11/2/2021

REVIEWED
By Sarah Collins at 11:55 am, Nov 03, 2021

Worklist: 5344

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2021-2160	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-2249	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-2279		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2286	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-2296		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2297		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2324		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2327		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 11/2/21 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	2249-2			
b	cal 2	2279-1	2286-2			
c	cal 3	2296-1	2160-1			
d	cal 4	2297-1				
e	Cal 5	2324-1				
f	cal 6	2327-1				
g	cal 7	urine positive control				
h	Internal control	urine negative control				

C2021-____-_

Toxicology AM method 27/26 external prep information

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**

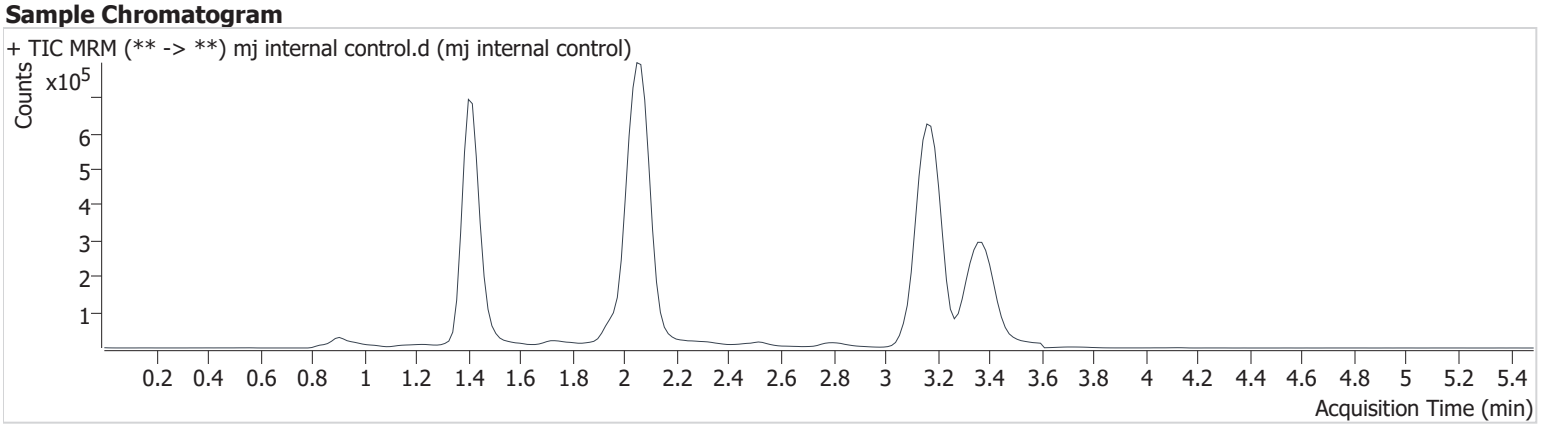
ppd 8/26/21 Exp 8/26/22 neg urine lot 5621	lot u82621	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	last used 11/1/21
ppd 11/2/21 Exp 8/26/22 neg urine lot 83121	lot u11221	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 PM

Instrument	69679	**Data File**	mj internal control.d
Type	QC	**Sample**	mj internal control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2021 12:04:28 PM		
Sample Info.			

Sample Chromatogram



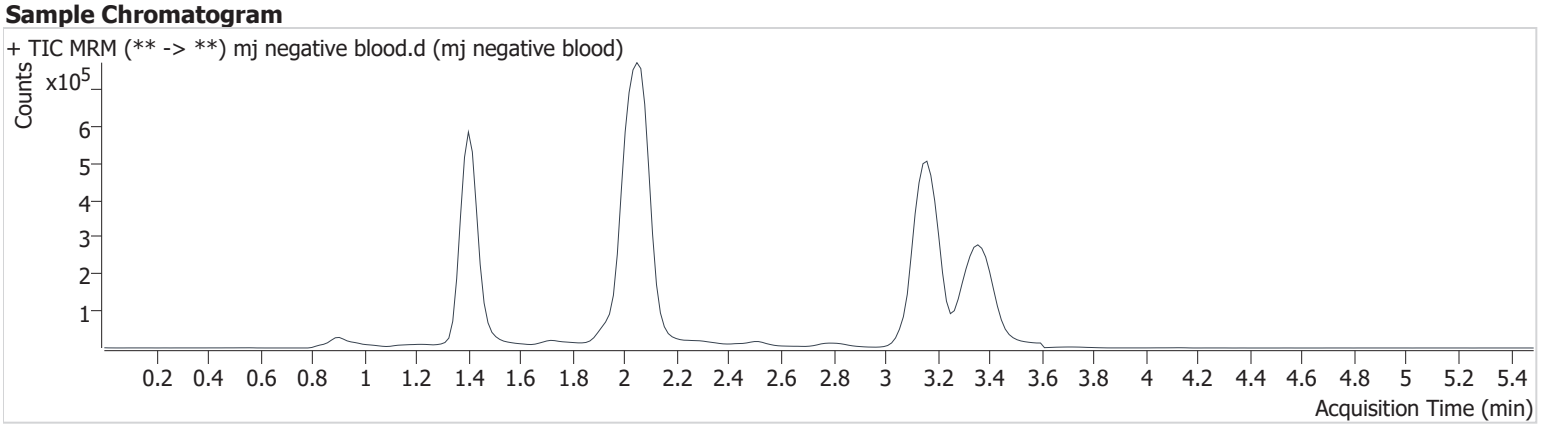
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	33813	∞	1105.8	∞	1962419	5.379 ng/ml
THC-COOH	1.431	120476	883.6	34.2	89.2	628662	15.086 ng/ml
THC	3.197	347298	11881.0	23.2	∞	2904937	4.745 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update	11/2/2021 4:29:09 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-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2021 12:11:12 PM		
Sample Info.			

