REVIEWED
By Amber Gerheart at 9:39 am, Oct 21, 2022

10/19/2022

Worklist: 6138

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
C2022-2126		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2212		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2220		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2295		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2343		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 10/19/22 Analyst: Anne Nord
Plate lot#: 220802 Plate re-test: 2/2/23

Mobile phase A: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
MTBE LCMS Methanol Hexane
Blank Blood Lot: 22B52016-1 **Urine Blank:** blood only **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 blood (calibrated pipette) Pipette ID: I41142J** 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
☒ 4. Case sample response for THC 1ng/ml, OH-THC 3ng/mL (quantitative blood), Carboxy-THC: 5 ng/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 is it 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 range 3-100 (cal 1 dropped due to ratio)*

	1	2	3	4	5	6
a	cal 1	blood control internal				
b	cal 2	negative blood				
c	cal 3	2126-1				
d	cal 4	2212-1				
e	Cal 5	2220-1				
f	cal 6	2295-1				
g	cal 7	2343-1				
h	Internal control (blood)					

Plate position 3

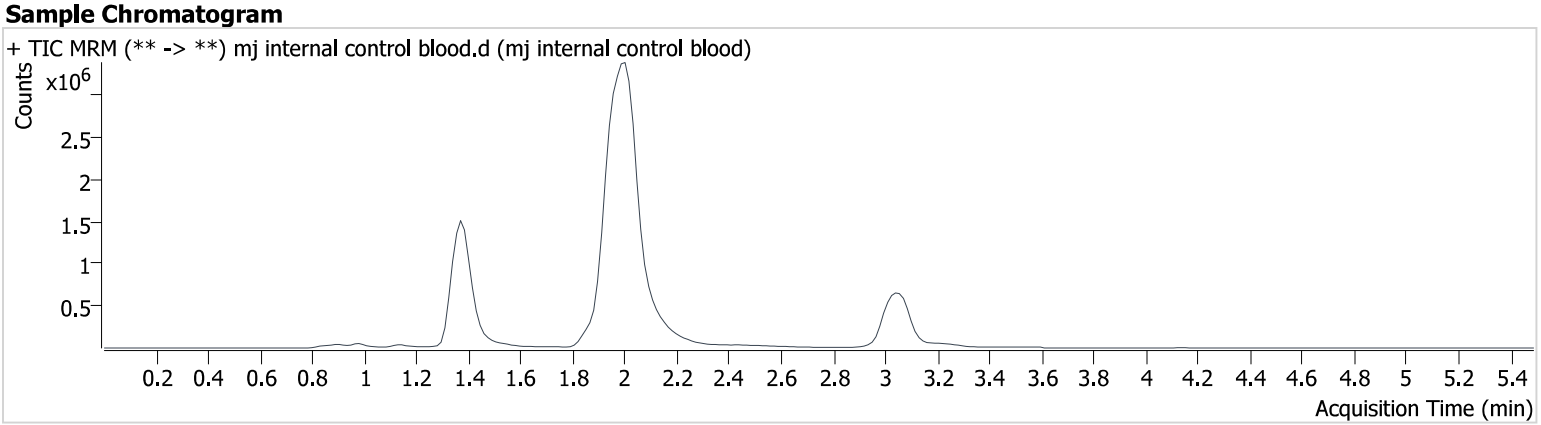
c2022-____-_

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 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**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:51:37 PM		
Sample Info.			

Sample Chromatogram



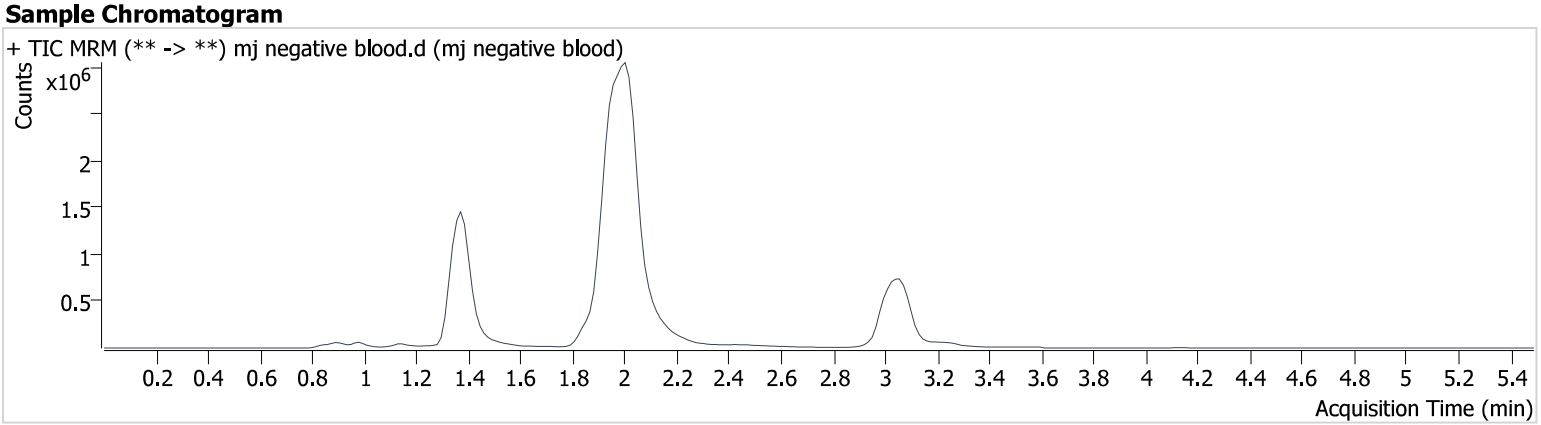
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	76707	∞	994.0	504.3	4875499	4.848 ng/ml
THC-COOH	1.403	100708	977.2	281.4	∞	1353158	13.775 ng/ml
THC	3.061	476440	2388.5	24.7	∞	3847868	4.673 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update	10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj negative blood.d
Type	Sample	**Sample**	mj negative blood
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B2	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:58:22 PM		
Sample Info.			

