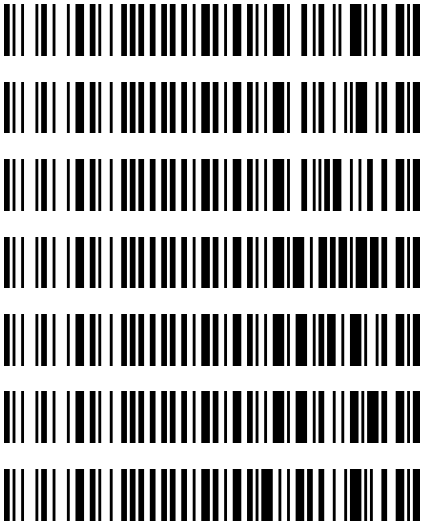
REVIEWED
By Anne Nord at 8:39 am, May 16, 2023

5/12/2023

Worklist: 6370

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2023-0949	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ
C2023-0949	2	BLOOD	AM 27 Blood THC Quant by LC-QQQ
C2023-0949	3	BLOOD	AM 27 Blood THC Quant by LC-QQQ
C2023-0989	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-1001	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-1003	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-1049	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 5/11/23
Plate lot#: 230113

Analyst: Britany Wylie
Plate re-test: 7/13/23

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE LCMS Methanol

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 22B52016-1 **Urine Blank:** 12522

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 69679

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 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette **1000µL (calibrated pipette) blood or 1000µL hydrolyzed urine Pipette ID: K52558G** in wells of analytical (standards) plate.
☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 4. Pipette **500µL 0.1% formic acid in water blood sample, 500 ul saturated phosphate buffer in urine** in wells 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) Manifold ID: 66792
☒ 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.
SPE Dry ID: 66819
☒ 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. SN > 10
☒ 4. Case sample response for THC 1ng/ml LOD ; 3ng/ml LOQ, OH-THC 3ng/mL LOD and LOQ, Carboxy-THC: 5 ng/mL (qualitative only). 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 is it describe in comments section)
☒ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: .*Blood only Run*

end of run control- incorrect plate location was selected. QC was reconstituted and reinjected 5/12/23

BW

	1	2	3	4	5	6
a	cal 1	Internal control urine	1049-1 (a2 on SLE and Ext)			
b	cal 2	negative blood				
c	cal 3	949-1				
d	cal 4	949-2				
e	Cal 5	949-3				
f	cal 6	989-1				
g	cal 7	1001-1				
h	Internal control (blood)	1003-1				

Plate position 3

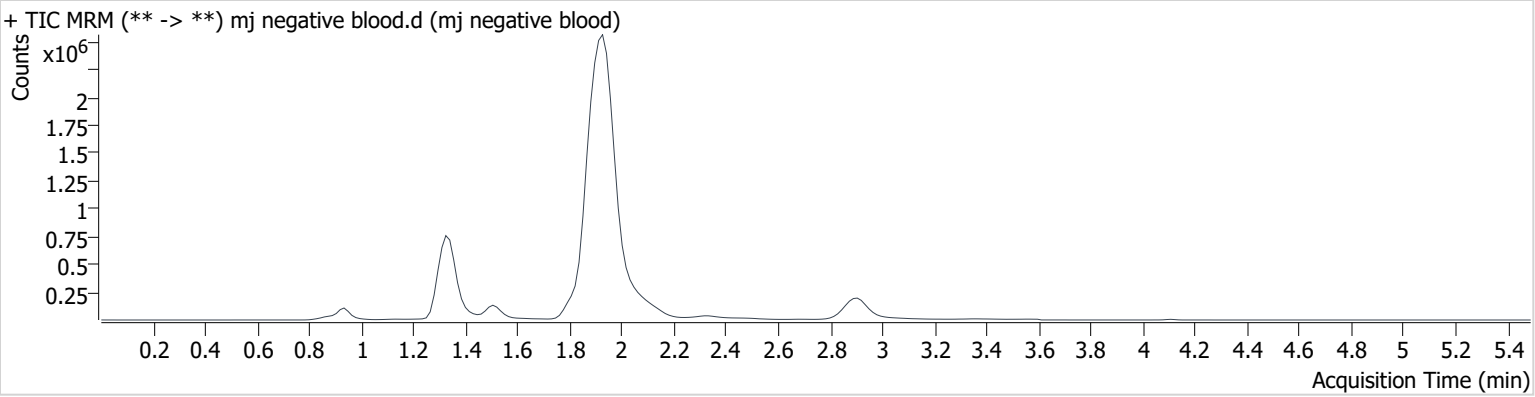
c2023-0____-_

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update	5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj negative blood.d
Type	Sample	**Sample**	mj negative blood
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-B2	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 11:18:45 PM		
Sample Info.			

