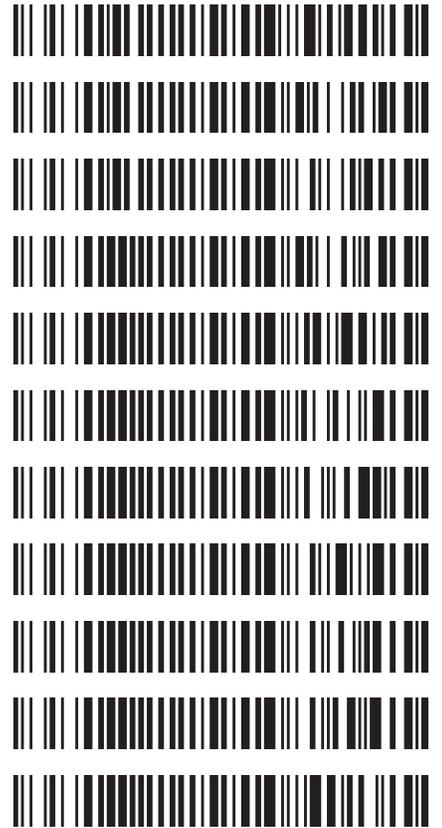




Worklist: 6640

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-4910	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-5344	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-5430	6	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3869	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3888	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3916	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3947	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3957	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3965	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3967	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0007	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: 01/05/2024

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: Curve limits: THC-OH 3-100

8

	1	2	3	4	5	6
a					P2023-3916-1	QC 1
b					P2023-3888-1	cal 100 ng
c					P2023-3869-1	cal 50 ng
d				P2024-0007-1	M2023-5430-6	cal 25 ng
e				P2023-3967-1	M2023-5344-1	cal 10ng
f				P2023-3965-1	M2023-4910-1	cal 5 ng
g				P2023-3957-1	NEG Blood	cal 3 ng
h				P2023-3947-3	QC 2	cal 1ng



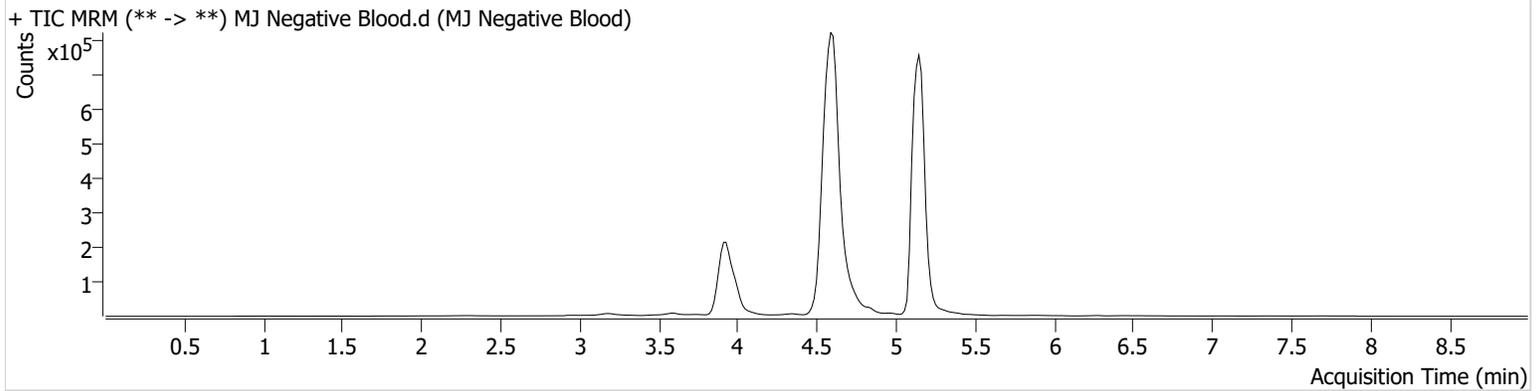
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-G5 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 4:19:57 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-OH	4.076 High	17017	∞	4.0 Low	12.86	1060053	1.5363 ng/ml



