

REVIEWED

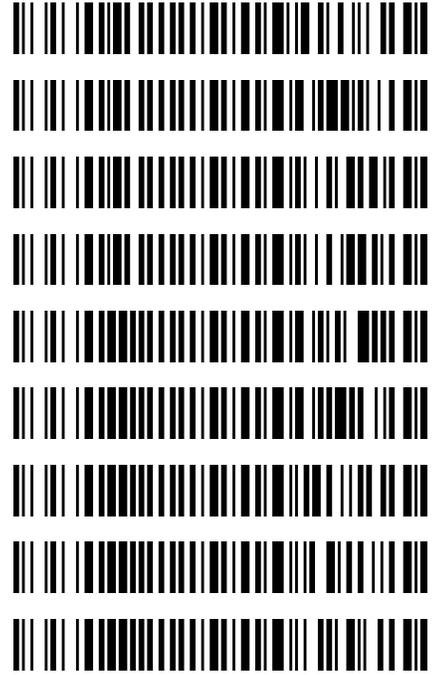
By Celena Shrum at 2:36 pm, Oct 11, 2023

TS

10/5/2023

Worklist: 6518

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-3012	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-3670	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-3684	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-3684	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2839	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2845	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2854	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2858	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2876	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: 10/03/2023

Analyst: Tamara Salazar

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

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

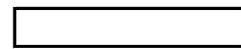
COMMENTS: Negative control did not inject properly with the initial injection. The control was re-injected.
THC - 3-100 -- calibrator 1 dropped due to accuracy/ratio

Analytical Plate Map

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1			P2023-2845-1	IS + QC_1
B	IS + Cal. 2				P2023-2839-1	IS + Cal. 7
C	IS + Cal. 3				M2023-3684-2	IS + Cal. 6
D	IS + Cal. 4				M2023-3684-1	IS + Cal. 5
E	IS + Cal. 5				M2023-3670-2	IS + Cal. 4
F	IS + Cal. 6			P2023-2876-1	M2023-3012-1	IS + Cal. 3
G	IS + Cal. 7			P2023-2858-1	Neg Blood	IS + Cal. 2
H	IS + QC_1			P2023-2854-1	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					P2023-2845-1	IS + QC_1
B					P2023-2839-1	IS + Cal. 7
C					M2023-3684-2	IS + Cal. 6
D				M2023-3670-2	M2023-3684-1	IS + Cal. 5
E				M2023-3012-1	M2023-3670-2*	IS + Cal. 4
F				P2023-2876-1	M2023-3012-1*	IS + Cal. 3
G				P2023-2858-1	Neg Blood	IS + Cal. 2
H				P2023-2854-1	IS + QC_1	IS + Cal. 1

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



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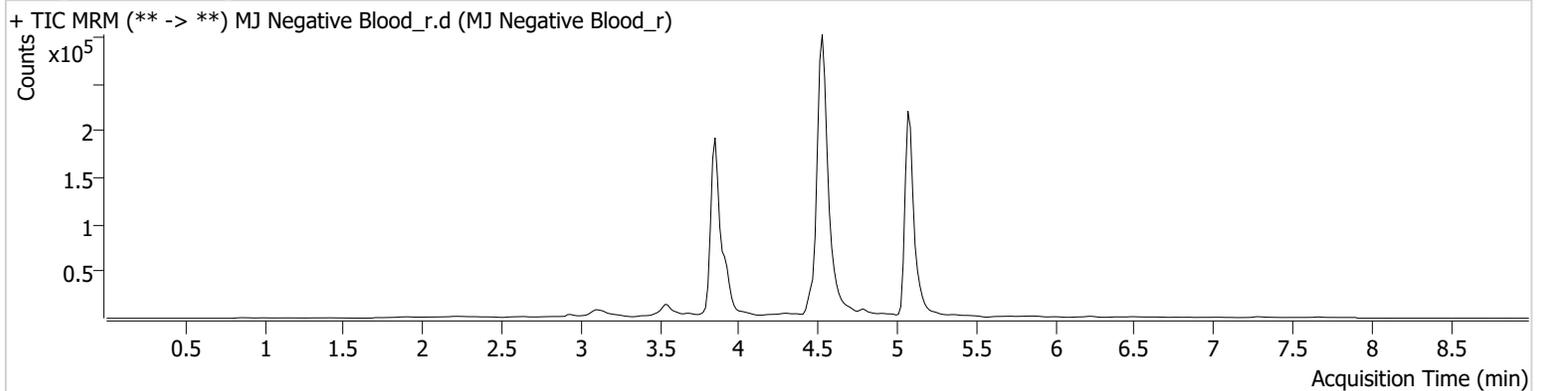
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

Instrument	Falco (069901)	Data File	MJ Negative Blood_r.d
Type	Sample	Sample	MJ Negative Blood_r
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-G5	Comment	
Injection Volume	10		
Acq. Date-Time	10/4/2023 11:57:35 AM		
Sample Info.	Negative control did not inject properly with initial injection. The control was re-injected.		

