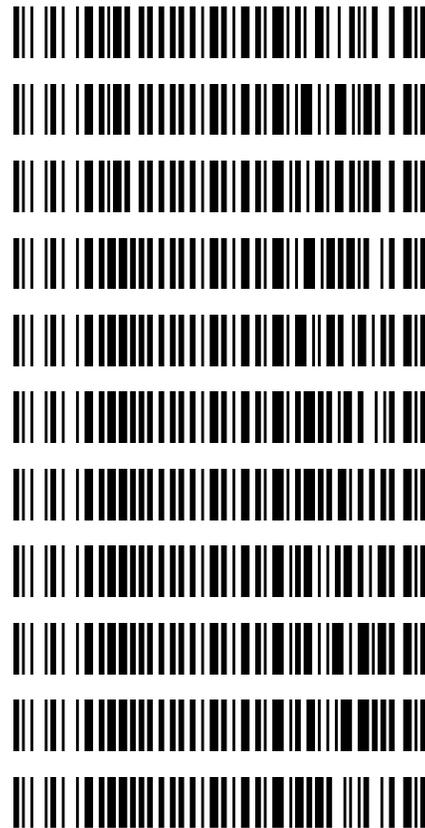


Worklist: 6485

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-3053	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-3397	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-3537	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2465	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2480	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2498	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2499	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2554	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2561	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2565	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2575	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: 08/30/23Analyst: Sarah Collins

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: 23A52594

Blank Urine Lot: N/A

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.** Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: 3382167**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. 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.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate. Amount transferred: 800uL
- 7. 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)**
- 8. Wait 5 minutes.
- 9. Add **2.25mL MTBE. (Add in 3 increments of 750uL)**
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 12. Add **2.25mL Hexane. (Add in 3 increments of 750uL)**
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
- 16. 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: *The end QC was not in the original worklist. It was injected 8/31/23 with no issues.*

8c

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_2 end	p2023-2499-1			IS + QC_1 blood
B	IS + Cal. 2	negative blood	p2023-2554-2			IS + Cal. 7
C	IS + Cal. 3	m2023-3053-1	p2023-2561-1			IS + Cal. 6
D	IS + Cal. 4	m2023-3397-2	p2023-2565-1			IS + Cal. 5
E	IS + Cal. 5	m2023-3537-1*	p2023-2575-1			IS + Cal. 4
F	IS + Cal. 6	p2023-2465-2	m2023-3537-1			IS + Cal. 3
G	IS + Cal. 7	p2023-2480-1				IS + Cal. 2
H	IS + QC_1 start	p2023-2498-1			IS + QC_1 blood	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Moved during analytical step 7 due to blood clot

SC

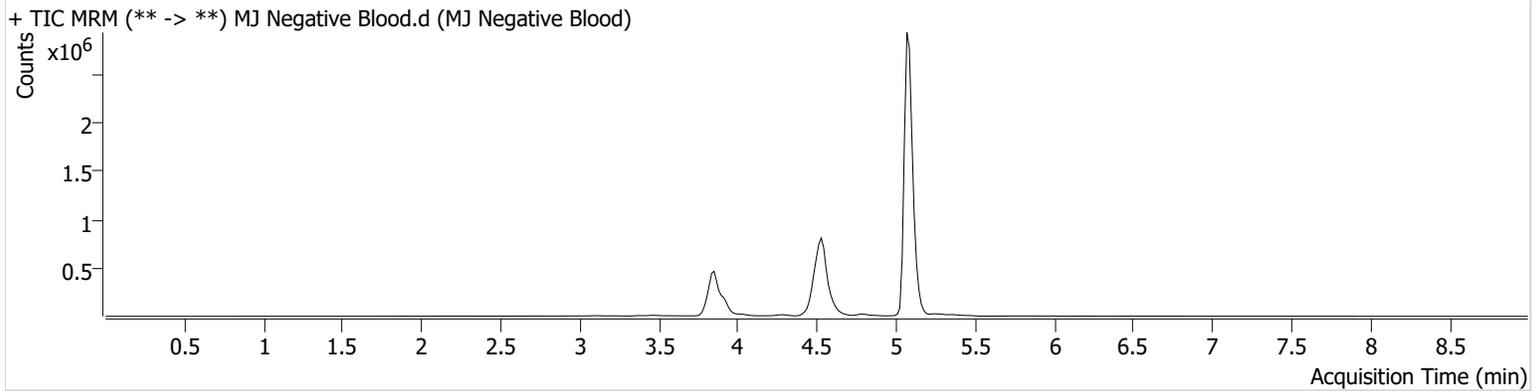


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
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	8/30/2023 4:00:40 PM		
Sample Info.			

