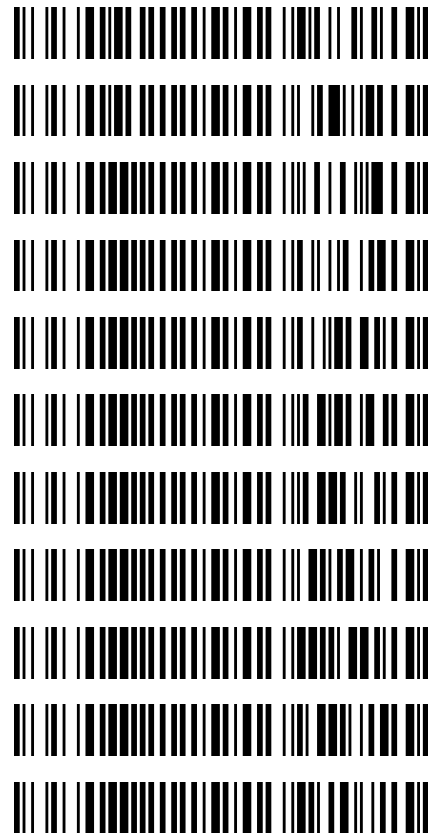


REVIEWED
By Tamara Salazar at 7:53 am, Jul 05, 2022

Worklist: 6012

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-2092	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-2207	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1398	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1467	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1476	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1503	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1506	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1590	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1593	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1598	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1648	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: 06/28/2022

Analyst: Celena Shrum

Plate lot#: 220309

Plate Retest Date: 09/09/2022

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 20L20723

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. Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: #42**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500µL 0.1% formic acid in water blood sample** of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
- 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. Case sample response for THC 1ng/mL and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- 5. Did all QCs pass for each analyte? (if not, describe in comments section)
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: The instrument stopped part way through the run due to a pressure issue. The issue was resolved, and the run was restarted with no further pressure issues. During the run, it appeared that the internal standard blank was running low, so the run was paused, the vial was refilled, and the run was restarted.

THC-OH not evaluated due to ratio issues with the calibrators.

	1	2	3	4	5	6
a	cal 1ng	QC 2	P2022-1506-1			
b	cal 3 ng	Blood NEG	P2022-1590-1			
c	cal 5 ng	M2022-2092-2	P2022-1593-1			
d	cal 10ng	M2022-2207-3	P2022-1598-1			
e	cal 25 ng	P2022-1398-1	P2022-1648-1			
f	cal 50 ng	P2022-1467-1				
g	cal 100 ng	P2022-1476-2				
h	QC 1	P2022-1503-1				

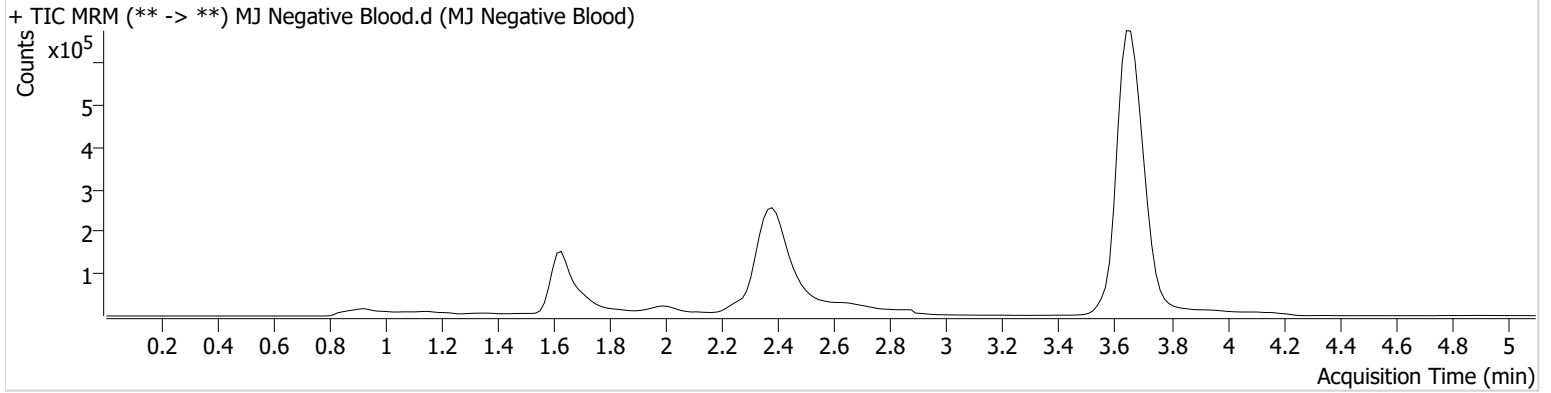


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-B2	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 2:13:06 PM		
Sample Info.			

