

REVIEWED

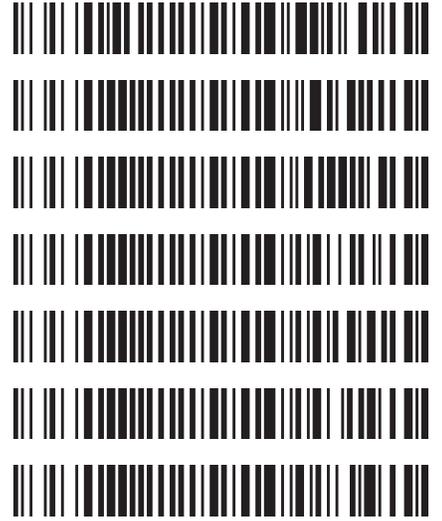
By Tamara Salazar at 4:15 pm, Jan 29, 2024

1/29/2024

9

Worklist: 6666

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-5537	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-4040	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0074	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0082	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0084	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0088	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0143	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: 01/26/2024

Analyst: Celena Shrum

Plate lot#: 231212

Plate Retest Date: 06/12/2024

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 23E52981

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

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 (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 r² 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:

	1	2	3	4	5	6
A	IS + Cal. 1	QC2	P2024-0143-1			
B	IS + Cal. 2	NEG Blood	P2024-0074-1			
C	IS + Cal. 3	M2023-5537-1	P2024-0082-1			
D	IS + Cal. 4	P2023-4040-1				
E	IS + Cal. 5	P2024-0074-1*				
F	IS + Cal. 6	P2024-0082-1*				
G	IS + Cal. 7	P2024-0084-1				
H	QC1	P2024-0088-1				

*Moved during SLE portion of the extraction due to blood clots



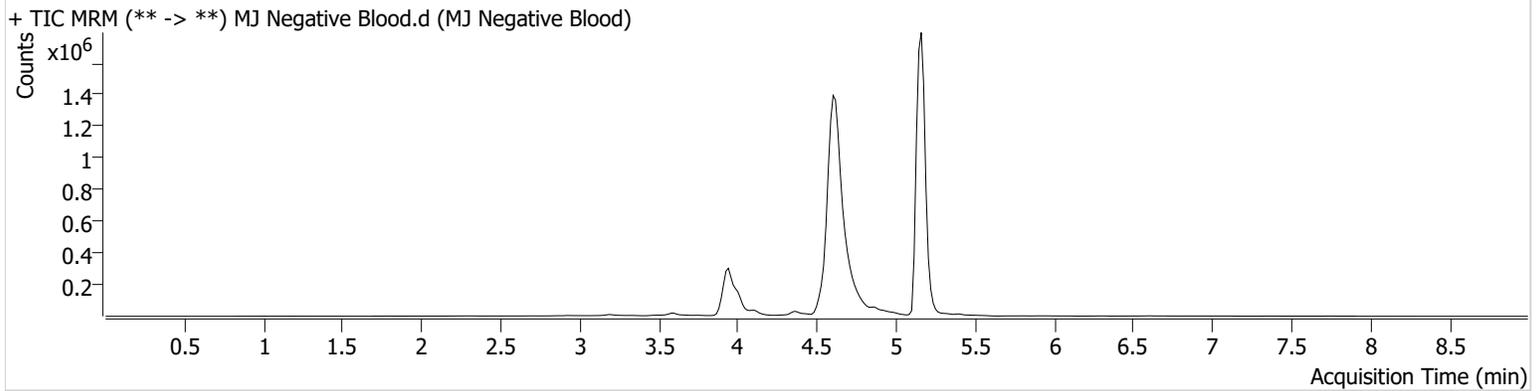
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/29/2024 1:27:05 PM

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**
Injection Volume 10
Acq. Date-Time 1/26/2024 2:39:13 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





