1/13/2022

REVIEWED
By Britany Wylie at 3:13 pm, Jan 13, 2022

Worklist: 5512

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
C2021-2770	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-2790		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-2793		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-0006		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-0012		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-0032		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2022-0045		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 1/12/22 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: 21D52496 **Urine Blank:** 83121 **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: *THC-OH not evaluated in this run, ratios indicate an interferant.*

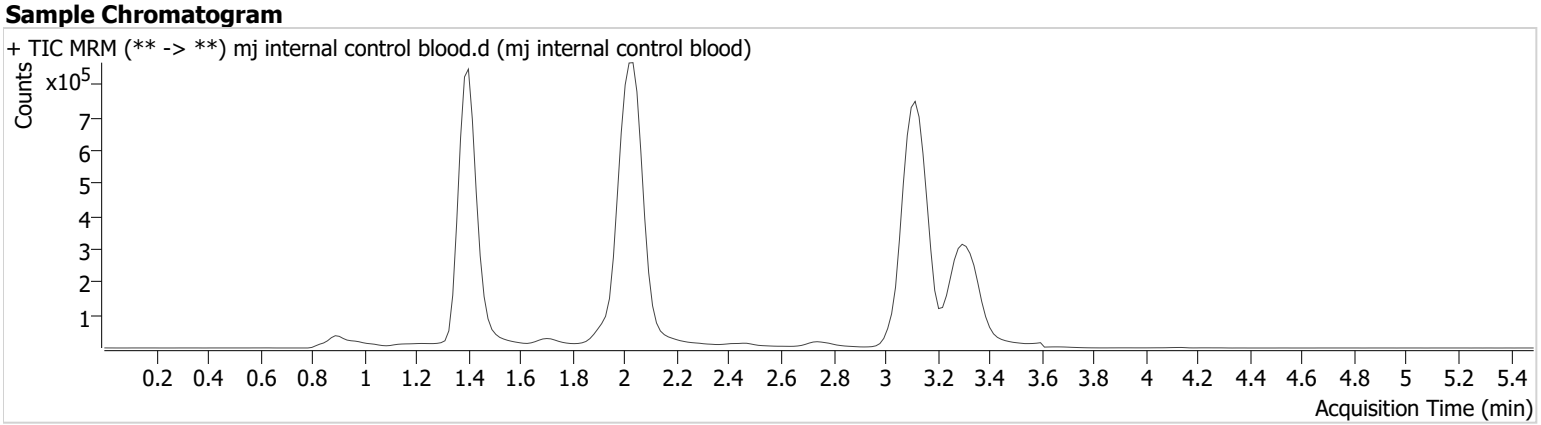
	1	2	3	4	5	6
a	cal 1	Internal control (urine)	c2021-2790-1			
b	cal 2	negative blood	c2022-0032-1			
c	cal 3	c2021-2793-1				
d	cal 4	c2022-0006-1				
e	Cal 5	c2022-0012-1				
f	cal 6	c2022-0045-1				
g	cal 7	negative urine				
h	Internal control (blood)	c2021-2770-3				

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 12:35:21 PM		
Sample Info.			

Sample Chromatogram



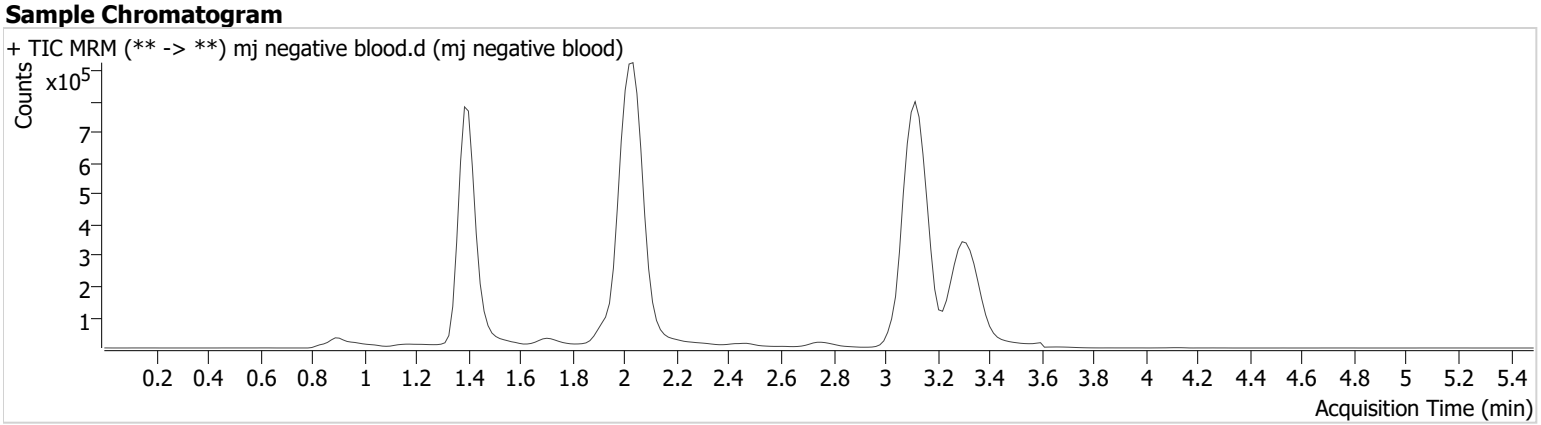
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	46813	∞	1191.8	∞	2437701	5.355 ng/ml
THC-COOH	1.416	167772	466.5	34.0	238.3	751463	16.170 ng/ml
THC	3.137	418729	∞	24.6	50842 68000 1341.8	3564313	4.656 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 12:42:05 PM		
Sample Info.			

