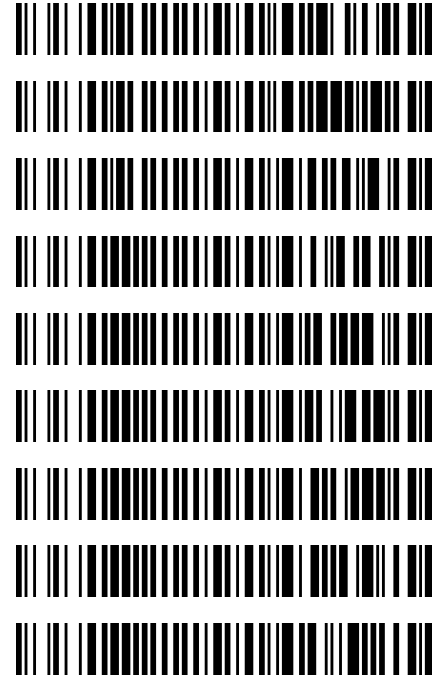


Worklist: 6303

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-0635	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-0638	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-0694	4	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0590	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0607	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0636	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0668	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0669	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0715	1	BCK	AM 27 Blood THC Quant by LC-QQQ



8C

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

Extraction Date: 03/30/2023

Plate lot#: 220802

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 23A52593

Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Sarah Collins

Plate Retest Date: 07/23/2023

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Urine Lot: POC021022

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: #27
- 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: 800 uL
- 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 r² 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: *THC-OH 3-100* (Calibrator 1 dropped due to accuracy)

8C

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_2			p2023-0636-1*	IS + QC_1 blood start
B	IS + Cal. 2				p2023-0607-1	IS + Cal. 7
C	IS + Cal. 3				p2023-0590-1*	IS + Cal. 6
D	IS + Cal. 4			p2023-0636-1	m2023-0694-4	IS + Cal. 5
E	IS + Cal. 5			p2023-0590-1	m2023-0638-1	IS + Cal. 4
F	IS + Cal. 6			p2023-0715-1	m2023-0635-1	IS + Cal. 3
G	IS + Cal. 7			p2023-0669-1	negative blood	IS + Cal. 2
H	IS + QC_1			p2023-0668-1	IS + QC_1 blood end	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Samples moved during analytical step 6 due to blood clot

SC

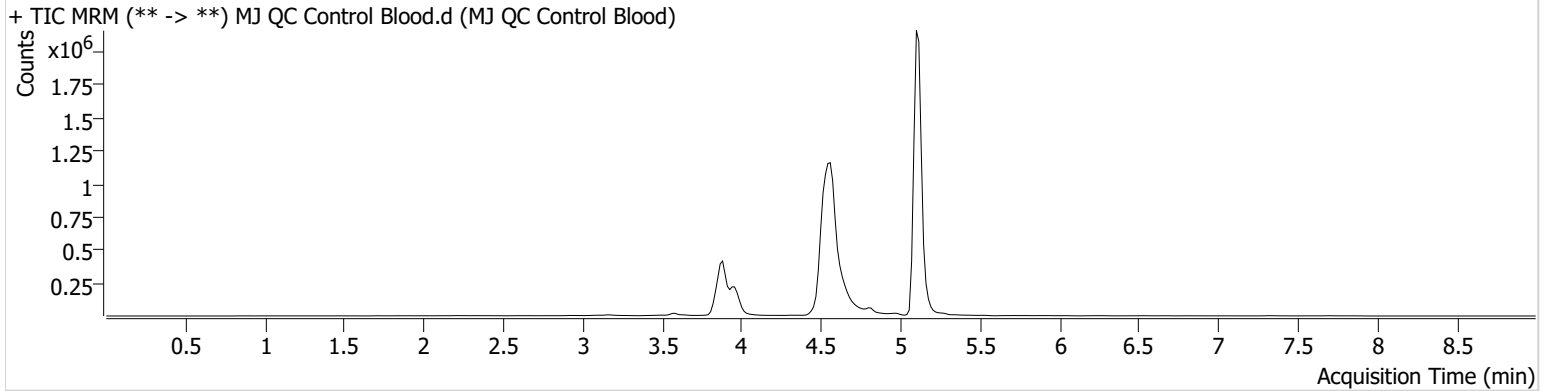


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-A6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 12:47:46 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	110933	∞	12.8	∞	1618674	4.9869 ng/ml
THC-COOH	3.969	45821	1241.94	244.5	412.80	545595	14.5173 ng/ml
THC	5.120	307511	3244.90	23.4	232.30	6892438	5.1489 ng/ml

