10/13/2020

Worklist: 4567

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2020-1912	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1916	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1918	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1925	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1935	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 10/07/20 Analyst: Anne Nord
Plate lot#: 200723 Plate Expiration: 01/23/2021

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: 20G20792 **Urine Blank:** 10120 **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 and 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 urine, the ratio in the control is off.*

THC-OH blood curve range 3-100

	1	2	3	4	5	6
a	cal 100 ng	neg blood				QC 1
b	cal 50 ng	1935				cal 100 ng
c	cal 25 ng	neg urine				cal 50 ng
d	cal 10ng	external urine				cal 25 ng
e	cal 5 ng	1925				cal 10ng
f	cal 3 ng	1918				cal 5 ng
g	cal 1ng	1916				cal 3 ng
h	QC 1	1912				cal 1ng

c2020-

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 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

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**

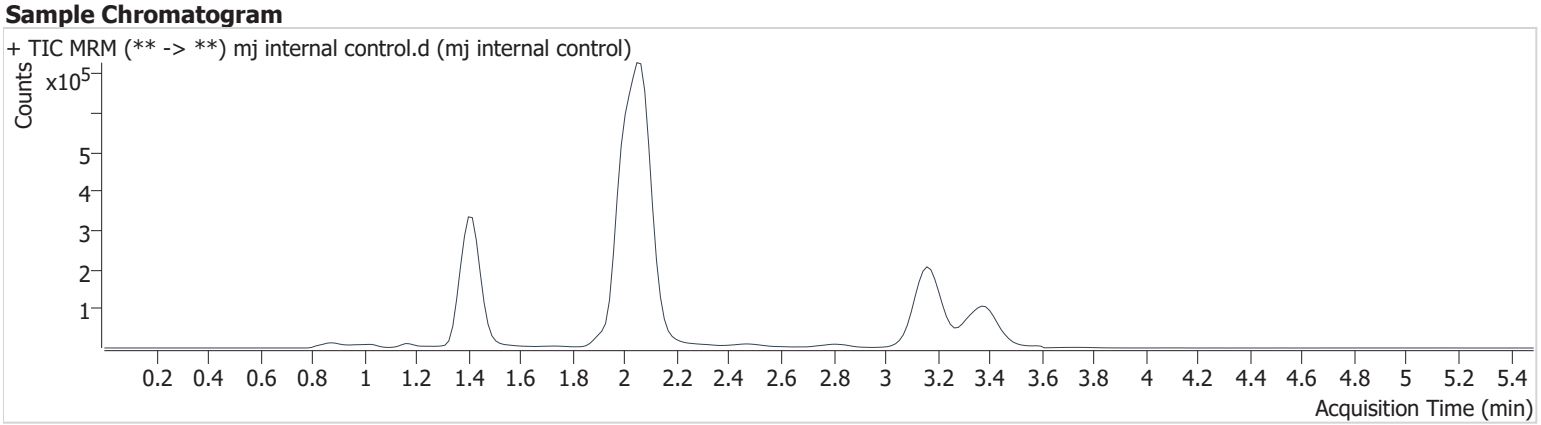
ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 AM

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	10/7/2020 7:07:28 PM		
Sample Info.			

Sample Chromatogram



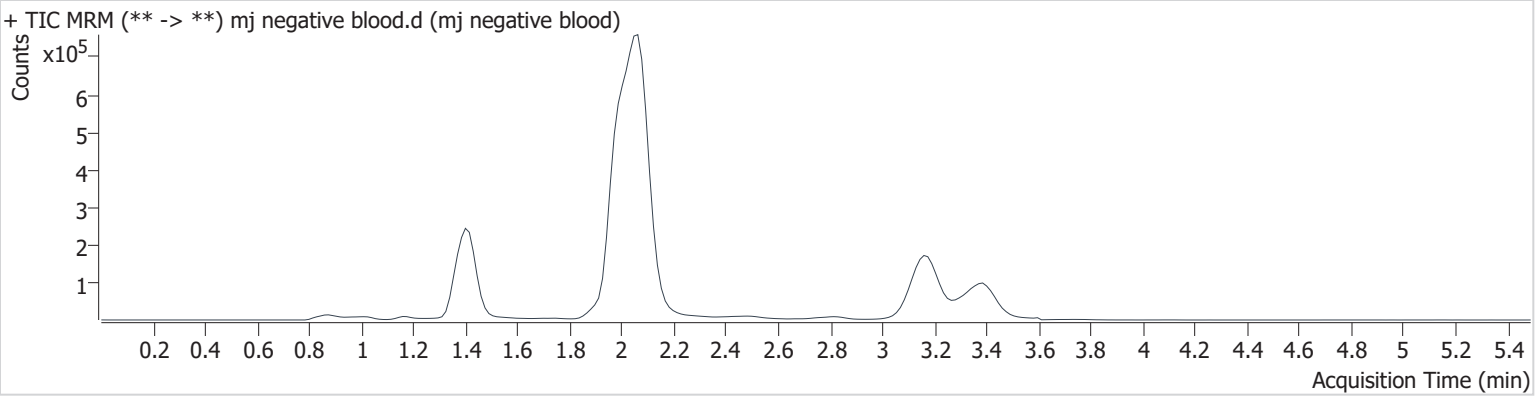
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	117417	∞	12.0	∞	908138	4.617 ng/ml
THC-COOH	1.431	99157	305.7	34.5	108.8	499980	15.030 ng/ml
THC	3.197	78834	1689378542 691600.0	25.0	∞	717849	4.436 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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	10/7/2020 7:15:10 PM		
Sample Info.			