Sample Chromatogram



SC

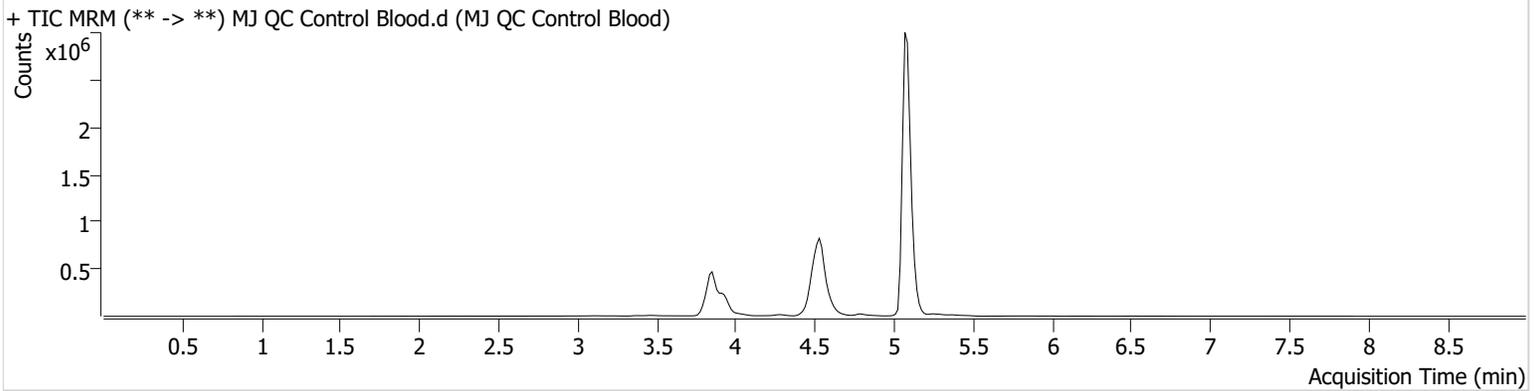


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 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	Sarah Collins
Sample Position	P1-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	8/30/2023 3:34:28 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	464342	∞	25.8	230.41	10672748	4.2539 ng/ml
THC-COOH	3.939	53127	897.61	250.9	939.08	508480	15.2526 ng/ml
THC-OH	3.850	115921	∞	13.5	197.62	1987037	4.6938 ng/ml

SC

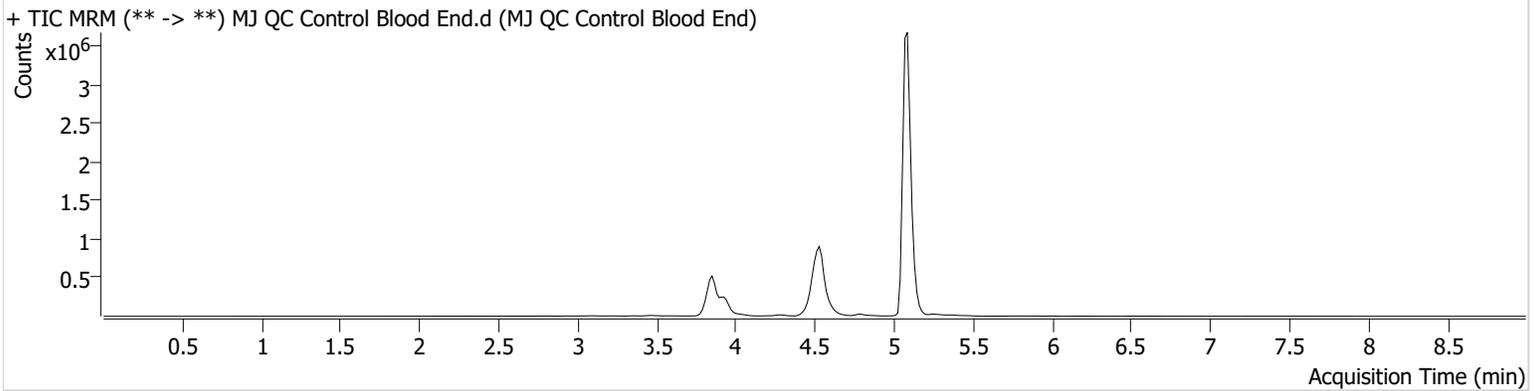


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	MJ QC Control Blood End.d
Type	QC	Sample	MJ QC Control Blood End
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
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	8/31/2023 10:56:29 AM		
Sample Info.			

