

Worklist: 6406

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
P2023-1258	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2023-1343	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1459	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1505	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1506	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1575	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1591	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: 06/07/2023

Analyst: Tamara Salazar

Plate lot#: 230113

Plate Retest Date: 07/13/2023

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 23A52594

Blank Urine Lot: POC021022

Column: UCT Selectra DA 100 x 2.1mm 3um

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: 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 and urine (if applicable) (calibrated pipette)** into the appropriate wells of 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, and 500µL of saturated phosphate buffer to urine samples** in the wells of the analytical plate.
- 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 7. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
Amount transferred: 750µL
- 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)
- 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.
- 17. 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. Did all QCs pass for each analyte? (if not, describe in comments section)
- 5. Enter QCs into control charting.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	Neg Urine	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	Neg Blood	P2023-1258-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	P2023-1343-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	P2023-1459-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2023-1505-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2023-1506-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2023-1575-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2023-1591-1	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	Neg Urine			
B	IS + Cal. 2	Neg Blood	P2023-1258-1			
C	IS + Cal. 3	P2023-1343-1	P2023-1459-1*			
D	IS + Cal. 4	P2023-1459-1*				
E	IS + Cal. 5	P2023-1505-1				
F	IS + Cal. 6	P2023-1506-1				
G	IS + Cal. 7	P2023-1575-1				
H	IS + QC_1	P2023-1591-1				

*Sample moved during step 7 of the extraction process due to a clot

TS

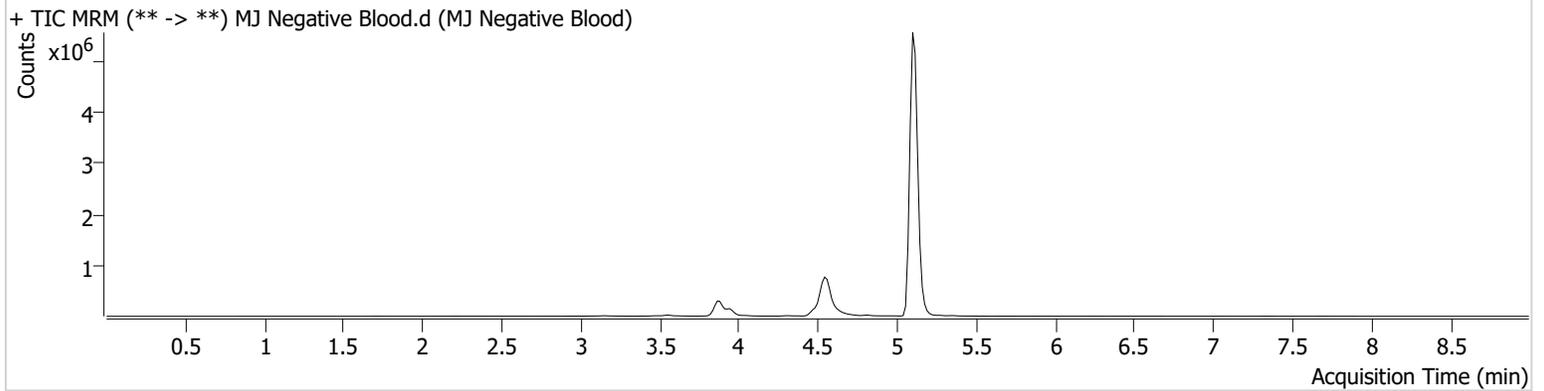


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
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	6/7/2023 3:58:32 PM		
Sample Info.			

Sample Chromatogram



TS



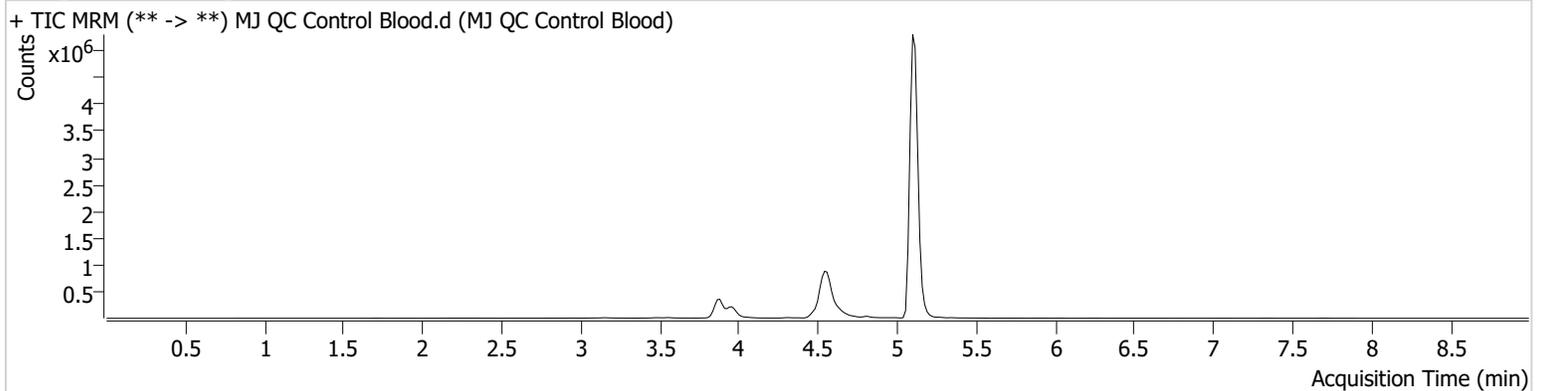
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 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** Tamara Salazar
Sample Position P1-H1 **Comment**
Injection Volume 10
Acq. Date-Time 6/7/2023 3:32:20 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	5.120	799107	∞	24.2	∞	17914178	5.0192 ng/ml
THC-COOH	3.969	53064	∞	203.3	∞	466010	15.0253 ng/ml
THC-OH	3.881	103588	∞	14.1	∞	1313864	4.7658 ng/ml