Sample Chromatogram

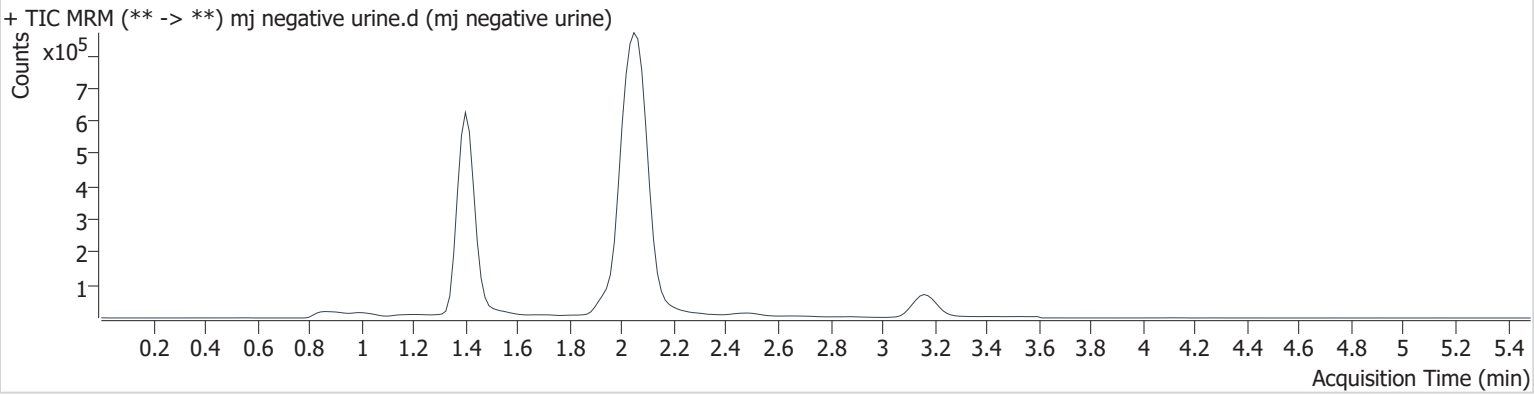


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update	11/2/2021 4:29:09 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-H2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2021 2:46:57 PM		
Sample Info.			

Sample Chromatogram

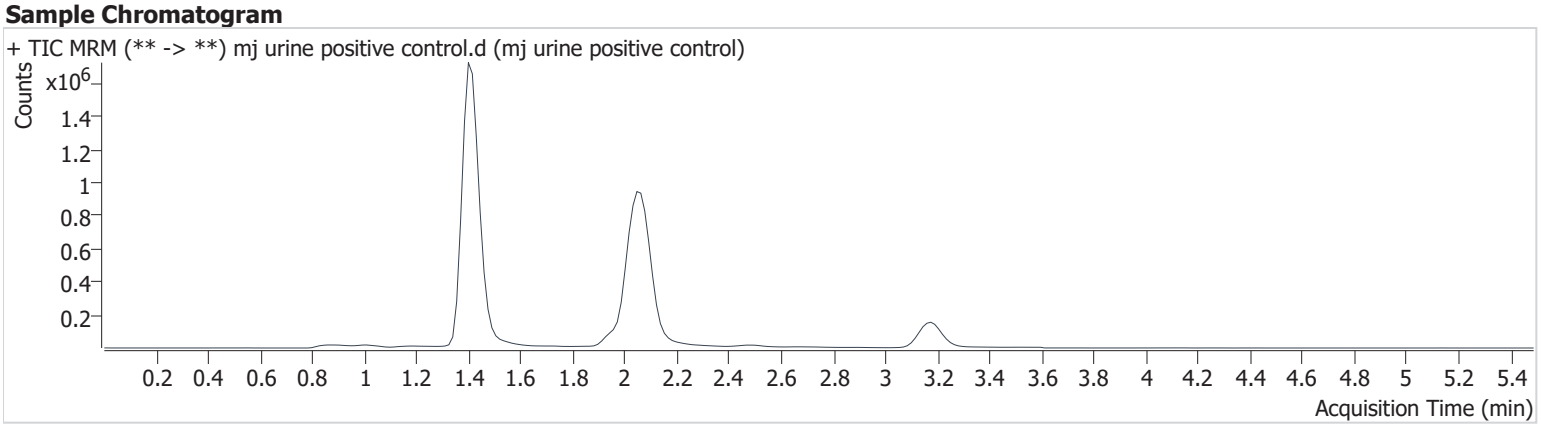


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 PM

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-G2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2021 3:46:53 PM		
Sample Info.			

