

REVIEWED

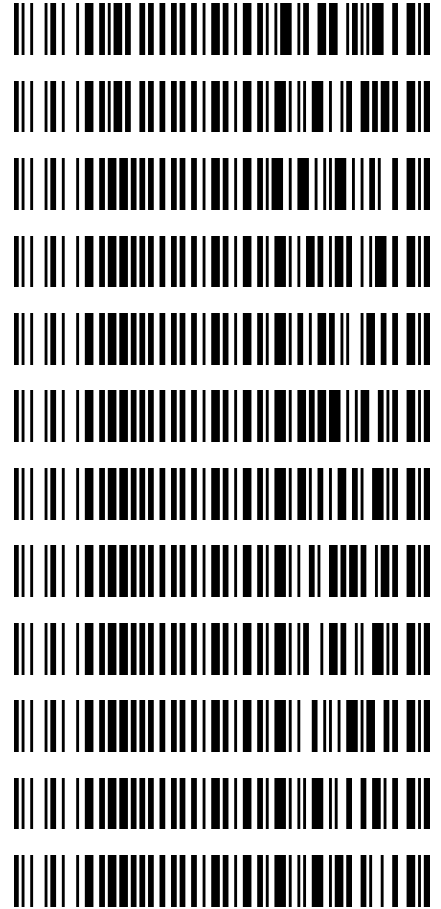
By Tamara Salazar at 2:49 pm, Sep 13, 2023

9/12/2023

CS

Worklist: 6492

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-0917	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-3224	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1958	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1992	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2029	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2048	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2049	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2123	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2349	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2383	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2404	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2409	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: 09/11/2023

Analyst: Celena Shrum

Plate lot#: 230627

Plate Retest Date: 12/27/2023

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	P2023-2049-1			
B	IS + Cal. 2	NEG Blood	P2023-2123-1			
C	IS + Cal. 3	M2023-0917-1	P2023-2349-1			
D	IS + Cal. 4	M2023-3224-2	P2023-2383-1			
E	IS + Cal. 5	P2023-1958-1	P2023-2404-1			
F	IS + Cal. 6	P2023-1992-2	P2023-2409-1			
G	IS + Cal. 7	P2023-2029-1				
H	QC1	P2023-2048-1				

Samples were moved to columns 4-6 during the SLE portion of the extraction (A1 moved to A4, D3 moved to D6, etc.)

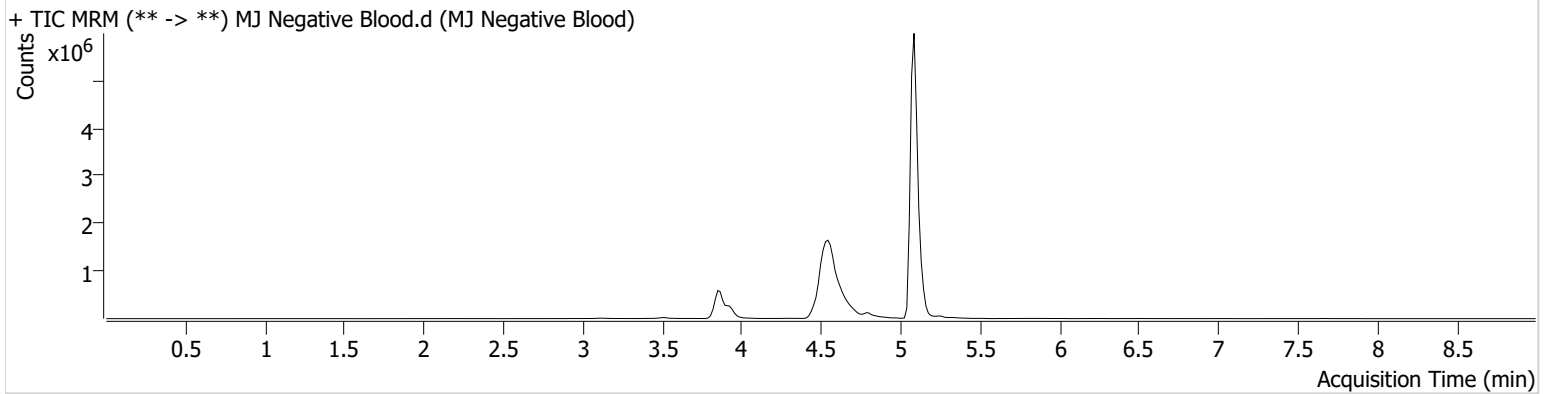


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-B5	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	9/11/2023 5:17:48 PM		
Sample Info.			

