

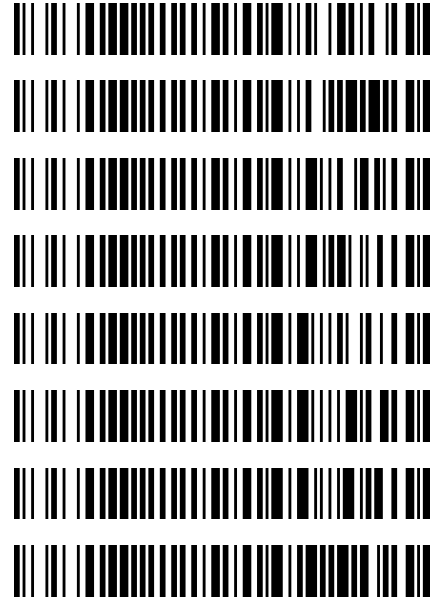
Worklist: 6433

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2023-2190	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2223	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2236	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2351	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2368	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2421	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2519	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2603	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2619	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2023-2757	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1497	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1520	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1607	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1788	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1789	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1790	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1794	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1795	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1796	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1797	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2023-1831	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

Worklist: 6433

TS

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
P2023-1835	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1867	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1919	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1923	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1931	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1934	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1945	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1985	1	BCK	AM 27 Blood THC Quant by LC-QQQ



TS

AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 07/13/2023

Analyst: Tamara Salazar

Plate lot#: 220802

Plate Retest Date: 07/23/2023

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:

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	M2023-2519-1	P2023-1789-1	P2023-1867-1	IS + QC_1
B	IS + Cal. 2	Neg Blood	M2023-2603-2	P2023-1790-1	P2023-1919-1	IS + Cal. 7
C	IS + Cal. 3	M2023-2190-1	M2023-2619-1	P2023-1794-1	P2023-1923-1	IS + Cal. 6
D	IS + Cal. 4	M2023-2223-2	M2023-2757-2	P2023-1795-1	P2023-1931-1	IS + Cal. 5
E	IS + Cal. 5	M2023-2236-1	P2023-1497-1	P2023-1796-1	P2023-1934-1	IS + Cal. 4
F	IS + Cal. 6	M2023-2351-2	P2023-1520-1	P2023-1797-1	P2023-1945-1	IS + Cal. 3
G	IS + Cal. 7	M2023-2368-1	P2023-1607-1	P2023-1831-1	P2023-1985-1	IS + Cal. 2
H	IS + QC_1	M2023-2421-1	P2023-1788-2	P2023-1835-1	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	M2023-2519-1	P2023-1789-1	P2023-1867-1	
B	IS + Cal. 2	Neg Blood	M2023-2603-2	P2023-1790-1	P2023-1919-1	
C	IS + Cal. 3	M2023-2190-1	M2023-2619-1	P2023-1794-1	P2023-1923-1	
D	IS + Cal. 4	M2023-2223-2	M2023-2757-2	P2023-1795-1	P2023-1931-1	
E	IS + Cal. 5	M2023-2236-1	P2023-1497-1	P2023-1796-1	P2023-1934-1	
F	IS + Cal. 6	M2023-2351-2	P2023-1520-1	P2023-1797-1	P2023-1945-1	
G	IS + Cal. 7	M2023-2368-1	P2023-1607-1	P2023-1831-1	P2023-1985-1	
H	IS + QC_1	M2023-2421-1	P2023-1788-2	P2023-1835-1*	P2023-1835-1*	

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

TS

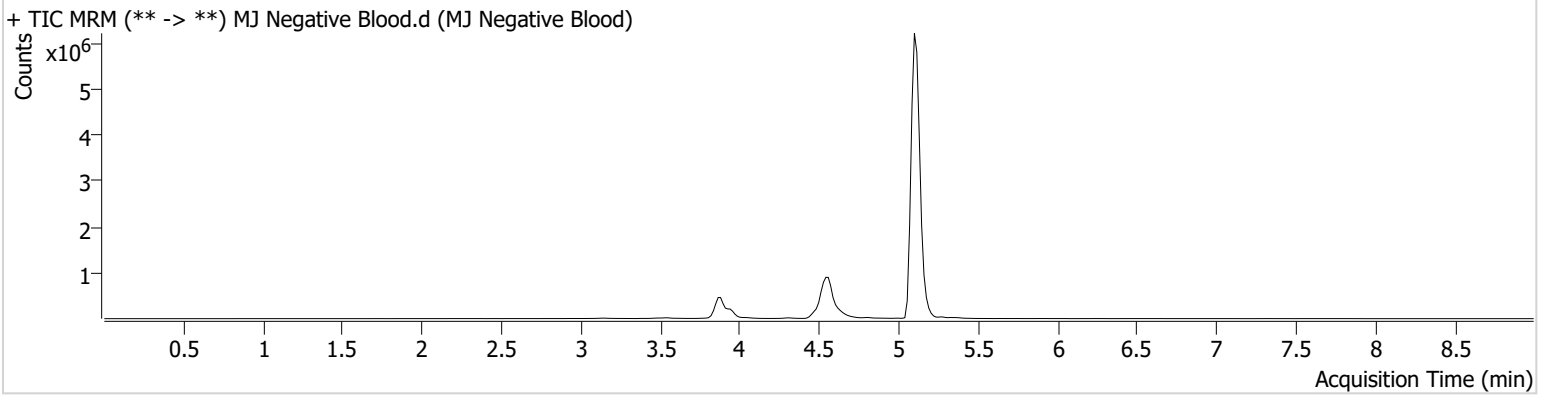


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\071323 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 7/14/2023 9:58:32 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	P5-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	7/13/2023 2:13:51 PM		
Sample Info.			