Sample Chromatogram



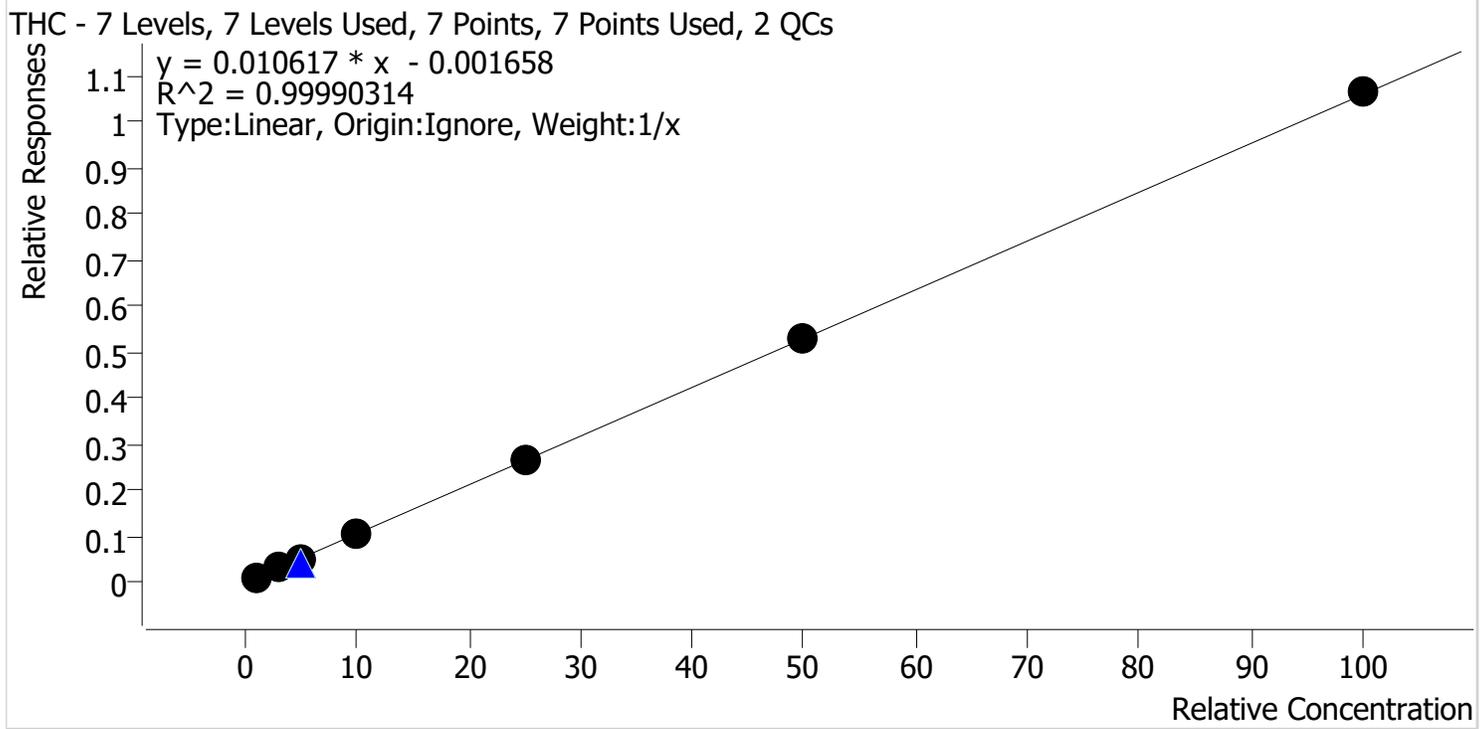
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	541334	5860.80	25.8	∞	12578397	4.2096 ng/ml
THC-COOH	3.939	55907	1371.79	246.5	∞	538363	15.1640 ng/ml
THC-OH	3.850	126429	∞	13.1	∞	2093172	4.8607 ng/ml

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 8/31/2023 11:10 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	105.8
Cal 2 MJ	2	✓	3.0	3.0	99.0
Cal 3 MJ	3	✓	5.0	4.9	97.9
Cal 4 MJ	4	✓	10.0	9.7	97.3
Cal 5 MJ	5	✓	25.0	24.9	99.6
Cal 6 MJ	6	✓	50.0	49.9	99.9
Cal 7 MJ	7	✓	100.0	100.5	100.5