Sample Chromatogram



CS

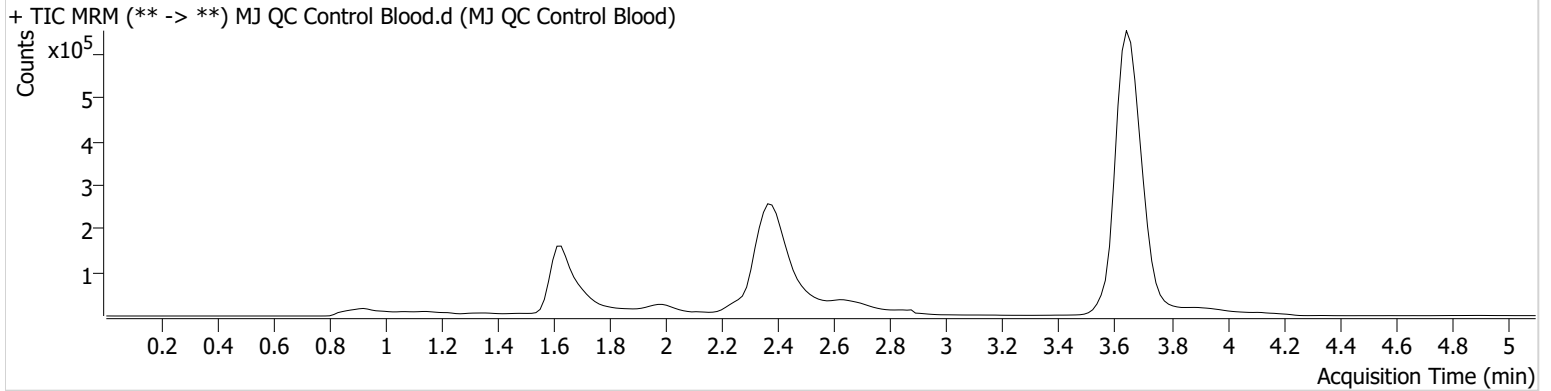


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 1:57:52 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	54320	∞	63.2	126.97	140751	19.5612 ng/ml
THC-OH	1.708	155566	∞	6.0	26.49	536441	2.3486 ng/ml Low *
THC	3.661	164876	∞	29.7	∞	3848689	4.7541 ng/ml

*THC-OH not evaluated

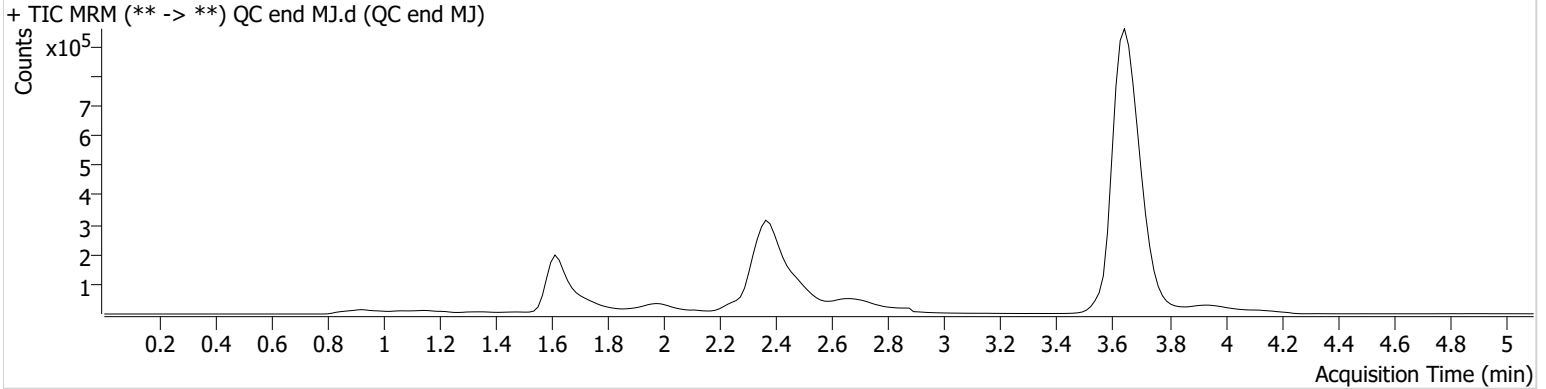


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument Falco (069901) **Data File** QC end MJ.d
Type QC **Sample** QC end MJ
Acq. Method AM 27 THCQ.m **Operator** Celena Shrum
Sample Position P1-A2 **Comment**
Injection Volume 10
Acq. Date-Time 6/29/2022 9:33:13 AM
Sample Info.