Sample Chromatogram

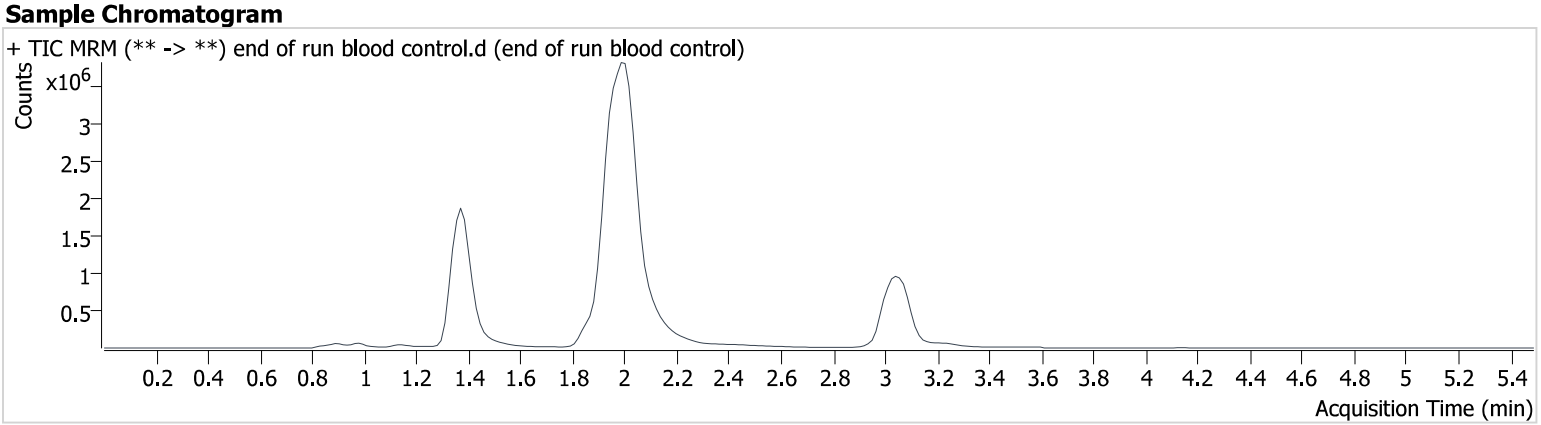


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	end of run blood control.d
Type	Sample	**Sample**	end of run blood control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 9:12:01 PM		
Sample Info.			