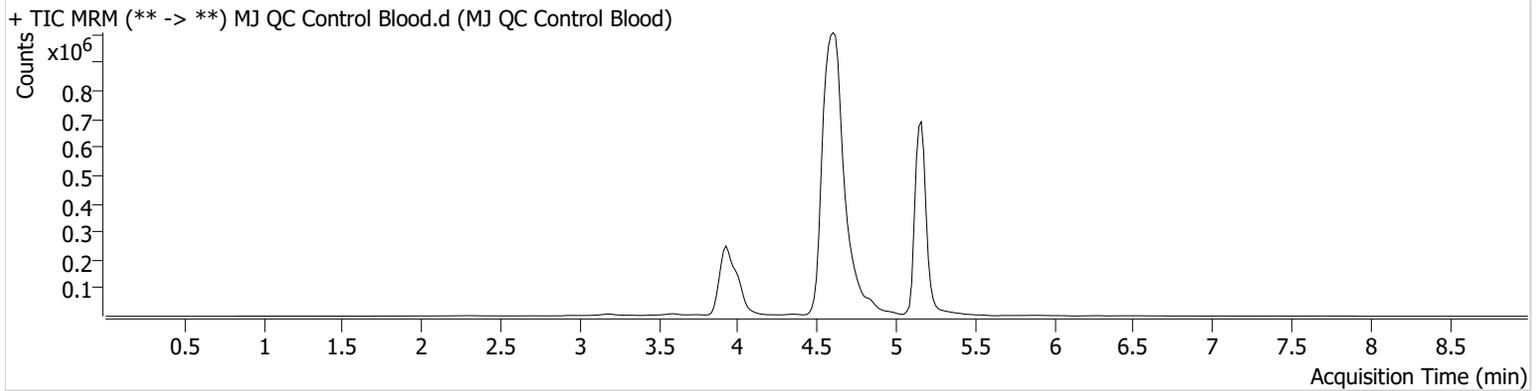
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-A6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 3:53:44 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	5.165	139092	∞	26.0	∞	2894477	5.1736 ng/ml
THC-COOH	4.000	38024	621.26	217.4	∞	377841	14.5724 ng/ml
THC-OH	3.926	77701	∞	14.4	∞	1097952	4.6230 ng/ml



AM #27 Cannabinoids Quant. Results

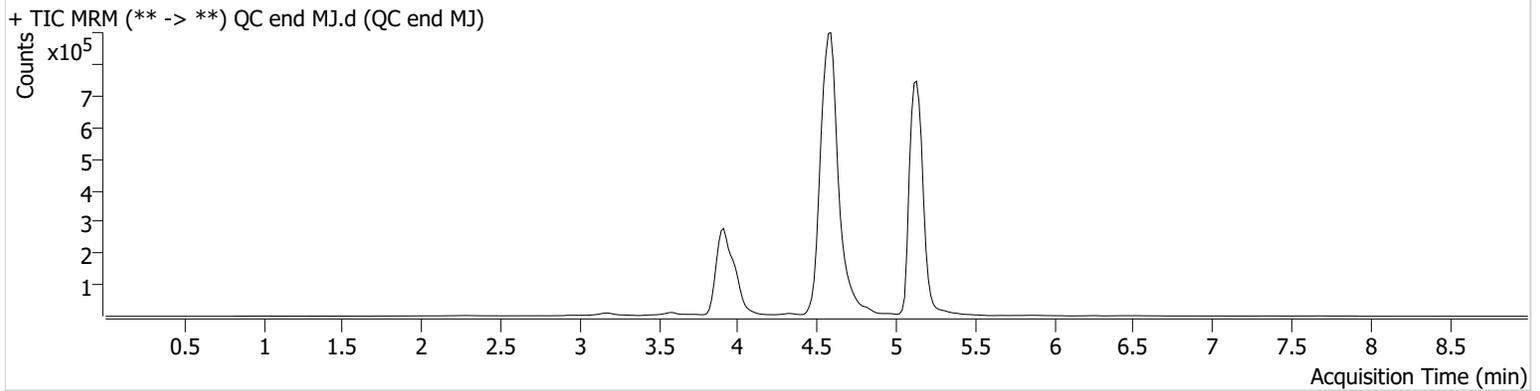
Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 AM

Instrument Falco (069901)
Type QC
Acq. Method AM 27 Agilent Method.m
Sample Position P1-H5
Injection Volume 10
Acq. Date-Time 1/5/2024 9:34:39 PM
Sample Info.

Data File QC end MJ.d
Sample QC end MJ
Operator Celena Shrum
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.

