REVIEWED
By Sarah Collins at 1:40 pm, Nov 18, 2021

11/17/2021

Worklist: 5391

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2021-2399		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2434		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-2435	4	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-2443		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-2459		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2461		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 11/17/21 Analyst: Anne Nord
Plate lot#: 210609 Plate Expiration: 12-9-21

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 3-100*

	1	2	3	4	5	6
a	cal 1	neg blood	2443-1			
b	cal 2	2399-1				
c	cal 3	2459-1				
d	cal 4	2461-1				
e	Cal 5	neg urine				
f	cal 6	Urine positive				
g	cal 7	2434-1				
h	Internal control	2435-4				

C2021-____-_

Toxicology AM method 27/26 external prep informati

working solution 15 ug/ml in meoh C-THC, THC-OH, 7.5 ug/ml THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH
Ppd 8/26/21 Exp: 8/26/22 lot 82621 by AMN

Drug	lot	expiration
C-THC	FE04151901	6/1/2024
THC-OH	FE06152002	6/1/2025
THC	FE04222001	5/1/2025

AM 27/26 blood control 100 ul working solution lot () in 9900 ul blood lot ()

		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	

AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine **out of use**

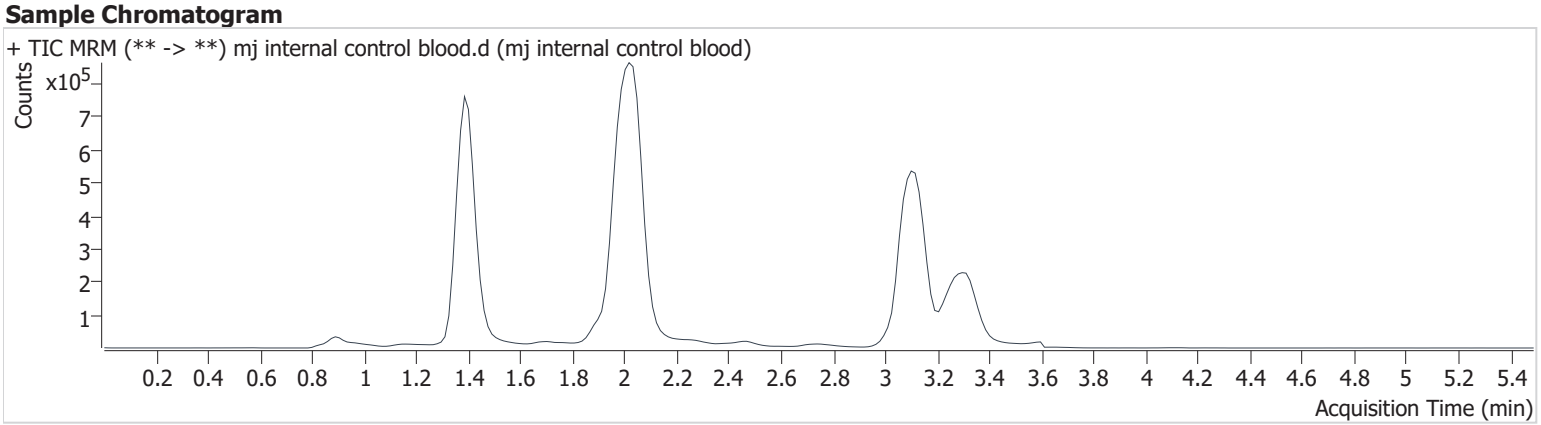
ppd 8/26/21 Exp 8/26/22 neg urine lot 5621	lot u82621	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	last used 11/1/21
ppd 11/2/21 Exp 8/26/22 neg urine lot 83121	lot u11221	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 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	11/17/2021 2:53:25 PM		
Sample Info.			

Sample Chromatogram



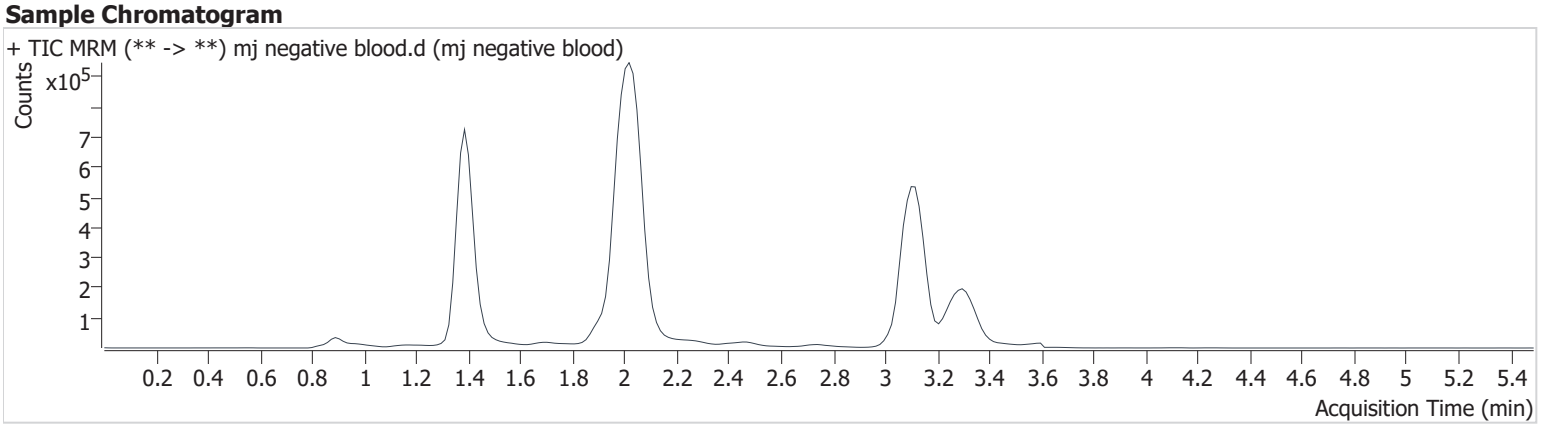
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	36920	∞	1072.3	2638.1	2259917	5.247 ng/ml
THC-COOH	1.416	140872	166.3	34.9	307.8	737766	14.970 ng/ml
THC	3.137	326369	∞	23.5	1376.7	2655260	4.666 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update	11/18/2021 8:04:14 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-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/17/2021 3:00:08 PM		
Sample Info.			