Sample Chromatogram

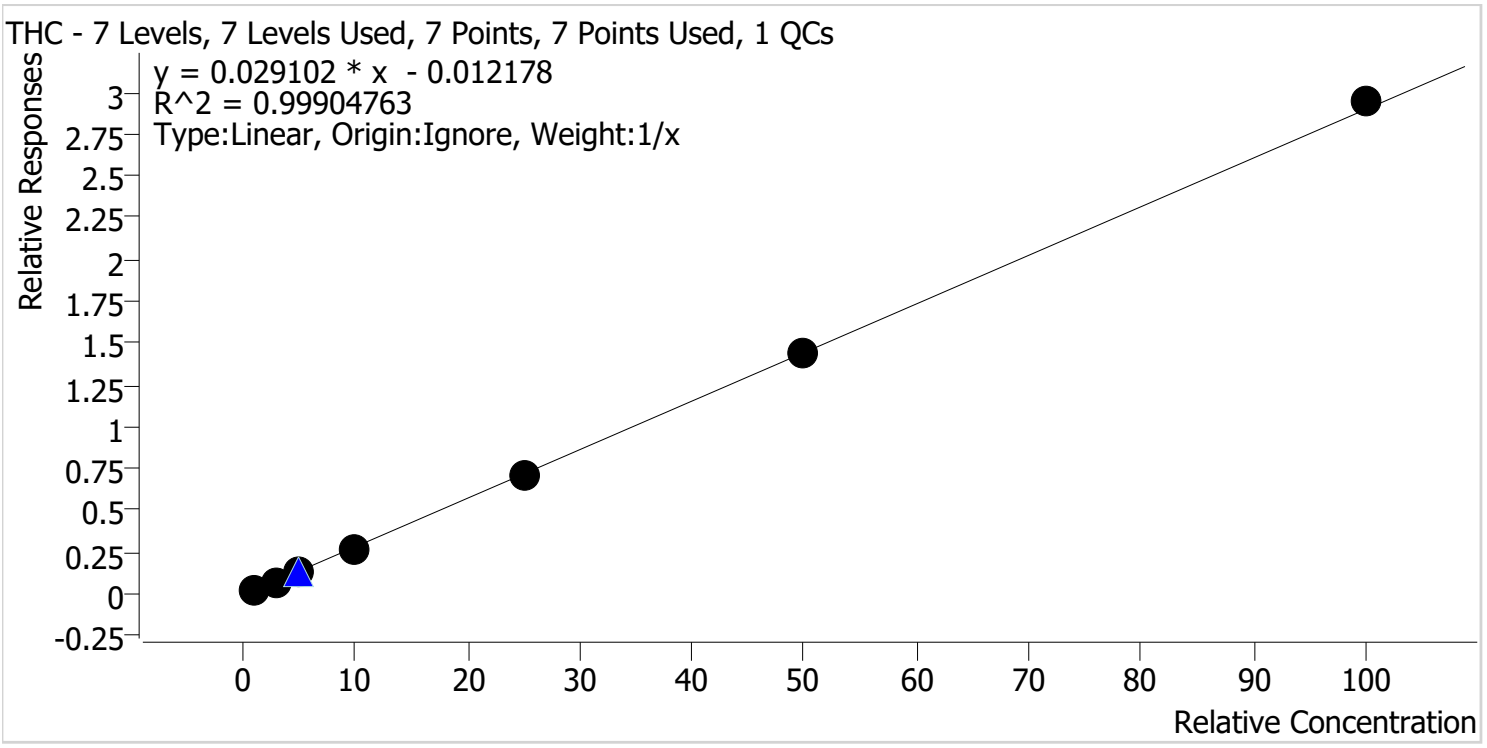


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	95468	∞	1014.3	∞	6094862	4.830 ng/ml
THC-COOH	1.403	137499	1044.7	262.6	∞	1682349	15.019 ng/ml
THC	3.061	736534	133210.2	23.4	1554.9	5780911	4.796 ng/ml

Compound Calibration Report

Agilent | Trusted Answers

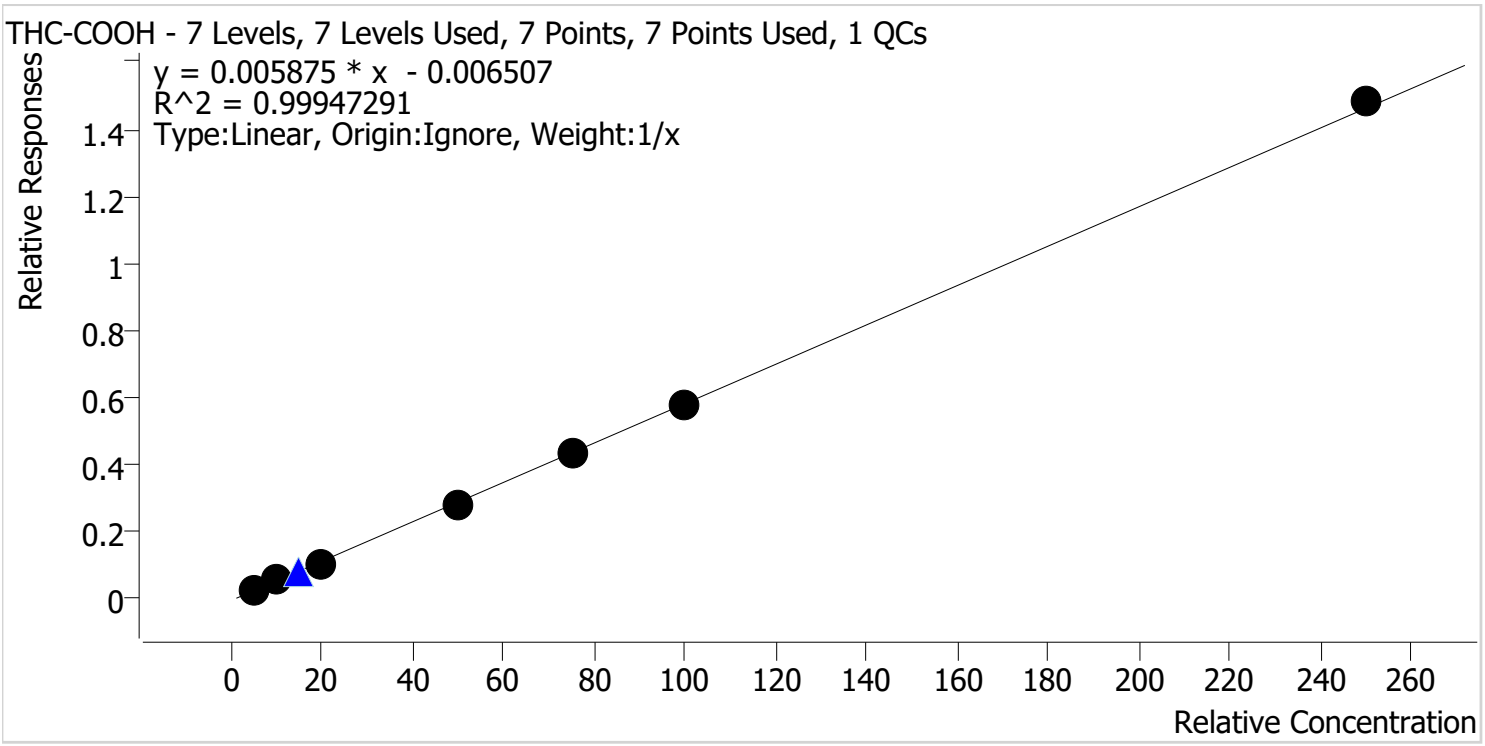
Batch results	D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Last Cal. Update	10/20/2022 9:52 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	119.8
mj cal 2	2	✔	3.0	2.9	96.0
mj cal 3	3	✔	5.0	4.5	91.0
mj cal 4	4	✔	10.0	9.4	94.0
mj cal 5	5	✔	25.0	24.4	97.8
mj cal 6	6	✔	50.0	50.0	99.9
mj cal 7	7	✔	100.0	101.6	101.6