Sample Chromatogram



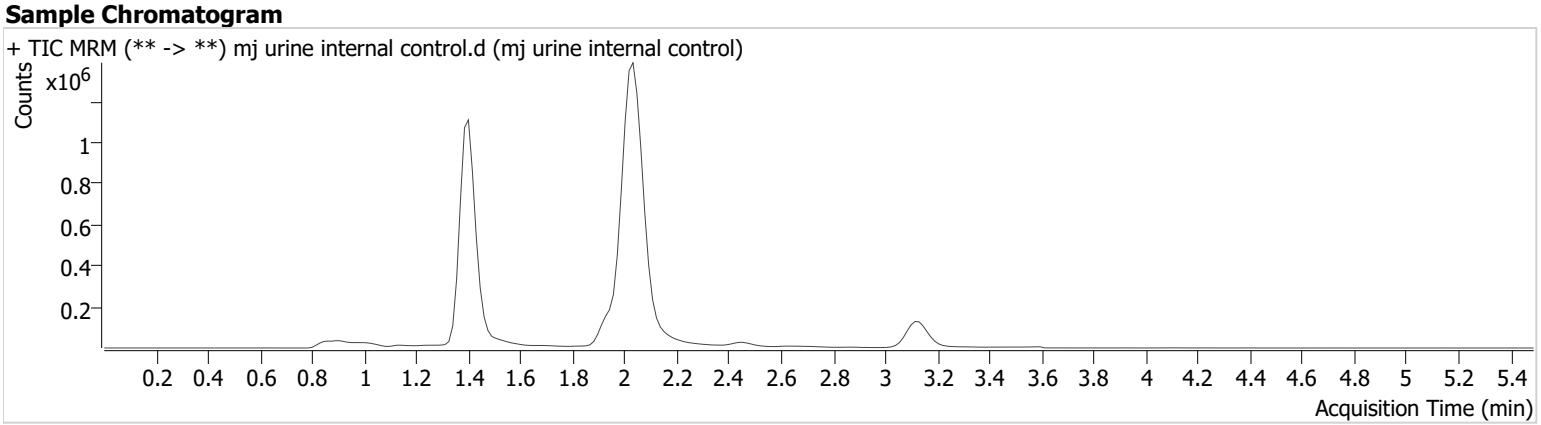
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.454	6801	∞	1600.3 **High**	∞	2714699	0.468 ng/ml	Low

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 PM

Instrument	69679	**Data File**	mj urine internal control.d
Type	Sample	**Sample**	mj urine internal control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	1/12/2022 1:42:16 PM		
Sample Info.			

Sample Chromatogram



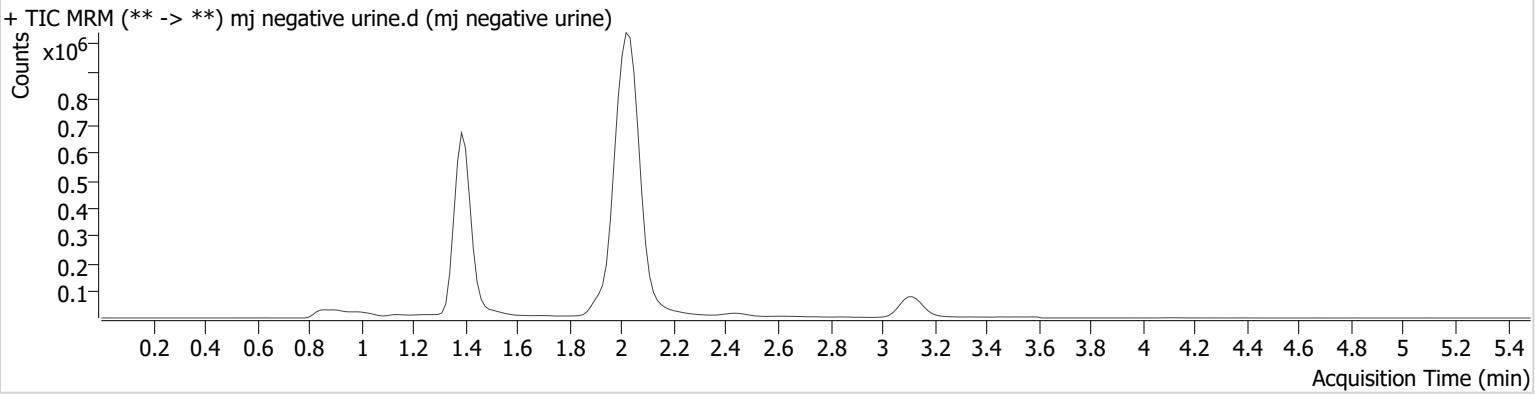
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	60185	∞	868.6	∞	3389819	4.931 ng/ml
THC-COOH	1.431	141706	446.7	32.8	378.7	578276	17.668 ng/ml
THC	3.137	75725	1695957797 3703.5	26.5	488.5	664892	4.524 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 PM

Instrument	69679	**Data File**	mj negative urine.d
Type	Sample	**Sample**	mj negative urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-G2	**Comment**	
Injection Volume	10		
Acq. Date-Time	1/12/2022 1:48:58 PM		
Sample Info.			