Sample Chromatogram



TS

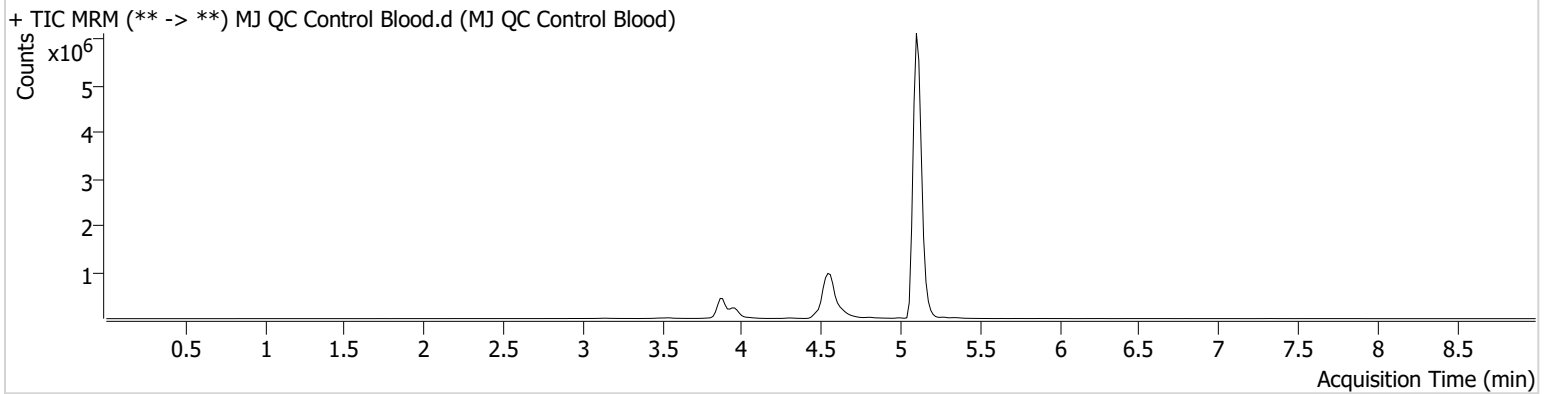


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\071323 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 7/14/2023 9:58:32 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	P5-H1	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	7/13/2023 1:47:40 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	949671	∞	23.3	∞	21401449	5.0473 ng/ml
THC-COOH	3.969	49258	867.50	239.8	1557.46	500573	15.0285 ng/ml
THC-OH	3.881	114679	∞	12.6	∞	1547834	4.7085 ng/ml

TS

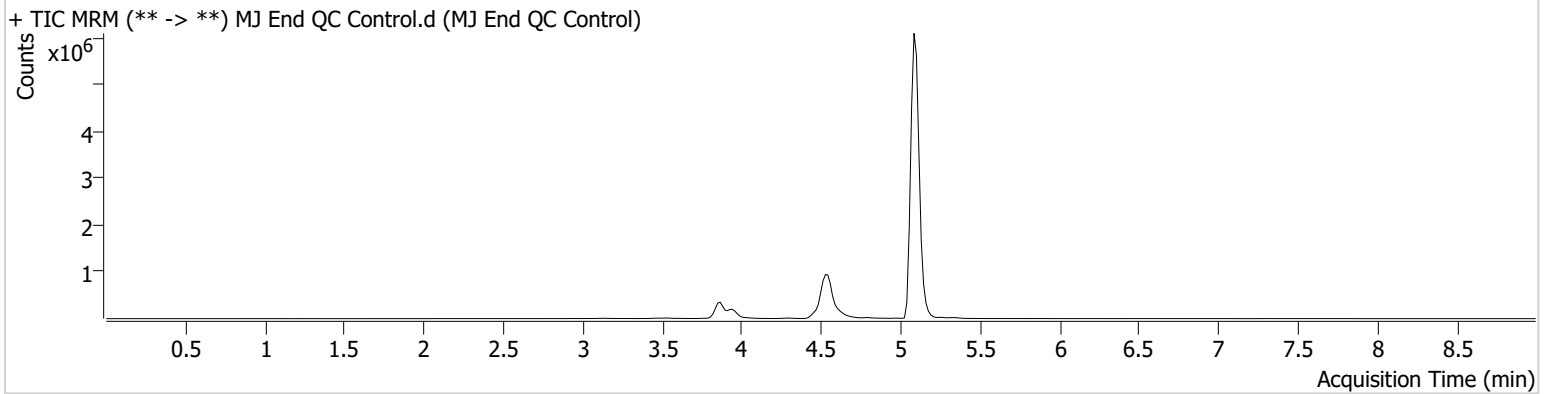


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\071323 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 7/14/2023 9:58:32 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	Tamara Salazar
Sample Position	P5-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	7/14/2023 3:19:49 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	920413	20672.46	23.6	∞	21192223	4.9447 ng/ml
THC-COOH	3.954	42579	634.03	237.7	∞	438068	14.8497 ng/ml
THC-OH	3.865	97469	∞	11.5	116.06	1262805	4.8972 ng/ml