Sample Chromatogram

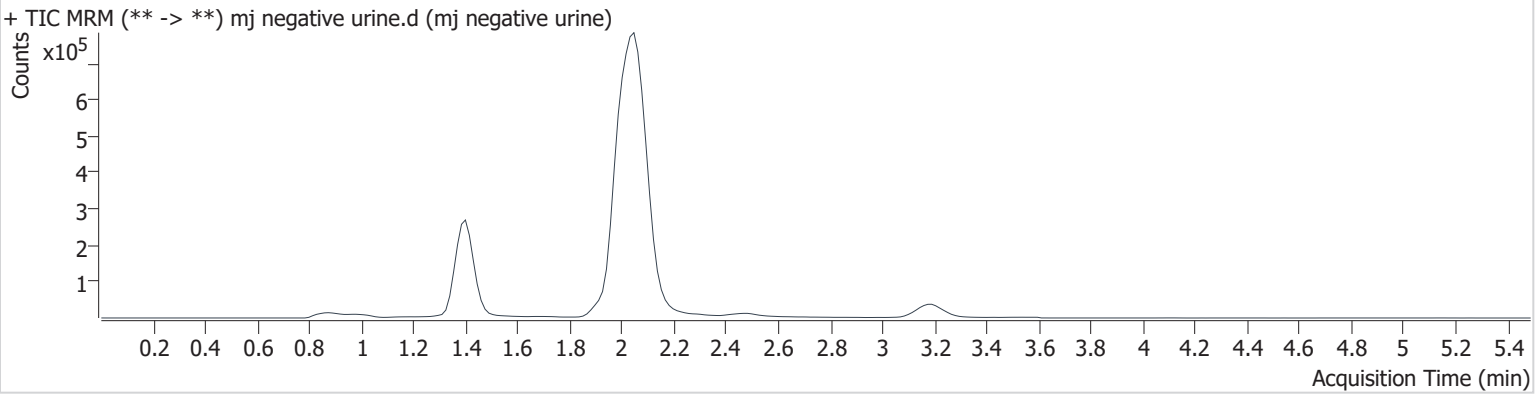


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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-C2	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/7/2020 7:45:53 PM		
Sample Info.			

Sample Chromatogram

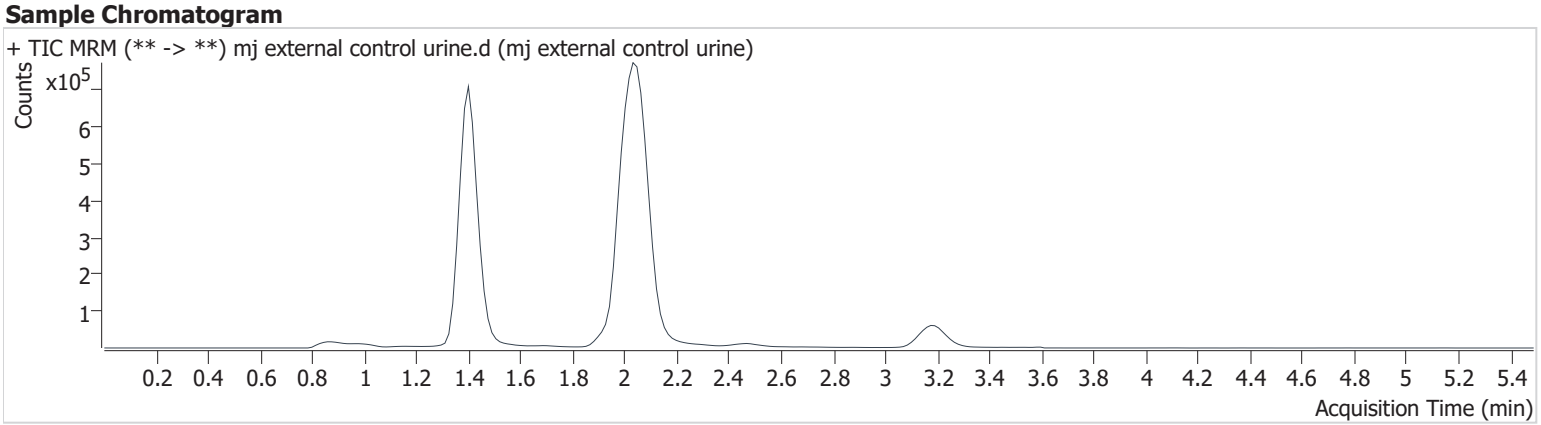


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 AM

Instrument	69679	**Data File**	mj external control urine.d
Type	Sample	**Sample**	mj external control urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-D2	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/7/2020 8:01:15 PM		
Sample Info.			