Sample Chromatogram



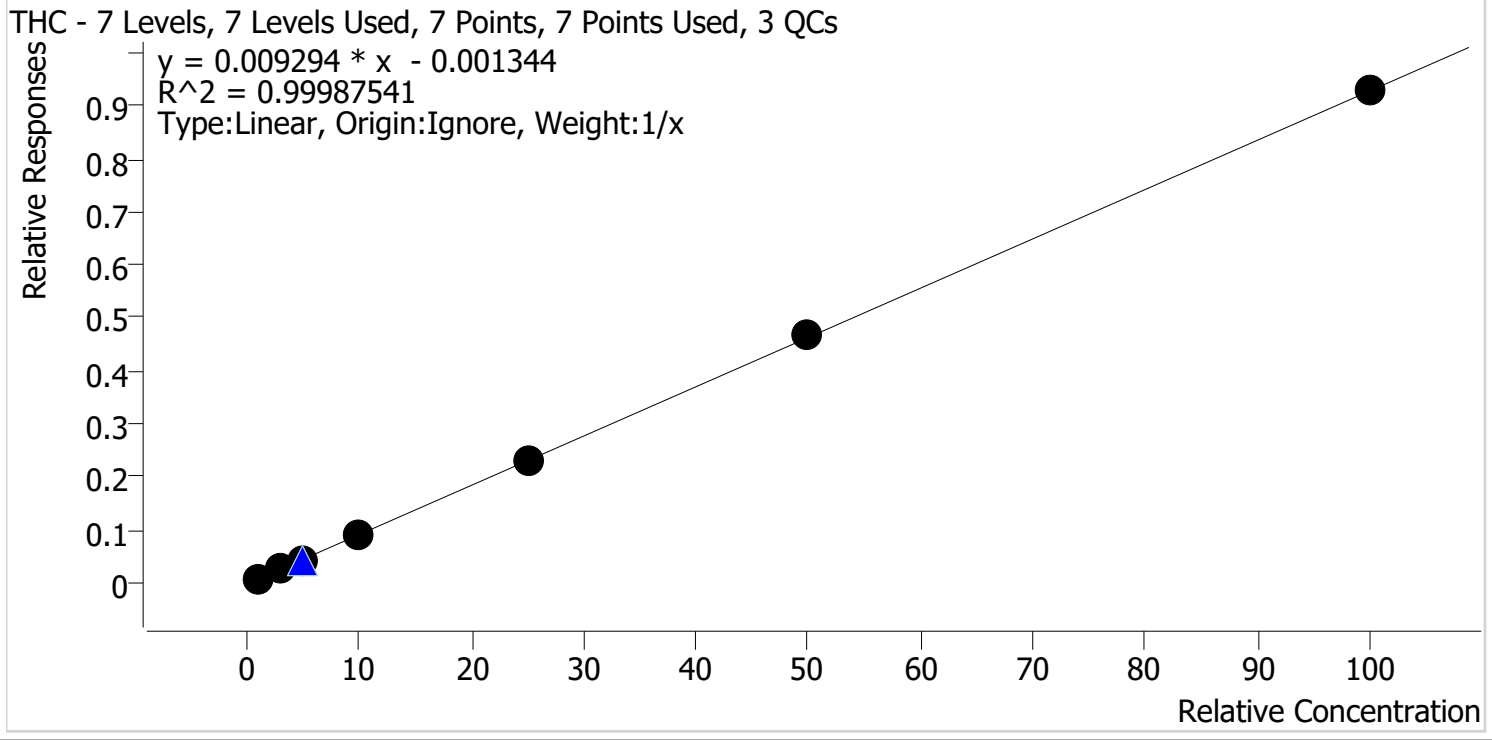
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	48110	∞	54.1	∞	172775	15.0633 ng/ml
THC-OH	1.693	162016	∞	6.2	85.05	627134	0.4913 ng/ml Low *
THC	3.661	258145	∞	29.1	∞	5963738	4.8022 ng/ml

*THC-OH not evaluated



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/1/2022 11:37 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