Sample Chromatogram

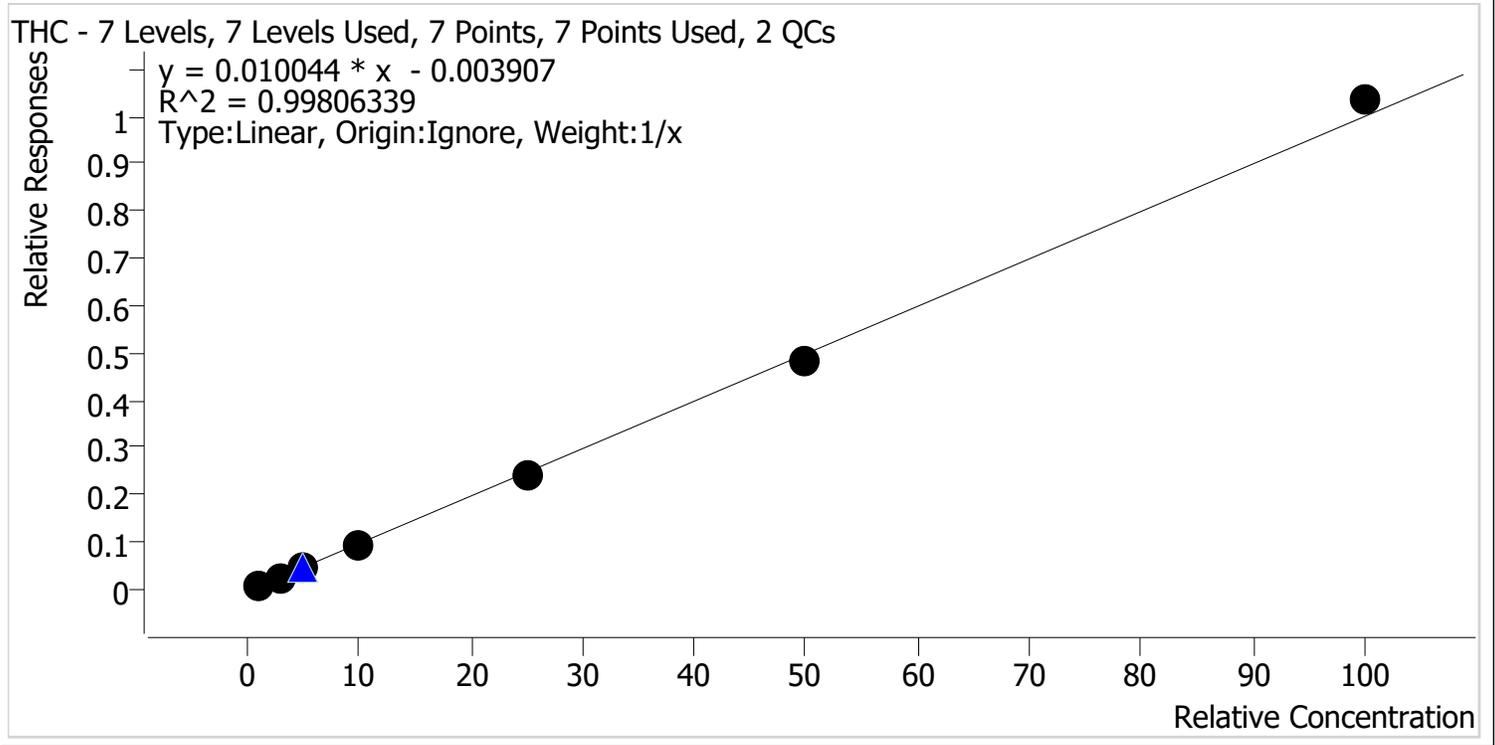


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.150	167789	∞	22.7	∞	3907385	4.6646 ng/ml
THC-COOH	3.985	43705	215.09	214.2	966.74	425523	14.8499 ng/ml
THC-OH	3.911	93637	∞	14.0	∞	1273016	4.7802 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/9/2024 10:50 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