Sample Chromatogram

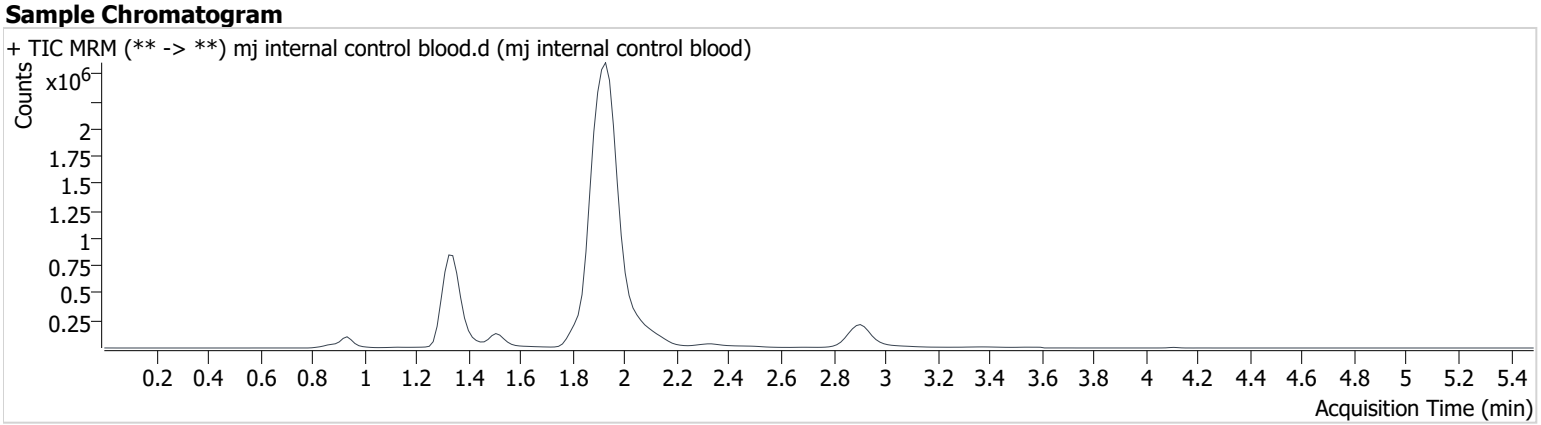


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj internal control blood.d
Type	QC	**Sample**	mj internal control blood
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 11:12:10 PM		
Sample Info.			

Sample Chromatogram



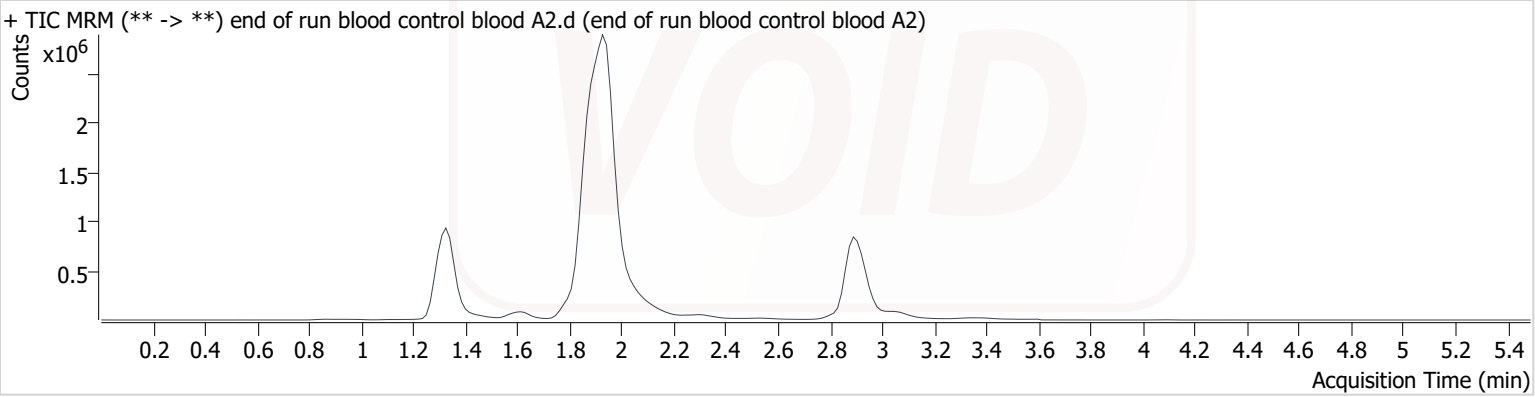
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	47933	1643.8	938.64	∞	2640588	4.310	ng/ml
THC-COOH	1.358	57258	∞	252.22	∞	722013	15.653	ng/ml
THC	2.911	157332	∞	24.94	∞	1087455	4.881	ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update	5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	end of run blood control blood A2.d
Type	Sample	**Sample**	end of run blood control blood A2
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/12/2023 12:57:34 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.358	17705	∞	303.14 **High**	∞	1010627	4.695	ng/ml **Low**
THC	2.911	37277	31629.9	33.20 **High**	∞	4484142	0.688	ng/ml **Low**

Incorrect plate location injected- sample was reinjected with the correct position.