SC

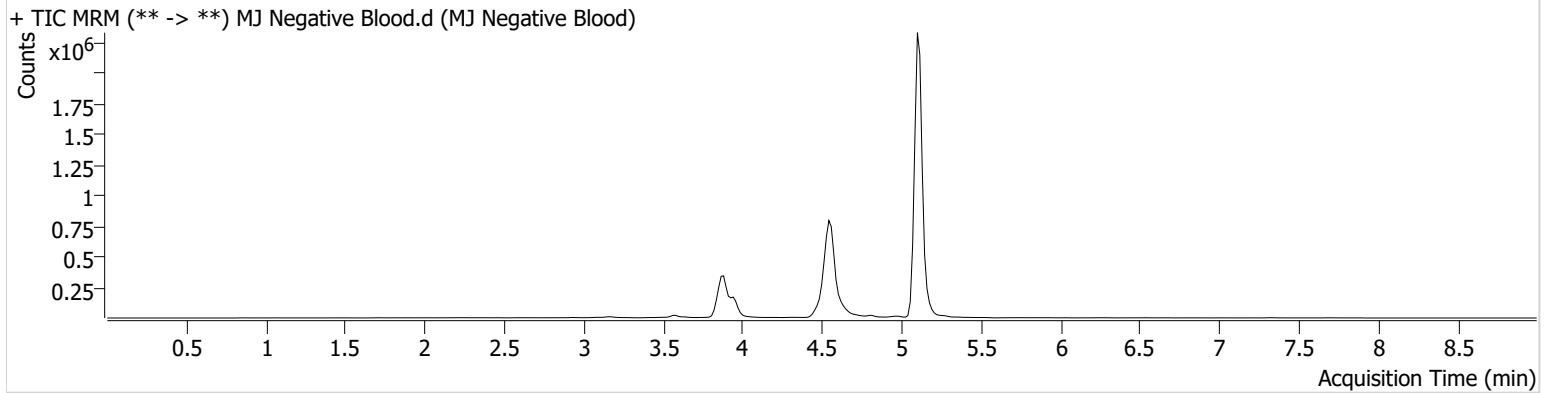


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-G5	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 1:13:58 PM		
Sample Info.			

Sample Chromatogram



SC

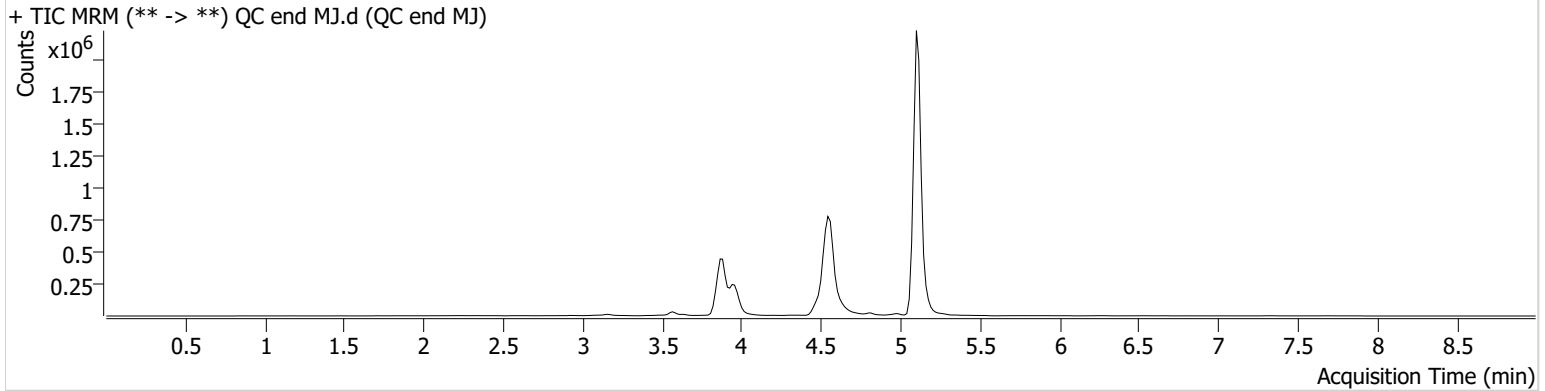


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 AM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Sarah Collins
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 5:35:56 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	111316	836.55	14.2	∞	1714519	4.7458 ng/ml
THC-COOH	3.969	48521	486.55	243.4	279.89	577891	14.5139 ng/ml
THC	5.120	290460	1186.41	22.8	271.31	6901663	4.8699 ng/ml