Sample Chromatogram





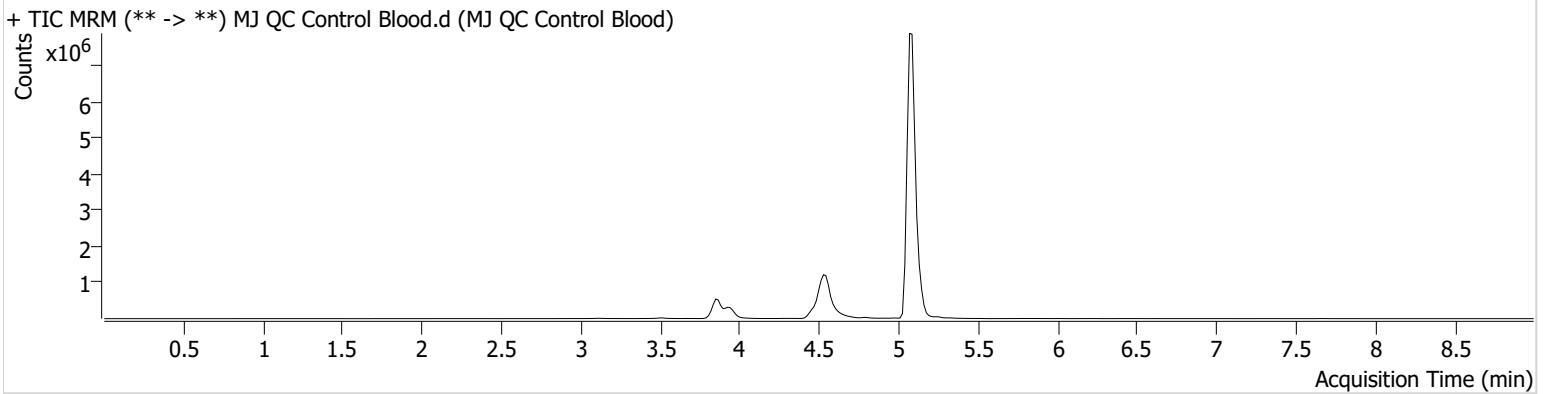
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-H4 Comment
Injection Volume 10
Acq. Date-Time 9/11/2023 4:51:35 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	1036026	1331.81	27.4	∞	27812757	4.2050 ng/ml
THC-COOH	3.954	71593	∞	249.8	∞	717392	14.8020 ng/ml
THC-OH	3.865	128117	∞	14.9	∞	2092506	4.5793 ng/ml



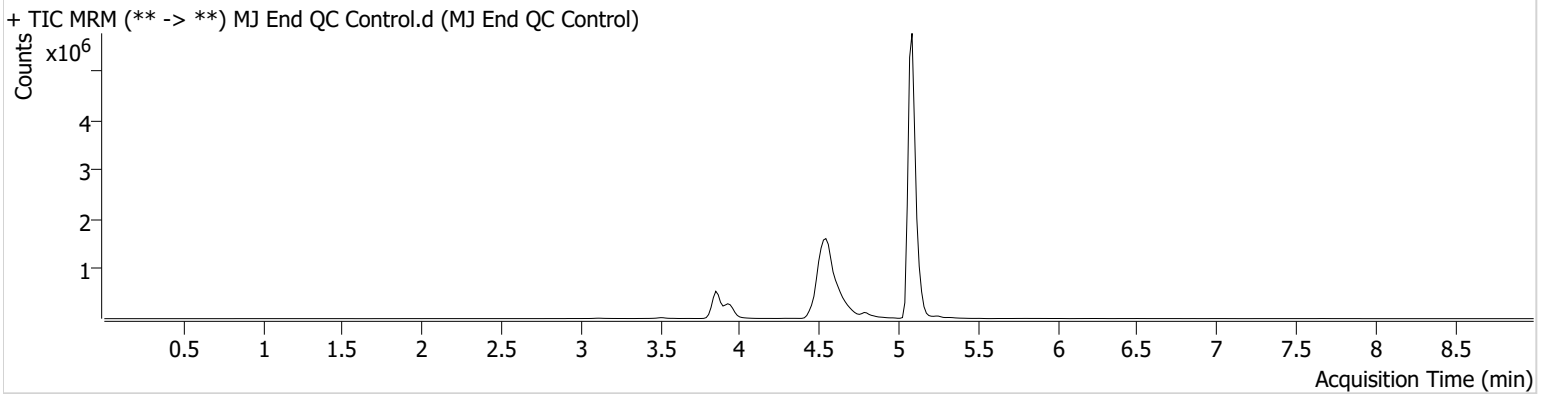
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 AM

Instrument Falco (069901) **Data File** MJ End QC Control.d
Type QC **Sample** MJ End QC Control
Acq. Method AM 27 Agilent Method.m **Operator** Celena Shrum
Sample Position P1-A5 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 10:58:32 PM
Sample Info.