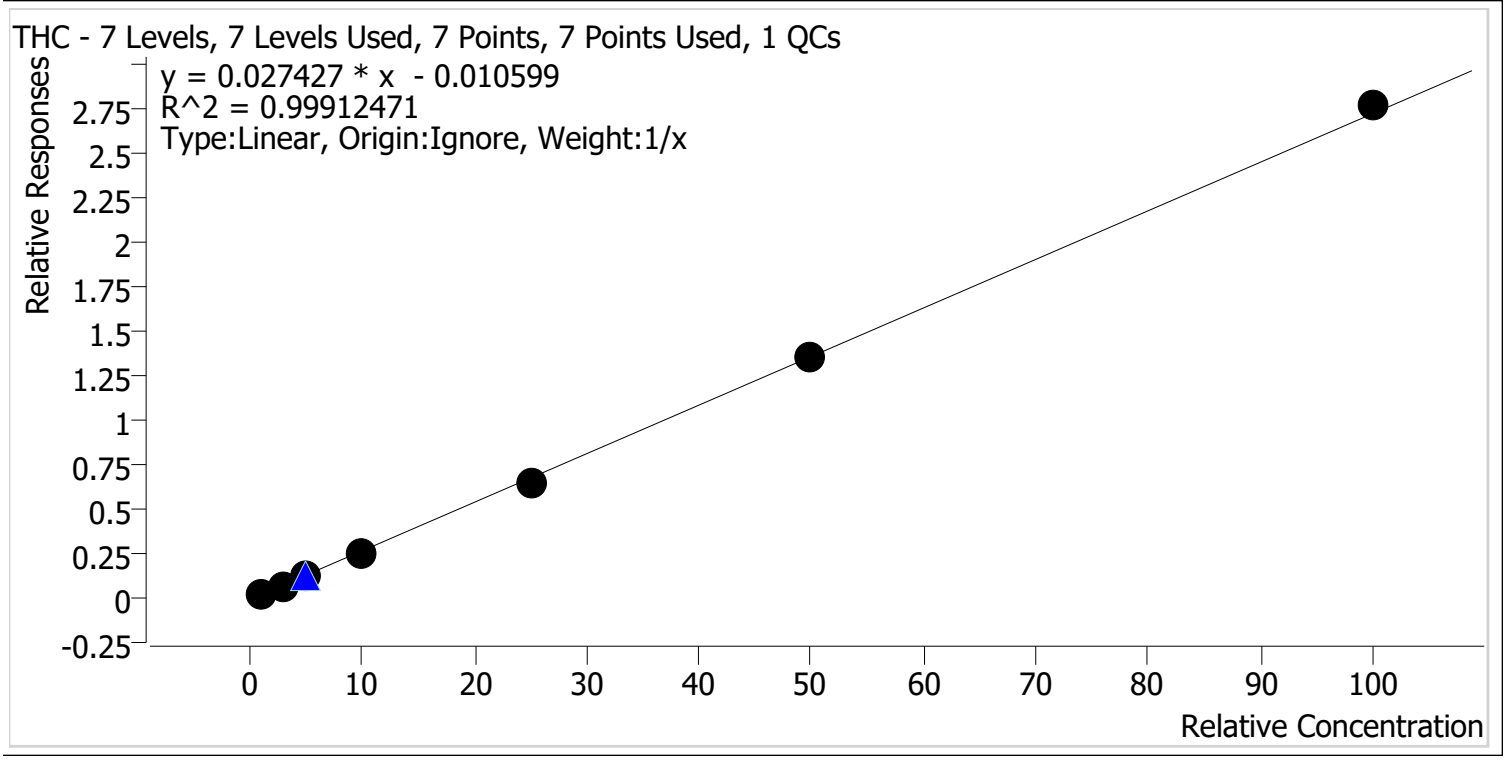
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	441090	14230.6	841.3	3526.7	2478476	52.193 ng/ml
THC-COOH	1.446	296025	1751.6	37.3	1381.2	525911	43.034 ng/ml
THC	3.182	312335	32135.7	22.9	1648.5	566987	20.472 ng/ml

Compound Calibration Report

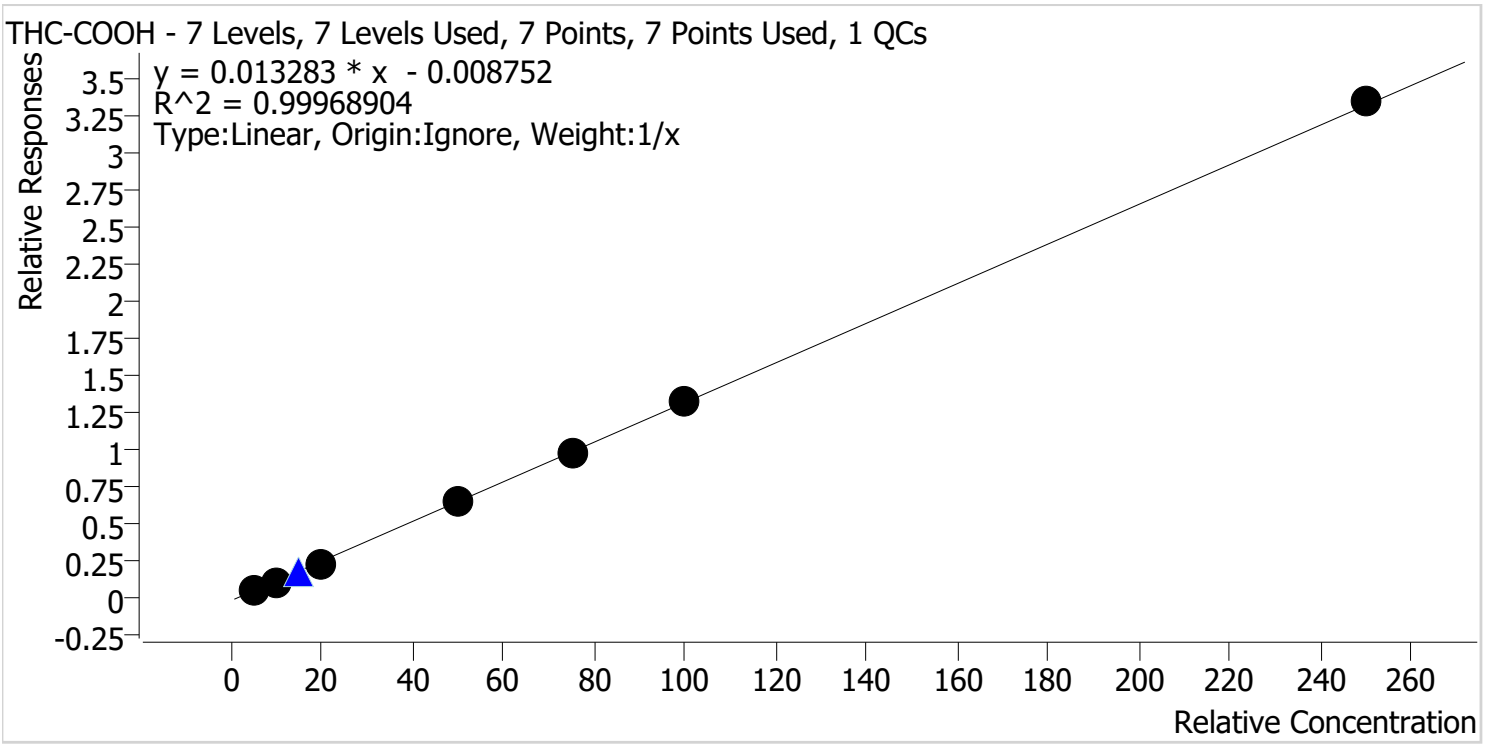
Batch results	D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Last Cal. Update	11/2/2021 4:29 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	117.9
mj cal2	2	✔	3.0	2.9	95.2
mj cal 3	3	✔	5.0	4.7	93.5
mj cal 4	4	✔	10.0	9.5	95.3
mj cal 5	5	✔	25.0	24.0	96.2
mj cal 6	6	✔	50.0	50.1	100.2
mj cal 7	7	✔	100.0	101.6	101.6

