3/30/2022

REVIEWED
By Britany Wylie at 7:59 am, Mar 31, 2022

Worklist: 5727

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2022-0438	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0553	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0587	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0614	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0638	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 3/30/22 Analyst: Anne Nord
Plate lot#: 211018 Plate re-test: 4/18/22

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: 22B52020 **Urine Blank:** **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: 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
☒ 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: *Blood only run*
Curve Limits: THC-OH 3-100
THC reported as less then 3 for values between 1 and 3.

	1	2	3	4	5	6
a	cal 1	Internal control				
b	cal 2	negative blood				
c	cal 3	553-1				
d	cal 4	587-1				
e	Cal 5	614-1				
f	cal 6	638-1				
g	cal 7	438-1				
h	Internal control (blood)					

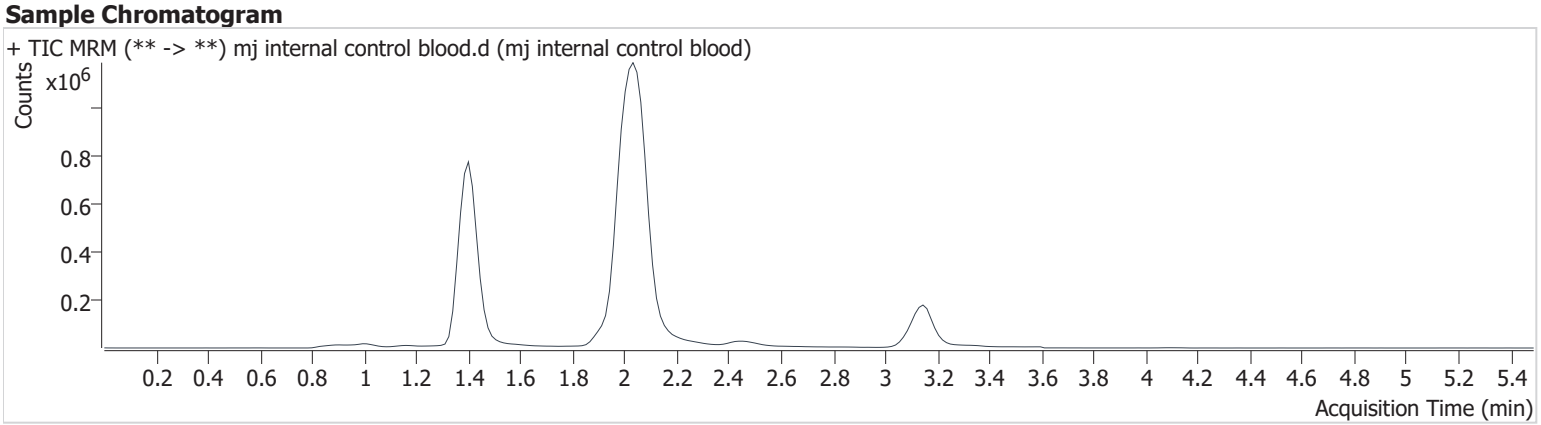
c2022-0____-_

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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	3/30/2022 11:46:20 AM		
Sample Info.			

Sample Chromatogram



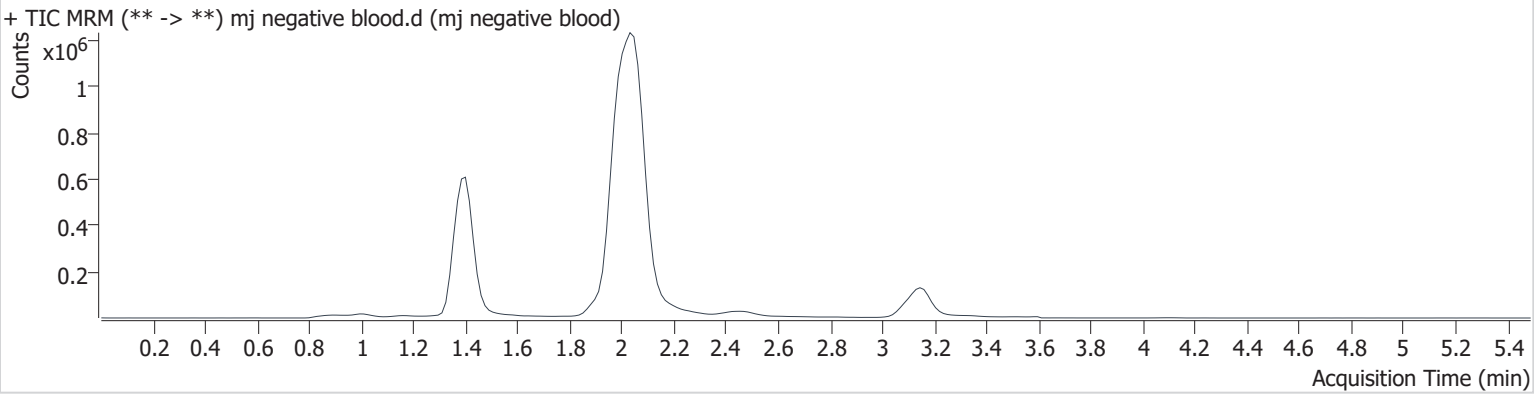
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	322589	∞	12.6	∞	2375978	5.440 ng/ml
THC-COOH	1.431	151963	482.0	35.6	226.1	695576	15.331 ng/ml
THC	3.152	109046	∞	24.4	∞	920042	4.592 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update	3/30/2022 1:39:43 PM