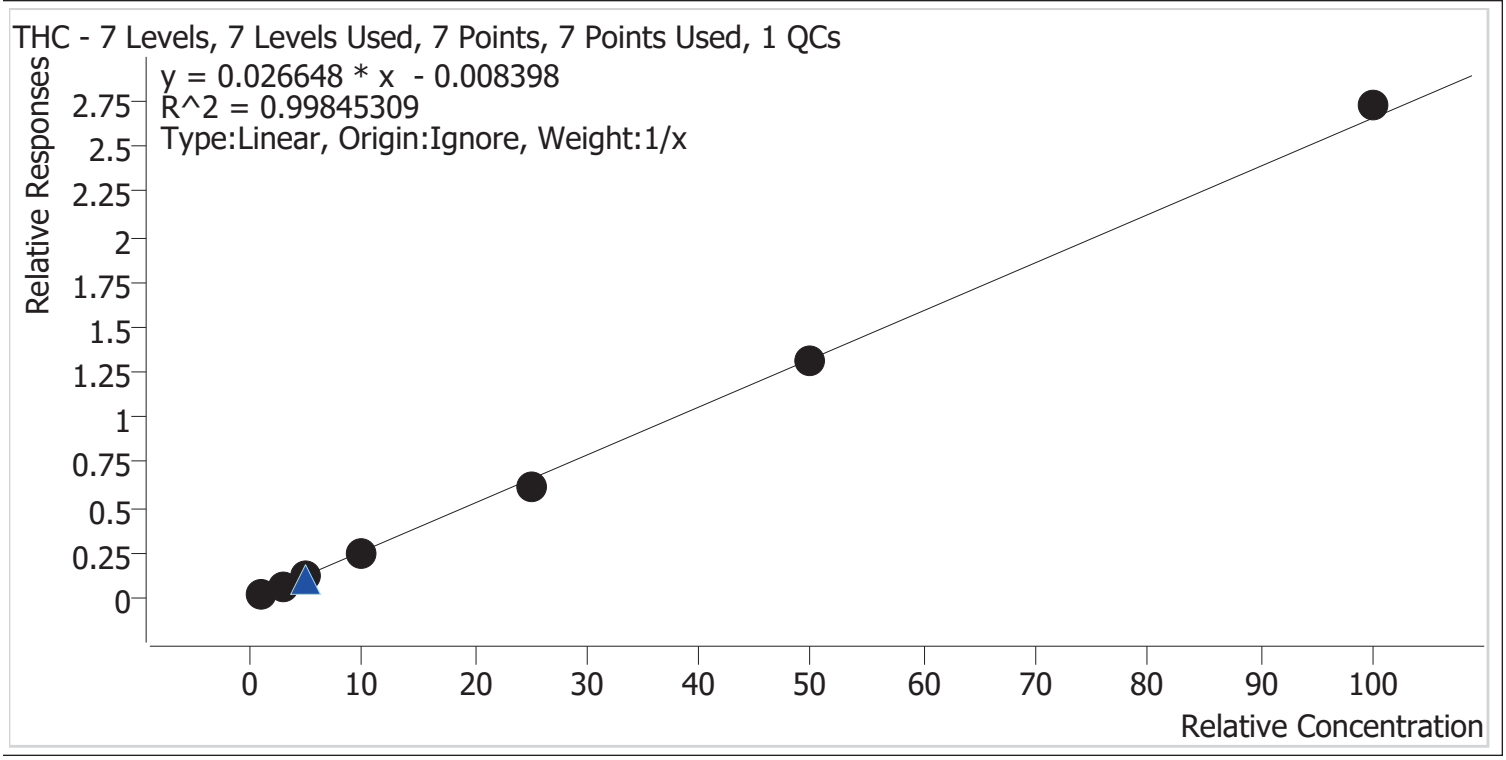
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1430202	∞	17.5 **High**	∞	875992	52.142 ng/ml
THC-COOH	1.416	223156	257.7	34.1	173.7	366729	44.167 ng/ml
THC	3.197	127650	∞	24.8	1695.0	283870	17.190 ng/ml

