

REVIEWED

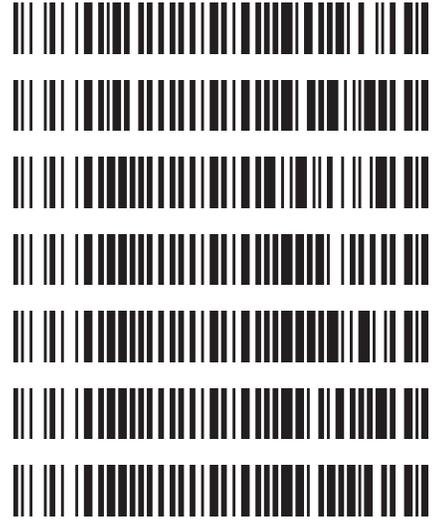
By Tamara Salazar at 8:34 am, Apr 05, 2024

4/3/2024



Worklist: 6754

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2024-0854	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2024-1071	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0534	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0642	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0810	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0880	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0883	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: 04/03/2024

Plate lot#: 231212

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 23A52595

LCMS-QQQ ID: 069901

Analyst: Celena Shrum

Plate Retest Date: 06/12/2024

Mobile phase B: 0.1% Formic acid in Acetonitrile

Column: UCT Selectra DA 100 x 2.1mm 3um

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:

9

	1	2	3	4	5	6
A	IS + Cal. 1	QC2	M2024-1071-2			
B	IS + Cal. 2	NEG Blood				
C	IS + Cal. 3	M2024-0854-1				
D	IS + Cal. 4	P2024-0534-2				
E	IS + Cal. 5	P2024-0642-2				
F	IS + Cal. 6	P2024-0810-1				
G	IS + Cal. 7	P2024-0880-1				
H	QC1	P2024-0883-1				

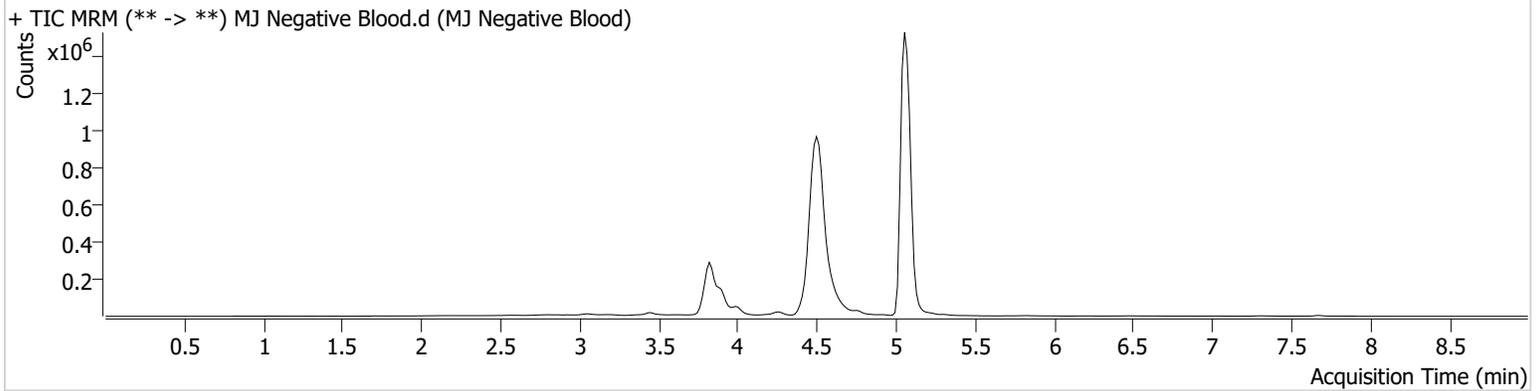
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 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	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	4/3/2024 4:19:01 PM		
Sample Info.			

