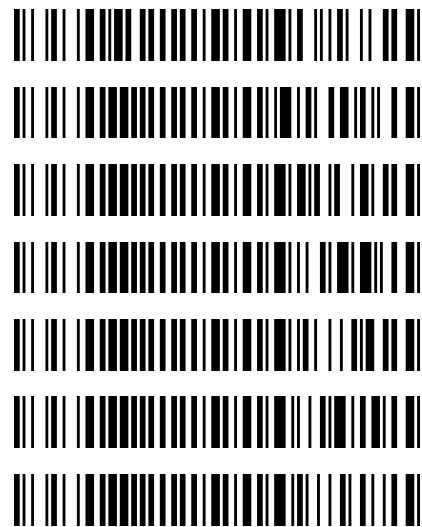


TS

9/20/2023

Worklist: 6503

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-3353	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0787	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2253	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2330	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2335	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2634	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2659	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

TS

Extraction Date: 09/18/2023

Plate lot#: 230627

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 23E52981

Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Tamara Salazar

Plate Retest Date: 12/27/2023

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Urine Lot:

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:

End QC and case sample M2023-3353-1 had the wrong well position specified and did not initially inject. The well position was corrected, and the samples were injected without issue.

TS

Analytical Plate Map

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1			P2023-2634-1	IS + QC_1
B	IS + Cal. 2				P2023-2335-1	IS + Cal. 7
C	IS + Cal. 3				P2023-2330-2	IS + Cal. 6
D	IS + Cal. 4				P2023-2253-1	IS + Cal. 5
E	IS + Cal. 5				P2023-0787-1	IS + Cal. 4
F	IS + Cal. 6				M2023-3353-1	IS + Cal. 3
G	IS + Cal. 7				Neg Blood	IS + Cal. 2
H	IS + QC_1			P2023-2659-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO



SLE Plate Map

TS

	1	2	3	4	5	6
A					P2023-2634-1	IS + QC_1
B					P2023-2335-1	IS + Cal. 7
C					P2023-2330-2	IS + Cal. 6
D					P2023-2253-1	IS + Cal. 5
E					P2023-0787-1	IS + Cal. 4
F					M2023-3353-1*	IS + Cal. 3
G				M2023-3353-1	Neg Blood	IS + Cal. 2
H				P2023-2659-1	IS + QC_1	IS + Cal. 1

*Moved during step 7 of the extraction due to a blood clot.



TS

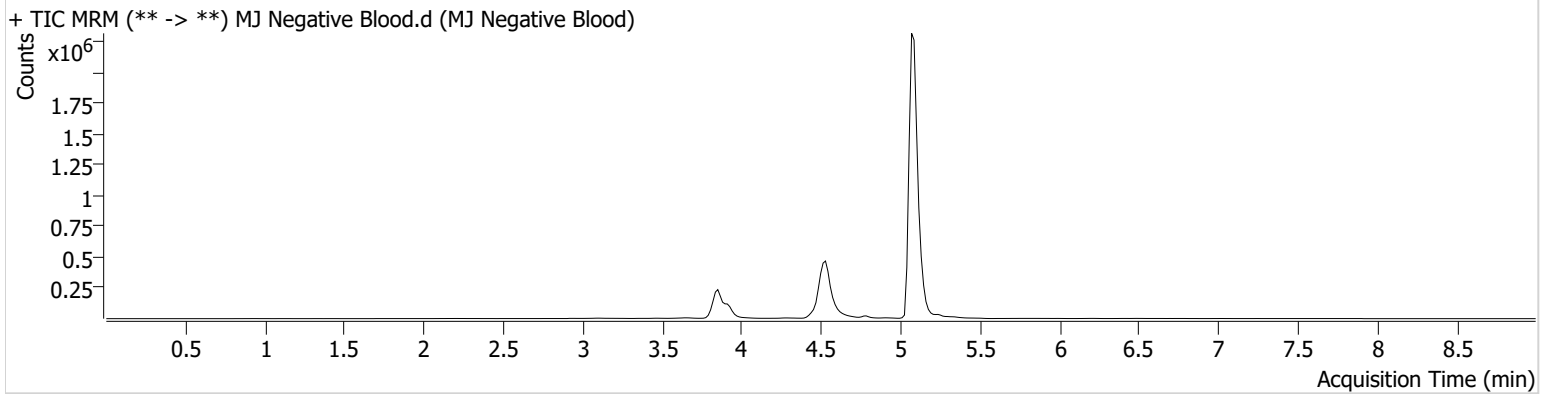


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091823 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/19/2023 1:39:33 PM

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-G5	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	9/18/2023 6:30:22 PM		
Sample Info.			