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Sample Chromatogram



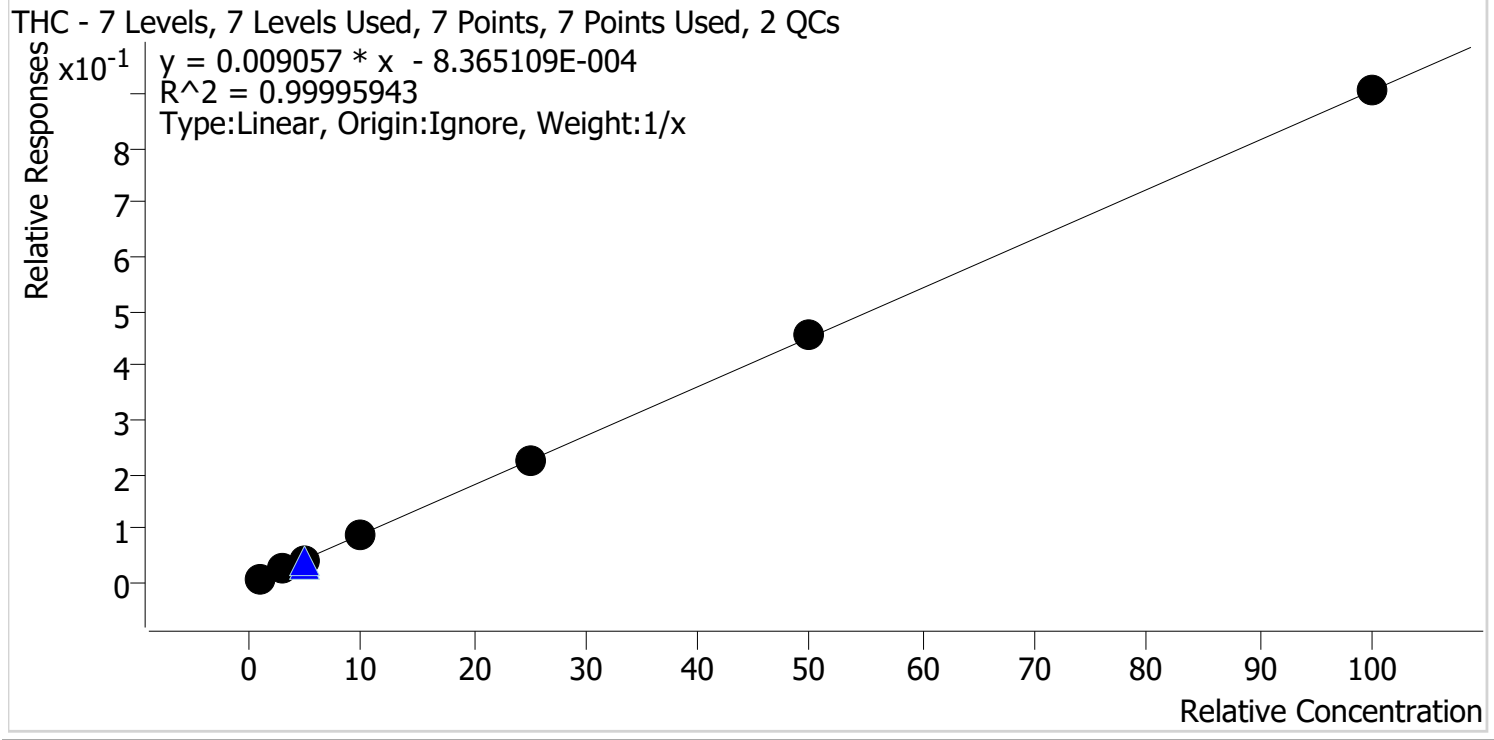
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	728847	2536.98	27.8	381.49	17840660	4.6029 ng/ml
THC-COOH	3.939	68378	1319.40	245.1	1470.36	663730	15.2741 ng/ml
THC-OH	3.865	122982	188.97	13.4	∞	1975108	4.6548 ng/ml

CS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 9/12/2023 8:20 AM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3