Sample Chromatogram





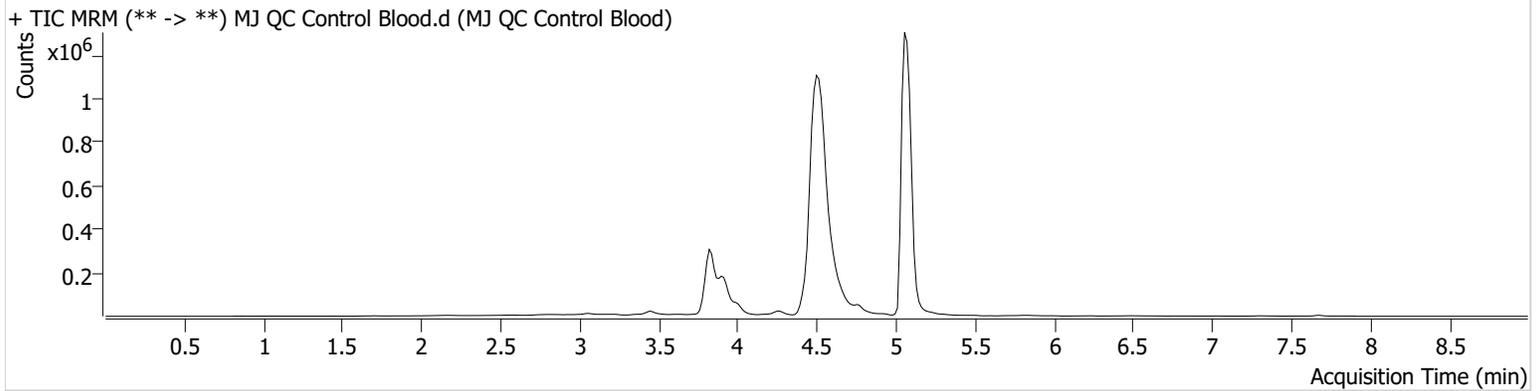
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 PM

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 4/3/2024 3:52:51 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	226951	∞	26.0	∞	5170571	5.0931 ng/ml
THC-COOH	3.924	39389	∞	225.4	∞	413437	14.4118 ng/ml
THC-OH	3.835	101201	∞	13.0	∞	1250972	5.0065 ng/ml



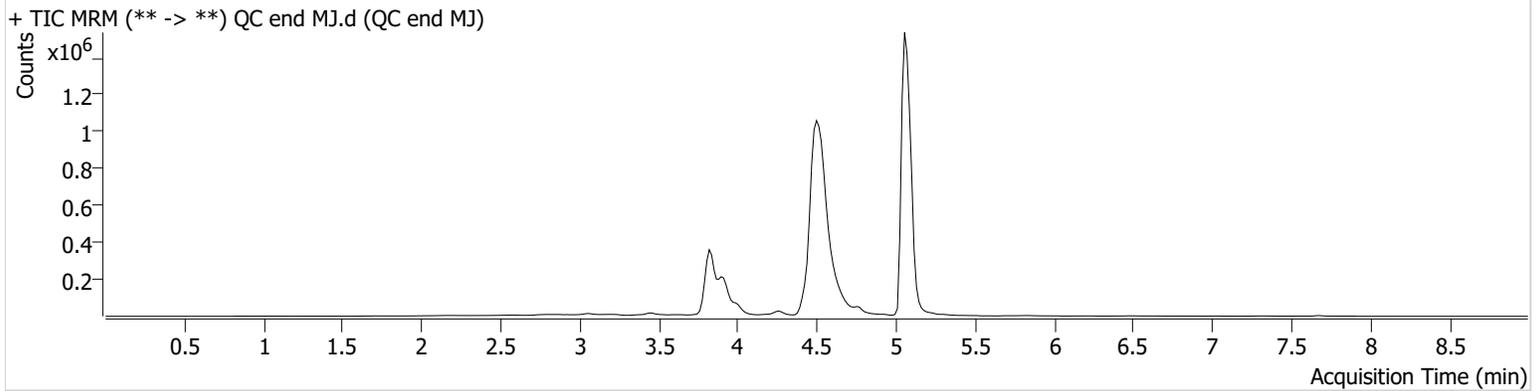
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 PM

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**
Injection Volume 10
Acq. Date-Time 4/3/2024 7:48:34 PM
Sample Info.