Sample Chromatogram

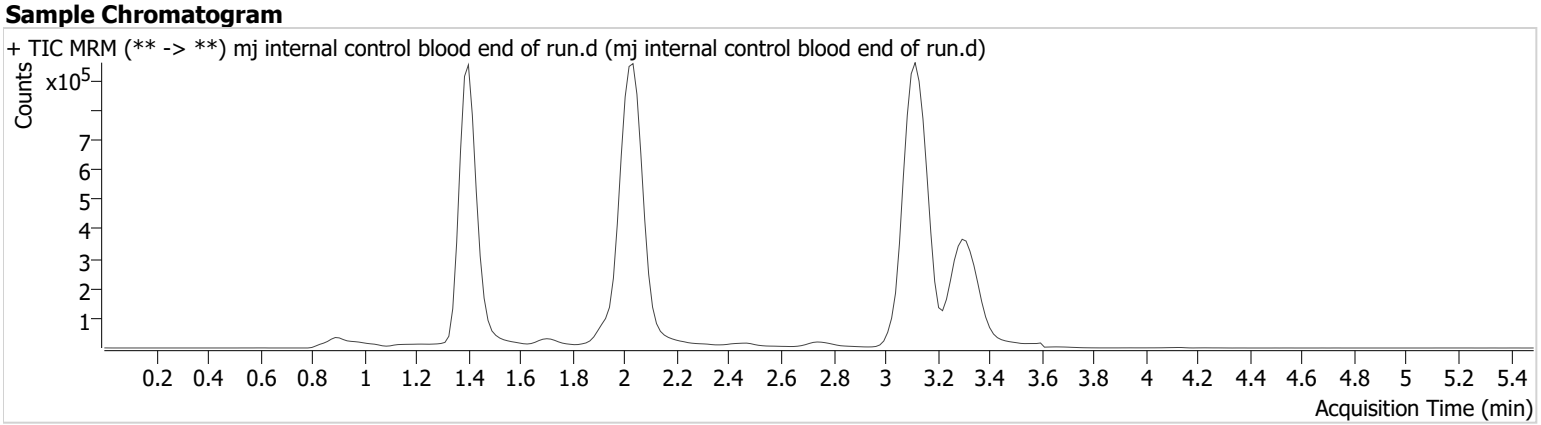


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 PM

Instrument	69679	**Data File**	mj internal control blood end of run.d
Type	QC	**Sample**	mj internal control blood end of run.d
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	1/12/2022 3:08:58 PM		
Sample Info.			

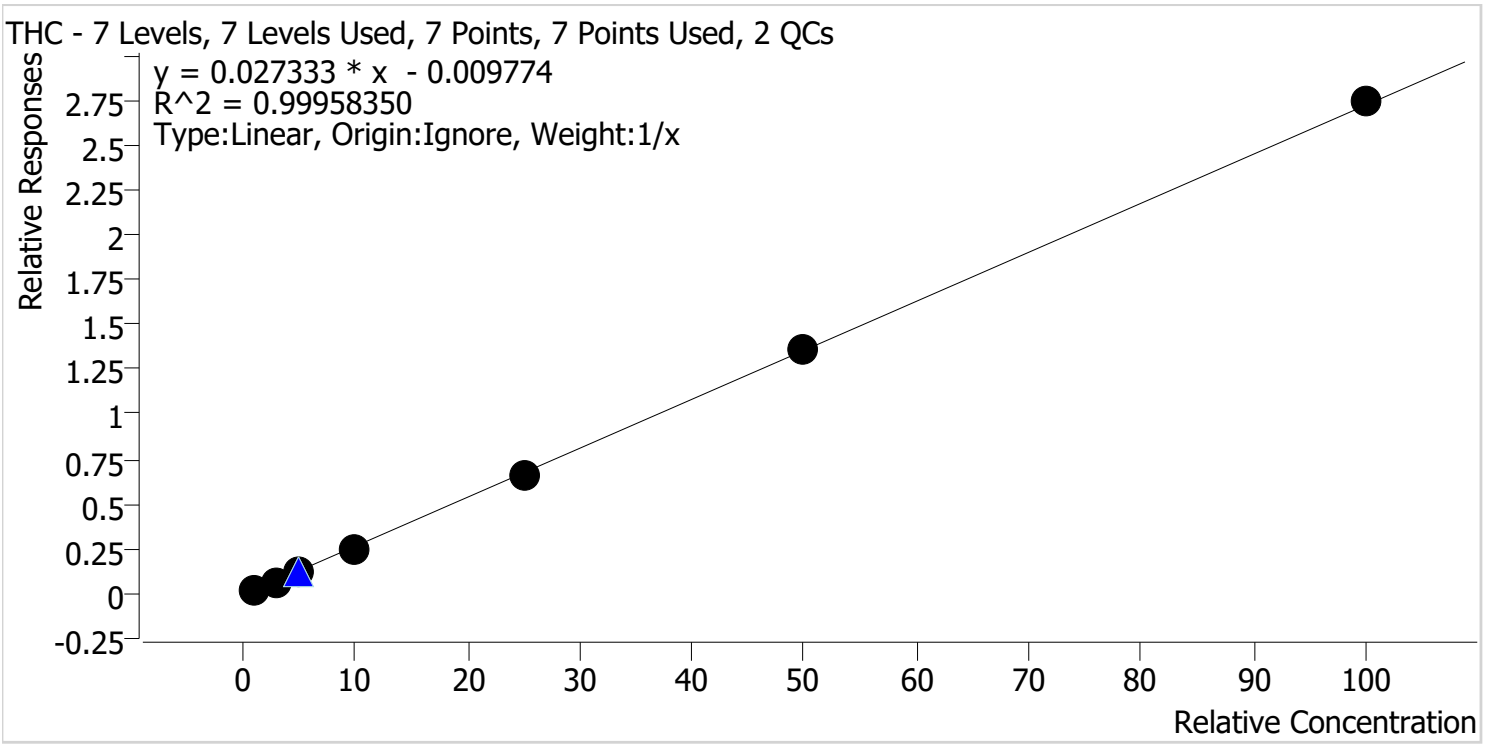
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	48096	∞	1208.2 **High**	∞	2604917	5.138 ng/ml
THC-COOH	1.416	175926	206.3	35.4	244.8	832862	15.343 ng/ml
THC	3.137	542134	∞	24.3	3191.1	4548848	4.718 ng/ml