Sample Chromatogram

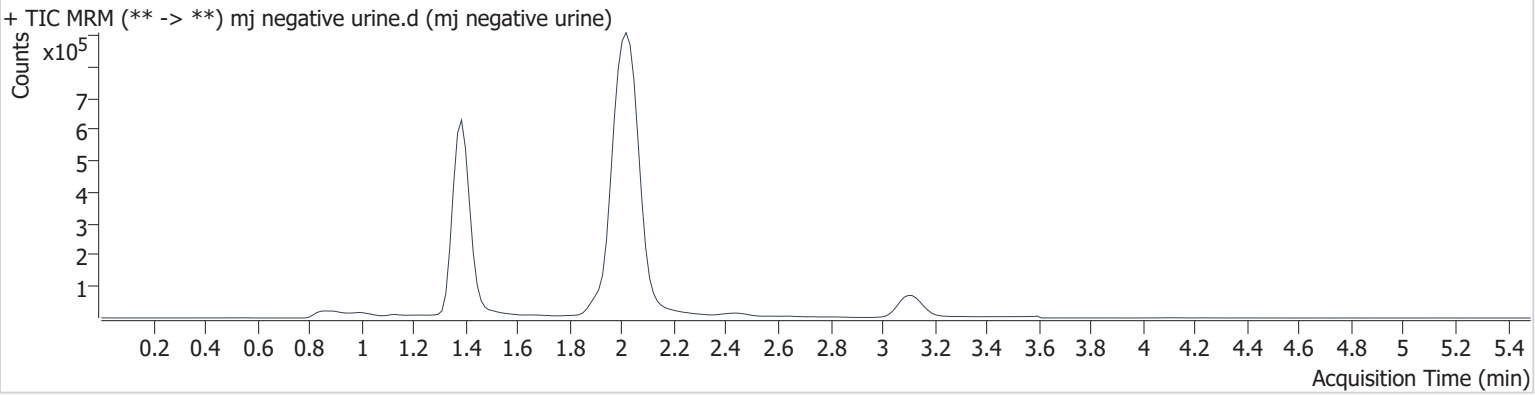


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update	11/18/2021 8:04:14 AM

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-E2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/17/2021 3:53:26 PM		
Sample Info.			

Sample Chromatogram

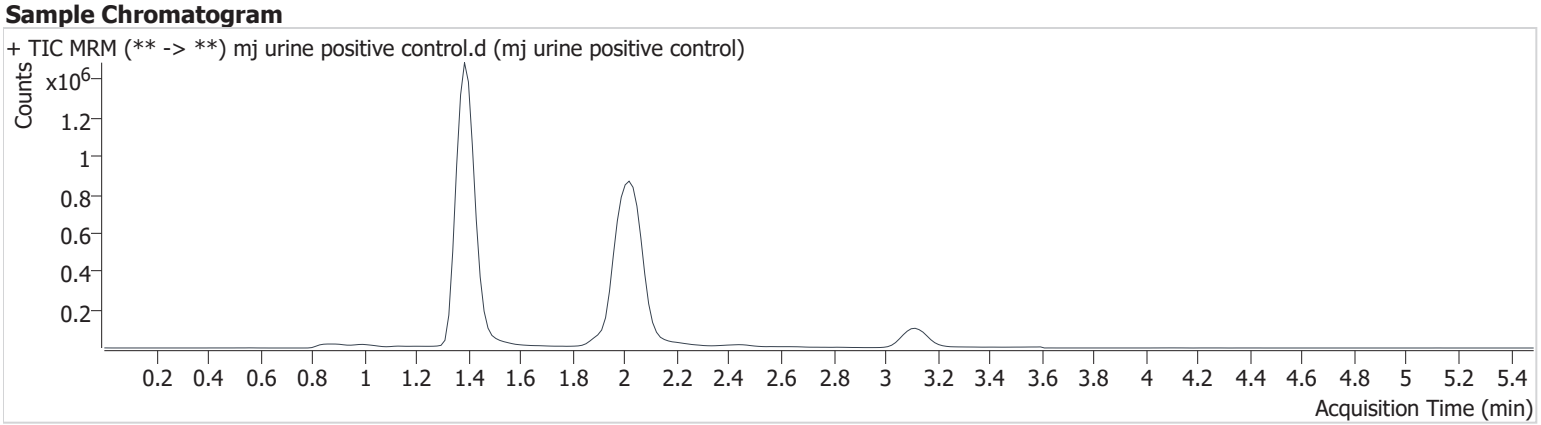


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 AM

Instrument	69679	**Data File**	mj urine positive control.d
Type	Sample	**Sample**	mj urine positive control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-F2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/17/2021 4:00:10 PM		
Sample Info.			