Compound Calibration Report

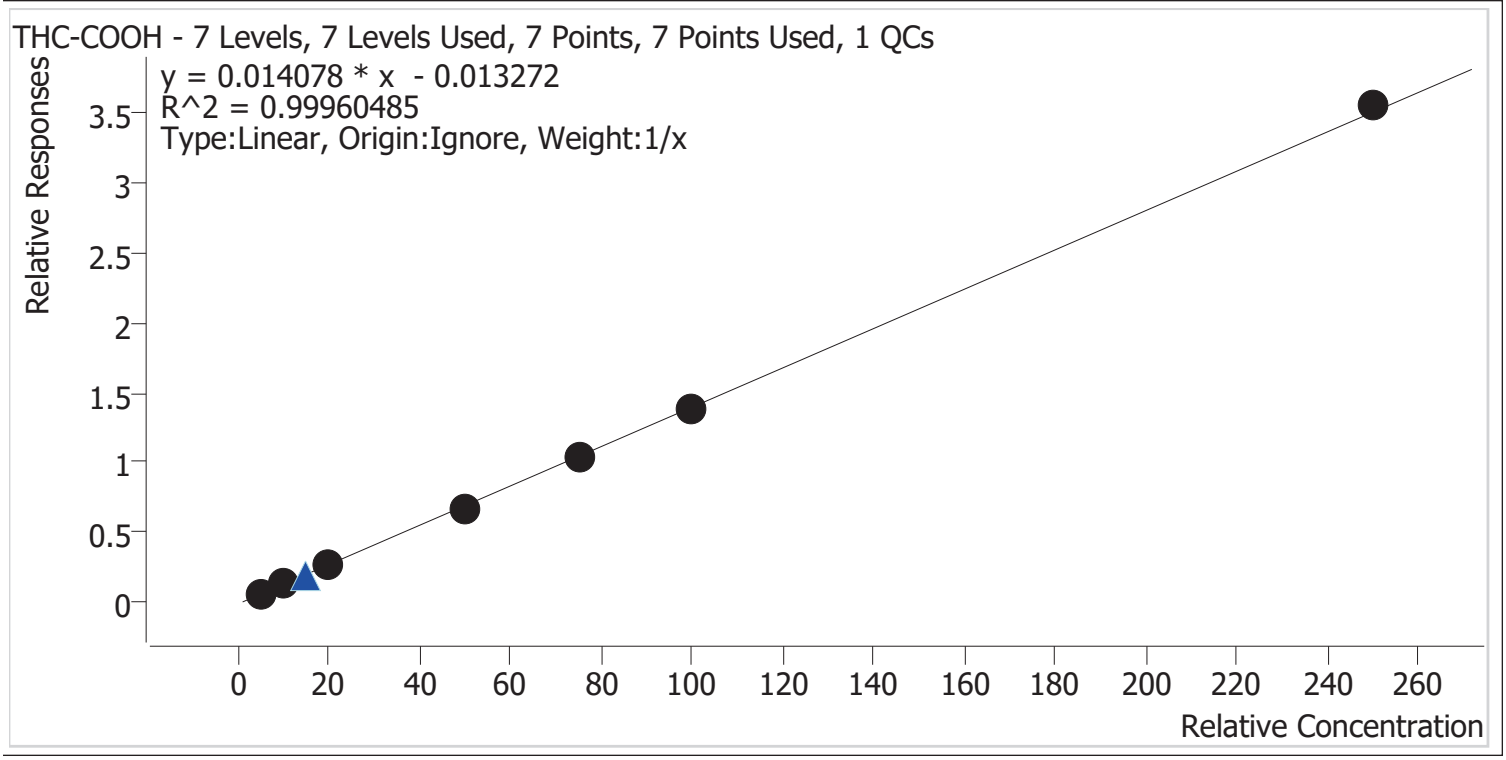
Batch results	D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Last Cal. Update	10/8/2020 11:25 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	1.2	120.8
mj cal2	2	✓	3.0	2.8	94.5
mj cal 3	3	✓	5.0	4.8	95.6
mj cal 4	4	✓	10.0	9.2	92.5
mj cal 5	5	✓	25.0	23.7	94.7
mj cal 6	6	✓	50.0	49.7	99.3
mj cal 7	7	✓	100.0	102.6	102.6