Compound Calibration Report

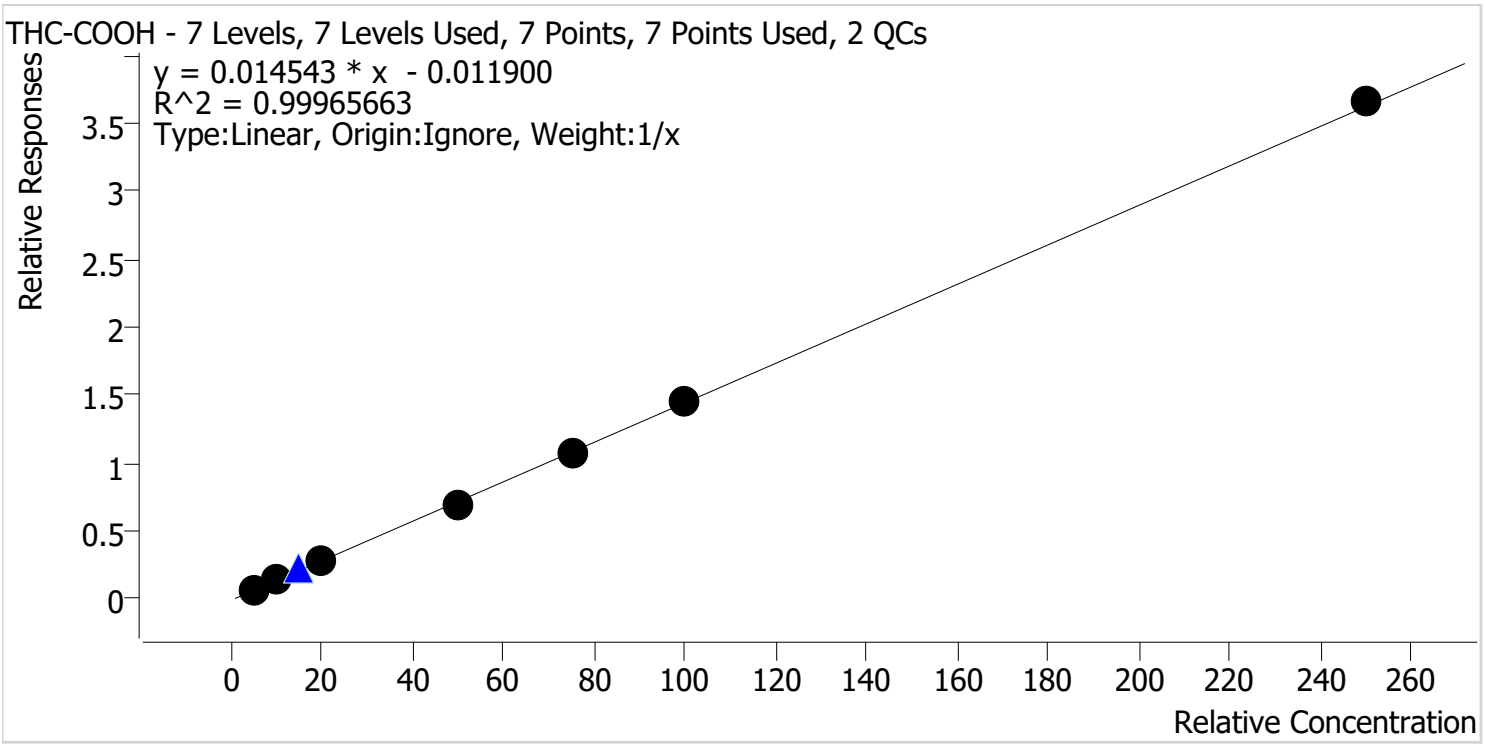
Batch results	D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Last Cal. Update	1/12/2022 3:20 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.1	114.5
mj cal2	2	✔	3.0	2.9	95.2
mj cal 3	3	✔	5.0	4.7	94.0
mj cal 4	4	✔	10.0	9.6	96.1
mj cal 5	5	✔	25.0	24.8	99.1
mj cal 6	6	✔	50.0	50.2	100.4
mj cal 7	7	✔	100.0	100.7	100.7