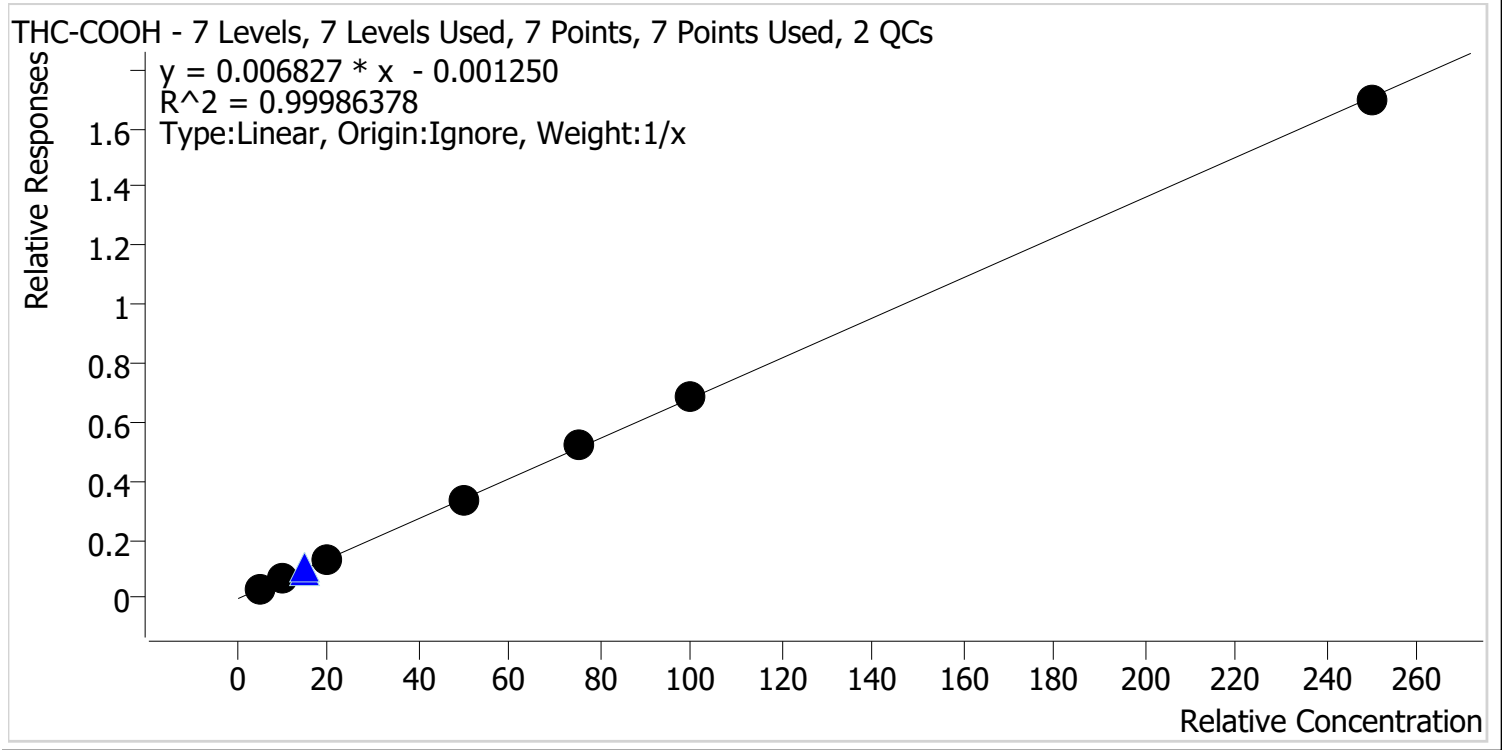
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.0	103.2
Cal 2 MJ	2	✓	3.0	3.0	99.3
Cal 3 MJ	3	✓	5.0	5.0	99.0
Cal 4 MJ	4	✓	10.0	9.8	98.3
Cal 5 MJ	5	✓	25.0	24.9	99.6
Cal 6 MJ	6	✓	50.0	50.3	100.6
Cal 7 MJ	7	✓	100.0	100.0	100.0

CS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 9/12/2023 8:20 AM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

