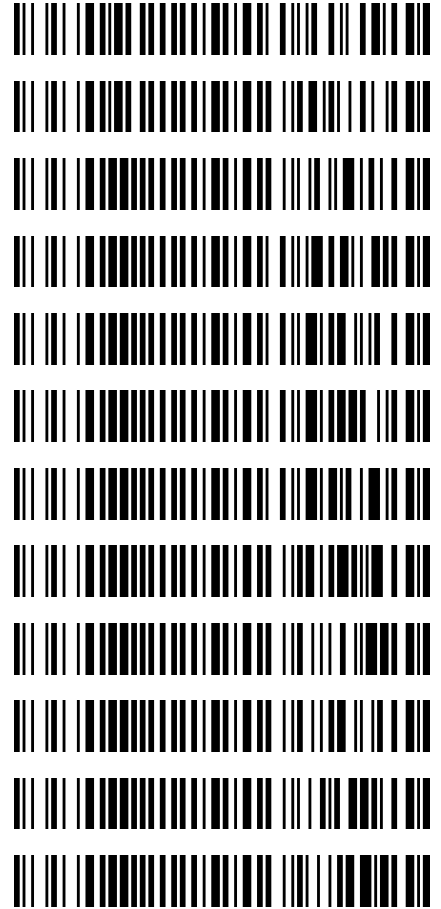


Worklist: 5993

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-1607	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-1910	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1205	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1268	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1322	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1322	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1322	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1368	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1381	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1385	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1426	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1452	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: 06/13/2022

Analyst: Tamara Salazar

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

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: 42**
- 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: 800µL
- 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 samples with calculated concentrations for THC at 1ng/mL or greater and OH-THC at 3ng/mL or greater may be reported quantitatively (blood only). Calculated concentrations for carboxy-THC of 5ng/mL may be reported qualitatively. 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: THC-OH not evaluated due to possible interfering peak.

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	P2022-1368-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	M2022-1607-1	P2022-1381-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2022-1910-1	P2022-1385-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	P2022-1205-3	P2022-1426-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2022-1268-1	P2022-1452-1	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2022-1322-1	Neg Blood	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2022-1322-2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2022-1322-3	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

TS

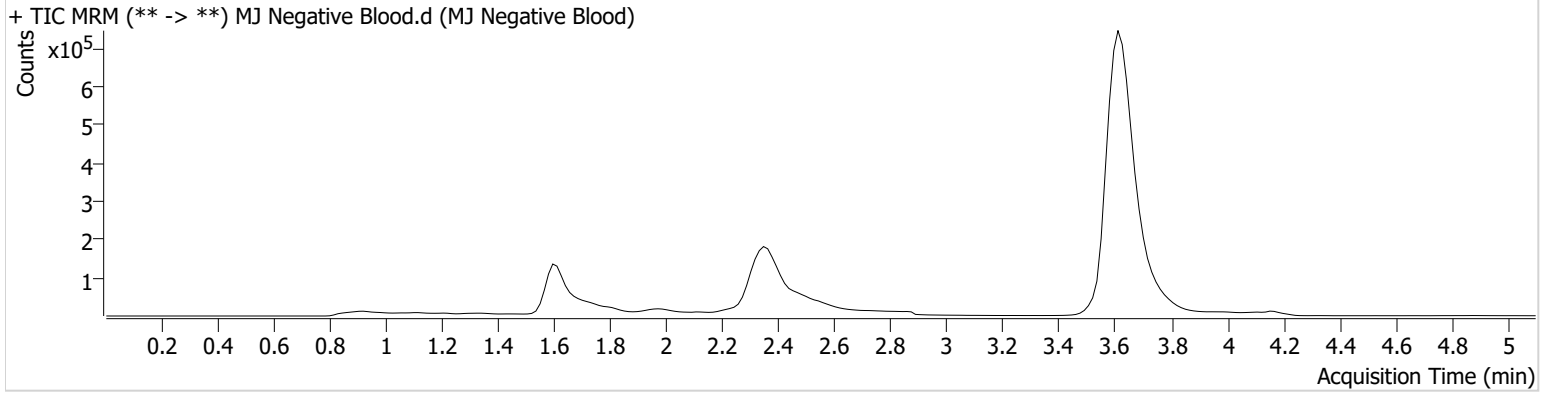


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F3	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 1:57:52 PM		
Sample Info.			