Compound Calibration Report

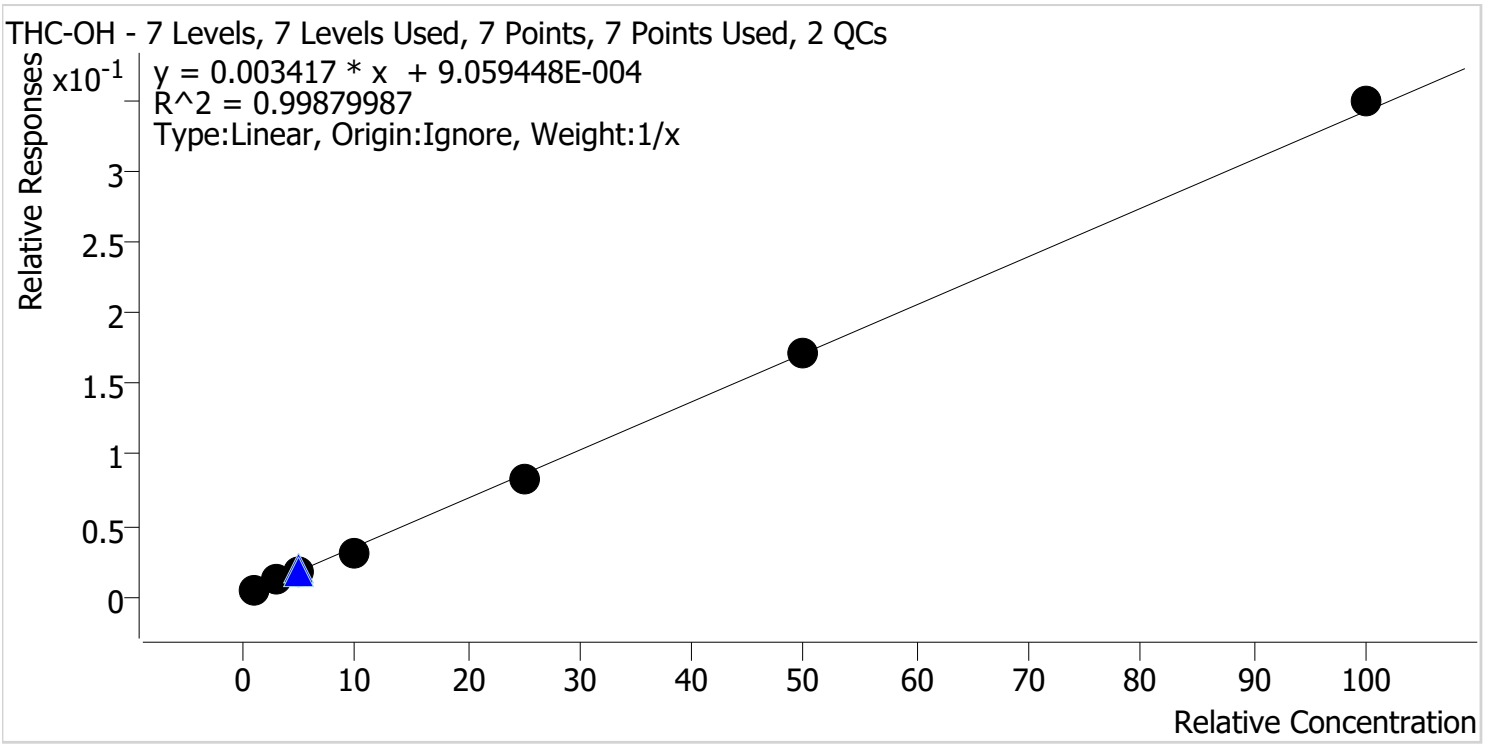
Batch results	D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Last Cal. Update	1/12/2022 3:20 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.3	105.6
mj cal2	2	✔	10.0	9.9	99.3
mj cal 3	3	✔	20.0	19.7	98.7
mj cal 4	4	✔	50.0	48.1	96.2
mj cal 5	5	✔	75.0	74.0	98.6
mj cal 6	6	✔	100.0	100.5	100.5
mj cal 7	7	✔	250.0	252.5	101.0

Compound Calibration Report

Batch results	D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Last Cal. Update	1/12/2022 3:20 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	1.1	114.9
mj cal2	2	✔	3.0	3.0	101.3
mj cal 3	3	✔	5.0	4.8	95.2
mj cal 4	4	✔	10.0	9.0	90.1
mj cal 5	5	✔	25.0	24.2	96.7
mj cal 6	6	✔	50.0	49.9	99.8
mj cal 7	7	✔	100.0	102.0	102.0

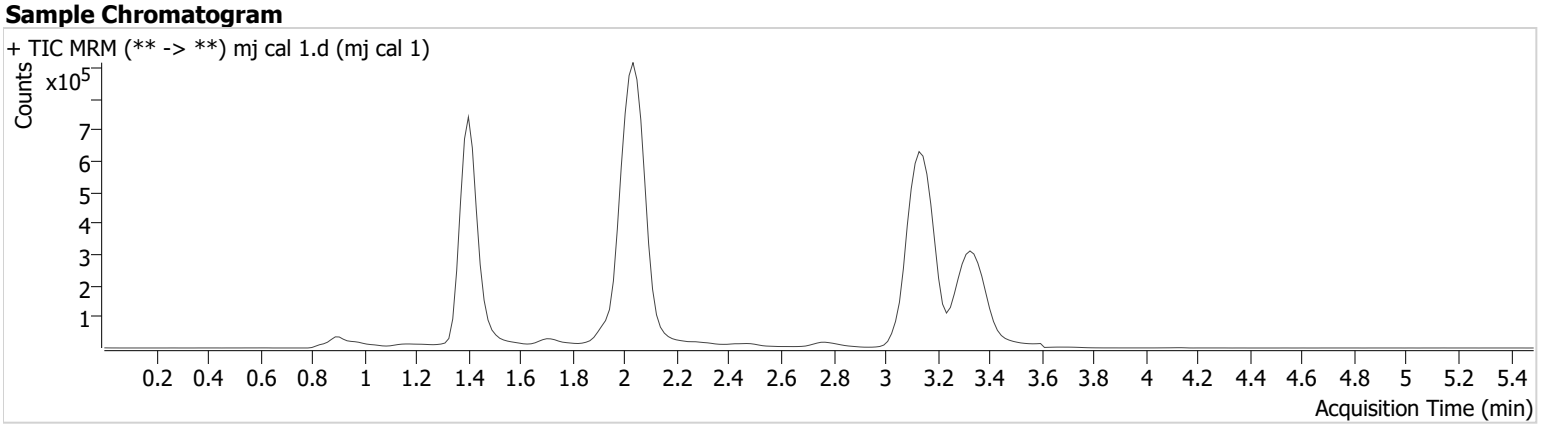
Not evaluated

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 11:41:46 AM		
Sample Info.			