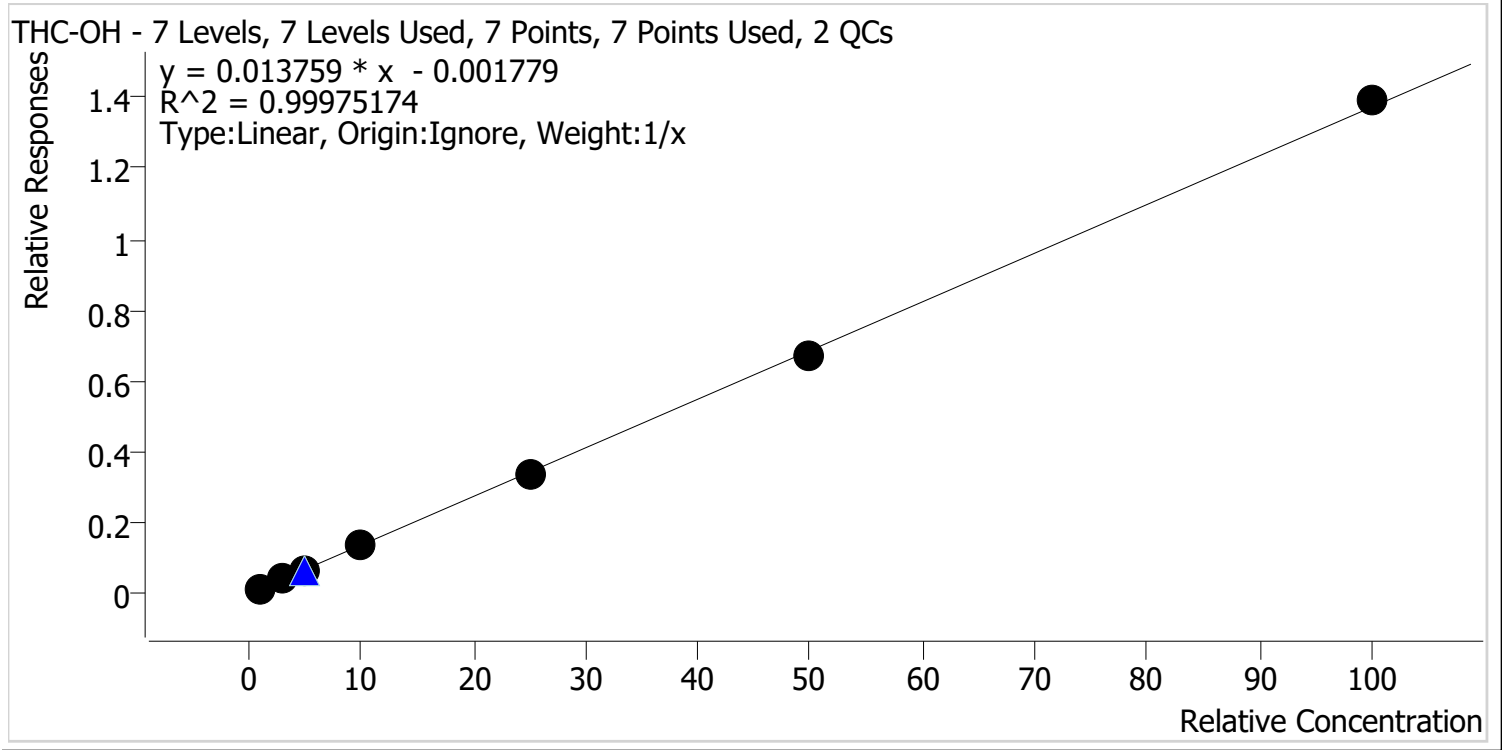


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.1	101.7
Cal 2 MJ	2	✓	10.0	9.7	97.0
Cal 3 MJ	3	✓	20.0	20.0	99.8
Cal 4 MJ	4	✓	50.0	49.8	99.7
Cal 5 MJ	5	✓	75.0	76.0	101.4
Cal 6 MJ	6	✓	100.0	101.2	101.2
Cal 7 MJ	7	✓	250.0	248.2	99.3



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 9/12/2023 8:20 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	1.0	104.7
Cal 2 MJ	2	✓	3.0	3.0	98.5
Cal 3 MJ	3	✓	5.0	5.0	100.0
Cal 4 MJ	4	✓	10.0	9.8	98.2
Cal 5 MJ	5	✓	25.0	24.8	99.0
Cal 6 MJ	6	✓	50.0	49.1	98.2
Cal 7 MJ	7	✓	100.0	101.3	101.3



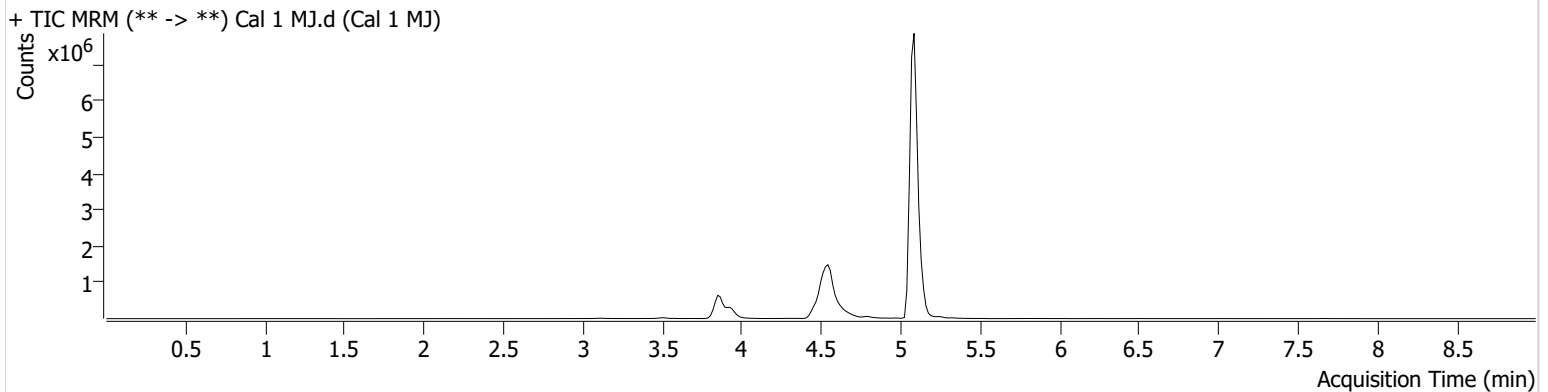
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-A4 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 3:06:35 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	5.090	230462	2678.32	25.1	∞	27078129	1.0320 ng/ml
THC-COOH	3.954	28549	∞	252.6	∞	852902	5.0865 ng/ml
THC-OH	3.865	31705	∞	12.5	31.00	2511085	1.0469 ng/ml