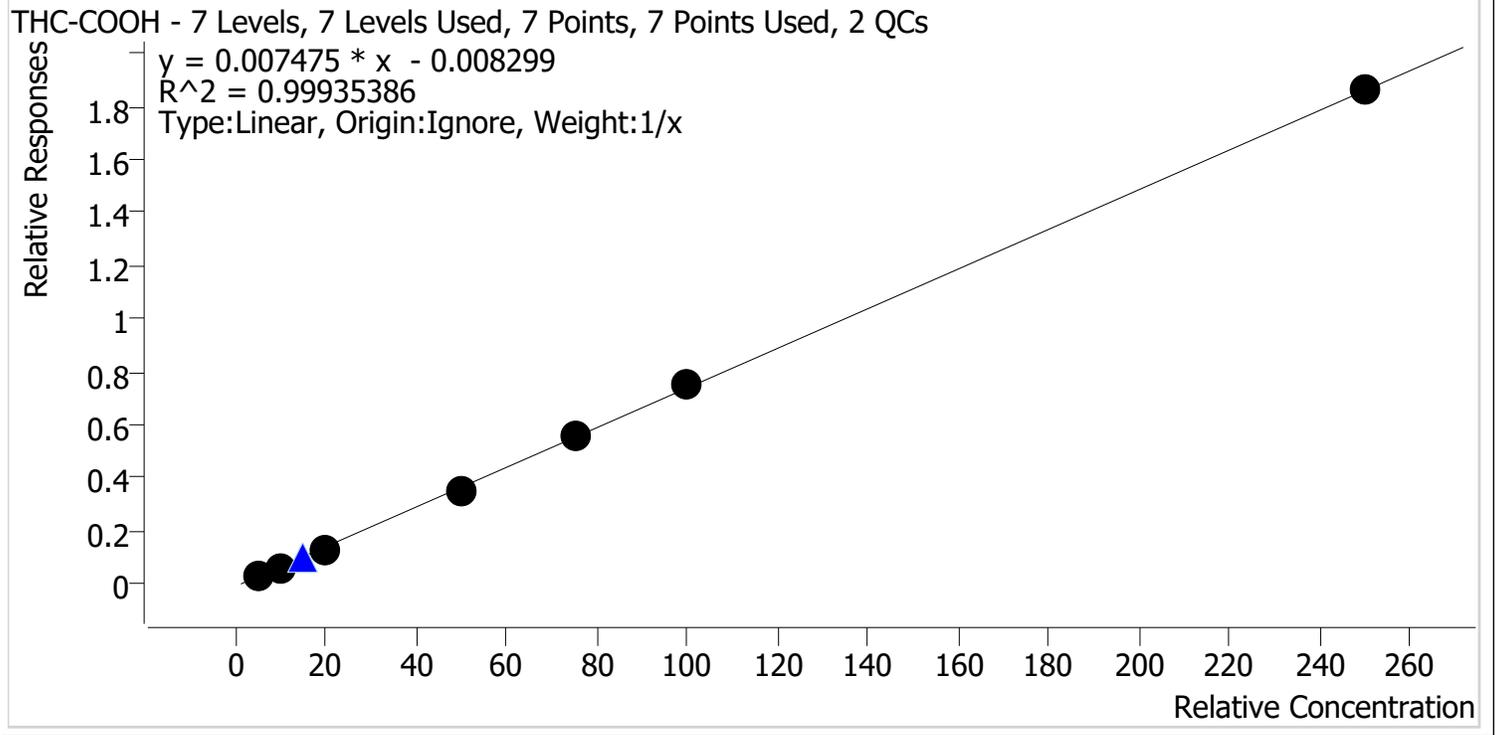


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.2	120.0
Cal 2 MJ	2	✓	3.0	2.9	97.3
Cal 3 MJ	3	✓	5.0	4.7	93.9
Cal 4 MJ	4	✓	10.0	9.2	92.4
Cal 5 MJ	5	✓	25.0	24.0	96.1
Cal 6 MJ	6	✓	50.0	48.5	97.0
Cal 7 MJ	7	✓	100.0	103.4	103.4



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/9/2024 10:50 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