TS

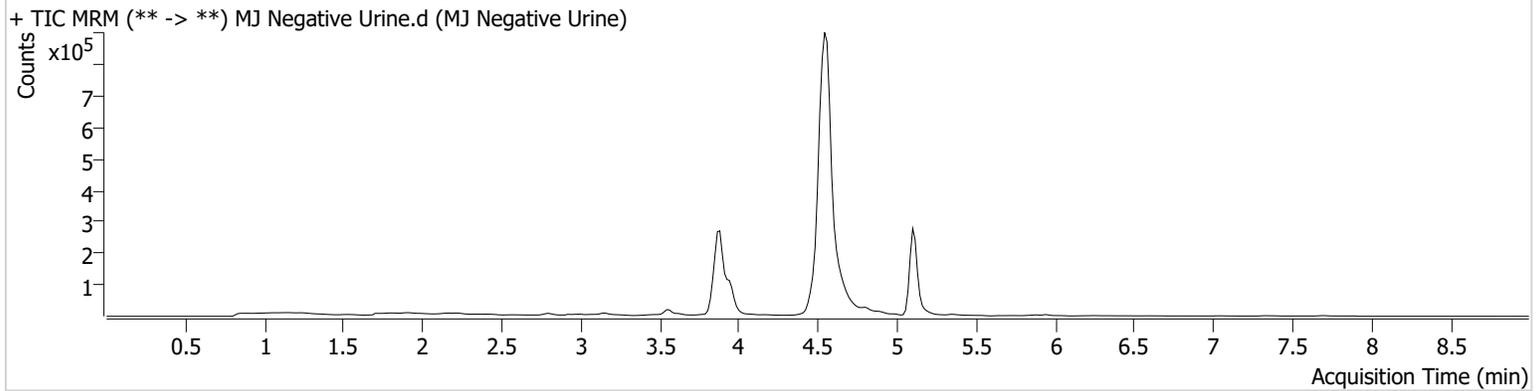


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	MJ Negative Urine.d
Type	Sample	Sample	MJ Negative Urine
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-A3	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	6/7/2023 7:01:52 PM		
Sample Info.			

Sample Chromatogram



TS

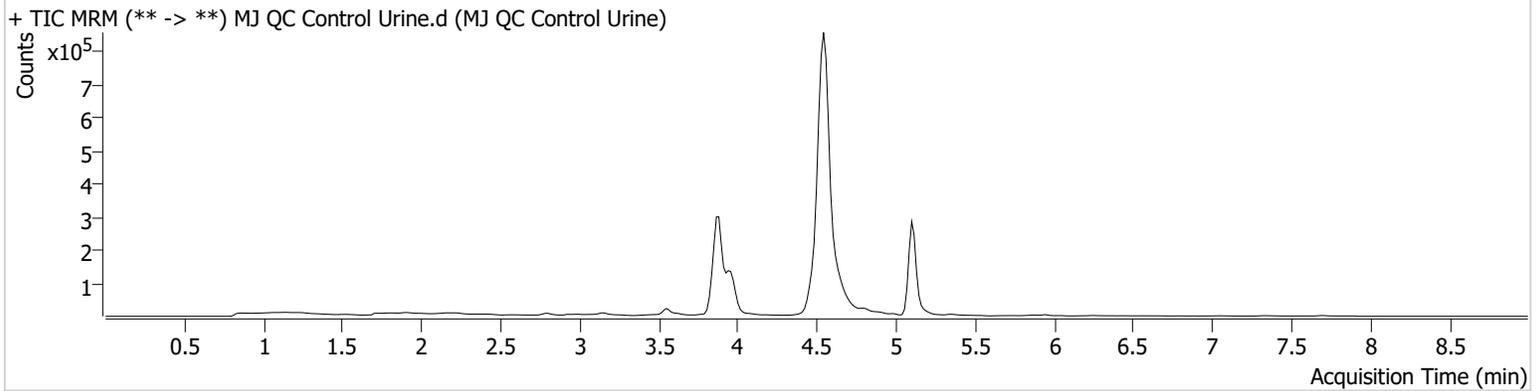


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	MJ QC Control Urine.d
Type	QC	Sample	MJ QC Control Urine
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-A2	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	6/7/2023 7:54:19 PM		
Sample Info.			