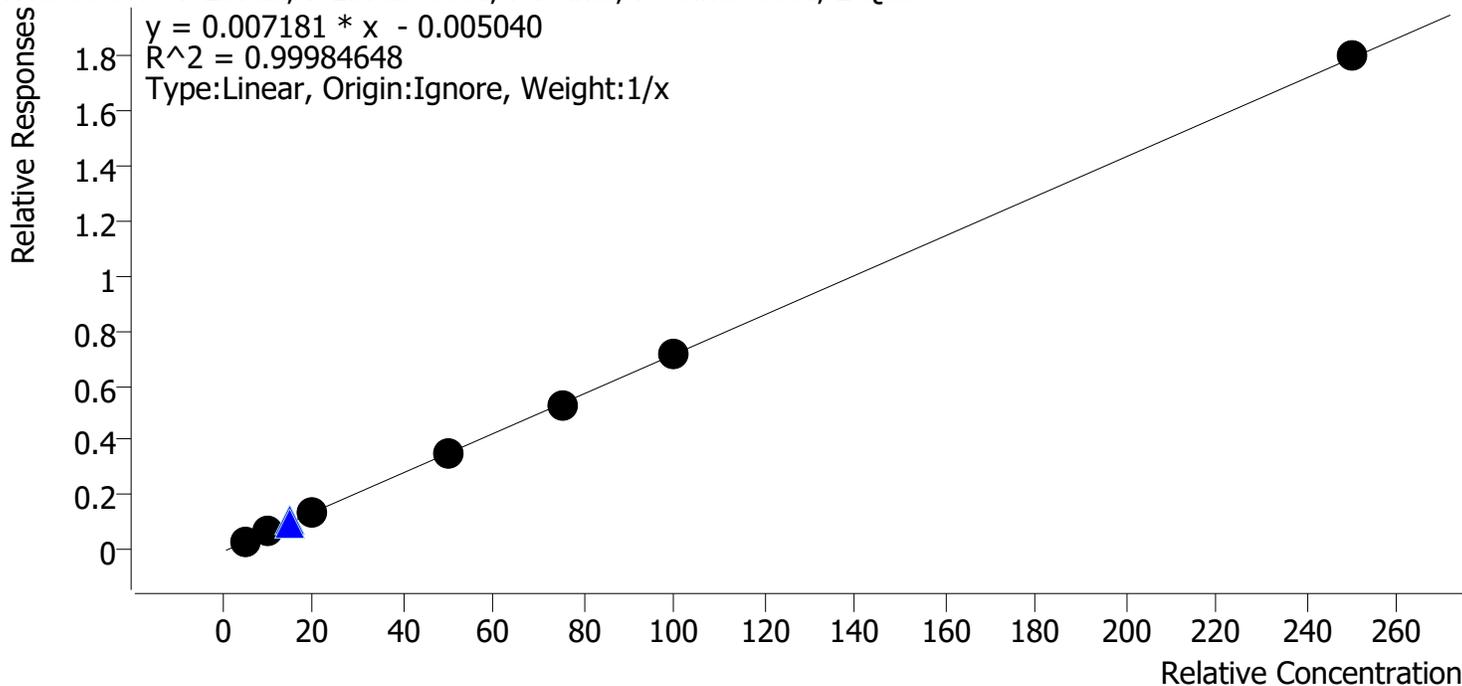
SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 8/31/2023 11:10 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	5.3	105.7
Cal 2 MJ	2	✓	10.0	9.7	97.3
Cal 3 MJ	3	✓	20.0	19.6	98.2
Cal 4 MJ	4	✓	50.0	49.3	98.6
Cal 5 MJ	5	✓	75.0	74.2	98.9
Cal 6 MJ	6	✓	100.0	100.8	100.8
Cal 7 MJ	7	✓	250.0	251.0	100.4