Sample Chromatogram



TS

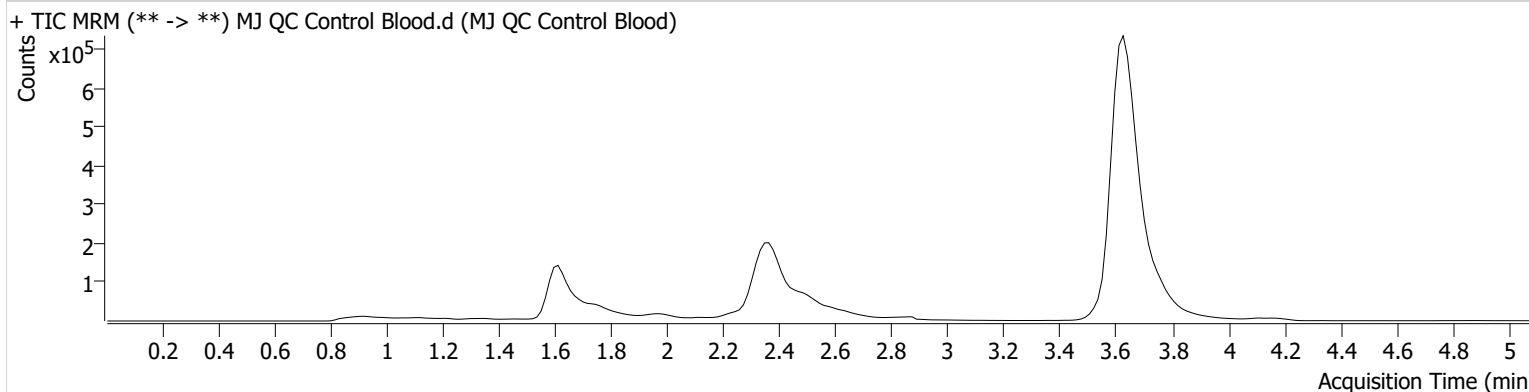


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 1:42:37 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	31672	∞	72.4	335.93	128289	14.2049 ng/ml
* THC-OH	1.693	148179	58.87	6.1	∞	491733	0.8237 ng/ml Low
THC	3.646	200737	∞	28.8	266.23	4888705	4.8213 ng/ml

*Compound not evaluated.