Sample Chromatogram



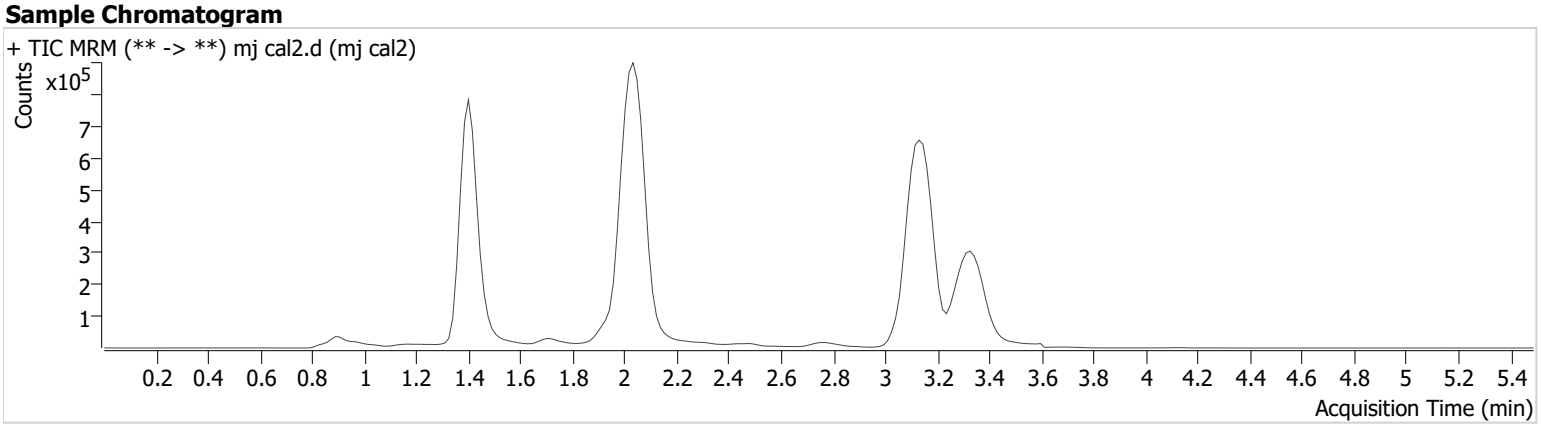
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.409	11877	∞	2236.1 **High**	∞	2458138	1.149 ng/ml	Low
THC-COOH	1.431	46172	135.5	37.1	370.4	711232	5.282 ng/ml	
THC	3.167	73850	∞	25.1	294.1	3433497	1.145 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 11:48:31 AM		
Sample Info.			

Sample Chromatogram



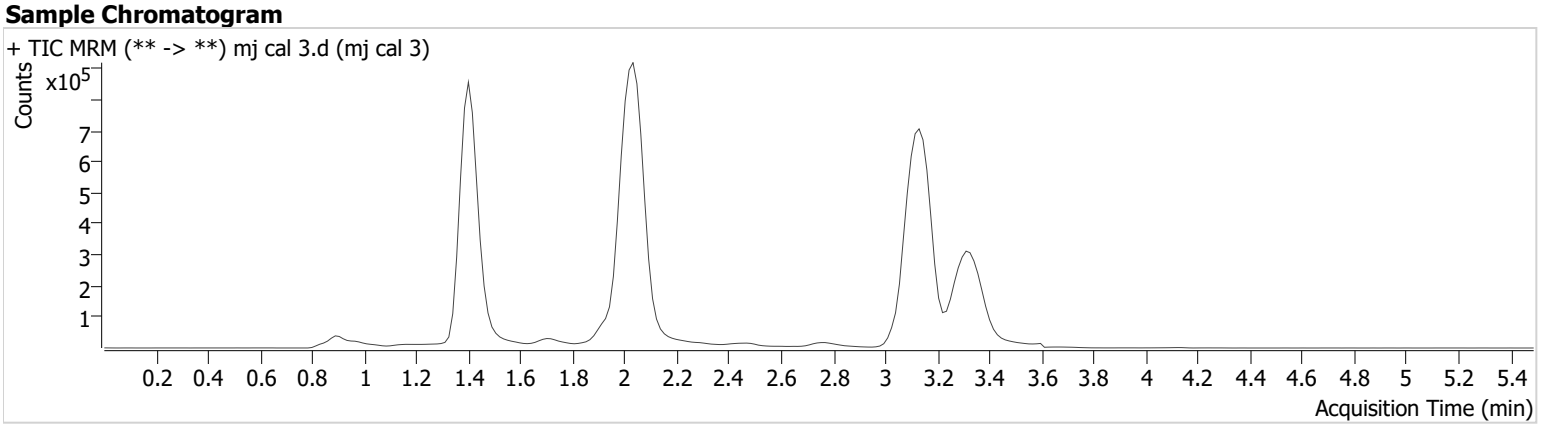
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	27587	∞	1439.3 **High**	∞	2442479	3.040 ng/ml
THC-COOH	1.431	93968	337.0	36.4	55.8	708809	9.934 ng/ml
THC	3.152	231009	36866.5	25.1	1076.7	3382812	2.856 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 11:55:13 AM		
Sample Info.			