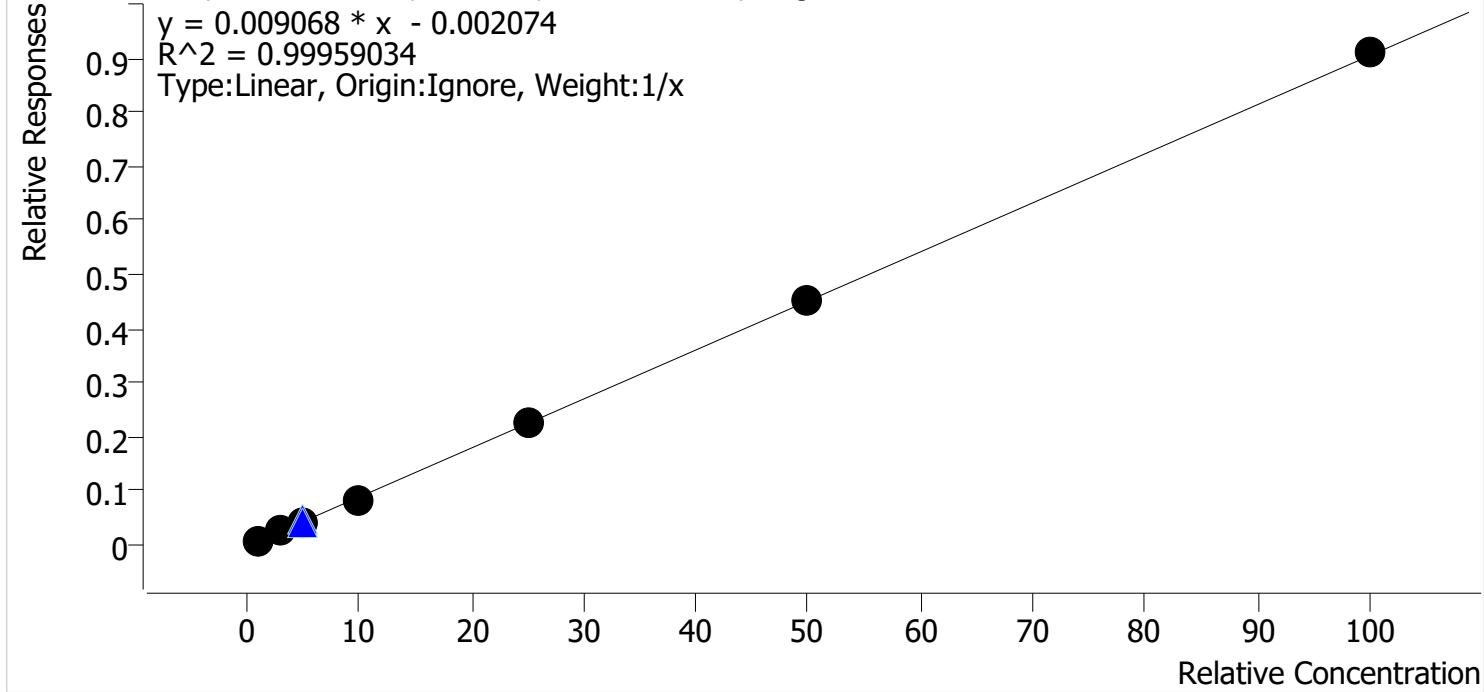
8c



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Last Cal. Update 3/31/2023 10:54 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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