Compound Calibration Report

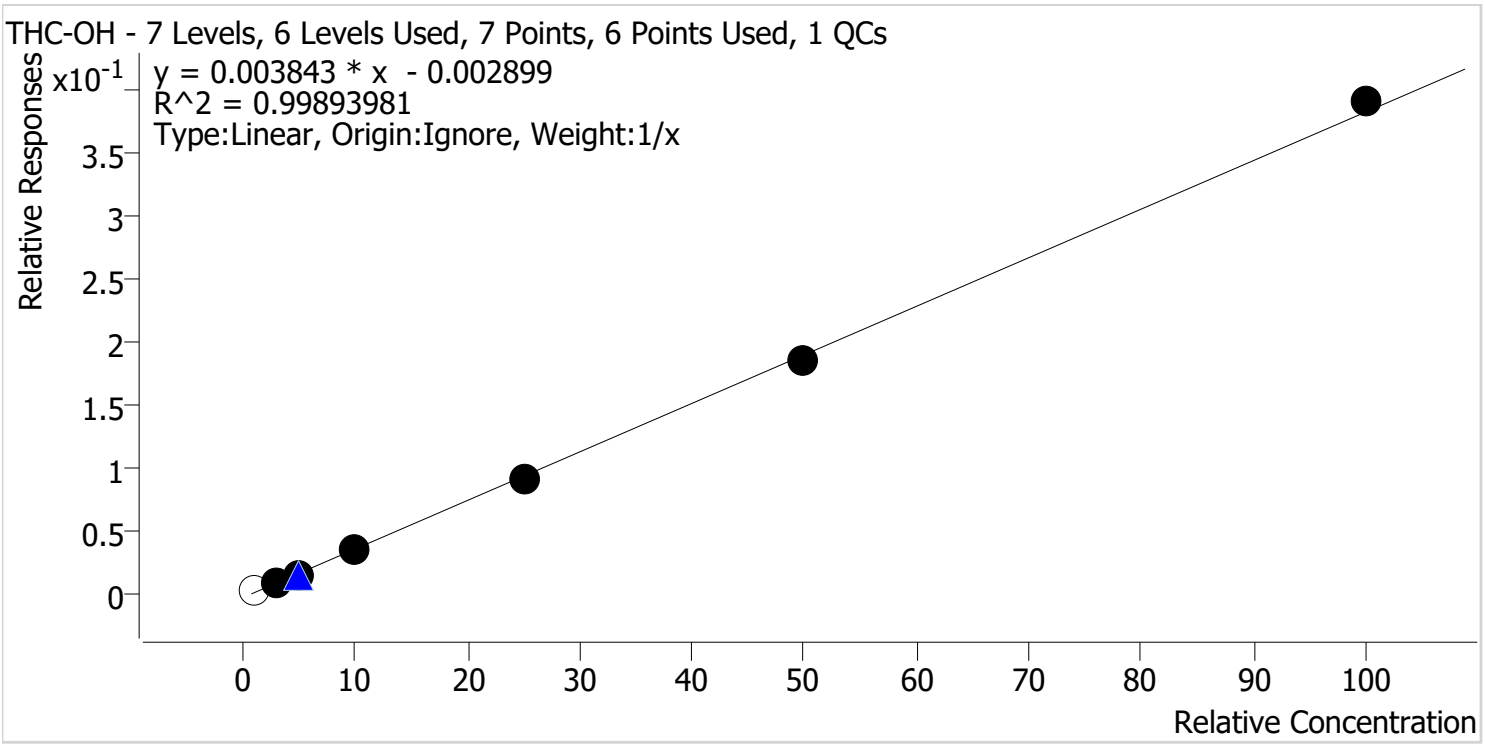
Batch results	D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Last Cal. Update	10/20/2022 9:52 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.5	109.1
mj cal 2	2	✔	10.0	9.9	99.4
mj cal 3	3	✔	20.0	18.9	94.6
mj cal 4	4	✔	50.0	48.4	96.8
mj cal 5	5	✔	75.0	74.4	99.2
mj cal 6	6	✔	100.0	99.6	99.6
mj cal 7	7	✔	250.0	253.3	101.3