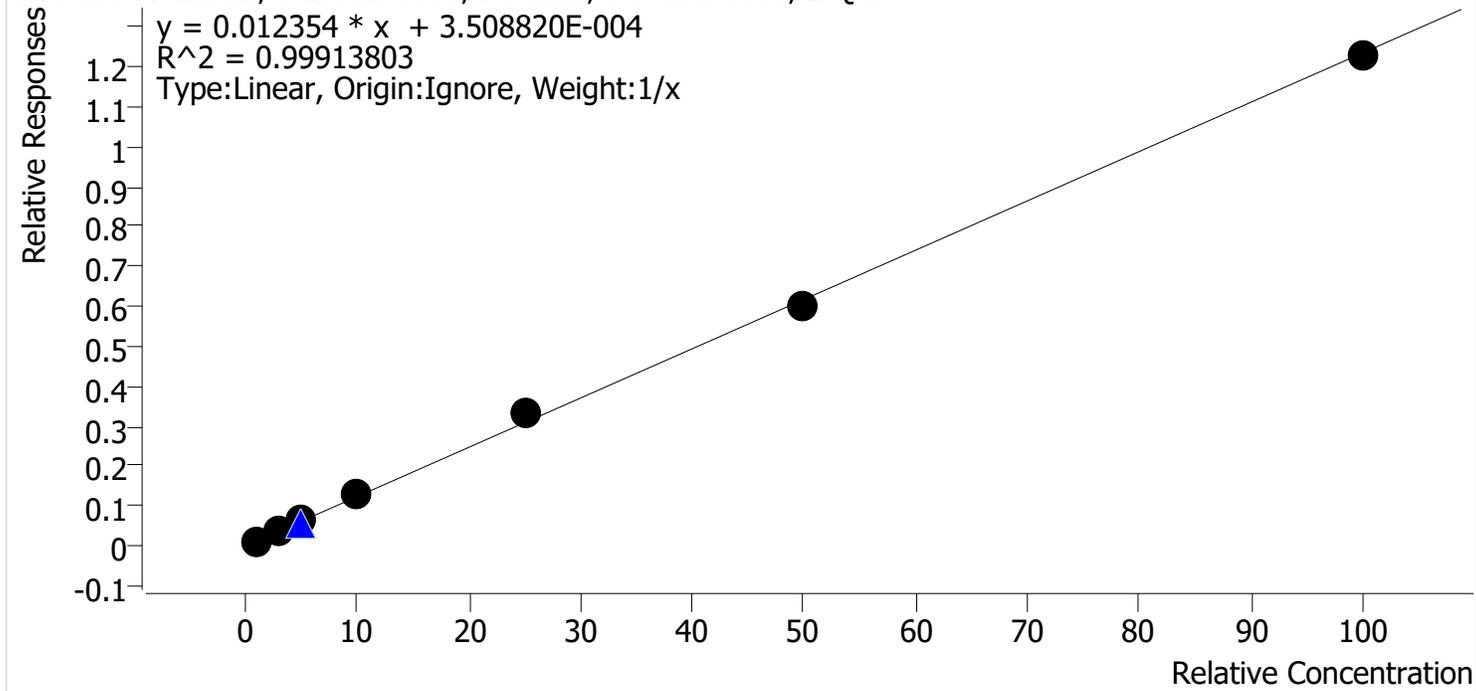
SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 8/31/2023 11:10 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	0.9	94.3
Cal 2 MJ	2	✓	3.0	3.0	100.8
Cal 3 MJ	3	✓	5.0	5.0	99.4
Cal 4 MJ	4	✓	10.0	10.2	101.7
Cal 5 MJ	5	✓	25.0	26.6	106.6
Cal 6 MJ	6	✓	50.0	48.9	97.8
Cal 7 MJ	7	✓	100.0	99.3	99.3

SC

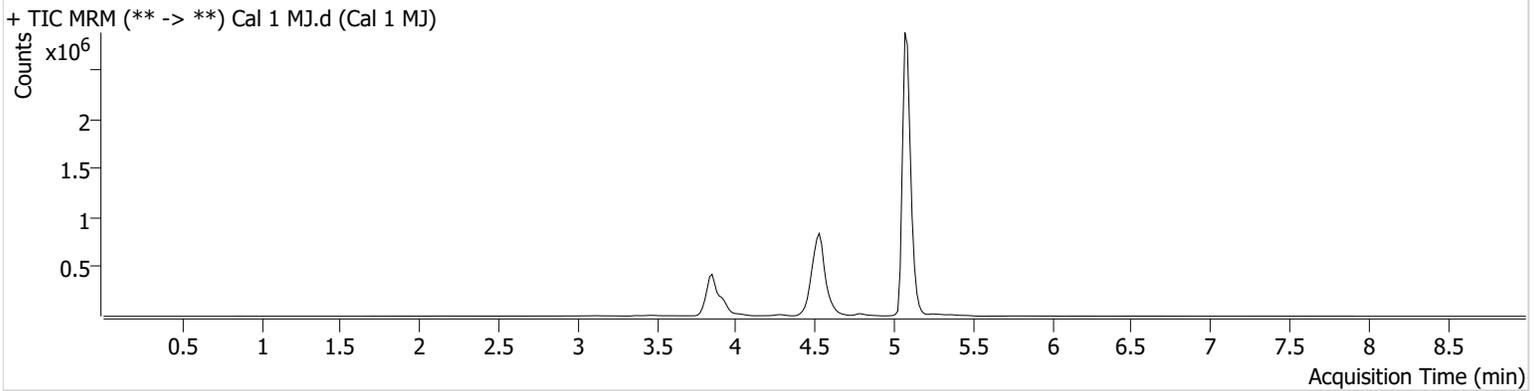


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
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	8/30/2023 1:49:27 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	98260	536.30	27.7	∞	10263234	1.0579 ng/ml
THC-COOH	3.939	15237	569.33	261.3	190.79	462965	5.2853 ng/ml
THC-OH	3.850	22276	∞	12.2	∞	1855361	0.9435 ng/ml