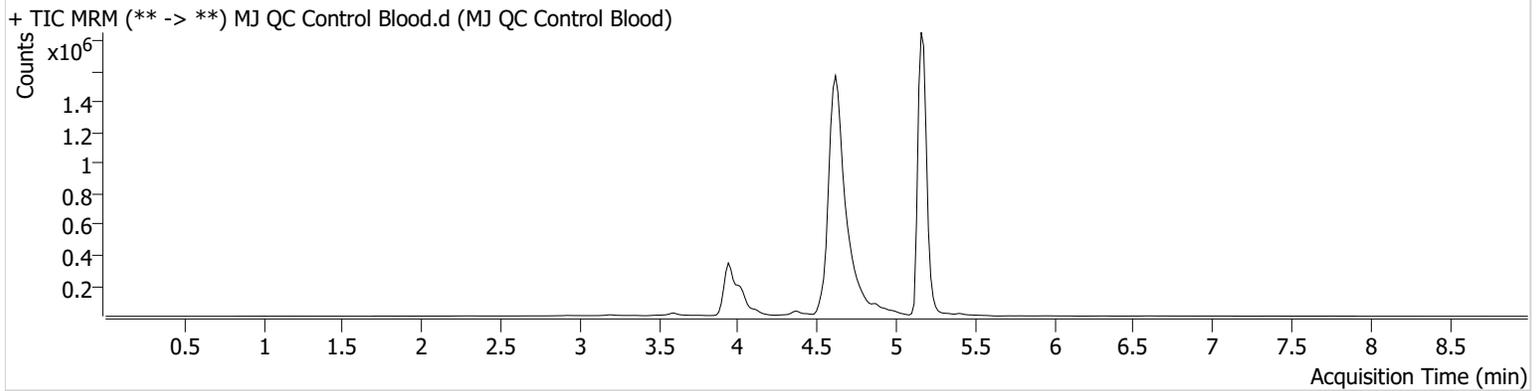
AM #27 Cannabinoids Quant. Results

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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 1/26/2024 2:13:00 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	310711	∞	24.8	∞	6723838	5.1436 ng/ml
THC-COOH	4.030	39763	173.88	236.4	1045.70	431978	14.3671 ng/ml
THC-OH	3.956	87707	∞	15.4	∞	1300842	5.0314 ng/ml



AM #27 Cannabinoids Quant. Results

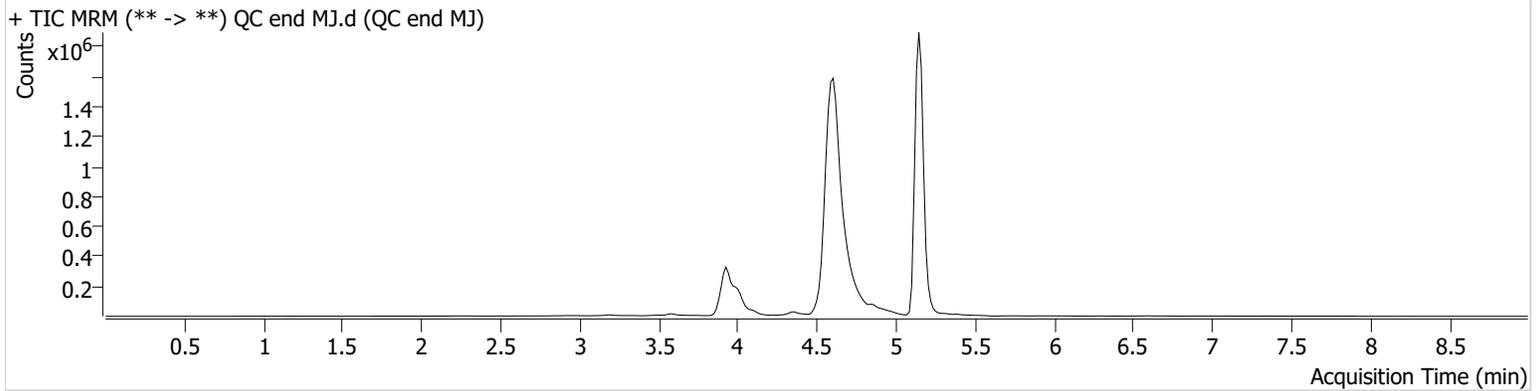
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Calibration Last Update 1/29/2024 1:27:05 PM

Instrument Falco (069901)
Type QC
Acq. Method AM 27 Agilent Method.m
Sample Position P1-A2
Injection Volume 10
Acq. Date-Time 1/26/2024 6:08:56 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

