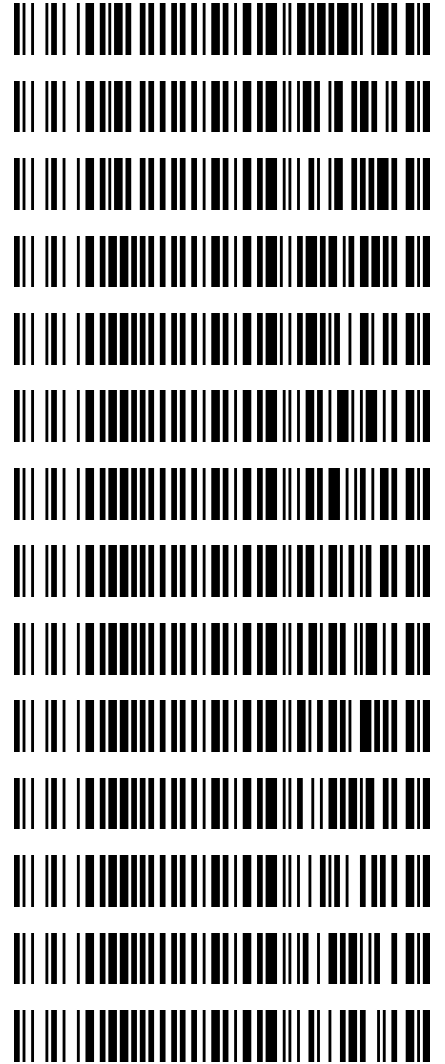


Worklist: 6624

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-5076	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-5127	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-5163	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3553	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ
P2023-3555	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ
P2023-3569	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3571	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3631	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3639	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3651	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3652	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3662	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3672	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3696	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: 12/27/2023

Analyst: Tamara Salazar

Plate lot#: 231212

Plate Retest Date: 06/12/2024

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 23E52981

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

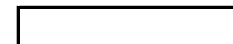
COMMENTS:

Analytical Plate Map

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	P2023-3571-1			IS + QC_1
B	IS + Cal. 2	Neg Blood	P2023-3631-1			IS + Cal. 7
C	IS + Cal. 3	M2023-5076-2	P2023-3639-1			IS + Cal. 6
D	IS + Cal. 4	M2023-5127-2	P2023-3651-1			IS + Cal. 5
E	IS + Cal. 5	M2023-5163-2	P2023-3652-1			IS + Cal. 4
F	IS + Cal. 6	P2023-3553-1	P2023-3662-1			IS + Cal. 3
G	IS + Cal. 7	P2023-3555-1	P2023-3672-1			IS + Cal. 2
H	IS + QC_1	P2023-3569-1	P2023-3696-1		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	IS + Cal. 1	IS + QC_1	P2023-3571-1	M2023-5163-2		
B	IS + Cal. 2	Neg Blood	P2023-3631-1			
C	IS + Cal. 3	M2023-5076-2	P2023-3639-1			
D	IS + Cal. 4	M2023-5127-2	P2023-3651-1			
E	IS + Cal. 5	M2023-5163-2*	P2023-3652-1			
F	IS + Cal. 6	P2023-3553-1	P2023-3662-1			
G	IS + Cal. 7	P2023-3555-1	P2023-3672-1			
H	IS + QC_1	P2023-3569-1	P2023-3696-1			

*Sample moved during step 7 of the extraction due to clotting.



TS

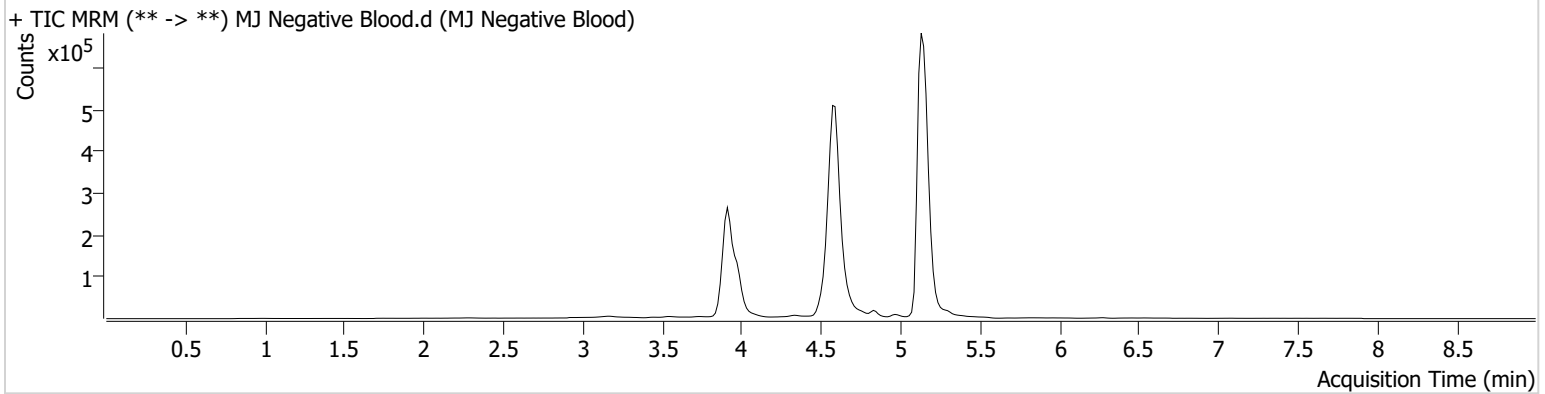


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 12/28/2023 8:16:51 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	12/27/2023 2:35:16 PM		
Sample Info.			