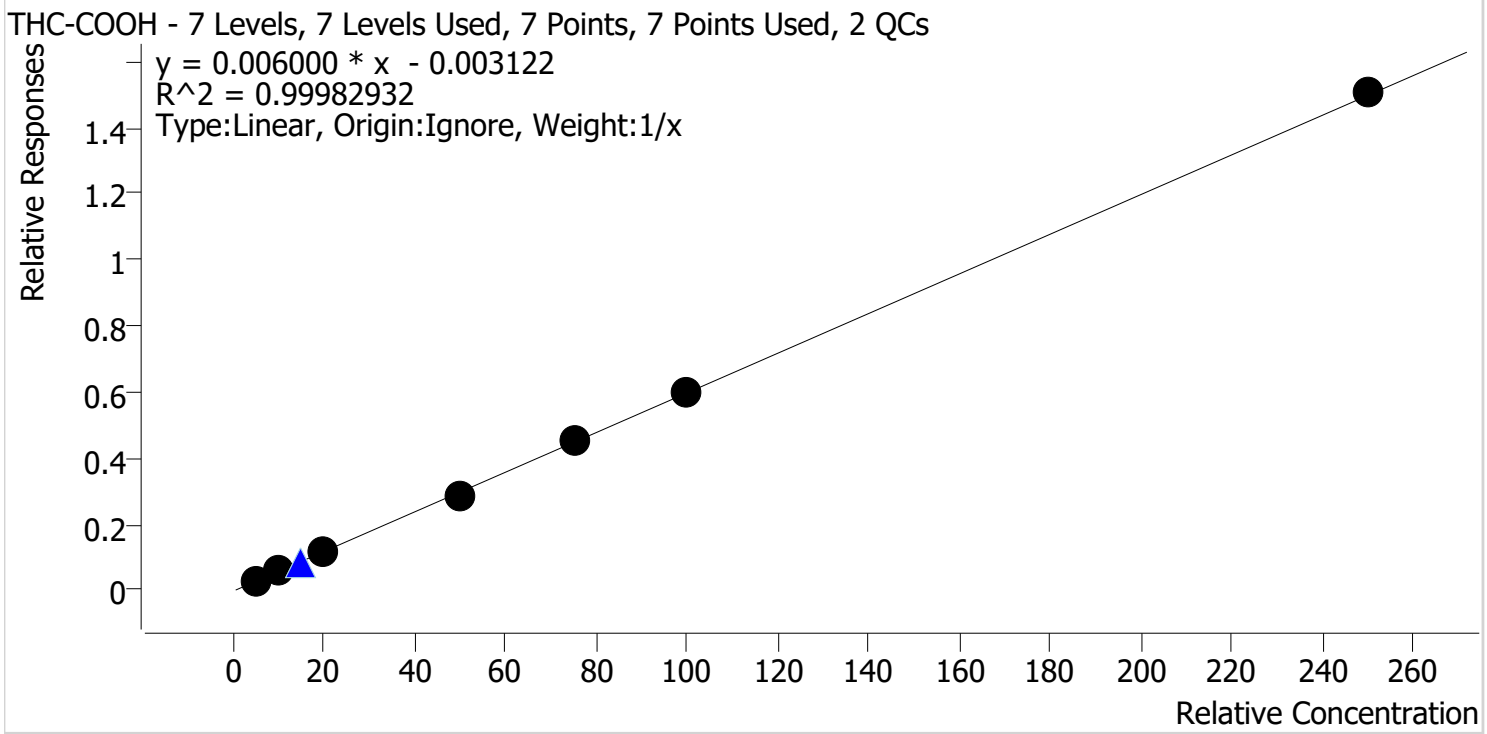
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	110.7
Cal 2 MJ	2	✓	3.0	3.0	100.3
Cal 3 MJ	3	✓	5.0	4.7	94.1
Cal 4 MJ	4	✓	10.0	9.4	94.1
Cal 5 MJ	5	✓	25.0	24.9	99.7
Cal 6 MJ	6	✓	50.0	50.3	100.5
Cal 7 MJ	7	✓	100.0	100.6	100.6

8c



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Last Cal. Update 3/31/2023 10:54 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