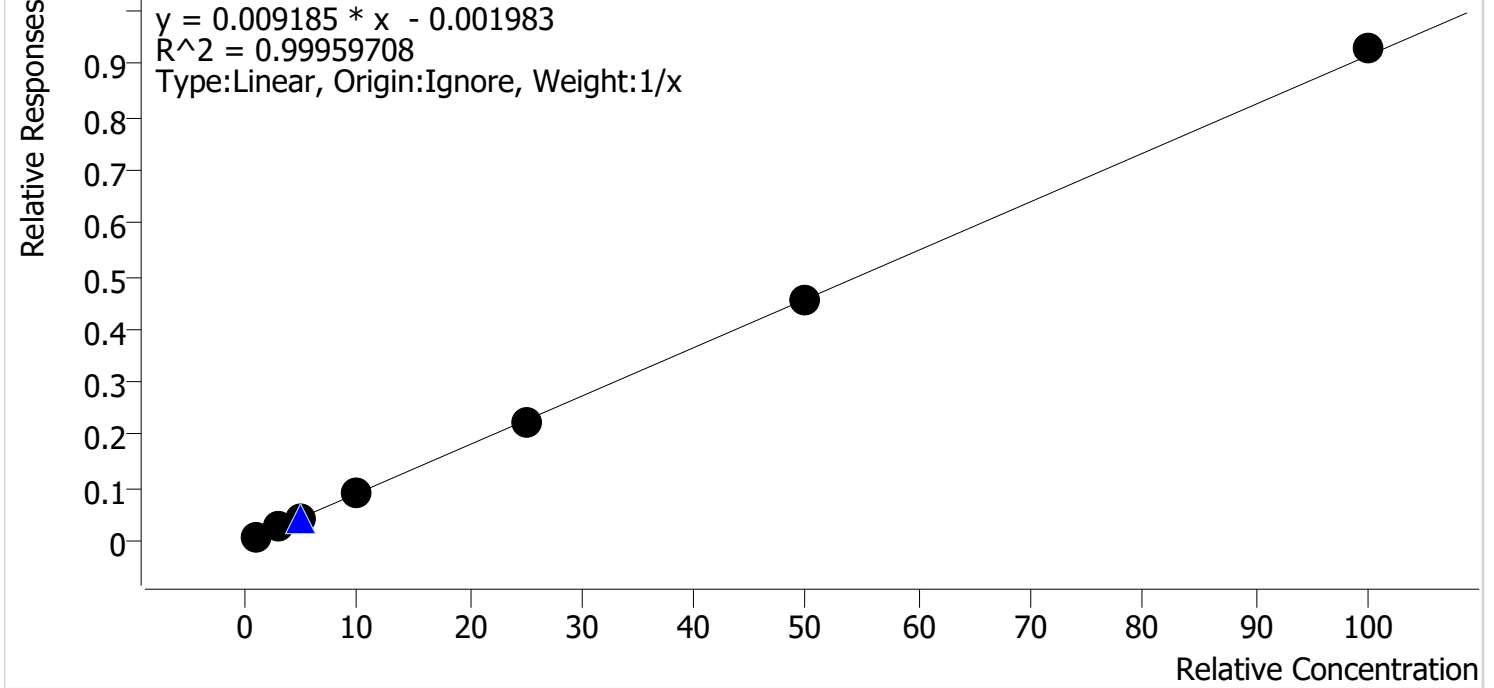
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\071323 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Last Cal. Update 7/14/2023 9:58 AM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3

THC - 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	109.7
Cal 2 MJ	2	✓	3.0	2.9	97.6
Cal 3 MJ	3	✓	5.0	4.9	97.5
Cal 4 MJ	4	✓	10.0	9.7	97.4
Cal 5 MJ	5	✓	25.0	24.2	97.0
Cal 6 MJ	6	✓	50.0	49.7	99.3
Cal 7 MJ	7	✓	100.0	101.4	101.4

