

REVIEWED

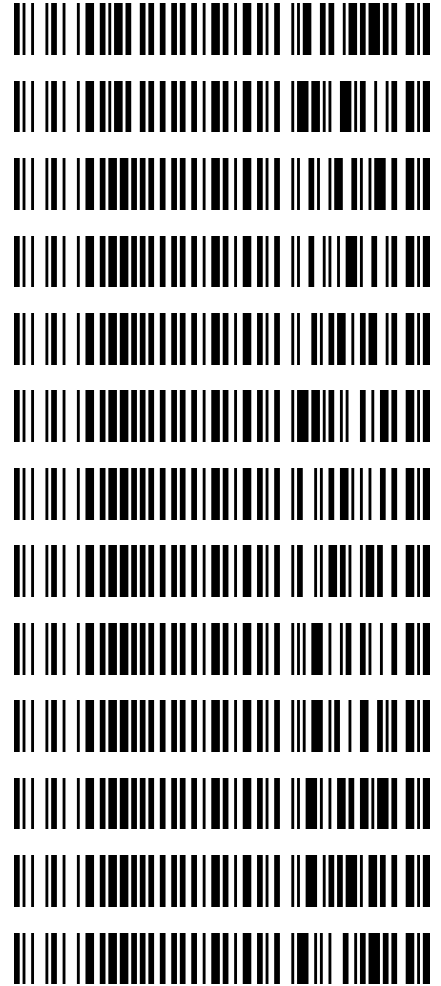
By Anne Nord at 12:58 pm, Feb 10, 2022

SC

2/9/2022

Worklist: 5580

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-0072	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-0342	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-4253	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-4261	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0242	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0273	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0277	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0284	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0308	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0309	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0320	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0329	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0334	1	BCK	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 02/08/2022
Plate lot#: IDP-108-3-211018
Mobile phase A: 0.1% Formic Acid in LCMS Water
Blank Blood Lot: Lampire 20L20725
LCMS-QQQ ID: 069901

Analyst: Sarah Collins
Retest Date: 04/18/2022
Mobile phase B: 0.1% Formic acid in Acetonitrile
Column: UCT Selectra DA 100 x 2.1mm 3um

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. Pipette **500µL 0.1% formic acid in water blood sample, 500 µL saturated phosphate buffer in urine** in wells of 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 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: Did not evaluate THC-OH due to interfering peak.

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	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1		p2022-0242-1	p2022-0273-1	IS + QC_1 blood
B	IS + Cal. 2			p2022-0334-1	p2022-0242-1*	IS + Cal. 7
C	IS + Cal. 3			p2022-0329-1	p2021-4261-1	IS + Cal. 6
D	IS + Cal. 4			p2022-0320-1	p2021-4253-2	IS + Cal. 5
E	IS + Cal. 5			p2022-0309-1*	m2022-0342-3	IS + Cal. 4
F	IS + Cal. 6			p2022-0308-1	m2022-0072-1	IS + Cal. 3
G	IS + Cal. 7			p2022-0284-1	negative blood	IS + Cal. 2
H	IS + QC_1		p2022-0309-1	p2022-0277-1	IS + QC_1 urine	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Samples moved during analytical step 6 due to blood clot

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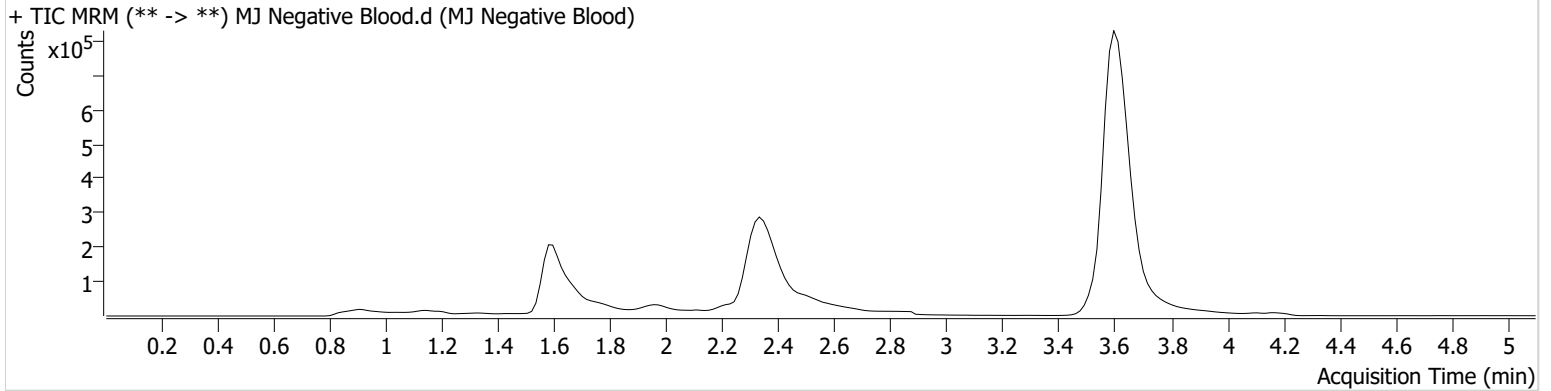