Sample Chromatogram



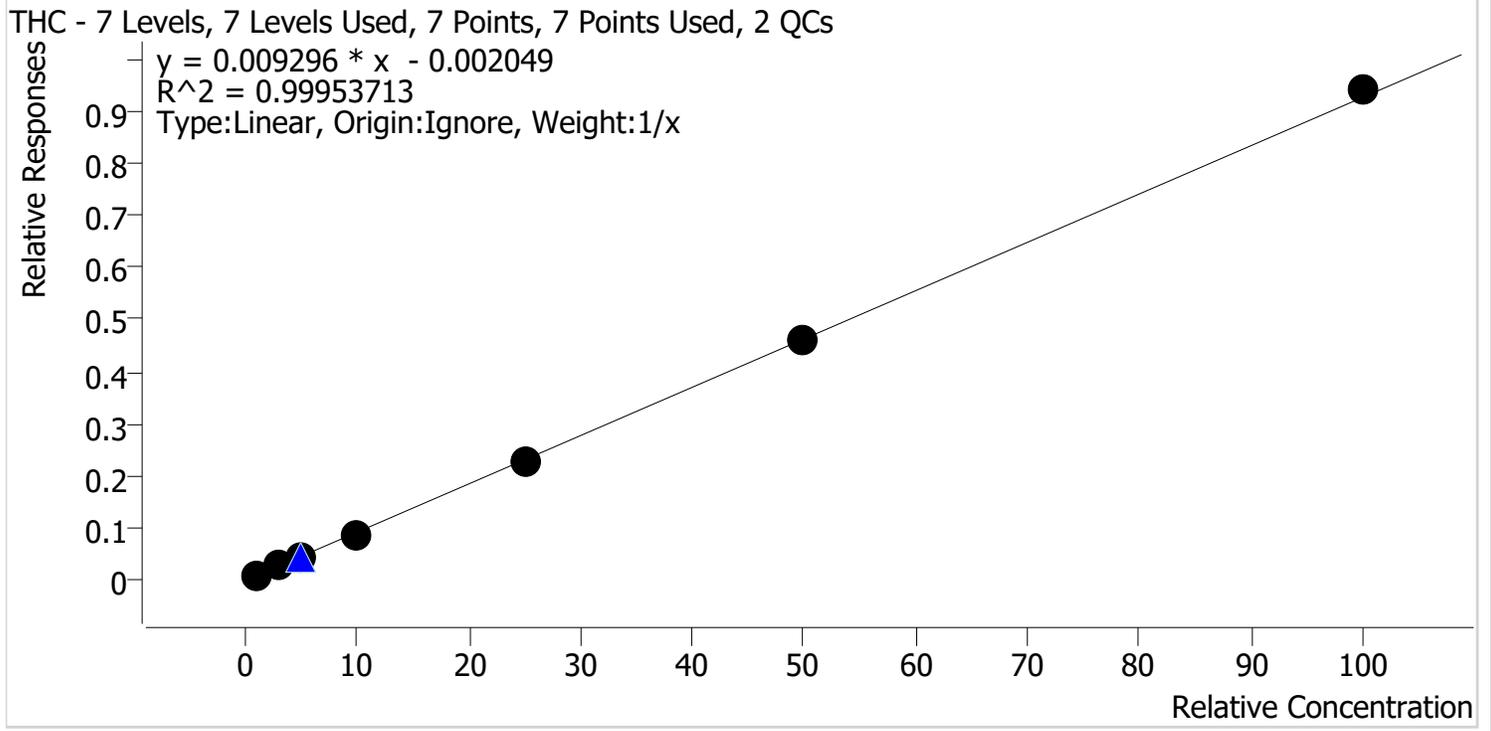
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	38692	808.67	28.0	∞	916717	4.7609 ng/ml
THC-COOH	3.969	32019	∞	213.6	274.80	296970	14.2570 ng/ml
THC-OH	3.881	88652	∞	15.2	∞	1134335	4.7250 ng/ml

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 6/8/2023 8:16 AM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3