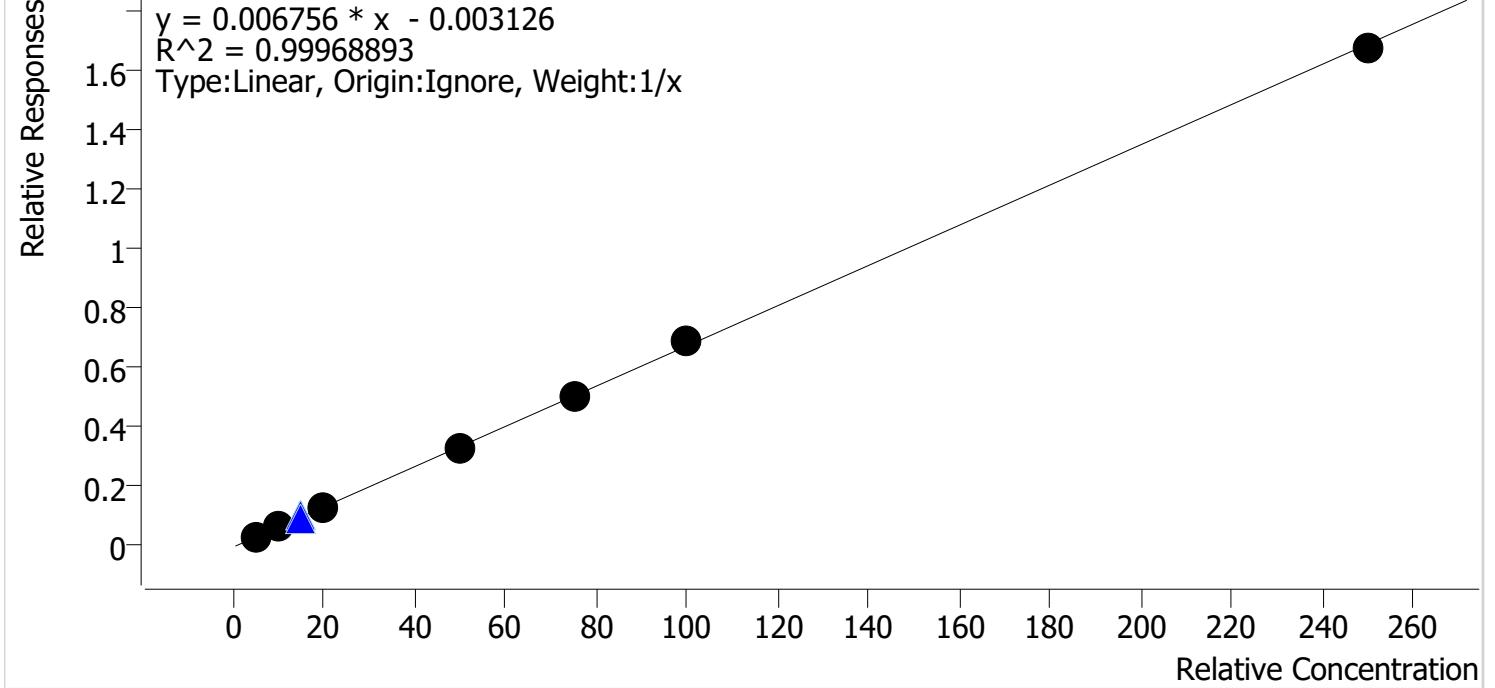
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\071323 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Last Cal. Update 7/14/2023 9:58 AM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.1	102.8
Cal 2 MJ	2	✓	10.0	9.7	97.4
Cal 3 MJ	3	✓	20.0	19.7	98.3
Cal 4 MJ	4	✓	50.0	49.4	98.9
Cal 5 MJ	5	✓	75.0	75.6	100.9
Cal 6 MJ	6	✓	100.0	102.7	102.7
Cal 7 MJ	7	✓	250.0	247.7	99.1

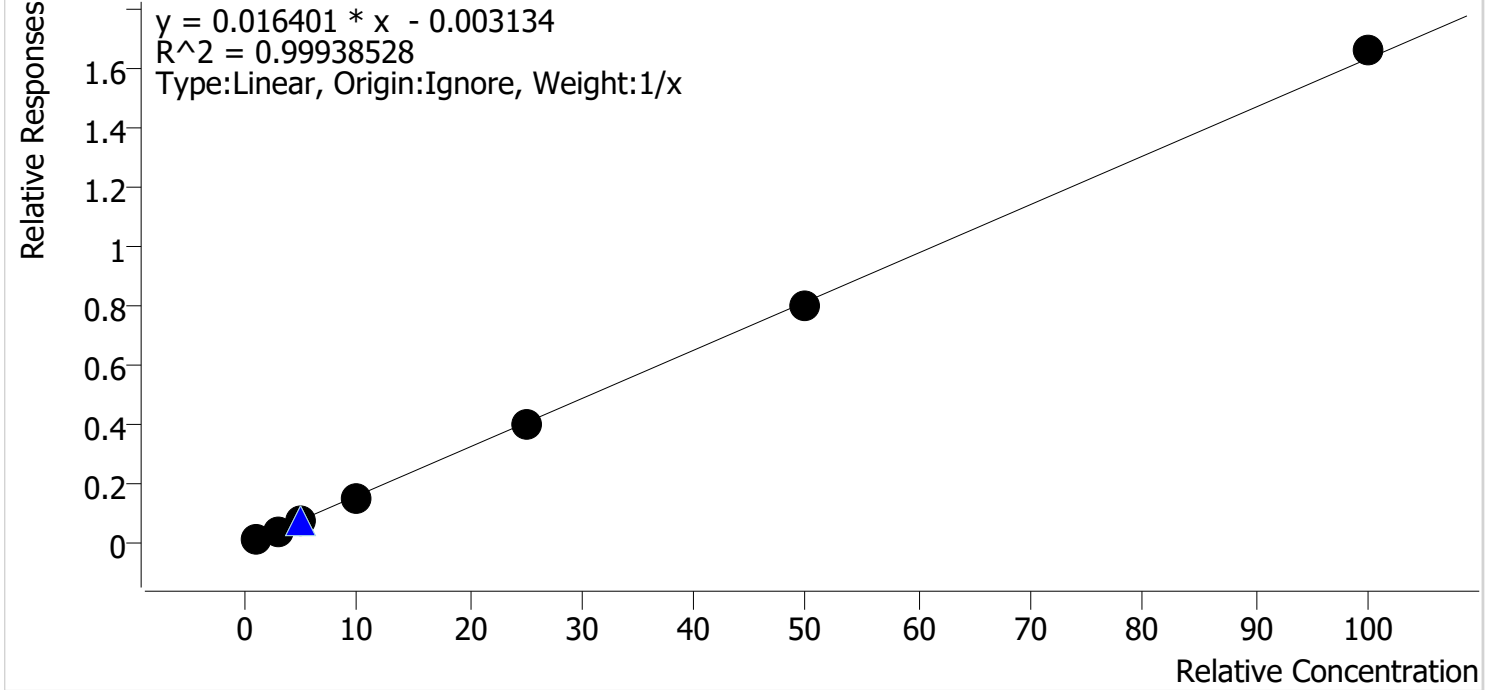
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AM #27 Cannabinoids Quant. Calibration Curve Report