Sample Chromatogram



TS



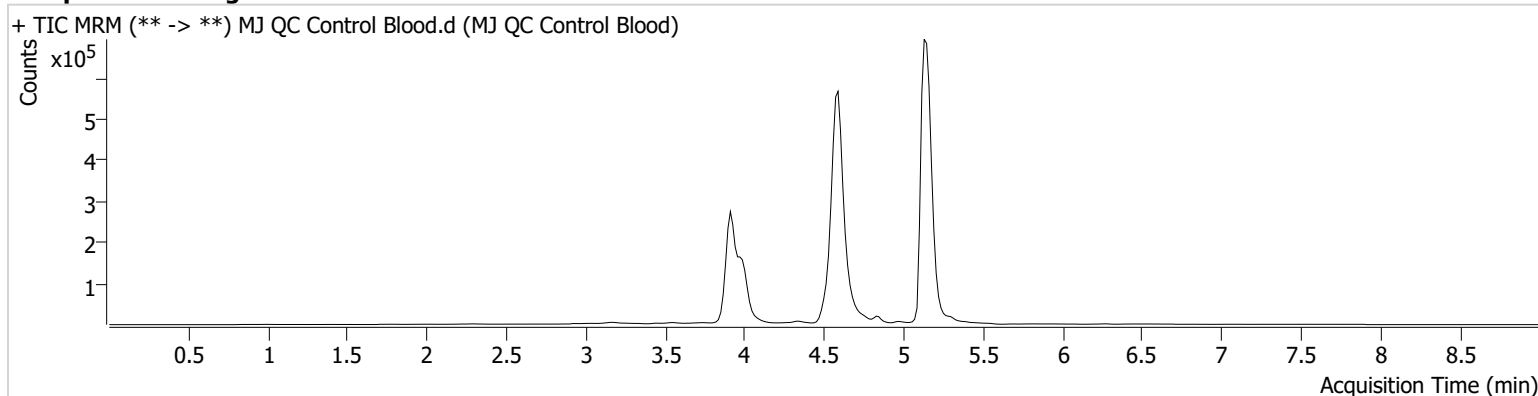
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 12/28/2023 8:16:51 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 12/27/2023 2:09:05 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	5.150	131640	∞	26.9	∞	2865760	4.9288 ng/ml
THC-COOH	4.000	34258	∞	237.7	566.61	359793	14.3296 ng/ml
THC-OH	3.911	78471	∞	13.1	∞	1063705	4.9795 ng/ml

TS



AM #27 Cannabinoids Quant. Results

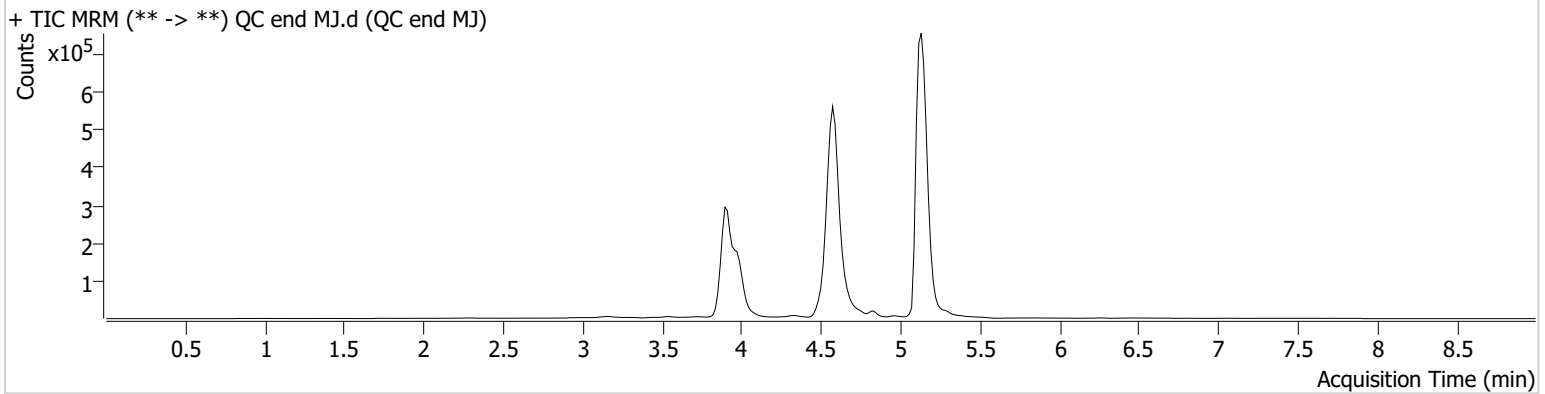
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Calibration Last Update 12/28/2023 8:16:51 AM

Instrument Falco (069901)
Type QC
Acq. Method AM 27 Agilent Method.m
Sample Position P1-A2
Injection Volume 10
Acq. Date-Time 12/27/2023 9:08:23 PM
Sample Info.