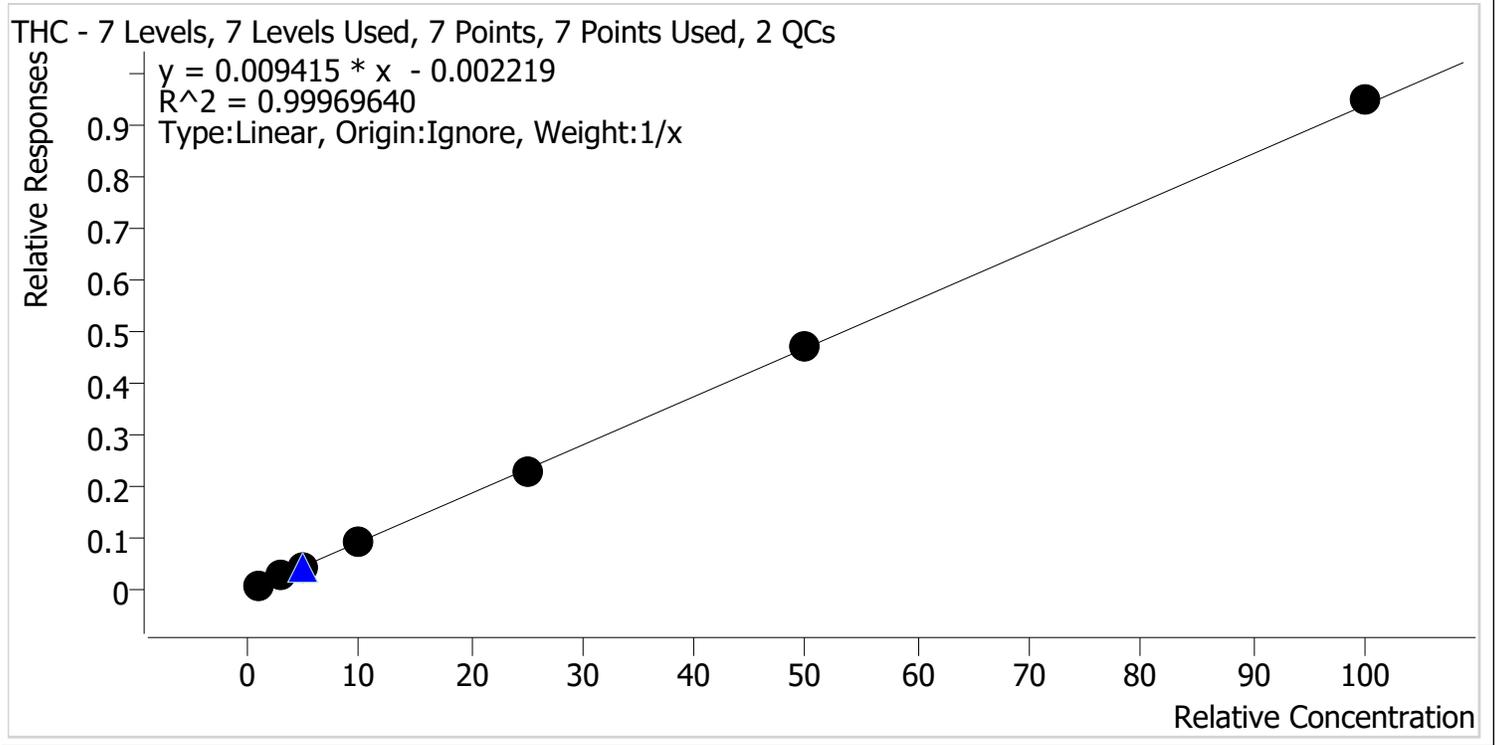


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.150	307724	∞	24.7	∞	6730579	5.0915 ng/ml
THC-COOH	4.015	39676	213.03	232.3	∞	423491	14.6098 ng/ml
THC-OH	3.926	70714	∞	16.0	∞	1183410	4.5190 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/29/2024 1:27 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