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Last Cal. Update 7/14/2023 9:58 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	1.2	115.1
Cal 2 MJ	2	✓	3.0	2.9	95.8
Cal 3 MJ	3	✓	5.0	4.7	94.1
Cal 4 MJ	4	✓	10.0	9.6	96.0
Cal 5 MJ	5	✓	25.0	24.7	98.7
Cal 6 MJ	6	✓	50.0	49.4	98.7
Cal 7 MJ	7	✓	100.0	101.6	101.6

TS



AM #27 Cannabinoids Quant. Results

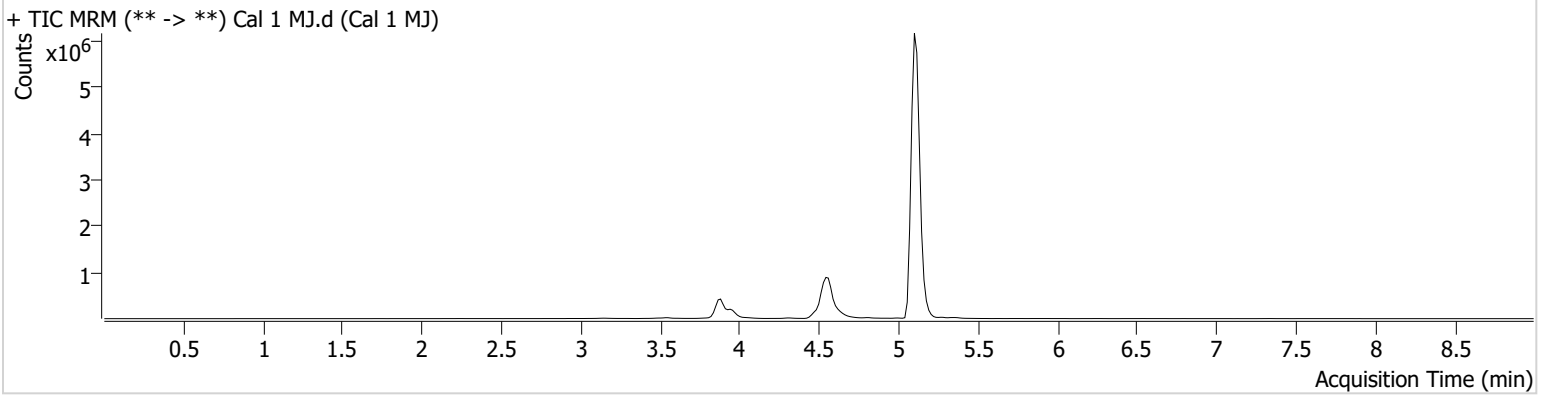
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Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P5-A1
Injection Volume 10
Acq. Date-Time 7/13/2023 12:02:44 PM
Sample Info.