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



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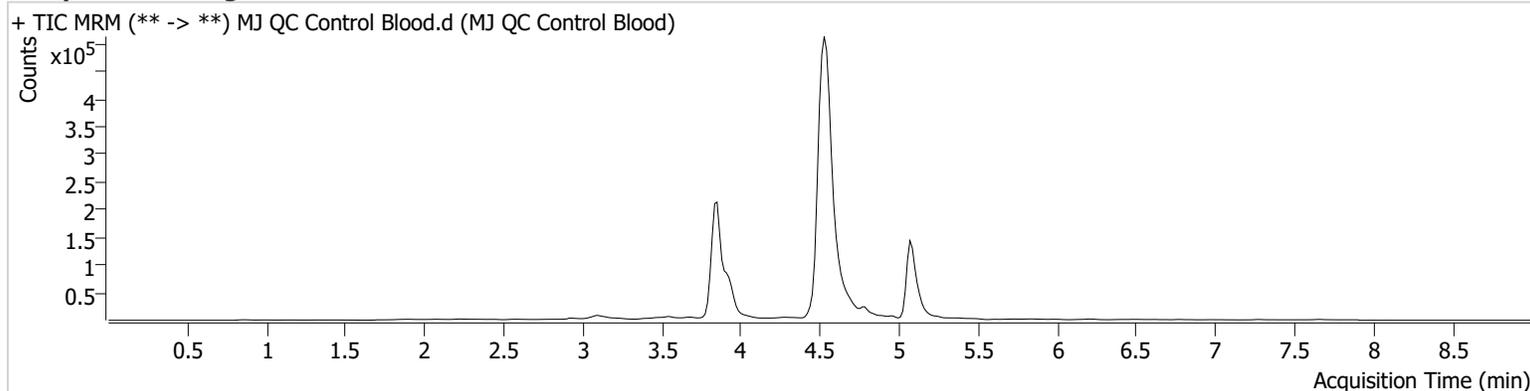
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 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 10/3/2023 5:56:28 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	5.075	21640	∞	34.1	∞	490783	4.8220 ng/ml
THC-COOH	3.939	16840	∞	202.3	∞	162910	15.0926 ng/ml
THC-OH	3.850	57106	∞	14.8	222.40	836079	4.5630 ng/ml

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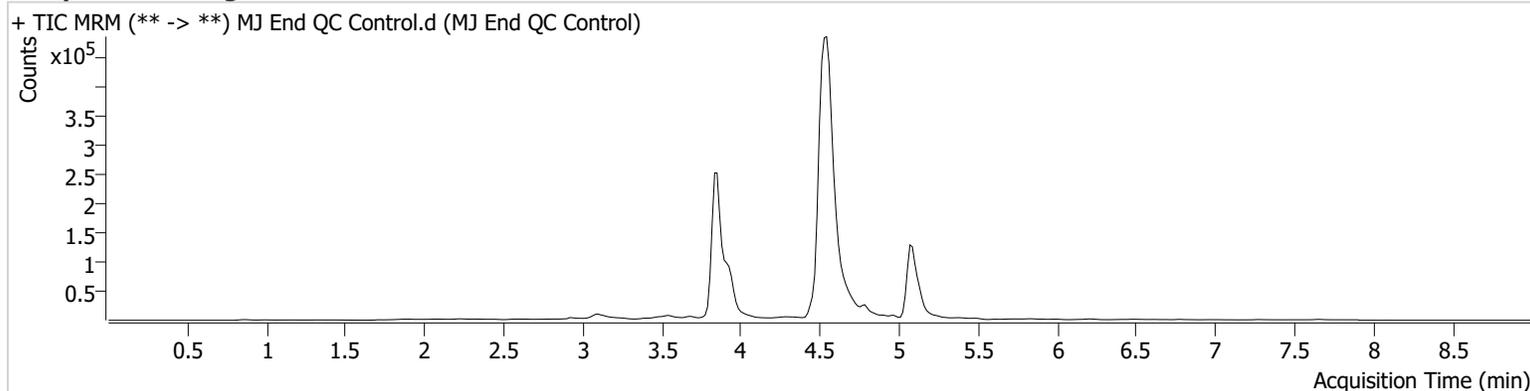
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

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 P1-A6 **Comment**
Injection Volume 10
Acq. Date-Time 10/3/2023 10:44:59 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	20576	∞	32.6	∞	508630	4.4747 ng/ml
THC-COOH	3.939	19702	759.91	214.7	365.36	188112	15.2871 ng/ml
THC-OH	3.850	66222	∞	14.7	142.61	955673	4.6263 ng/ml

