REVIEWED
By Anne Nord at 9:09 am, Jul 29, 2021

7/28/2021

Worklist: 5129

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2021-1425	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1488	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1501	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1517	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1549	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1553	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1590	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1679	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1682	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1712	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1714	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1715	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ

AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 7/27/21 Analyst: Britany Wylie
Plate lot#: 210609 Plate Expiration: 12-9-2021
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: 21D52496 **Neg Urine Lot**: 5621
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 250ul 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** 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: 10ng/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: Hydroxy-THC not evaluated in Urine samples.

am 27

7/27/21 extraction

	1	2	3	4	5	6
A	IS + Cal. 1	neg blood	1553-1			IS + QC_1
B	IS + Cal. 2	1425-1	1590-1			IS + Cal. 7
C	IS + Cal. 3	1488-1	1679-2			IS + Cal. 6
D	IS + Cal. 4	1501-1 (did not flow through SLE)	1682-1			IS + Cal. 5
E	IS + Cal. 5	1517-1	1712-1			IS + Cal. 4
F	IS + Cal. 6	1549-1	1714-2			IS + Cal. 3
G	IS + Cal. 7	neg urine	1715-1			IS + Cal. 2
H	IS + QC_1	urine control	1501-1			IS + Cal. 1

All wells to contain 100 µl of residual DMSO

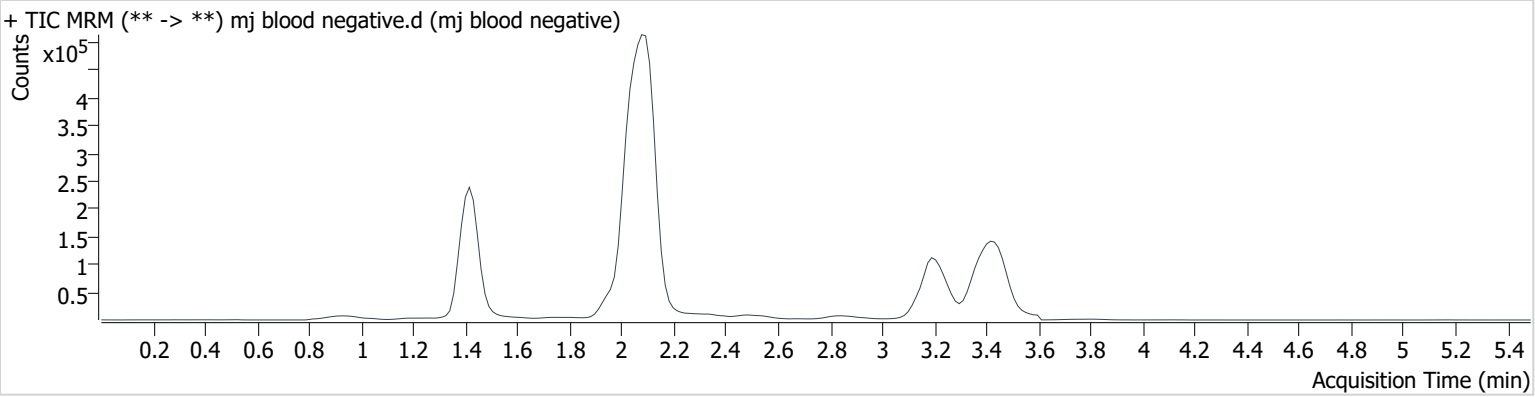
Case #: C2021-____

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj blood negative.d
Type	Sample	**Sample**	mj blood negative
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/28/2021 12:00:08 AM		
Sample Info.			

Sample Chromatogram

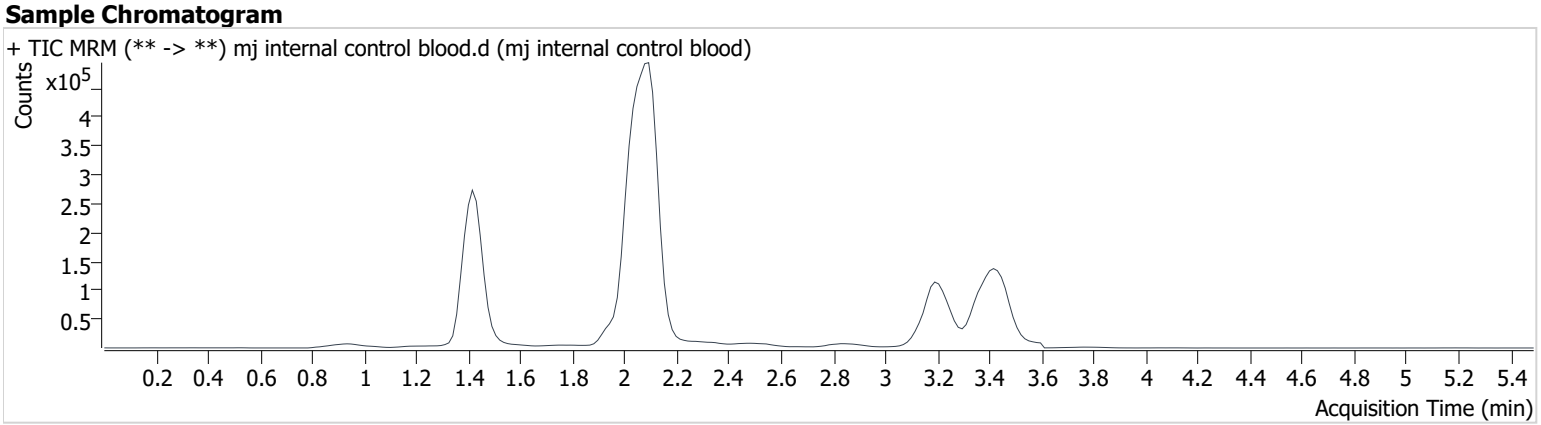


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 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**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:53:26 PM		
Sample Info.			