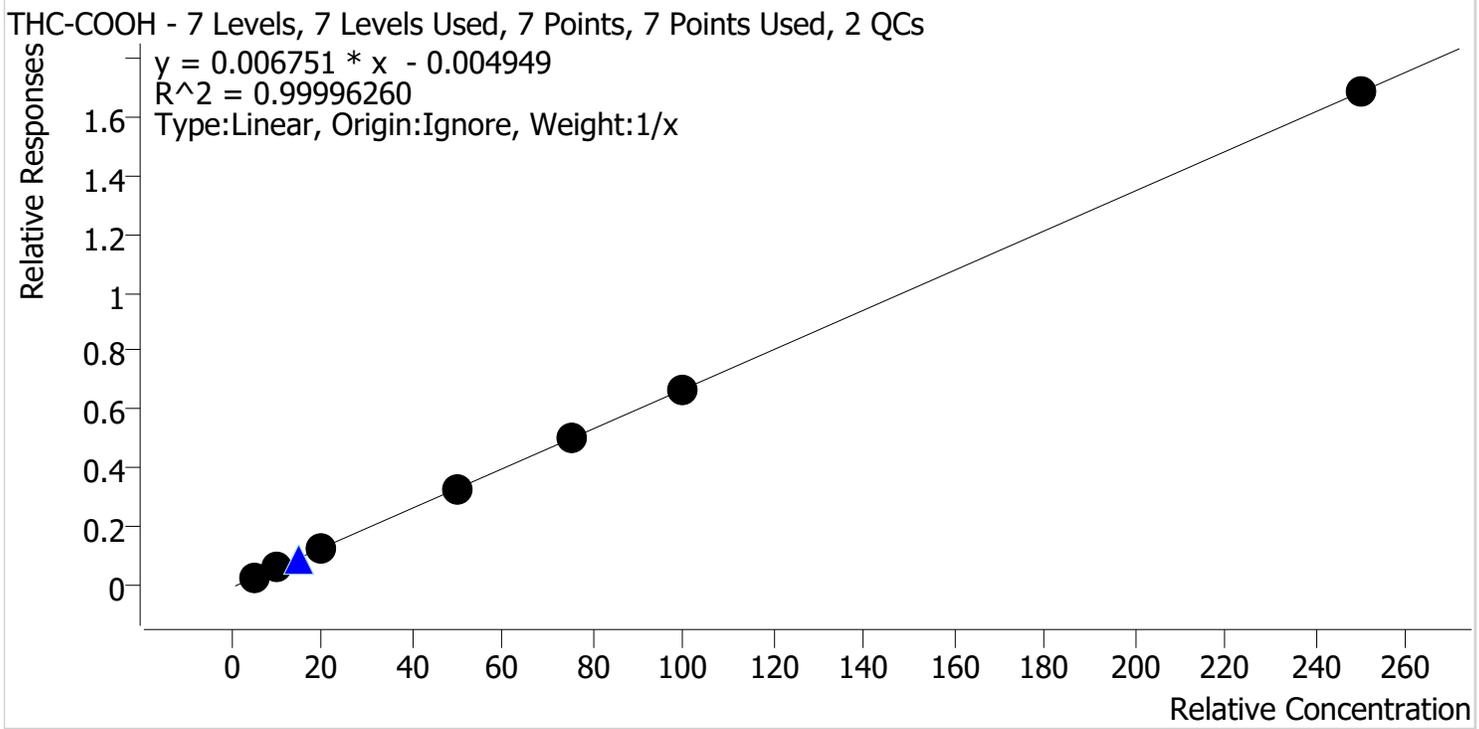
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	109.5
Cal 2 MJ	2	✓	3.0	2.9	97.3
Cal 3 MJ	3	✓	5.0	4.9	97.3
Cal 4 MJ	4	✓	10.0	9.8	97.5
Cal 5 MJ	5	✓	25.0	24.3	97.3
Cal 6 MJ	6	✓	50.0	50.0	99.9
Cal 7 MJ	7	✓	100.0	101.1	101.1

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/29/2024 1:27 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