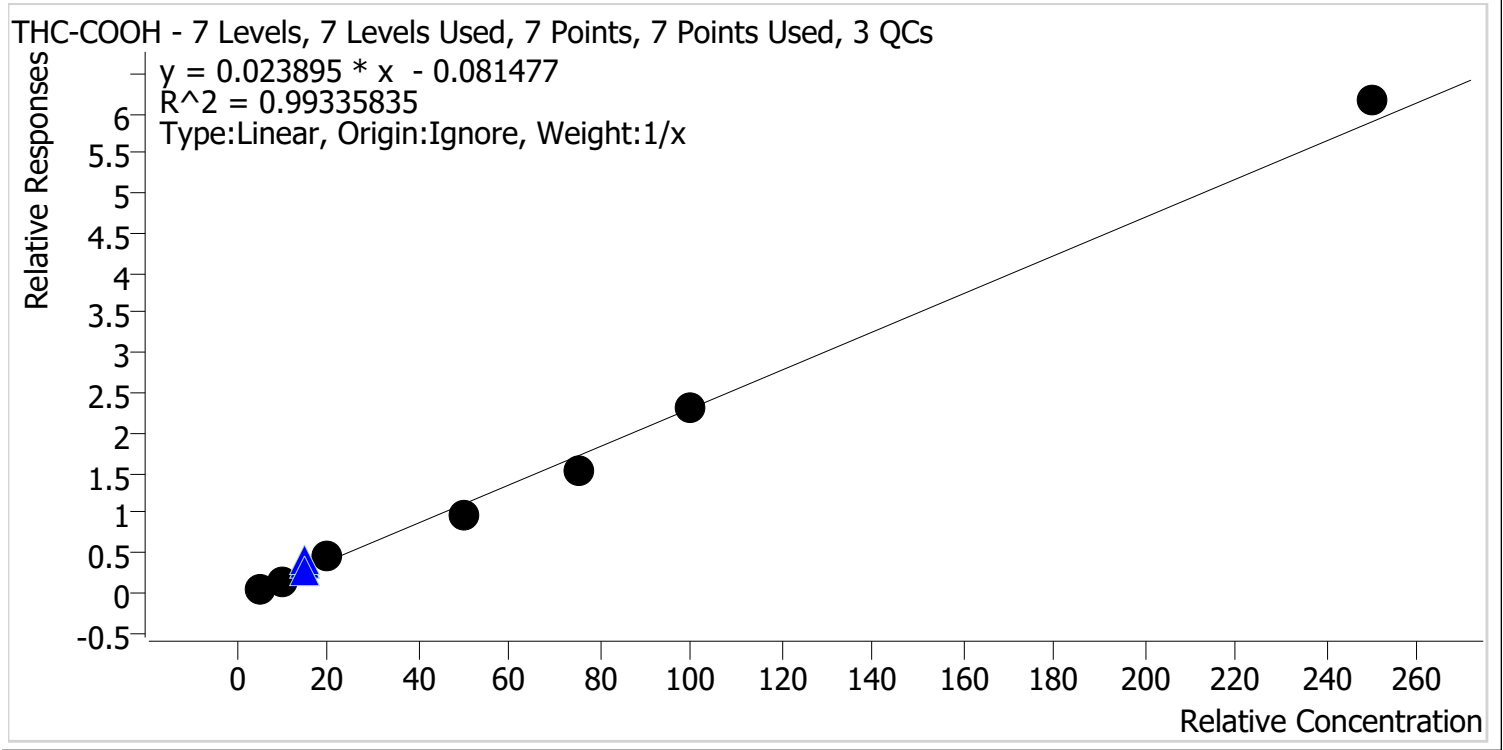


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	106.4
Cal 2 MJ	2	✓	3.0	2.9	97.8
Cal 3 MJ	3	✓	5.0	4.9	98.2
Cal 4 MJ	4	✓	10.0	9.7	97.3
Cal 5 MJ	5	✓	25.0	24.8	99.4
Cal 6 MJ	6	✓	50.0	50.5	101.0
Cal 7 MJ	7	✓	100.0	100.0	100.0



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/1/2022 11:37 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