Sample Chromatogram



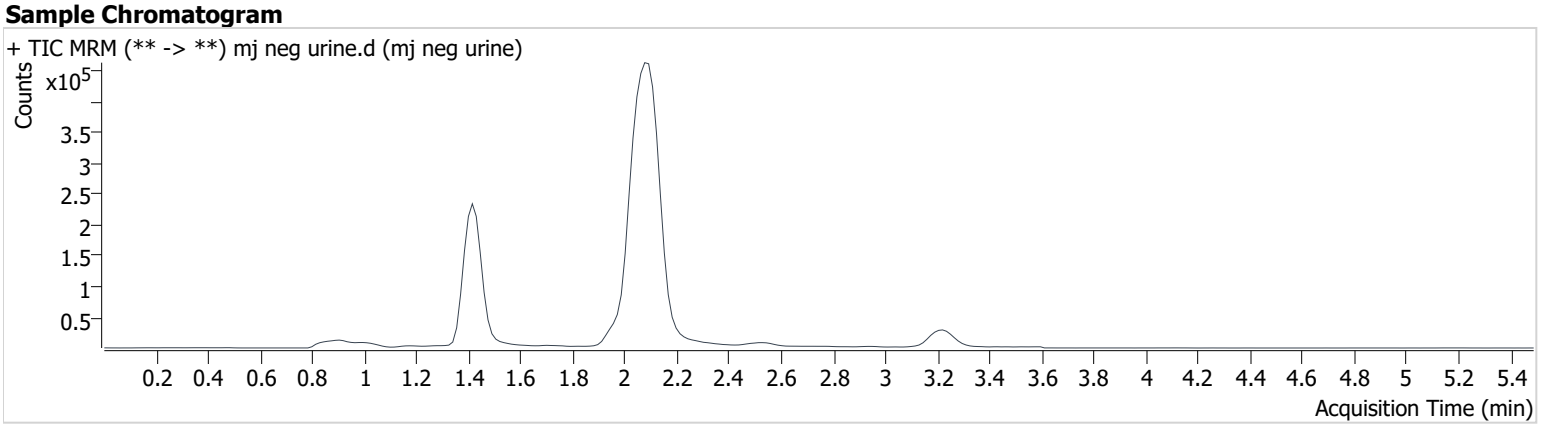
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	13847	∞	936.7	6242.6	896412	4.870	ng/ml
THC-COOH	1.446	50650	534.0	36.0	26612.7	284947	14.407	ng/ml
THC	3.227	41327	467.8	28.2	∞	380681	4.638	ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update	7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj neg urine.d
Type	Sample	**Sample**	mj neg urine
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-G2	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/28/2021 1:20:06 AM		
Sample Info.			

Sample Chromatogram

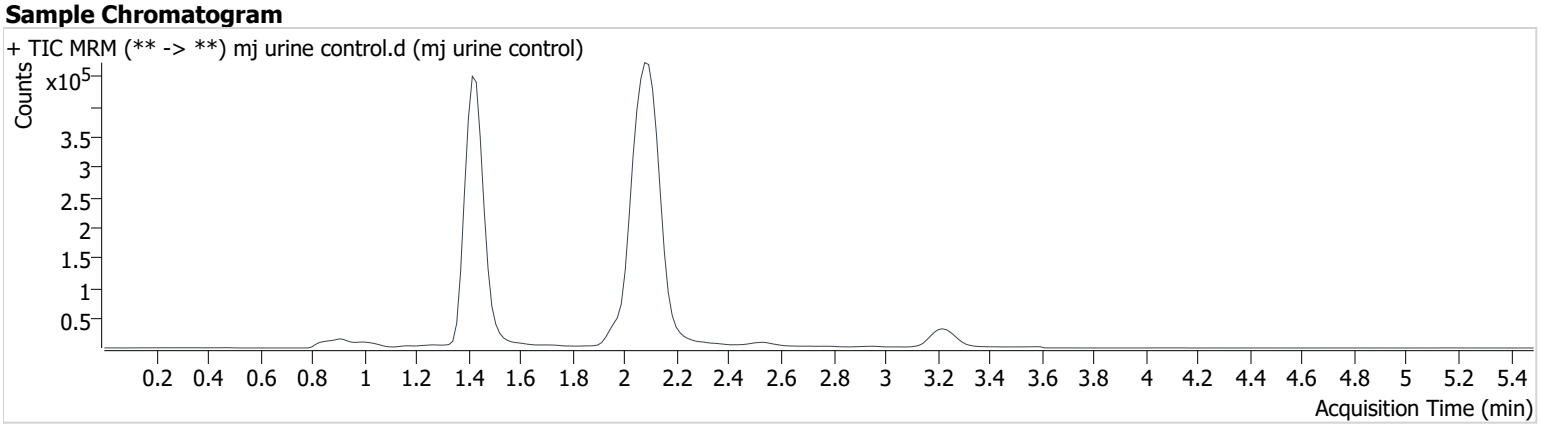


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj urine control.d
Type	Sample	**Sample**	mj urine control
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-H2	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/28/2021 1:33:28 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-OH	1.424	89091	∞	897.0	∞	923556	29.730	ng/ml	NE
THC-COOH	1.446	108732	508.3	34.0	36579.8	191781	43.695	ng/ml	
THC	3.242	30822	∞	27.5	∞	164447	7.757	ng/ml	