Sample Chromatogram



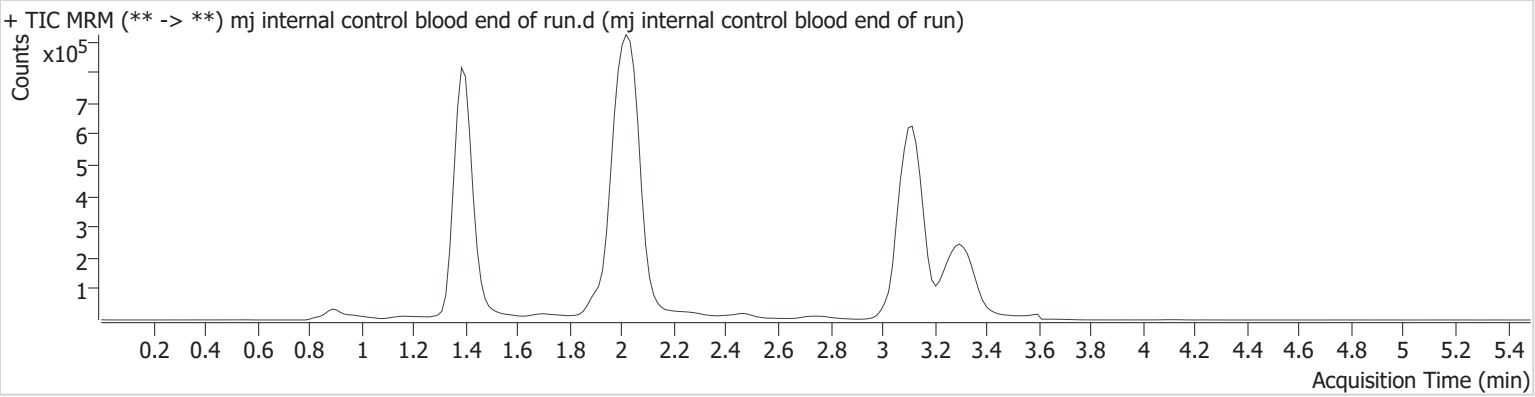
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	408807	∞	832.2	∞	2584307	47.008 ng/ml
THC-COOH	1.416	251500	1488.3	36.3	655.2	517931	37.063 ng/ml
THC	3.122	153371	∞	25.0	484.2	509880	10.861 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 AM

Instrument	69679	**Data File**	mj internal control blood end of run.d
Type	QC	**Sample**	mj internal control blood end of run
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/17/2021 4:53:30 PM		
Sample Info.			

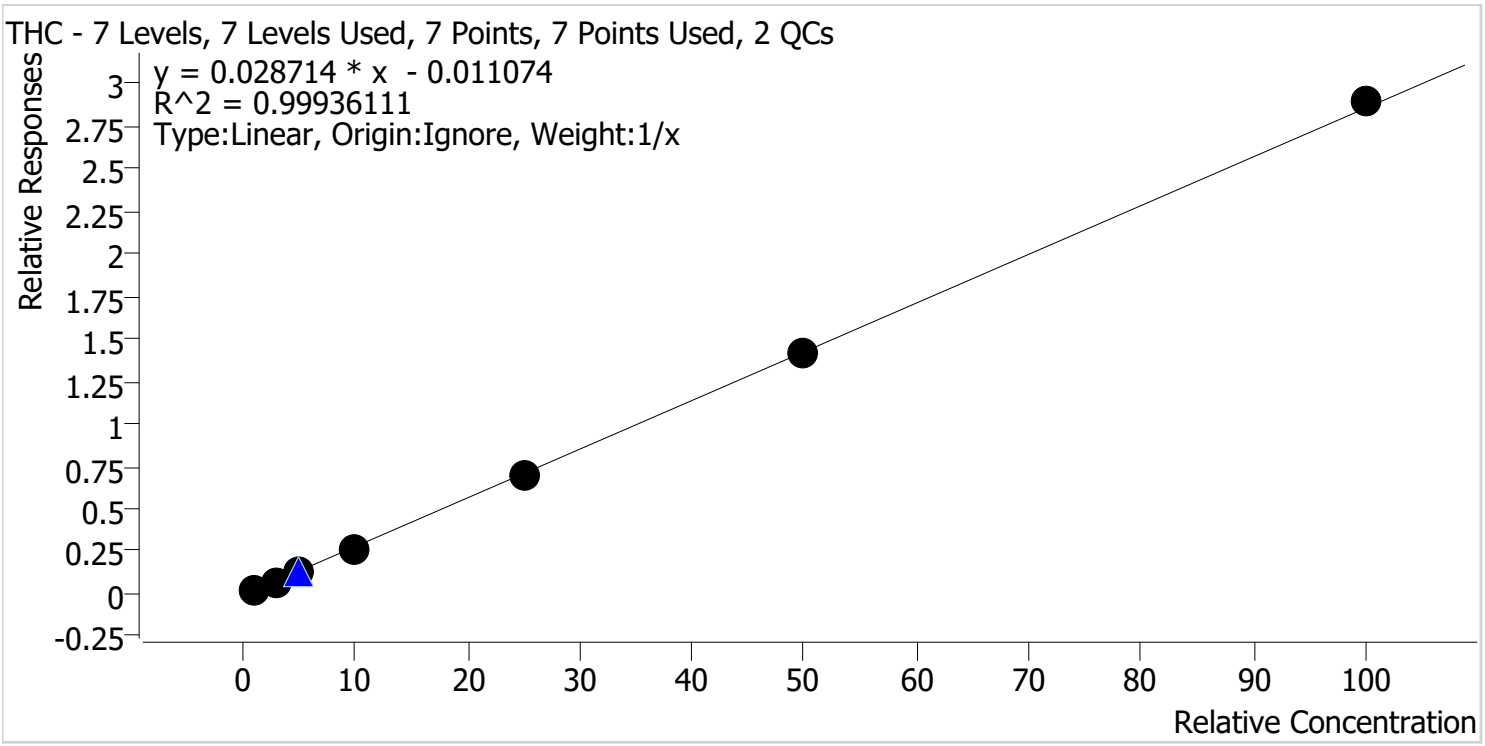
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	38315	∞	1021.1	∞	2386258	5.164 ng/ml
THC-COOH	1.416	154475	2067.3	32.8	244.2	779000	15.521 ng/ml
THC	3.137	380357	99855.1	23.0	1122.5	3029184	4.759 ng/ml