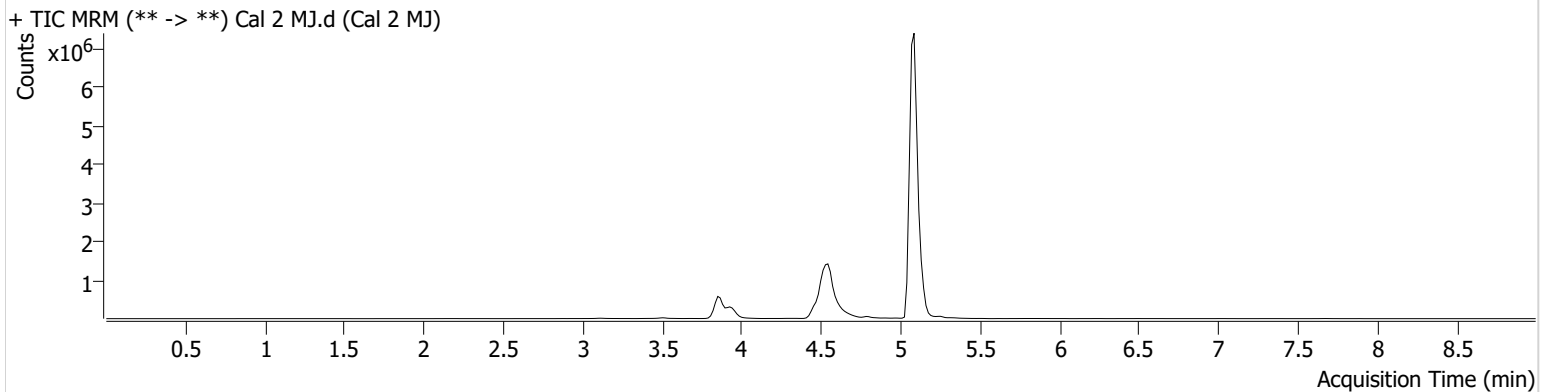
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-B4 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 3:19:52 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	664849	4132.74	25.1	∞	25435570	2.9783 ng/ml
THC-COOH	3.954	48601	911.34	242.8	2985.56	748219	9.6982 ng/ml
THC-OH	3.865	83092	∞	13.4	161.01	2136683	2.9557 ng/ml



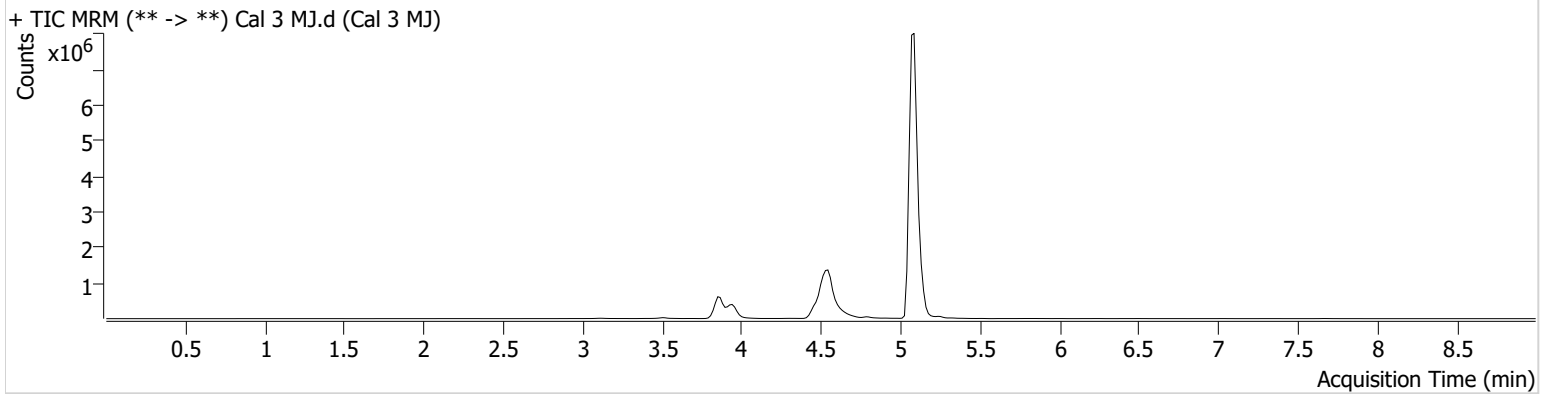
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-C4 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 3:32:58 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	5.090	1217900	21601.73	25.7	∞	27673550	4.9513 ng/ml
THC-COOH	3.954	108794	1758.61	245.1	11177.15	806154	19.9521 ng/ml
THC-OH	3.865	154157	∞	13.3	∞	2300656	4.9993 ng/ml