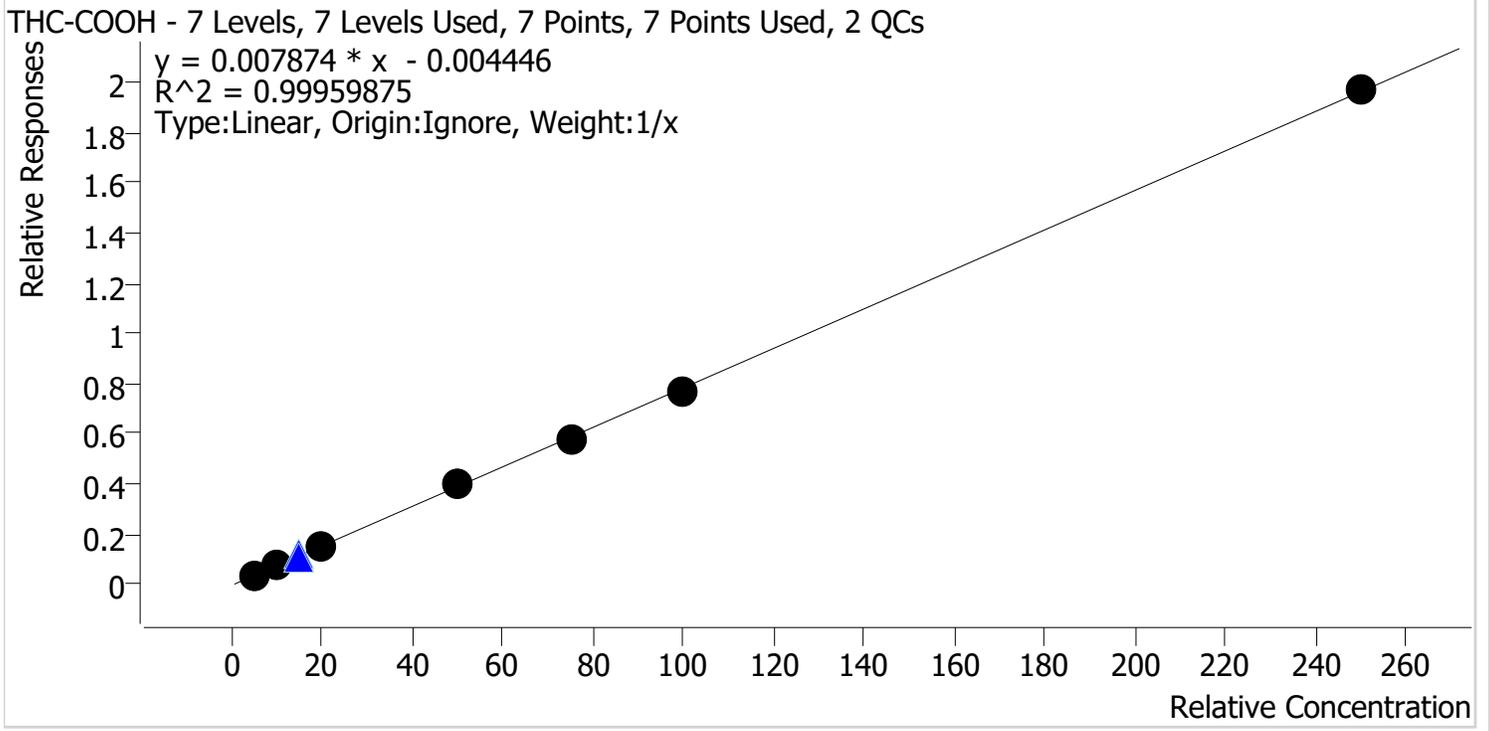
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	108.5
Cal 2 MJ	2	✓	3.0	3.0	99.6
Cal 3 MJ	3	✓	5.0	4.9	98.7
Cal 4 MJ	4	✓	10.0	9.5	95.3
Cal 5 MJ	5	✓	25.0	24.2	97.0
Cal 6 MJ	6	✓	50.0	49.7	99.5
Cal 7 MJ	7	✓	100.0	101.5	101.5

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 6/8/2023 8:16 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	5.0	100.7
Cal 2 MJ	2	✓	10.0	9.9	98.9
Cal 3 MJ	3	✓	20.0	19.7	98.4
Cal 4 MJ	4	✓	50.0	52.2	104.3
Cal 5 MJ	5	✓	75.0	74.7	99.6
Cal 6 MJ	6	✓	100.0	97.7	97.7
Cal 7 MJ	7	✓	250.0	250.8	100.3