Sample Chromatogram



TS



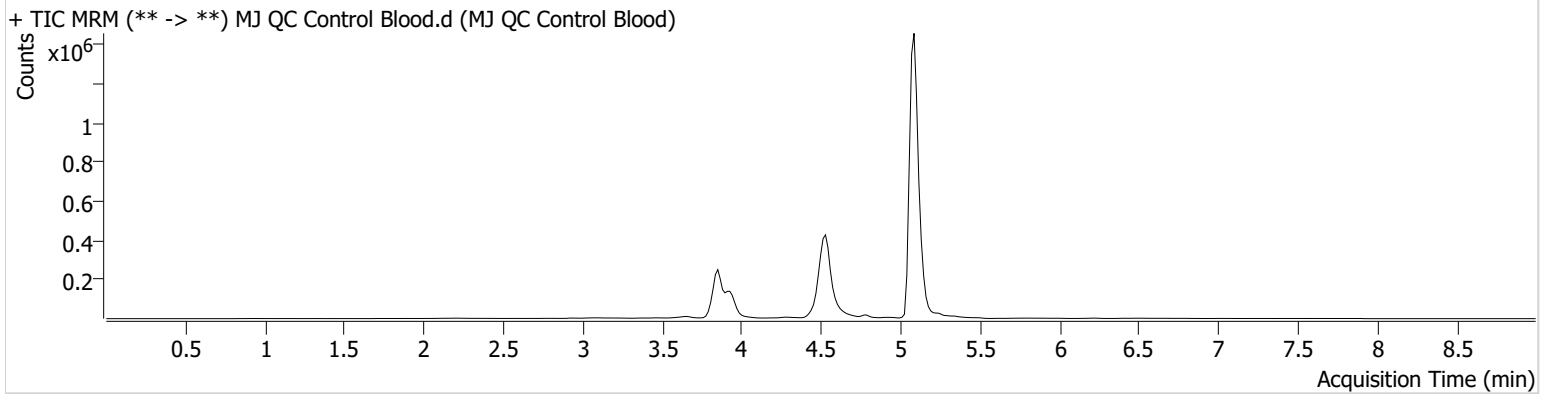
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091823 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/19/2023 1:39:33 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** Tamara Salazar
Sample Position P1-A6 **Comment**
Injection Volume 10
Acq. Date-Time 9/18/2023 6:04:09 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	229328	∞	27.9	∞	5174099	4.2455 ng/ml
THC-COOH	3.939	37330	804.32	191.8	∞	318167	14.9427 ng/ml
THC-OH	3.850	72331	∞	13.9	141.55	933699	4.6891 ng/ml

TS



AM #27 Cannabinoids Quant. Results

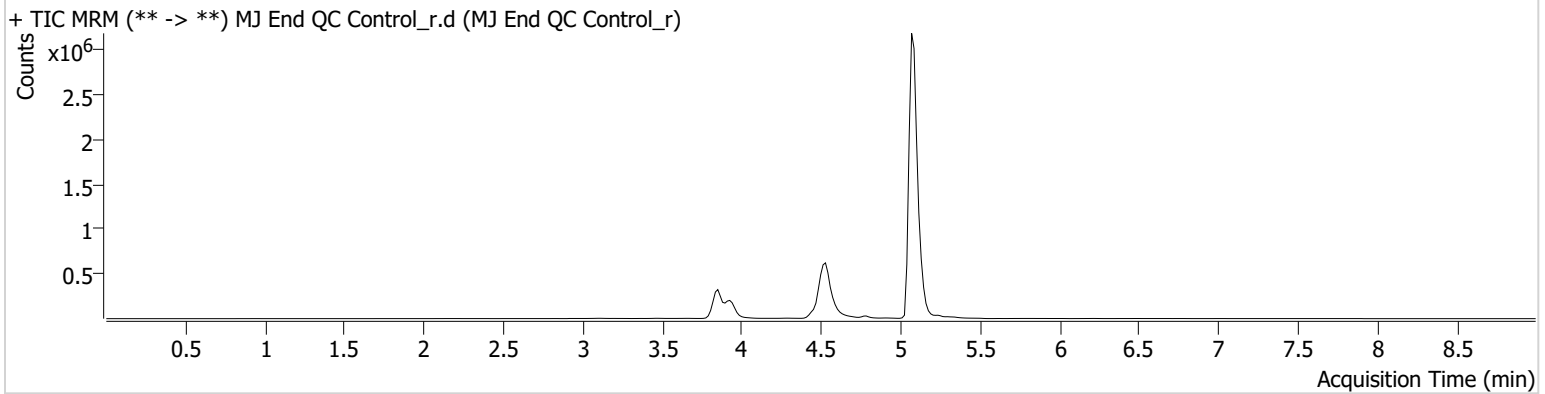
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Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type QC
Acq. Method AM 27 Agilent Method.m
Sample Position P1-H5
Injection Volume 10
Acq. Date-Time 9/19/2023 12:05:42 PM
Sample Info.

Data File MJ End QC Control_r.d
Sample MJ End QC Control_r
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	5.090	477659	∞	26.0	485.40	10954629	4.1789 ng/ml
THC-COOH	3.939	56350	614.85	190.1	249.60	458947	15.6106 ng/ml
THC-OH	3.850	92301	∞	13.7	253.84	1179332	4.7357 ng/ml

