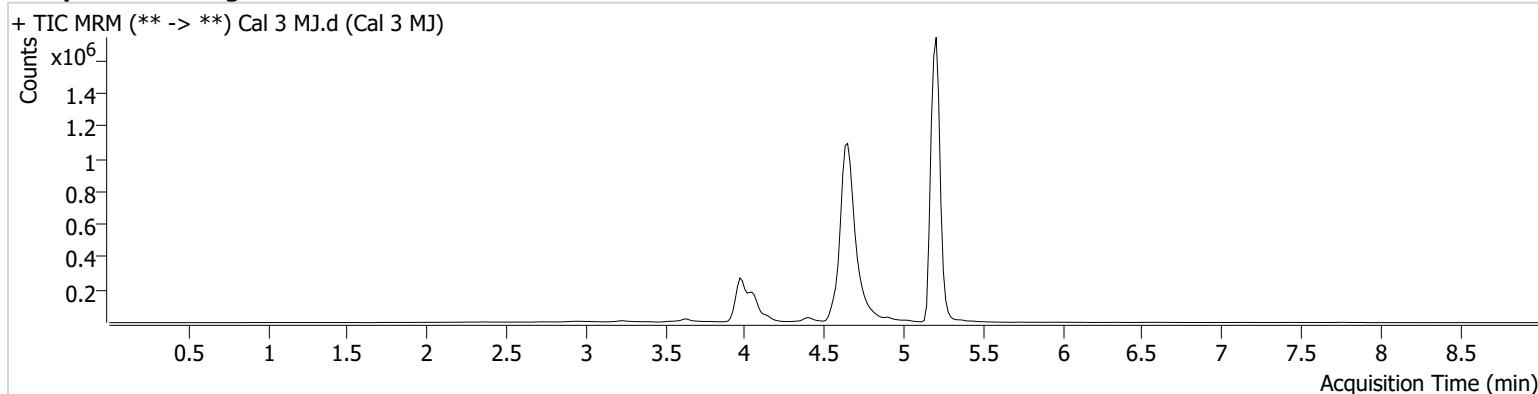
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument Falco (069901) **Data File** Cal 3 MJ.d
Type Cal **Sample** Cal 3 MJ
Acq. Method AM 27 Agilent Method.m **Operator** Celena Shrum
Sample Position P1-C1 **Comment**
Injection Volume 10
Acq. Date-Time 2/12/2024 4:07:45 PM
Sample Info.

Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.210	277445	2226.66	25.2	∞	6607663	4.8213 ng/ml
THC-COOH	4.060	48074	418.48	216.1	∞	377578	18.8958 ng/ml
THC-OH	3.986	86048	183.54	13.4	∞	1067068	4.7100 ng/ml