TS

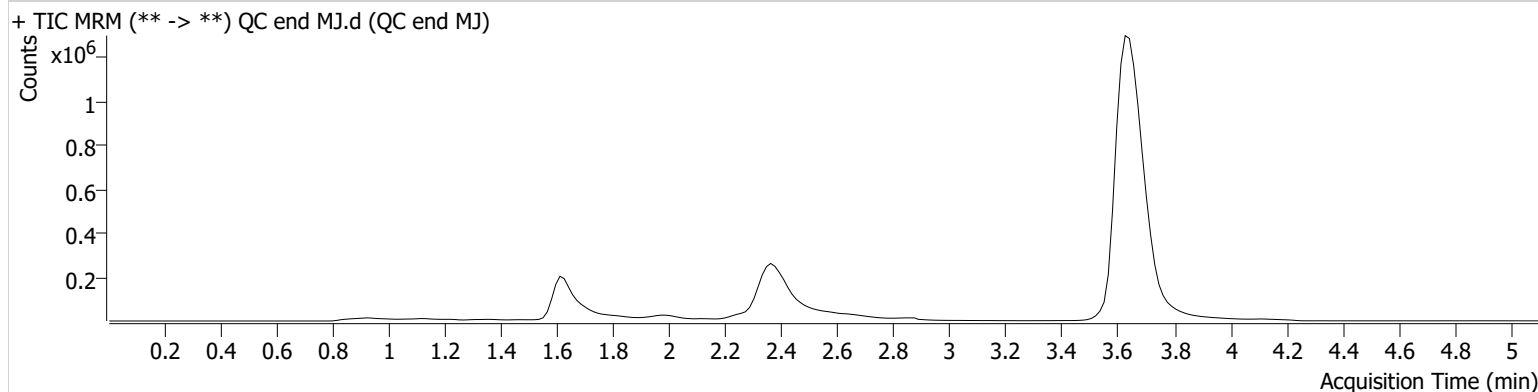


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 5:15:50 PM		

Sample Chromatogram



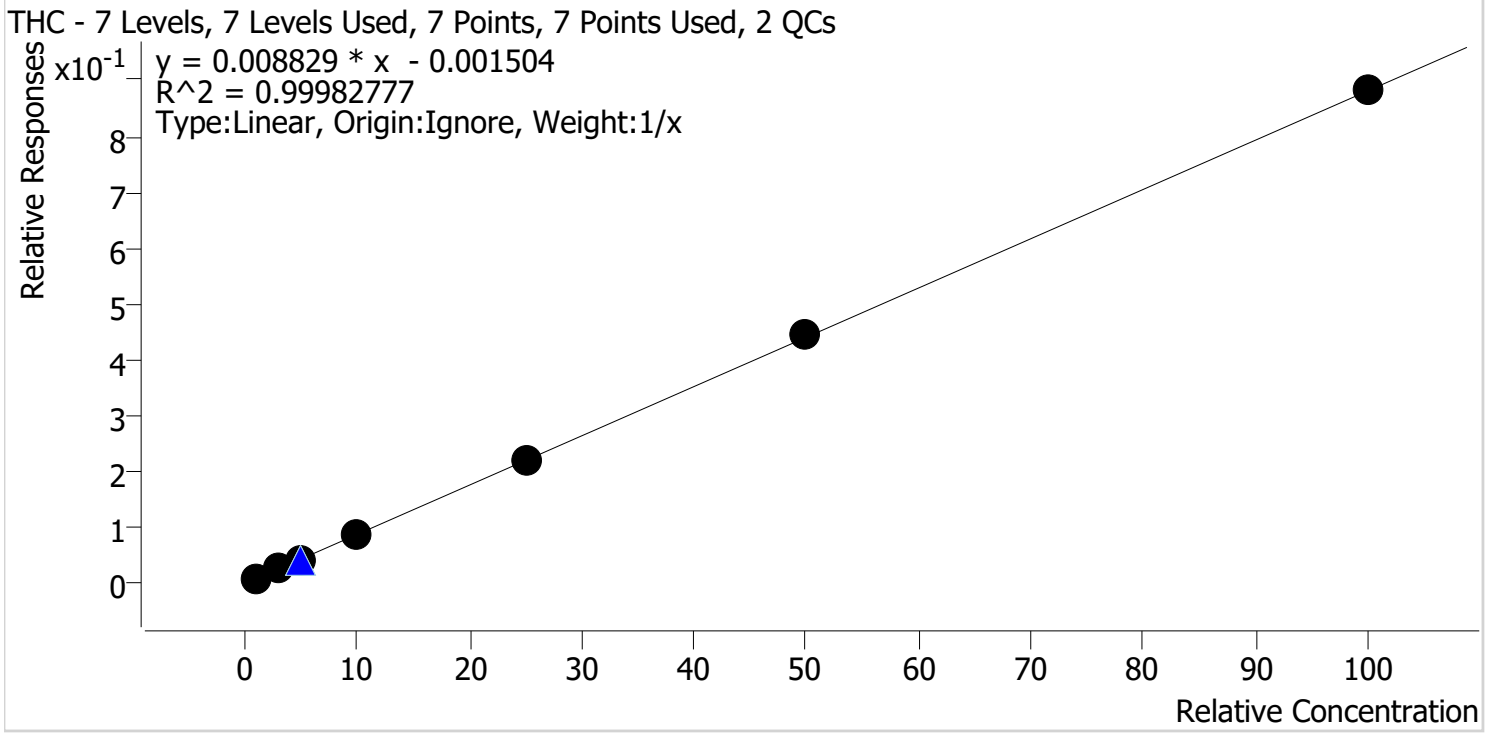
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.655	44015	∞	58.2	∞	166670	14.9893 ng/ml
THC	3.646	257679	∞	29.4	559.64	6429521	4.7098 ng/ml

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 6/16/2022 3:39 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