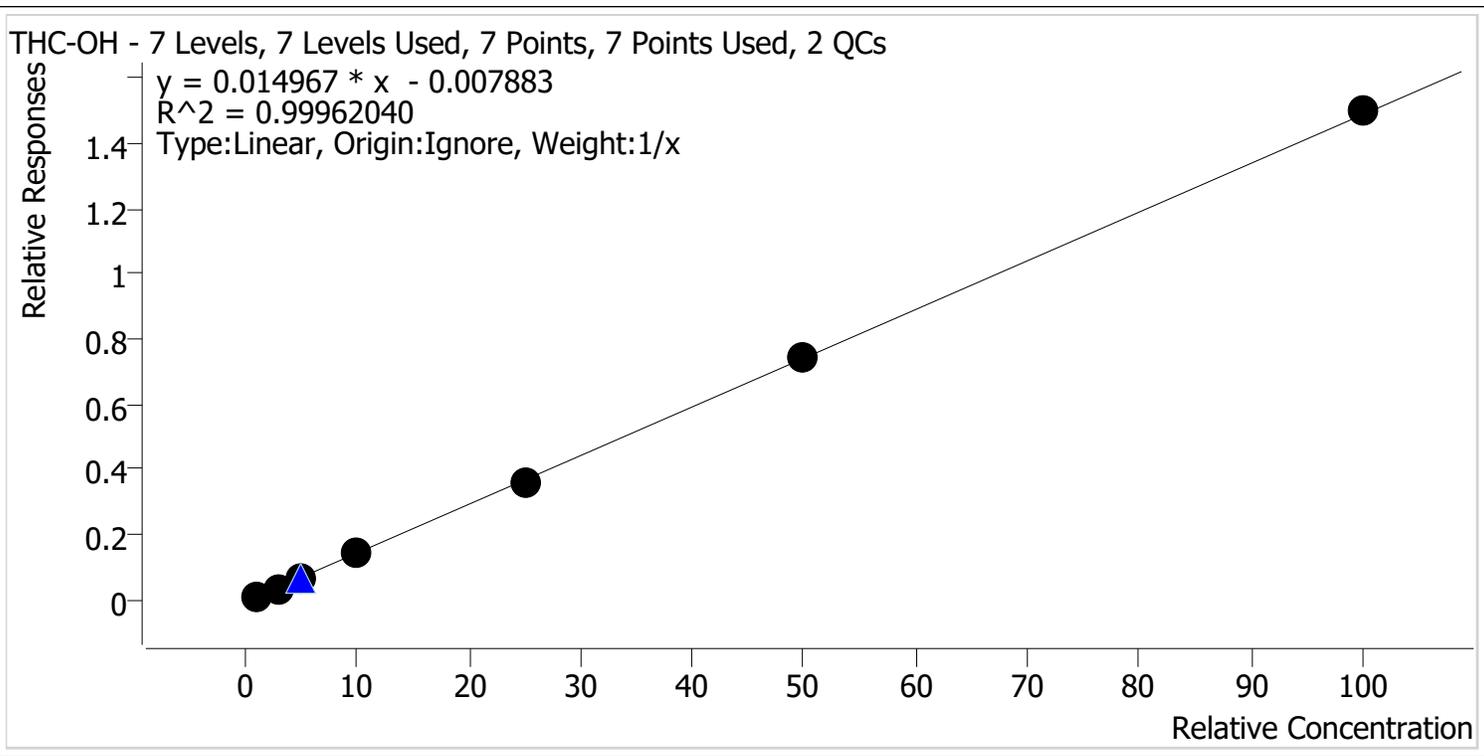


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.1	102.2
Cal 2 MJ	2	✓	10.0	9.9	99.4
Cal 3 MJ	3	✓	20.0	19.6	98.2
Cal 4 MJ	4	✓	50.0	49.7	99.4
Cal 5 MJ	5	✓	75.0	75.4	100.5
Cal 6 MJ	6	✓	100.0	100.3	100.3
Cal 7 MJ	7	✓	250.0	249.9	100.0



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/29/2024 1:27 PM
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	111.4
Cal 2 MJ	2	✓	3.0	2.8	94.0
Cal 3 MJ	3	✓	5.0	4.9	97.0
Cal 4 MJ	4	✓	10.0	9.9	99.4
Cal 5 MJ	5	✓	25.0	24.2	96.8
Cal 6 MJ	6	✓	50.0	50.4	100.7
Cal 7 MJ	7	✓	100.0	100.7	100.7



AM #27 Cannabinoids Quant. Results

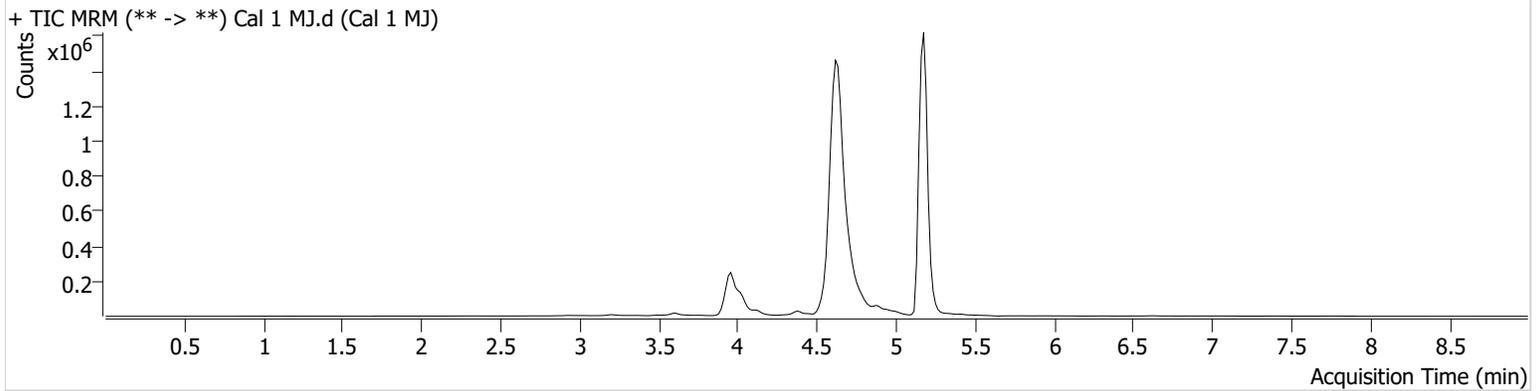
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Calibration Last Update 1/29/2024 1:27:05 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-A1
Injection Volume 10
Acq. Date-Time 1/26/2024 12:27:59 PM
Sample Info.