Compound Calibration Report

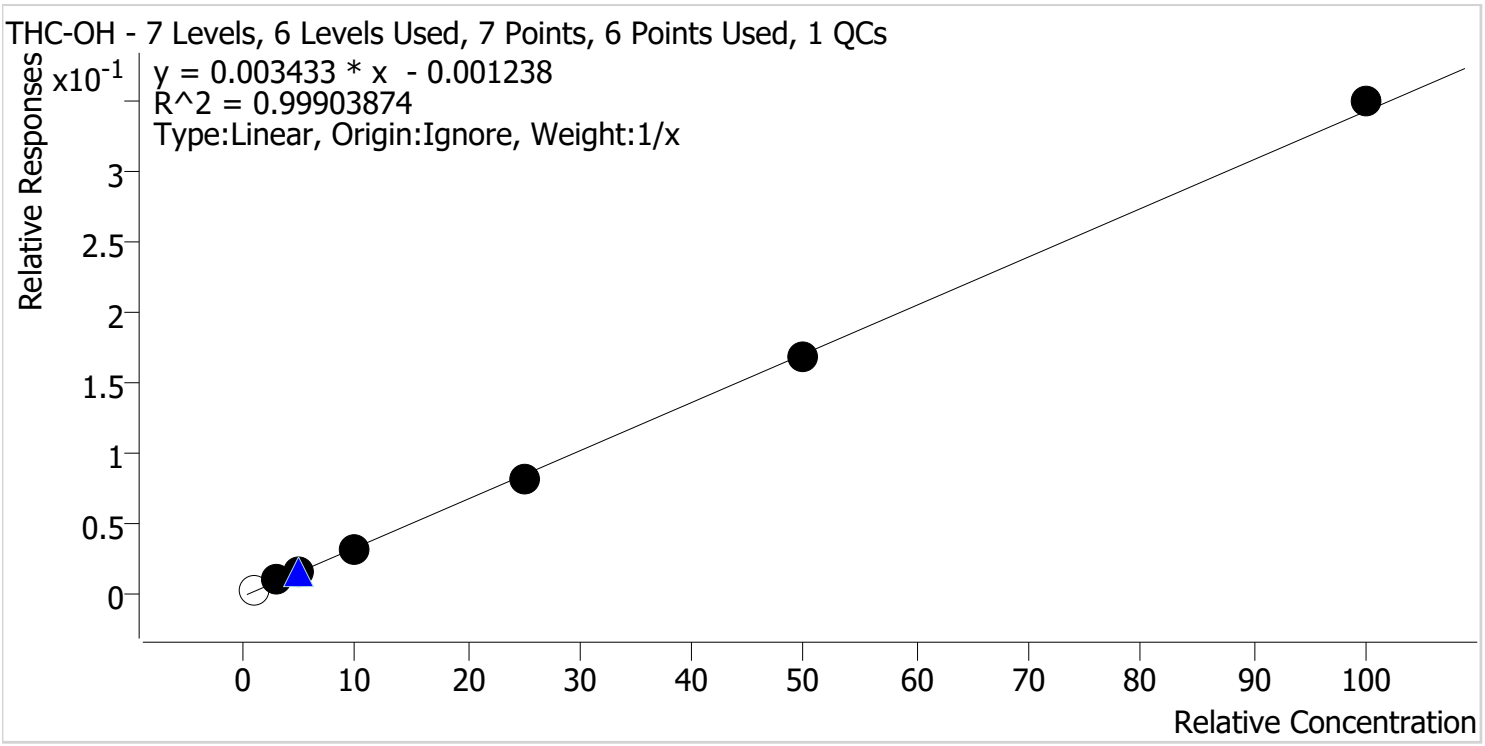
Batch results	D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Last Cal. Update	11/2/2021 4:29 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.5	109.3
mj cal2	2	✔	10.0	9.6	96.1
mj cal 3	3	✔	20.0	19.1	95.5
mj cal 4	4	✔	50.0	49.4	98.8
mj cal 5	5	✔	75.0	74.8	99.7
mj cal 6	6	✔	100.0	99.9	99.9
mj cal 7	7	✔	250.0	251.8	100.7

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Last Cal. Update	11/2/2021 4:29 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	1.4	137.6
mj cal2	2	✔	3.0	3.2	107.9
mj cal 3	3	✔	5.0	5.0	100.3
mj cal 4	4	✔	10.0	9.6	95.7
mj cal 5	5	✔	25.0	23.9	95.4
mj cal 6	6	✔	50.0	49.4	98.7
mj cal 7	7	✔	100.0	102.0	102.0