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-G5	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 3:24:51 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.663 High	205778	∞	2.0 Low	12.15	860555	2.8436 ng/ml Low

*Compound not evaluated in this batch

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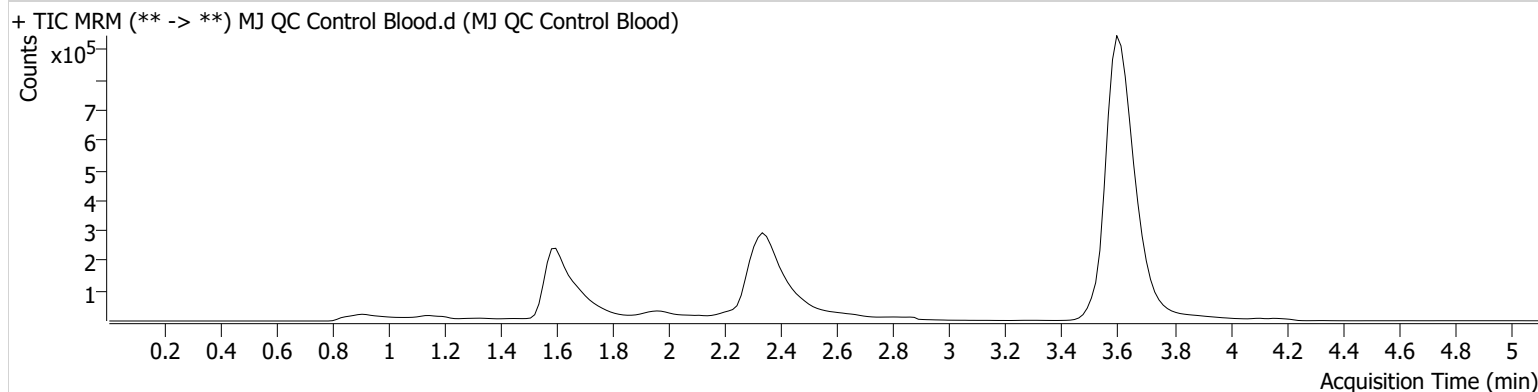
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 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	Sarah Collins
Sample Position	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 3:09:38 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663 High	264624	771.90	5.9 Low	65.43	966965	5.1071 ng/ml
THC-COOH	1.625	89531	∞	54.3	137.57	257860	16.2105 ng/ml
THC	3.616	271717	1354.33	25.2	251.45	6319055	4.6375 ng/ml

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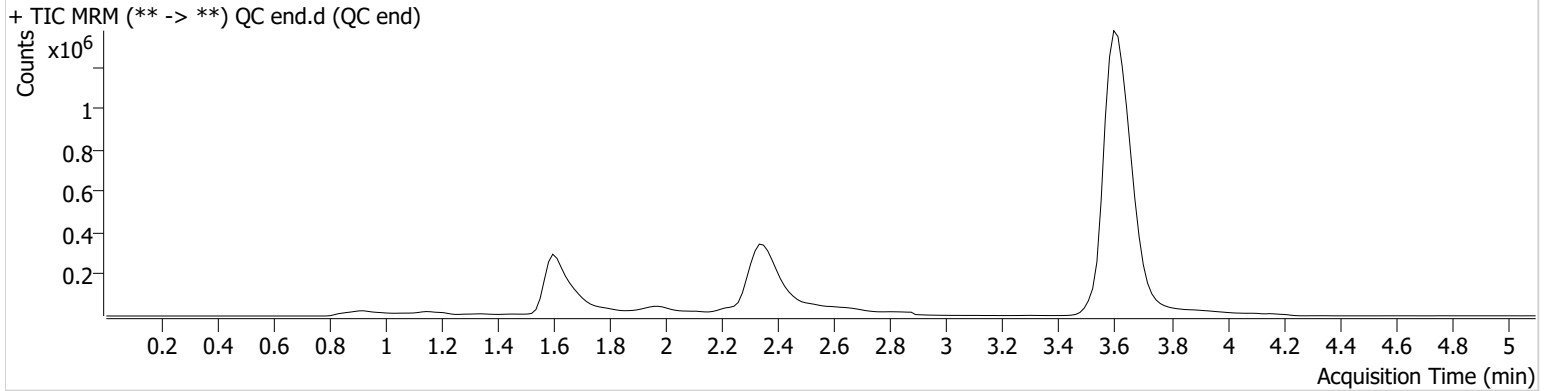


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	QC end.d
Type	QC	Sample	QC end
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 6:58:13 PM		

