REVIEWED
By Celena Shrum at 11:52 am, Feb 08, 2021

2/3/2021

4/2/21- reviewed corrections to control/calibration positions on am 27 plate map BW

Worklist: 4771

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2021-0106	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0117	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-0143	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0216	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 02/02/21 Analyst: Anne Nord
Plate lot#: 201206 Plate Expiration: 06/06/2021

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: 20J20793 **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 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 urine, the ratio in the control is off.*

	1	2	3	4	5	6
a	cal 1 ~~cal 100 ng~~	neg blood				QC 1
b	cal 2 ~~cal 50 ng~~	106-1				cal 100 ng
c	cal 3 ~~cal 25 ng~~	143-1				cal 50 ng
d	cal 4 ~~cal 10ng~~	216-1				cal 25 ng
e	cal 5 ~~cal 5 ng~~	neg urine				cal 10ng
f	cal 6 ~~cal 3 ng~~	urine control				cal 5 ng
g	cal 7 ~~cal 1ng~~	117-1				cal 3 ng
h	QC 1					cal 1ng

C2021-0___-_

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**

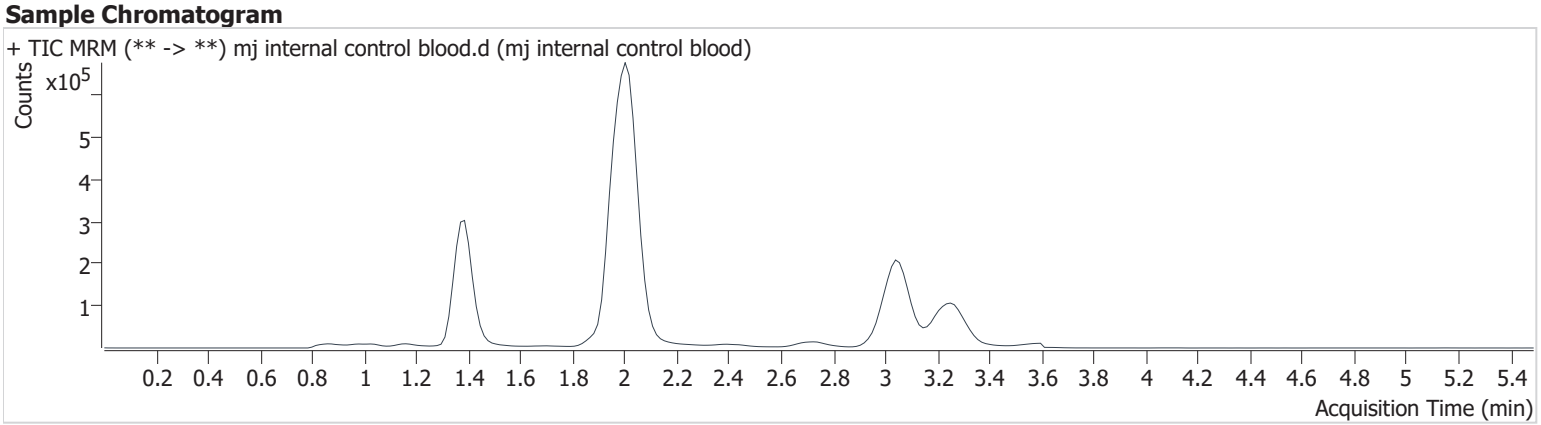
ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	1/12/2021
ppd 1/13/21 Exp 7/1/21 neg urine lot 10120	lot 11321	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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	2/2/2021 12:01:41 PM		
Sample Info.			