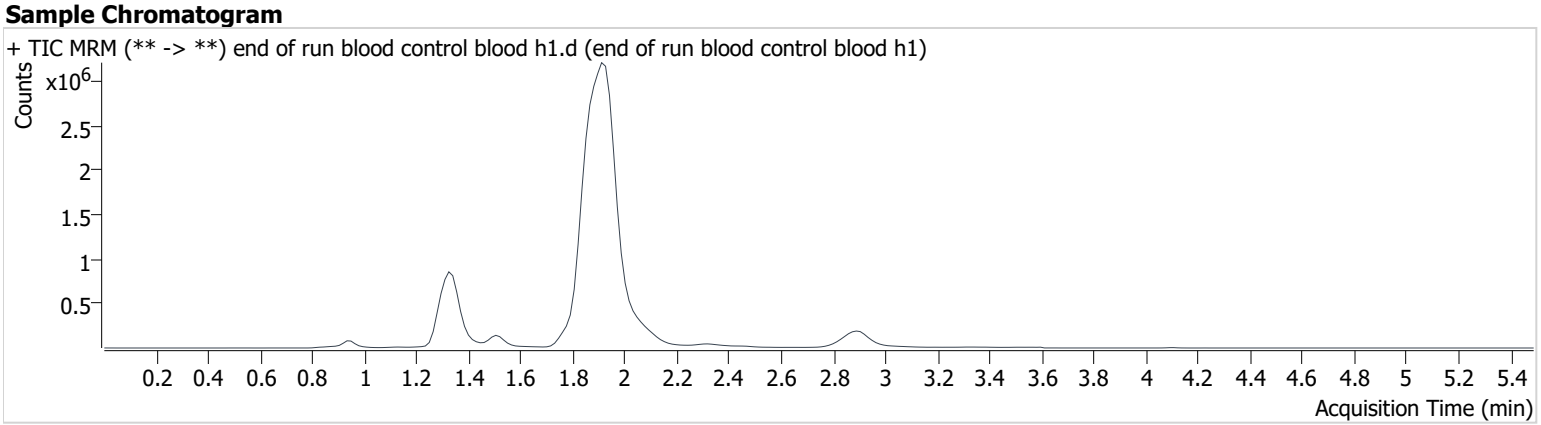
This sample was not evaluated

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/15/2023 10:44:18 AM

Instrument	69679	**Data File**	end of run blood control blood h1.d
Type	Sample	**Sample**	end of run blood control blood h1
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/12/2023 9:03:33 AM		
Sample Info.			

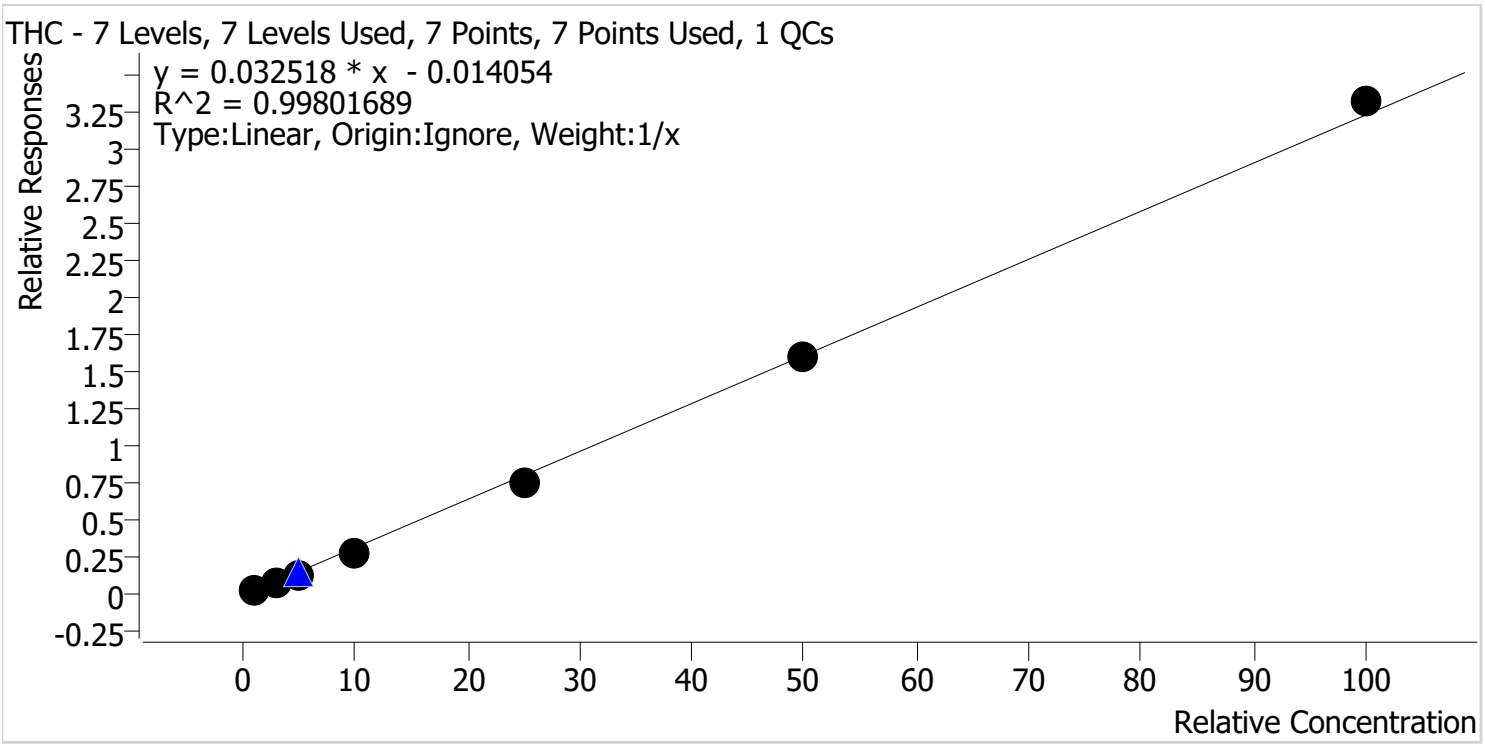
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	52255	∞	936.75	∞	2801211	4.419	ng/ml
THC-COOH	1.358	55826	19.6	261.62	∞	837667	13.408	ng/ml
THC	2.911	159345	∞	25.23	∞	734618	7.103	ng/ml