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 ()

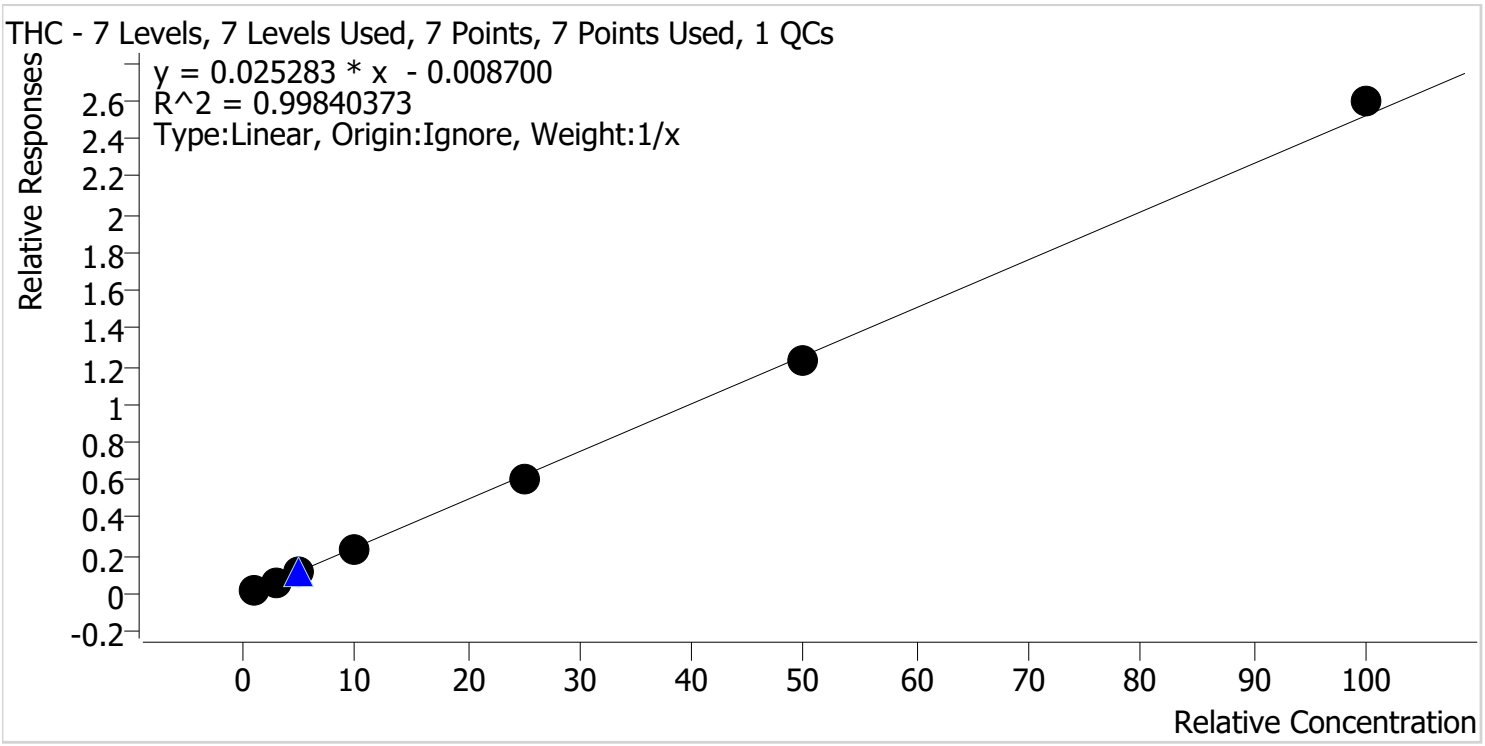
		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	

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	1/12/2021
ppd 1/13/21 Exp 7/1/21 neg urine lot 10120	lot 11321	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	3/28/2021
ppd 3/29/21 Exp 7/1/21 neg urine lot 2121	lot 32921	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	5/27/2021
ppd 5/28/21 Exp 7/1/21 neg urine lot 5621	lot 52821	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

Hydroxy THC not evaluated in Urine control or samples in this batch.