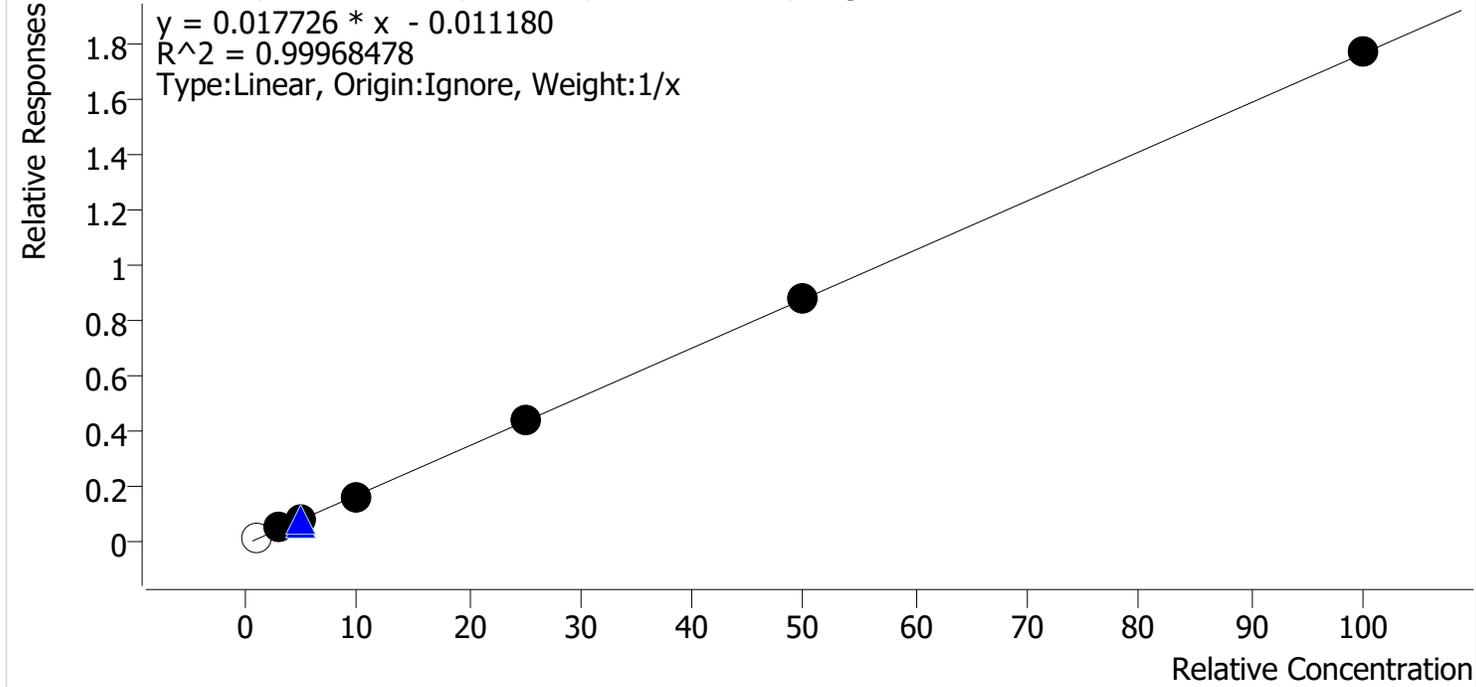
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.5	109.3
Cal 2 MJ	2	✓	10.0	9.7	97.4
Cal 3 MJ	3	✓	20.0	18.9	94.4
Cal 4 MJ	4	✓	50.0	48.1	96.2
Cal 5 MJ	5	✓	75.0	75.1	100.1
Cal 6 MJ	6	✓	100.0	102.5	102.5
Cal 7 MJ	7	✓	250.0	250.2	100.1



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/9/2024 10:50 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	1.4	140.1
Cal 2 MJ	2	✓	3.0	3.2	105.9
Cal 3 MJ	3	✓	5.0	4.9	98.6
Cal 4 MJ	4	✓	10.0	9.4	94.5
Cal 5 MJ	5	✓	25.0	25.2	100.8
Cal 6 MJ	6	✓	50.0	49.9	99.9
Cal 7 MJ	7	✓	100.0	100.3	100.3



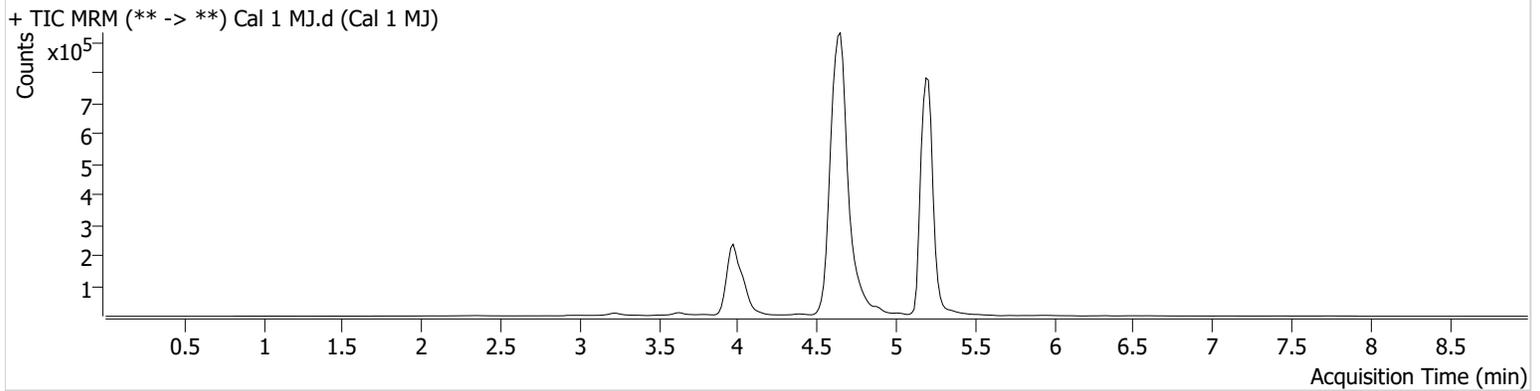
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-H6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 2:08:43 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	31446	296.54	28.5	∞	3862138	1.1997 ng/ml
THC-COOH	4.045	12374	188.29	209.1	∞	380222	5.4638 ng/ml
THC-OH	3.971	14657	∞	13.7	10.74	1072804	1.4014 ng/ml