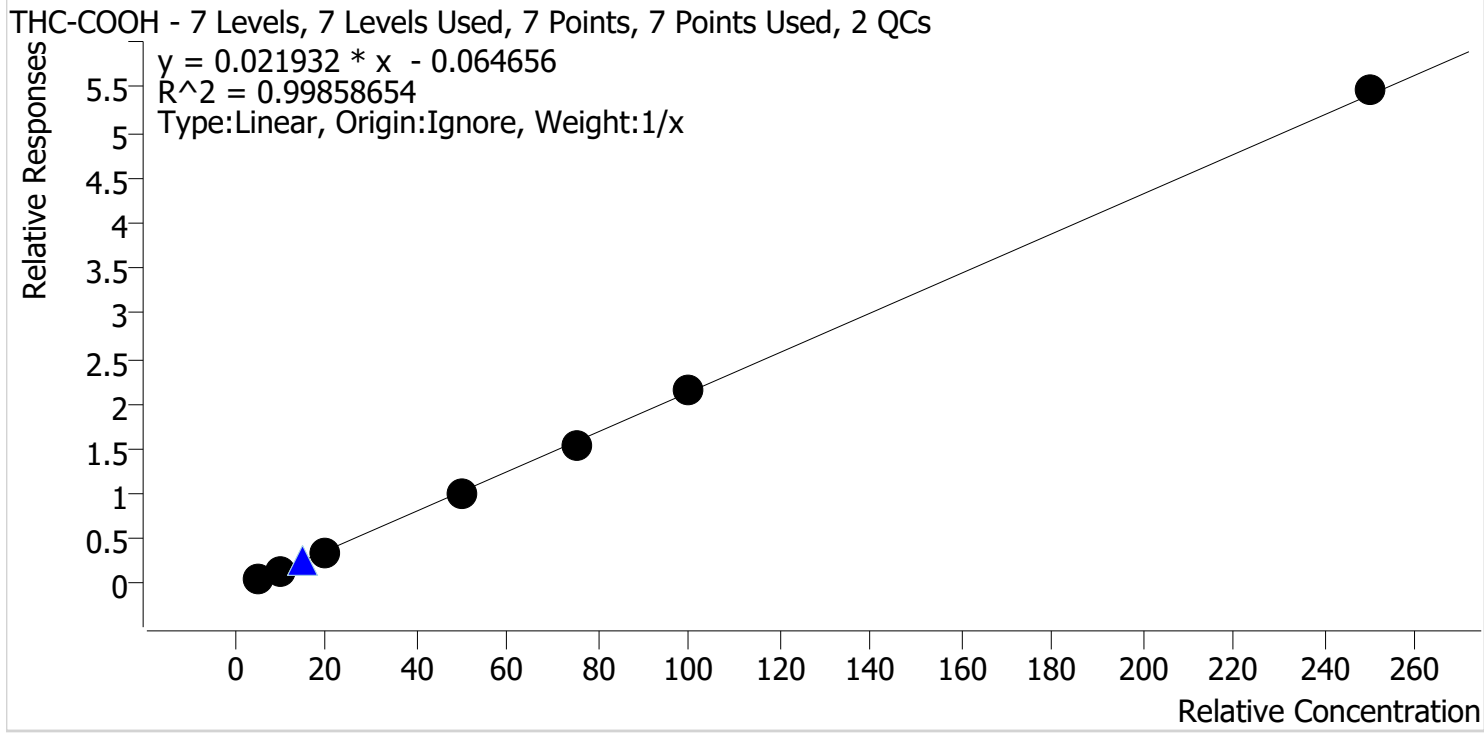
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	108.1
Cal 2 MJ	2	✓	3.0	2.9	96.6
Cal 3 MJ	3	✓	5.0	4.9	98.0
Cal 4 MJ	4	✓	10.0	9.7	96.7
Cal 5 MJ	5	✓	25.0	24.9	99.5
Cal 6 MJ	6	✓	50.0	50.5	101.0
Cal 7 MJ	7	✓	100.0	100.1	100.1

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 6/16/2022 3:39 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