Compound Calibration Report

Batch results	D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Last Cal. Update	10/20/2022 9:52 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



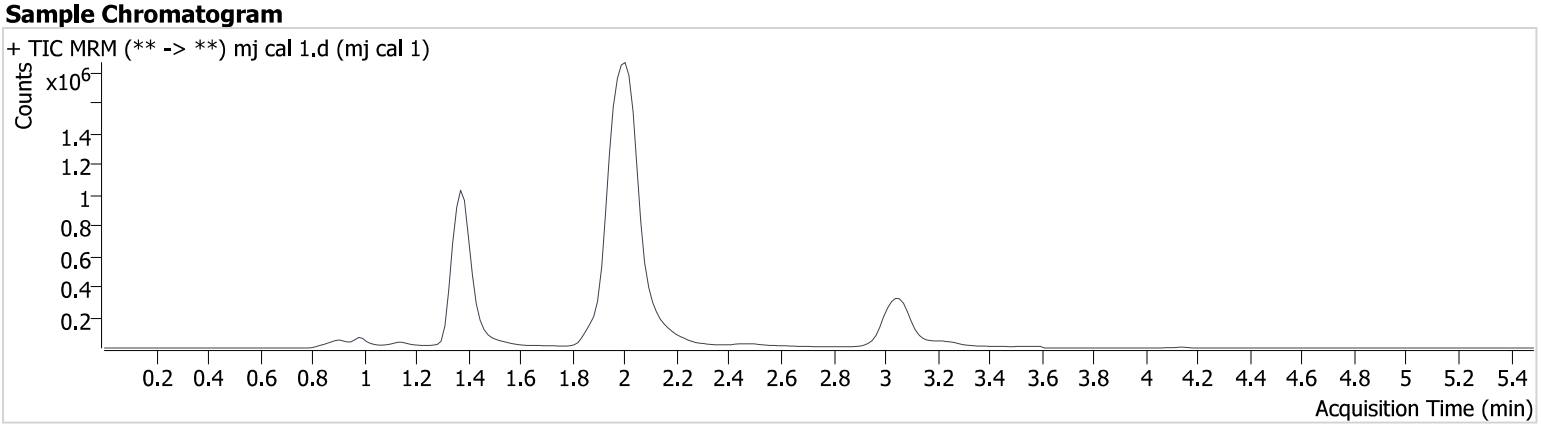
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✗	1.0	1.6	162.8
mj cal 2	2	✔	3.0	3.3	111.1
mj cal 3	3	✔	5.0	4.8	95.0
mj cal 4	4	✔	10.0	9.7	96.8
mj cal 5	5	✔	25.0	24.4	97.5
mj cal 6	6	✔	50.0	48.8	97.5
mj cal 7	7	✔	100.0	102.1	102.1

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj cal 1.d
Type	Cal	**Sample**	mj cal 1
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 6:57:49 PM		
Sample Info.			