~~A~~ 11/3/21

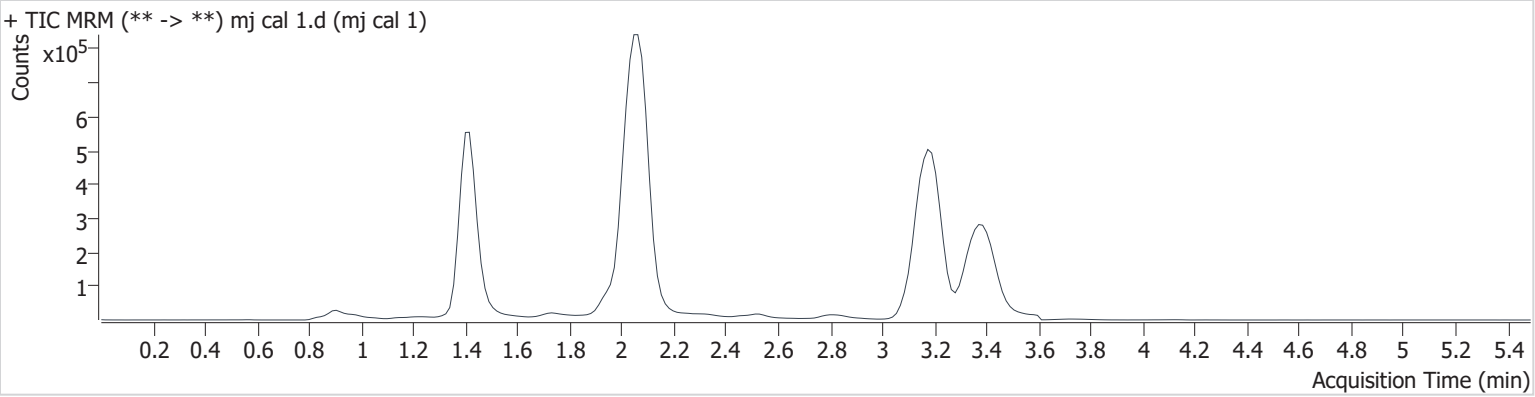
Dropped cal 1 due to ratio being ~~our~~ out of range.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 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	11/2/2021 11:10:56 AM		
Sample Info.			

Sample Chromatogram



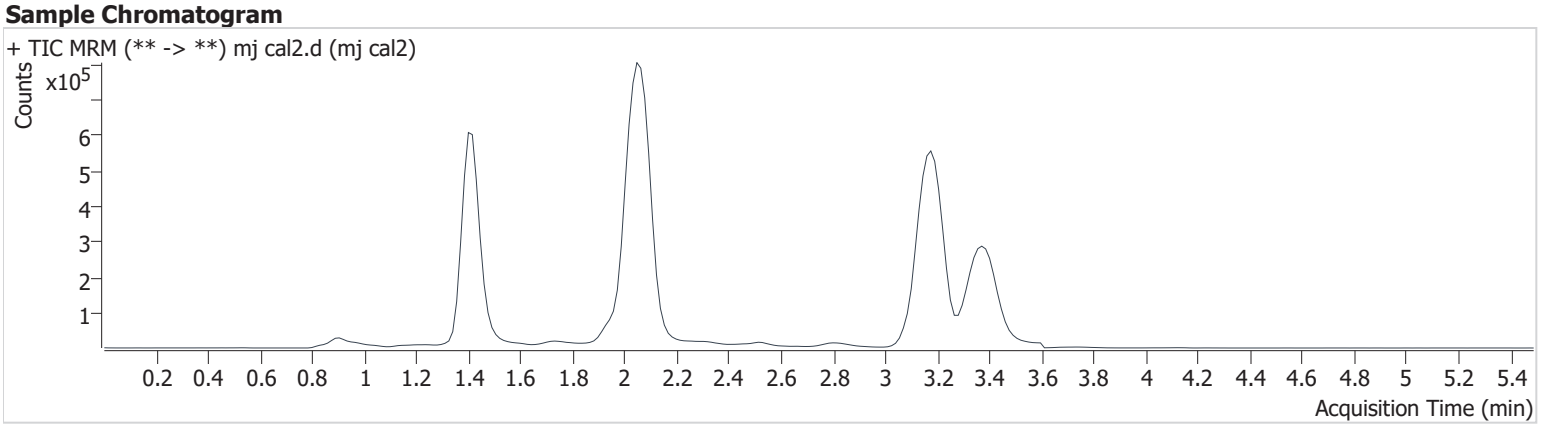
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.409	6509	∞	1338.3 **High**	149.2	1867398	1.376 ng/ml	**Low**
THC-COOH	1.446	35783	91.9	34.2	78.5	560368	5.466 ng/ml	
THC	3.197	56079	1979943058 84484.0	23.5	55.3	2578476	1.179 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 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	11/2/2021 11:17:40 AM		
Sample Info.			