Compound Calibration Report

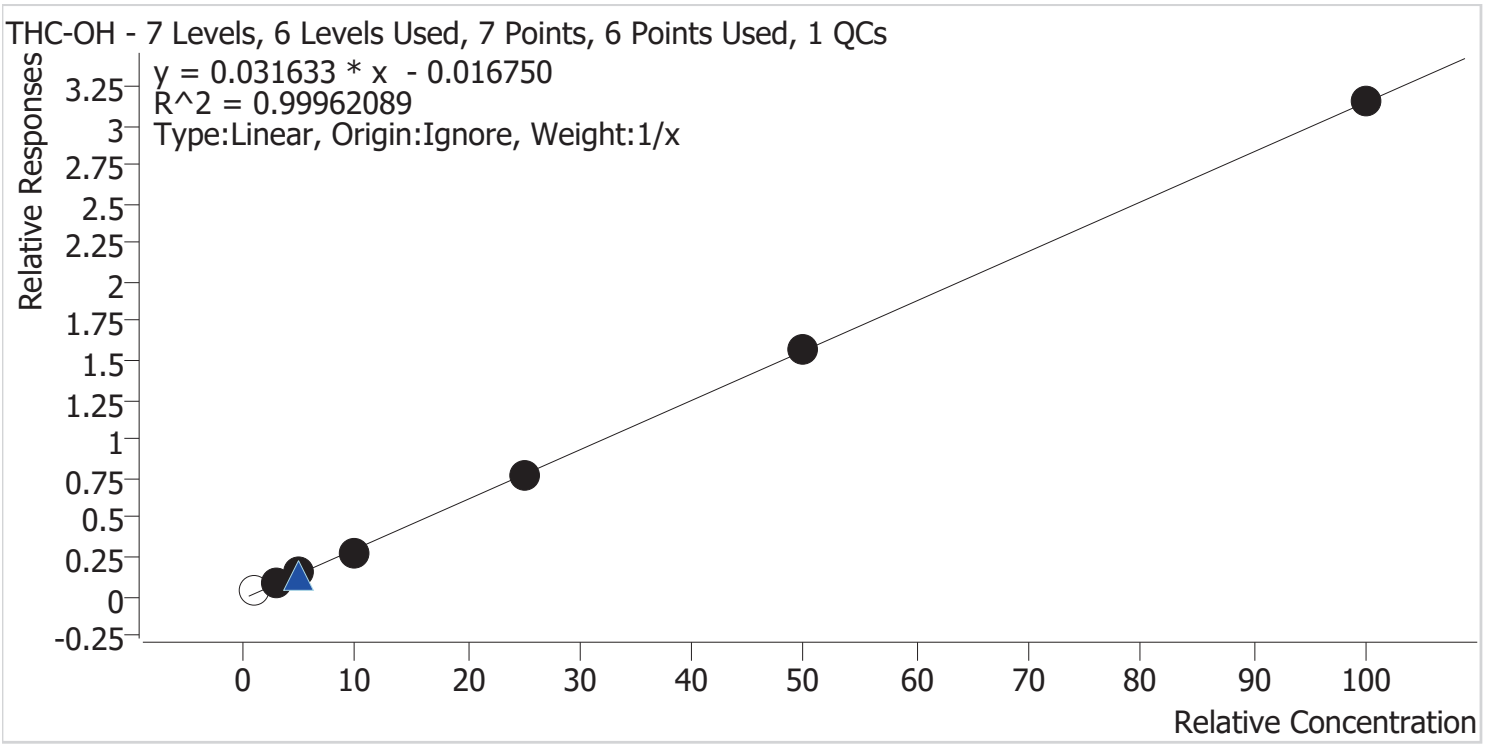
Batch results	D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Last Cal. Update	10/8/2020 11:25 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.1	102.8
mj cal2	2	✓	10.0	10.5	104.6
mj cal 3	3	✓	20.0	19.2	95.8
mj cal 4	4	✓	50.0	48.8	97.6
mj cal 5	5	✓	75.0	74.1	98.9
mj cal 6	6	✓	100.0	99.0	99.0
mj cal 7	7	✓	250.0	253.3	101.3

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Last Cal. Update	10/8/2020 11:25 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



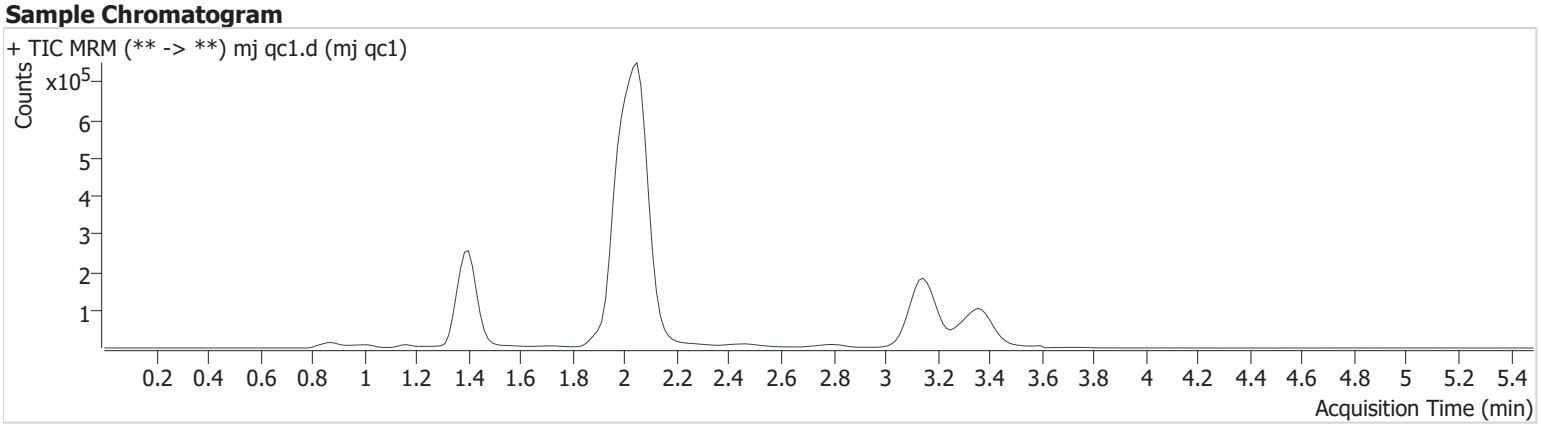
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	×	1.0	1.8	184.1
mj cal2	2	✓	3.0	3.0	101.5
mj cal 3	3	✓	5.0	5.2	104.3
mj cal 4	4	✓	10.0	9.4	93.5
mj cal 5	5	✓	25.0	25.0	99.9
mj cal 6	6	✓	50.0	50.3	100.6
mj cal 7	7	✓	100.0	100.1	100.1

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 AM

Instrument	69679	**Data File**	mj qc1.d
Type	Cal	**Sample**	mj qc1
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/7/2020 6:13:25 PM		
Sample Info.			