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Sample Chromatogram

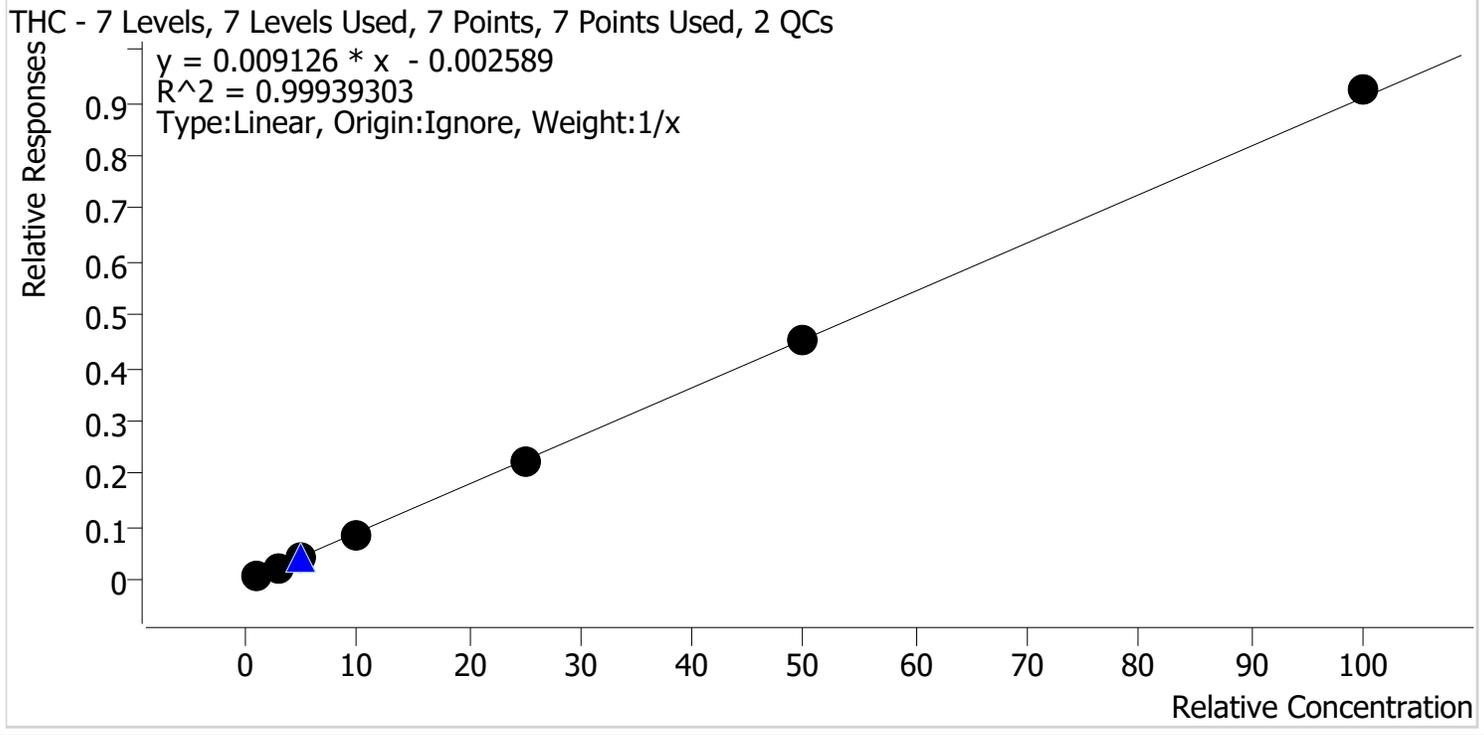


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	260549	∞	25.3	∞	5946896	5.0843 ng/ml
THC-COOH	3.924	44266	365.75	220.1	724.77	479778	13.9733 ng/ml
THC-OH	3.835	116113	∞	13.3	∞	1463946	4.9140 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 4/4/2024 3:04 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