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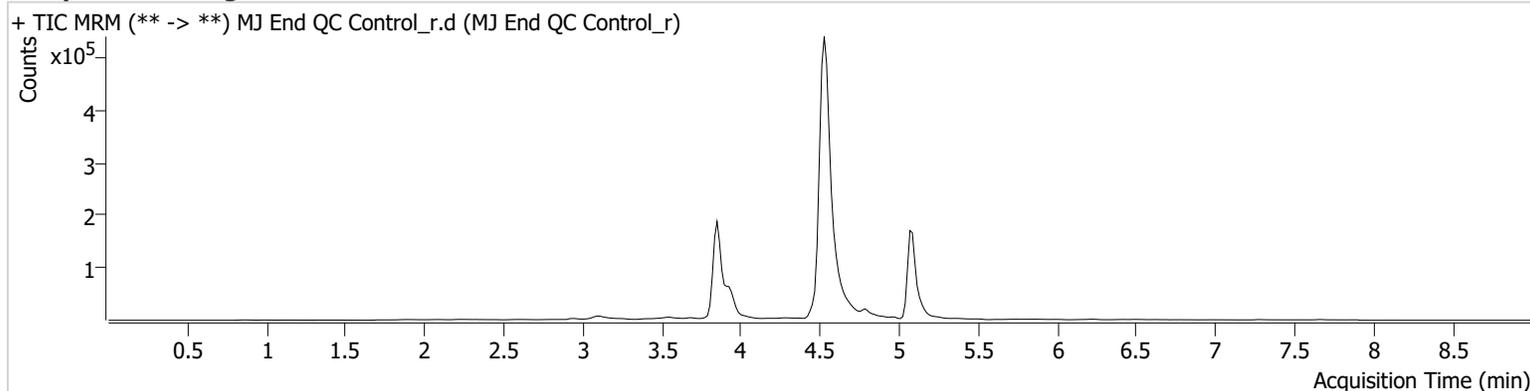


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

Instrument	Falco (069901)	Data File	MJ End QC Control_r.d
Type	QC	Sample	MJ End QC Control_r
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-A6	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	10/4/2023 12:23:59 PM		
Sample Info.	QC followed re-injected samples.		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	22761	∞	31.0	∞	568592	4.4343 ng/ml
THC-COOH	3.939	11921	143.83	214.5	∞	123418	14.1283 ng/ml
THC-OH	3.850	46661	∞	13.1	110.76	638131	4.8711 ng/ml

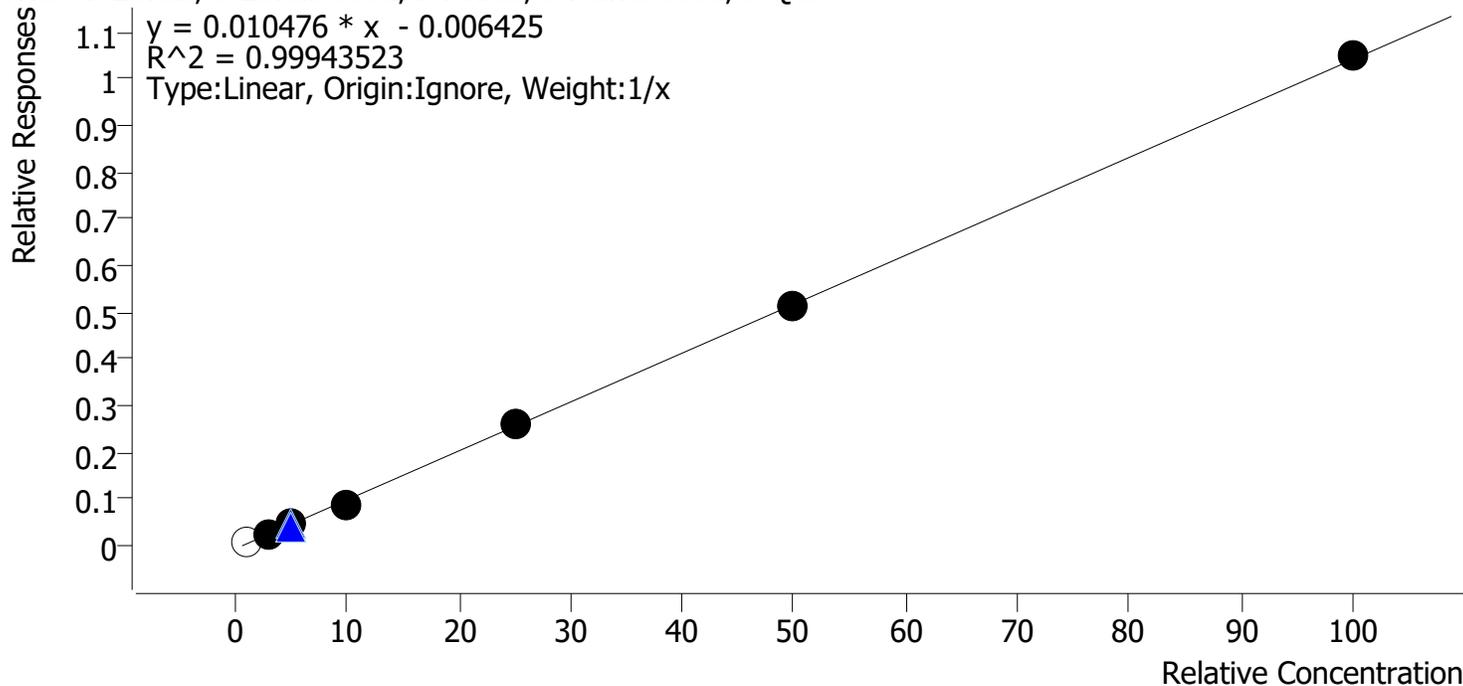
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
 Last Cal. Update 10/4/2023 2:45 PM
 Analyst Name ISP\Datastor
 Analyte THC Internal Standard THC-D3

THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 3 QCs



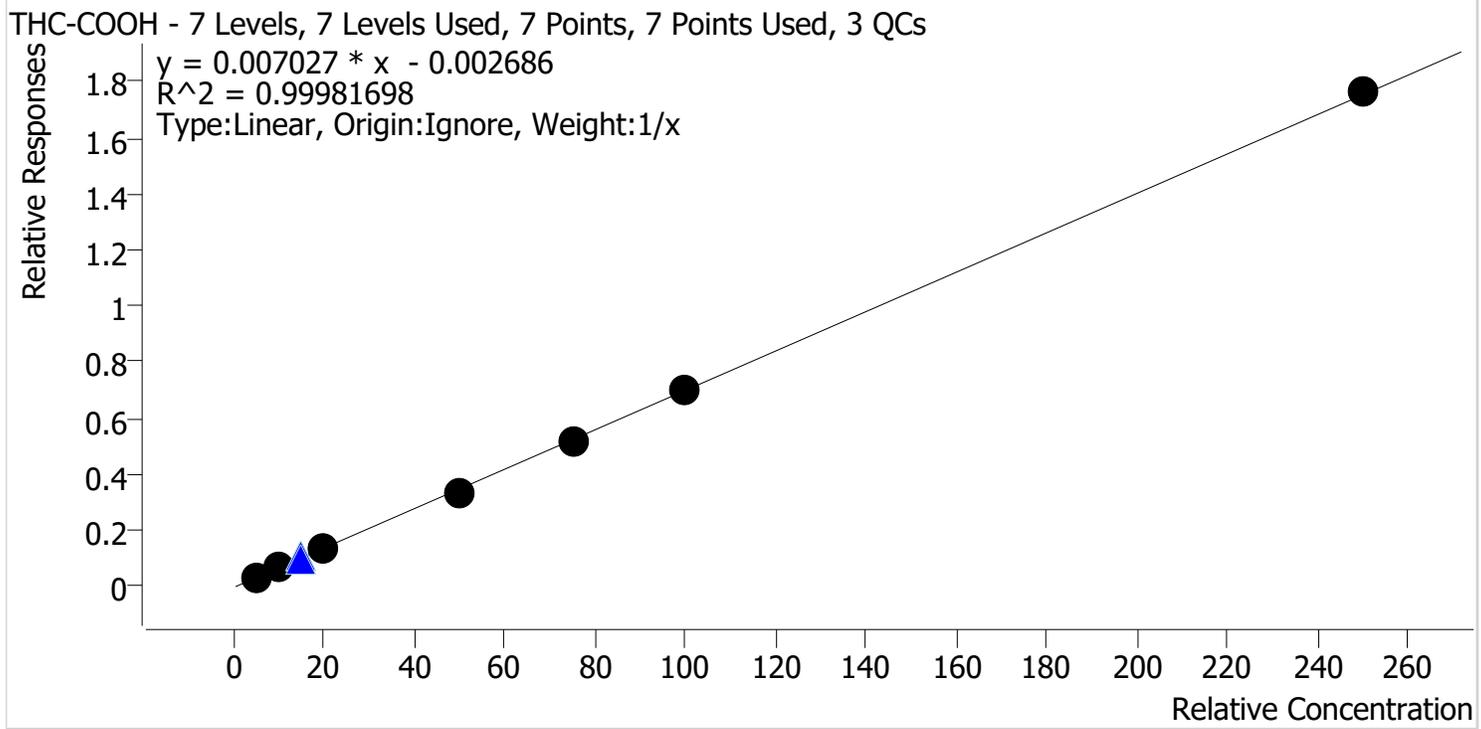
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	1.5	153.1
Cal 2 MJ	2	✓	3.0	3.2	105.5
Cal 3 MJ	3	✓	5.0	5.1	101.5
Cal 4 MJ	4	✓	10.0	9.2	92.2
Cal 5 MJ	5	✓	25.0	25.2	100.8
Cal 6 MJ	6	✓	50.0	49.6	99.3
Cal 7 MJ	7	✓	100.0	100.7	100.7

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 10/4/2023 2:45 PM
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	102.1
Cal 2 MJ	2	✓	10.0	10.0	99.6
Cal 3 MJ	3	✓	20.0	20.2	101.1
Cal 4 MJ	4	✓	50.0	48.6	97.3
Cal 5 MJ	5	✓	75.0	73.9	98.6
Cal 6 MJ	6	✓	100.0	100.7	100.7
Cal 7 MJ	7	✓	250.0	251.4	100.6