Compound Calibration Report

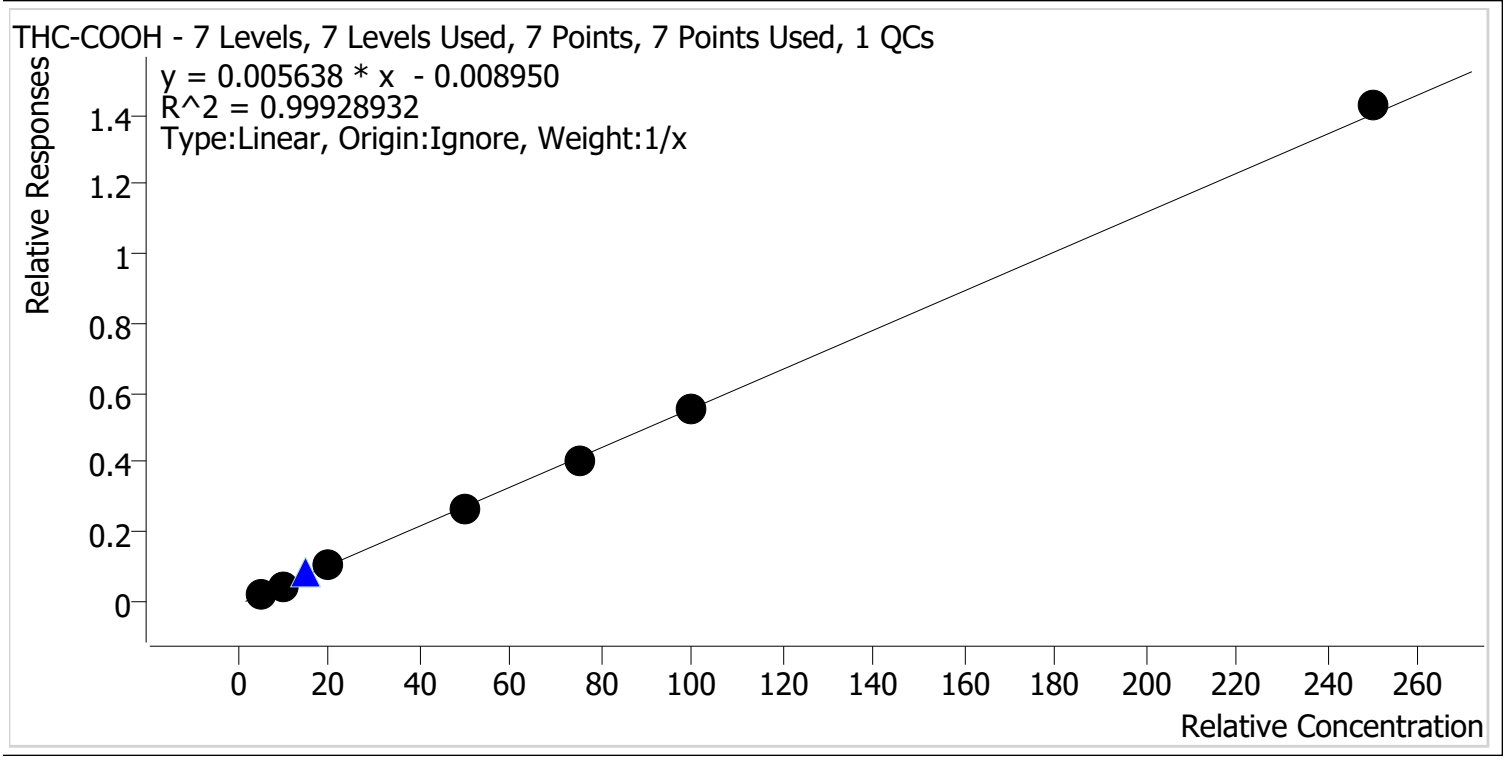
Batch results	D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Last Cal. Update	5/12/2023 9:17 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	123.9
mj cal 2	2	✔	3.0	2.9	97.6
mj cal 3	3	✔	5.0	4.5	90.4
mj cal 4	4	✔	10.0	9.1	91.1
mj cal 5	5	✔	25.0	23.7	94.8
mj cal 6	6	✔	50.0	49.7	99.5
mj cal 7	7	✔	100.0	102.8	102.8