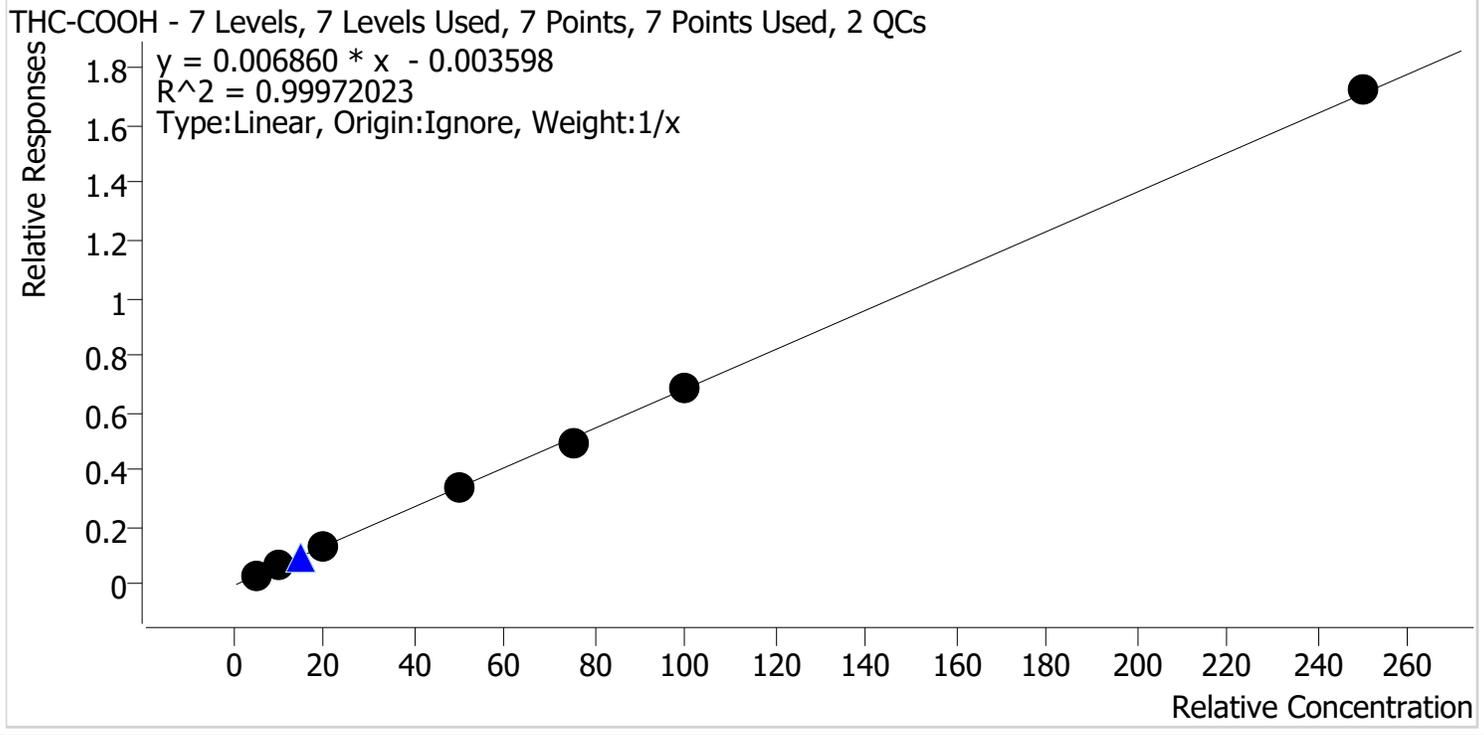


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	114.0
Cal 2 MJ	2	✓	3.0	2.9	97.2
Cal 3 MJ	3	✓	5.0	4.7	95.0
Cal 4 MJ	4	✓	10.0	9.5	95.4
Cal 5 MJ	5	✓	25.0	24.3	97.4
Cal 6 MJ	6	✓	50.0	49.7	99.4
Cal 7 MJ	7	✓	100.0	101.6	101.6



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 4/4/2024 3:04 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



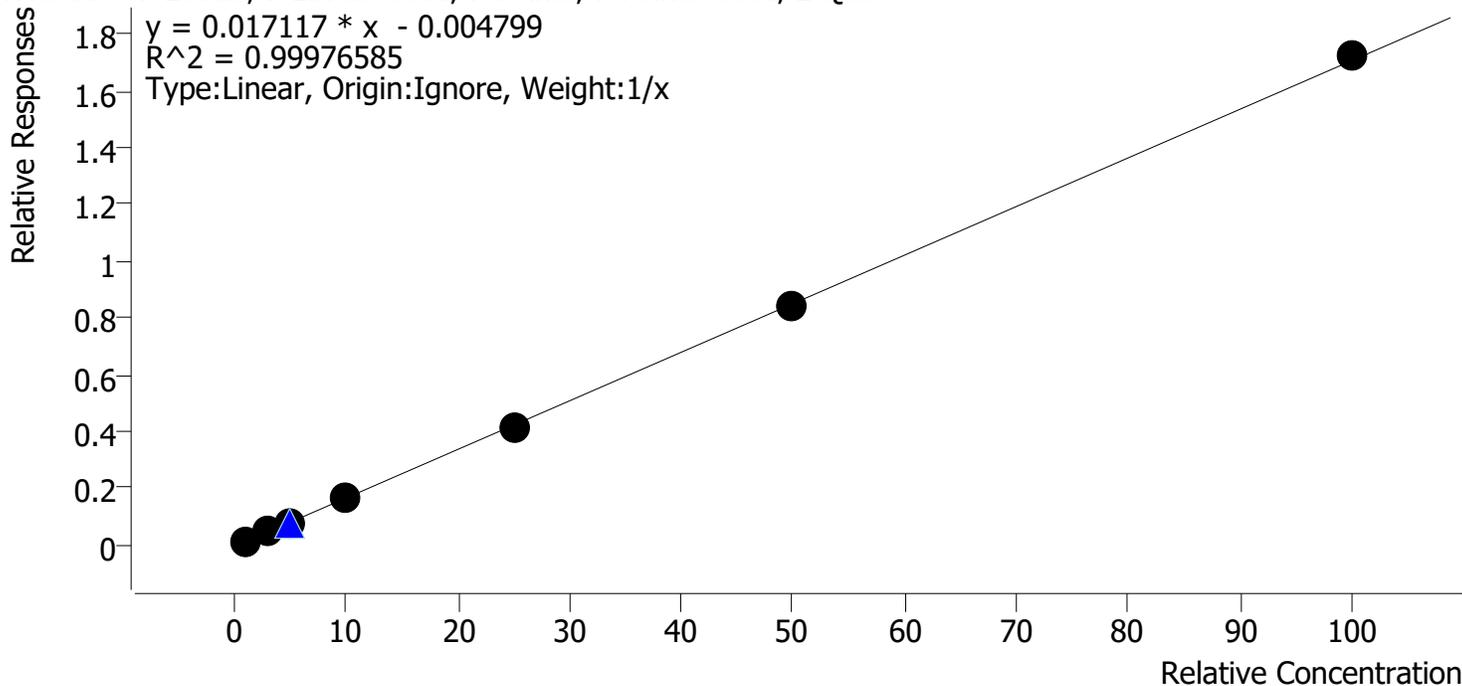
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.2	103.2
Cal 2 MJ	2	✓	10.0	10.0	99.8
Cal 3 MJ	3	✓	20.0	19.9	99.4
Cal 4 MJ	4	✓	50.0	49.5	99.0
Cal 5 MJ	5	✓	75.0	72.6	96.8
Cal 6 MJ	6	✓	100.0	101.0	101.0
Cal 7 MJ	7	✓	250.0	251.9	100.7