Sample Chromatogram



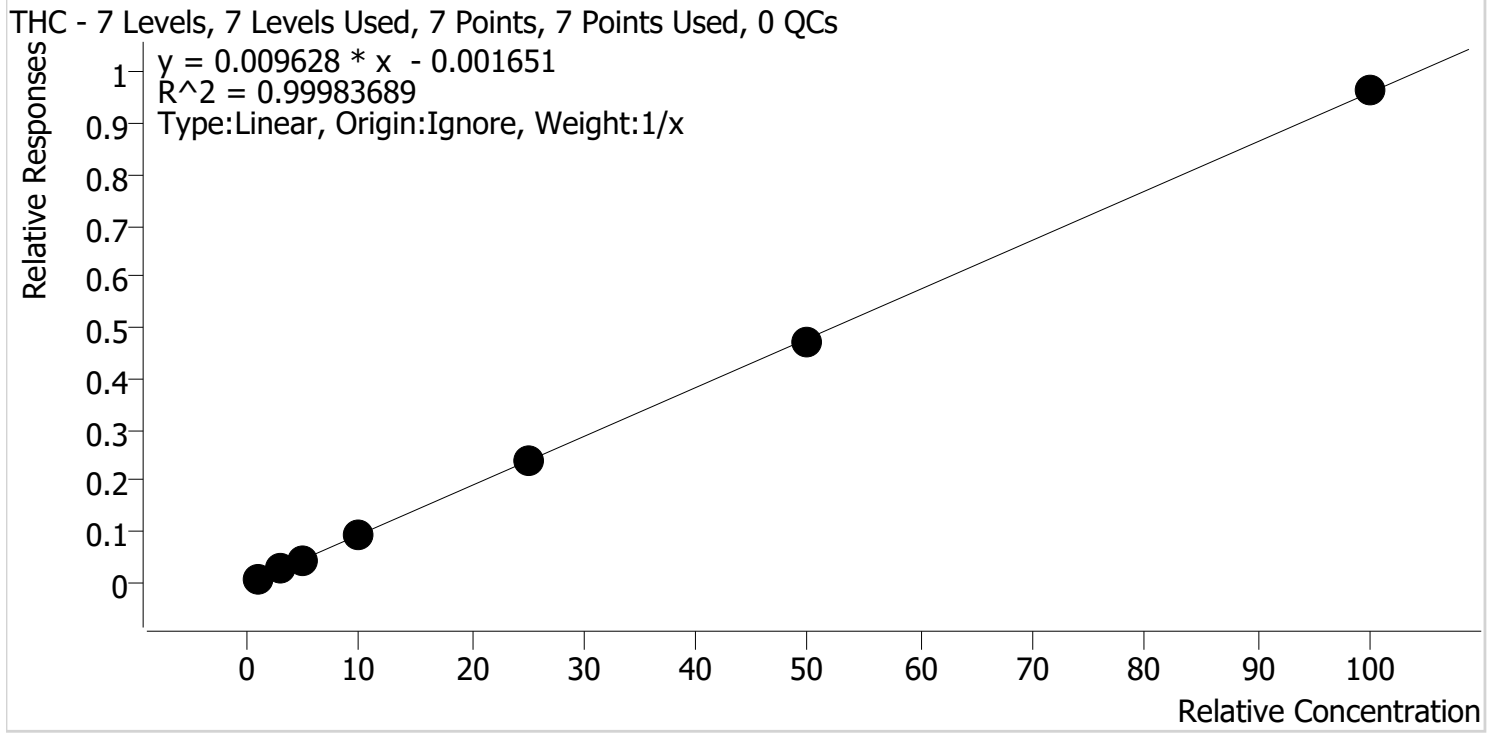
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678 High	265179	181.02	6.7 Low	∞	1045067	3.8017 ng/ml
THC-COOH	1.640	90274	∞	71.7	∞	284847	14.7183 ng/ml
THC	3.616	384498	2470.50	25.8	∞	8811559	4.7036 ng/ml

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 2/9/2022 8:30 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