Data File QC end MJ.d
Sample QC end 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	5.135	146595	∞	26.4	∞	3231519	4.8698 ng/ml
THC-COOH	3.985	38571	∞	230.0	∞	399223	14.5337 ng/ml
THC-OH	3.911	87844	∞	13.5	∞	1209361	4.9060 ng/ml

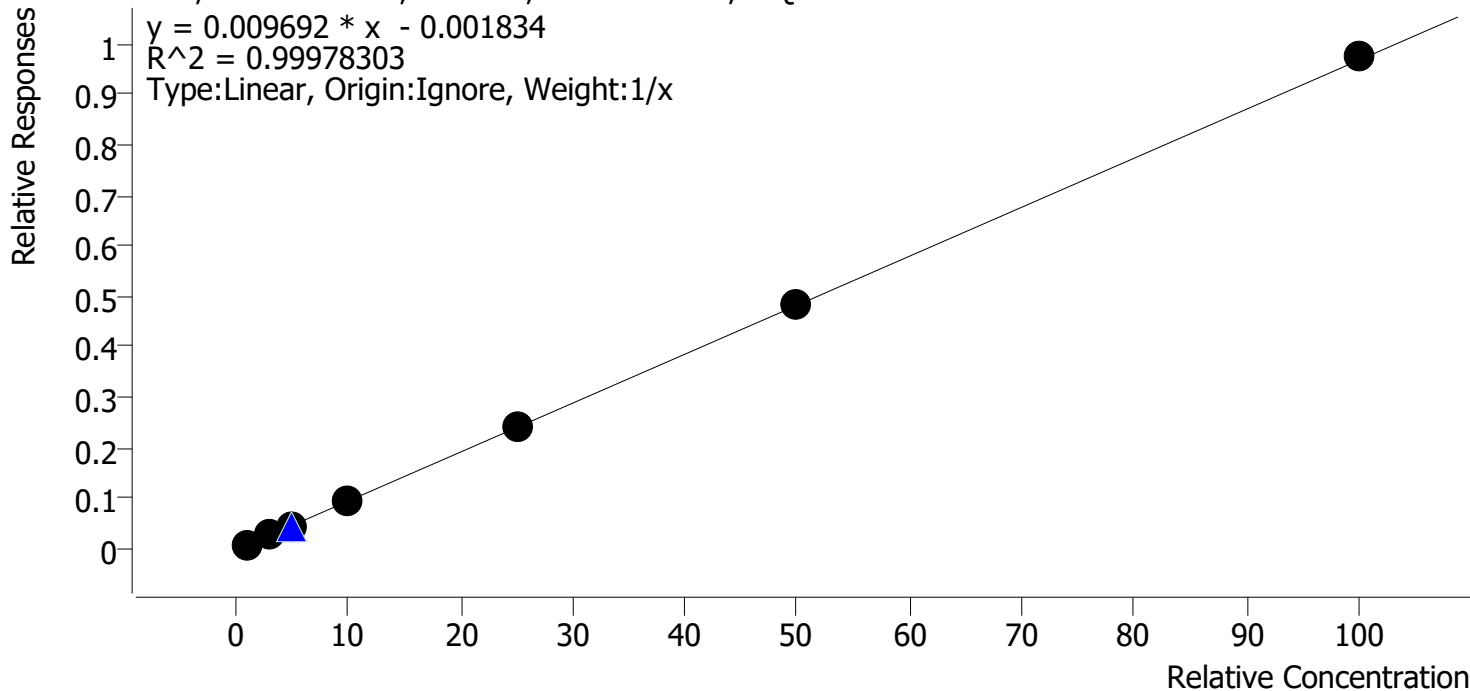
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
 Last Cal. Update 12/28/2023 8:16 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	109.6
Cal 2 MJ	2	✓	3.0	2.9	97.9
Cal 3 MJ	3	✓	5.0	4.7	94.8
Cal 4 MJ	4	✓	10.0	9.8	97.6
Cal 5 MJ	5	✓	25.0	24.9	99.8
Cal 6 MJ	6	✓	50.0	49.8	99.6
Cal 7 MJ	7	✓	100.0	100.7	100.7