Sample Chromatogram



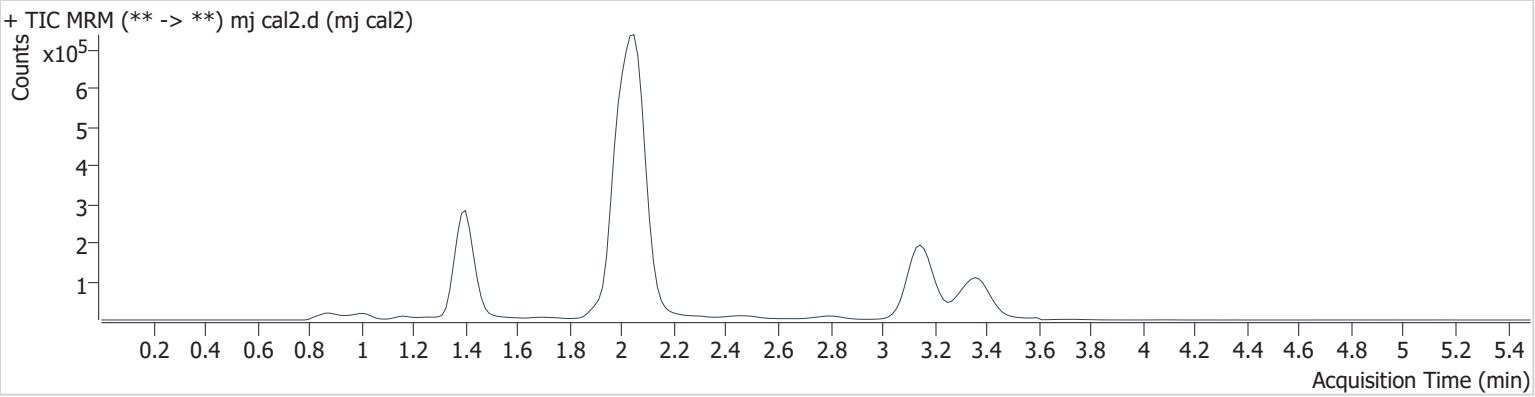
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.391	34517	∞	8.5	∞	831984	1.841 ng/ml	Low
THC-COOH	1.431	26092	62.3	35.8	145.5	441402	5.142 ng/ml	Low
THC	3.197	15275	130.0	25.5	∞	642251	1.208 ng/ml	Low

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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	10/7/2020 6:21:09 PM		
Sample Info.			

Sample Chromatogram



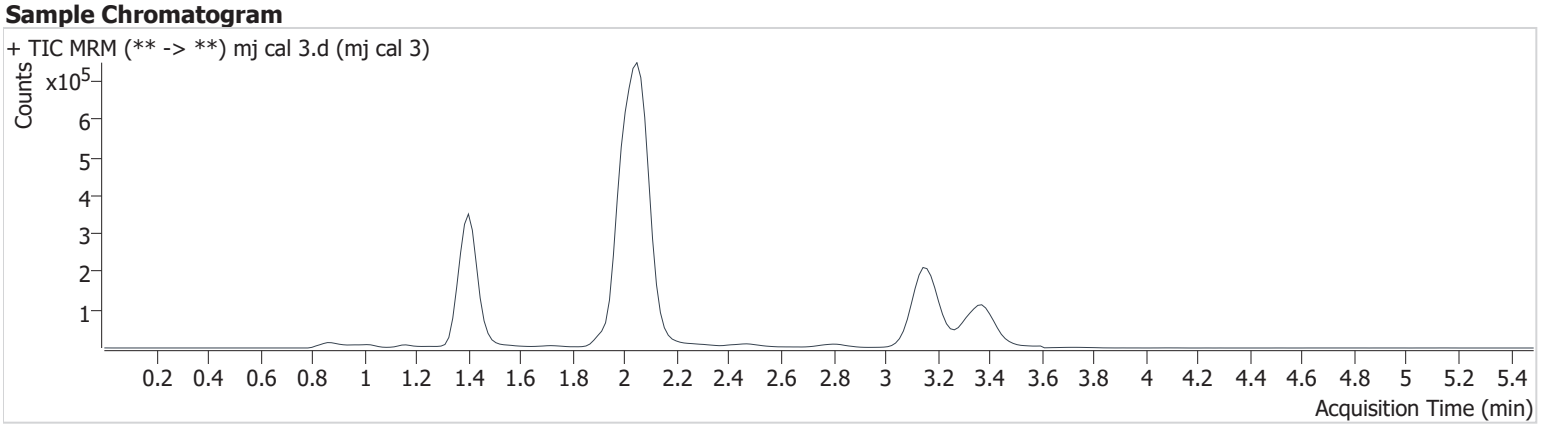
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.391	63314	∞	11.8	∞	795334	3.046 ng/ml	
THC-COOH	1.431	59239	48.4	33.0	23523.9	442129	10.460 ng/ml	
THC	3.182	42468	∞	26.2	148.5	632710	2.834 ng/ml	Low

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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	10/7/2020 6:28:51 PM		
Sample Info.			