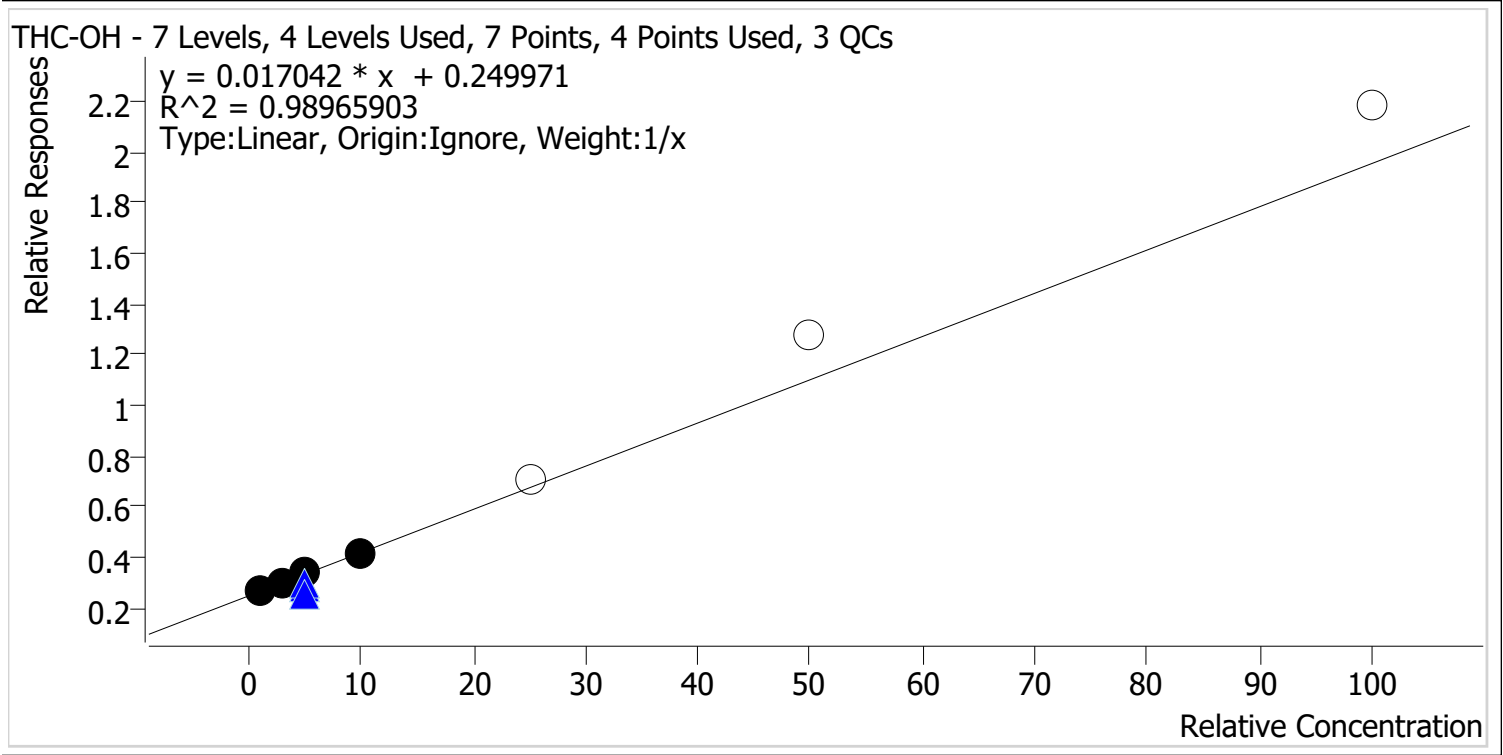


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.5	110.5
Cal 2 MJ	2	✓	10.0	9.2	92.4
Cal 3 MJ	3	✓	20.0	22.9	114.7
Cal 4 MJ	4	✓	50.0	44.6	89.2
Cal 5 MJ	5	✓	75.0	66.5	88.6
Cal 6 MJ	6	✓	100.0	100.2	100.2
Cal 7 MJ	7	✓	250.0	261.1	104.4



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/1/2022 11:37 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	✓	1.0	1.1	107.1
Cal 2 MJ	2	✓	3.0	2.6	85.5
Cal 3 MJ	3	✓	5.0	5.4	107.5
Cal 4 MJ	4	✓	10.0	10.0	99.9
Cal 5 MJ	5	✗	25.0	27.3	109.3
Cal 6 MJ	6	✗	50.0	60.0	120.0
Cal 7 MJ	7	✗	100.0	113.4	113.4

THC-OH not evaluated

CS

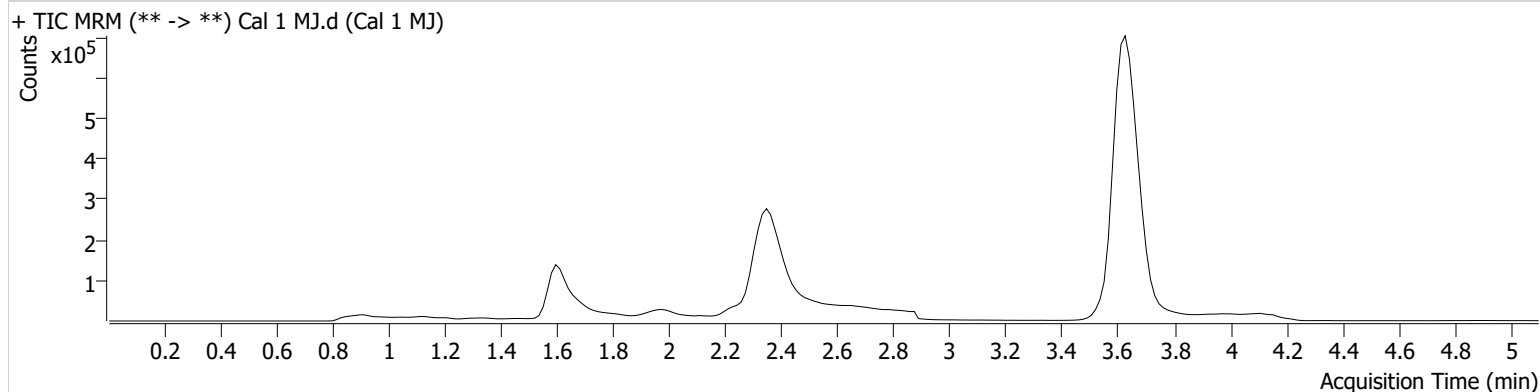


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 12:56:52 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	6462	∞	68.3	25.38	127811	5.5258 ng/ml
THC-OH	1.678	113386	∞	4.6	34.27	422729	1.0710 ng/ml Low
THC	3.631	36320	202.51	31.8	∞	4251325	1.0638 ng/ml