Sample Chromatogram



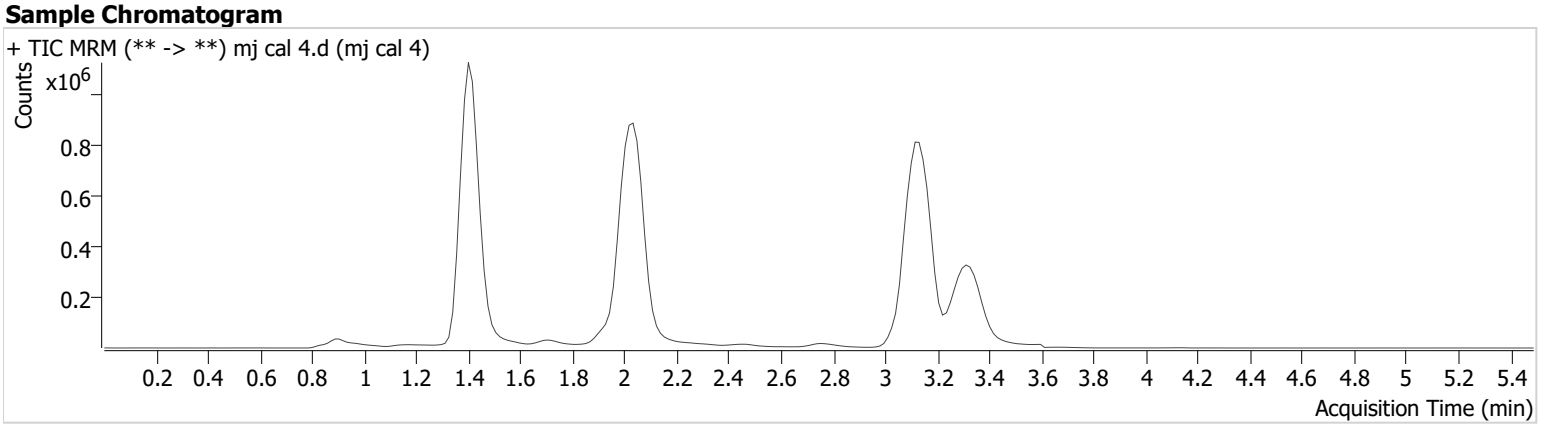
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	41147	∞	1281.9 **High**	∞	2396011	4.761 ng/ml
THC-COOH	1.431	197446	169.4	34.7	151.2	717544	19.739 ng/ml
THC	3.152	407128	4618471638 55621.0	23.1	1375.3	3428688	4.702 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 12:01:55 PM		
Sample Info.			

Sample Chromatogram



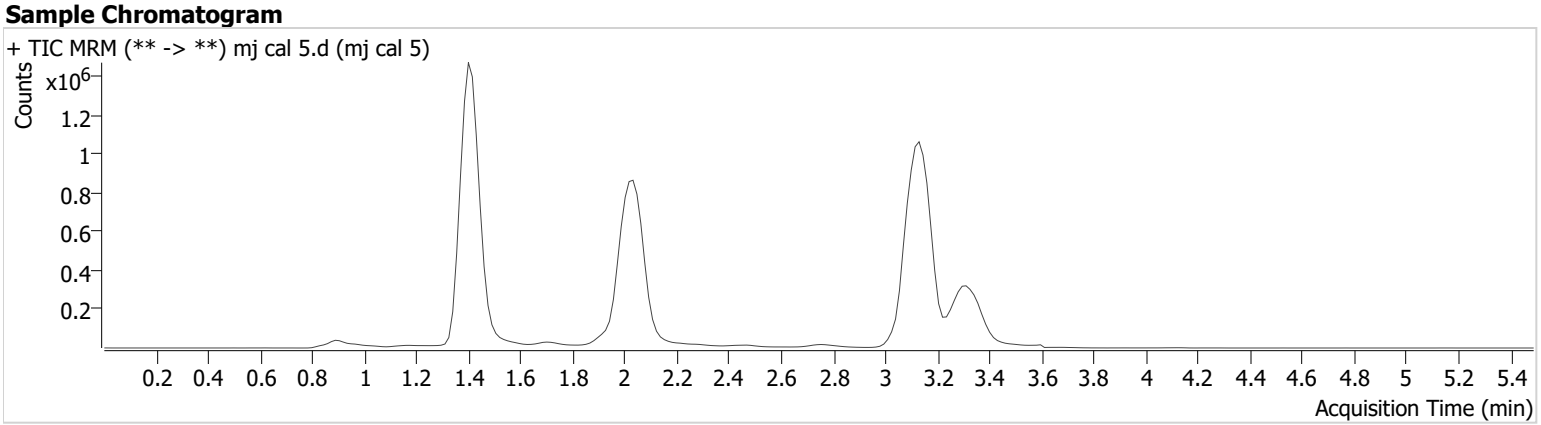
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	79535	∞	1139.1	∞	2510238	9.008 ng/ml
THC-COOH	1.431	516754	719.4	37.9	2098.4	751425	48.106 ng/ml
THC	3.152	900426	∞	22.7	∞	3560556	9.610 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 12:08:37 PM		
Sample Info.			