Data File Cal 1 MJ.d
Sample Cal 1 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	5.180	48558	∞	27.6	∞	6001758	1.0949 ng/ml
THC-COOH	4.045	10293	215.16	231.6	175.69	348212	5.1113 ng/ml
THC-OH	3.956	8956	∞	19.2	41.13	1019276	1.1138 ng/ml

AM #27 Cannabinoids Quant. Results



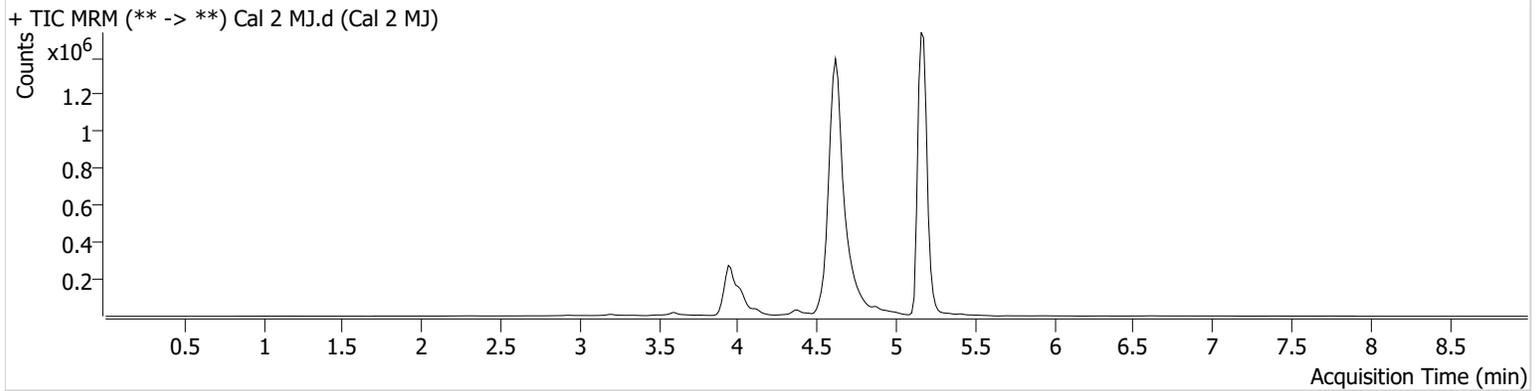
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Calibration Last Update 1/29/2024 1:27:05 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-B1
Injection Volume 10
Acq. Date-Time 1/26/2024 12:41:15 PM
Sample Info.

Data File Cal 2 MJ.d
Sample Cal 2 MJ
Operator Celena Shrum
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	152251	∞	24.8	∞	6023734	2.9201 ng/ml
THC-COOH	4.030	22855	1054.04	223.3	790.25	367484	9.9448 ng/ml
THC-OH	3.956	36973	∞	17.6	∞	1076563	2.8213 ng/ml

AM #27 Cannabinoids Quant. Results

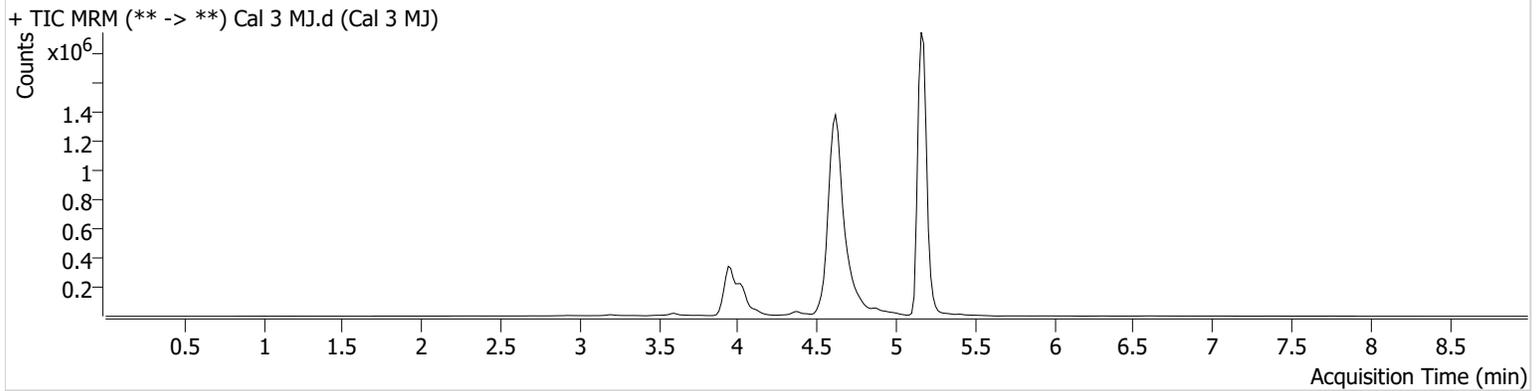


Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/29/2024 1:27:05 PM

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 1/26/2024 12:54:21 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	314054	∞	24.3	∞	7203048	4.8663 ng/ml
THC-COOH	4.030	57640	308.44	237.2	∞	451707	19.6334 ng/ml
THC-OH	3.956	85056	∞	15.7	∞	1314340	4.8504 ng/ml