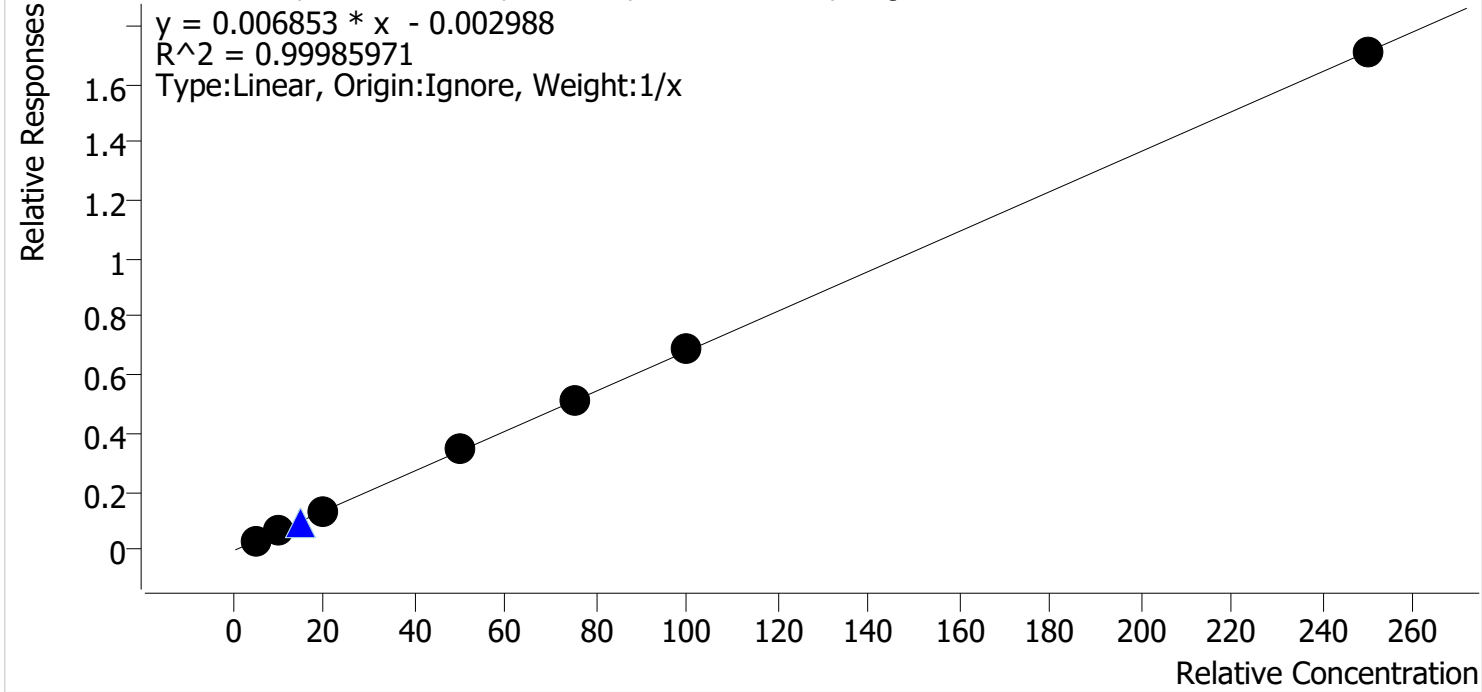
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 12/28/2023 8:16 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	5.0	101.0
Cal 2 MJ	2	✓	10.0	10.1	101.2
Cal 3 MJ	3	✓	20.0	19.3	96.4
Cal 4 MJ	4	✓	50.0	50.8	101.6
Cal 5 MJ	5	✓	75.0	74.4	99.2
Cal 6 MJ	6	✓	100.0	100.9	100.9
Cal 7 MJ	7	✓	250.0	249.5	99.8