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 4/4/2024 3:04 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	109.6
Cal 2 MJ	2	✓	3.0	2.9	97.4
Cal 3 MJ	3	✓	5.0	4.8	95.3
Cal 4 MJ	4	✓	10.0	9.9	98.7
Cal 5 MJ	5	✓	25.0	24.6	98.5
Cal 6 MJ	6	✓	50.0	49.8	99.6
Cal 7 MJ	7	✓	100.0	100.9	100.9



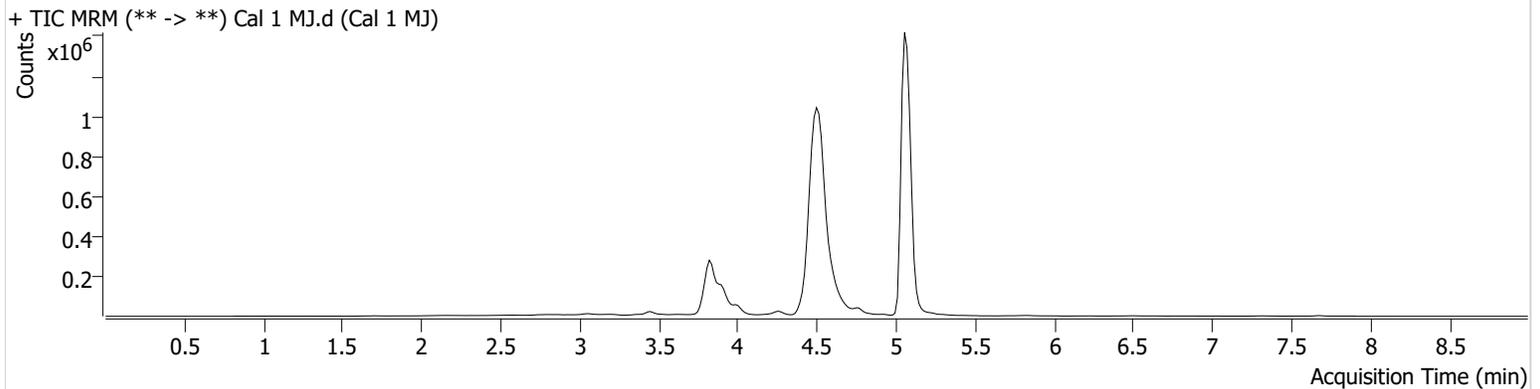
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 PM

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**
Injection Volume 10
Acq. Date-Time 4/3/2024 2:08:00 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	5.075	45221	∞	29.1	∞	5787061	1.1399 ng/ml
THC-COOH	3.924	13607	235.45	223.0	112.07	427899	5.1597 ng/ml
THC-OH	3.820	17973	∞	12.5	∞	1287872	1.0957 ng/ml