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	3/30/2022 11:53:04 AM		
Sample Info.			

Sample Chromatogram

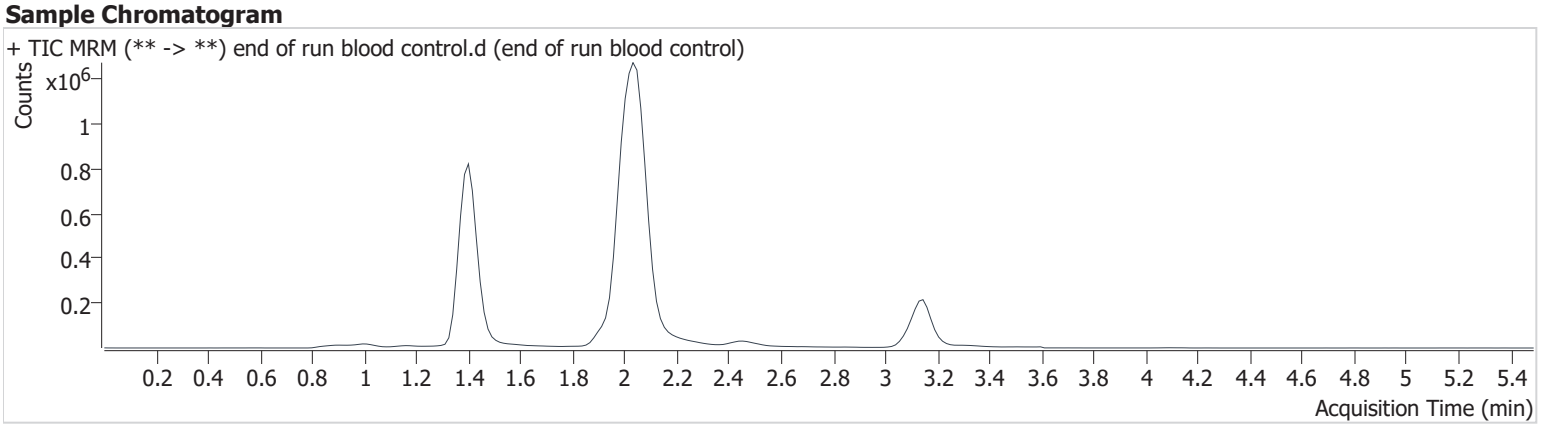


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	3/30/2022 1:06:35 PM		
Sample Info.			