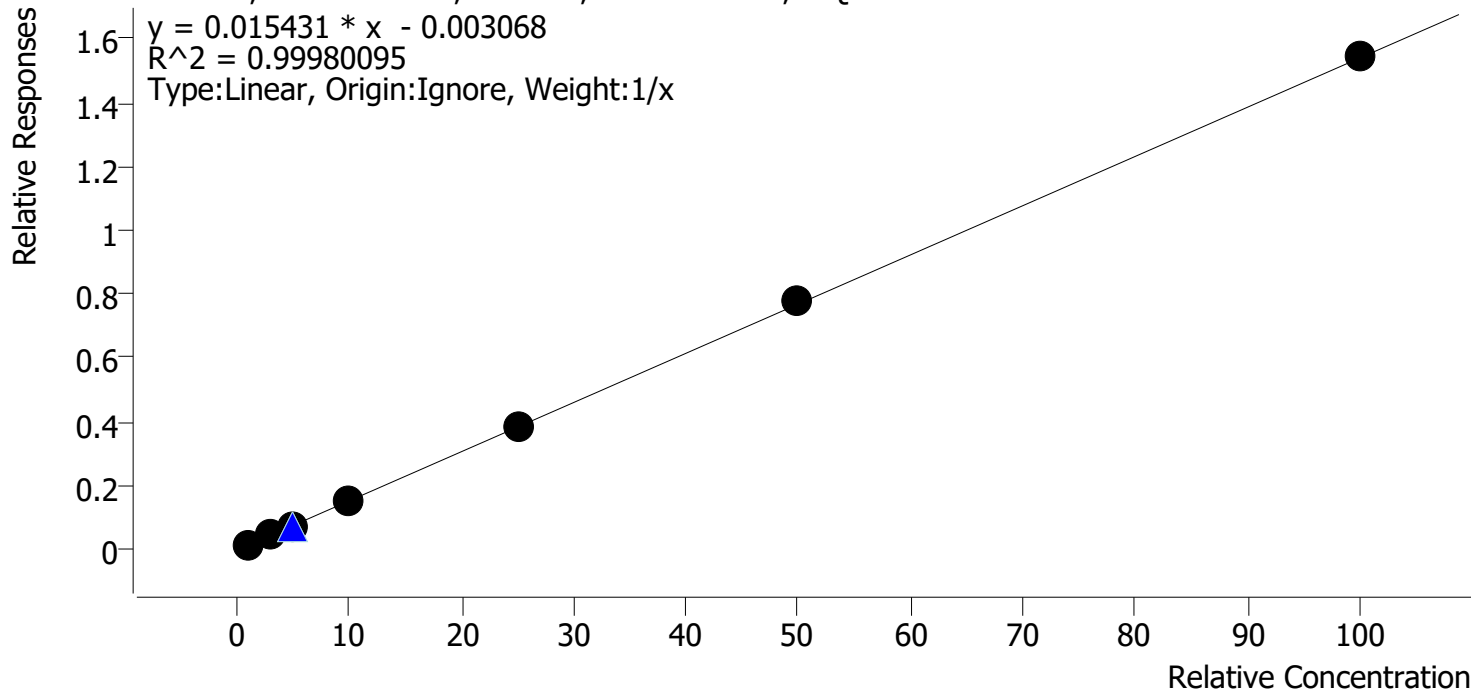
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 12/28/2023 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	1.1	110.1
Cal 2 MJ	2	✓	3.0	2.9	95.6
Cal 3 MJ	3	✓	5.0	4.8	96.7
Cal 4 MJ	4	✓	10.0	9.7	97.0
Cal 5 MJ	5	✓	25.0	24.9	99.7
Cal 6 MJ	6	✓	50.0	50.3	100.6
Cal 7 MJ	7	✓	100.0	100.3	100.3

TS



AM #27 Cannabinoids Quant. Results

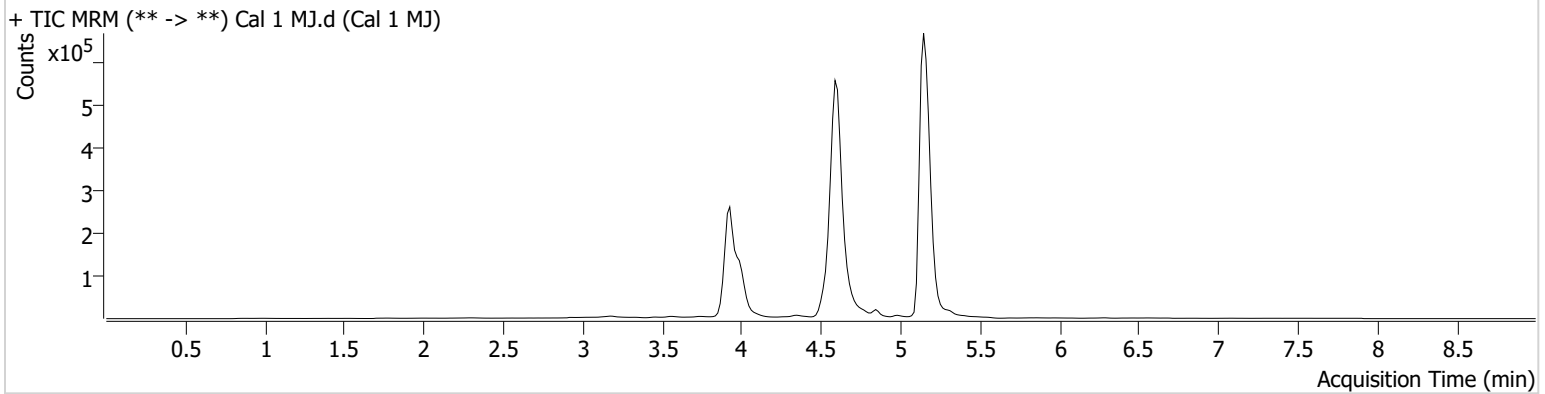
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Calibration Last Update 12/28/2023 8:16:51 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-A1
Injection Volume 10
Acq. Date-Time 12/27/2023 11:57:36 AM
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	5.165	24333	144.00	30.1	∞	2767886	1.0963 ng/ml
THC-COOH	4.015	11073	231.39	233.3	275.55	350231	5.0494 ng/ml
THC-OH	3.926	14745	∞	11.9	∞	1059557	1.1006 ng/ml