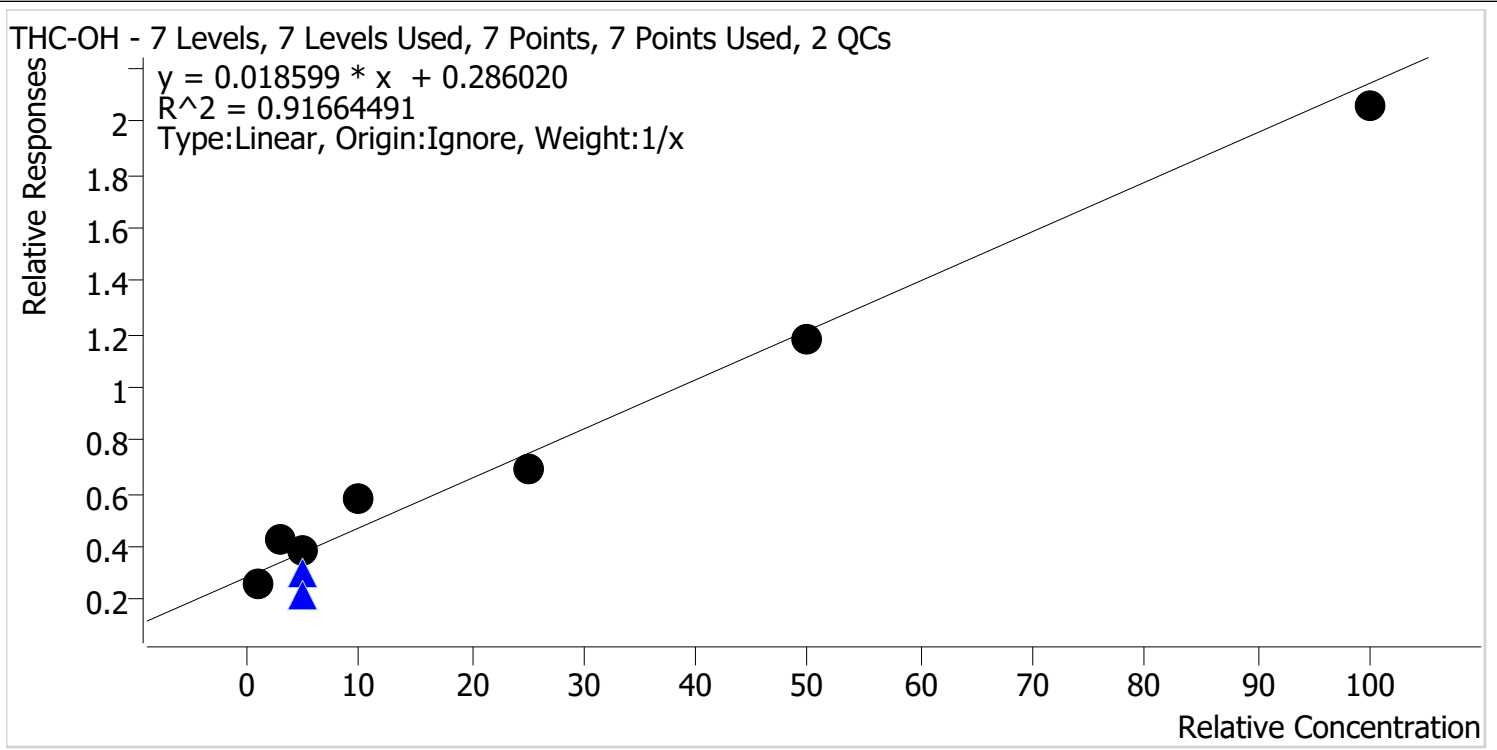
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	6.0	120.6
Cal 2 MJ	2	✓	10.0	8.7	87.1
Cal 3 MJ	3	✓	20.0	18.7	93.3
Cal 4 MJ	4	✓	50.0	49.3	98.5
Cal 5 MJ	5	✓	75.0	73.6	98.2
Cal 6 MJ	6	✓	100.0	101.4	101.4
Cal 7 MJ	7	✓	250.0	252.3	100.9

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 6/16/2022 3:39 PM
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	0.0	0.0
Cal 2 MJ	2	✓	3.0	7.4	246.4
Cal 3 MJ	3	✓	5.0	5.6	111.9
Cal 4 MJ	4	✓	10.0	16.2	162.1
Cal 5 MJ	5	✓	25.0	22.0	87.9
Cal 6 MJ	6	✓	50.0	48.5	97.0
Cal 7 MJ	7	✓	100.0	95.3	95.3

*Compound not evaluated due to interfering peak.

TS

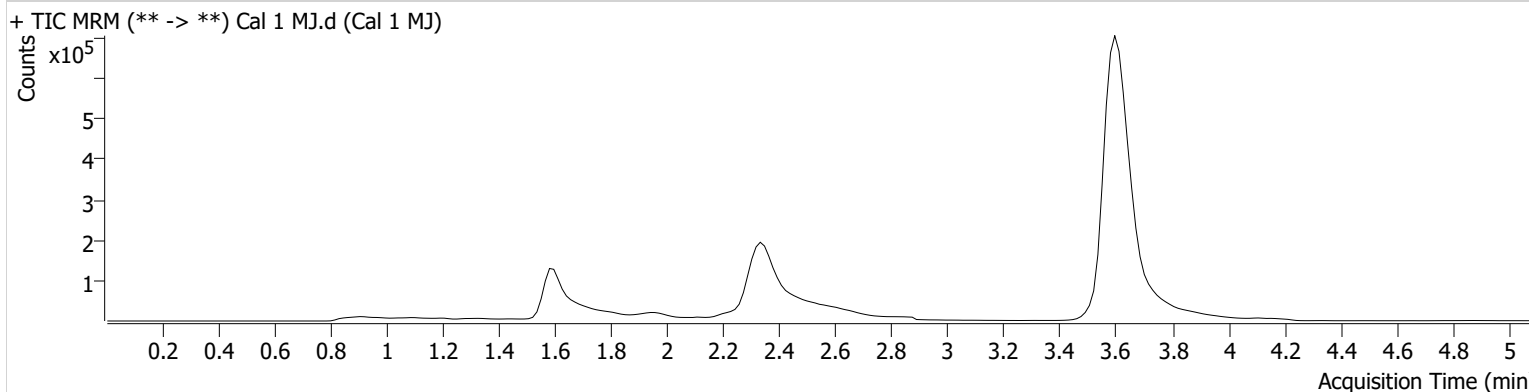


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 12:41:37 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.625	8824	∞	67.5	79.22	130511	6.0309 ng/ml
THC	3.601	37702	∞	30.8	∞	4688750	1.0812 ng/ml