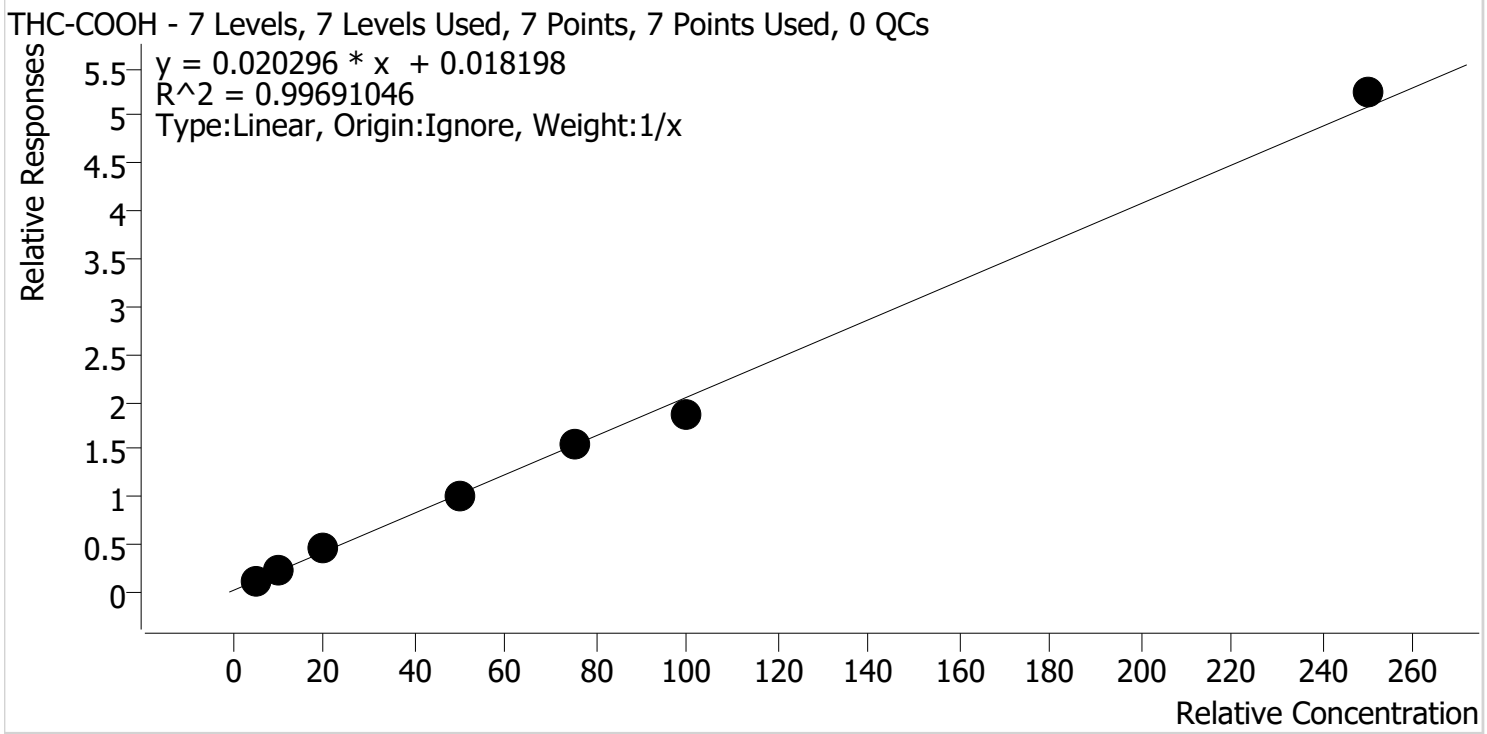
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.1	106.2
MJ Cal 2	2	✓	3.0	2.9	95.8
MJ Cal 3	3	✓	5.0	4.8	96.0
MJ Cal 4	4	✓	10.0	10.2	102.2
MJ Cal 5	5	✓	25.0	25.1	100.2
MJ Cal 6	6	✓	50.0	49.6	99.2
MJ Cal 7	7	✓	100.0	100.4	100.4

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 2/9/2022 8:30 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