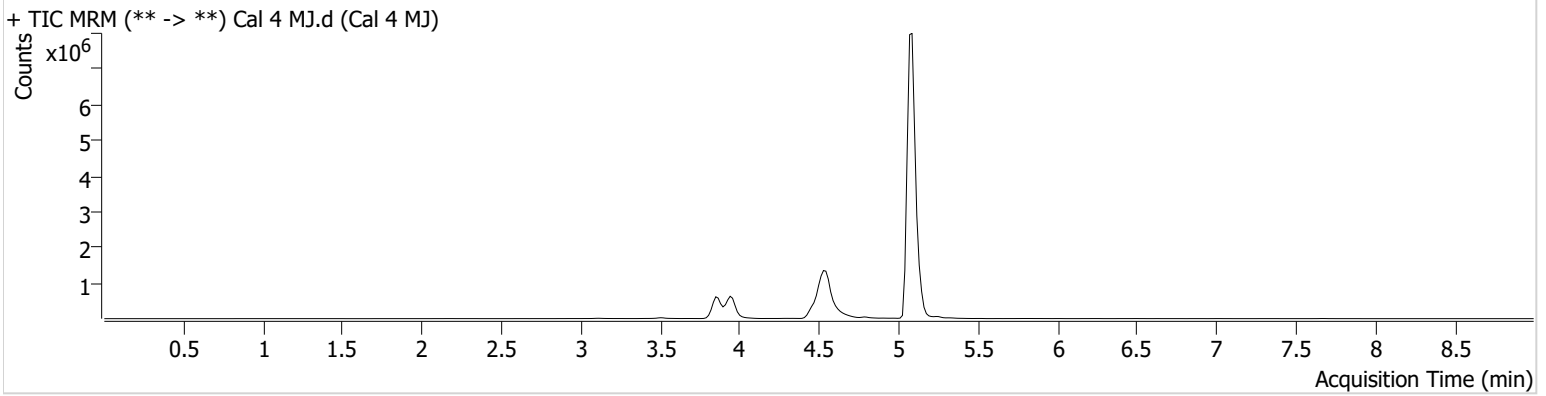
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-D4 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 3:46:04 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	2309453	∞	26.6	∞	26191863	9.8275 ng/ml
THC-COOH	3.954	249965	∞	248.0	13456.79	737427	49.8375 ng/ml
THC-OH	3.865	287294	∞	14.2	∞	2154039	9.8231 ng/ml



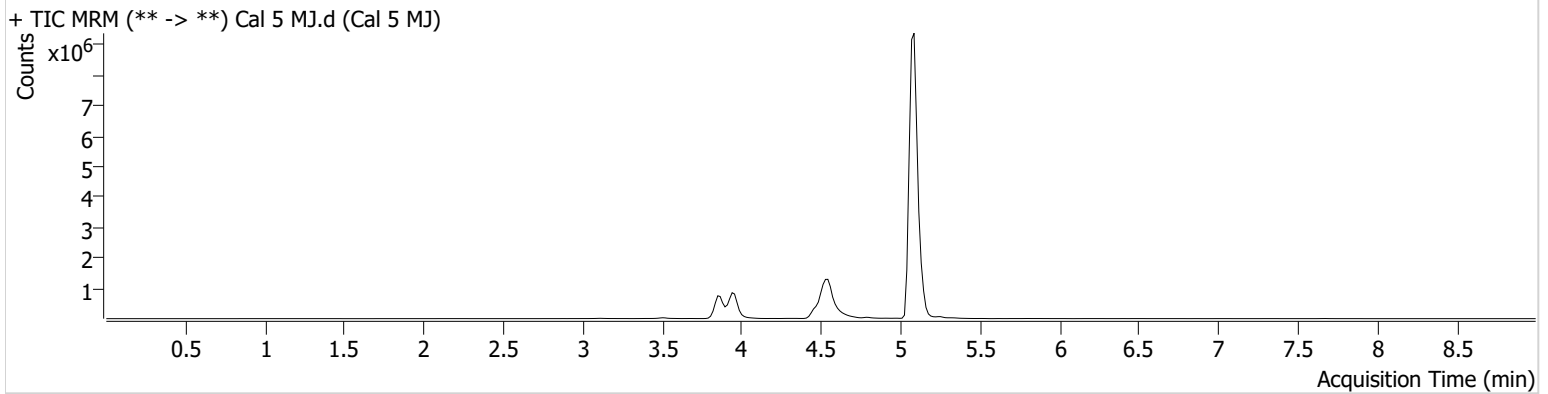
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-E4 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 3:59:10 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	6010333	∞	26.2	∞	26743525	24.9054 ng/ml
THC-COOH	3.954	368815	∞	249.6	∞	712153	76.0464 ng/ml
THC-OH	3.865	736373	∞	13.9	2639.70	2172916	24.7599 ng/ml