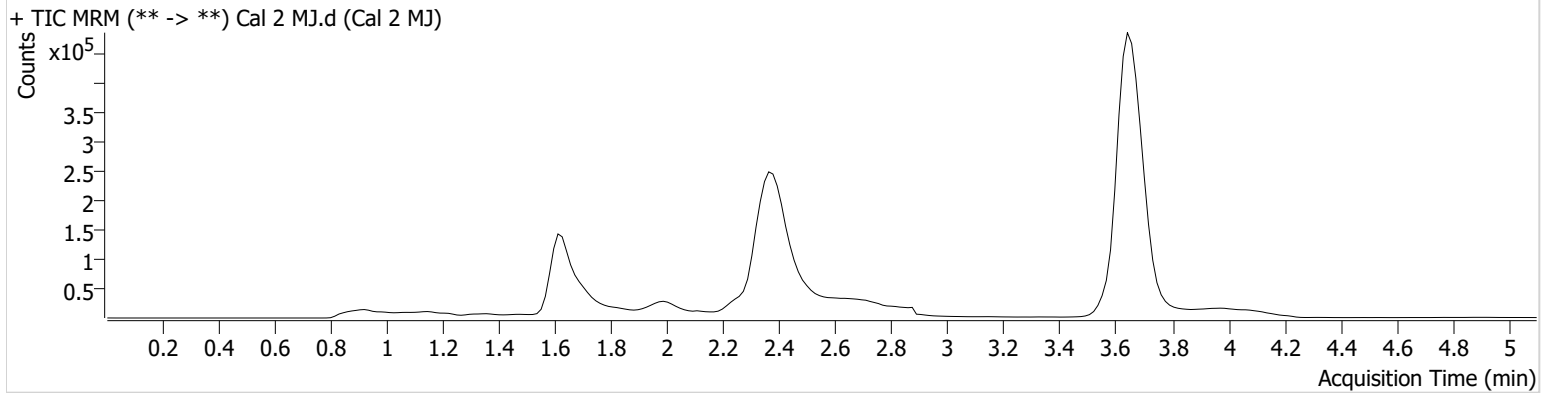
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-B1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 1:04:38 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	18281	∞	68.2	∞	131181	9.2420 ng/ml
THC-OH	1.693	133997	∞	5.0	26.64	456252	2.5653 ng/ml Low
THC	3.661	75953	∞	29.7	75.36	2929561	2.9343 ng/ml

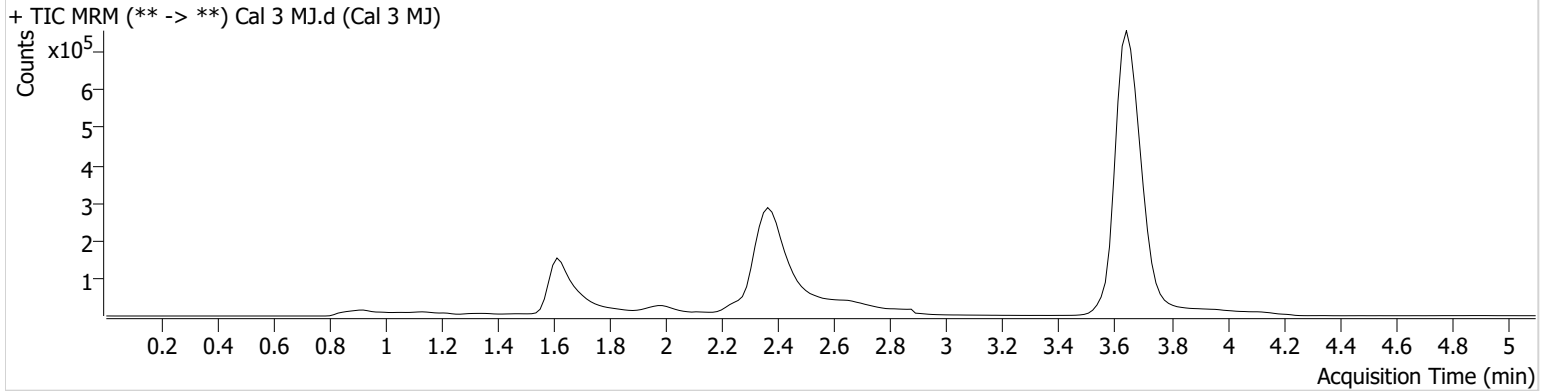
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 1:12:16 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	60022	∞	55.9	149.40	128677	22.9310 ng/ml
THC-OH	1.693	153564	∞	5.9	87.32	449579	5.3750 ng/ml
THC	3.661	195416	∞	28.2	∞	4414342	4.9079 ng/ml