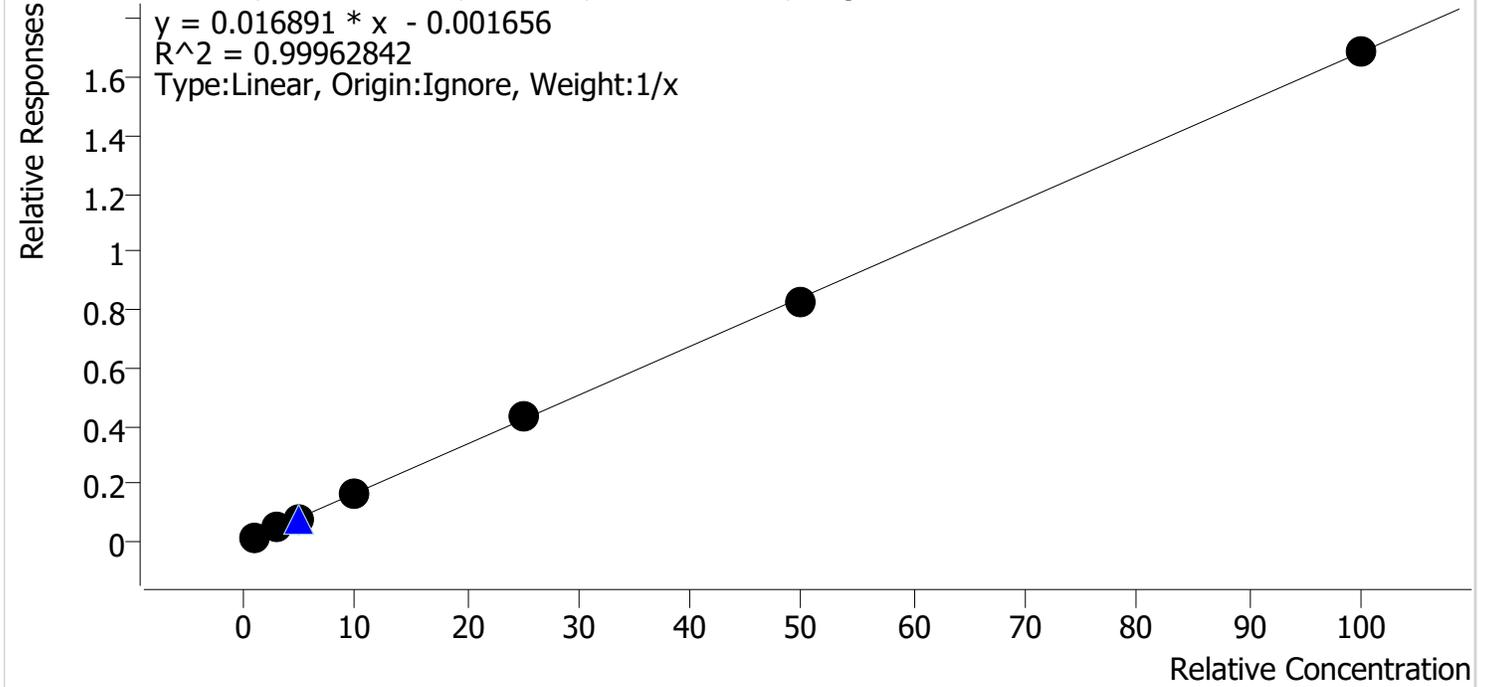
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 6/8/2023 8:16 AM
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	1.0	101.2
Cal 2 MJ	2	✓	3.0	2.9	96.6
Cal 3 MJ	3	✓	5.0	4.9	98.6
Cal 4 MJ	4	✓	10.0	10.2	101.8
Cal 5 MJ	5	✓	25.0	25.9	103.7
Cal 6 MJ	6	✓	50.0	49.0	98.1
Cal 7 MJ	7	✓	100.0	100.0	100.0

TS

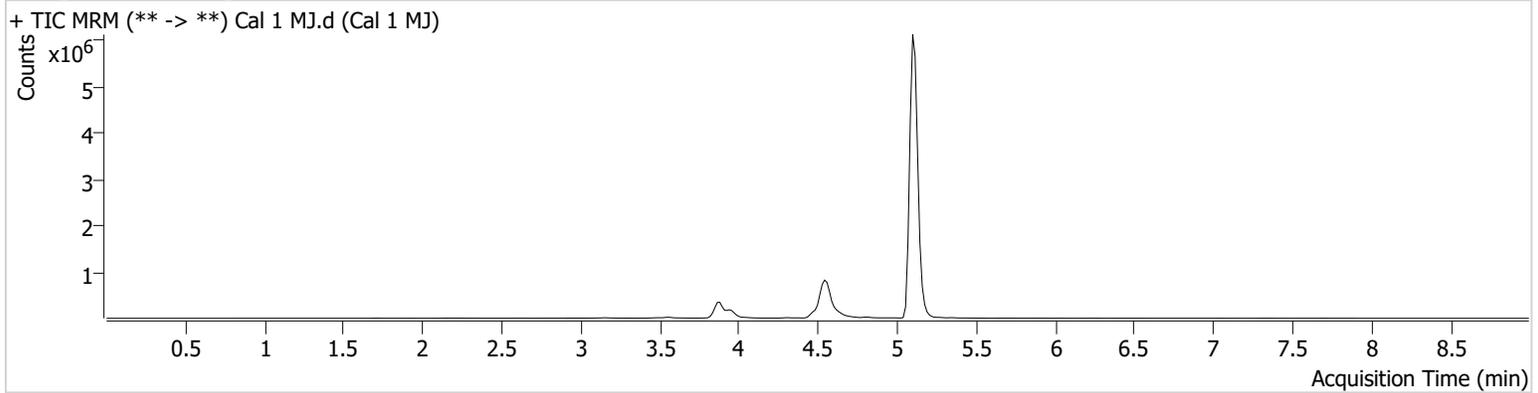


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
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	6/7/2023 1:47:30 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	173299	∞	25.9	∞	21568320	1.0848 ng/ml
THC-COOH	3.969	17145	∞	215.6	∞	486838	5.0370 ng/ml
THC-OH	3.881	20613	∞	11.2	59.63	1335271	1.0120 ng/ml