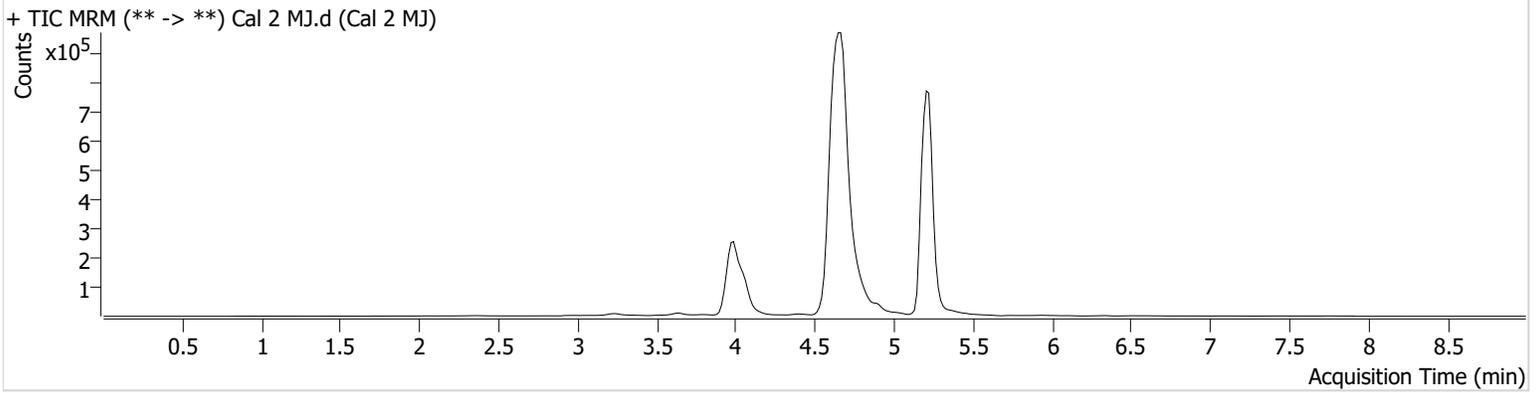
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-G6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 2:22:00 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	5.225	91012	∞	25.7	∞	3583585	2.9177 ng/ml
THC-COOH	4.060	25771	∞	219.6	∞	399391	9.7419 ng/ml
THC-OH	3.986	51575	∞	11.5	31.89	1142245	3.1779 ng/ml



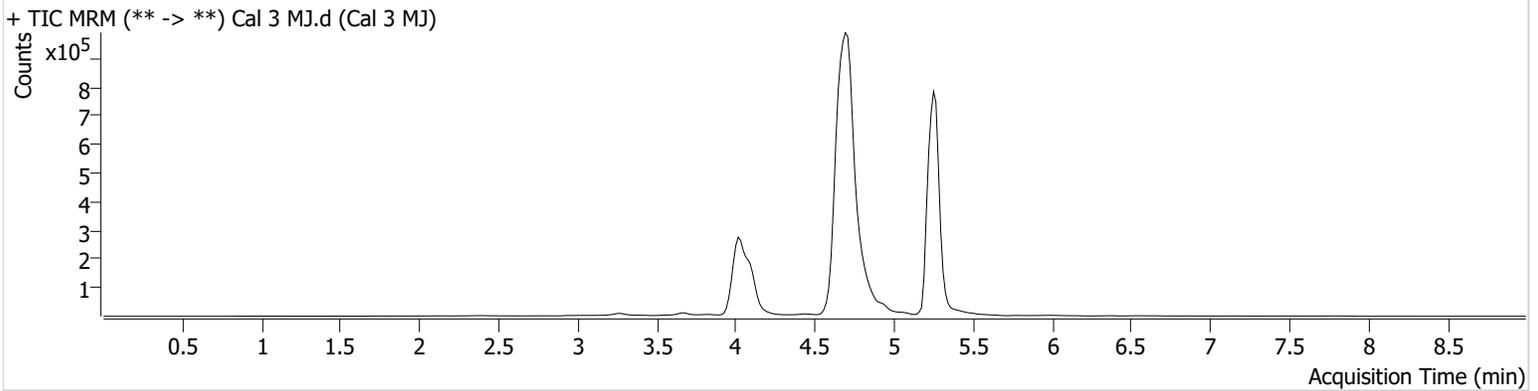
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-F6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 2:35:06 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	5.255	151350	∞	25.5	∞	3500150	4.6944 ng/ml
THC-COOH	4.105	56122	∞	213.8	∞	422397	18.8840 ng/ml
THC-OH	4.016	92367	∞	14.2	∞	1211584	4.9315 ng/ml