Compound Calibration Report

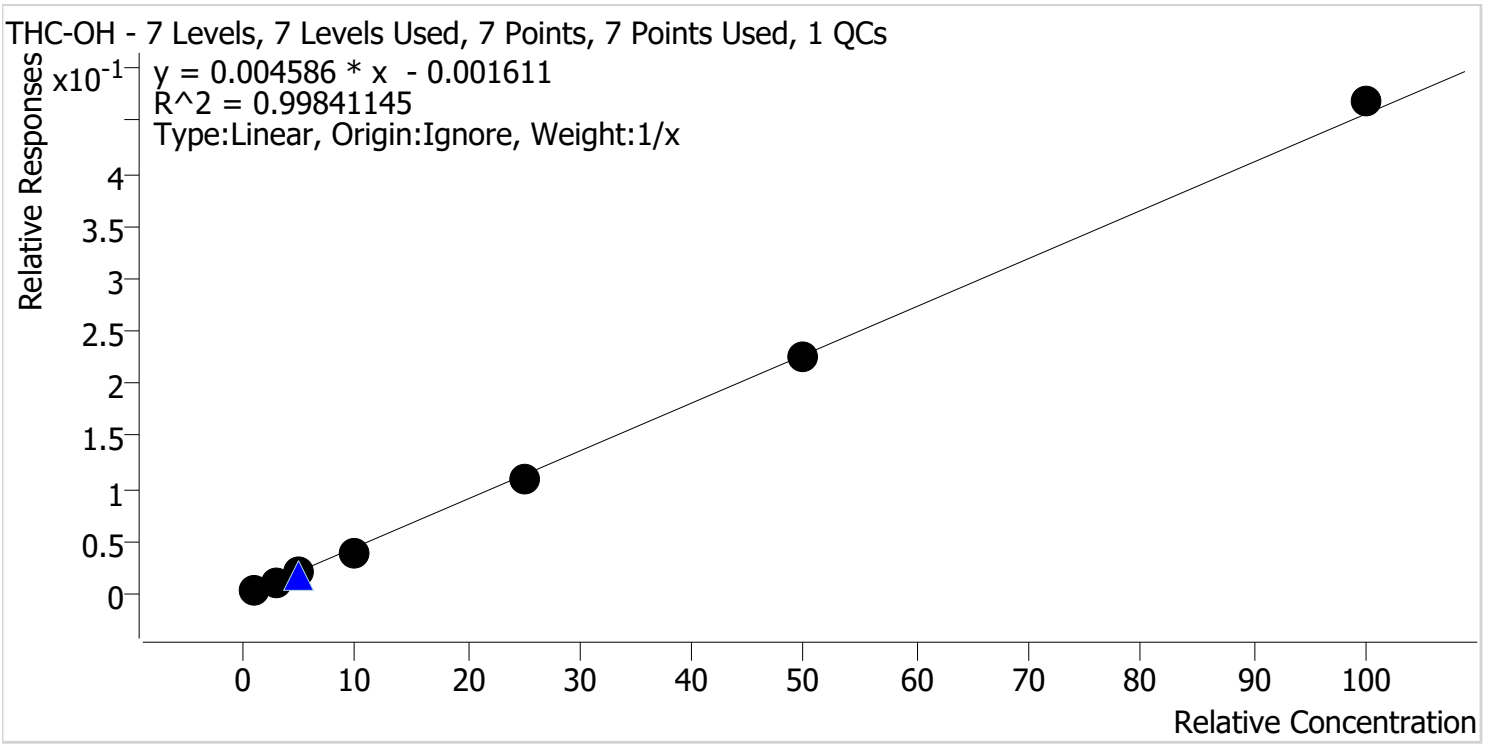
Batch results	D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Last Cal. Update	5/12/2023 9:17 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.5	110.2
mj cal 2	2	✔	10.0	9.7	97.4
mj cal 3	3	✔	20.0	19.5	97.3
mj cal 4	4	✔	50.0	47.8	95.7
mj cal 5	5	✔	75.0	73.6	98.1
mj cal 6	6	✔	100.0	99.4	99.4
mj cal 7	7	✔	250.0	254.4	101.8

Compound Calibration Report

Batch results	D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Last Cal. Update	5/12/2023 9:17 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



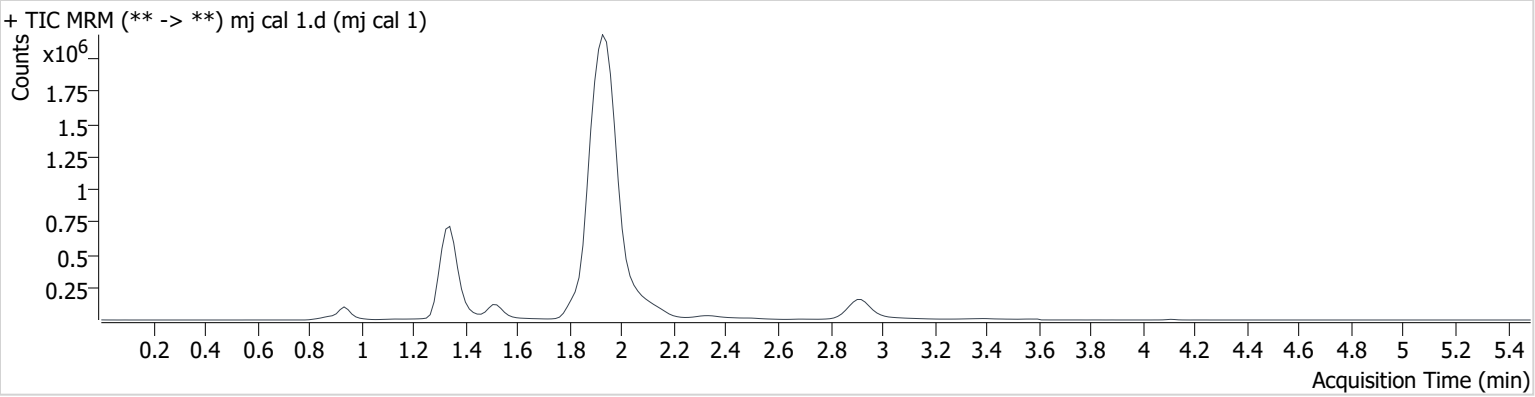
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	120.3
mj cal 2	2	✔	3.0	3.0	98.4
mj cal 3	3	✔	5.0	4.6	92.8
mj cal 4	4	✔	10.0	9.1	90.9
mj cal 5	5	✔	25.0	23.9	95.8
mj cal 6	6	✔	50.0	49.6	99.2
mj cal 7	7	✔	100.0	102.5	102.5

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update	5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj cal 1.d
Type	Cal	**Sample**	mj cal 1
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 10:25:58 PM		
Sample Info.			