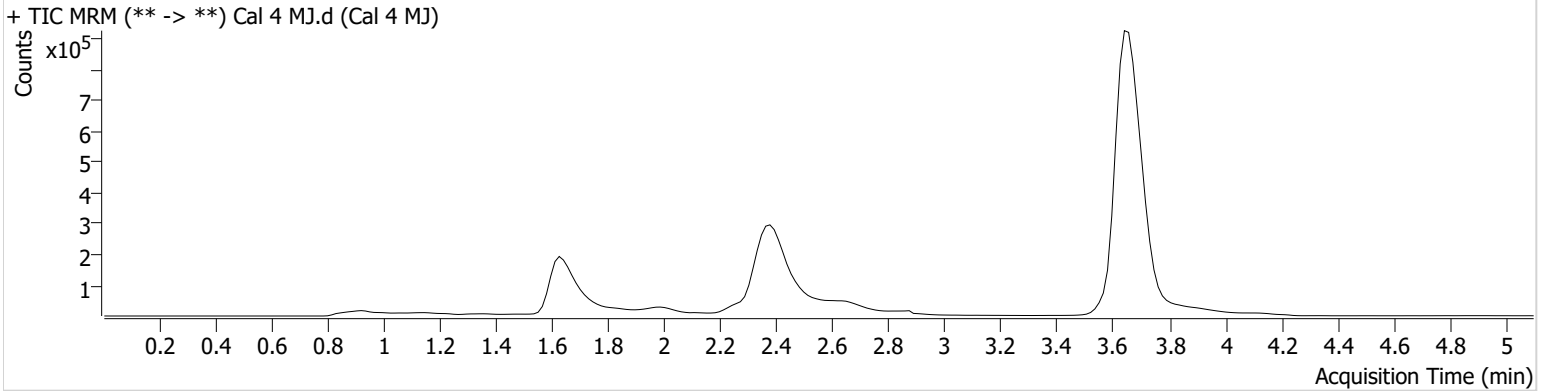
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 1:19:52 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	140913	∞	58.5	1025.30	143222	44.5853 ng/ml
THC-OH	1.708	210325	∞	6.4	42.12	500537	9.9886 ng/ml
THC	3.661	466746	∞	28.1	∞	5241604	9.7260 ng/ml

CS

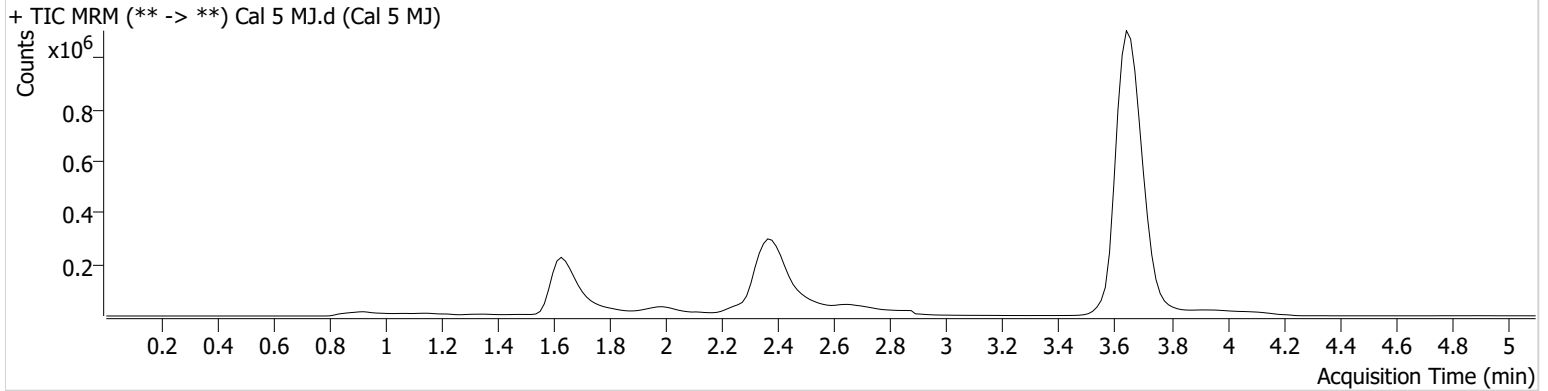


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 1:27:27 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	204526	∞	57.4	1148.55	135701	66.4861 ng/ml
THC-OH	1.618	342182	∞	10.0 High	∞	478256	27.3151 ng/ml
THC	3.661	1259076	∞	27.3	∞	5485390	24.8424 ng/ml