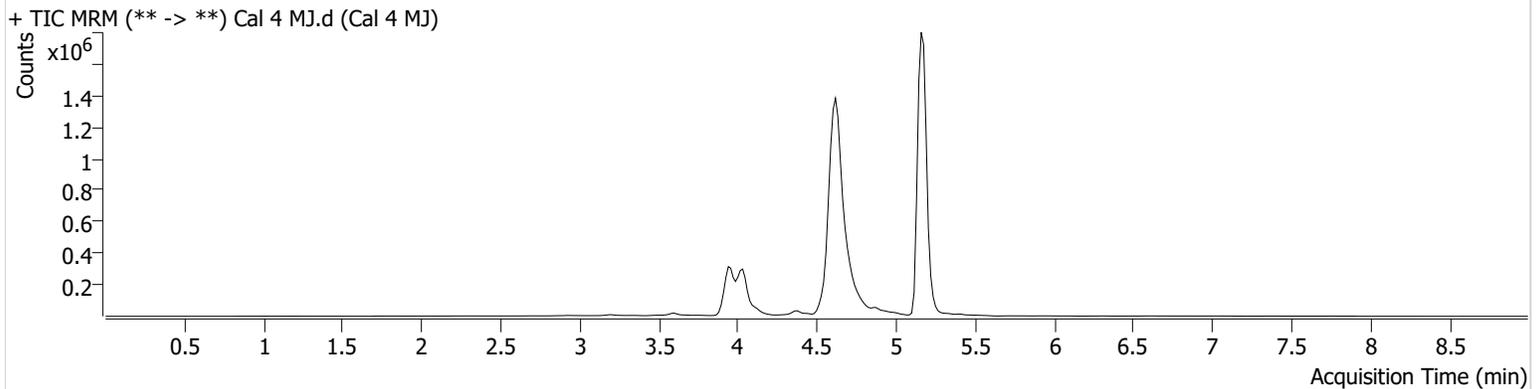
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/29/2024 1:27:05 PM

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**
Injection Volume 10
Acq. Date-Time 1/26/2024 1:07:28 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	5.180	575142	∞	24.5	∞	6419356	9.7514 ng/ml
THC-COOH	4.030	124948	4414.62	228.9	528.14	378086	49.6820 ng/ml
THC-OH	3.956	158383	∞	15.0	∞	1124669	9.9356 ng/ml

AM #27 Cannabinoids Quant. Results

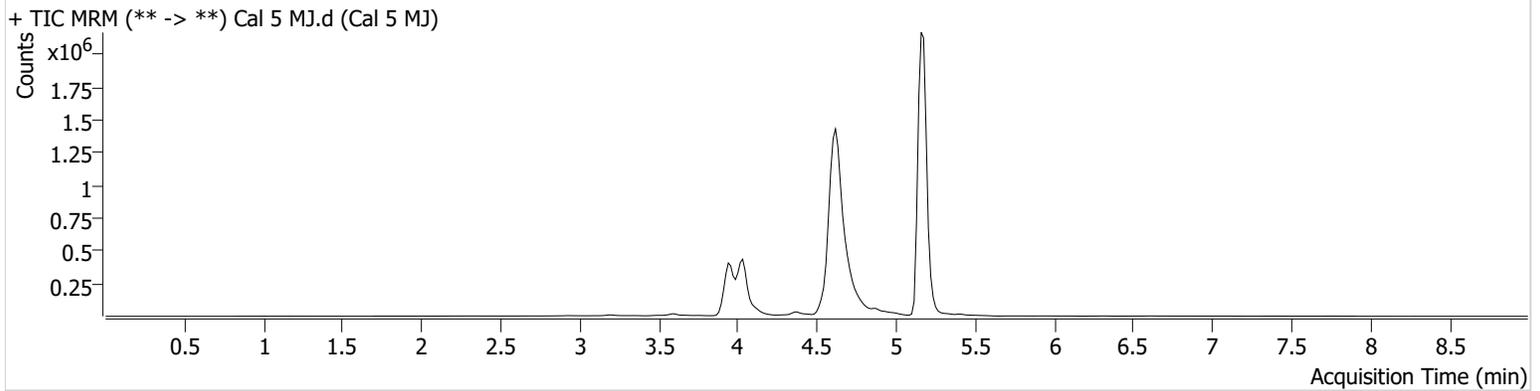


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Calibration Last Update 1/29/2024 1:27:05 PM

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**
Injection Volume 10
Acq. Date-Time 1/26/2024 1:20:34 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	1484502	13380.22	24.8	∞	6543426	24.3311 ng/ml
THC-COOH	4.030	197297	∞	229.8	∞	391555	75.3664 ng/ml
THC-OH	3.956	422257	∞	15.3	∞	1192289	24.1887 ng/ml