Compound Calibration Report

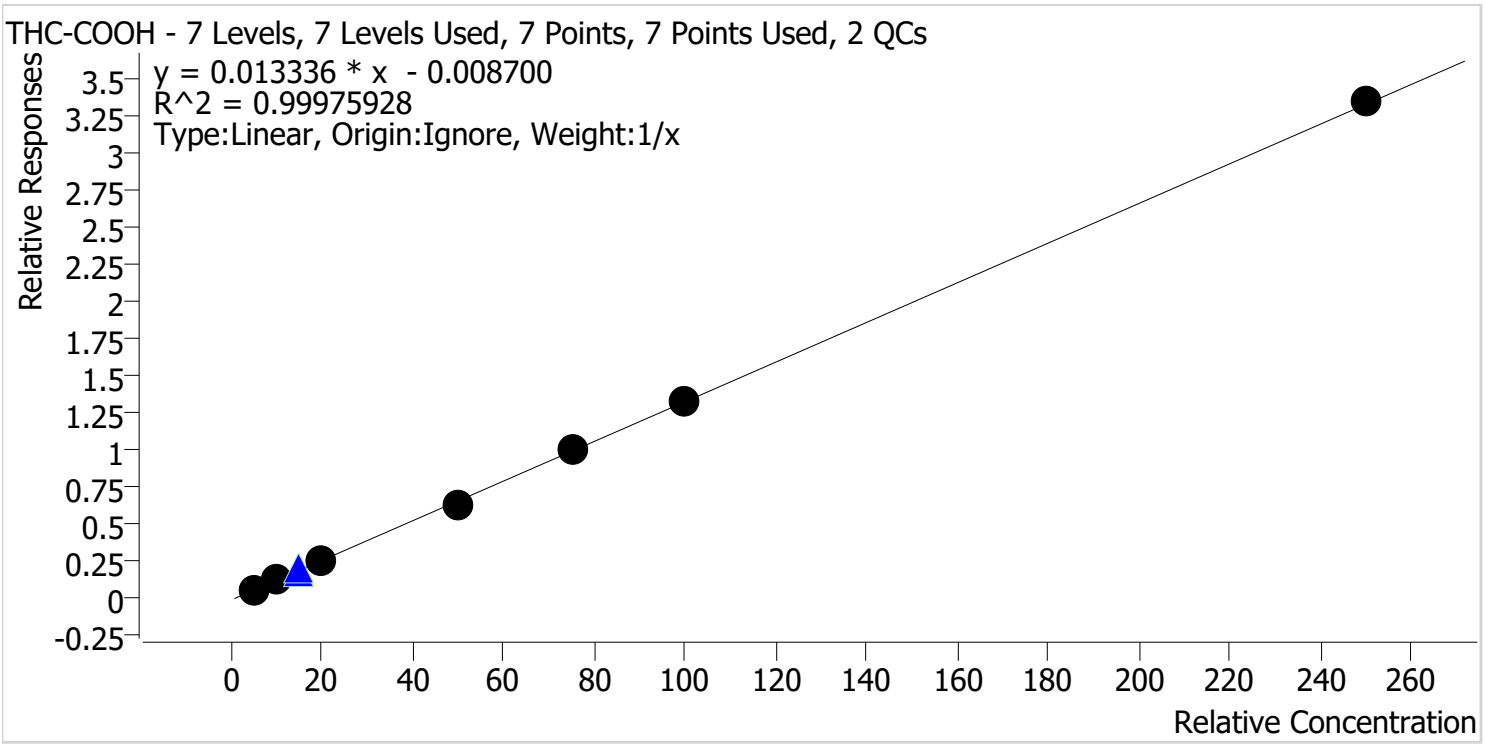
Batch results	D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Last Cal. Update	11/18/2021 8:04 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	116.6
mj cal2	2	✔	3.0	2.9	95.4
mj cal 3	3	✔	5.0	4.7	93.9
mj cal 4	4	✔	10.0	9.4	94.5
mj cal 5	5	✔	25.0	24.6	98.5
mj cal 6	6	✔	50.0	49.9	99.8
mj cal 7	7	✔	100.0	101.3	101.3