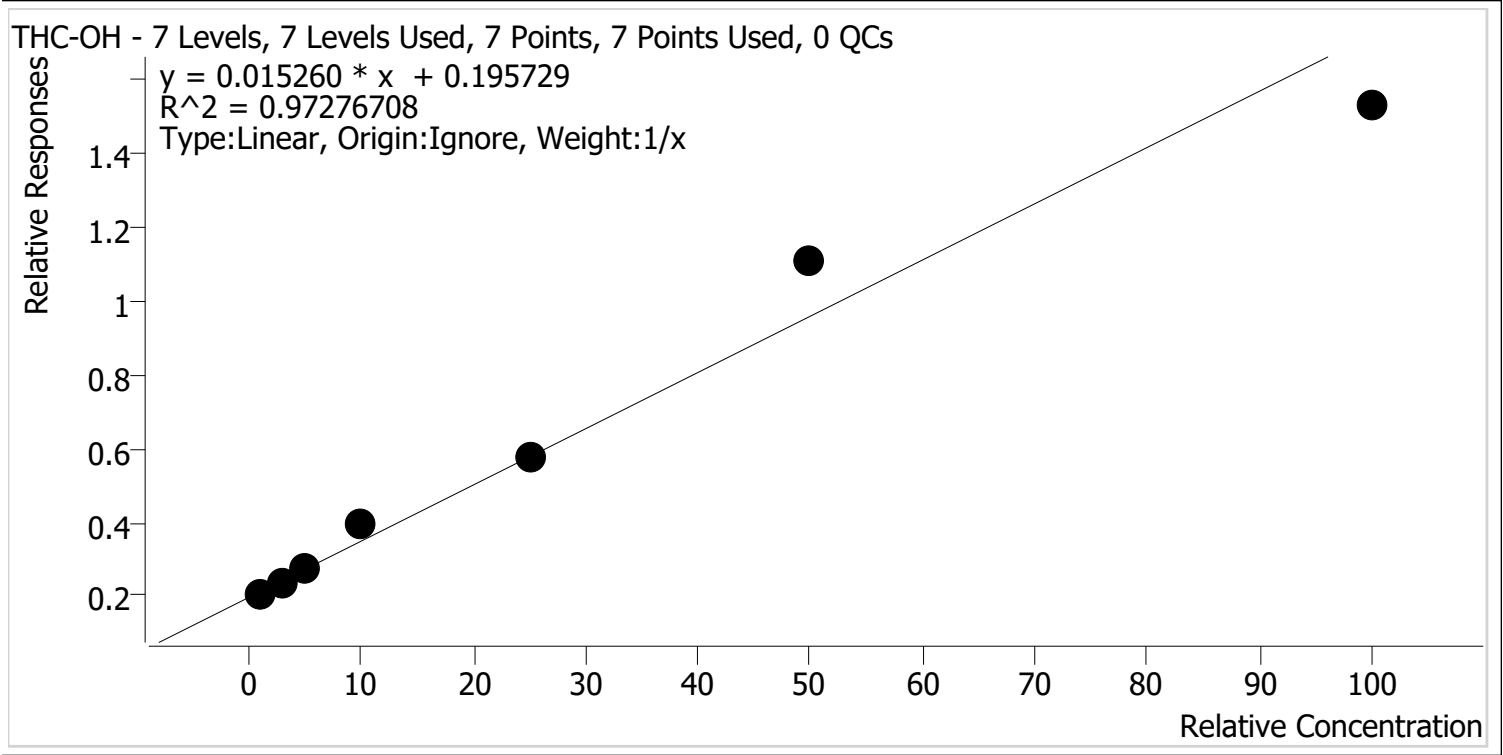
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	5.0	5.0	100.2
MJ Cal 2	2	✓	10.0	9.7	97.4
MJ Cal 3	3	✓	20.0	21.9	109.5
MJ Cal 4	4	✓	50.0	48.7	97.5
MJ Cal 5	5	✓	75.0	75.9	101.2
MJ Cal 6	6	✓	100.0	91.3	91.3
MJ Cal 7	7	✓	250.0	257.4	102.9

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 2/9/2022 8:30 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	0.7	66.6
MJ Cal 2	2	✓	3.0	2.6	87.3
MJ Cal 3	3	✓	5.0	5.3	106.6
MJ Cal 4	4	✓	10.0	13.2	132.2
MJ Cal 5	5	✓	25.0	25.2	100.9
MJ Cal 6	6	✓	50.0	59.5	119.1
MJ Cal 7	7	✓	100.0	87.4	87.4

THC-OH not evaluated due to interfering peak.

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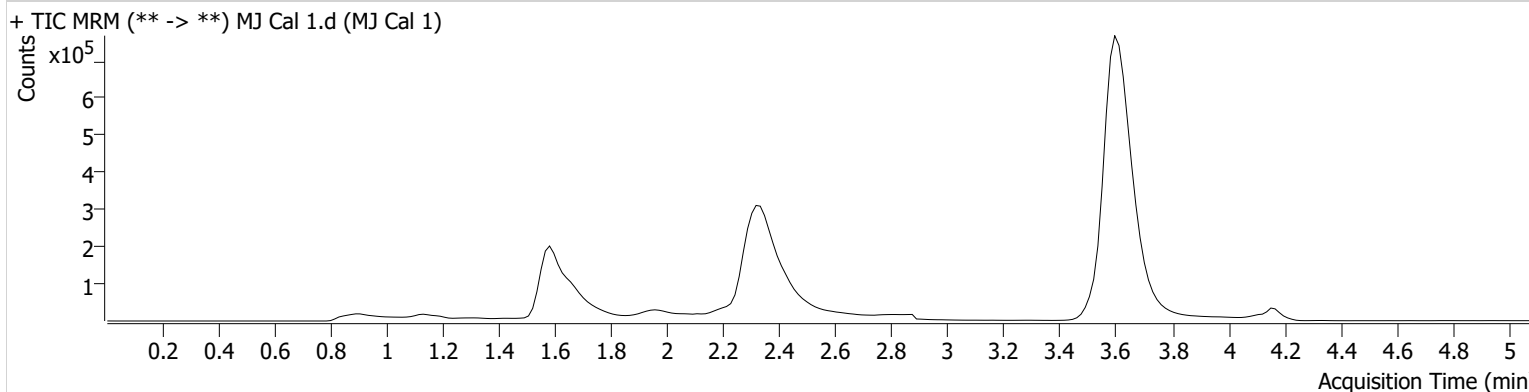