TS

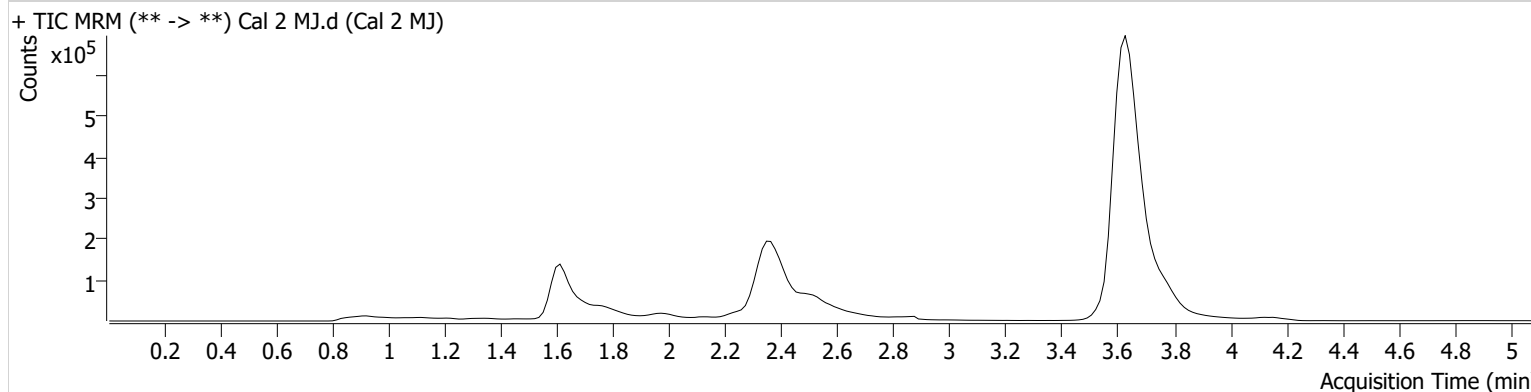


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-B1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 12:49:23 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	16516	∞	71.9	∞	130735	8.7082 ng/ml
THC-OH	1.693	131373	∞	3.9 Low	85.86	310184	7.3935 ng/ml
THC	3.631	114161	∞	30.3	∞	4740665	2.8980 ng/ml

TS



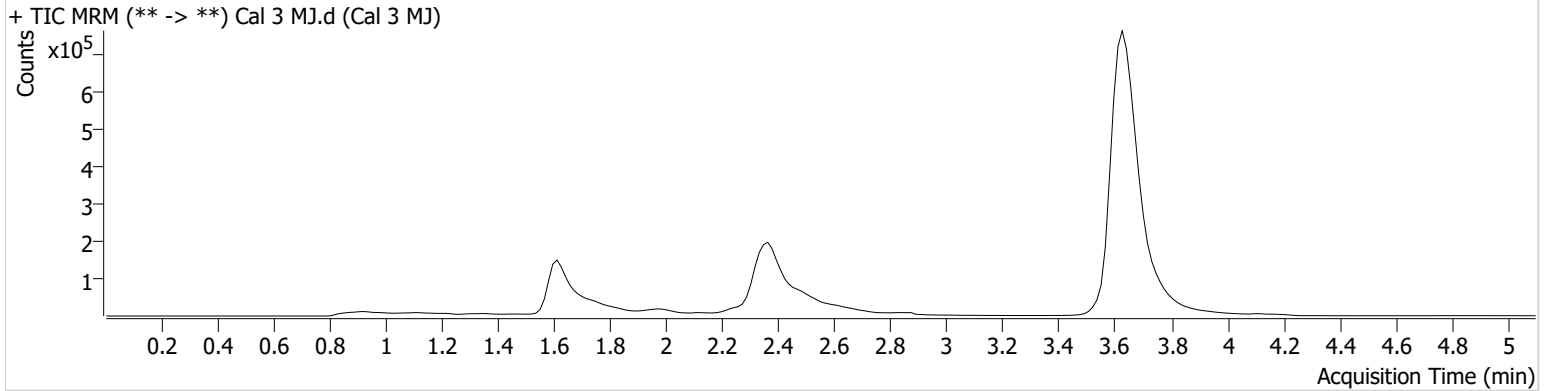
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 12:56:59 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	44735	32.24	66.2	∞	129886	18.6521 ng/ml
THC-OH	1.693	147737	∞	3.9 Low	∞	378715	5.5960 ng/ml
THC	3.631	205181	451.60	28.6	219.75	4911998	4.9017 ng/ml