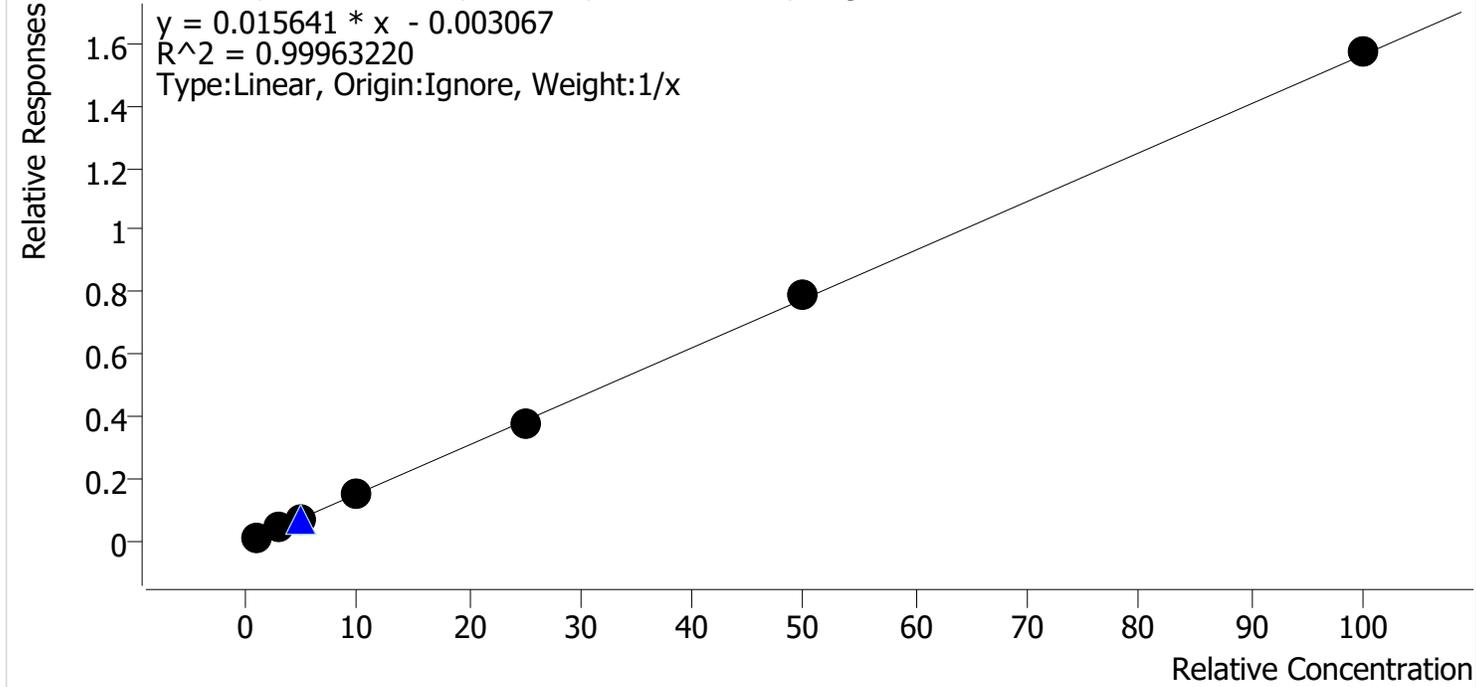
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 10/4/2023 2:45 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	1.1	110.6
Cal 2 MJ	2	✓	3.0	2.9	97.3
Cal 3 MJ	3	✓	5.0	4.8	95.4
Cal 4 MJ	4	✓	10.0	9.8	98.0
Cal 5 MJ	5	✓	25.0	24.2	97.0
Cal 6 MJ	6	✓	50.0	50.5	101.1
Cal 7 MJ	7	✓	100.0	100.6	100.6

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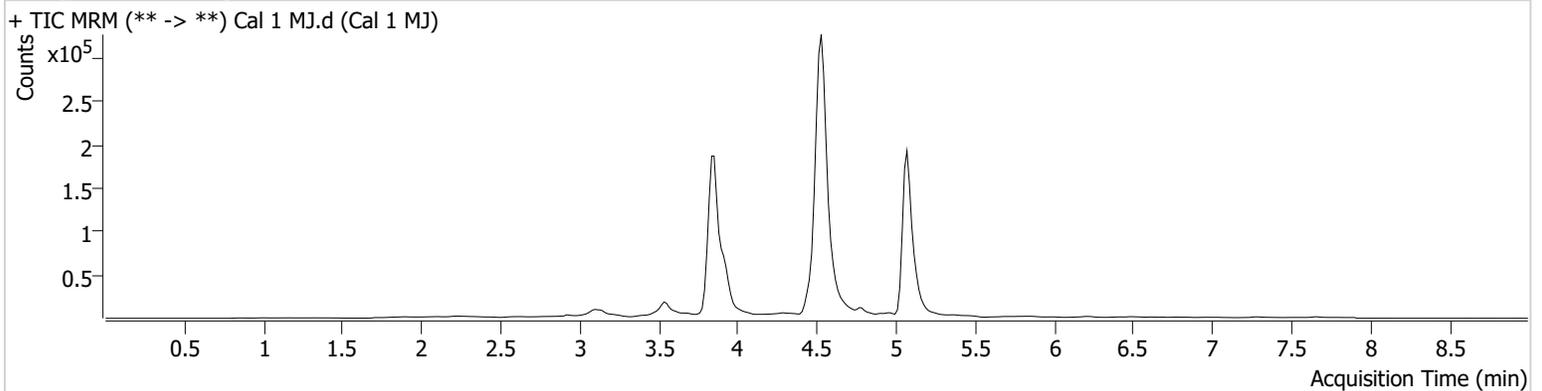
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