Data File Cal 1 MJ.d
Sample Cal 1 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	184296	∞	25.0	∞	22765052	1.0974 ng/ml
THC-COOH	3.969	16499	∞	253.7	∞	522317	5.1383 ng/ml
THC-OH	3.881	25442	∞	10.3	∞	1615747	1.1512 ng/ml

TS



AM #27 Cannabinoids Quant. Results

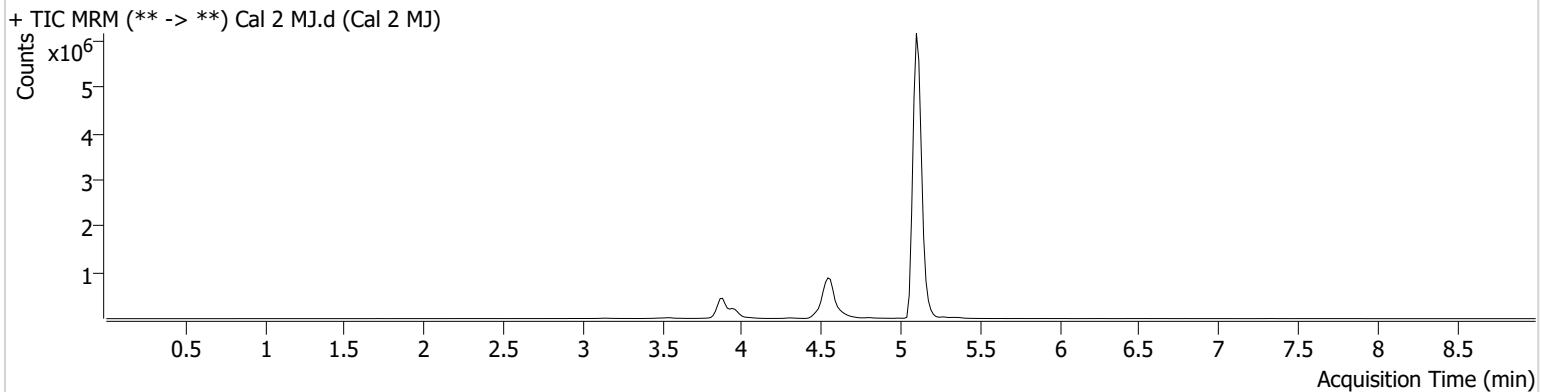
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Calibration Last Update 7/14/2023 9:58:32 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P5-B1
Injection Volume 10
Acq. Date-Time 7/13/2023 12:16:00 PM
Sample Info.

Data File Cal 2 MJ.d
Sample Cal 2 MJ
Operator Tamara Salazar
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	564556	∞	23.6	∞	22677104	2.9265 ng/ml
THC-COOH	3.969	33475	∞	248.6	5904.09	533955	9.7424 ng/ml
THC-OH	3.881	74286	400.25	12.9	∞	1689041	2.8727 ng/ml

TS



AM #27 Cannabinoids Quant. Results

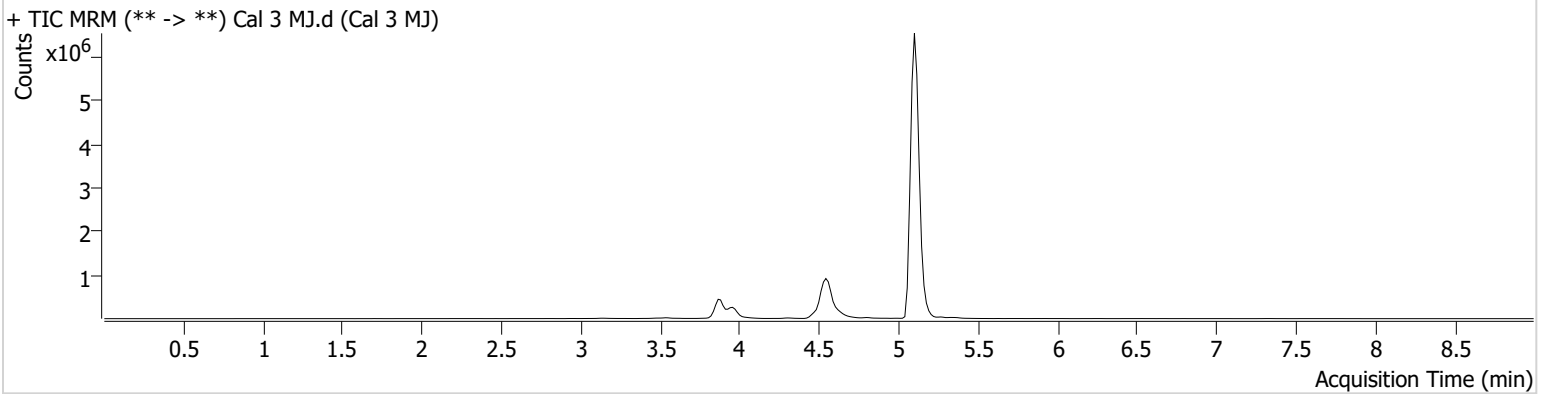
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Calibration Last Update 7/14/2023 9:58:32 AM

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Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P5-C1
Injection Volume 10
Acq. Date-Time 7/13/2023 12:29:05 PM
Sample Info.