Sample Chromatogram



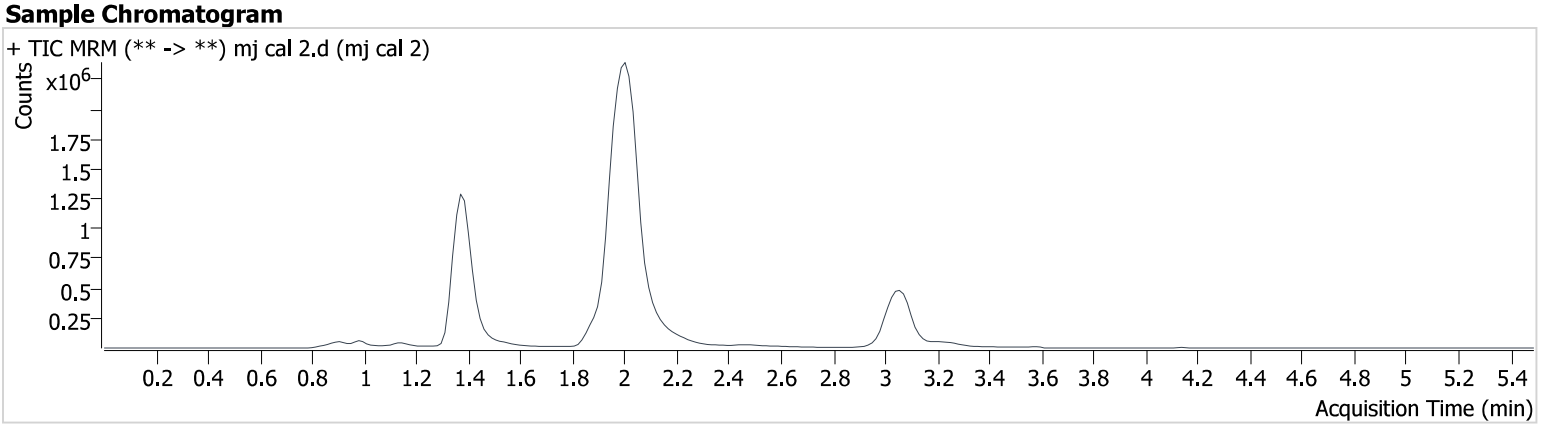
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.379	13219	109.3	1645.5 **High**	∞	3935637	1.628 ng/ml	**Low**
THC-COOH	1.403	22558	∞	259.6	132.3	883097	5.456 ng/ml	
THC	3.061	48764	423.4	30.1	64.1	2150716	1.198 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj cal 2.d
Type	Cal	**Sample**	mj cal 2
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:04:33 PM		
Sample Info.			

Sample Chromatogram



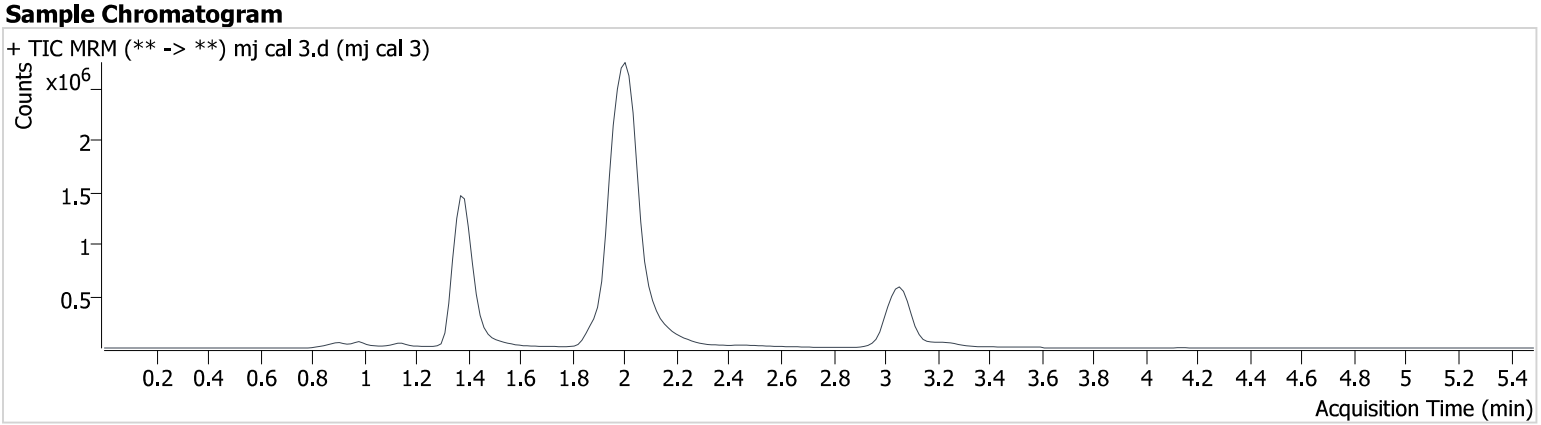
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	43703	2315.7	1087.1	∞	4411522	3.332 ng/ml
THC-COOH	1.403	59768	423.4	257.8	∞	1151353	9.943 ng/ml
THC	3.077	207276	2442.0	23.9	372.7	2893487	2.880 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update	10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj cal 3
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:11:18 PM		
Sample Info.			