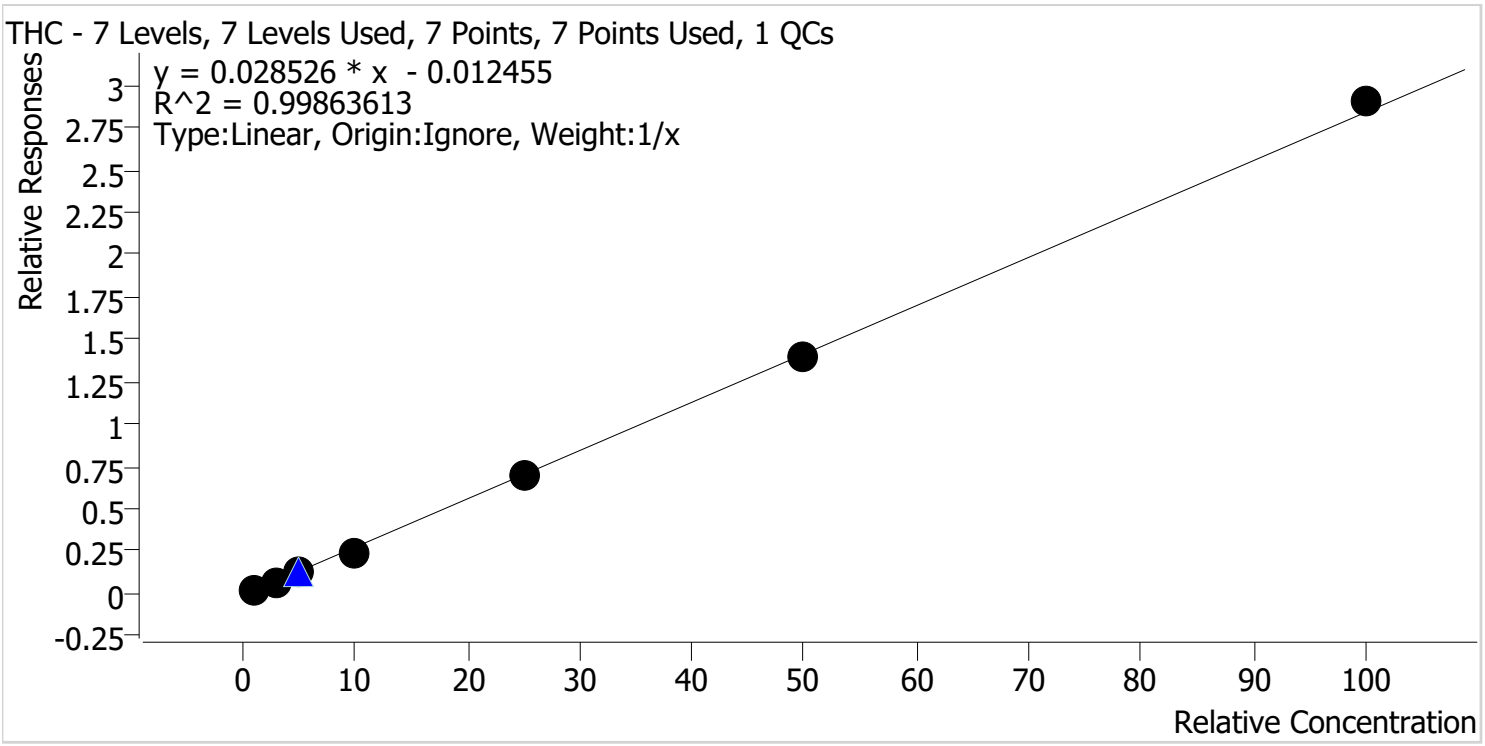
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	321279	∞	13.8	∞	2464780	5.244 ng/ml
THC-COOH	1.431	163929	311.1	35.2	103.3	738621	15.562 ng/ml
THC	3.152	124003	∞	25.2	∞	1063115	4.526 ng/ml

Compound Calibration Report

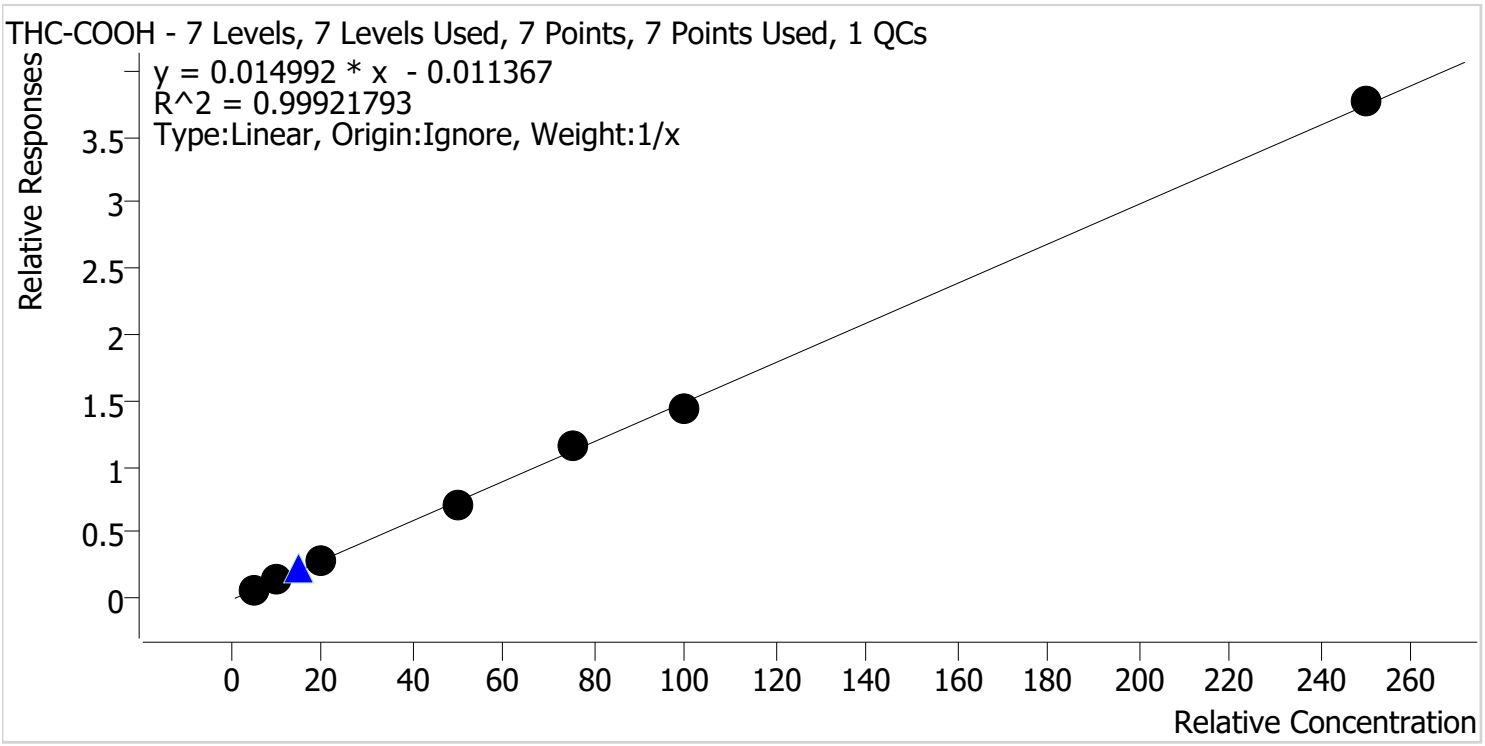
Batch results	D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Last Cal. Update	3/30/2022 1:39 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	120.6
mj cal2	2	✔	3.0	2.9	97.7
mj cal 3	3	✔	5.0	4.6	91.1
mj cal 4	4	✔	10.0	9.1	90.9
mj cal 5	5	✔	25.0	24.7	98.7
mj cal 6	6	✔	50.0	49.3	98.7
mj cal 7	7	✔	100.0	102.2	102.2