Data File Cal 3 MJ.d
Sample Cal 3 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	1000143	∞	23.7	∞	23363572	4.8768 ng/ml
THC-COOH	3.969	67574	∞	237.9	∞	520879	19.6654 ng/ml
THC-OH	3.881	119516	247.70	12.2	∞	1613772	4.7066 ng/ml

TS



AM #27 Cannabinoids Quant. Results

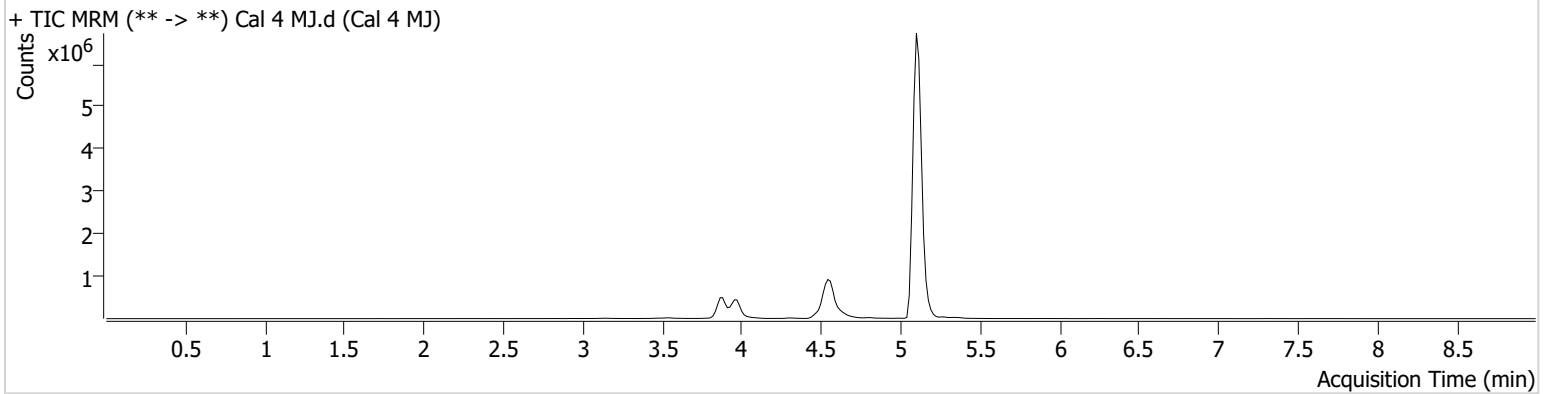
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Calibration Last Update 7/14/2023 9:58:32 AM

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Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P5-D1
Injection Volume 10
Acq. Date-Time 7/13/2023 12:42:11 PM
Sample Info.

Data File Cal 4 MJ.d
Sample Cal 4 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	1995476	∞	24.3	∞	22811807	9.7402 ng/ml
THC-COOH	3.969	175628	4779.56	238.8	∞	530851	49.4340 ng/ml
THC-OH	3.881	260437	553.21	12.6	∞	1688398	9.5960 ng/ml

TS



AM #27 Cannabinoids Quant. Results

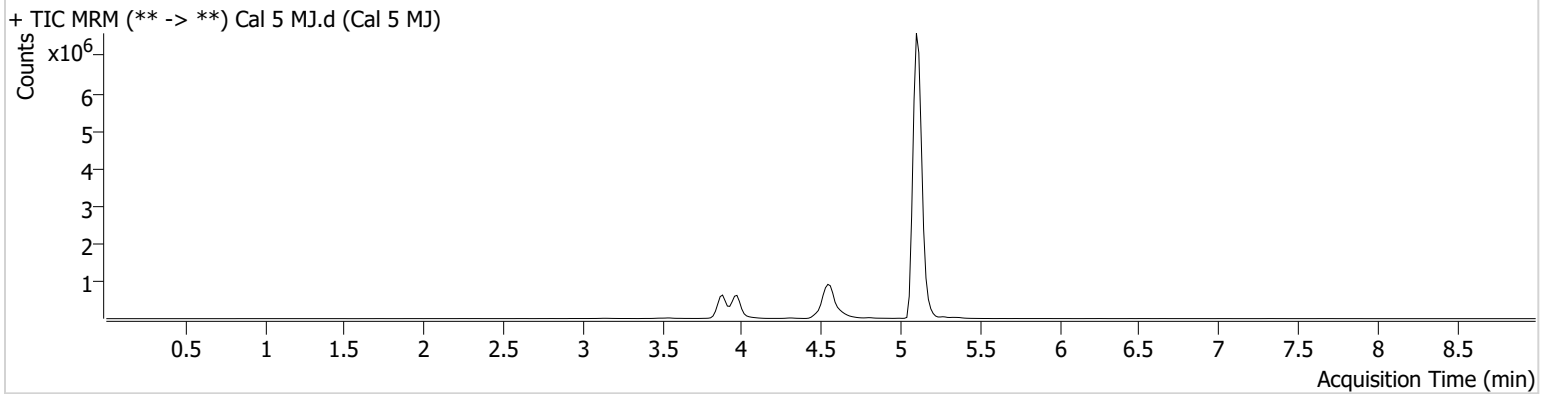
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Calibration Last Update 7/14/2023 9:58:32 AM

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Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P5-E1
Injection Volume 10
Acq. Date-Time 7/13/2023 12:55:17 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	5078535	59054.01	24.6	∞	23007464	24.2492 ng/ml
THC-COOH	3.969	266485	∞	239.2	∞	524629	75.6494 ng/ml
THC-OH	3.881	688087	∞	13.1	∞	1714046	24.6674 ng/ml