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-H6 **Comment**
Injection Volume 10
Acq. Date-Time 10/3/2023 4:11:27 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	6743	∞	46.3 High	∞	701588	1.5307 ng/ml
THC-COOH	3.939	5376	74.78	216.1	51.99	161963	5.1056 ng/ml
THC-OH	3.850	11249	∞	14.7	17.68	790148	1.1063 ng/ml

TS



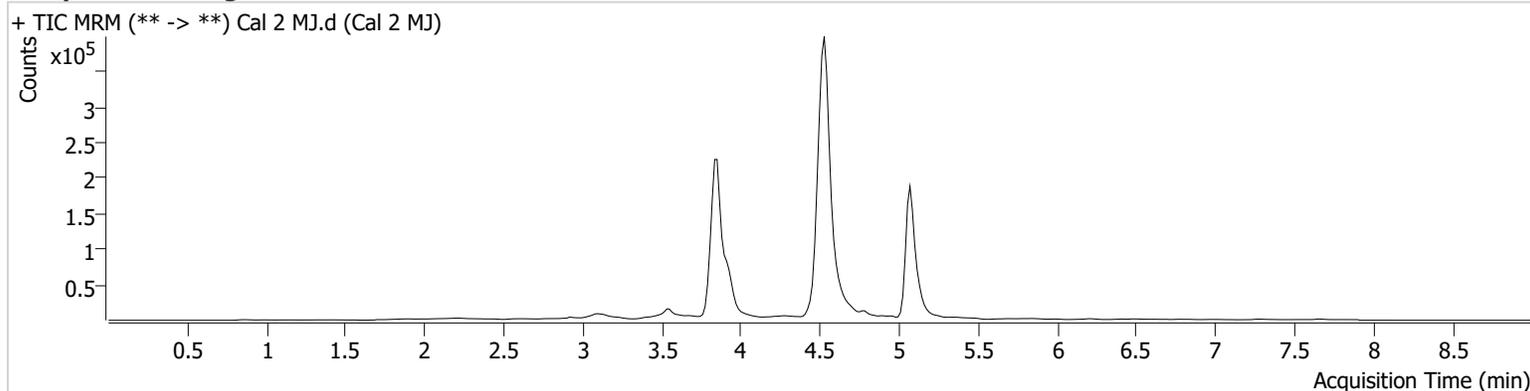
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

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-G6 **Comment**
Injection Volume 10
Acq. Date-Time 10/3/2023 4:24:43 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	18773	∞	37.2	∞	702146	3.1654 ng/ml
THC-COOH	3.939	11433	218.16	205.1	347.49	169851	9.9613 ng/ml
THC-OH	3.850	40445	∞	13.1	66.29	950097	2.9177 ng/ml

TS



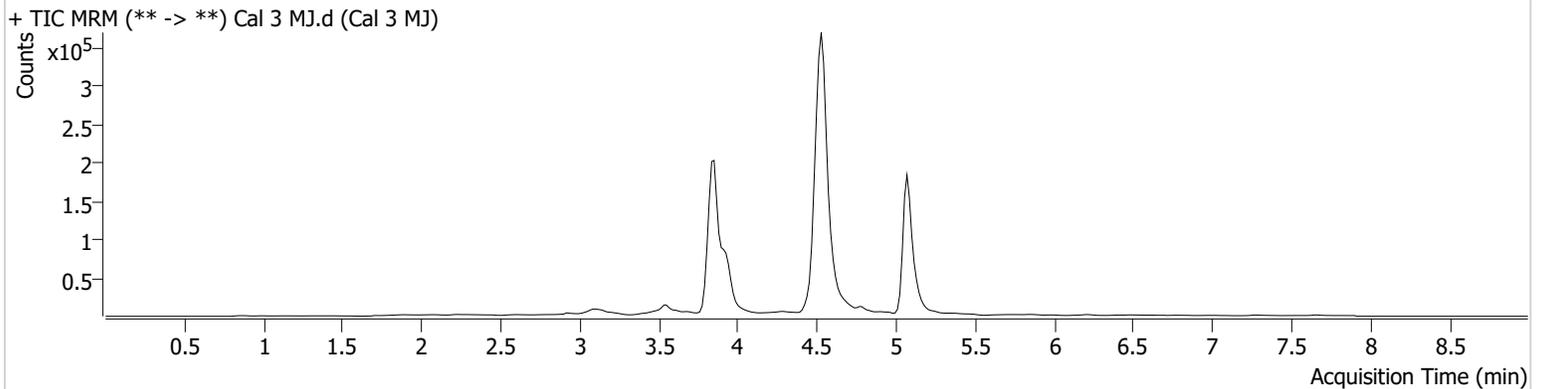
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