Compound Calibration Report

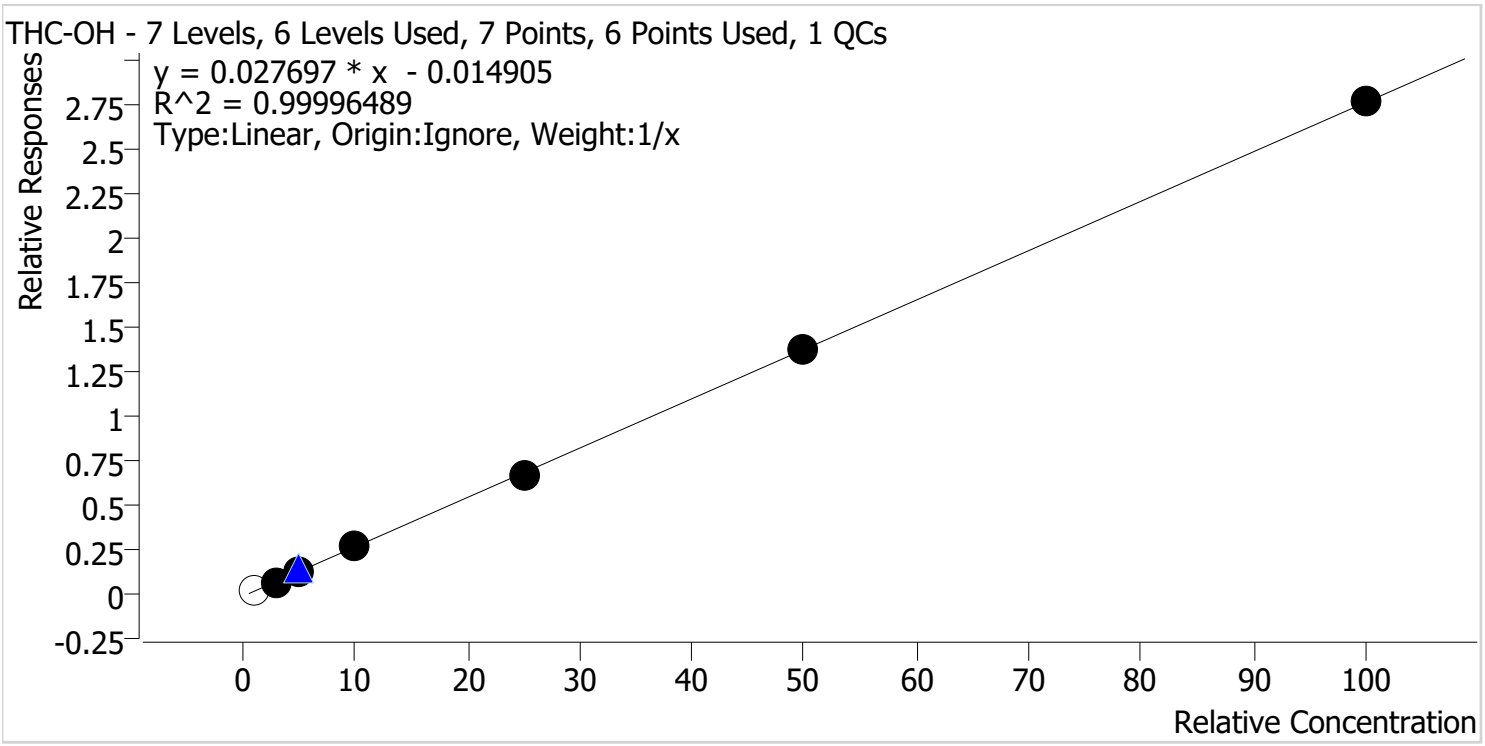
Batch results	D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Last Cal. Update	3/30/2022 1:39 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.1	102.9
mj cal2	2	✔	10.0	10.2	102.0
mj cal 3	3	✔	20.0	19.3	96.3
mj cal 4	4	✔	50.0	48.5	97.0
mj cal 5	5	✔	75.0	78.0	104.0
mj cal 6	6	✔	100.0	97.0	97.0
mj cal 7	7	✔	250.0	251.9	100.7