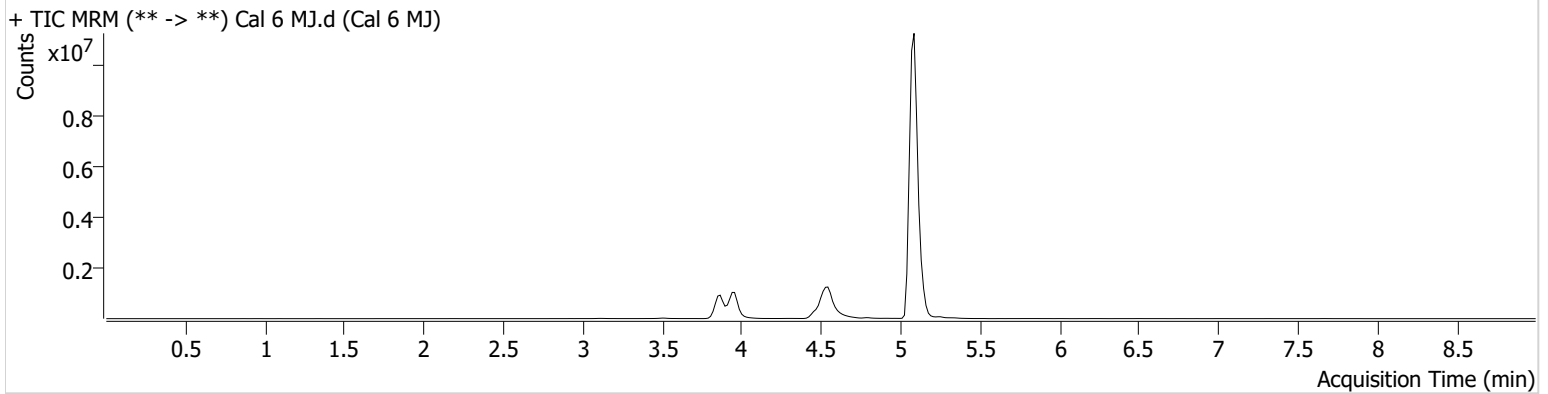
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-F4 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 4:12:16 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	11900735	∞	25.9	∞	26173938	50.2924 ng/ml
THC-COOH	3.954	479082	1770.95	244.5	30402.64	694872	101.1785 ng/ml
THC-OH	3.865	1428127	∞	14.0	∞	2119058	49.1122 ng/ml



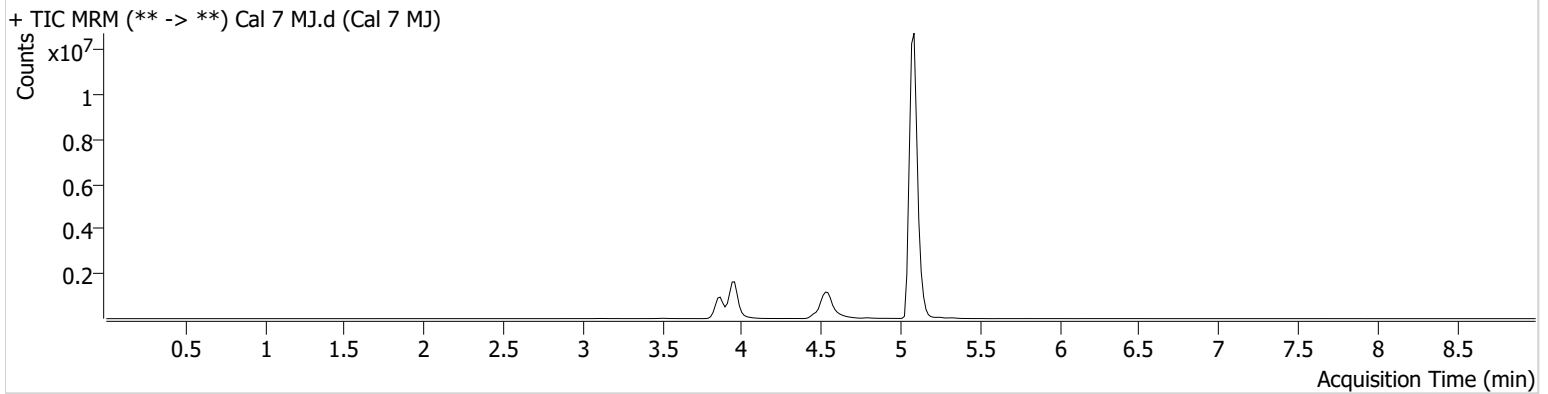
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091123 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/12/2023 8:20:44 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-G4 **Comment**
Injection Volume 10
Acq. Date-Time 9/11/2023 4:25:22 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	5.090	19093540	122251.82	25.6	∞	21097457	100.0131 ng/ml
THC-COOH	3.954	854448	14353.29	240.6	24956.78	504660	248.2010 ng/ml
THC-OH	3.865	2063837	∞	14.8	∞	1482617	101.3029 ng/ml