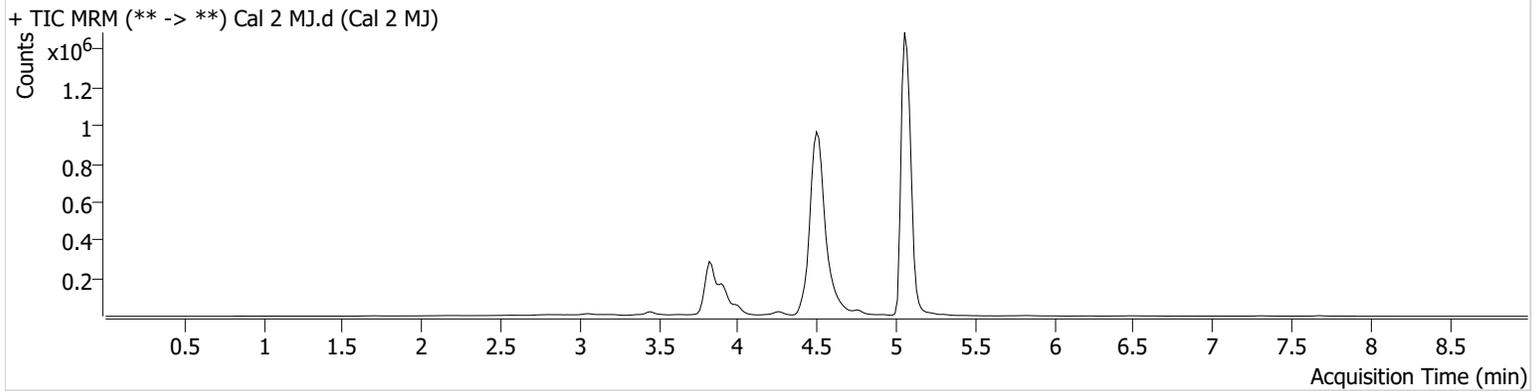
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 PM

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**
Injection Volume 10
Acq. Date-Time 4/3/2024 2:21:16 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	144359	∞	25.7	∞	6006312	2.9172 ng/ml
THC-COOH	3.924	26716	∞	221.6	∞	411781	9.9817 ng/ml
THC-OH	3.835	55669	∞	13.2	202.76	1231093	2.9221 ng/ml



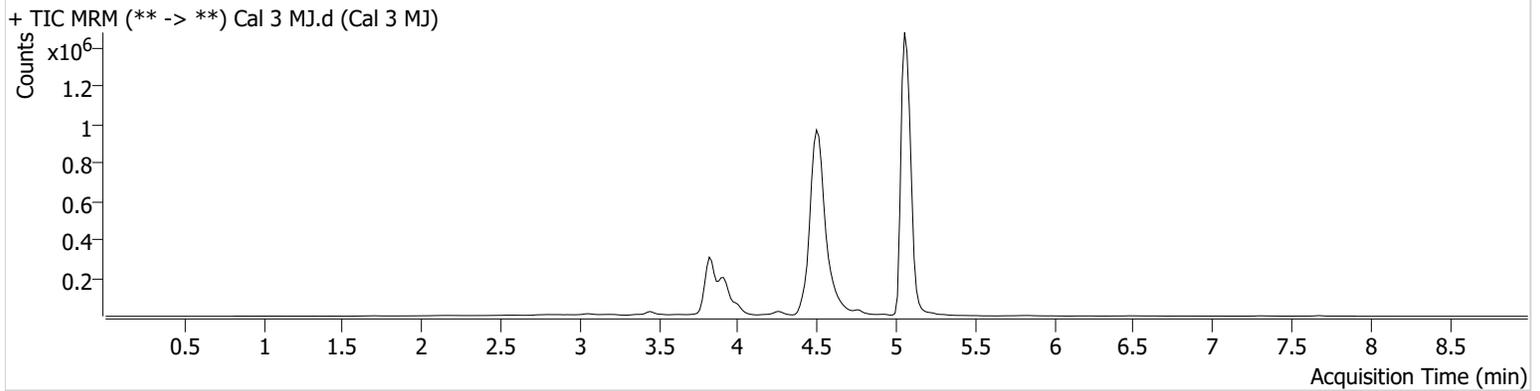
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 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 4/3/2024 2:34:22 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	240069	∞	25.6	∞	5892317	4.7479 ng/ml
THC-COOH	3.924	55040	333.18	220.2	392.50	414512	19.8796 ng/ml
THC-OH	3.835	98057	∞	13.9	∞	1277242	4.7655 ng/ml