Compound Calibration Report

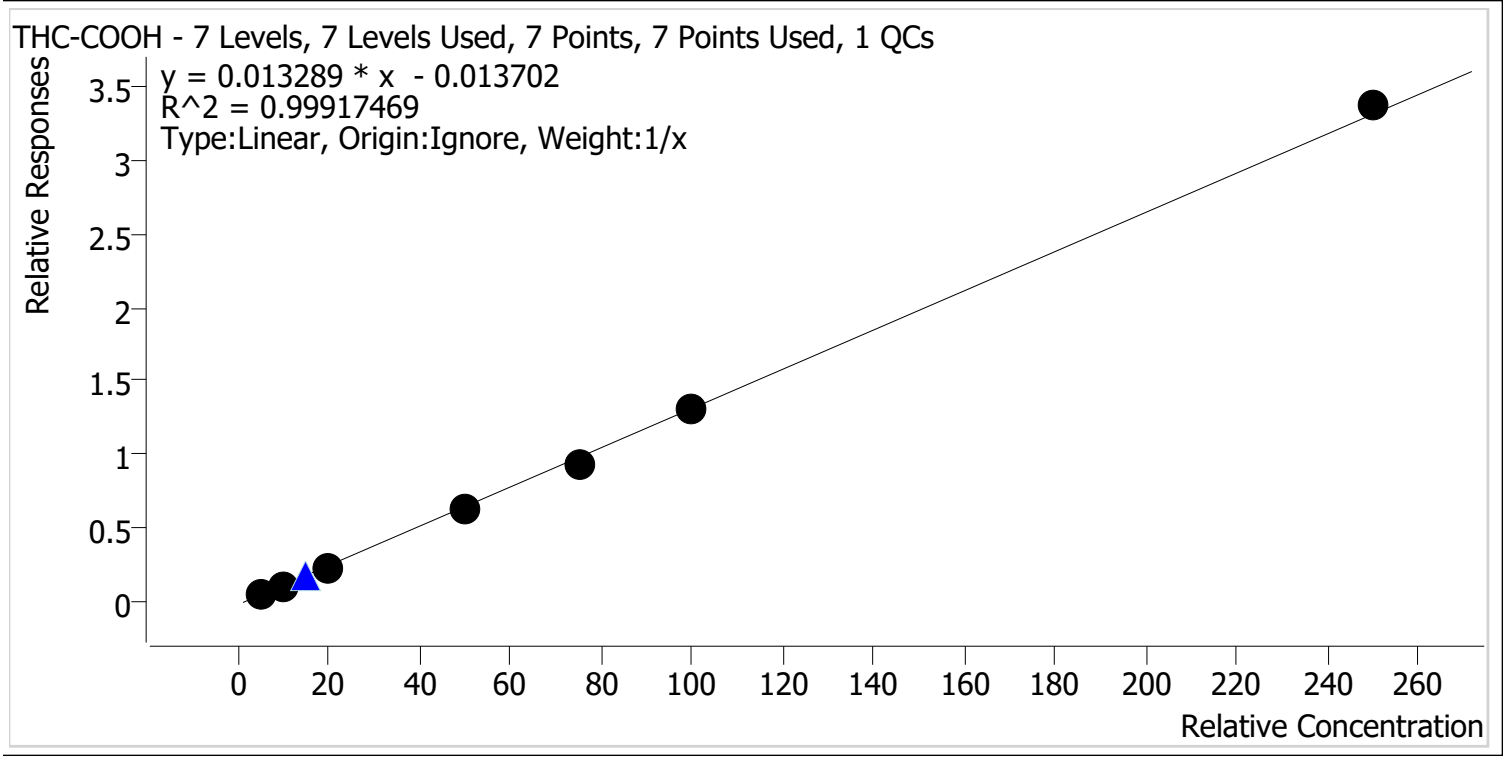
Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Last Cal. Update 7/28/2021 8:02 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	1.0	1.2	120.6
mj cal2	2	✔	3.0	2.9	95.9
mj cal 3	3	✔	5.0	4.7	93.8
mj cal 4	4	✔	10.0	9.3	92.8
mj cal 5	5	✔	25.0	23.9	95.7
mj cal 6	6	✔	50.0	49.1	98.2
mj cal 7	7	✔	100.0	102.9	102.9