Compound Calibration Report

Batch results	D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Last Cal. Update	3/30/2022 1:39 PM
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	1.3	133.1
mj cal2	2	✔	3.0	3.1	101.8
mj cal 3	3	✔	5.0	4.9	98.9
mj cal 4	4	✔	10.0	10.0	99.9
mj cal 5	5	✔	25.0	24.7	98.9
mj cal 6	6	✔	50.0	50.1	100.3
mj cal 7	7	✔	100.0	100.1	100.1

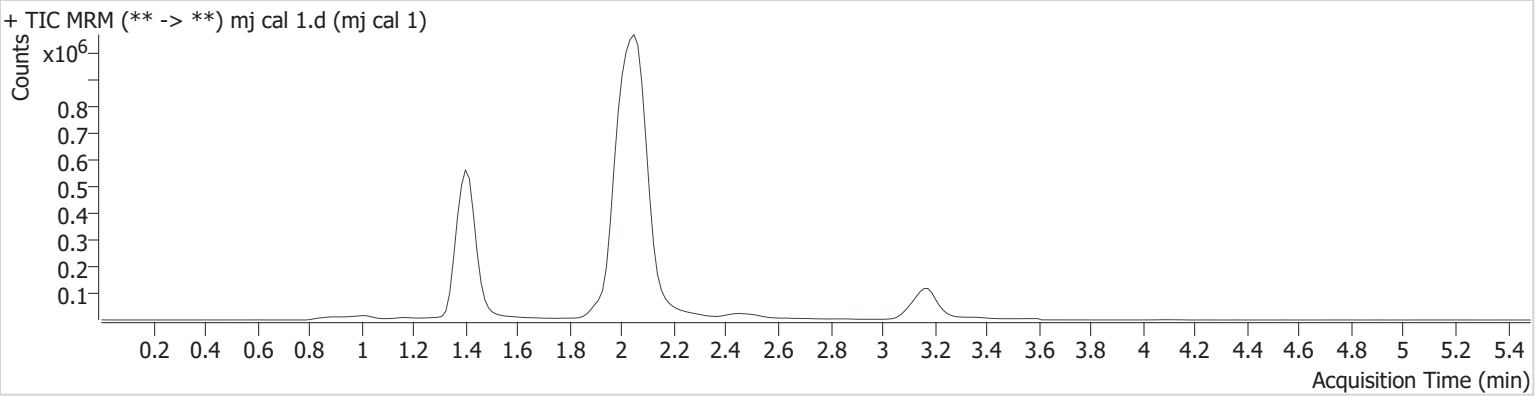
dropped cal 1 due to ratio being out of range.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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	3/30/2022 10:52:41 AM		
Sample Info.			

Sample Chromatogram



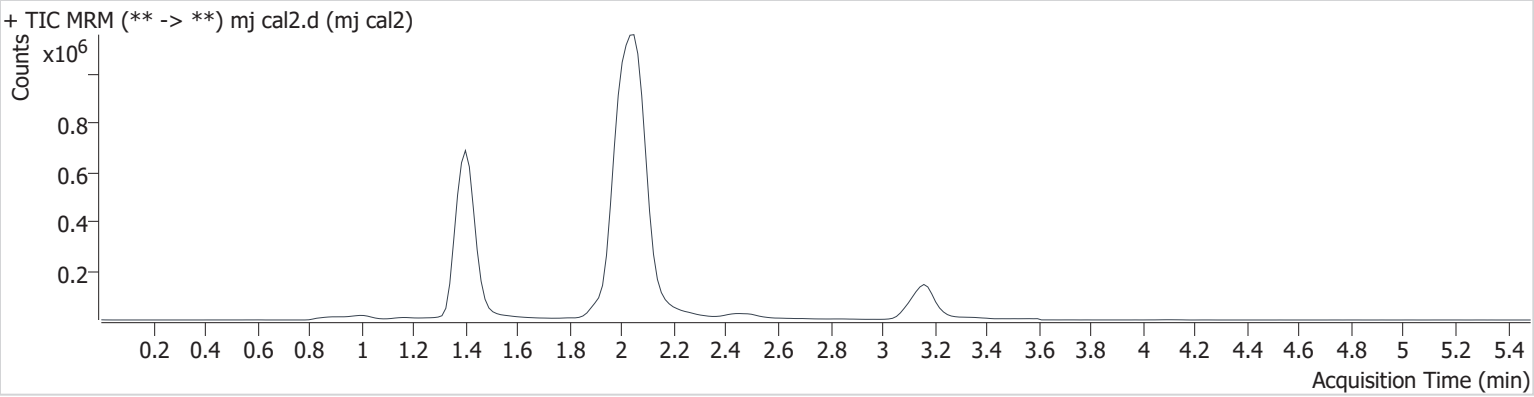
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.406	46712	∞	21.4 **High**	139.0	2128417	1.331 ng/ml	**Low**
THC-COOH	1.431	40260	137.7	36.6	66.6	612252	5.144 ng/ml	
THC	3.182	16264	∞	28.5	26.2	740977	1.206 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