Sample Chromatogram



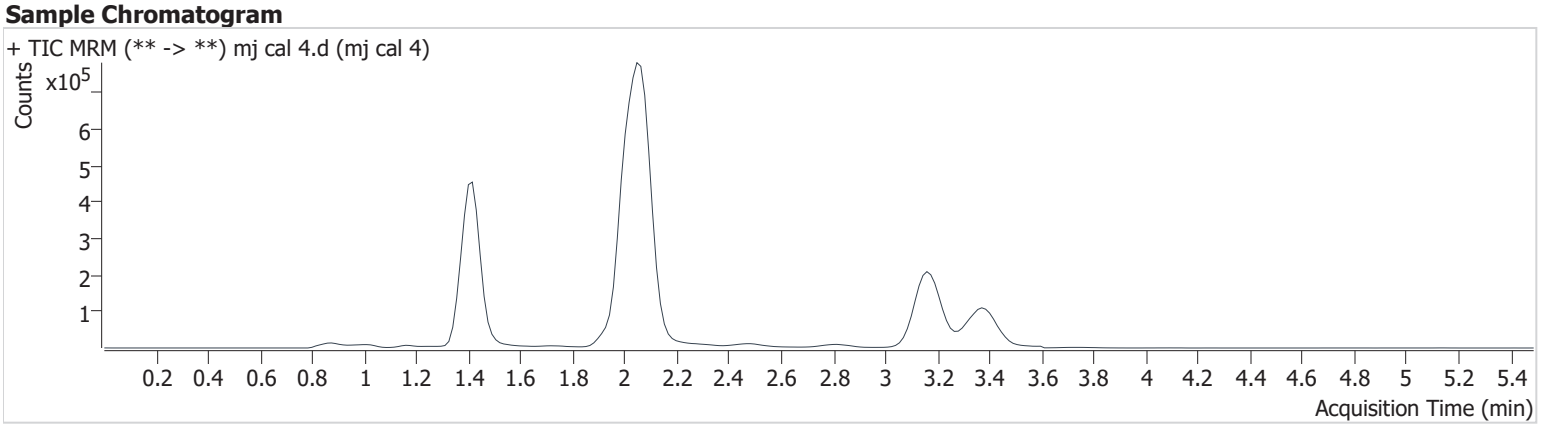
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	124311	∞	10.7	∞	838400	5.217 ng/ml
THC-COOH	1.416	118814	1061.1	35.6	264.4	463290	19.160 ng/ml
THC	3.182	80601	5740794823 8946.5	26.5	1030.8	677191	4.782 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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	10/7/2020 6:36:35 PM		
Sample Info.			

Sample Chromatogram



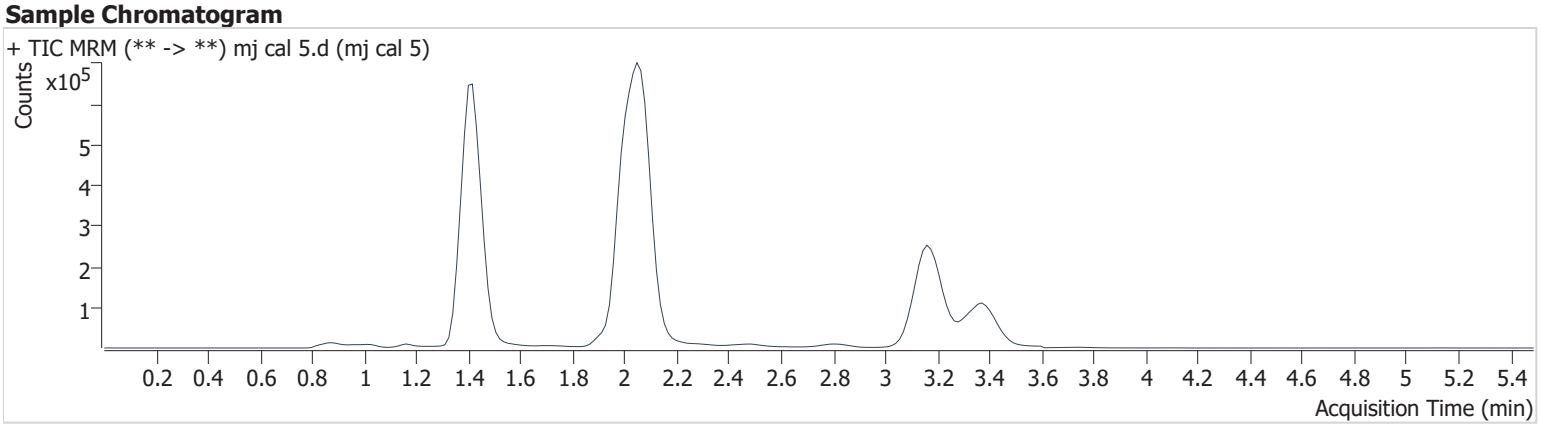
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	226743	∞	12.2	∞	812327	9.353 ng/ml
THC-COOH	1.431	289762	340.1	34.9	661.3	430233	48.784 ng/ml
THC	3.197	145786	∞	25.2	5205.8	612432	9.248 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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	10/7/2020 6:44:19 PM		
Sample Info.			