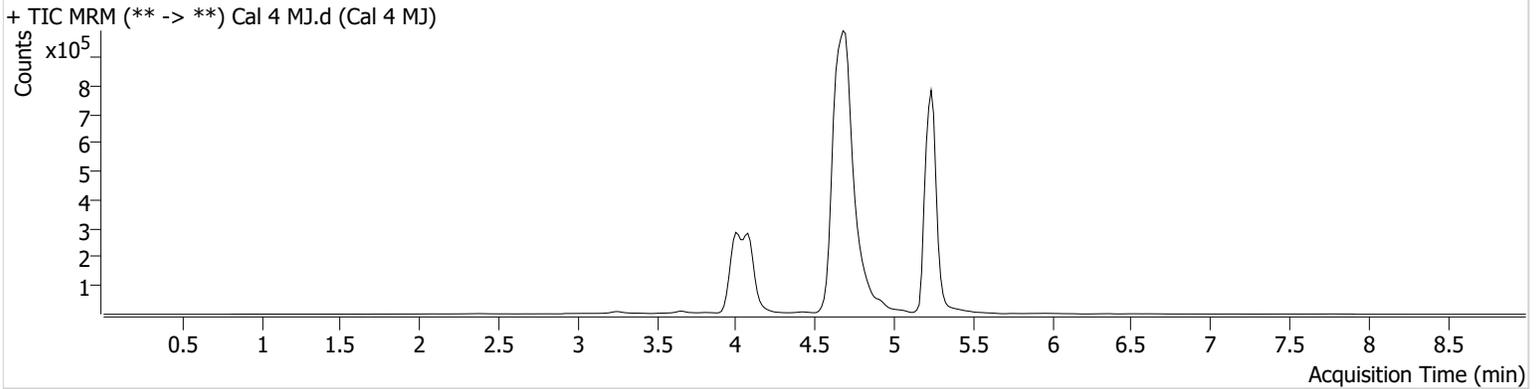
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-E6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 2:48:12 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	5.240	290191	∞	24.8	82.82	3265037	9.2384 ng/ml
THC-COOH	4.090	138388	2729.09	214.2	∞	394085	48.0861 ng/ml
THC-OH	4.001	184427	∞	13.9	∞	1180128	9.4469 ng/ml



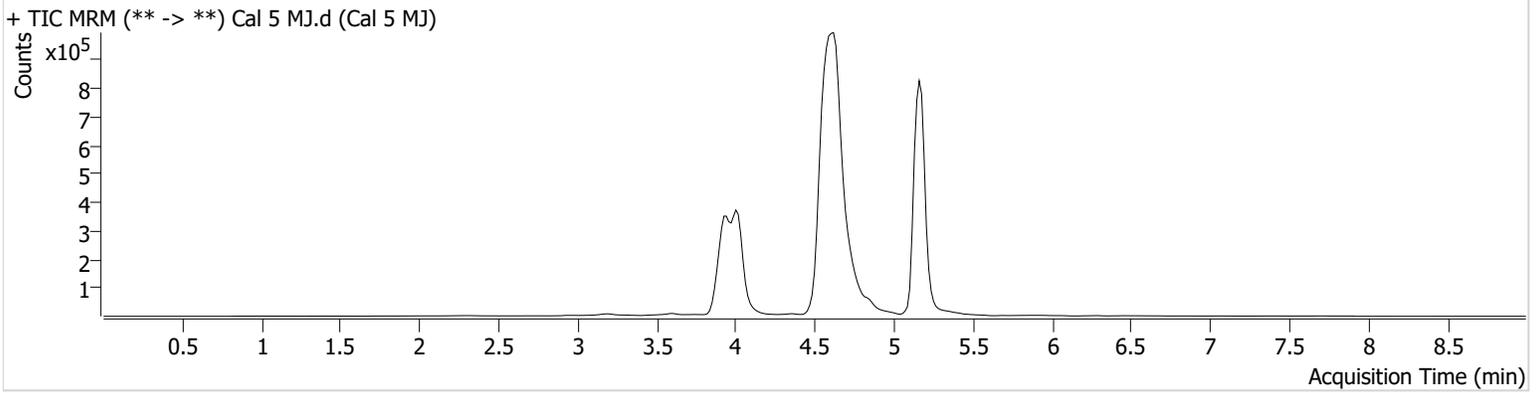
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-D6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 3:01:19 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	5.165	715875	∞	24.3	∞	3016753	24.0162 ng/ml
THC-COOH	4.015	211776	10078.95	212.1	∞	383085	75.0623 ng/ml
THC-OH	3.926	513481	∞	13.1	∞	1179081	25.1985 ng/ml