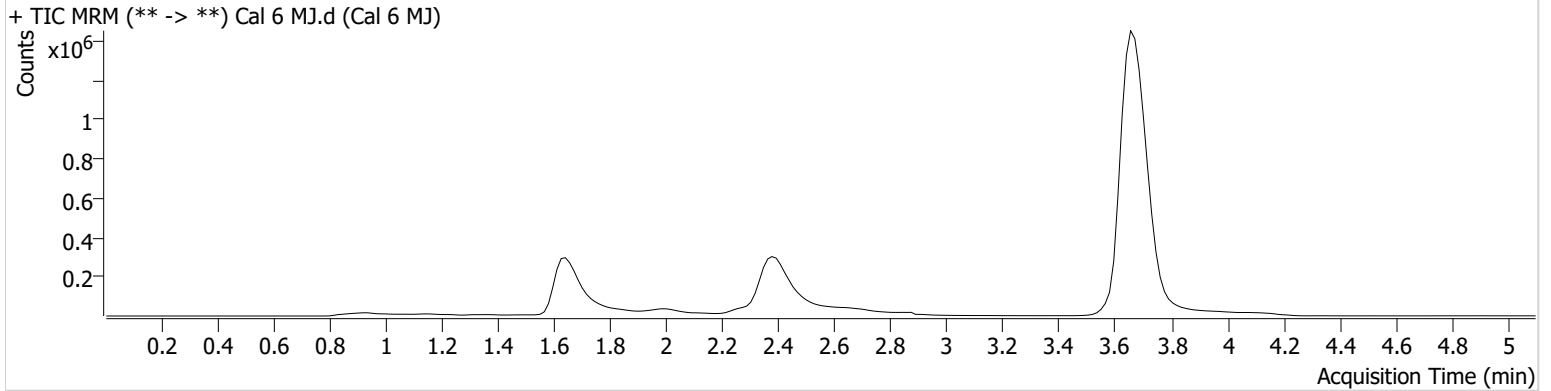
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 1:35:03 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.670	331175	∞	61.2	∞	143250	100.1626 ng/ml
THC-OH	1.633	604343	∞	11.5 High	∞	474986	59.9904 ng/ml
THC	3.676	2710287	∞	27.8	∞	5792164	50.4933 ng/ml

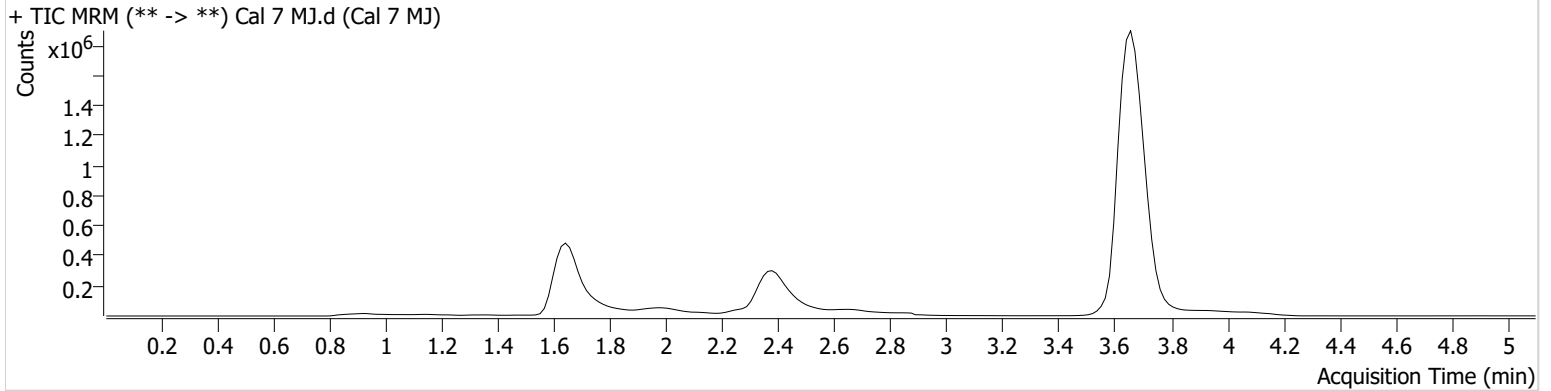
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\062822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/1/2022 11:37:10 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	6/28/2022 1:42:39 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	820521	∞	56.3	∞	133274	261.0672 ng/ml
THC-OH	1.633	1055737	∞	12.3 High	∞	483837	113.3680 ng/ml
THC	3.661	5212927	∞	27.8	∞	5615433	100.0323 ng/ml