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Cal 1.d
Type	Cal	Sample	MJ Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-H6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 2:08:34 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663 High	184879	110.15	4.1 Low	26.45	897944	0.6659 ng/ml Low
THC-COOH	1.625	28470	∞	53.1	70.29	237558	5.0083 ng/ml
THC	3.616	45879	∞	29.4	64.72	5353059	1.0616 ng/ml

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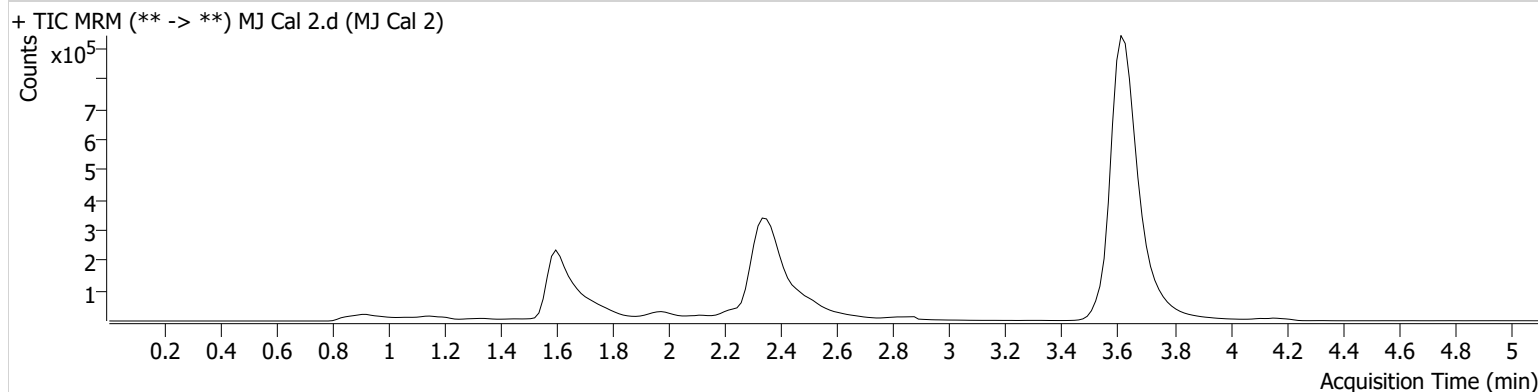
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Cal 2.d
Type	Cal	Sample	MJ Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-G6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 2:16:21 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678 High	221480	∞	5.1 Low	66.53	939709	2.6187 ng/ml Low
THC-COOH	1.640	54531	∞	62.9	∞	252674	9.7368 ng/ml
THC	3.631	161056	2002.64	26.2	∞	6188070	2.8747 ng/ml

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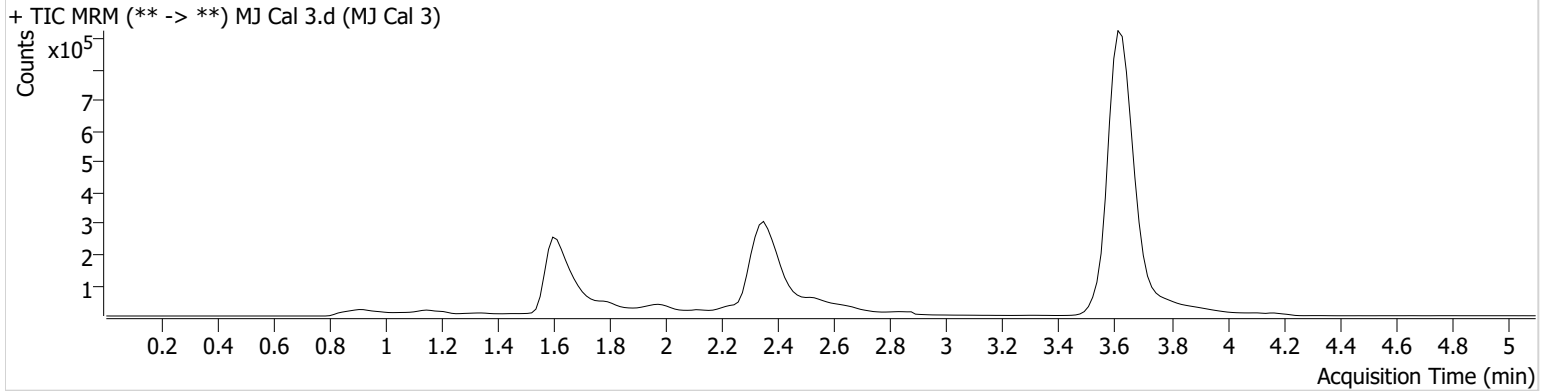