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-F6 **Comment**
Injection Volume 10
Acq. Date-Time 10/3/2023 4:37:48 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	30910	∞	33.5	∞	661239	5.0754 ng/ml
THC-COOH	3.939	22021	268.52	202.4	185.43	157929	20.2256 ng/ml
THC-OH	3.850	58584	42.97	14.4	130.45	818865	4.7701 ng/ml

TS



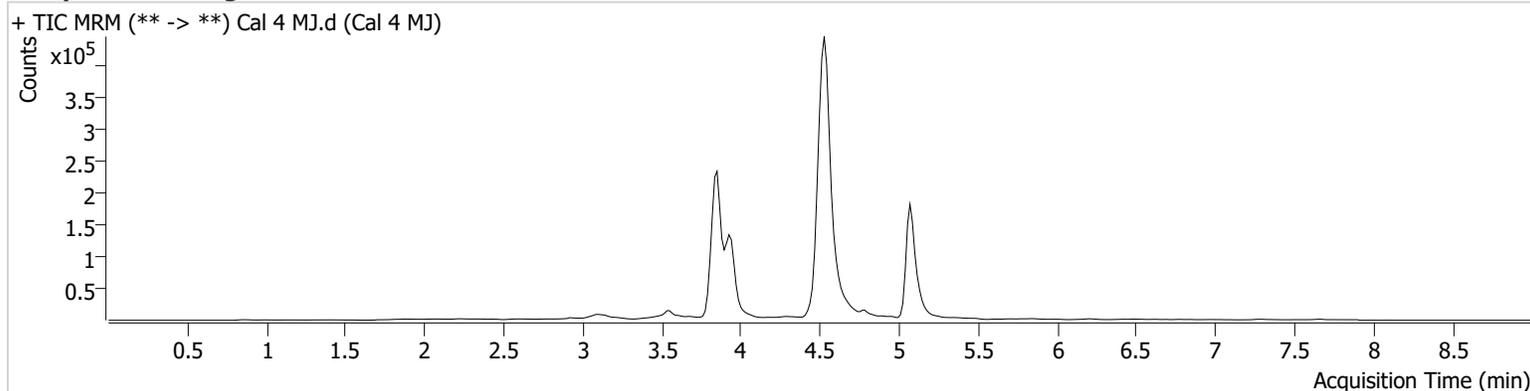
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

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-E6 **Comment**
Injection Volume 10
Acq. Date-Time 10/3/2023 4:50:54 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	56035	400.04	29.0	∞	621513	9.2193 ng/ml
THC-COOH	3.939	54762	294.78	212.6	1041.97	161536	48.6269 ng/ml
THC-OH	3.850	128554	∞	14.3	∞	855411	9.8044 ng/ml

TS



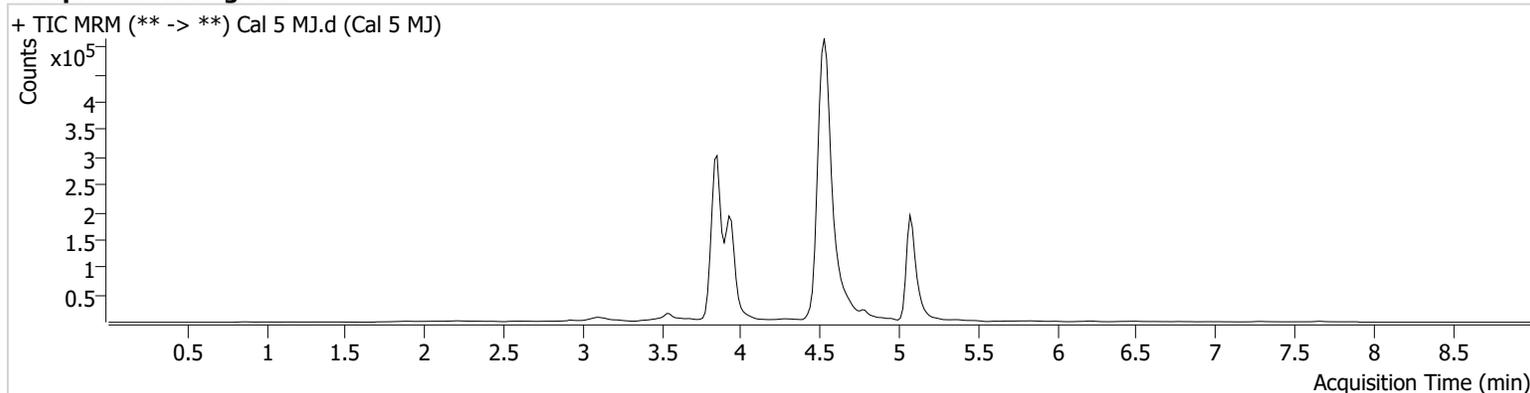
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 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**
Injection Volume 10
Acq. Date-Time 10/3/2023 5:04:00 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	137446	4926.82	26.9	∞	533612	25.2000 ng/ml
THC-COOH	3.939	87891	∞	212.6	∞	170031	73.9443 ng/ml
THC-OH	3.850	344359	∞	13.9	467.77	915684	24.2399 ng/ml