Compound Calibration Report

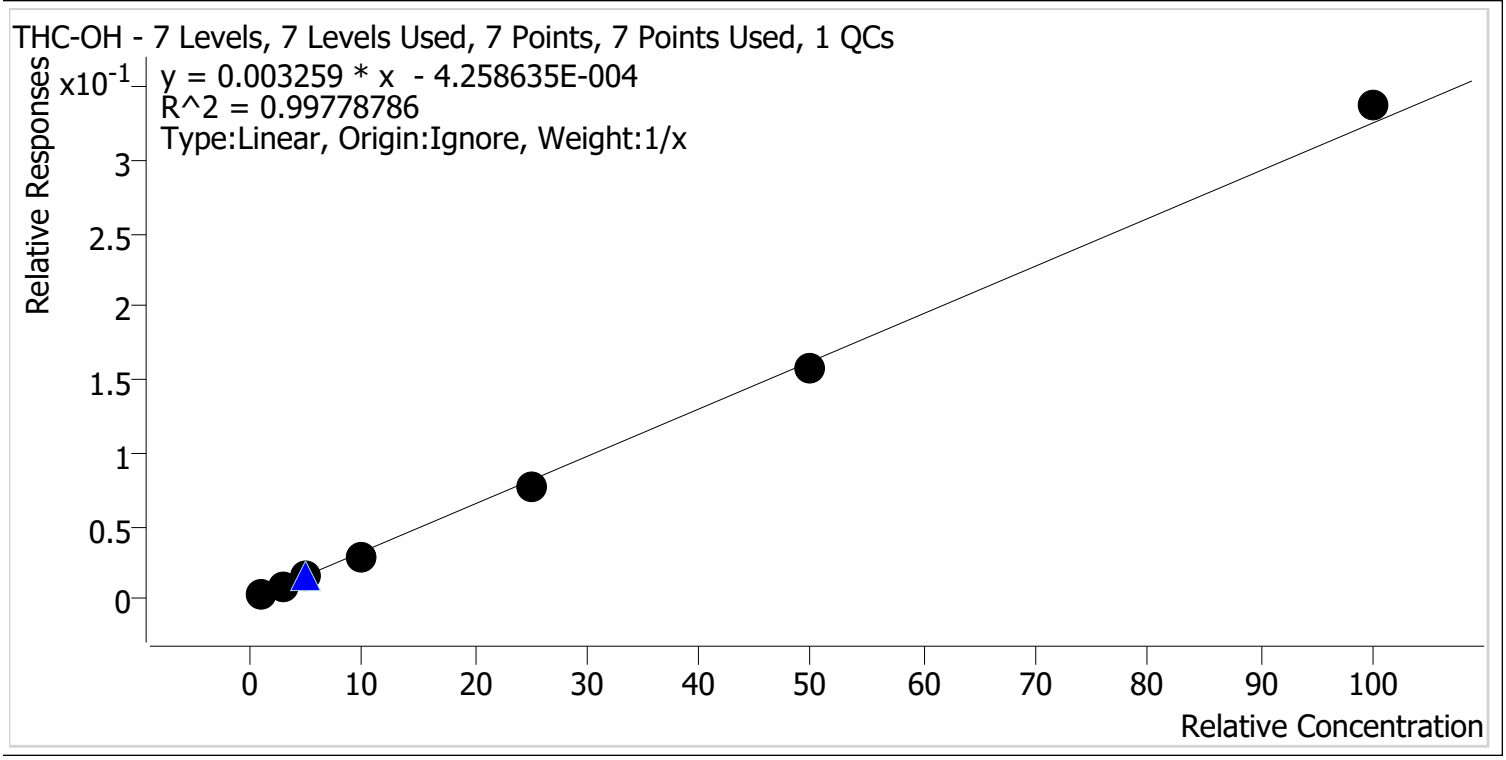
Batch results	D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Last Cal. Update	7/28/2021 8:02 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	5.0	5.5	110.1
mj cal2	2	✔	10.0	9.8	98.1
mj cal 3	3	✔	20.0	19.3	96.4
mj cal 4	4	✔	50.0	48.6	97.3
mj cal 5	5	✔	75.0	72.0	95.9
mj cal 6	6	✔	100.0	100.5	100.5
mj cal 7	7	✔	250.0	254.4	101.7

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Last Cal. Update	7/28/2021 8:02 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



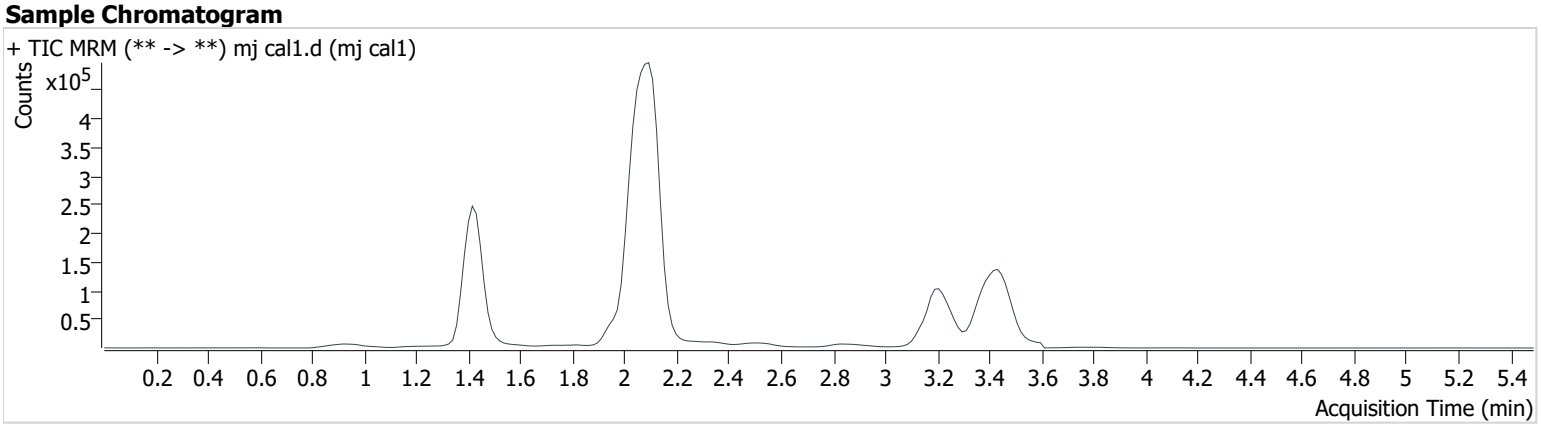
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	1.0	1.2	121.3
mj cal2	2	✔	3.0	2.9	96.5
mj cal 3	3	✔	5.0	4.7	94.9
mj cal 4	4	✔	10.0	9.1	91.1
mj cal 5	5	✔	25.0	23.9	95.4
mj cal 6	6	✔	50.0	48.5	97.1
mj cal 7	7	✔	100.0	103.6	103.6

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj cal1.d
Type	Cal	**Sample**	mj cal1
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:06:31 PM		
Sample Info.			