TS



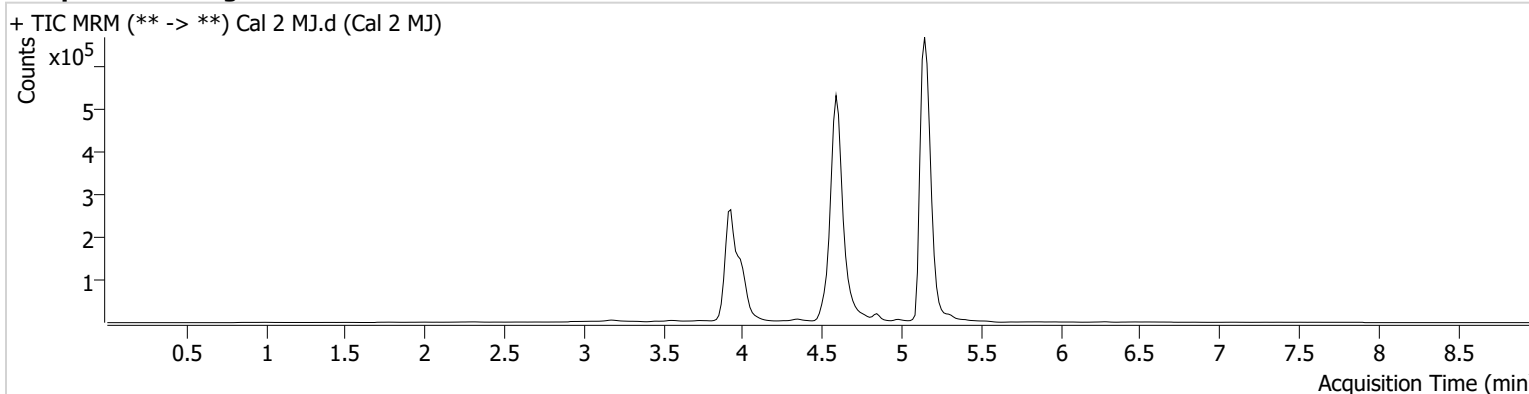
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 12/28/2023 8:16:51 AM

Instrument Falco (069901) **Data File** Cal 2 MJ.d
Type Cal **Sample** Cal 2 MJ
Acq. Method AM 27 Agilent Method.m **Operator** Tamara Salazar
Sample Position P1-B1 **Comment**
Injection Volume 10
Acq. Date-Time 12/27/2023 12:10:54 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	5.150	73003	∞	26.8	∞	2741531	2.9367 ng/ml
THC-COOH	4.000	23509	396.74	232.1	∞	354182	10.1211 ng/ml
THC-OH	3.926	44198	∞	14.4	∞	1072660	2.8690 ng/ml

TS



AM #27 Cannabinoids Quant. Results

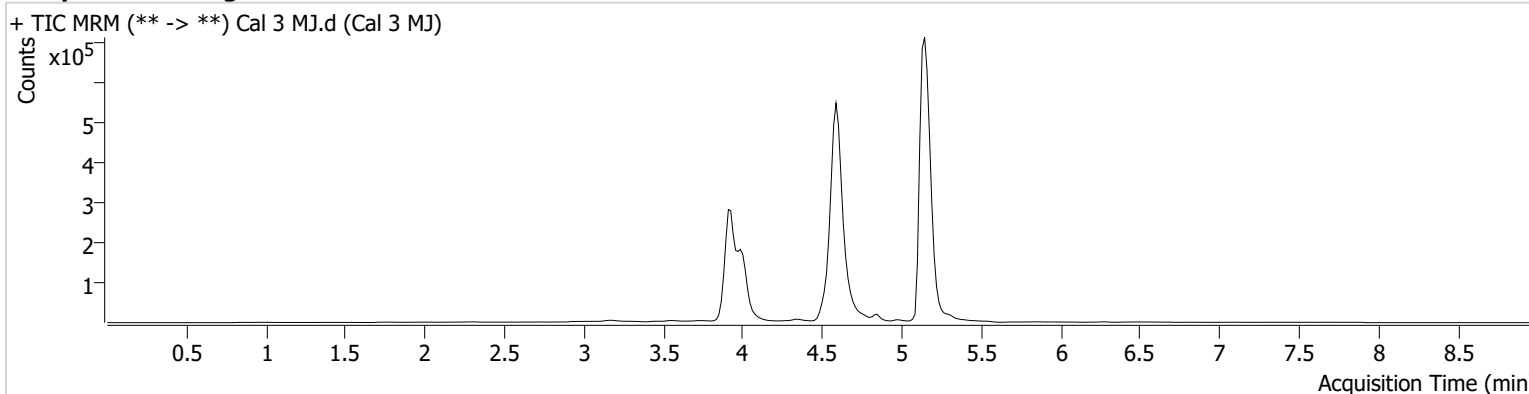
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Calibration Last Update 12/28/2023 8:16:51 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-C1
Injection Volume 10
Acq. Date-Time 12/27/2023 12:24:00 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	5.150	129925	∞	25.5	∞	2946837	4.7383 ng/ml
THC-COOH	4.000	47238	408.57	239.5	1215.66	365852	19.2761 ng/ml
THC-OH	3.926	80514	∞	13.9	∞	1125745	4.8337 ng/ml