Instrument	69679	**Data File**	mj cal2.d
Type	Cal	**Sample**	mj cal2
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	3/30/2022 10:59:25 AM		
Sample Info.			

Sample Chromatogram



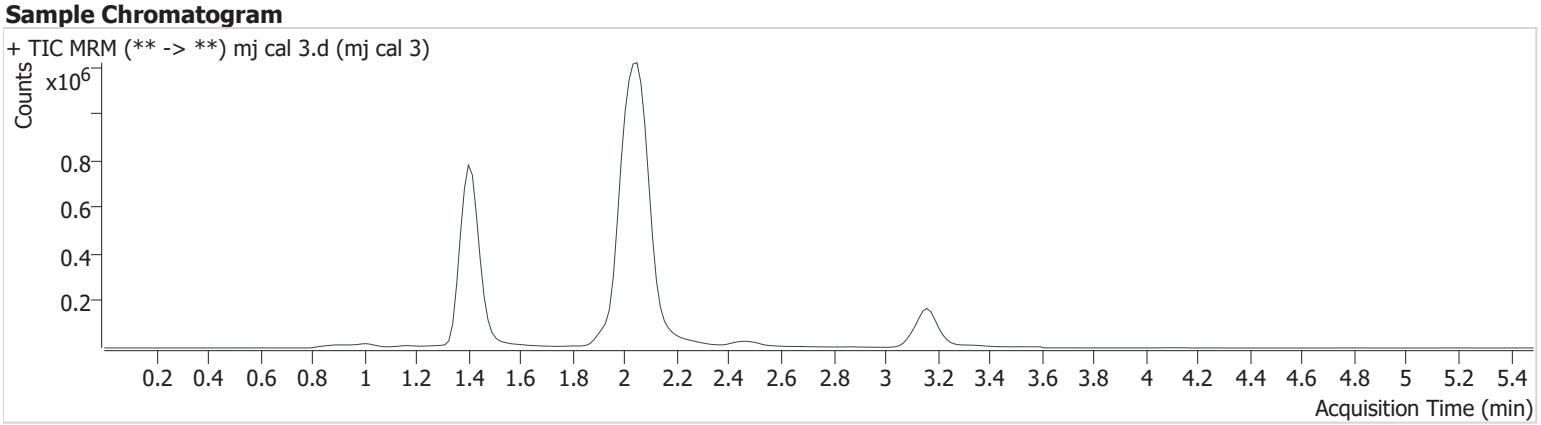
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	171260	∞	13.0	∞	2457894	3.054 ng/ml
THC-COOH	1.431	96026	571.1	32.9	194.1	678128	10.204 ng/ml
THC	3.182	59730	∞	29.1	1439.5	838951	2.932 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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	3/30/2022 11:06:09 AM		
Sample Info.			