Sample Chromatogram



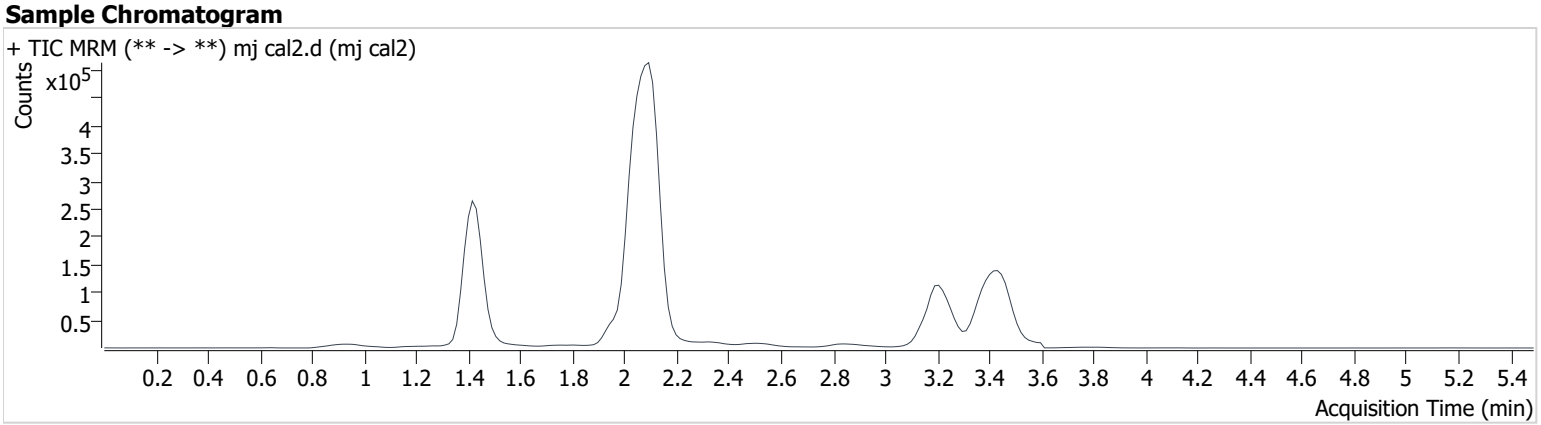
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	3287	456.2	853.9	∞	931933	1.213	ng/ml Low
THC-COOH	1.446	15456	56.7	36.6	532.4	260080	5.503	ng/ml
THC	3.242	8272	171.9	30.2	13.7	379670	1.206	ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update	7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj cal2.d
Type	Cal	**Sample**	mj cal2
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:13:15 PM		
Sample Info.			

Sample Chromatogram



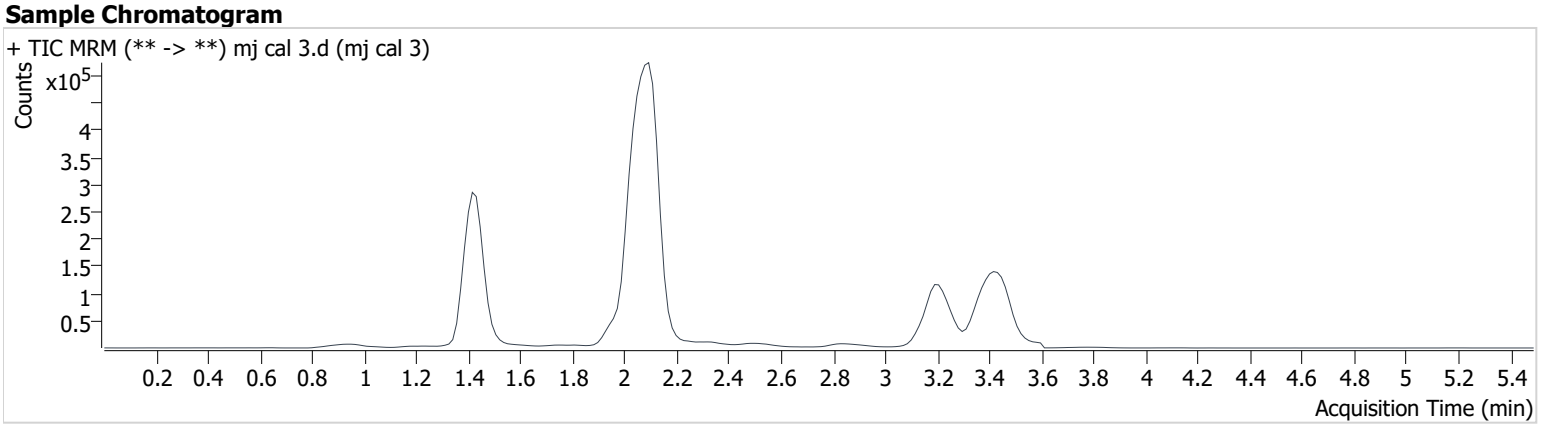
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	8411	∞	812.4	153.7	933689	2.895	ng/ml Low
THC-COOH	1.446	31949	117.0	35.3	56.4	273756	9.813	ng/ml
THC	3.242	25857	∞	25.8	153.4	403665	2.878	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj cal 3
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:19:57 PM		
Sample Info.			