Sample Chromatogram



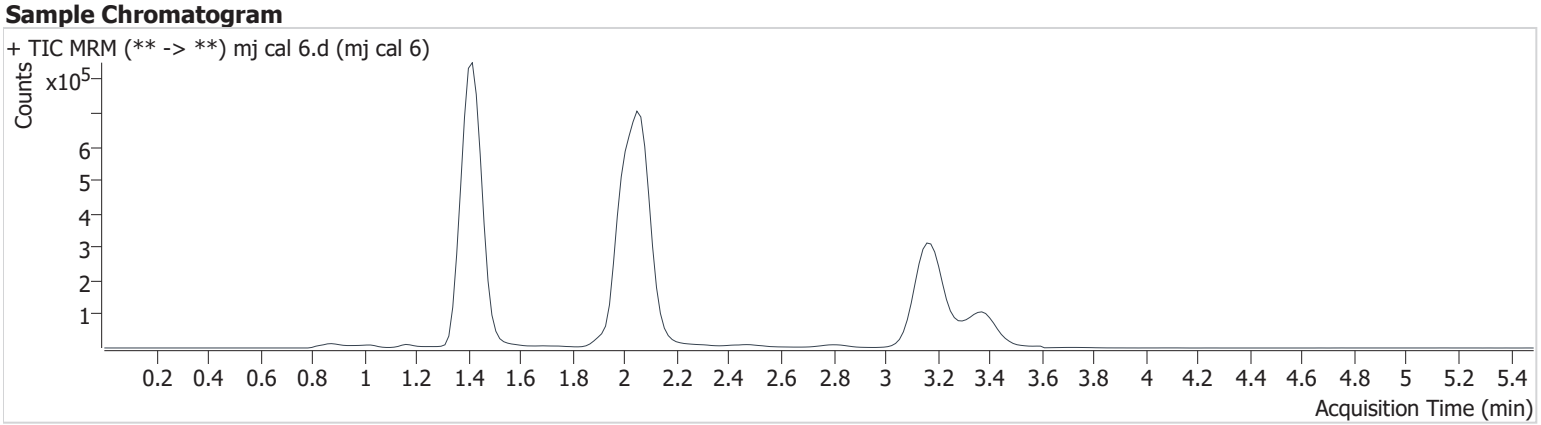
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	688044	∞	11.0	∞	889834	24.973 ng/ml
THC-COOH	1.431	501731	1032519.7	35.6	563.3	486845	74.149 ng/ml
THC	3.197	425976	∞	24.6	∞	683988	23.686 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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	10/7/2020 6:52:01 PM		
Sample Info.			

Sample Chromatogram



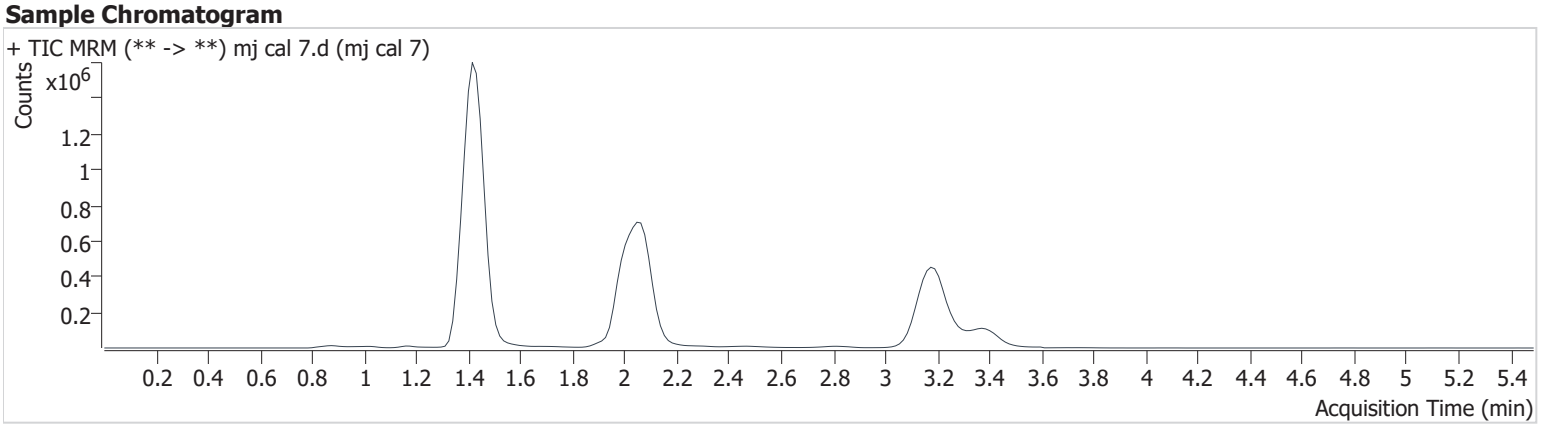
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1372136	∞	11.4	∞	871678	50.292 ng/ml
THC-COOH	1.431	667298	1705.2	35.4	611.8	483336	99.013 ng/ml
THC	3.197	884527	∞	24.5	∞	672602	49.666 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 100720\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2020 11:25:20 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	10/7/2020 6:59:46 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	2746737	∞	11.8	∞	871891	100.119 ng/ml
THC-COOH	1.431	1625631	1735.3	36.3	4576.5	457597	253.293 ng/ml
THC	3.197	1824814	∞	24.7	∞	669655	102.576 ng/ml