TS

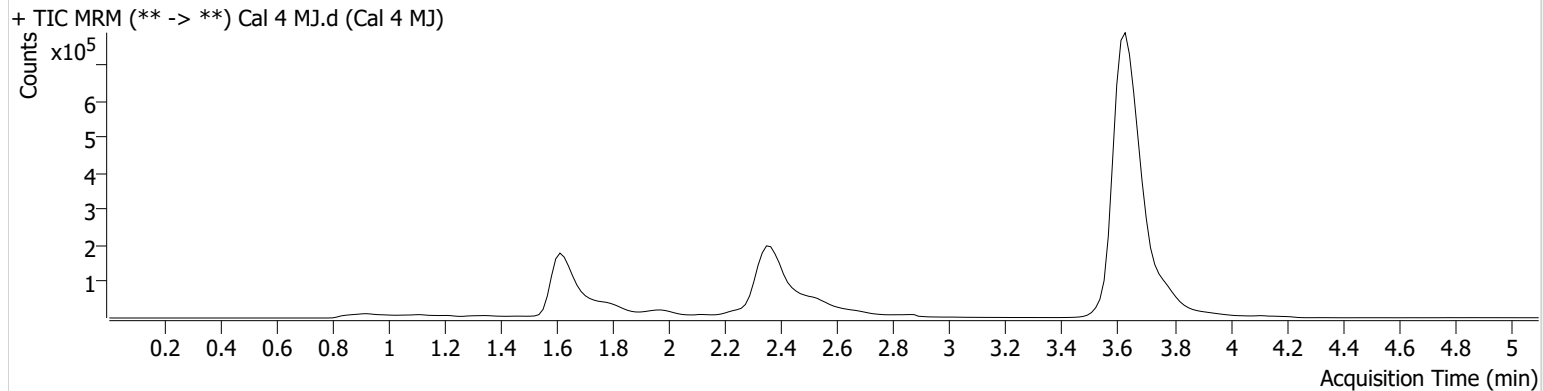


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 1:04:35 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	135423	∞	60.8	∞	133321	49.2634 ng/ml
THC-OH	1.693	200530	∞	5.3	∞	341283	16.2136 ng/ml
THC	3.631	413476	∞	28.2	∞	4929412	9.6712 ng/ml

TS

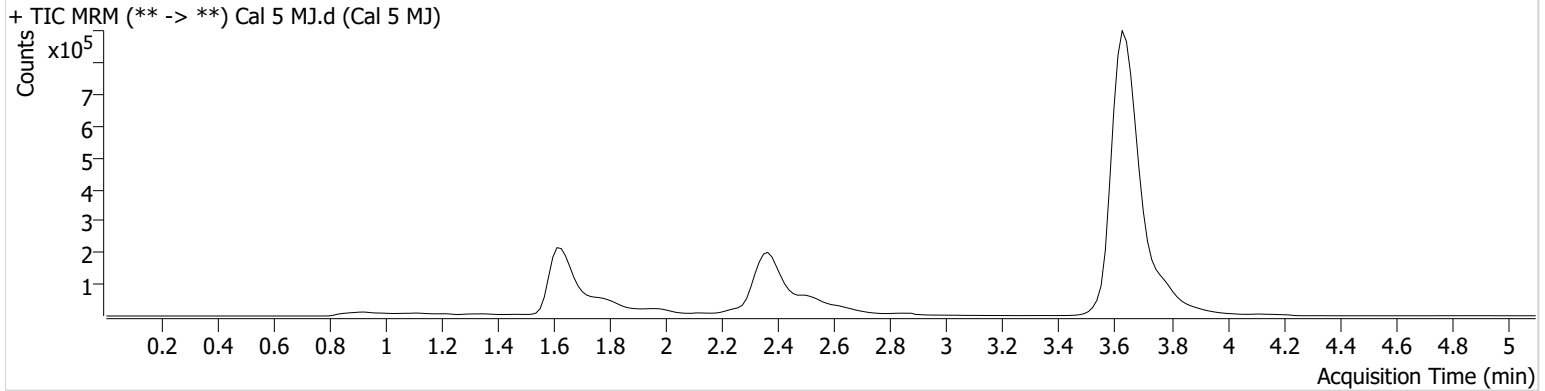


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 1:12:11 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	201889	155.26	61.1	2175.07	130235	73.6308 ng/ml
THC-OH	1.618 Low	354872	∞	6.8	∞	510816	21.9742 ng/ml
THC	3.646	1049261	∞	27.6	4641.26	4812483	24.8659 ng/ml