SC

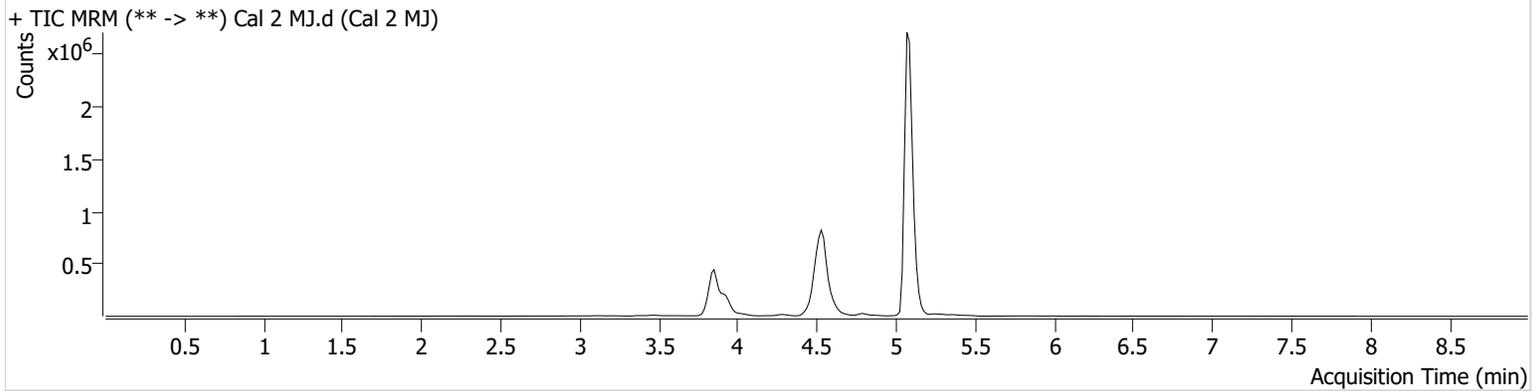


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
Sample Position	P1-B1	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	8/30/2023 2:02:43 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	283660	7023.04	26.4	∞	9494851	2.9699 ng/ml
THC-COOH	3.939	30445	1187.84	254.5	1445.87	469480	9.7331 ng/ml
THC-OH	3.850	69941	85.82	13.2	172.74	1853978	3.0253 ng/ml

SC

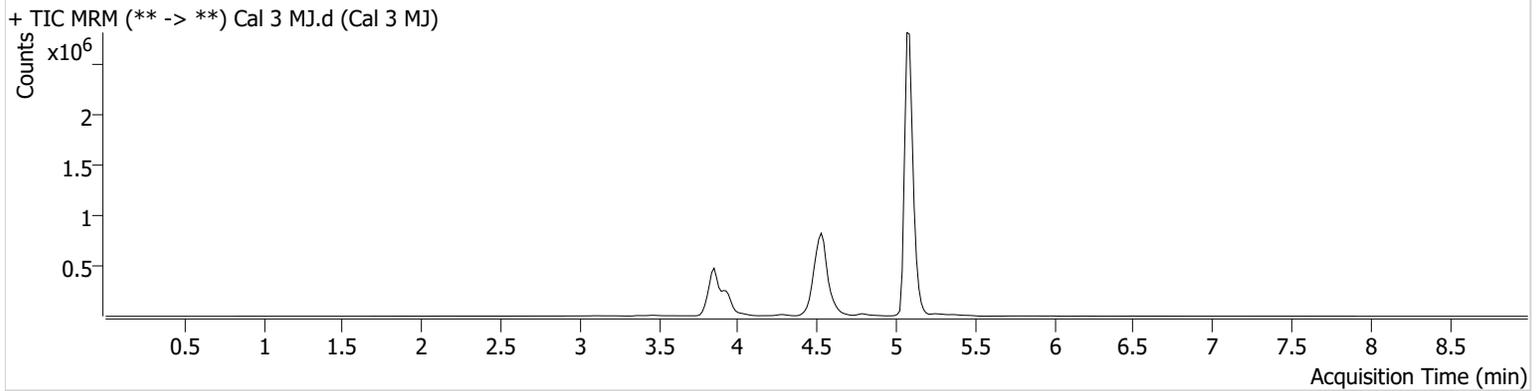


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
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	8/30/2023 2:15:49 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	499185	∞	25.9	∞	9920474	4.8954 ng/ml
THC-COOH	3.939	66200	1041.74	254.3	1240.74	486734	19.6431 ng/ml
THC-OH	3.850	121801	128.06	13.3	∞	1973045	4.9685 ng/ml