Sample Chromatogram



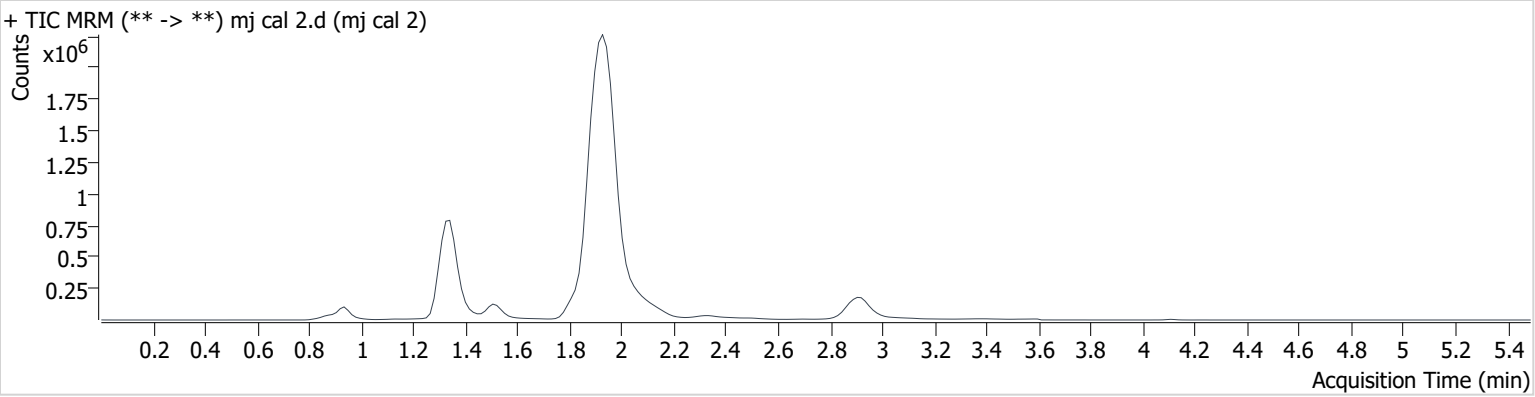
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.349	10126	∞	895.94	∞	2592778	1.203	ng/ml Low
THC-COOH	1.358	14866	∞	284.68	∞	671892	5.512	ng/ml
THC	2.926	24487	∞	30.01	71.6	933754	1.239	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj cal 2.d
Type	Cal	**Sample**	mj cal 2
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 10:32:42 PM		
Sample Info.			

Sample Chromatogram



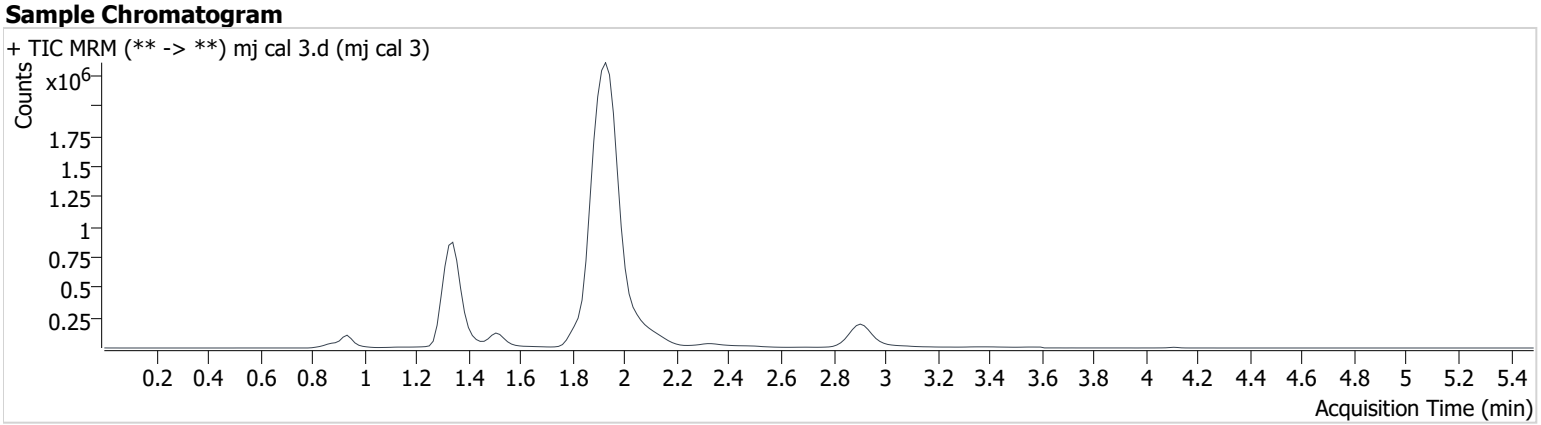
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	31343	1254.8	913.07	∞	2627795	2.952	ng/ml Low
THC-COOH	1.358	31777	∞	277.40	∞	691047	9.743	ng/ml
THC	2.926	81474	925.0	25.81	∞	1004327	2.927	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj cal 3
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 10:39:19 PM		
Sample Info.			