Sample Chromatogram



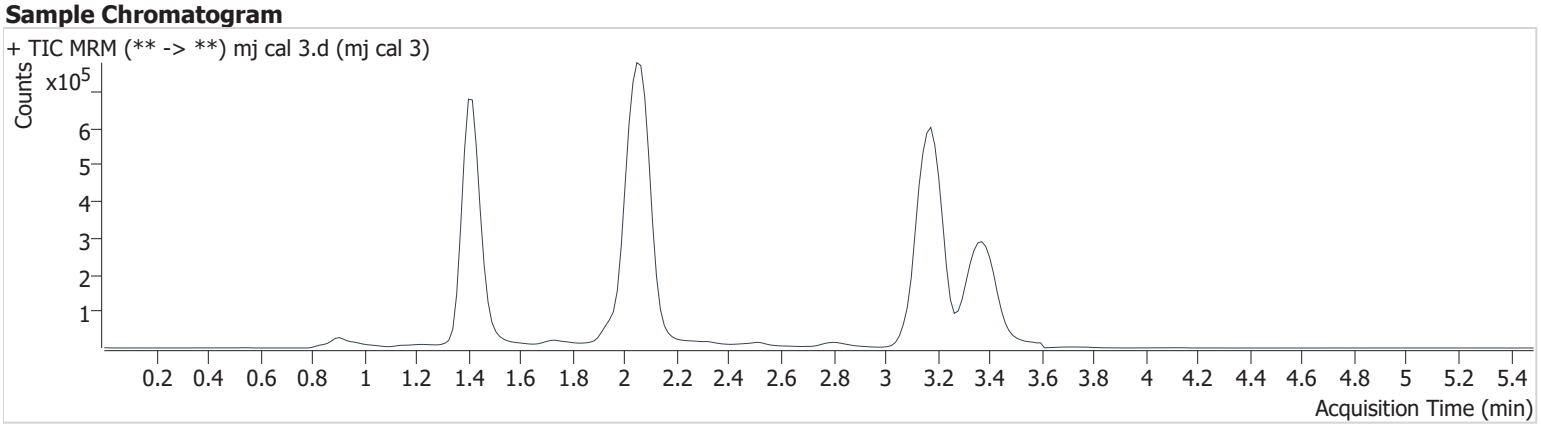
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	18974	∞	1058.0	781.6	1921940	3.236 ng/ml
THC-COOH	1.446	70830	51.0	35.3	190.5	595530	9.613 ng/ml
THC	3.197	187243	1665.7	24.7	∞	2764228	2.856 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 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	11/2/2021 11:24:22 AM		
Sample Info.			

Sample Chromatogram



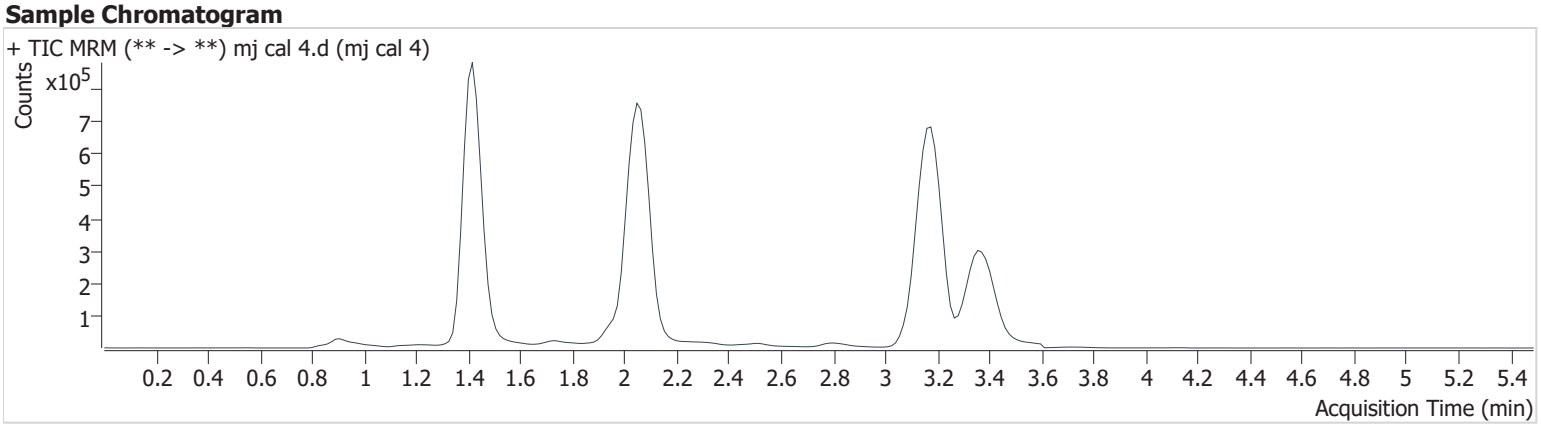
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	31483	1051913005 532190.0	1013.2	∞	1969730	5.016 ng/ml
THC-COOH	1.431	148948	84.9	36.6	441.4	607968	19.102 ng/ml
THC	3.197	334941	∞	23.3	534.3	2846034	4.677 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 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	11/2/2021 11:31:04 AM		
Sample Info.			

Sample Chromatogram



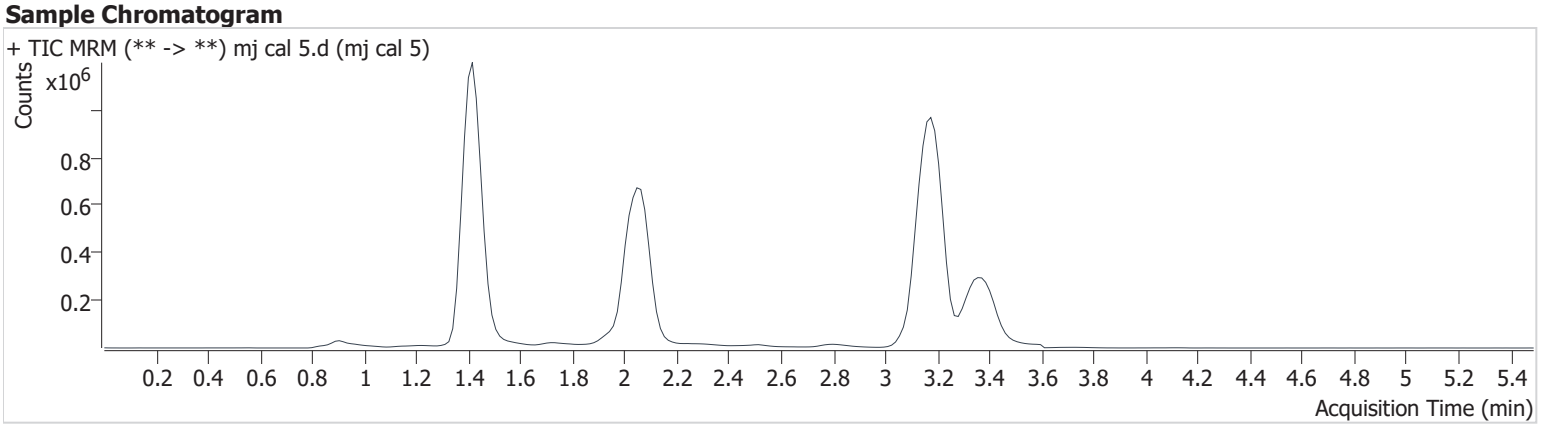
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	62590	∞	1051.3	3518.7	1978355	9.575 ng/ml
THC-COOH	1.431	390945	716.1	36.5	681.1	604040	49.383 ng/ml
THC	3.197	704866	∞	23.3	∞	2810022	9.532 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 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	11/2/2021 11:37:46 AM		
Sample Info.			