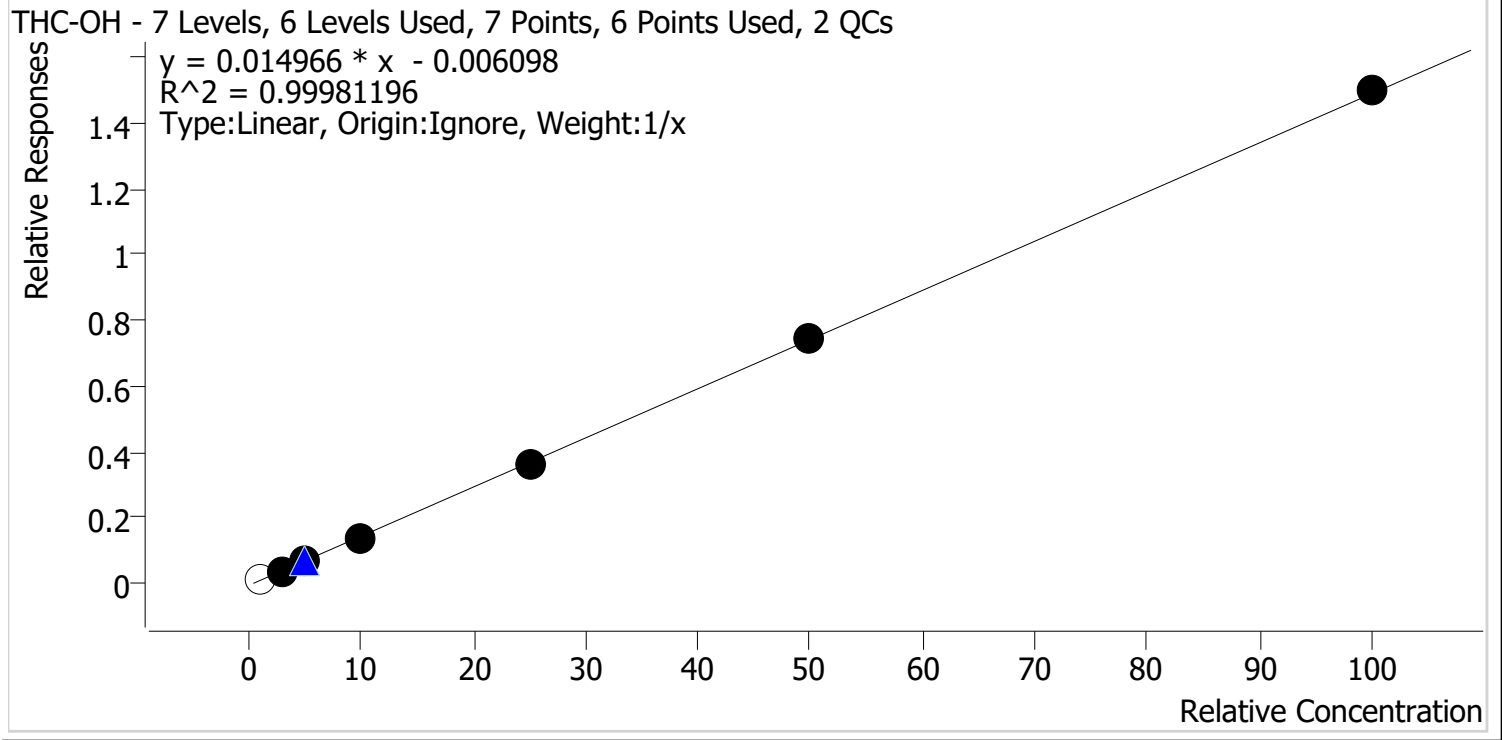
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.3	105.0
Cal 2 MJ	2	✓	10.0	9.8	97.8
Cal 3 MJ	3	✓	20.0	19.8	98.9
Cal 4 MJ	4	✓	50.0	48.7	97.5
Cal 5 MJ	5	✓	75.0	75.6	100.8
Cal 6 MJ	6	✓	100.0	99.5	99.5
Cal 7 MJ	7	✓	250.0	251.3	100.5

8c



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Last Cal. Update 3/31/2023 10:54 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	1.4	135.9
Cal 2 MJ	2	✓	3.0	3.1	101.8
Cal 3 MJ	3	✓	5.0	5.1	101.8
Cal 4 MJ	4	✓	10.0	9.8	98.2
Cal 5 MJ	5	✓	25.0	24.3	97.4
Cal 6 MJ	6	✓	50.0	50.1	100.2
Cal 7 MJ	7	✓	100.0	100.6	100.6

SC

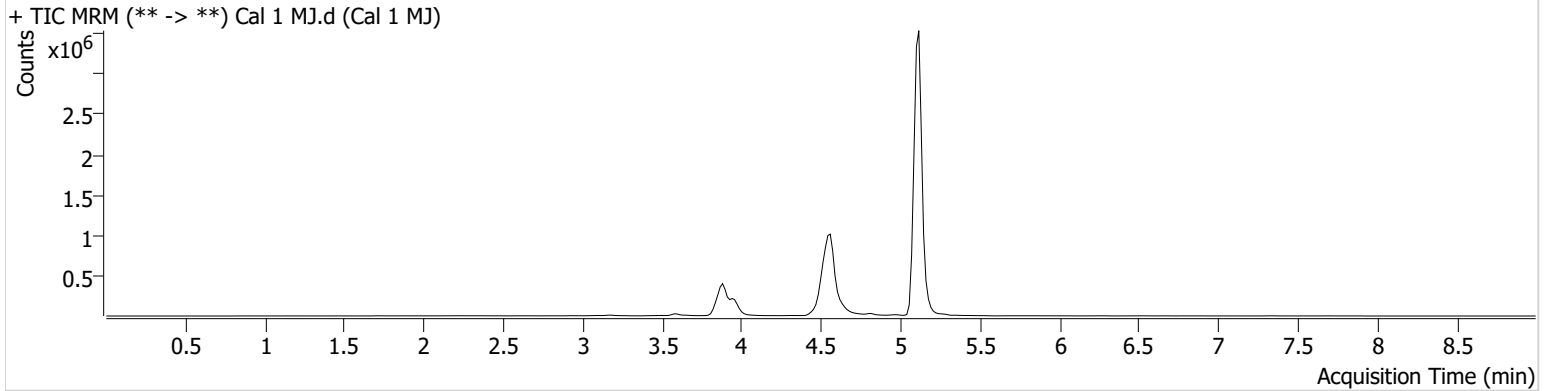


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-H6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 11:02:50 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	23582	67.01	11.1	34.06	1656805	1.3585 ng/ml Low
THC-COOH	3.969	18018	236.86	244.2	242.98	634838	5.2507 ng/ml
THC	5.120	96400	312.92	24.7	∞	12109037	1.1066 ng/ml