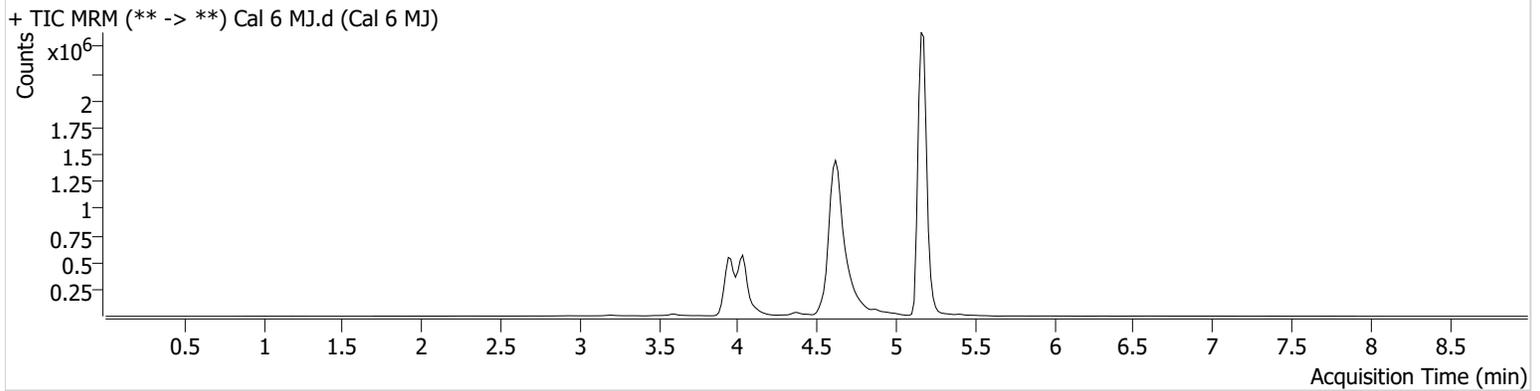
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/29/2024 1:27:05 PM

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	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	1/26/2024 1:33:42 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	2997198	∞	25.0	∞	6400644	49.9695 ng/ml
THC-COOH	4.030	262456	750.15	232.9	3000.21	390293	100.3354 ng/ml
THC-OH	3.956	909308	∞	15.0	∞	1218798	50.3732 ng/ml



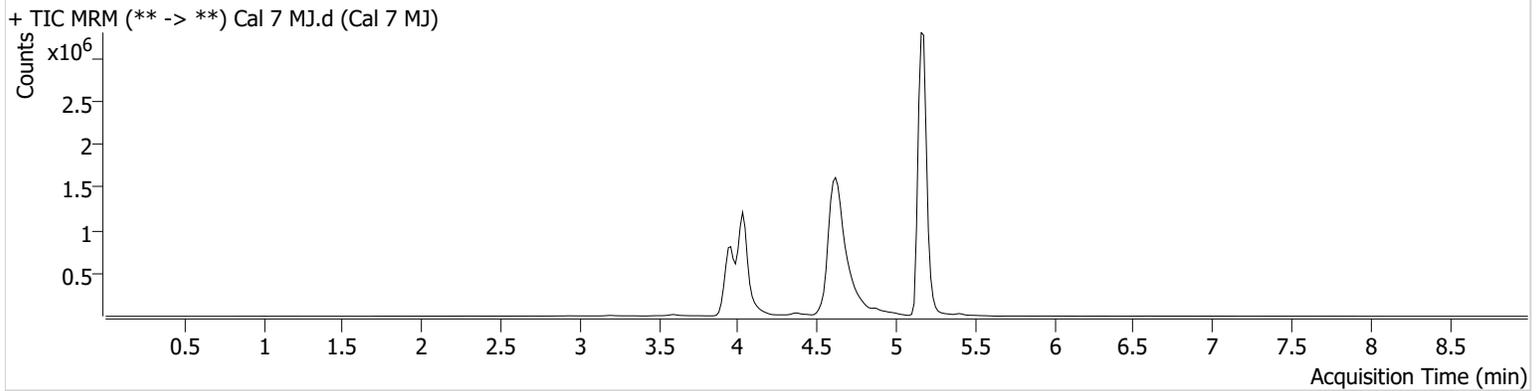
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\012624 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/29/2024 1:27:05 PM

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**
Injection Volume 10
Acq. Date-Time 1/26/2024 1:46:48 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	5.180	5491919	∞	25.5	∞	5784837	101.0666 ng/ml
THC-COOH	4.030	638088	5582.32	233.1	17104.91	379271	249.9266 ng/ml
THC-OH	3.956	1853060	∞	15.3	∞	1235717	100.7171 ng/ml