TS



AM #27 Cannabinoids Quant. Results

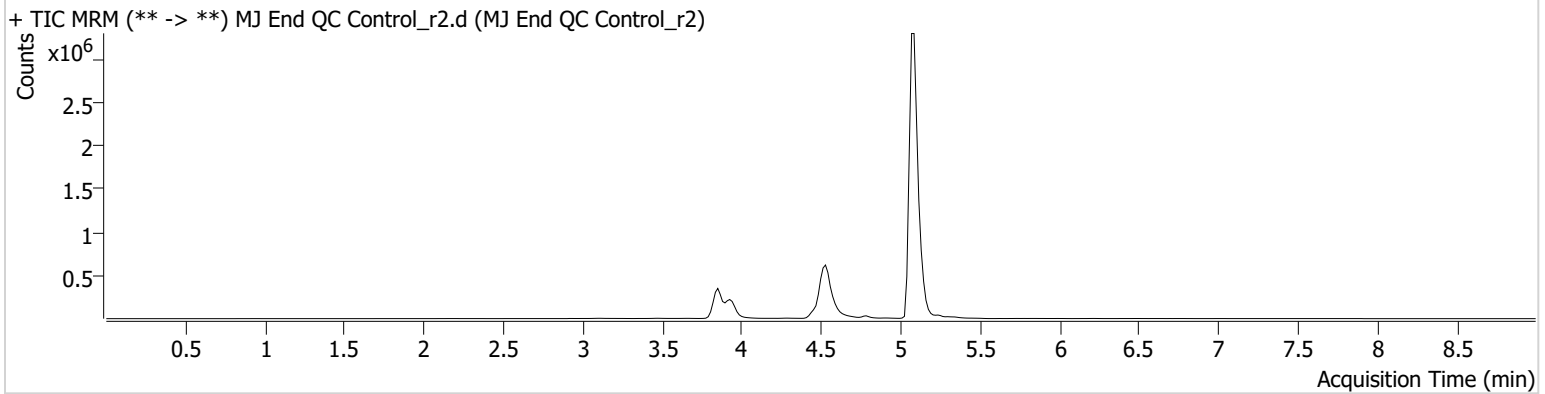
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Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type QC
Acq. Method AM 27 Agilent Method.m
Sample Position P1-H5
Injection Volume 10
Acq. Date-Time 9/19/2023 12:58:21 PM
Sample Info. QC followed re-injected samples.

Data File MJ End QC Control_r2.d
Sample MJ End QC Control_r2
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



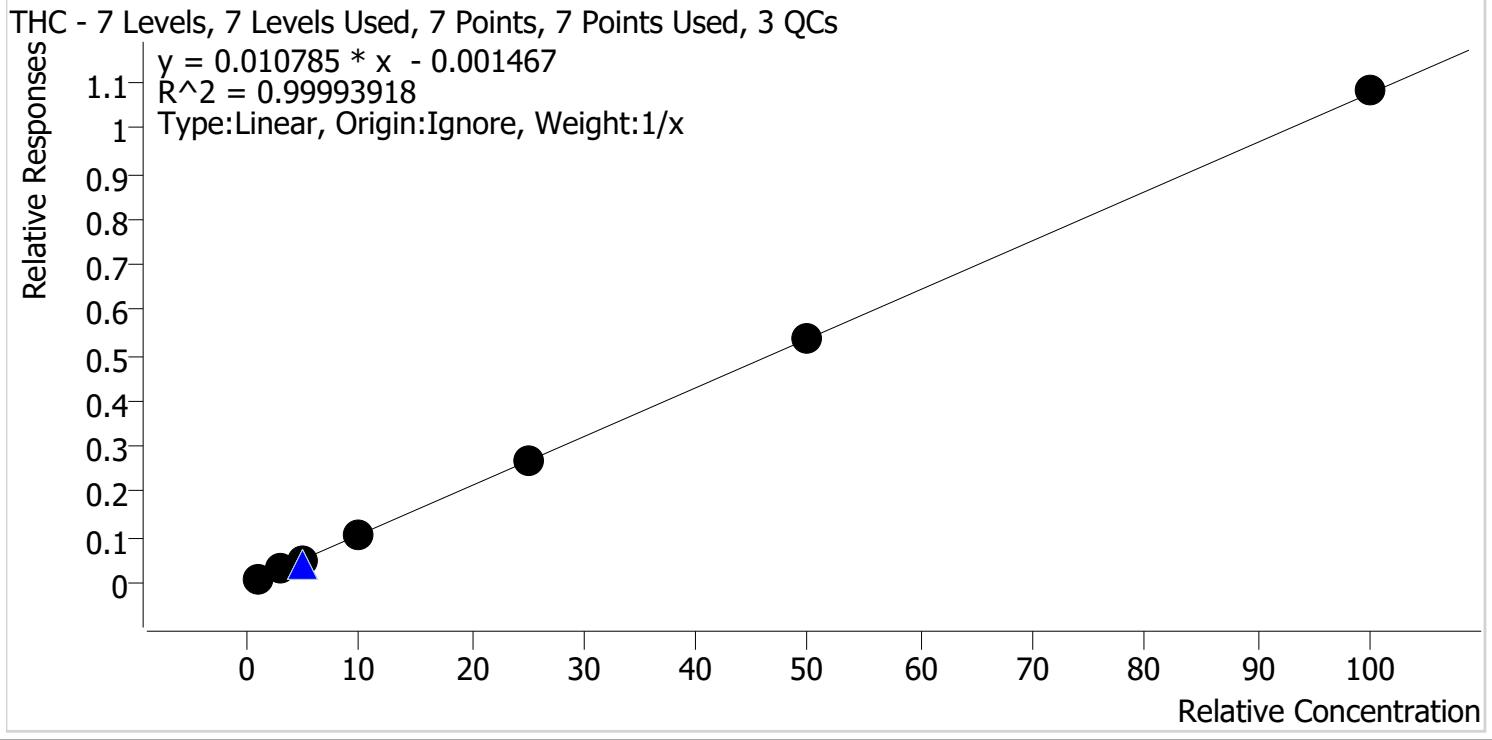
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	506803	∞	26.2	∞	11646541	4.1707 ng/ml
THC-COOH	3.939	60911	846.57	192.7	1462.01	495175	15.6384 ng/ml
THC-OH	3.850	97188	∞	13.6	∞	1244614	4.7253 ng/ml