SC

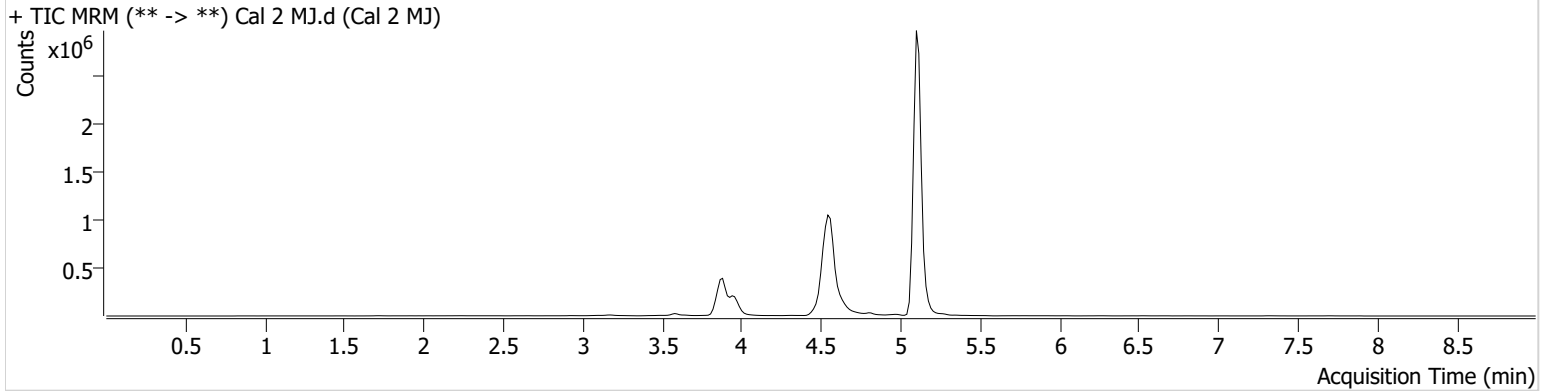


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-G6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 11:16:06 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	61551	∞	13.9	∞	1554417	3.0534 ng/ml
THC-COOH	3.969	30460	195.48	248.2	293.30	548500	9.7759 ng/ml
THC	5.120	244585	1320.93	22.8	∞	9706312	3.0076 ng/ml

SC

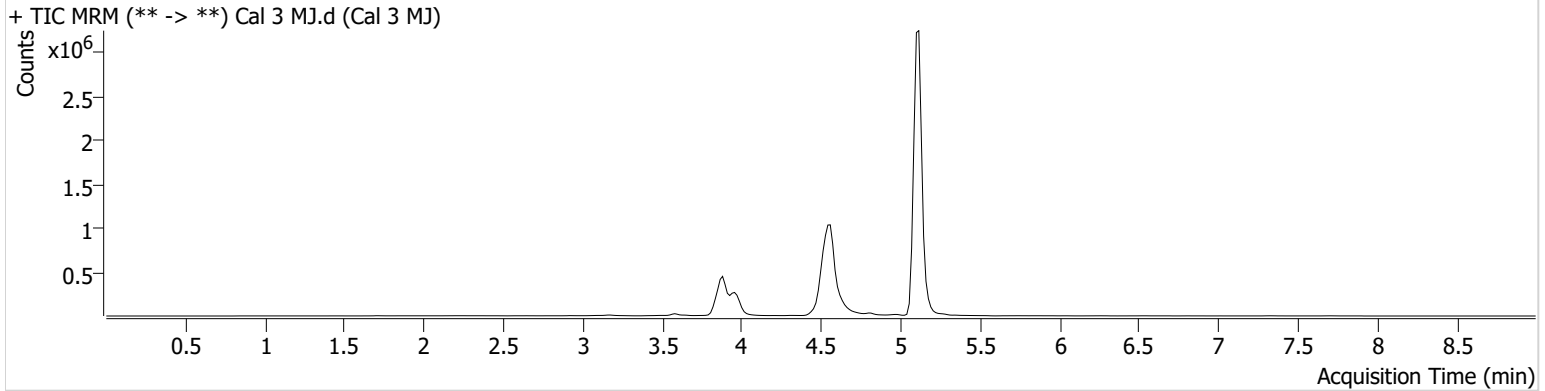


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-F6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 11:29:11 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	124867	∞	12.9	∞	1781142	5.0919 ng/ml
THC-COOH	3.969	69252	529.42	246.3	343.85	599048	19.7872 ng/ml
THC	5.120	448770	∞	22.7	∞	11052301	4.7065 ng/ml