Sample Chromatogram



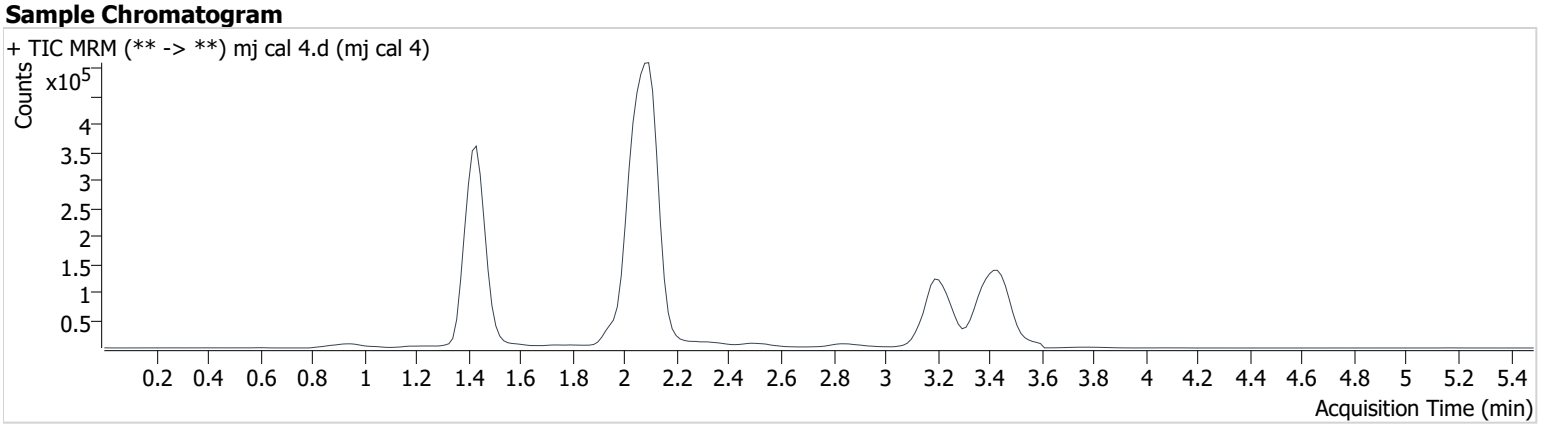
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	13854	∞	827.2	378.9	921107	4.746	ng/ml
THC-COOH	1.446	65231	133.3	36.2	26259.2	268968	19.281	ng/ml
THC	3.227	43817	∞	25.0	∞	398658	4.691	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj cal 4.d
Type	Cal	**Sample**	mj cal 4
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:26:39 PM		
Sample Info.			

Sample Chromatogram



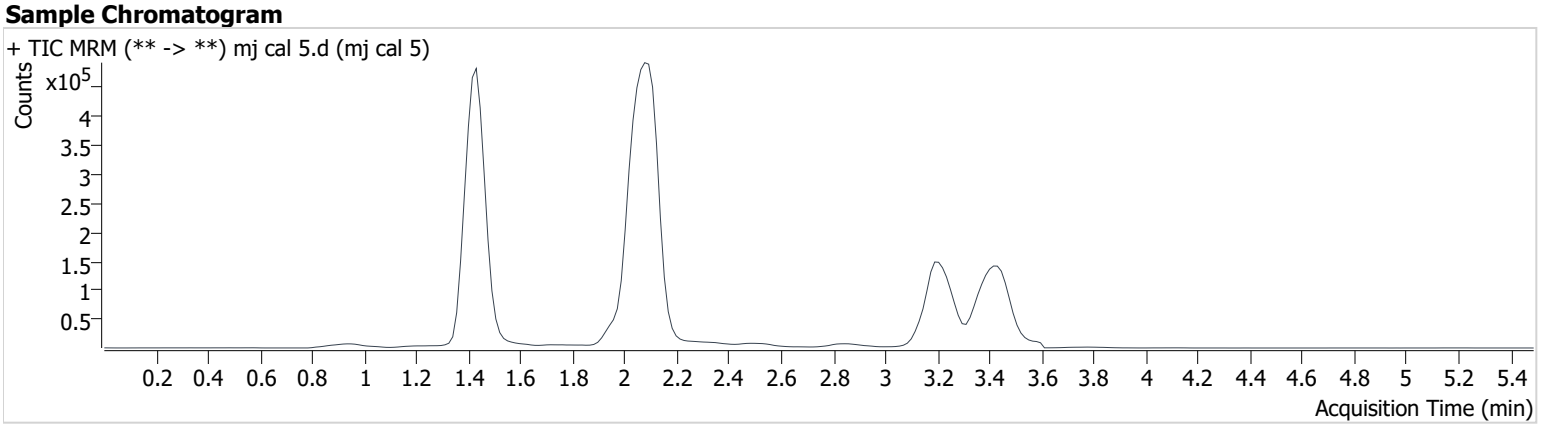
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	26955	∞	908.4	618.2	920754	9.113	ng/ml
THC-COOH	1.446	172340	1294.0	35.9	680.5	272452	48.630	ng/ml
THC	3.242	89532	∞	23.9	∞	396185	9.282	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:33:21 PM		
Sample Info.			