Sample Chromatogram



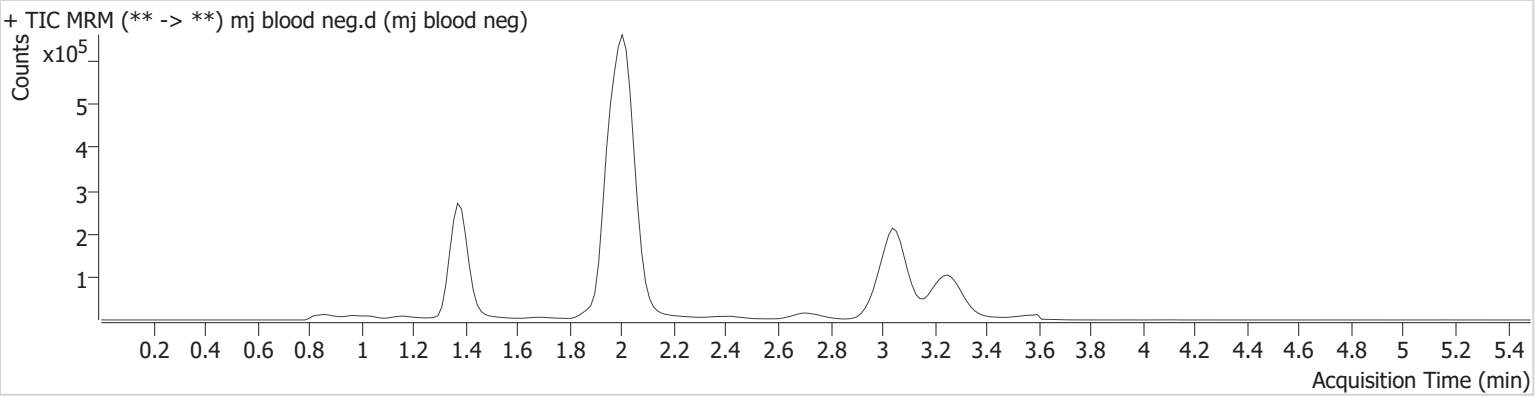
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	111277	∞	9.3	∞	833153	4.793 ng/ml
THC-COOH	1.401	68057	192106.3	34.1	82.8	384701	14.112 ng/ml
THC	3.077	36718	674.0	24.6	74.5	383066	4.052 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 PM

Instrument	69679	**Data File**	mj blood neg.d
Type	Sample	**Sample**	mj blood neg
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/2/2021 12:08:25 PM		
Sample Info.			

Sample Chromatogram



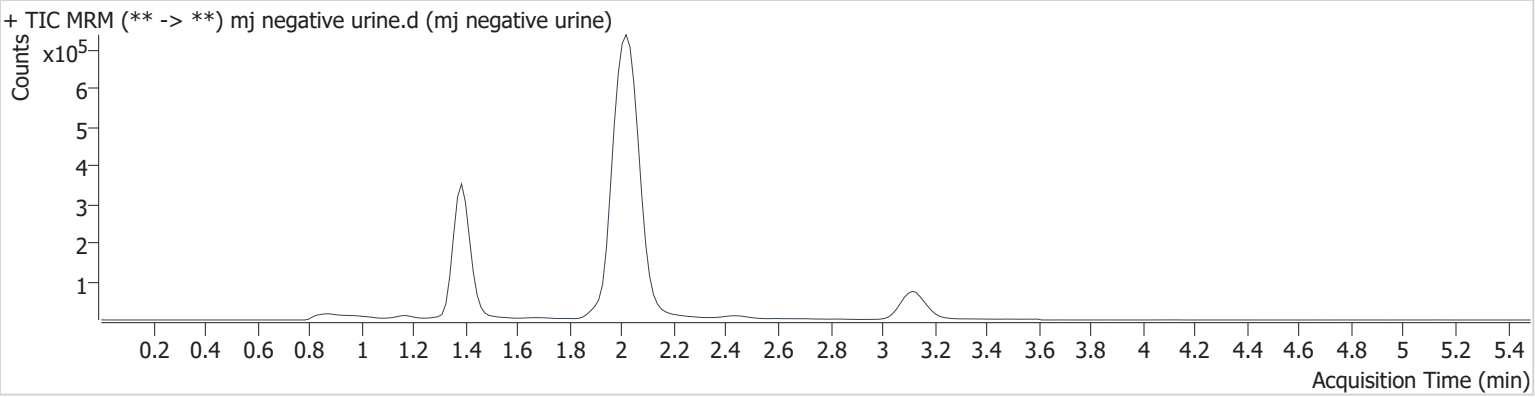
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.436	27464	∞			889669	0.884 ng/ml	Low

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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-E2	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/2/2021 1:15:19 PM		
Sample Info.			