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\091823 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 9/19/2023 1:39 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	1.0	104.3
Cal 2 MJ	2	✓	3.0	3.0	98.9
Cal 3 MJ	3	✓	5.0	4.9	98.8
Cal 4 MJ	4	✓	10.0	9.8	98.4
Cal 5 MJ	5	✓	25.0	24.9	99.5
Cal 6 MJ	6	✓	50.0	49.8	99.6
Cal 7 MJ	7	✓	100.0	100.6	100.6

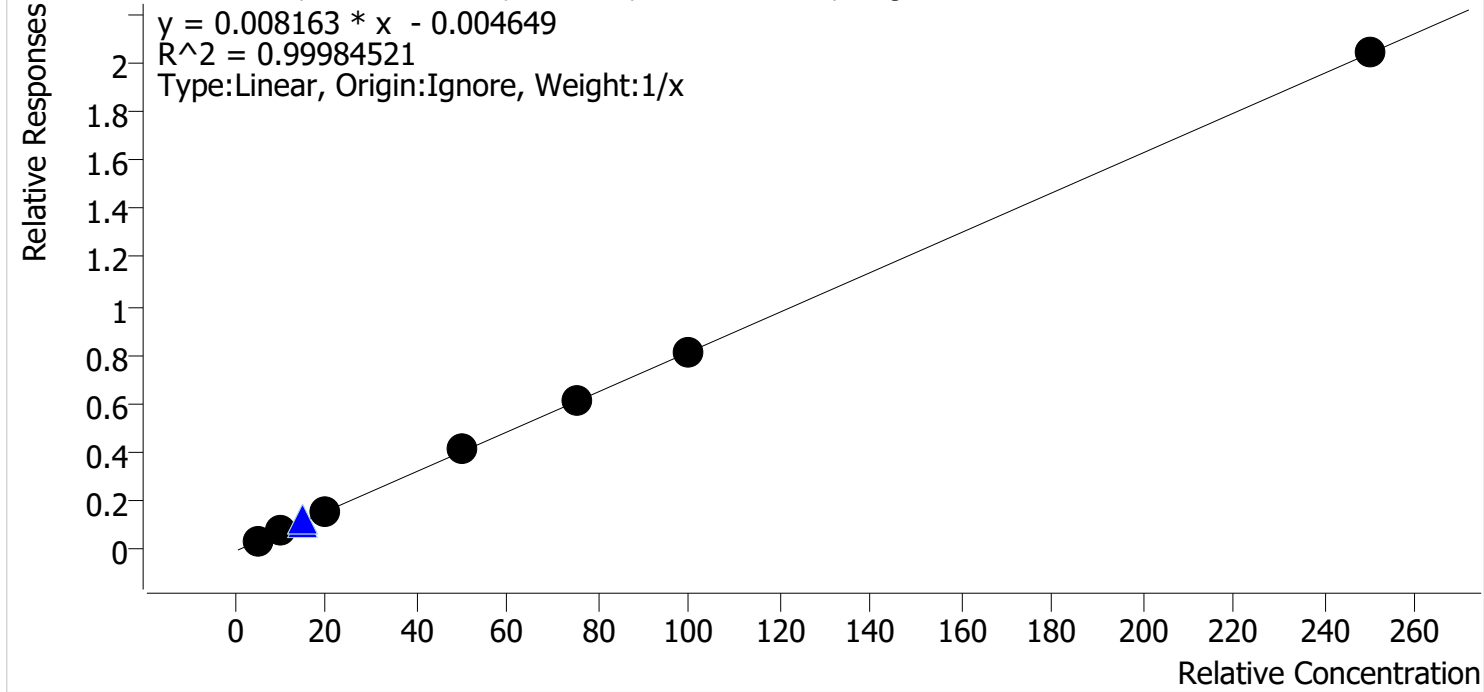
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\091823 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 9/19/2023 1:39 PM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 3 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.3	105.0
Cal 2 MJ	2	✓	10.0	9.6	96.4
Cal 3 MJ	3	✓	20.0	19.4	97.2
Cal 4 MJ	4	✓	50.0	50.8	101.6
Cal 5 MJ	5	✓	75.0	75.2	100.3
Cal 6 MJ	6	✓	100.0	99.3	99.3
Cal 7 MJ	7	✓	250.0	250.3	100.1