Compound Calibration Report

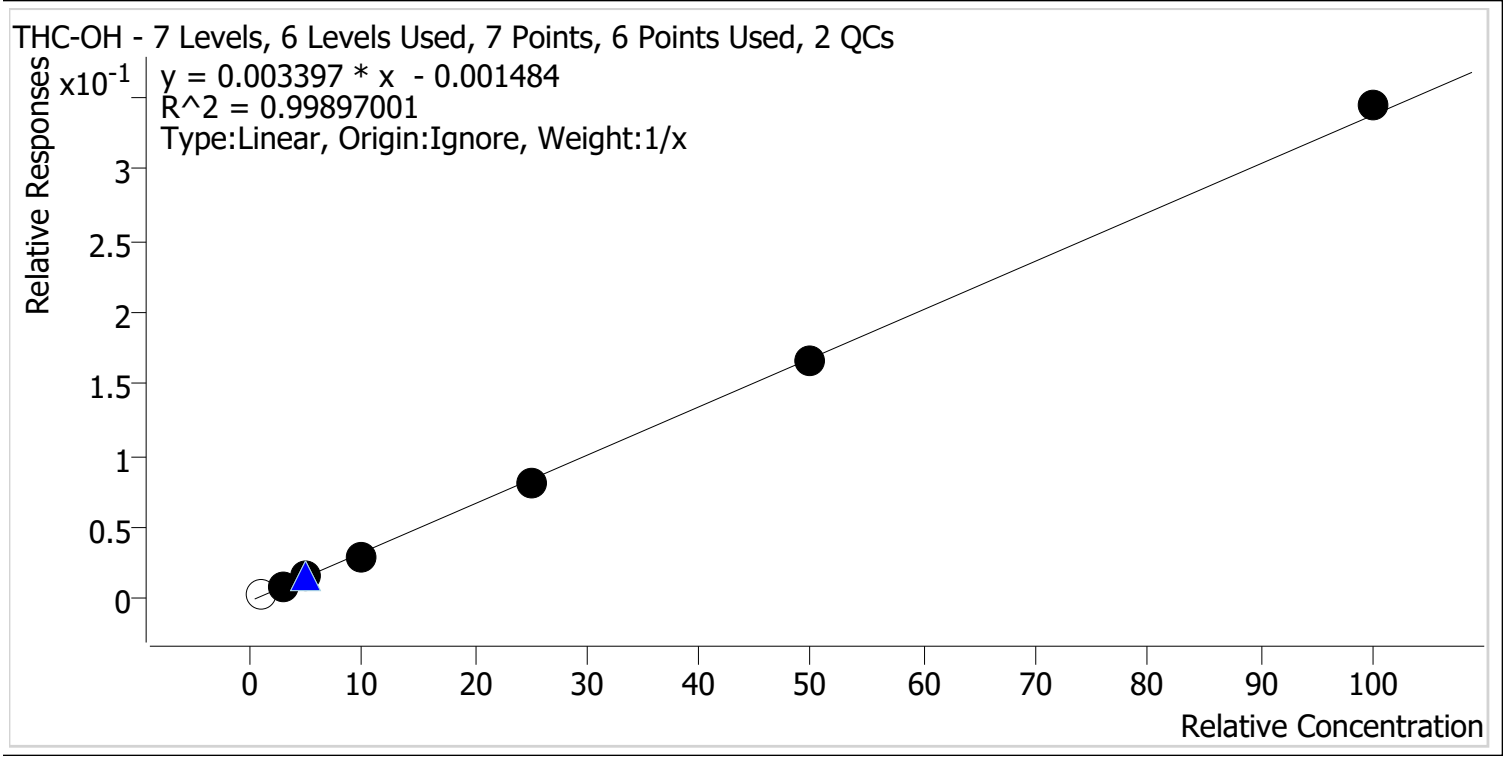
Batch results	D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Last Cal. Update	11/18/2021 8:04 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.2	103.6
mj cal2	2	✔	10.0	10.1	100.6
mj cal 3	3	✔	20.0	19.5	97.7
mj cal 4	4	✔	50.0	48.4	96.8
mj cal 5	5	✔	75.0	76.0	101.4
mj cal 6	6	✔	100.0	99.5	99.5
mj cal 7	7	✔	250.0	251.3	100.5

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Last Cal. Update	11/18/2021 8:04 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	1.5	153.4
mj cal2	2	✔	3.0	3.3	109.2
mj cal 3	3	✔	5.0	5.0	100.0
mj cal 4	4	✔	10.0	9.4	94.0
mj cal 5	5	✔	25.0	24.0	96.1
mj cal 6	6	✔	50.0	49.3	98.7
mj cal 7	7	✔	100.0	102.0	102.0

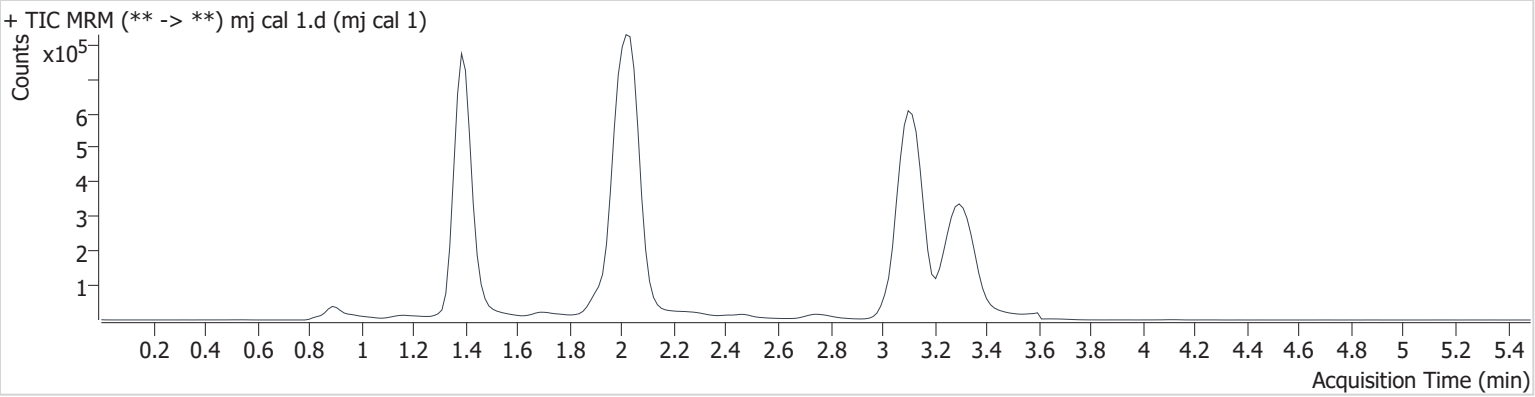
Cal 1 dropped ratio out of range

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 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	11/17/2021 1:59:53 PM		
Sample Info.			