TS



AM #27 Cannabinoids Quant. Results

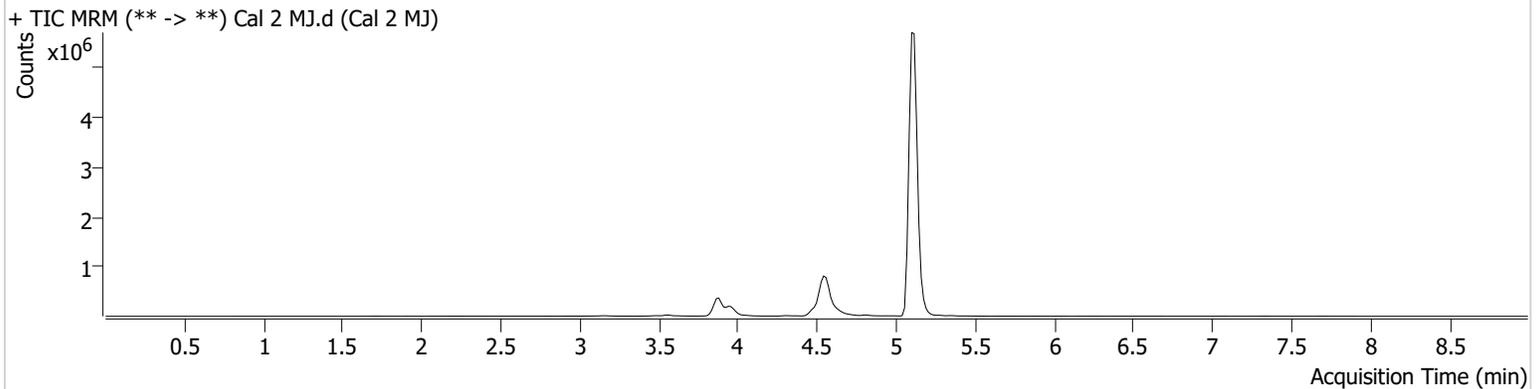
Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-B1
Injection Volume 10
Acq. Date-Time 6/7/2023 2:00:44 PM
Sample Info.

Data File Cal 2 MJ.d
Sample Cal 2 MJ
Operator Tamara Salazar
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	5.120	529200	∞	24.0	∞	20568731	2.9882 ng/ml
THC-COOH	3.969	36083	609.82	207.7	∞	491583	9.8863 ng/ml
THC-OH	3.881	64998	∞	13.1	∞	1374706	2.8973 ng/ml

TS

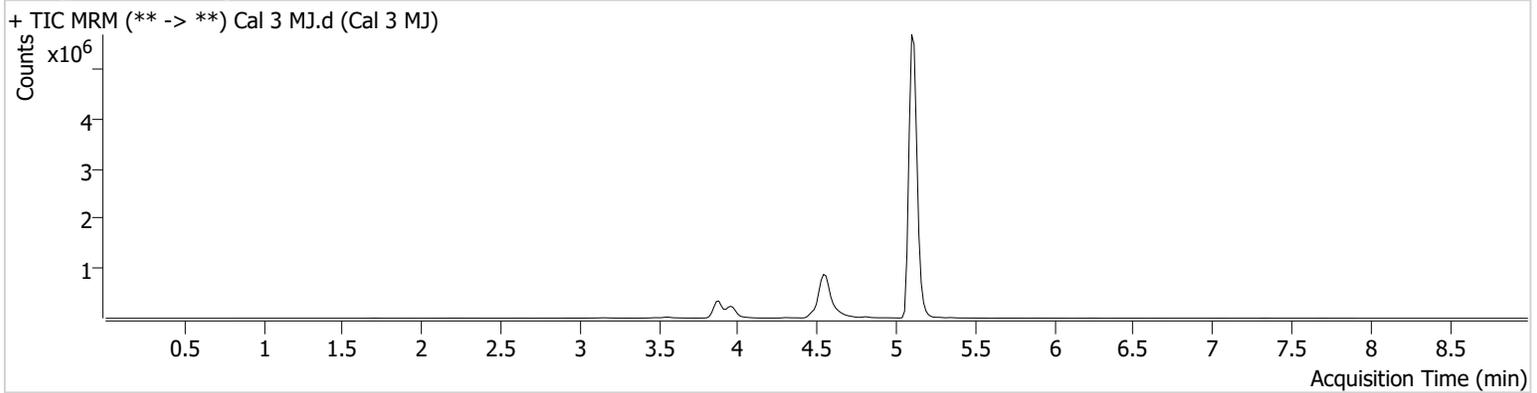


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-C1	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	6/7/2023 2:13:50 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	848457	∞	23.7	∞	19369521	4.9327 ng/ml
THC-COOH	3.969	69368	1074.88	216.5	∞	460780	19.6828 ng/ml
THC-OH	3.881	102230	∞	13.7	∞	1252398	4.9307 ng/ml