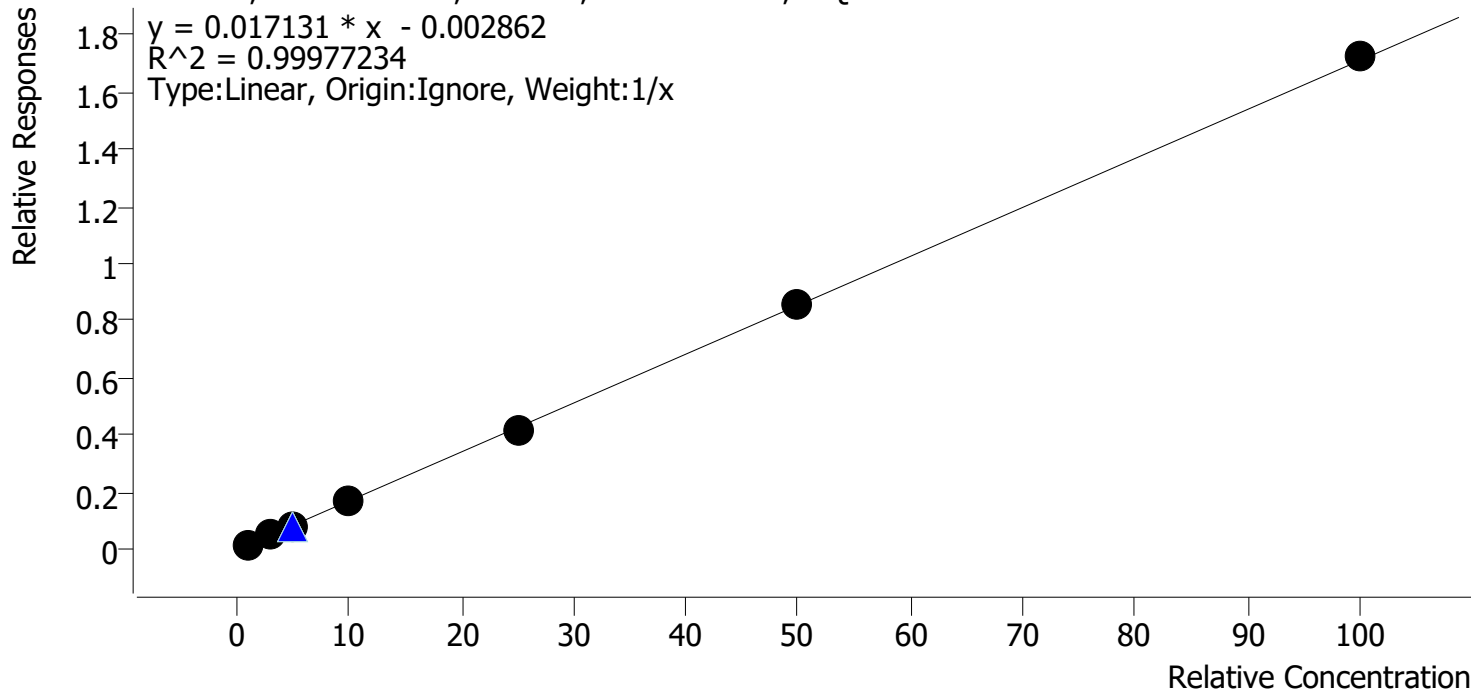
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\091823 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 9/19/2023 1:39 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, 3 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.0	103.7
Cal 2 MJ	2	✓	3.0	3.0	100.4
Cal 3 MJ	3	✓	5.0	5.0	99.4
Cal 4 MJ	4	✓	10.0	9.9	98.7
Cal 5 MJ	5	✓	25.0	24.2	96.7
Cal 6 MJ	6	✓	50.0	50.2	100.3
Cal 7 MJ	7	✓	100.0	100.8	100.8

TS



AM #27 Cannabinoids Quant. Results

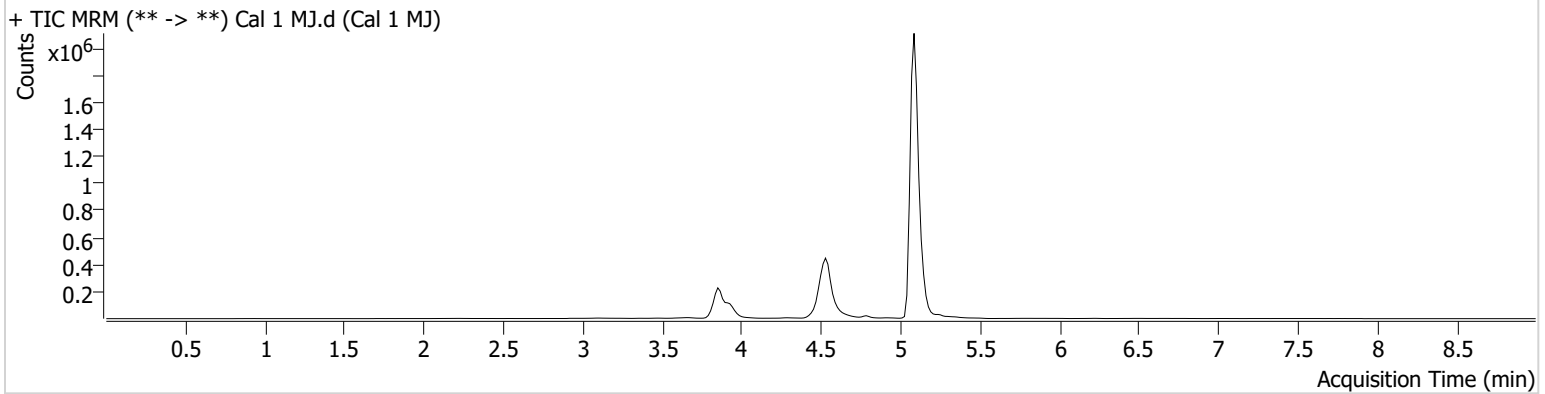
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Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-H6
Injection Volume 10
Acq. Date-Time 9/18/2023 4:19:10 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	5.090	73994	∞	29.4	∞	7566072	1.0428 ng/ml
THC-COOH	3.939	12522	85.69	194.8	∞	327724	5.2503 ng/ml
THC-OH	3.865	13315	71.51	15.5	49.54	893098	1.0374 ng/ml