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Cal 3.d
Type	Cal	Sample	MJ Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-F6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 2:23:56 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678 High	271783	∞	5.9 Low	56.38	981014	5.3285 ng/ml
THC-COOH	1.640	100605	∞	60.6	673.24	217477	21.8960 ng/ml
THC	3.631	247812	206.39	25.5	137.70	5561034	4.7998 ng/ml

SC

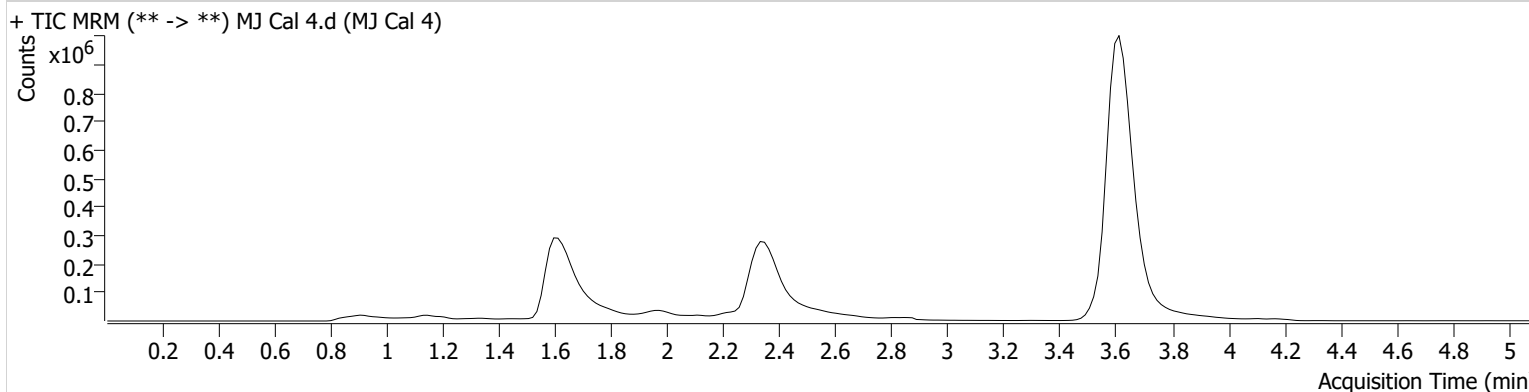


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Cal 4.d
Type	Cal	Sample	MJ Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-E6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 2:31:33 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678 High	385559	∞	7.1	303.19	970050	13.2196 ng/ml
THC-COOH	1.640	252202	∞	73.4	∞	250356	48.7374 ng/ml
THC	3.616	573761	3727.11	23.3	517.15	5929075	10.2224 ng/ml

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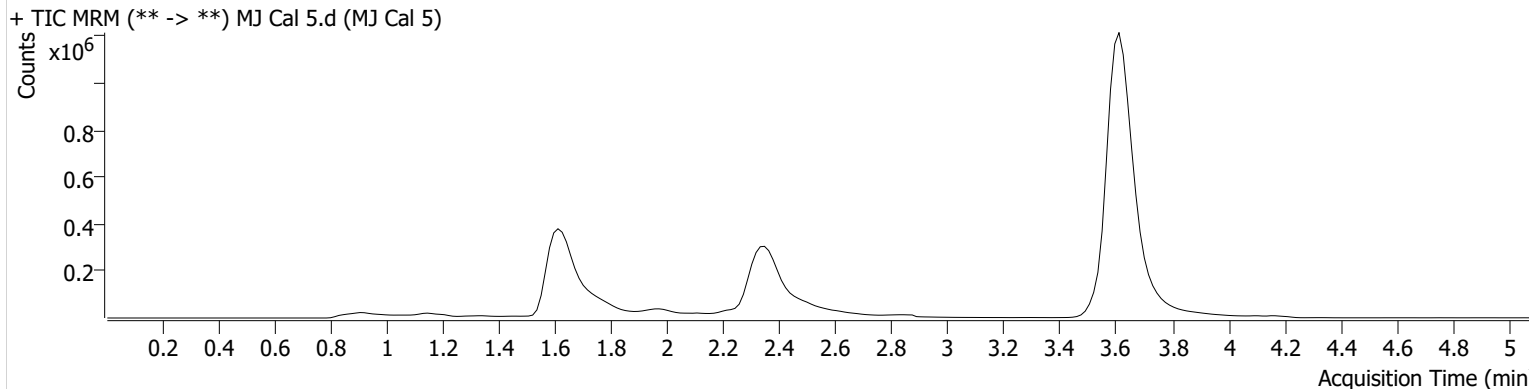


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Cal 5.d
Type	Cal	Sample	MJ Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-D6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 2:39:09 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	579575	790.95	10.8 High	∞	998408	25.2138 ng/ml
THC-COOH	1.640	409133	∞	61.1	2930.91	262399	75.9261 ng/ml
THC	3.616	1494519	∞	24.6	∞	6238282	25.0543 ng/ml