TS

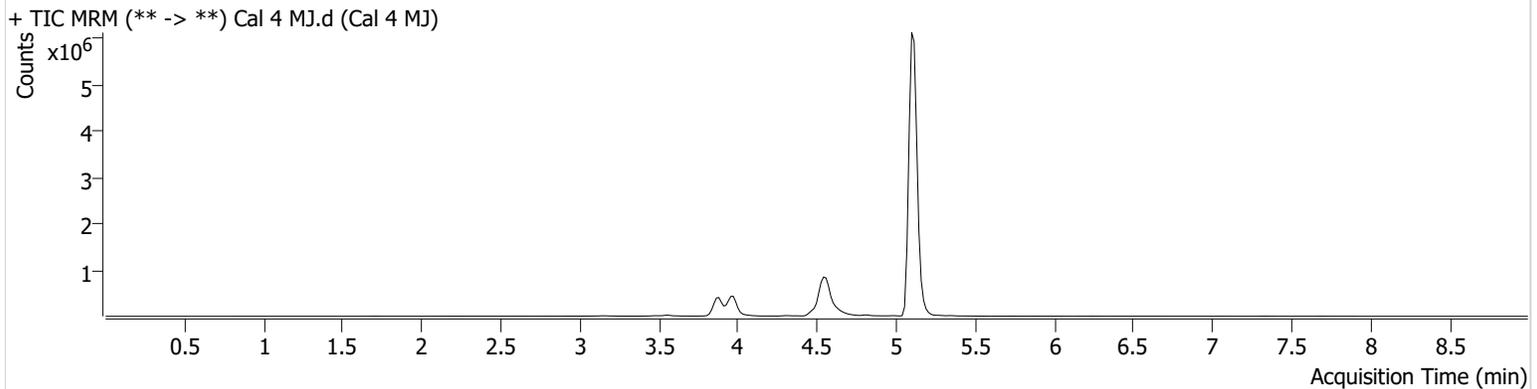


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
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	6/7/2023 2:26:55 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	1750566	∞	25.2	∞	20218598	9.5347 ng/ml
THC-COOH	3.969	199507	∞	199.3	∞	491057	52.1601 ng/ml
THC-OH	3.881	236096	∞	13.0	∞	1386151	10.1820 ng/ml

TS



AM #27 Cannabinoids Quant. Results

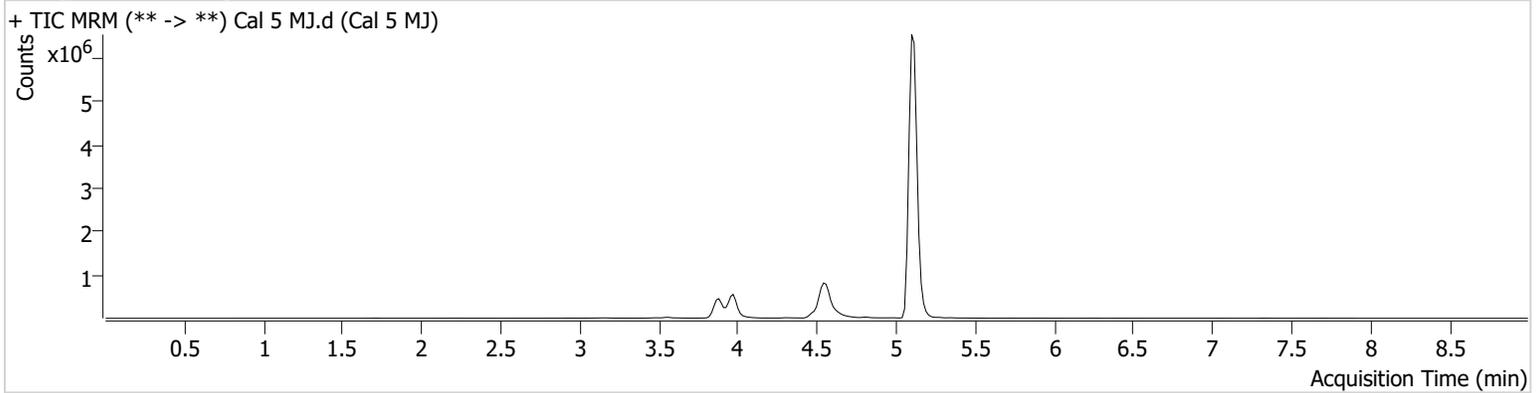
Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-E1
Injection Volume 10
Acq. Date-Time 6/7/2023 2:39:59 PM
Sample Info.