Sample Chromatogram



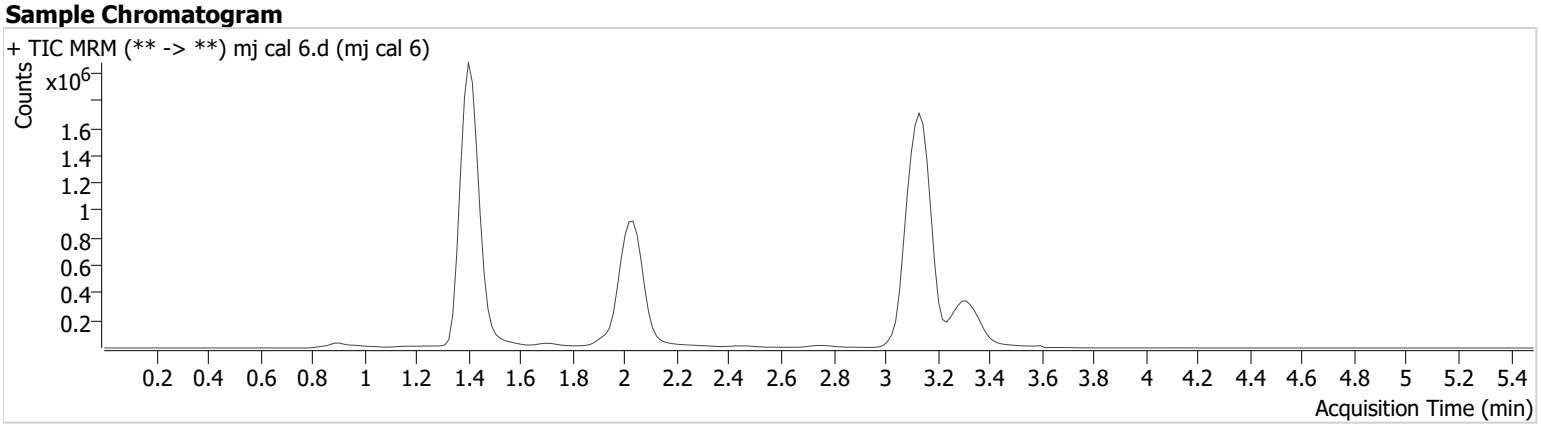
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	202323	∞	969.9	∞	2421822	24.184 ng/ml
THC-COOH	1.431	773516	2774.2	37.8	3371.2	727139	73.966 ng/ml
THC	3.152	2291536	∞	23.7	∞	3434828	24.766 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 12:15:19 PM		
Sample Info.			

Sample Chromatogram



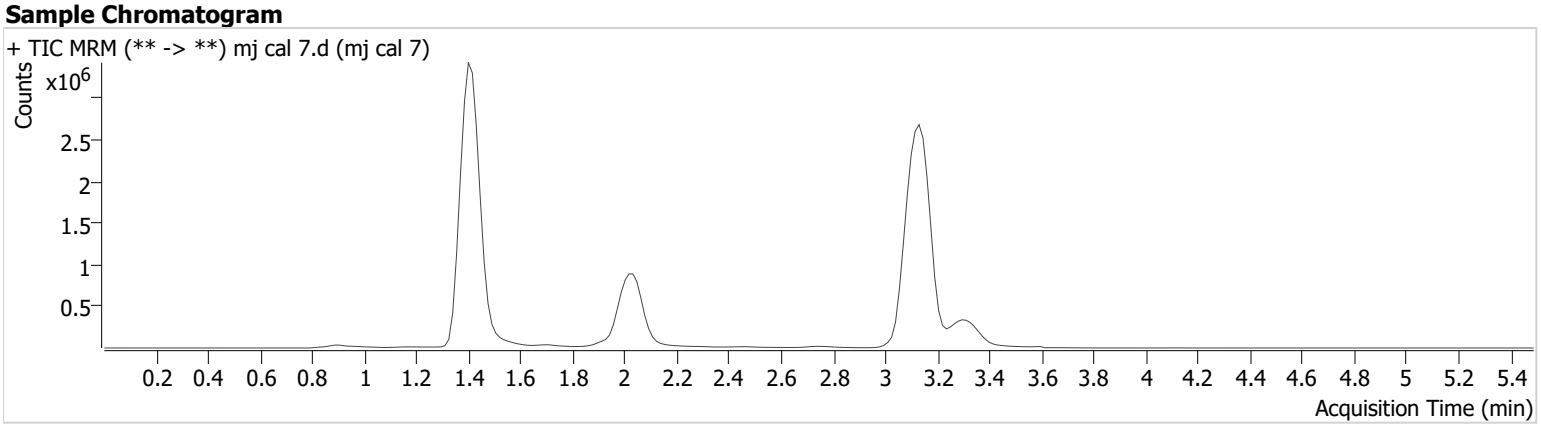
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	424473	∞	900.8	∞	2477158	49.883 ng/ml
THC-COOH	1.431	1043642	10625.2	37.8	302.3	719951	100.495 ng/ml
THC	3.152	5186164	∞	24.7	∞	3805085	50.223 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\011222\QuantResults\cann.batch.bin
Calibration Last Update 1/12/2022 3:20:10 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	1/12/2022 12:22:03 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	827584	∞	858.5	∞	2368938	101.975 ng/ml
THC-COOH	1.431	2396980	1635.4	38.9	2037.5	654933	252.478 ng/ml
THC	3.136	10359047	∞	24.4	∞	3777114	100.698 ng/ml