TS



AM #27 Cannabinoids Quant. Results

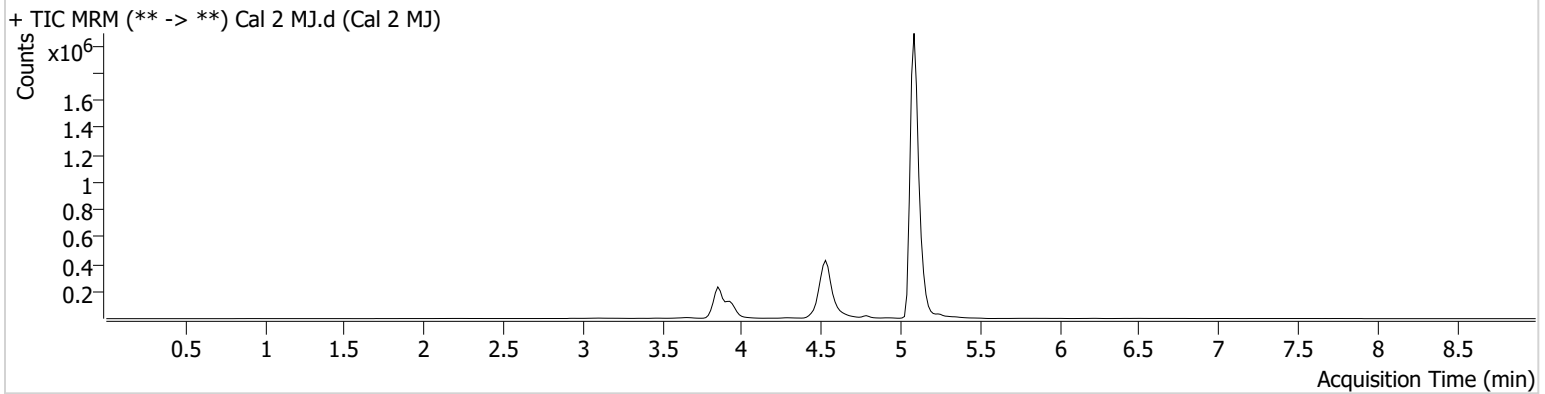
Batch results D:\MassHunter\Data\2023\AM 27 28\091823 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-G6
Injection Volume 10
Acq. Date-Time 9/18/2023 4:32:25 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	5.090	219412	∞	26.7	∞	7185685	2.9672 ng/ml
THC-COOH	3.939	24509	∞	200.6	∞	330931	9.6423 ng/ml
THC-OH	3.865	42986	∞	14.2	74.65	881681	3.0130 ng/ml

TS



AM #27 Cannabinoids Quant. Results

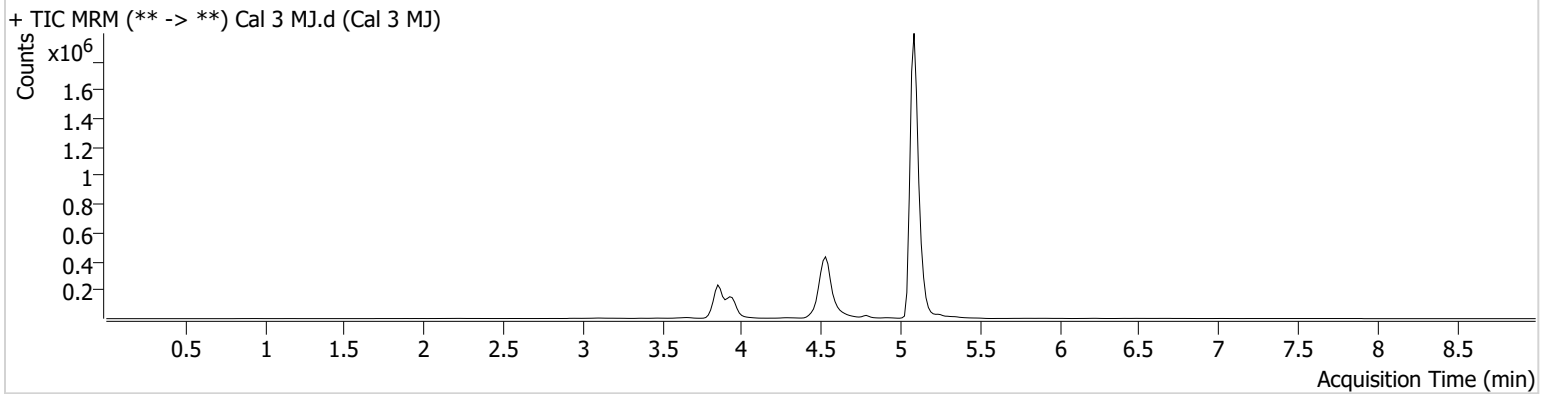
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Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-F6
Injection Volume 10
Acq. Date-Time 9/18/2023 4:45:32 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	5.090	342680	∞	26.0	∞	6614050	4.9399 ng/ml
THC-COOH	3.939	50479	458.67	188.1	1077.07	327706	19.4394 ng/ml
THC-OH	3.865	71800	279.61	13.7	140.17	872898	4.9686 ng/ml