TS

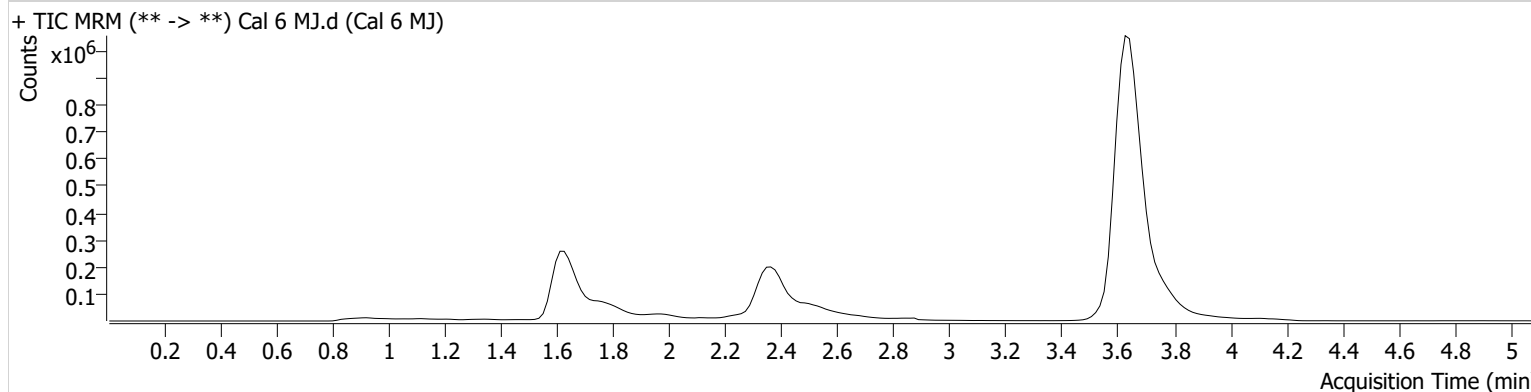


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 1:19:47 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	262309	∞	61.0	∞	121459	101.4204 ng/ml
THC-OH	1.618 Low	579159	∞	8.0 High	∞	487401	48.5104 ng/ml
THC	3.646	2068768	∞	27.7	∞	4656411	50.4932 ng/ml

TS

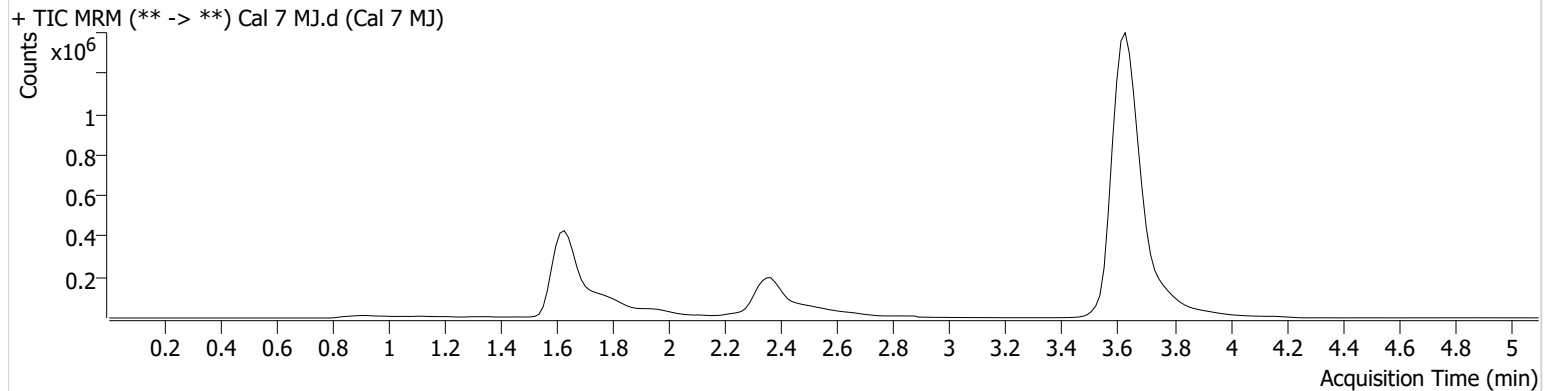


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\061322 AM 27 28 P1 and P2 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/16/2022 3:39:40 PM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	6/13/2022 1:27:23 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	656538	∞	59.8	∞	120057	252.2942 ng/ml
THC-OH	1.603 Low	724663	∞	12.4 High	∞	351972	95.3198 ng/ml
THC	3.631	4050176	∞	28.7	∞	4591271	100.0888 ng/ml