TS



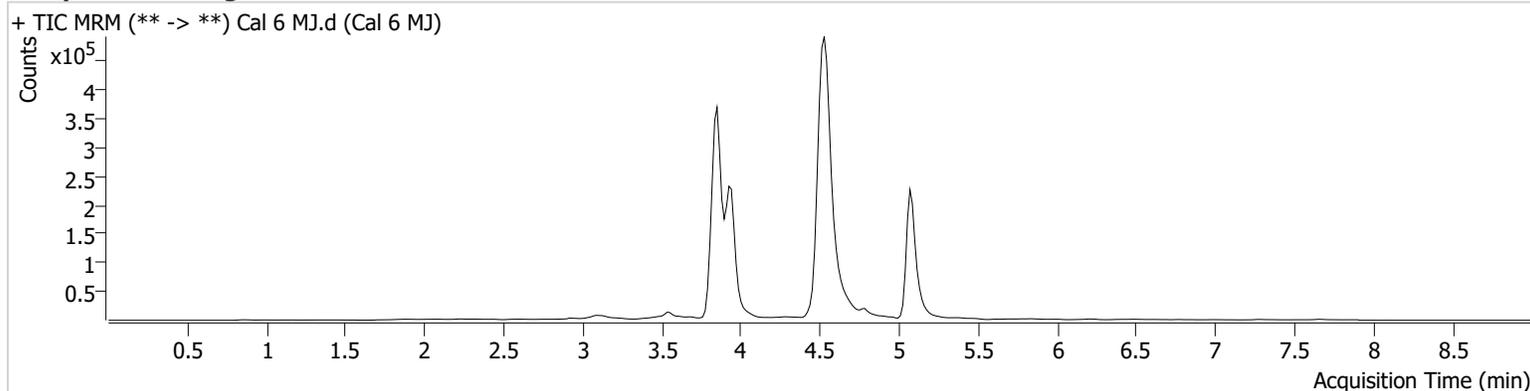
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

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-C6 **Comment**
Injection Volume 10
Acq. Date-Time 10/3/2023 5:17:07 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	263081	∞	25.9	∞	512156	49.6455 ng/ml
THC-COOH	3.939	114575	∞	205.2	1943.30	162465	100.7435 ng/ml
THC-OH	3.850	669633	∞	14.3	1077.15	850347	50.5436 ng/ml

TS



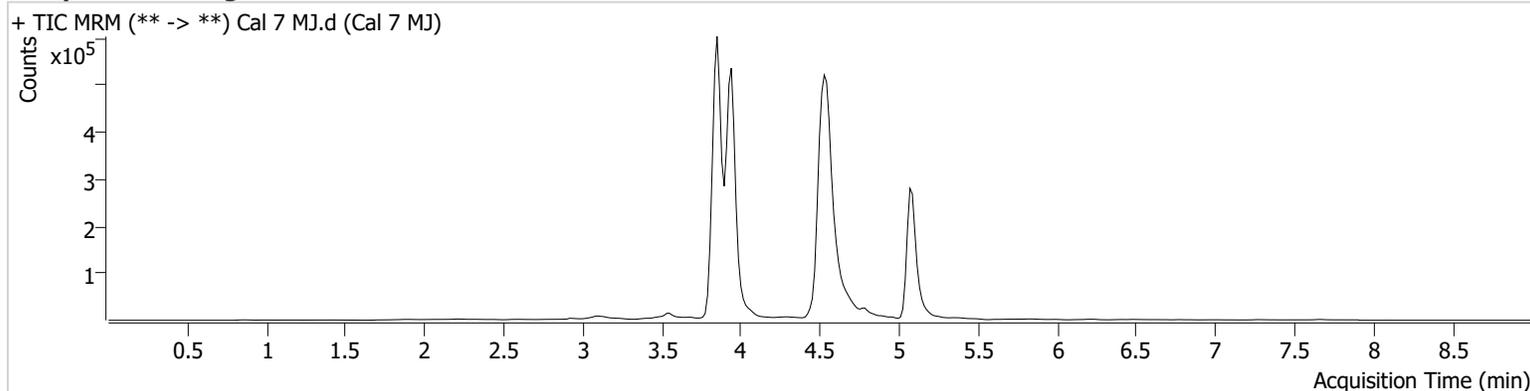
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\100323 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/4/2023 2:45:46 PM

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-B6 **Comment**
Injection Volume 10
Acq. Date-Time 10/3/2023 5:30:14 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	5.075	489840	∞	25.8	∞	467193	100.6943 ng/ml
THC-COOH	3.939	294109	2551.25	204.3	4416.68	166746	251.3928 ng/ml
THC-OH	3.850	1400571	∞	13.9	2436.89	891690	100.6180 ng/ml