TS



AM #27 Cannabinoids Quant. Results

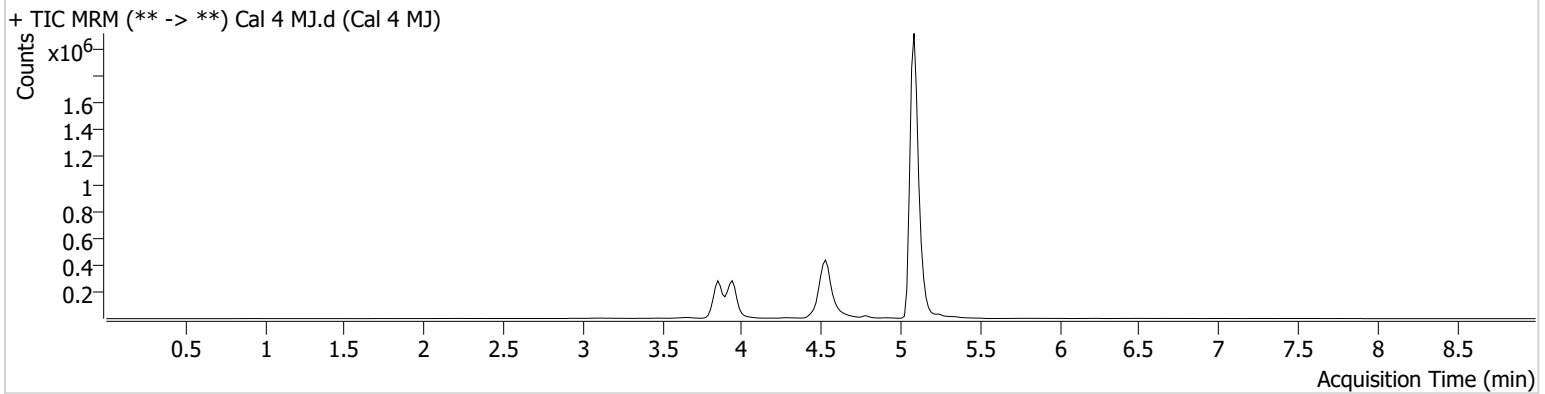
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Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-E6
Injection Volume 10
Acq. Date-Time 9/18/2023 4:58:38 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	5.090	700083	∞	26.3	∞	6689008	9.8401 ng/ml
THC-COOH	3.939	137181	1985.93	184.4	282.66	334444	50.8173 ng/ml
THC-OH	3.850	157128	∞	13.7	∞	945642	9.8664 ng/ml

TS

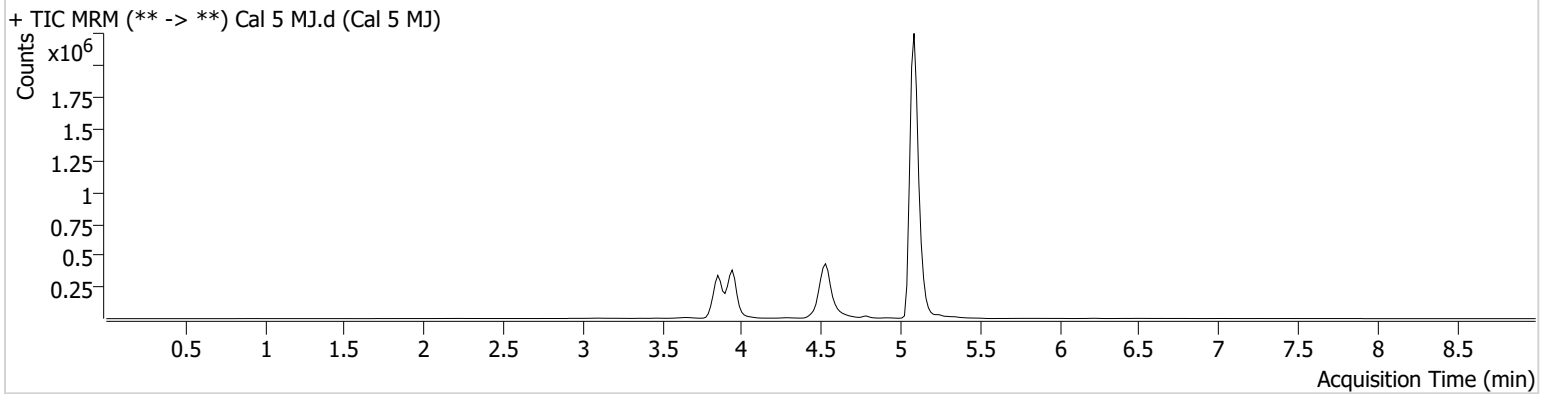


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\091823 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 9/19/2023 1:39:33 PM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-D6	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	9/18/2023 5:11:44 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	1640376	∞	26.4	∞	6148310	24.8735 ng/ml
THC-COOH	3.939	199571	∞	182.6	2887.63	327519	75.2152 ng/ml
THC-OH	3.850	399380	∞	14.3	1186.29	971297	24.1691 ng/ml