Sample Chromatogram

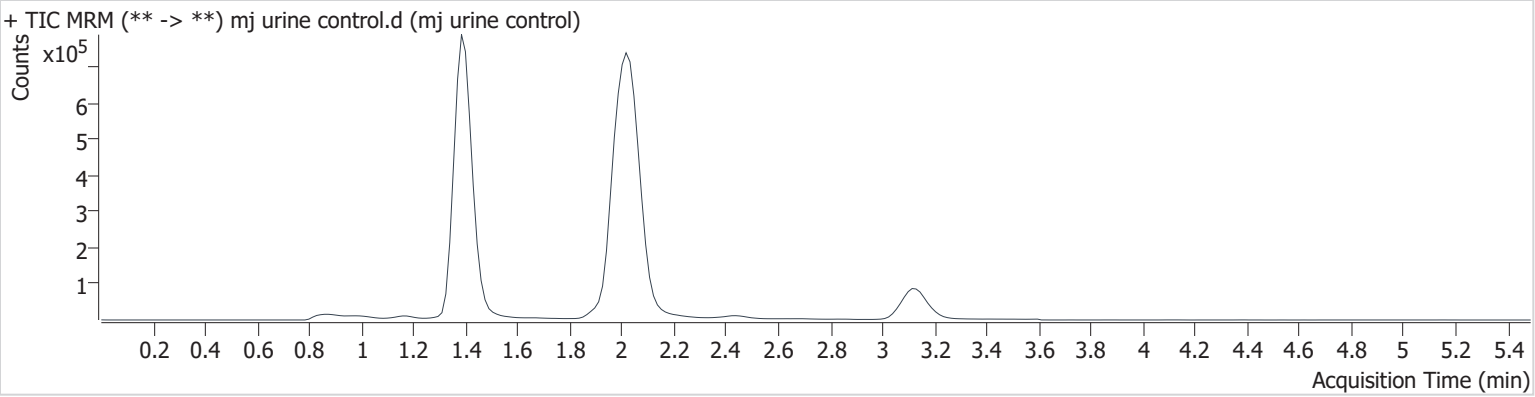


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 PM

Instrument	69679	**Data File**	mj urine control.d
Type	Sample	**Sample**	mj urine control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-F2	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/2/2021 1:22:03 PM		
Sample Info.			

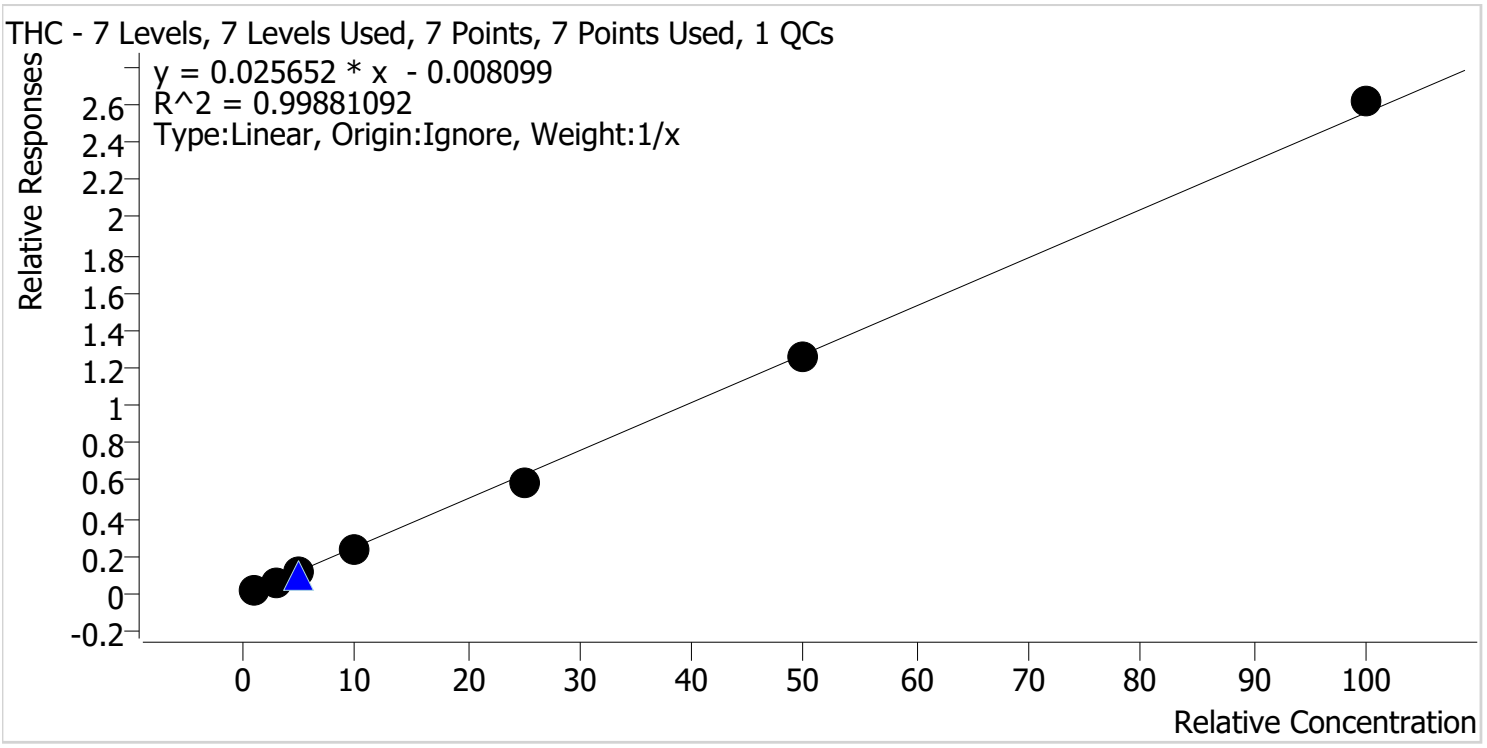
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	1494588	∞	14.8 **High**	∞	1224060	46.192 ng/ml
THC-COOH	1.416	197114	1349.1	34.9	1454.1	408120	36.472 ng/ml
THC	3.137	141398	∞	23.7	1292.3	431653	13.086 ng/ml