TS



AM #27 Cannabinoids Quant. Results

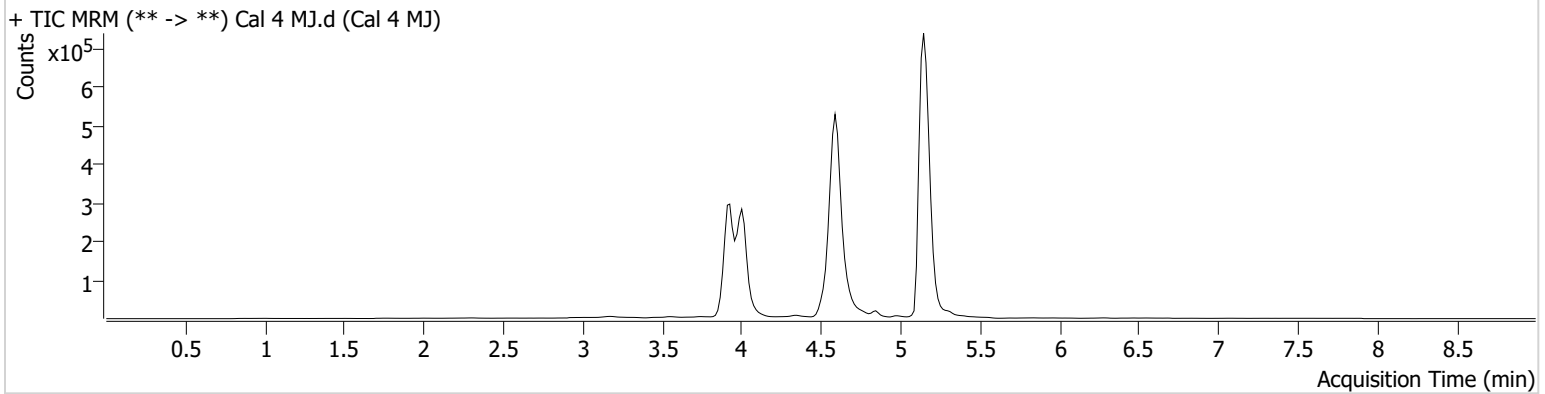
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Calibration Last Update 12/28/2023 8:16:51 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-D1
Injection Volume 10
Acq. Date-Time 12/27/2023 12:37:06 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	5.150	261287	∞	24.8	∞	2816708	9.7603 ng/ml
THC-COOH	4.000	120974	3221.04	228.8	1562.19	350650	50.7766 ng/ml
THC-OH	3.926	160727	∞	13.7	∞	1096005	9.7022 ng/ml

TS



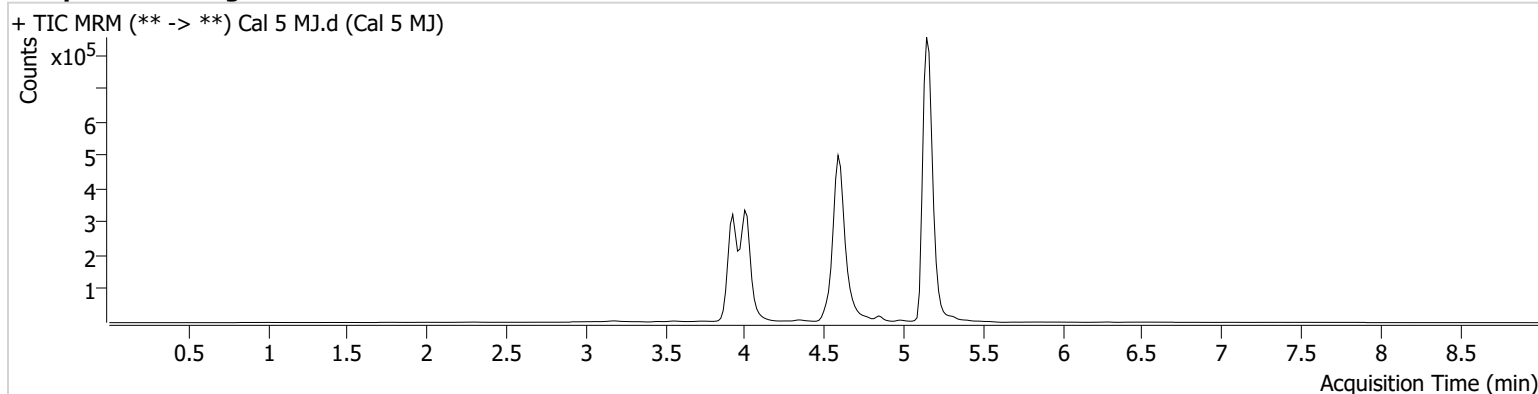
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 12/28/2023 8:16:51 AM

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-E1 **Comment**
Injection Volume 10
Acq. Date-Time 12/27/2023 12:50:12 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	5.165	635352	∞	24.7	∞	2648264	24.9429 ng/ml
THC-COOH	4.015	153891	2988.57	232.5	∞	303673	74.3802 ng/ml
THC-OH	3.926	355142	∞	14.3	∞	930353	24.9365 ng/ml