Sample Chromatogram



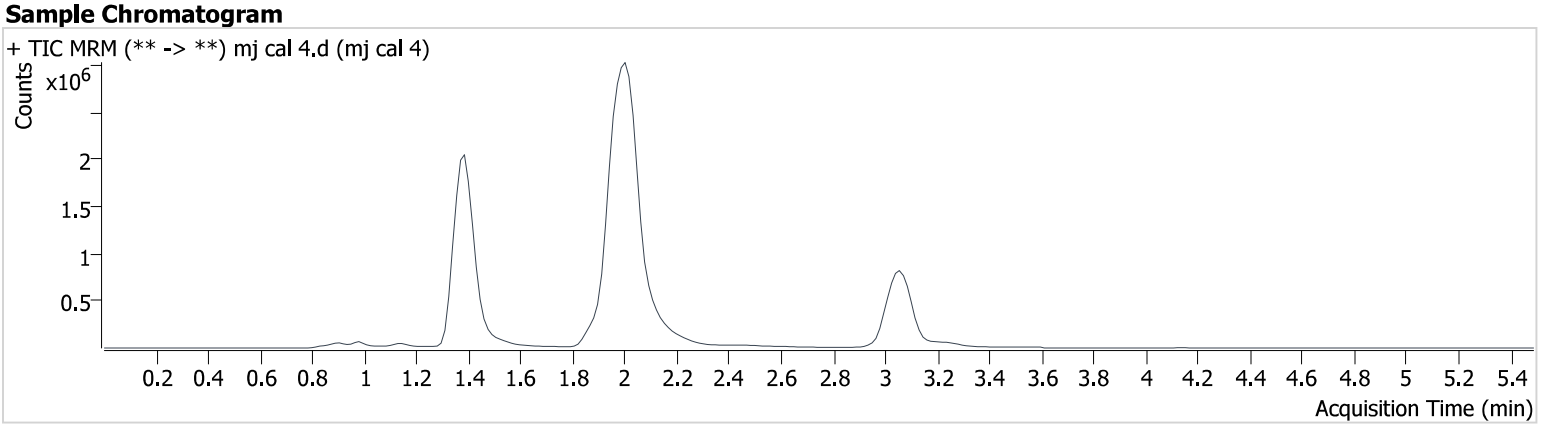
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	70028	∞	1061.5	∞	4560041	4.750 ng/ml
THC-COOH	1.403	128722	1662.3	273.1	∞	1229917	18.921 ng/ml
THC	3.061	391957	2081.3	23.9	457.1	3261397	4.548 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj cal 4.d
Type	Cal	**Sample**	mj cal 4
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:18:02 PM		
Sample Info.			

Sample Chromatogram



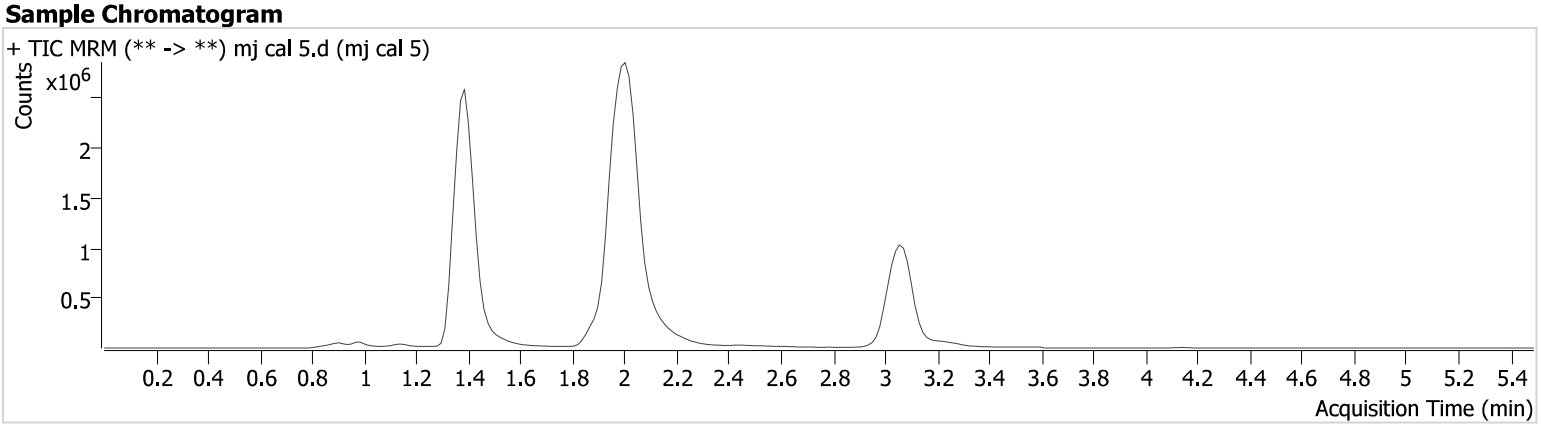
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	172280	∞	907.0	∞	5021383	9.682 ng/ml
THC-COOH	1.403	382140	∞	267.7	∞	1375853	48.383 ng/ml
THC	3.077	1058383	8058.2	23.8	1405.1	4047805	9.403 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:24:47 PM		
Sample Info.			