SC

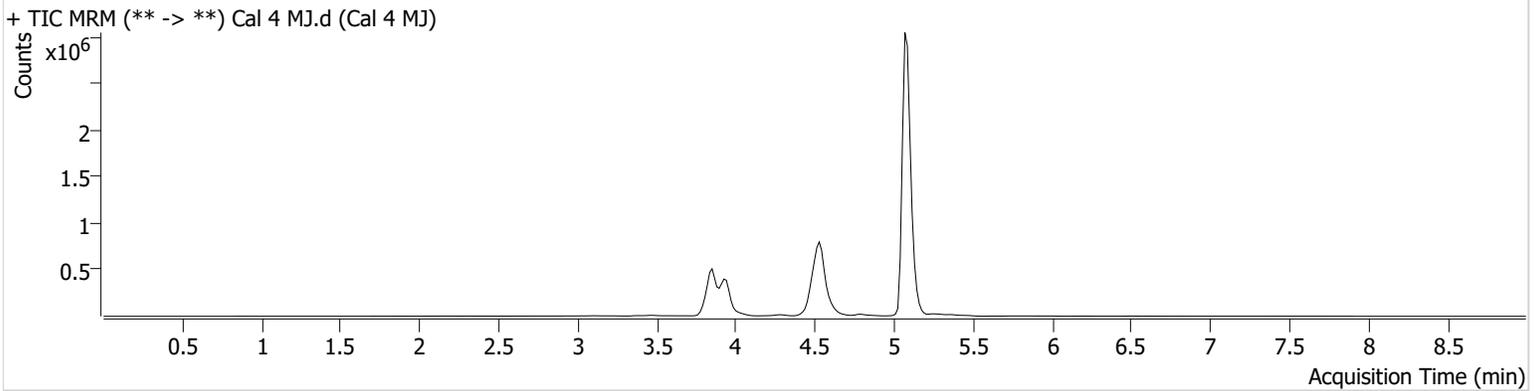


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
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	8/30/2023 2:28:55 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	1014697	8697.27	26.3	∞	9979916	9.7323 ng/ml
THC-COOH	3.939	165564	2158.73	242.5	∞	474627	49.2817 ng/ml
THC-OH	3.850	249719	∞	13.4	482.98	1981750	10.1714 ng/ml

SC

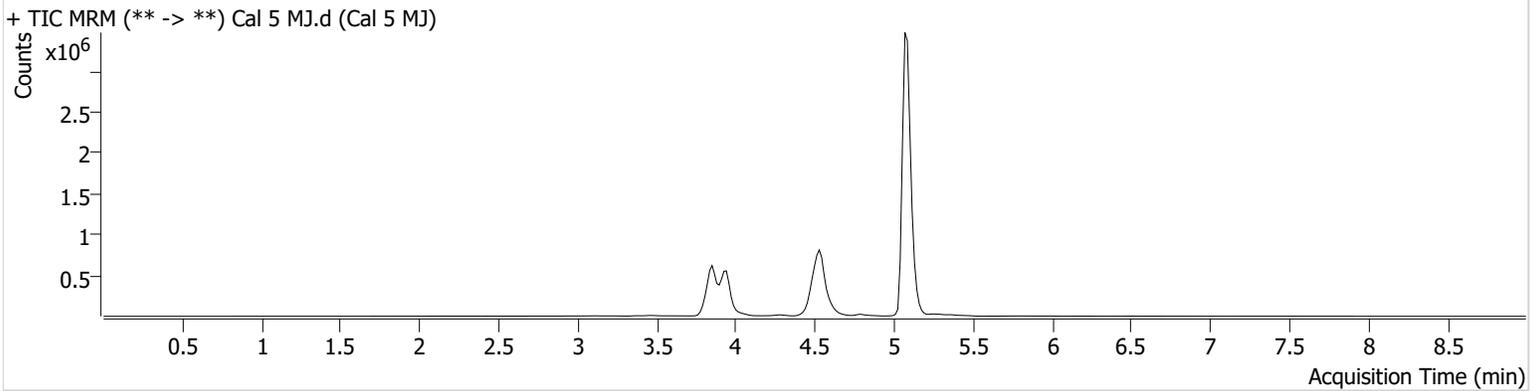


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
Sample Position	P1-E1	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	8/30/2023 2:42:01 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	2555337	∞	26.4	∞	9726589	24.9001 ng/ml
THC-COOH	3.939	256807	1826.90	241.5	∞	486523	74.2118 ng/ml
THC-OH	3.850	667421	825.28	13.8	1247.38	2025613	26.6422 ng/ml