Sample Chromatogram



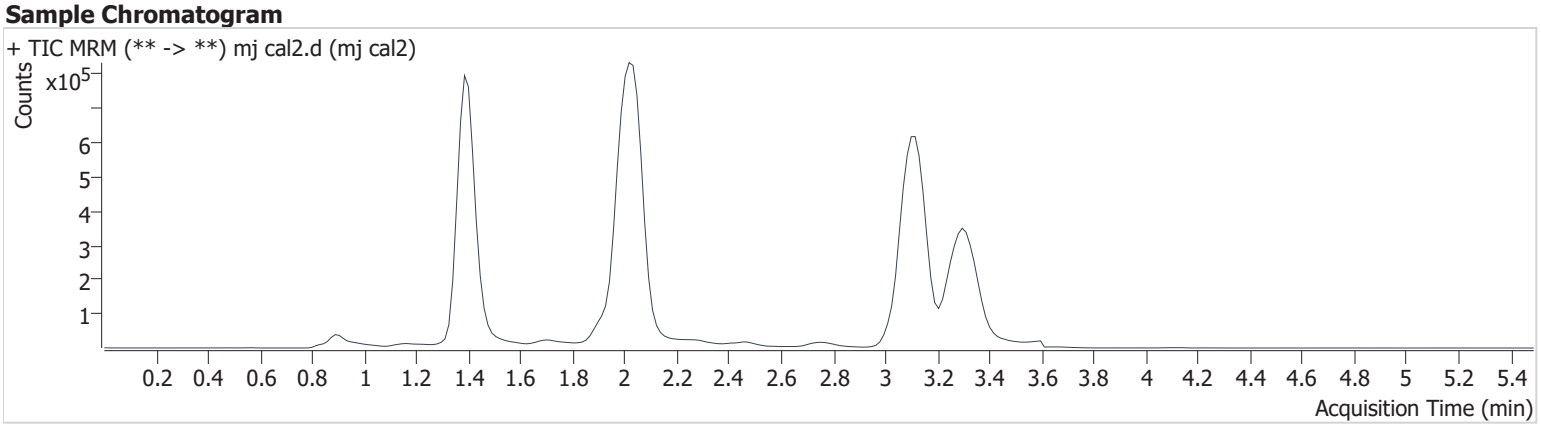
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.394	9830	∞	1273.6 **High**	∞	2637463	1.534 ng/ml	**Low**
THC-COOH	1.416	45363	24.7	35.7	37230.9	751427	5.179 ng/ml	
THC	3.137	70055	591.0	25.7	∞	3125405	1.166 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 AM

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	11/17/2021 2:06:37 PM		
Sample Info.			

Sample Chromatogram



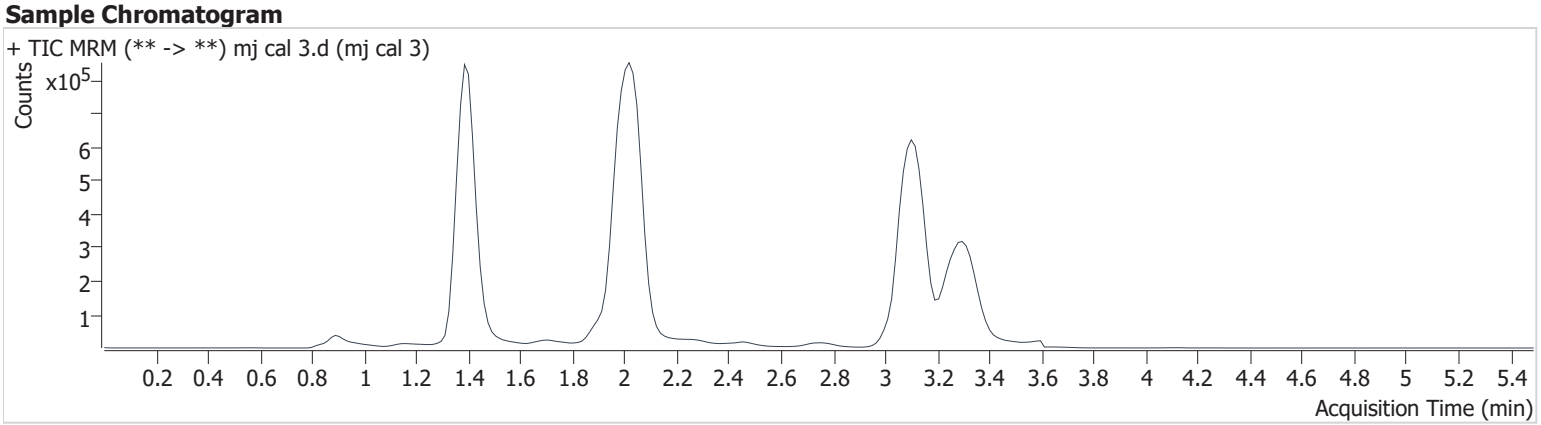
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	24432	∞	1100.3	∞	2532368	3.277 ng/ml
THC-COOH	1.416	90491	138.8	34.9	871.7	721407	10.058 ng/ml
THC	3.137	213236	4459.2	25.2	363.2	2999944	2.861 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 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	11/17/2021 2:13:19 PM		
Sample Info.			