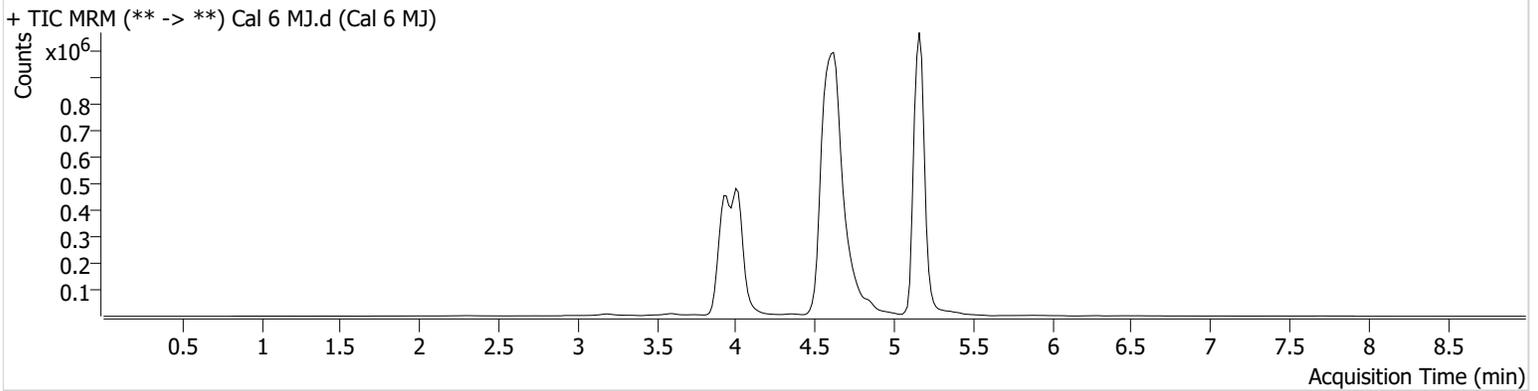
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-C6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 3:14:25 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	5.165	1473871	∞	24.9	∞	3050295	48.4987 ng/ml
THC-COOH	4.015	283346	∞	209.8	3783.08	373695	102.5406 ng/ml
THC-OH	3.926	1000666	∞	13.5	∞	1144987	49.9337 ng/ml



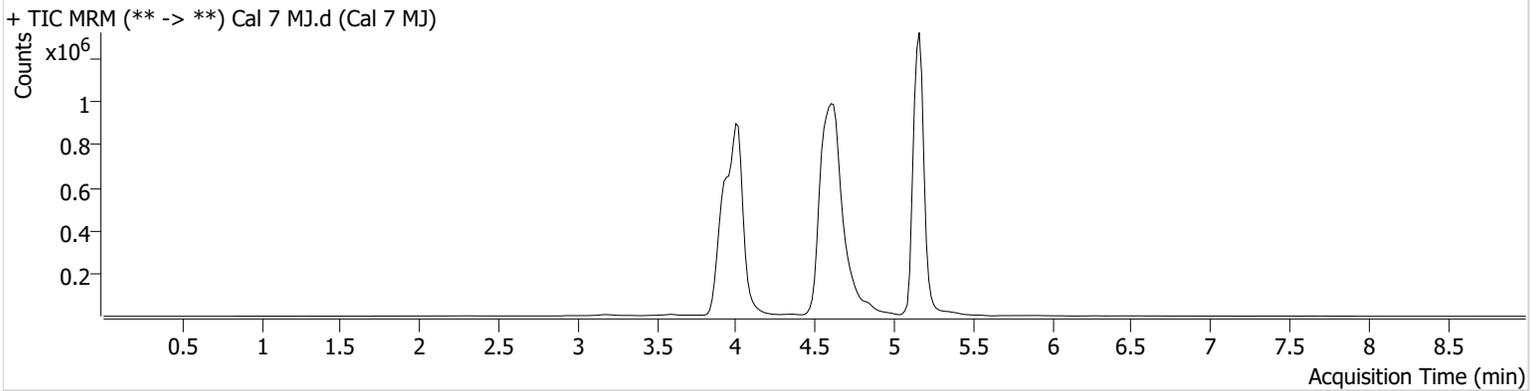
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\010523 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/9/2024 10:50:44 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-B6 **Comment**
Injection Volume 10
Acq. Date-Time 1/5/2024 3:27:31 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	5.165	2677386	∞	25.6	∞	2586989	103.4349 ng/ml
THC-COOH	4.015	623290	3130.21	217.3	∞	334707	250.2213 ng/ml
THC-OH	3.926	1847854	∞	13.7	∞	1045781	100.3116 ng/ml