SC

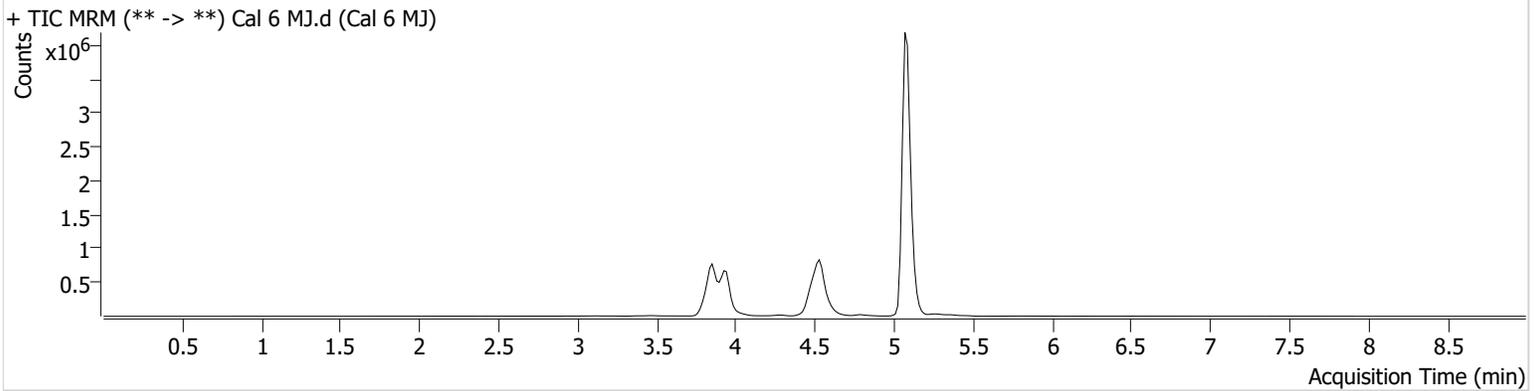


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
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	8/30/2023 2:55:07 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	4928862	∞	26.3	5041.97	9324576	49.9412 ng/ml
THC-COOH	3.939	338773	695.50	245.9	∞	471194	100.8291 ng/ml
THC-OH	3.850	1256000	∞	15.9	∞	2077440	48.9102 ng/ml

SC

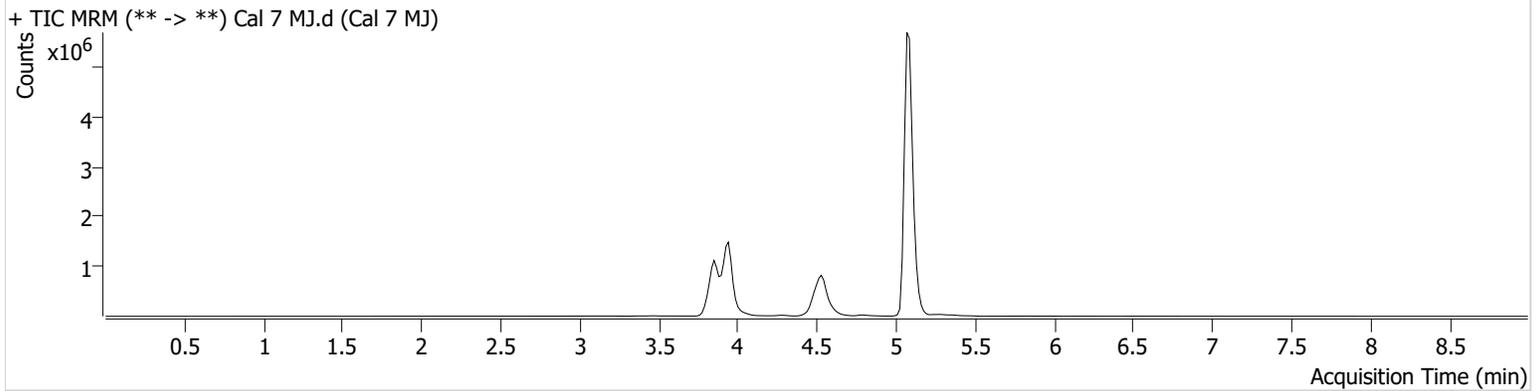


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\083023 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 8/31/2023 11:10:08 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
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	8/30/2023 3:08:13 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	9744258	∞	26.1	∞	9145884	100.5032 ng/ml
THC-COOH	3.939	813867	∞	239.4	∞	452804	251.0159 ng/ml
THC-OH	3.850	2461352	∞	14.9	∞	2005026	99.3390 ng/ml