TS



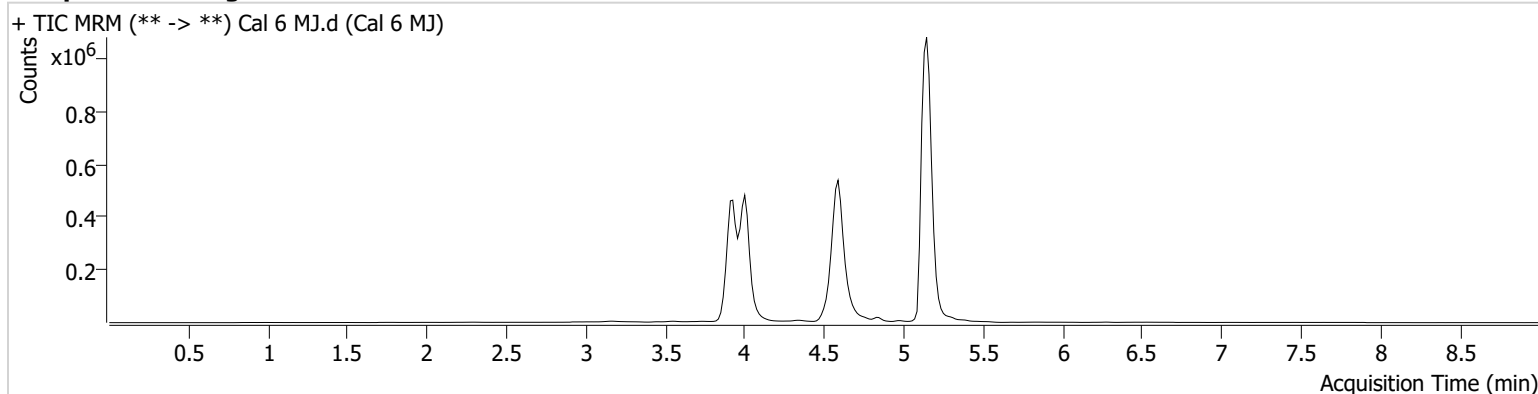
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 12/28/2023 8:16:51 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**
Injection Volume 10
Acq. Date-Time 12/27/2023 1:03:17 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	5.150	1387655	∞	25.3	∞	2884963	49.8174 ng/ml
THC-COOH	4.000	234901	2619.62	233.6	∞	341203	100.8909 ng/ml
THC-OH	3.926	844489	∞	14.0	∞	1092233	50.3042 ng/ml

TS



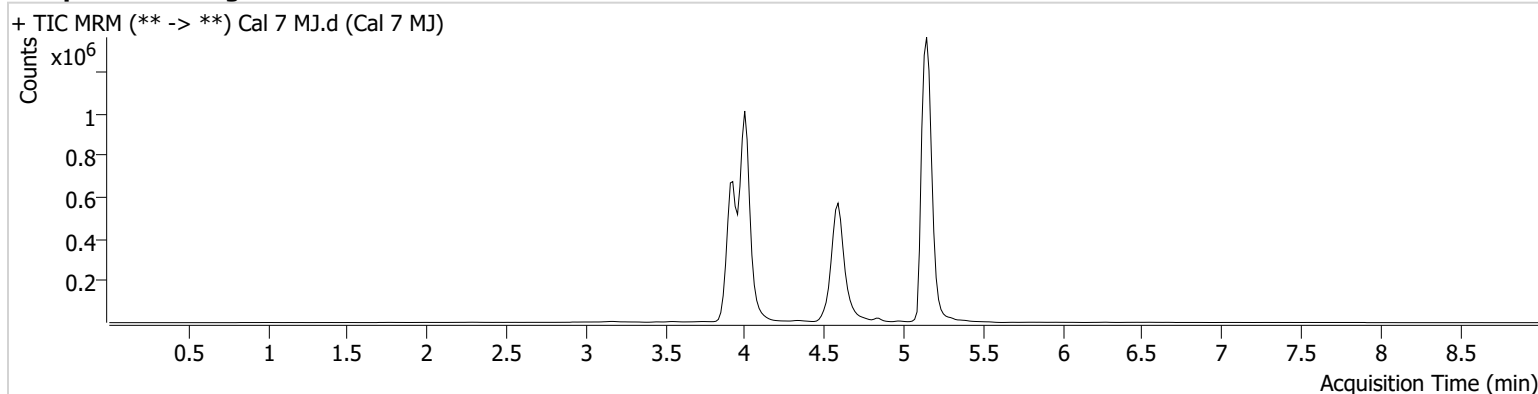
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\122723 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 12/28/2023 8:16:51 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**
Injection Volume 10
Acq. Date-Time 12/27/2023 1:16:23 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	5.150	2581331	∞	25.4	∞	2649614	100.7081 ng/ml
THC-COOH	4.000	558565	∞	234.0	∞	327229	249.5057 ng/ml
THC-OH	3.926	1671173	∞	14.2	∞	1082402	100.2537 ng/ml