SC



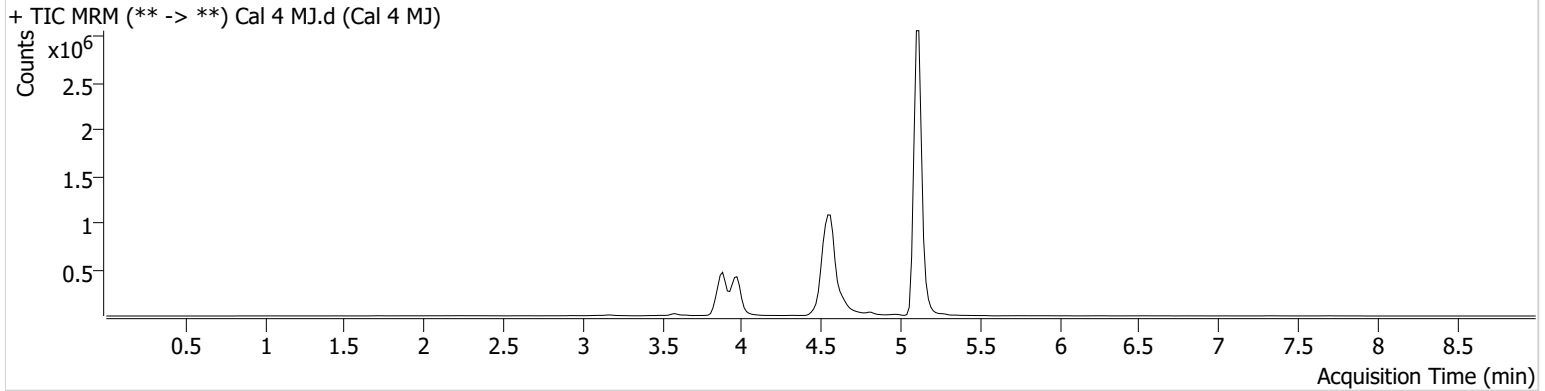
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-E6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 11:42:17 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	238315	∞	13.0	89.58	1691460	9.8220 ng/ml
THC-COOH	3.969	167229	516.68	245.9	1597.40	578173	48.7258 ng/ml
THC	5.120	808256	5081.00	21.2	∞	9703182	9.4148 ng/ml

SC

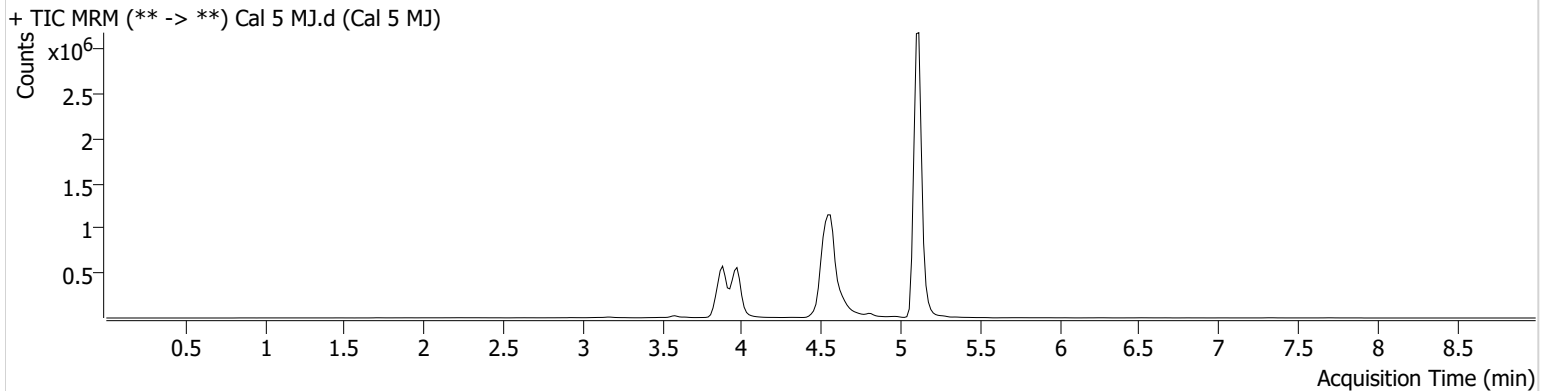


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-D6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 11:55:23 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	623390	∞	13.2	∞	1740186	24.3446 ng/ml
THC-COOH	3.969	248539	∞	243.4	950.57	551949	75.5681 ng/ml
THC	5.120	1945763	14548.74	23.0	∞	8687076	24.9298 ng/ml