Sample Chromatogram



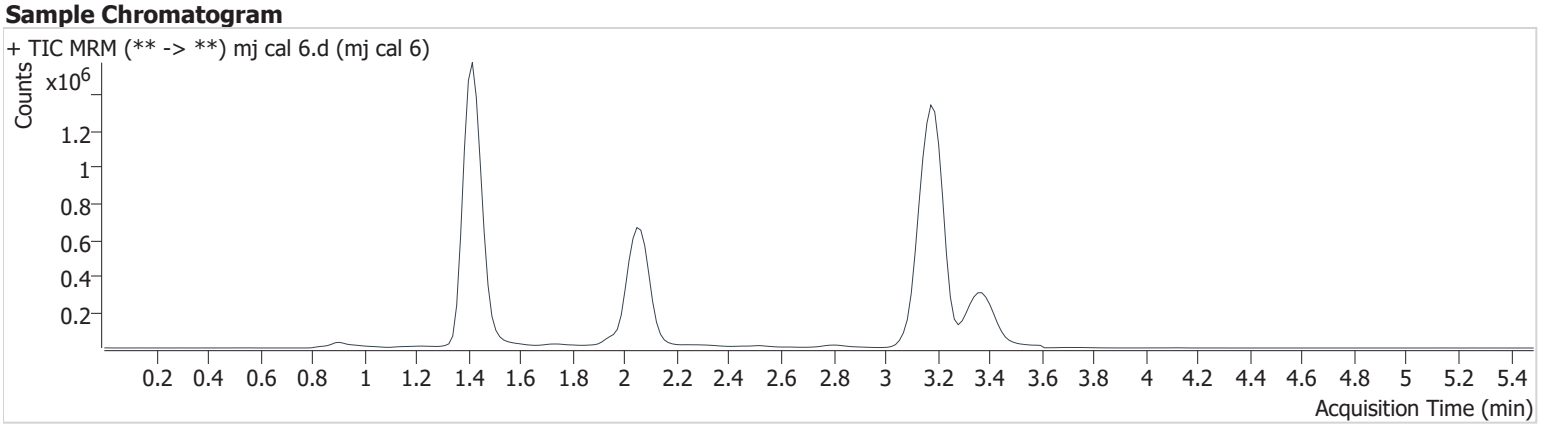
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	167283	∞	948.1	∞	2073974	23.852 ng/ml
THC-COOH	1.431	610827	743.4	37.2	575.4	620641	74.751 ng/ml
THC	3.182	2031427	∞	23.8	∞	3131271	24.041 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 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	11/2/2021 11:44:28 AM		
Sample Info.			

Sample Chromatogram



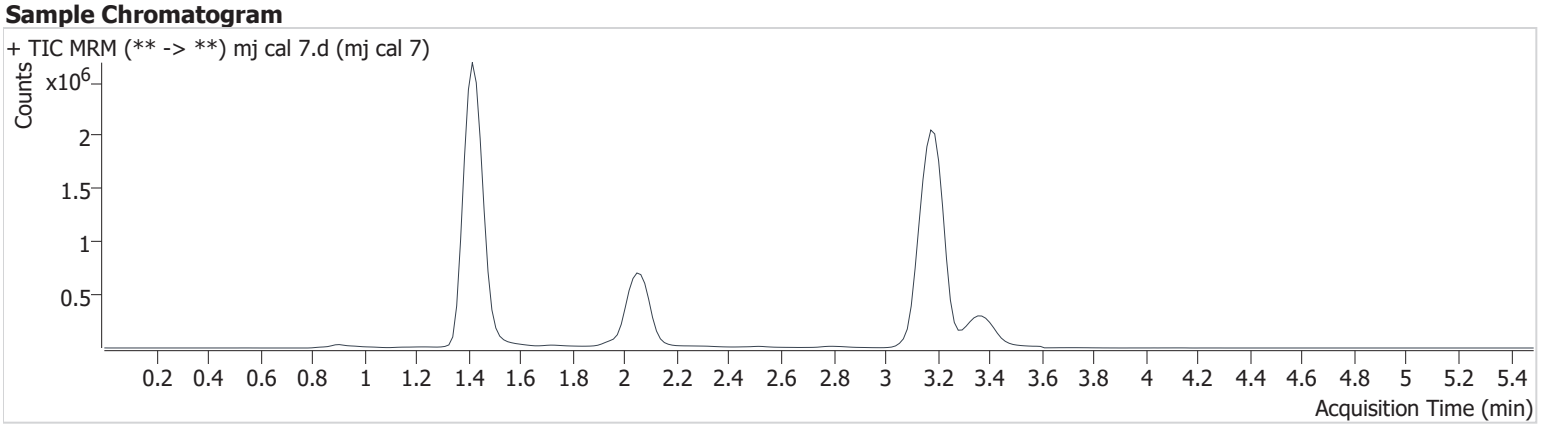
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	324780	10030.9	884.0	16465.9	1930718	49.354 ng/ml
THC-COOH	1.431	768103	1695.0	37.2	506540.5	582751	99.885 ng/ml
THC	3.197	3843851	∞	24.0	∞	2818980	50.103 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 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	11/2/2021 11:51:10 AM		
Sample Info.			