Sample Chromatogram



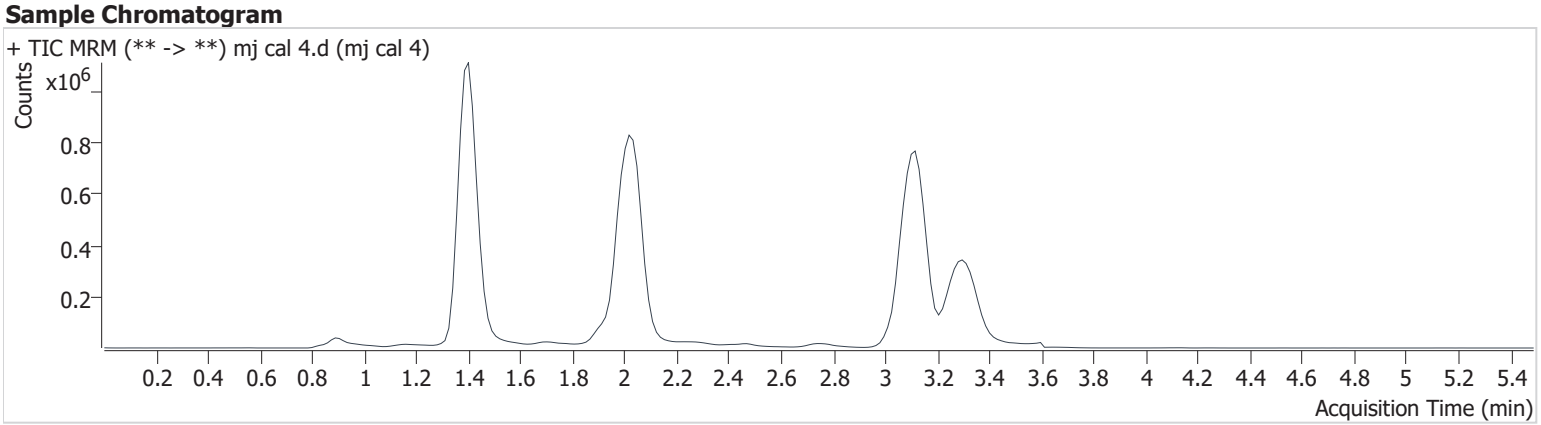
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	40055	7753.4	1043.6	17558 57127 31558.0	2583393	5.002 ng/ml
THC-COOH	1.416	183743	513.7	34.8	366.0	729610	19.536 ng/ml
THC	3.137	370559	∞	23.4	4855.7	2994172	4.696 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 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	11/17/2021 2:20:01 PM		
Sample Info.			

Sample Chromatogram



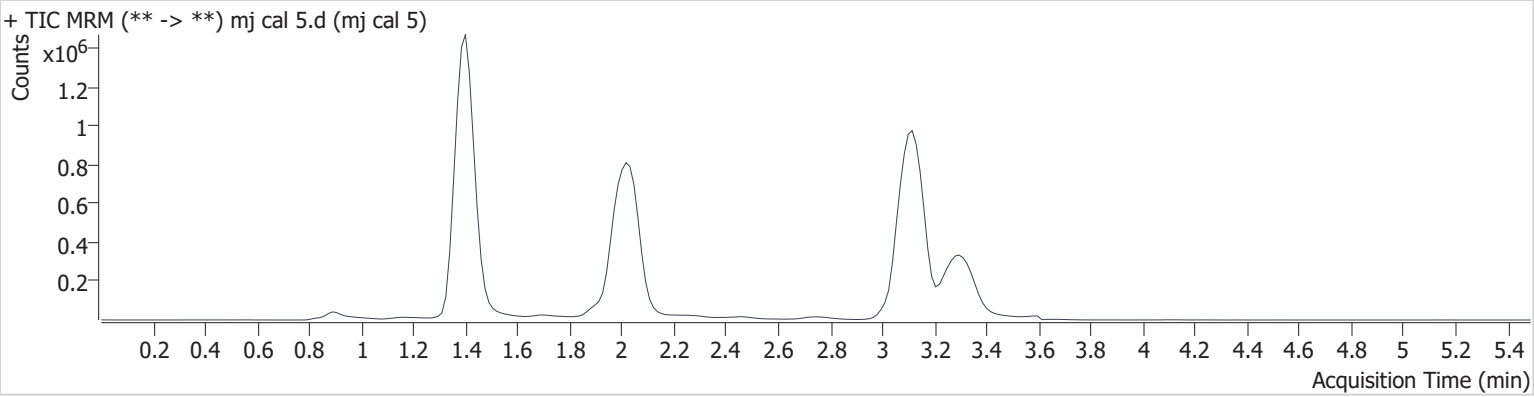
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	80081	∞	992.2	1907.7	2631117	9.397 ng/ml
THC-COOH	1.416	466366	690.0	36.5	756.2	732749	48.376 ng/ml
THC	3.137	819769	4586237653 743590.0	23.3	6610.7	3149873	9.449 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 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	11/17/2021 2:26:43 PM		
Sample Info.			