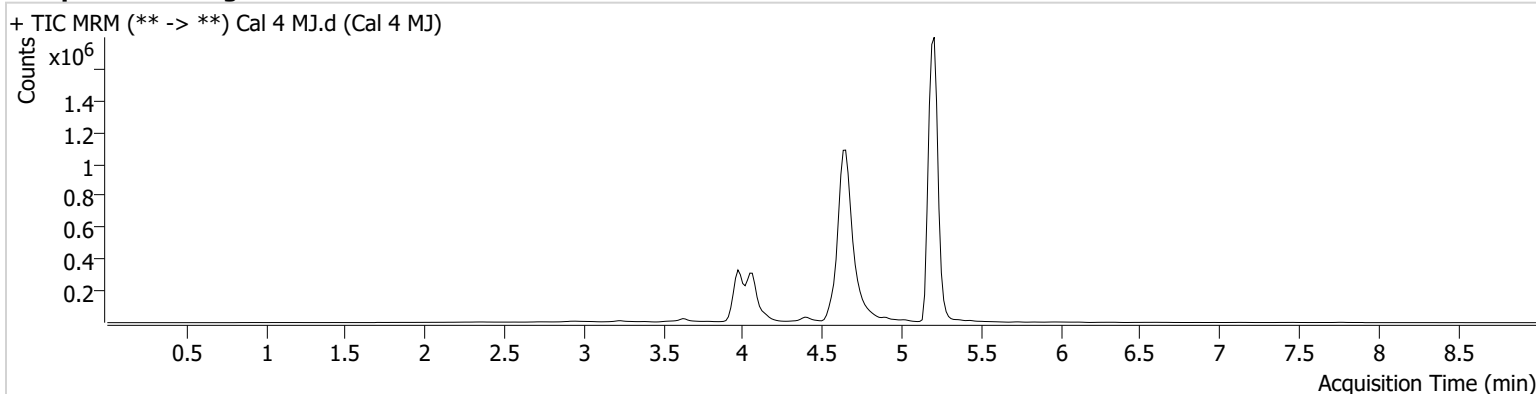


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Celena Shrum
Sample Position	P1-D1	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.
Injection Volume	10		
Acq. Date-Time	2/12/2024 4:20:53 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.210	582939	∞	24.7	∞	6714154	9.6730 ng/ml
THC-COOH	4.060	134471	∞	213.7	1428.58	391999	49.3232 ng/ml
THC-OH	3.986	199255	146.47	13.2	∞	1179805	9.8567 ng/ml

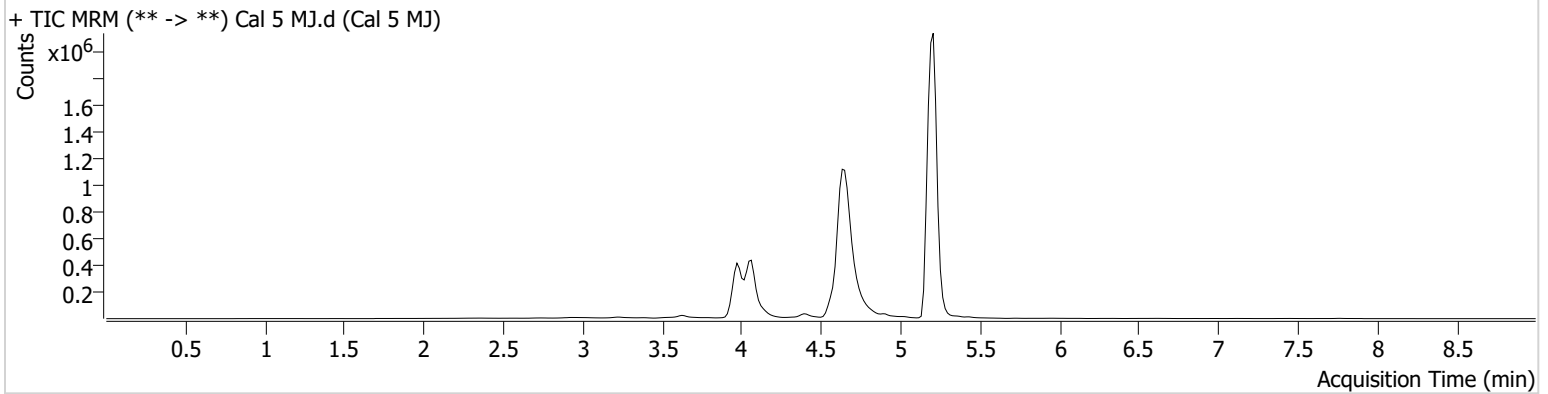


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Celena Shrum
Sample Position	P1-E1	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.
Injection Volume	10		
Acq. Date-Time	2/12/2024 4:33:59 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.210	1539698	∞	25.1	∞	6869142	24.5338 ng/ml
THC-COOH	4.060	204301	1925.62	215.4	∞	391821	74.4834 ng/ml
THC-OH	3.971	502618	∞	13.4	1168.43	1202618	24.3811 ng/ml