SC

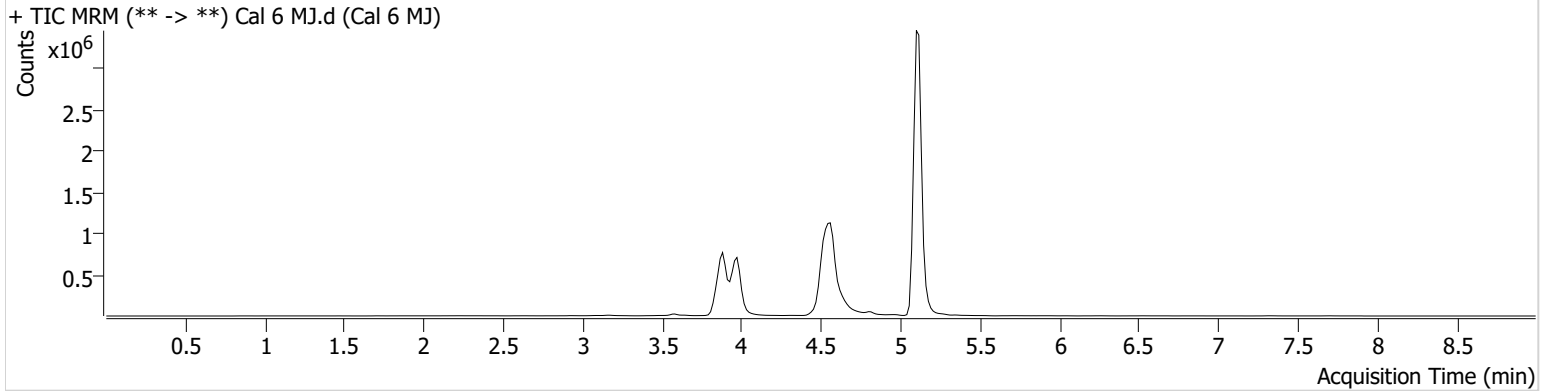


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-C6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 12:08:29 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	1311486	∞	13.2	∞	1763616	50.0972 ng/ml
THC-COOH	3.969	333677	1513.81	243.1	4112.92	561612	99.5426 ng/ml
THC	5.120	3515706	32249.61	23.6	1650.92	7750840	50.2509 ng/ml

SC

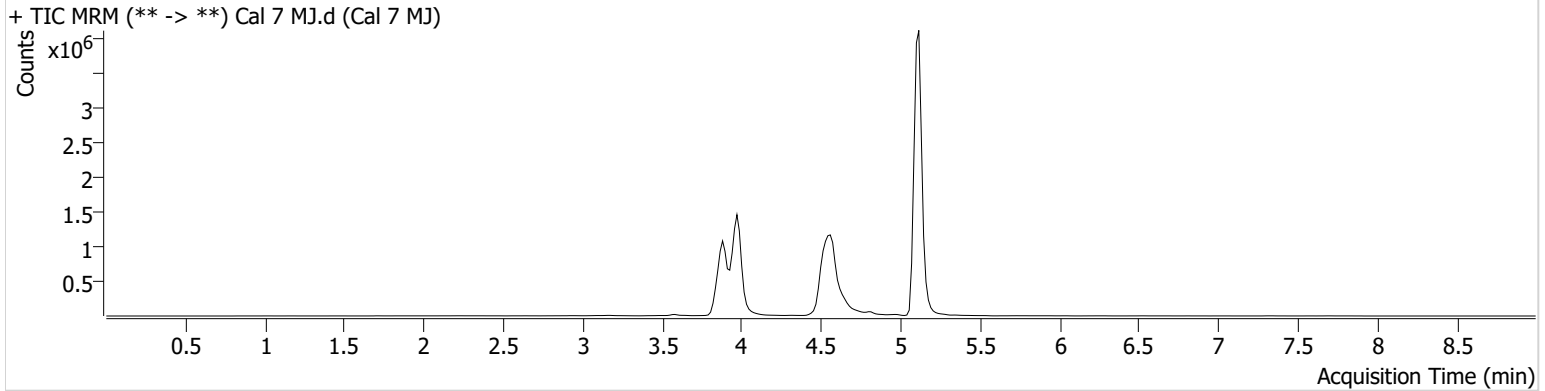


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\033023 AM 27 28 SC TS\QuantResults\AM 27 SC.batch.bin
Calibration Last Update 3/31/2023 10:54:58 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-B6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2023 12:21:34 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	2556717	∞	13.4	∞	1705272	100.5910 ng/ml
THC-COOH	3.969	753875	2262.27	243.8	2881.82	500915	251.3497 ng/ml
THC	5.120	6144084	∞	23.1	3125.66	6751767	100.5837 ng/ml