Sample Chromatogram



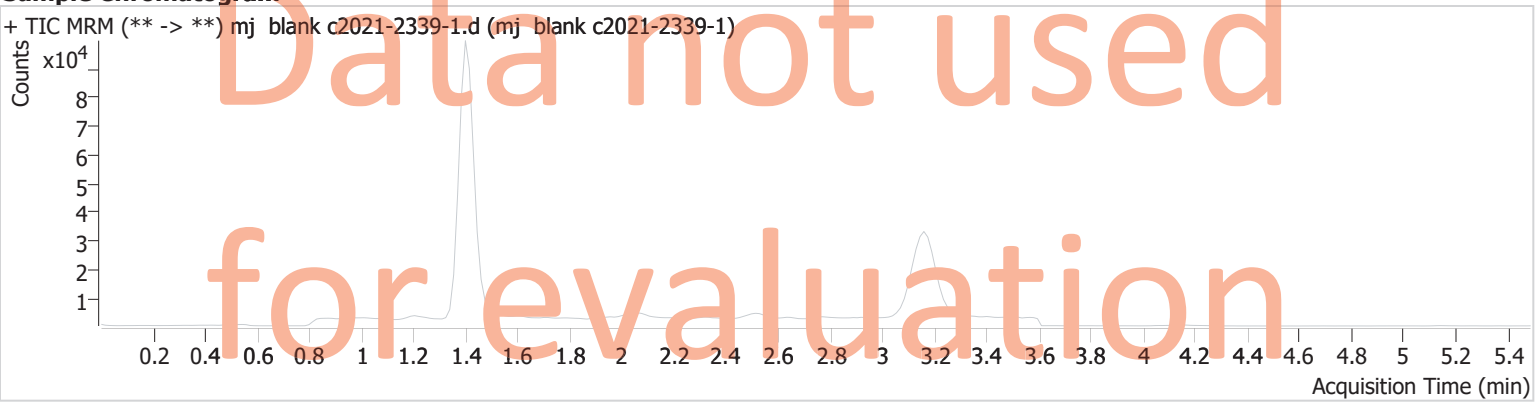
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	651490	∞	856.3	∞	1867434	101.968 ng/ml
THC-COOH	1.431	1829251	1319.9	37.3	1112.6	548336	251.800 ng/ml
THC	3.197	7446004	∞	24.3	∞	2682034	101.611 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update	11/2/2021 4:29:09 PM

Instrument	69679	**Data File**	mj blank c2021-2339-1.d
Type	Sample	**Sample**	mj blank c2021-2339-1
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	Vial 4	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2021 1:24:34 PM		
Sample Info.			

Sample Chromatogram

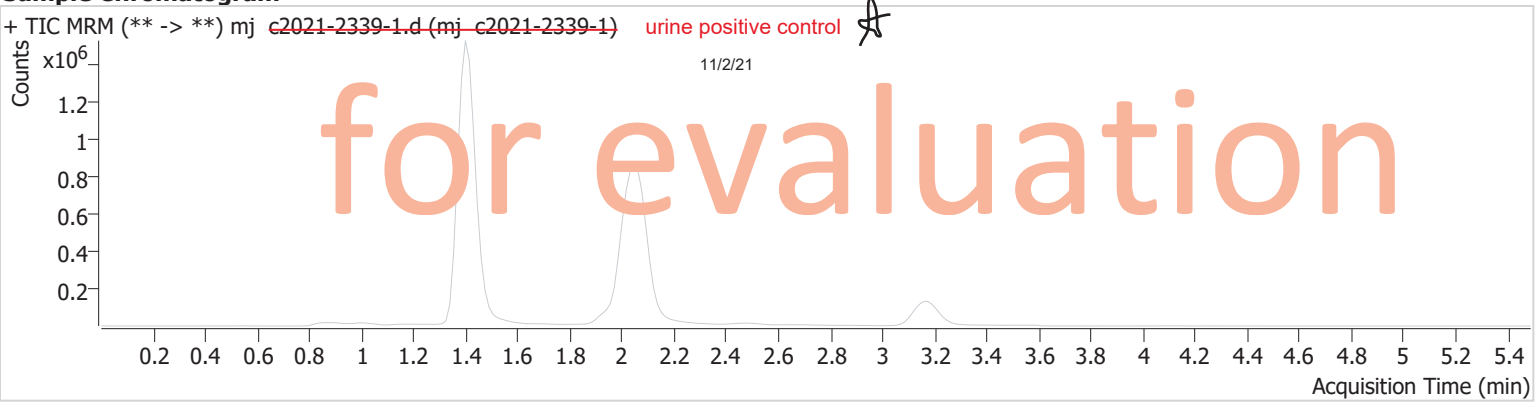


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\110221\QuantResults\cann.batch.bin
Calibration Last Update 11/2/2021 4:29:09 PM

Instrument	69679	**Data File**	mj c2021-2339-1.d
Type	Sample	**Sample**	mj ~~c2021-2339-1~~ urine positive control
~~Acq. Method~~	AM 27 THC quant.m	**Operator**	Anne Nord 11/2/21
Sample Position	P3-G2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2021 1:31:10 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	408138	15384.6	854.5	∞	2279289	52.513 ng/ml
THC-COOH	1.431	277917	626.0	36.1	1210.3	485912	43.716 ng/ml
THC	3.182	282954	∞	23.3	∞	518000	20.303 ng/ml

This sample is actually the urine positive control. I failed to delete this line from the worklist C2021-2339 was not extracted or run there was not enough sample.