Sample Chromatogram



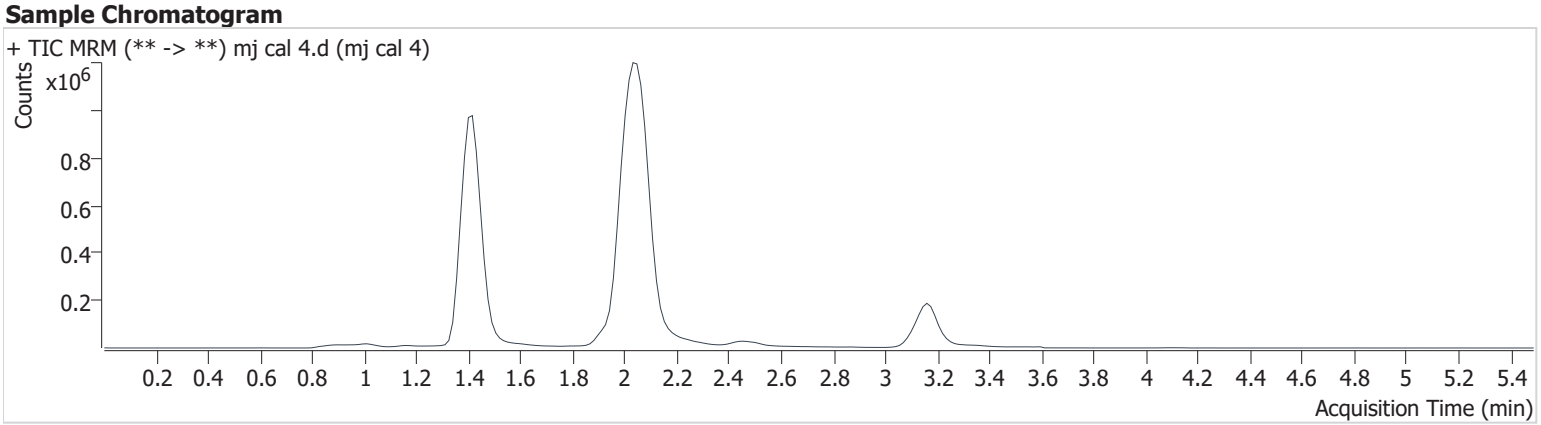
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	297207	∞	13.5	∞	2435048	4.945 ng/ml
THC-COOH	1.431	189824	12407.1	37.1	249.9	684518	19.256 ng/ml
THC	3.167	103666	∞	26.7	∞	882547	4.554 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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	3/30/2022 11:12:53 AM		
Sample Info.			

Sample Chromatogram



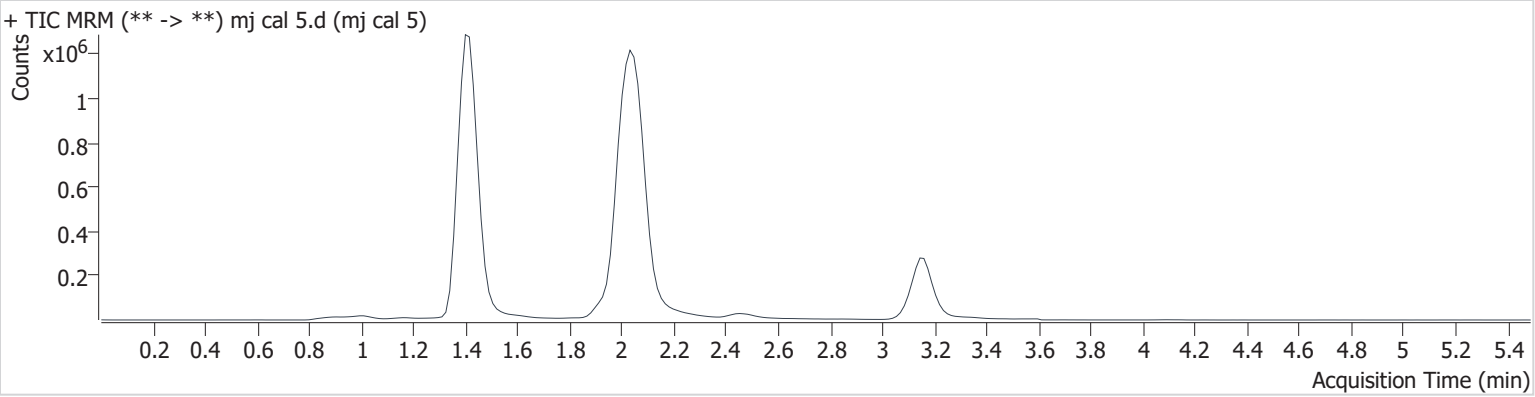
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	635724	∞	11.8	∞	2427110	9.995 ng/ml
THC-COOH	1.431	494253	1310.8	38.5	929.9	690272	48.520 ng/ml
THC	3.167	211899	∞	25.2	∞	858060	9.094 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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	3/30/2022 11:19:35 AM		
Sample Info.			