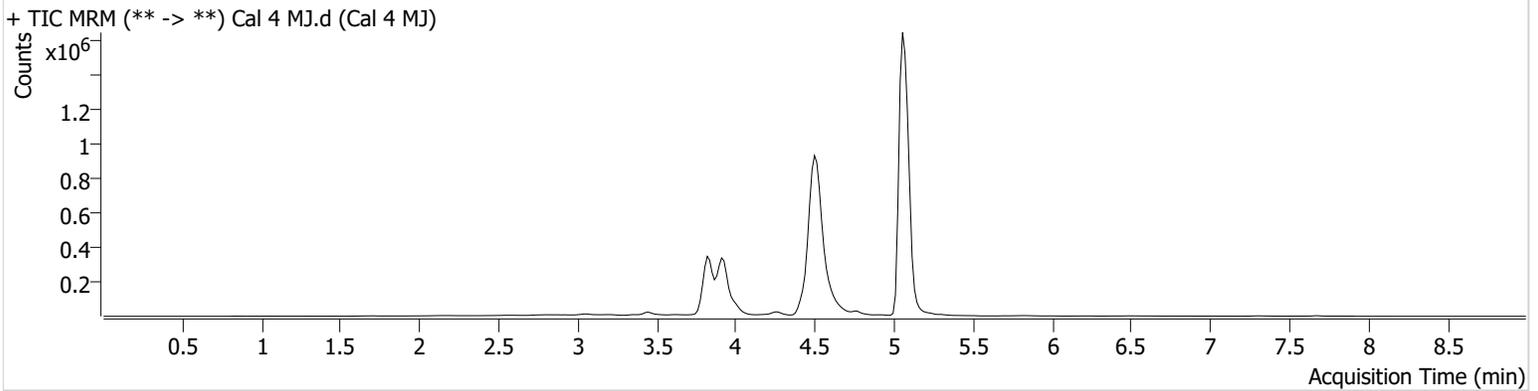
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 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 4/3/2024 2:47:26 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	530093	∞	25.0	∞	6274154	9.5412 ng/ml
THC-COOH	3.924	145151	2696.46	214.5	∞	431841	49.5193 ng/ml
THC-OH	3.835	217797	∞	13.0	∞	1327184	9.8675 ng/ml

AM #27 Cannabinoids Quant. Results

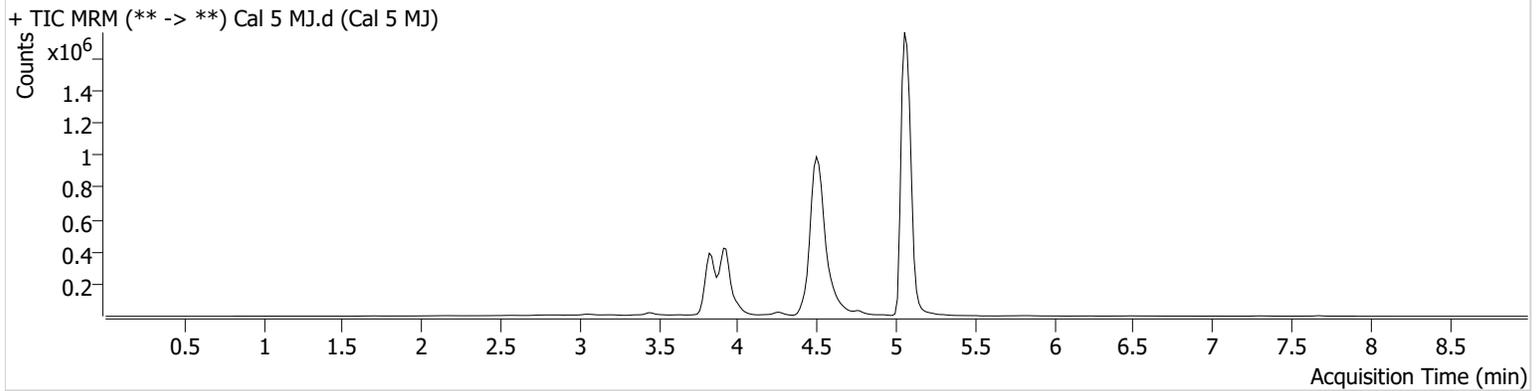


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Calibration Last Update 4/4/2024 3:04:39 PM

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 4/3/2024 3:00:32 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	1285855	∞	24.9	1145.70	5854764	24.3483 ng/ml
THC-COOH	3.924	196112	882.89	212.3	∞	396424	72.6346 ng/ml
THC-OH	3.835	505420	∞	12.9	∞	1212339	24.6360 ng/ml