TS



AM #27 Cannabinoids Quant. Results

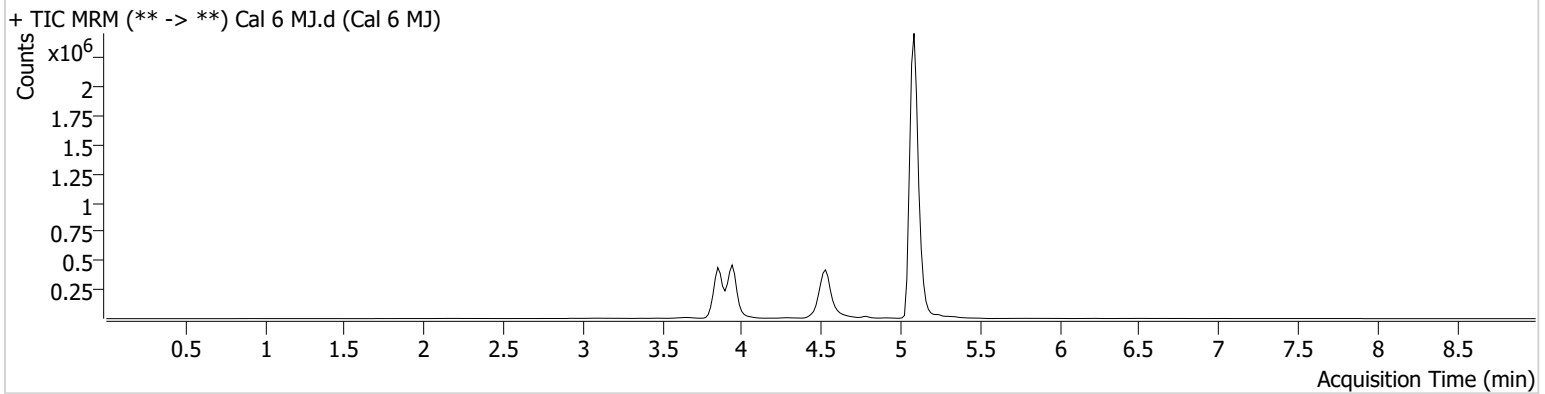
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Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-C6
Injection Volume 10
Acq. Date-Time 9/18/2023 5:24:50 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	5.090	2899898	∞	26.3	∞	5415363	49.7864 ng/ml
THC-COOH	3.939	245137	10109.27	189.5	∞	304064	99.3311 ng/ml
THC-OH	3.850	774408	∞	14.0	2919.10	904009	50.1719 ng/ml

TS



AM #27 Cannabinoids Quant. Results

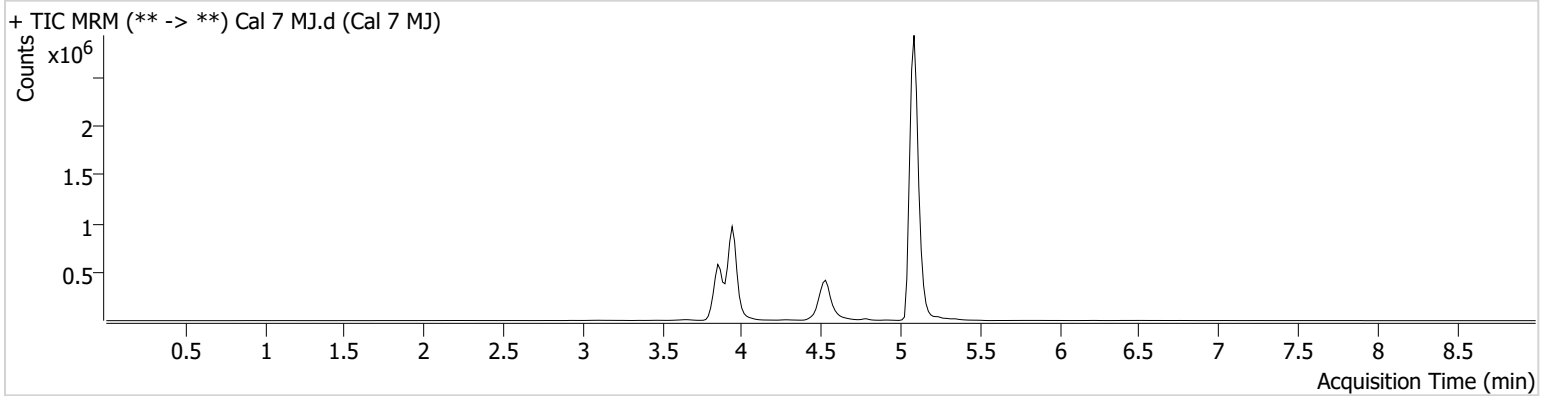
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Calibration Last Update 9/19/2023 1:39:33 PM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-B6
Injection Volume 10
Acq. Date-Time 9/18/2023 5:37:55 PM
Sample Info.

Data File Cal 7 MJ.d
Sample Cal 7 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	5.090	5036932	∞	26.5	∞	4650915	100.5502 ng/ml
THC-COOH	3.939	579017	5805.78	185.7	15087.56	284025	250.3044 ng/ml
THC-OH	3.850	1403816	∞	14.5	5690.78	814514	100.7736 ng/ml