Sample Chromatogram



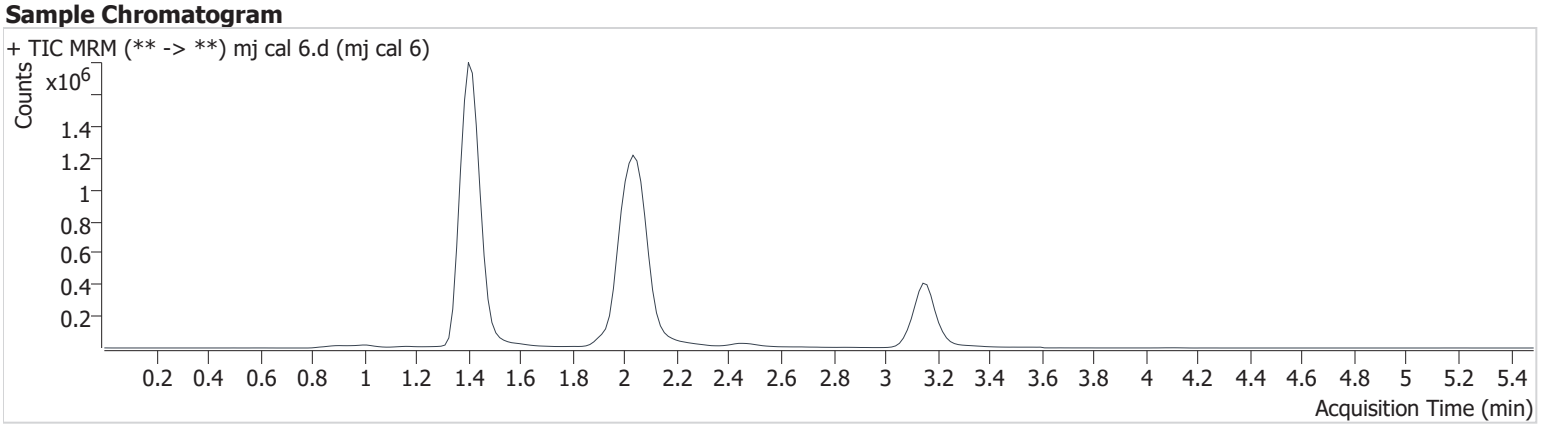
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1550741	∞	12.1	∞	2313942	24.735 ng/ml
THC-COOH	1.431	726474	1937.1	37.9	22707	627384	77.997 ng/ml
					9.6		
THC	3.167	604840	∞	24.2	∞	874361	24.686 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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	3/30/2022 11:26:18 AM		
Sample Info.			

Sample Chromatogram



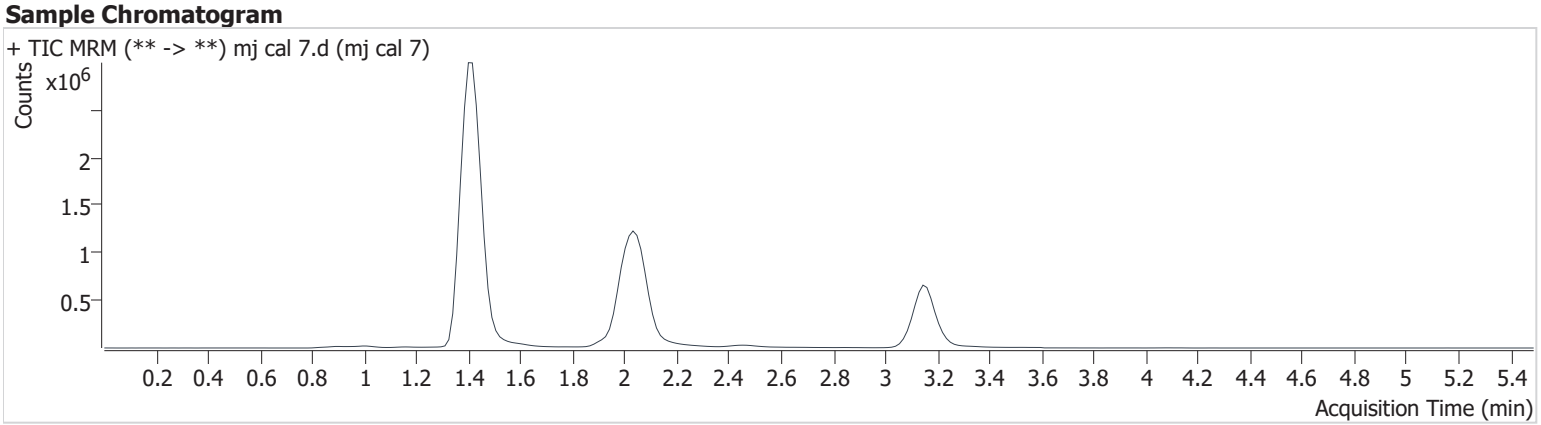
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	3255713	∞	12.4	6164.1	2369472	50.147 ng/ml
THC-COOH	1.431	954171	354.7	38.9	2462.3	661220	97.014 ng/ml
THC	3.167	1266959	∞	24.0	∞	908027	49.349 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\033022\QuantResults\cann.batch.bin
Calibration Last Update 3/30/2022 1:39:43 PM

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	3/30/2022 11:33:00 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	6239158	∞	12.5	∞	2262010	100.124 ng/ml
THC-COOH	1.431	2246332	8136.0	39.0	10862.2	596711	251.865 ng/ml
THC	3.152	2522578	∞	24.5	∞	869176	102.178 ng/ml