Sample Chromatogram



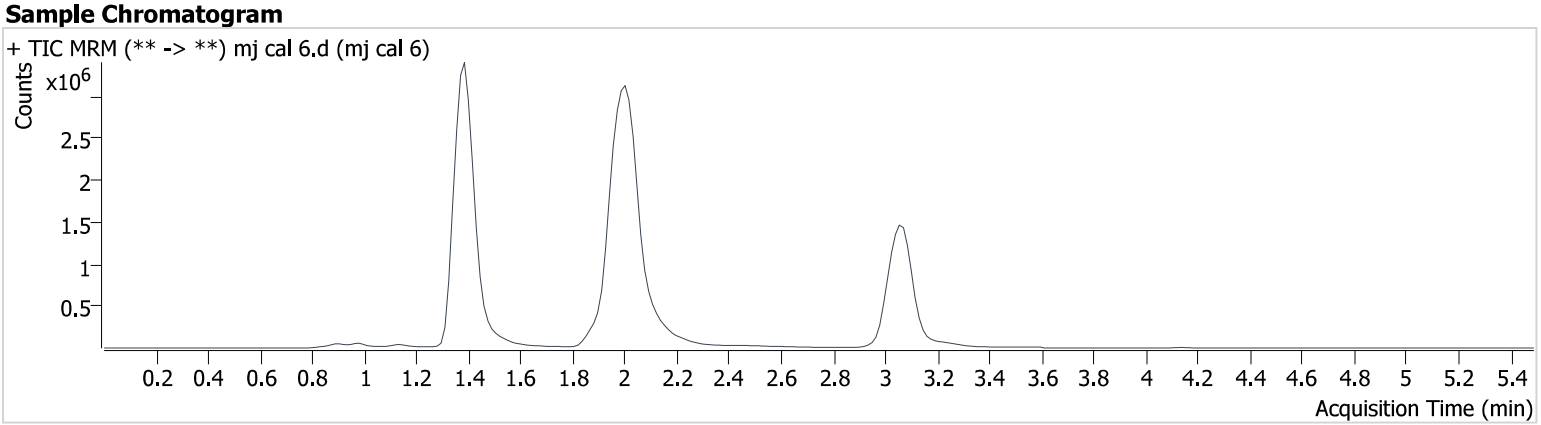
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	416728	∞	852.4	∞	4592058	24.368 ng/ml
THC-COOH	1.403	540385	∞	267.1	∞	1255400	74.374 ng/ml
THC	3.077	2530668	∞	24.7	3261.1	3619877	24.441 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:31:31 PM		
Sample Info.			

Sample Chromatogram



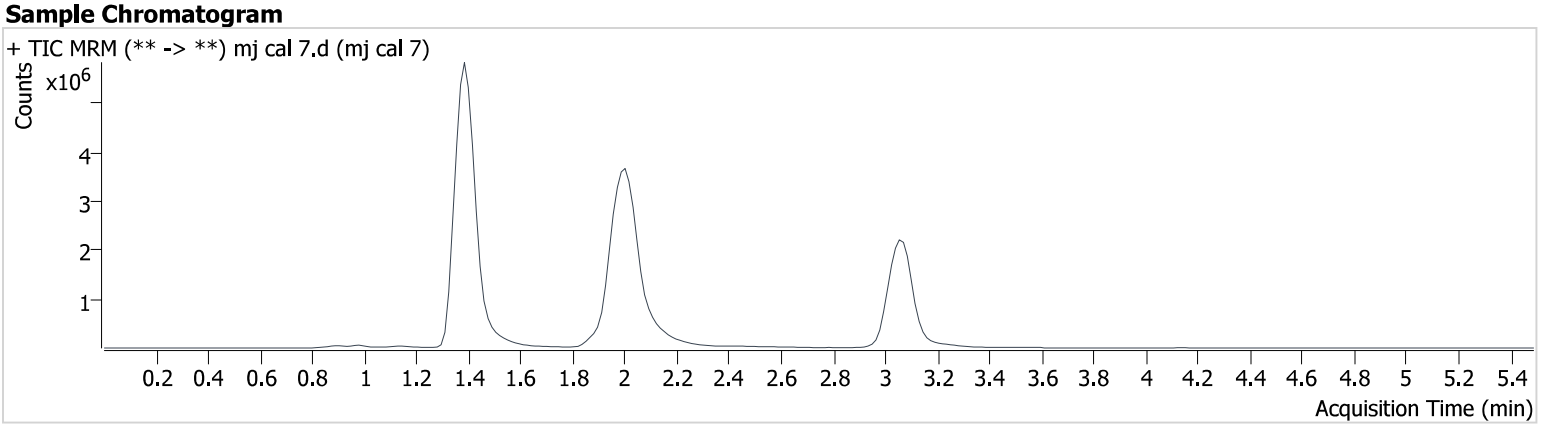
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	813361	∞	833.1	5173.4	4408825	48.759 ng/ml
THC-COOH	1.403	682768	∞	266.4	∞	1180127	99.582 ng/ml
THC	3.061	4945440	∞	24.8	3476.0	3430117	49.960 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\101922\QuantResults\cann.batch.bin
Calibration Last Update 10/20/2022 9:52:17 AM

Instrument	69679	**Data File**	mj cal 7.d
Type	Cal	**Sample**	mj cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/19/2022 7:38:15 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	1608598	64427.9	811.8	∞	4129855	102.108 ng/ml
THC-COOH	1.403	1614582	∞	261.9	11335.3	1089529	253.341 ng/ml
THC	3.077	9129665	195053.7	24.8	9160.3	3101382	101.570 ng/ml