Sample Chromatogram



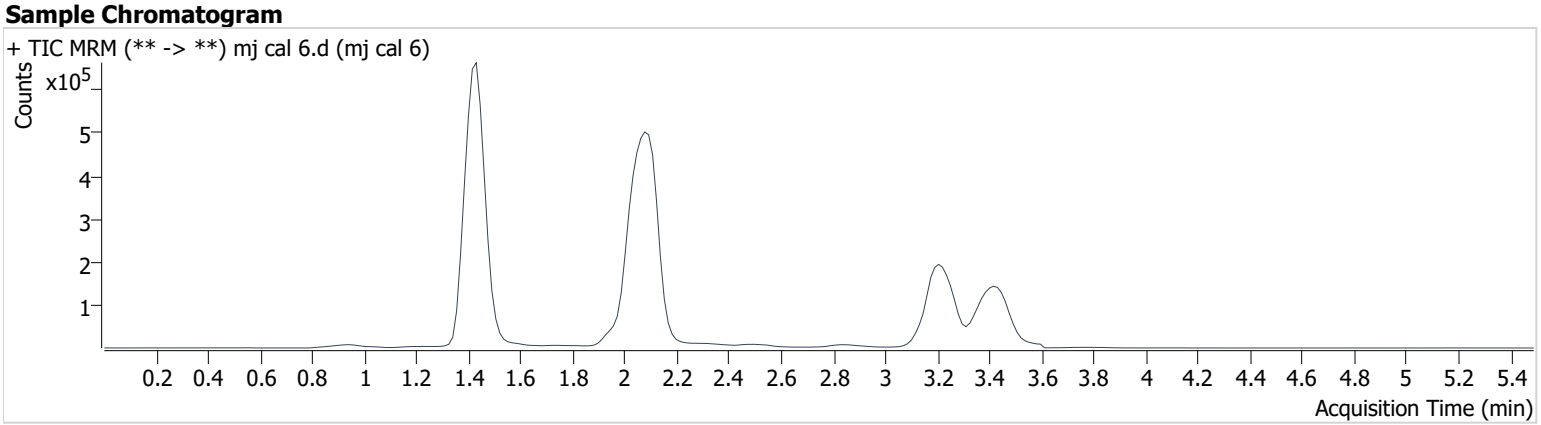
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	69607	∞	881.1	∞	900019	23.862	ng/ml
THC-COOH	1.446	255102	584.4	36.1	376.8	270679	71.950	ng/ml
THC	3.227	231983	∞	24.9	∞	388999	23.932	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:40:02 PM		
Sample Info.			

Sample Chromatogram



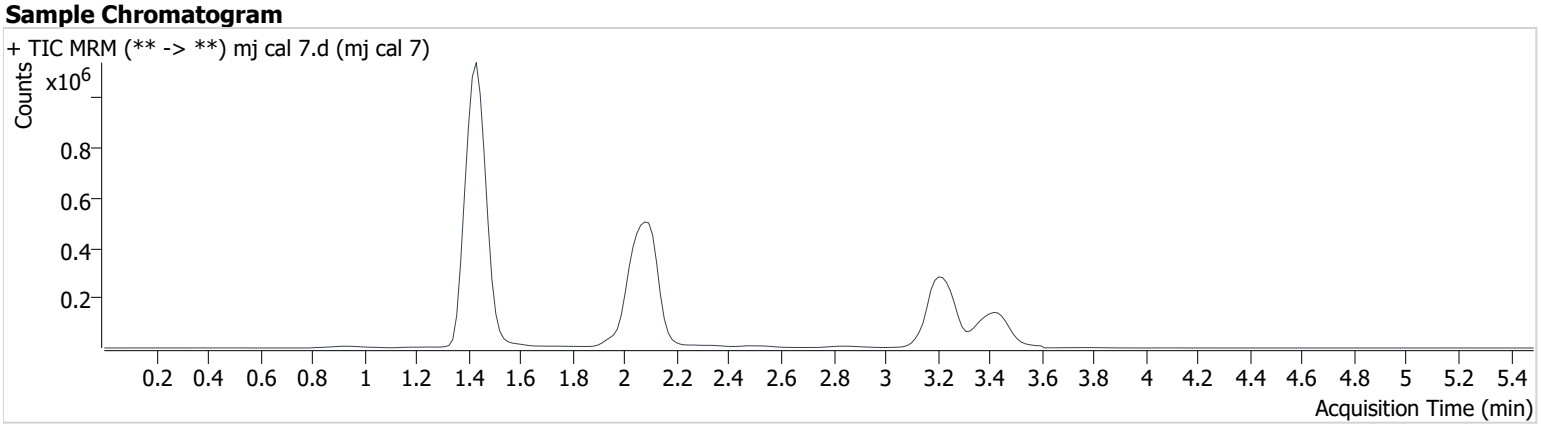
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	143397	∞	912.5	61779.5	908833	48.544	ng/ml
THC-COOH	1.446	347918	1495.3	35.7	1515.8	263280	100.472	ng/ml
THC	3.227	485830	∞	24.2	∞	394103	49.103	ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072721\QuantResults\cann.batch.bin
Calibration Last Update 7/28/2021 8:02:39 AM

Instrument	69679	**Data File**	mj cal 7.d
Type	Cal	**Sample**	mj cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/27/2021 11:46:44 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	291973	∞	861.6	∞	865624	103.627	ng/ml
THC-COOH	1.446	829642	1016.7	36.7	243412.0	246448	254.351	ng/ml
THC	3.227	984396	∞	23.3	∞	379624	102.908	ng/ml