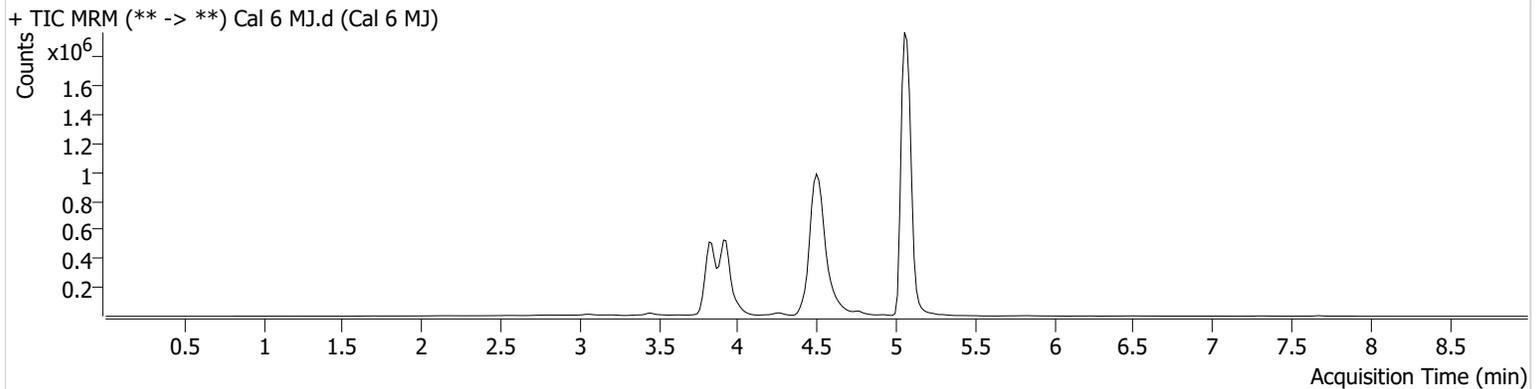
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\040324 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/4/2024 3:04:39 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**
Injection Volume 10
Acq. Date-Time 4/3/2024 3:13:36 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	2455784	∞	25.0	∞	5445661	49.6961 ng/ml
THC-COOH	3.924	266292	∞	216.2	∞	386474	100.9606 ng/ml
THC-OH	3.835	1054972	∞	13.5	680.83	1244925	49.7875 ng/ml

AM #27 Cannabinoids Quant. Results

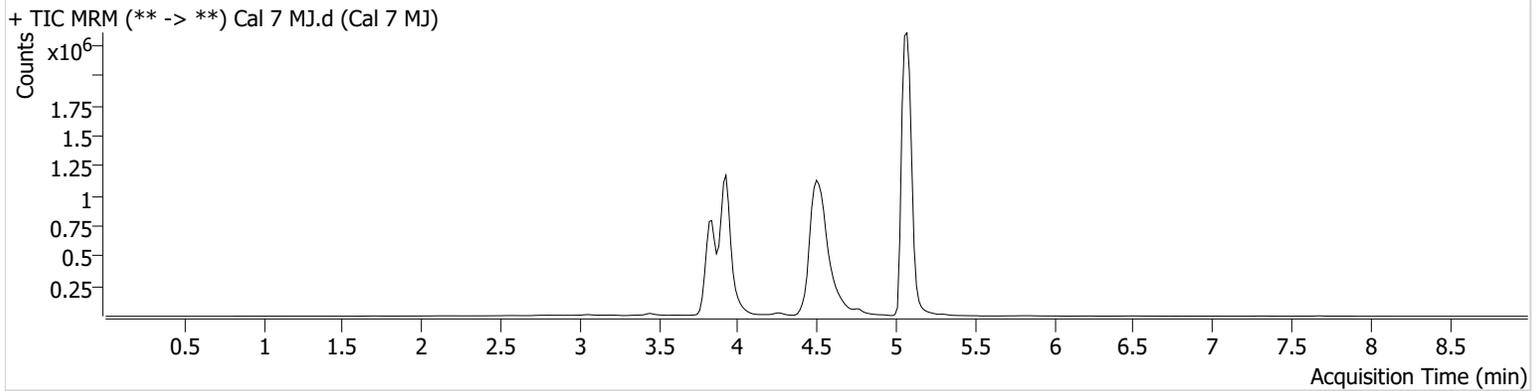


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Calibration Last Update 4/4/2024 3:04:39 PM

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 4/3/2024 3:26:40 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	4400096	∞	25.2	∞	4758161	101.6093 ng/ml
THC-COOH	3.924	664285	∞	214.5	∞	385252	251.8644 ng/ml
THC-OH	3.835	2184472	∞	13.4	∞	1268013	100.9257 ng/ml