TS



AM #27 Cannabinoids Quant. Results

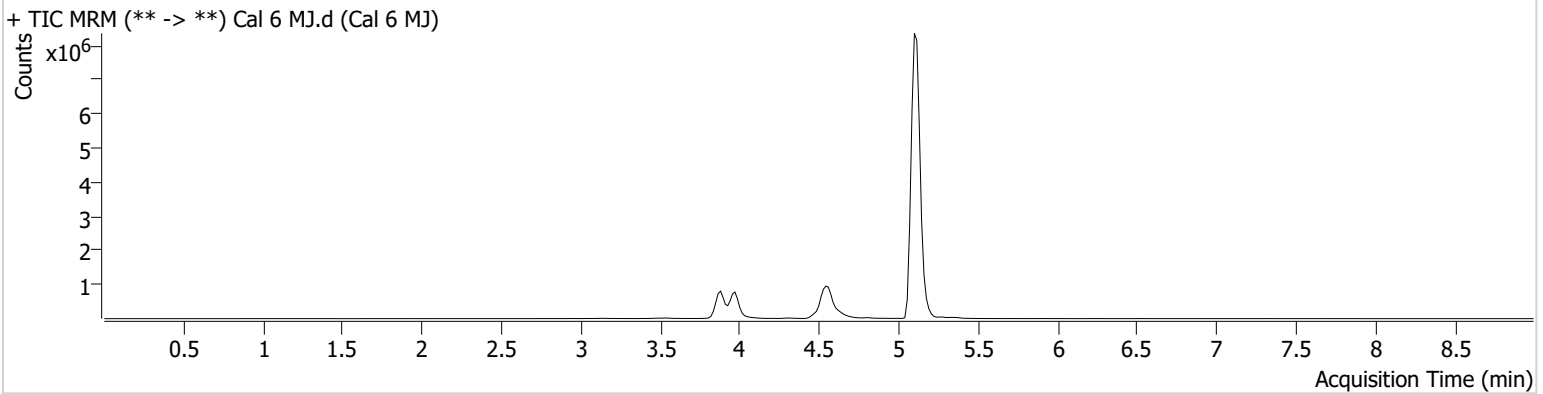
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Calibration Last Update 7/14/2023 9:58:32 AM

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Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P5-F1
Injection Volume 10
Acq. Date-Time 7/13/2023 1:08:23 PM
Sample Info.

Data File Cal 6 MJ.d
Sample Cal 6 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	9428939	178911.40	24.9	∞	20759552	49.6683 ng/ml
THC-COOH	3.969	342380	3079.39	239.3	∞	495861	102.6671 ng/ml
THC-OH	3.881	1333903	∞	14.6	∞	1654154	49.3580 ng/ml

TS

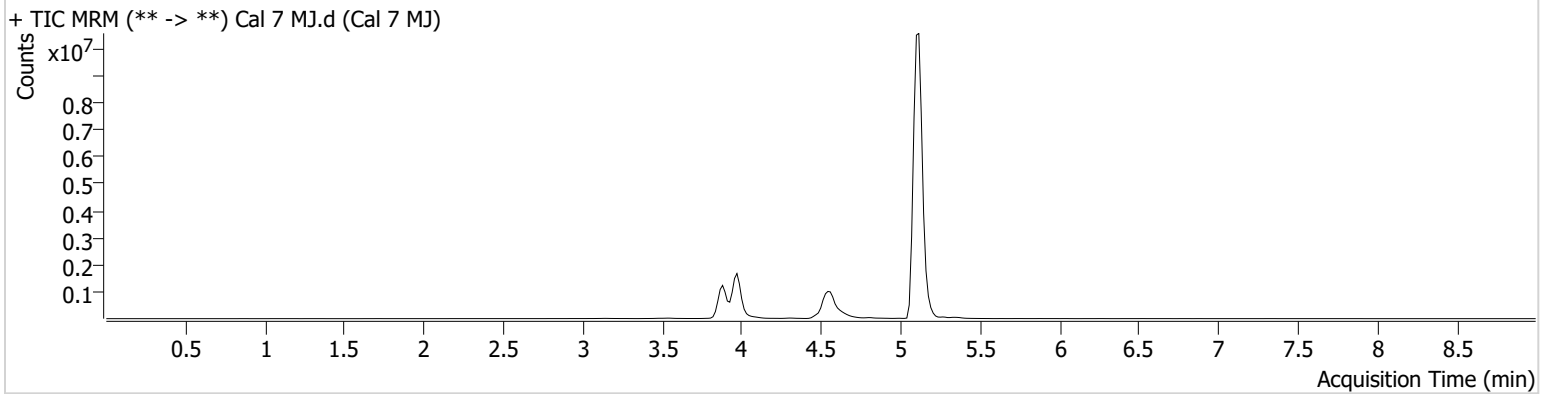


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\071323 AM 27 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 7/14/2023 9:58:32 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	P5-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	7/13/2023 1:21:28 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	17970221	∞	25.1	∞	19328815	101.4416 ng/ml
THC-COOH	3.969	786112	∞	240.9	∞	470636	247.7033 ng/ml
THC-OH	3.881	2741451	∞	14.3	∞	1647489	101.6482 ng/ml