Sample Chromatogram



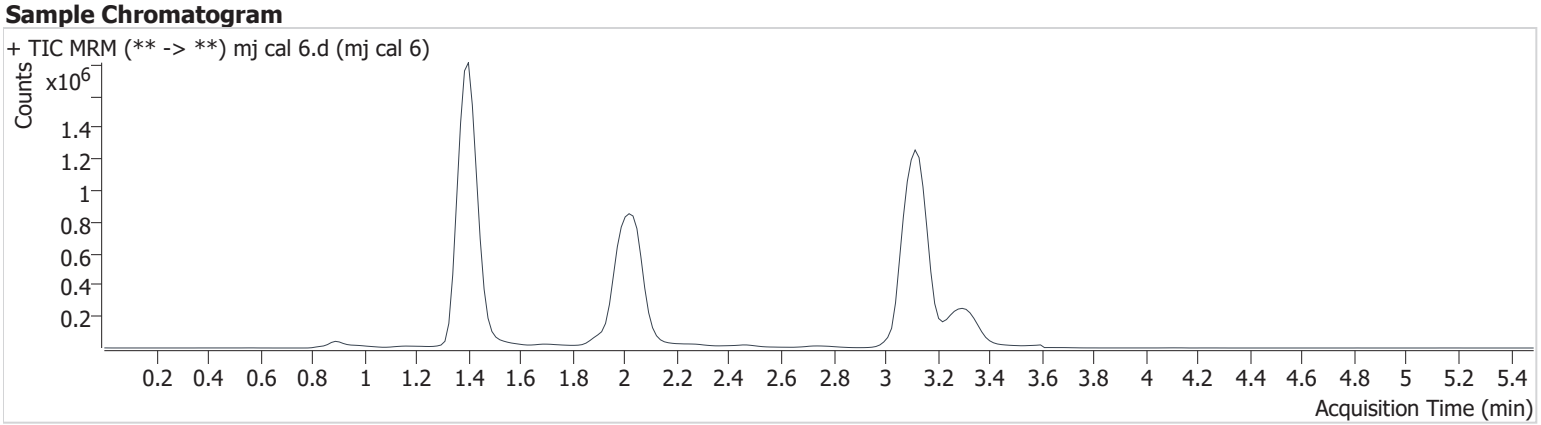
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	213763	∞	954.9	∞	2667381	24.030 ng/ml
THC-COOH	1.416	723889	3573.7	36.2	6804.0	720195	76.020 ng/ml
THC	3.137	2157370	∞	23.7	16160.8	3100376	24.619 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 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	11/17/2021 2:33:25 PM		
Sample Info.			

Sample Chromatogram



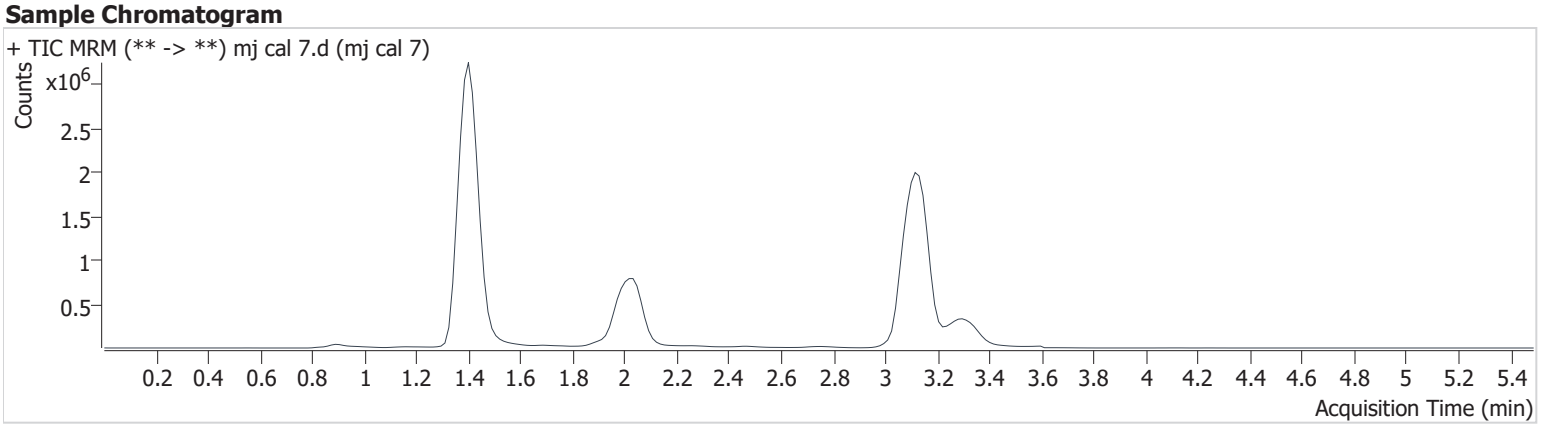
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	382017	∞	905.8	∞	2299665	49.343 ng/ml
THC-COOH	1.416	945433	106812.4	36.5	3408.6	716947	99.531 ng/ml
THC	3.137	3953840	∞	24.3	46877 73445 41163.0	2780092	49.915 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\111721\QuantResults\cann.batch.bin
Calibration Last Update 11/18/2021 8:04:14 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	11/17/2021 2:40:07 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	845131	∞	860.3	26301 8.3	2451005	101.951 ng/ml
THC-COOH	1.416	2148348	2901.3	37.6	2112.0	642689	251.301 ng/ml
THC	3.137	7995943	∞	24.7	11501 33690 41732 0.0	2759583	101.294 ng/ml