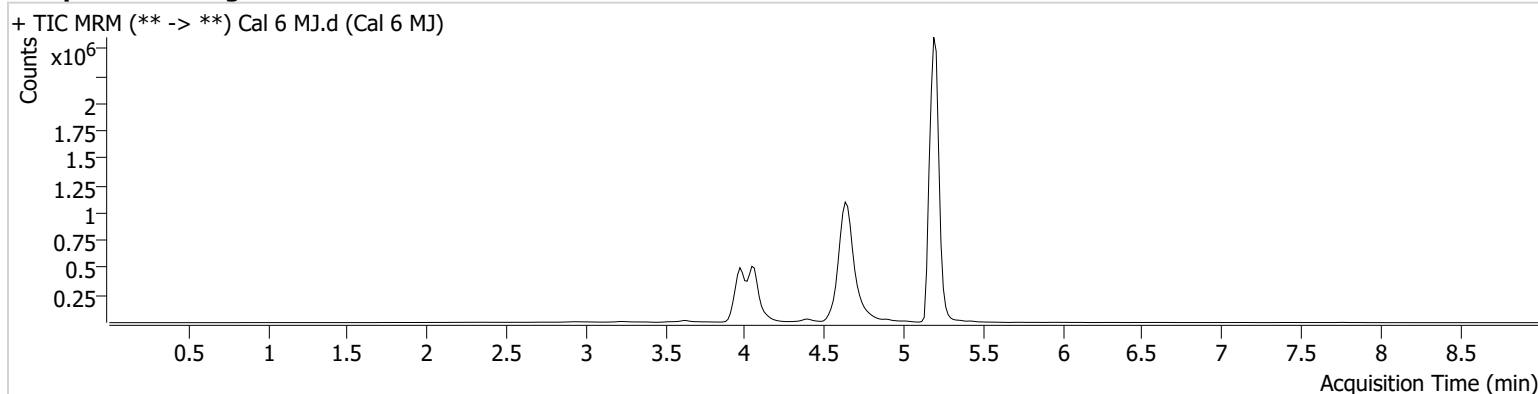
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument Falco (069901) **Data File** Cal 6 MJ.d
Type Cal **Sample** Cal 6 MJ
Acq. Method AM 27 Agilent Method.m **Operator** Celena Shrum
Sample Position P1-F1 **Comment**
Injection Volume 10
Acq. Date-Time 2/12/2024 4:47:05 PM
Sample Info.

Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.210	3067126	∞	25.5	∞	6786529	49.1850 ng/ml
THC-COOH	4.060	257609	1340.91	229.2	∞	370466	99.0195 ng/ml
THC-OH	3.971	990585	∞	13.3	∞	1146409	50.4000 ng/ml

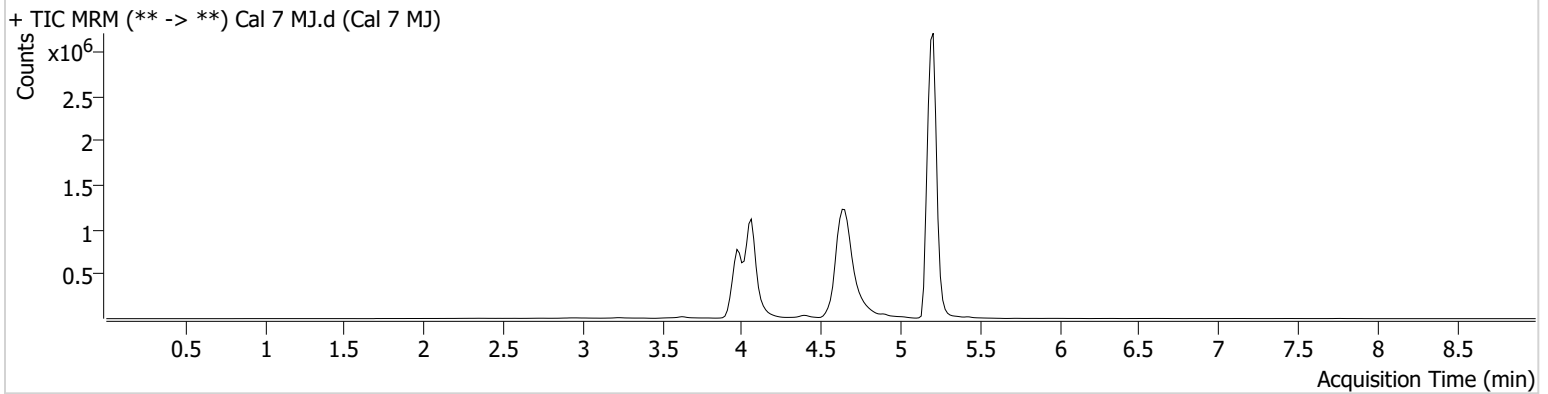


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\021224 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 2/15/2024 9:31:43 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Celena Shrum
Sample Position	P1-G1	Comment	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.
Injection Volume	10		
Acq. Date-Time	2/12/2024 5:00:12 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.210	5633678	∞	25.0	∞	6006628	101.7745 ng/ml
THC-COOH	4.060	635253	∞	216.5	∞	355530	252.9659 ng/ml
THC-OH	3.971	2048572	1589.58	13.8	∞	1187162	100.6443 ng/ml