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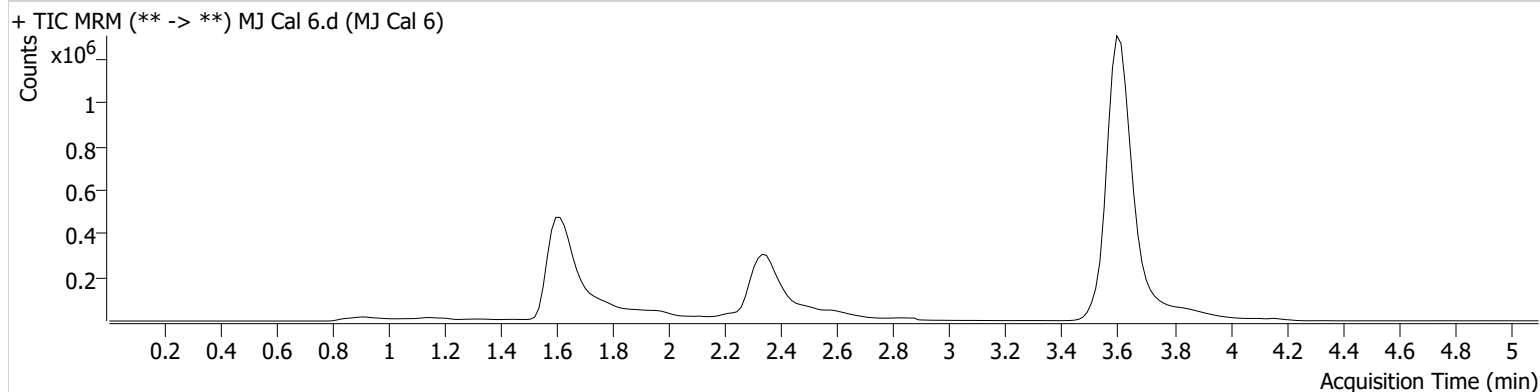
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Cal 6.d
Type	Cal	Sample	MJ Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-C6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 2:46:46 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	991287	∞	12.6 High	∞	897616	59.5420 ng/ml
THC-COOH	1.625	519285	∞	60.1	∞	277416	91.3314 ng/ml
THC	3.601	2429349	38759.27	25.2	6044.91	5105400	49.5938 ng/ml

SC

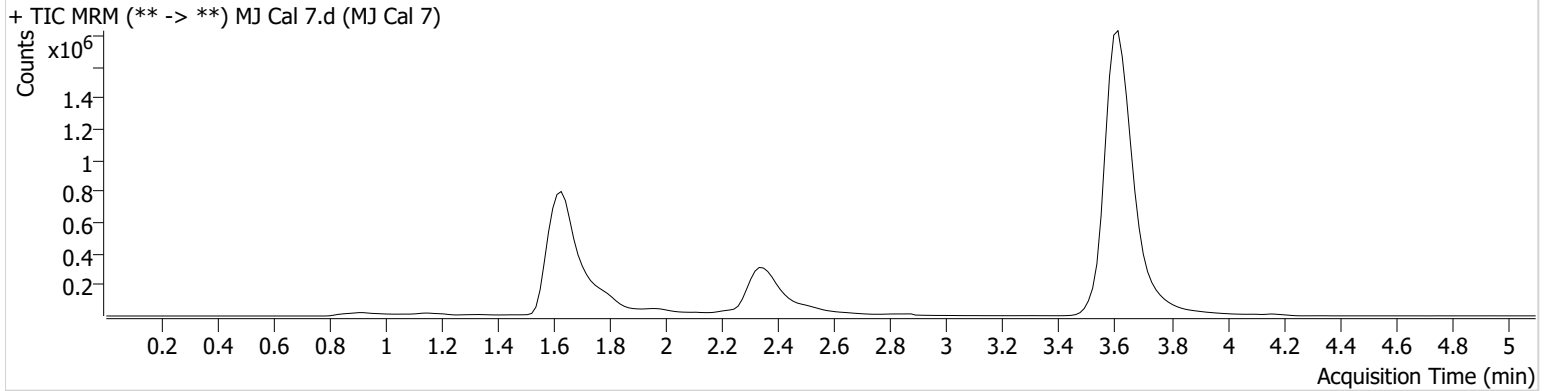


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\020822 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 2/9/2022 8:30:03 AM

Instrument	Falco (069901)	Data File	MJ Cal 7.d
Type	Cal	Sample	MJ Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-B6	Comment	
Injection Volume	10		
Acq. Date-Time	2/8/2022 2:54:22 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	1507784	∞	16.0 High	3683.33	985704	87.4115 ng/ml
THC-COOH	1.640	1325706	1179.92	61.5	7172.94	252916	257.3640 ng/ml
THC	3.616	5512754	∞	25.2	∞	5713058	100.3935 ng/ml