Sample Chromatogram



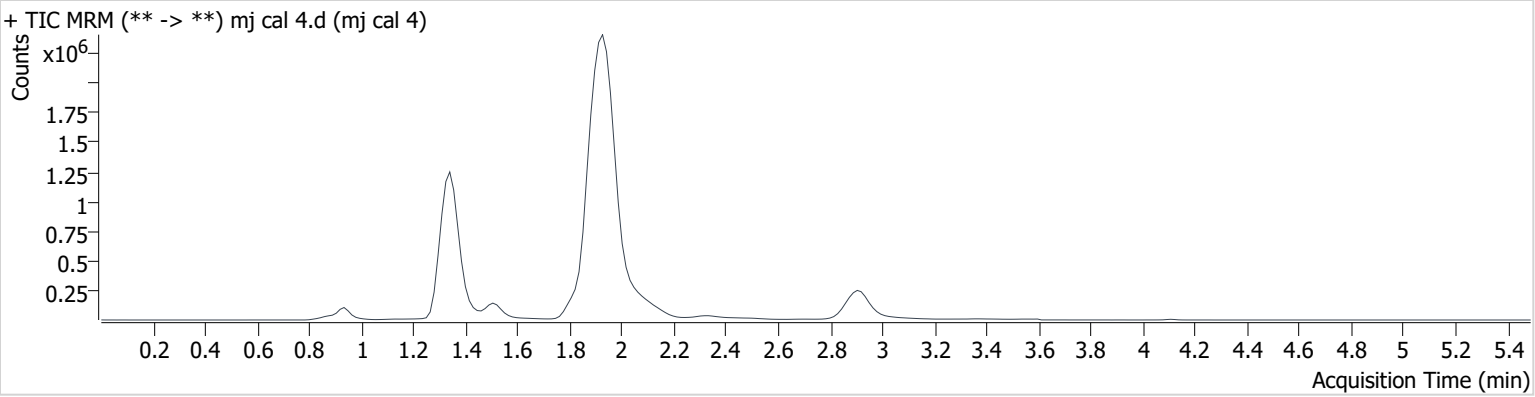
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	51266	∞	902.37	∞	2606092	4.641	ng/ml
THC-COOH	1.358	69402	∞	256.43	∞	688379	19.469	ng/ml
THC	2.926	135393	∞	26.66	574.3	1018324	4.521	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj cal 4.d
Type	Cal	**Sample**	mj cal 4
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 10:45:53 PM		
Sample Info.			

Sample Chromatogram



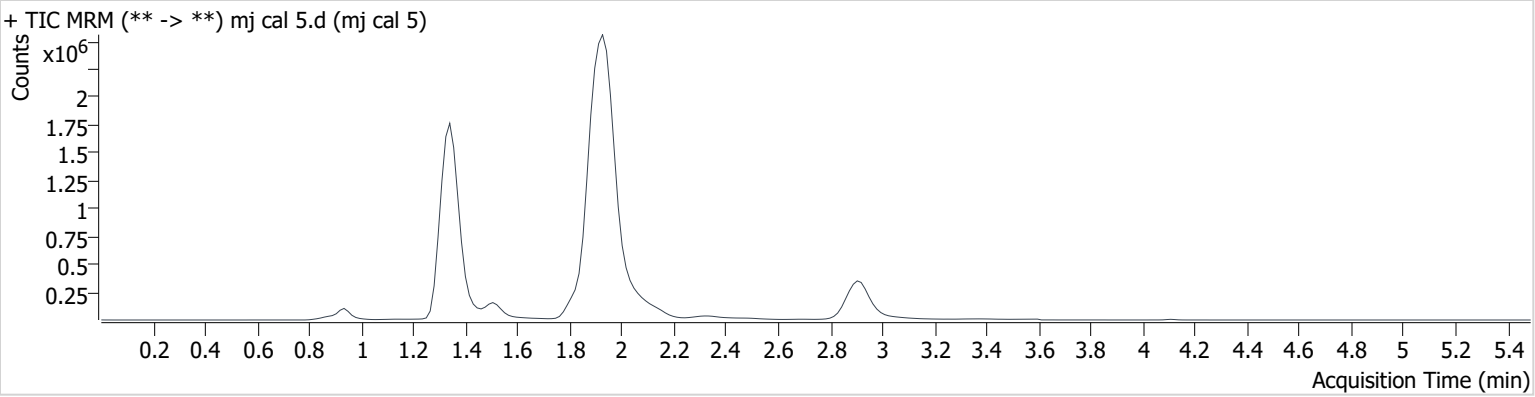
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	115122	∞	918.70	∞	2872864	9.090	ng/ml
THC-COOH	1.358	198962	∞	254.90	∞	762835	47.848	ng/ml
THC	2.926	323500	2268.5	25.14	∞	1146595	9.109	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 10:52:28 PM		
Sample Info.			