Data File Cal 5 MJ.d
Sample Cal 5 MJ
Operator Tamara Salazar
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	5.120	4148450	52394.04	25.0	∞	18578893	24.2411 ng/ml
THC-COOH	3.969	251227	∞	211.7	∞	430270	74.7144 ng/ml
THC-OH	3.881	529496	∞	14.1	∞	1213611	25.9287 ng/ml

TS

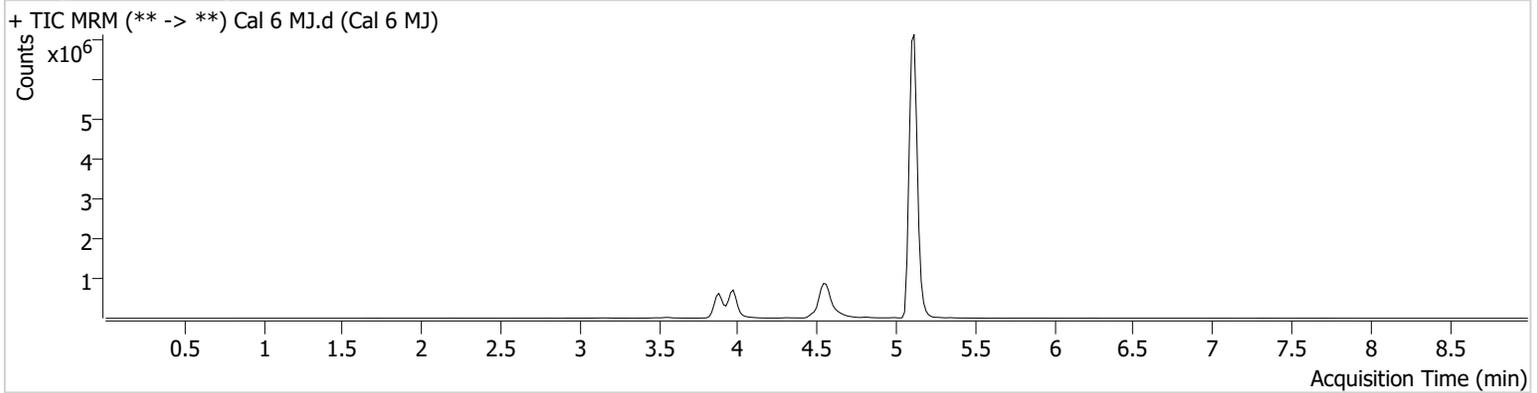


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
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	6/7/2023 2:53:03 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	7568922	61027.36	25.1	∞	16446745	49.7282 ng/ml
THC-COOH	3.969	331269	4518.91	211.5	5550.43	432983	97.7263 ng/ml
THC-OH	3.881	1018221	∞	14.7	∞	1232083	49.0257 ng/ml

TS

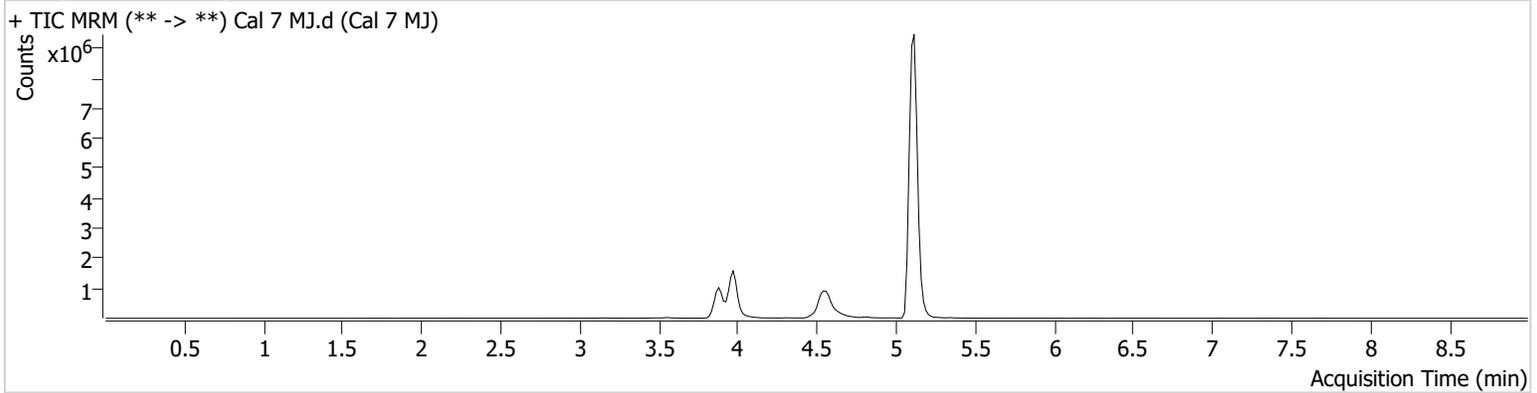


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\060623 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/8/2023 8:16:48 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
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	6/7/2023 3:06:07 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	14925749	410784.03	25.5	∞	15855348	101.4902 ng/ml
THC-COOH	3.969	820002	10002.50	209.2	∞	416163	250.7930 ng/ml
THC-OH	3.881	2262894	∞	14.9	∞	1340723	100.0237 ng/ml