Sample Chromatogram



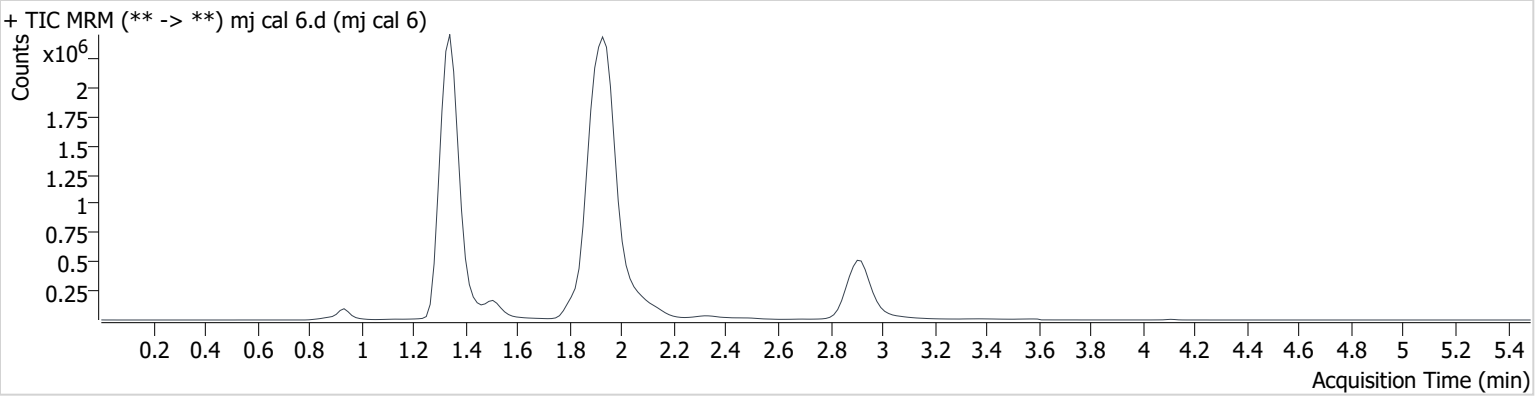
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	306360	∞	870.68	122207.1	2831781	23.943	ng/ml
THC-COOH	1.358	306973	∞	255.89	∞	756209	73.587	ng/ml
THC	2.926	860065	19534.7	23.94	4478.1	1136383	23.707	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 10:59:02 PM		
Sample Info.			

Sample Chromatogram



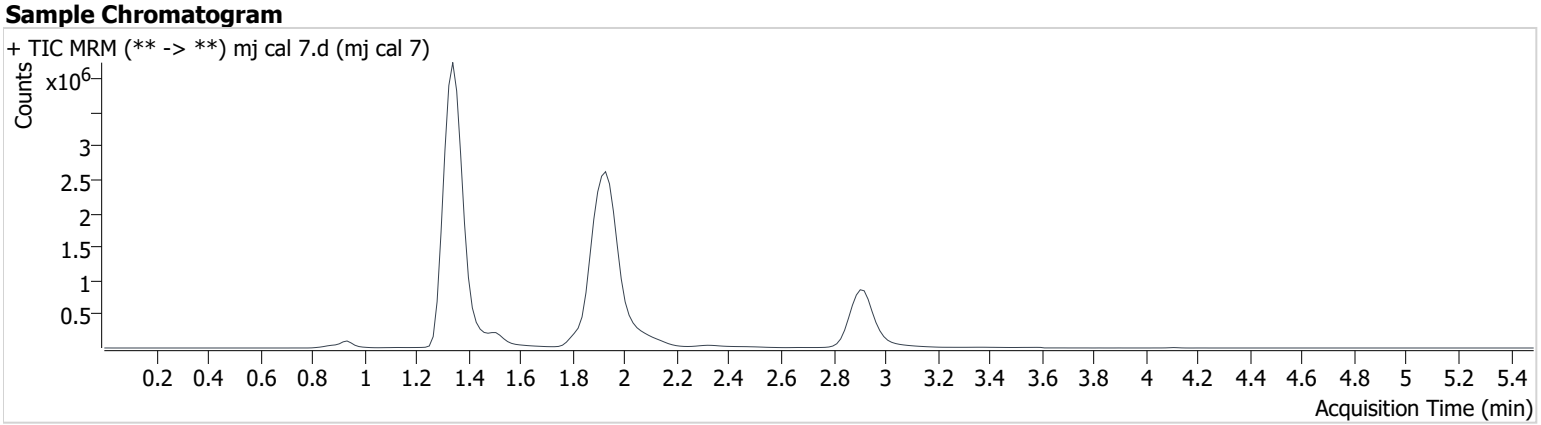
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	632702	∞	856.66	∞	2800091	49.625	ng/ml
THC-COOH	1.358	403061	∞	250.39	∞	730936	99.393	ng/ml
THC	2.926	1813463	∞	23.94	∞	1131000	49.741	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\51123r\QuantResults\thcq.batch.bin
Calibration Last Update 5/12/2023 9:17:44 AM

Instrument	69679	**Data File**	mj cal 7.d
Type	Cal	**Sample**	mj cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	5/11/2023 11:05:36 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.334	1246388	∞	835.59	∞	2659576	102.546	ng/ml
THC-COOH	1.358	952794	∞	250.65	∞	668336	254.447	ng/ml
THC	2.926	3697370	∞	24.25	17357.5	1111194	102.757	ng/ml