Compound Calibration Report

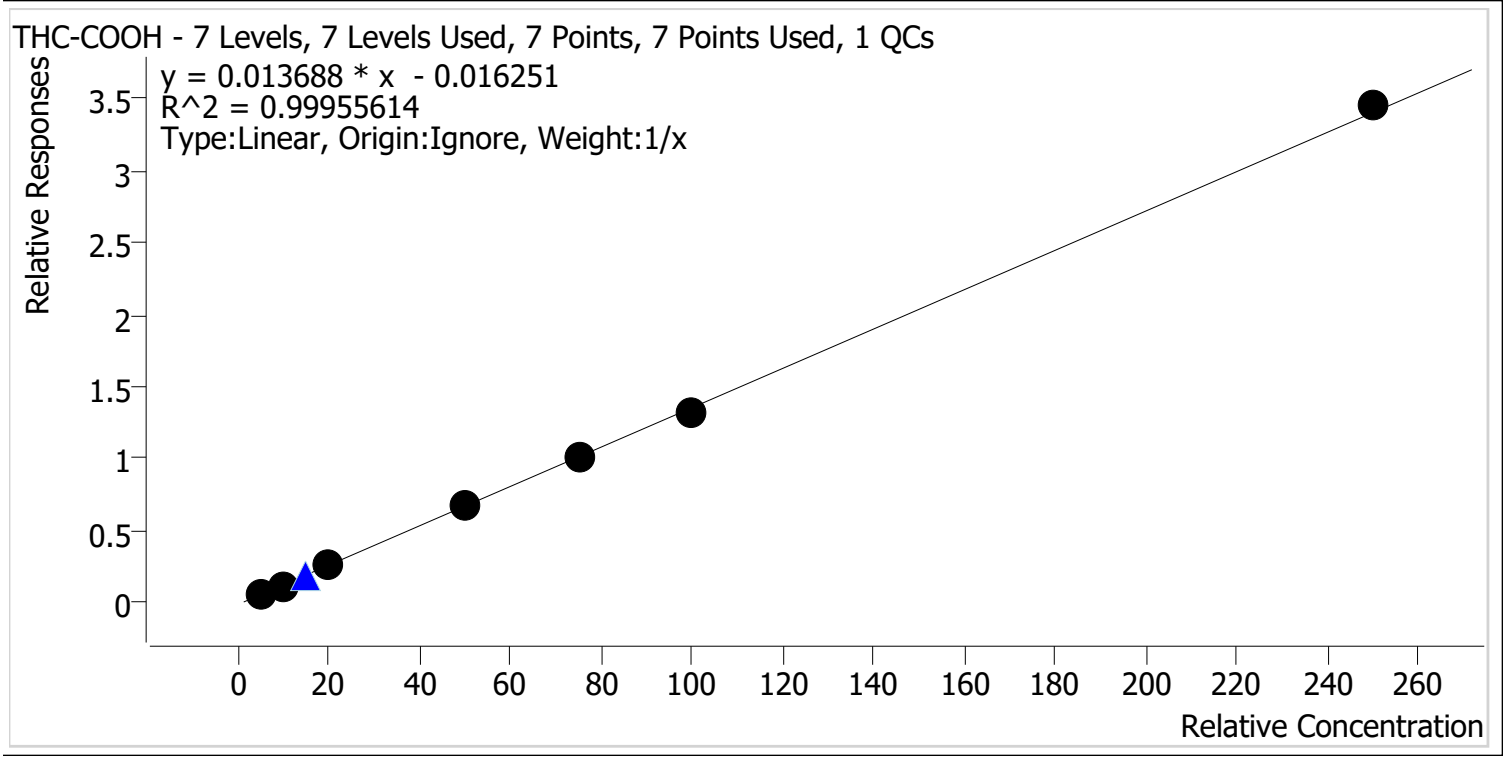
Batch results	D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Last Cal. Update	2/4/2021 12:06 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	1.0	1.2	116.5
mj cal2	2	✔	3.0	2.9	96.3
mj cal 3	3	✔	5.0	4.8	95.5
mj cal 4	4	✔	10.0	9.6	95.6
mj cal 5	5	✔	25.0	23.6	94.3
mj cal 6	6	✔	50.0	49.7	99.5
mj cal 7	7	✔	100.0	102.3	102.3

Compound Calibration Report

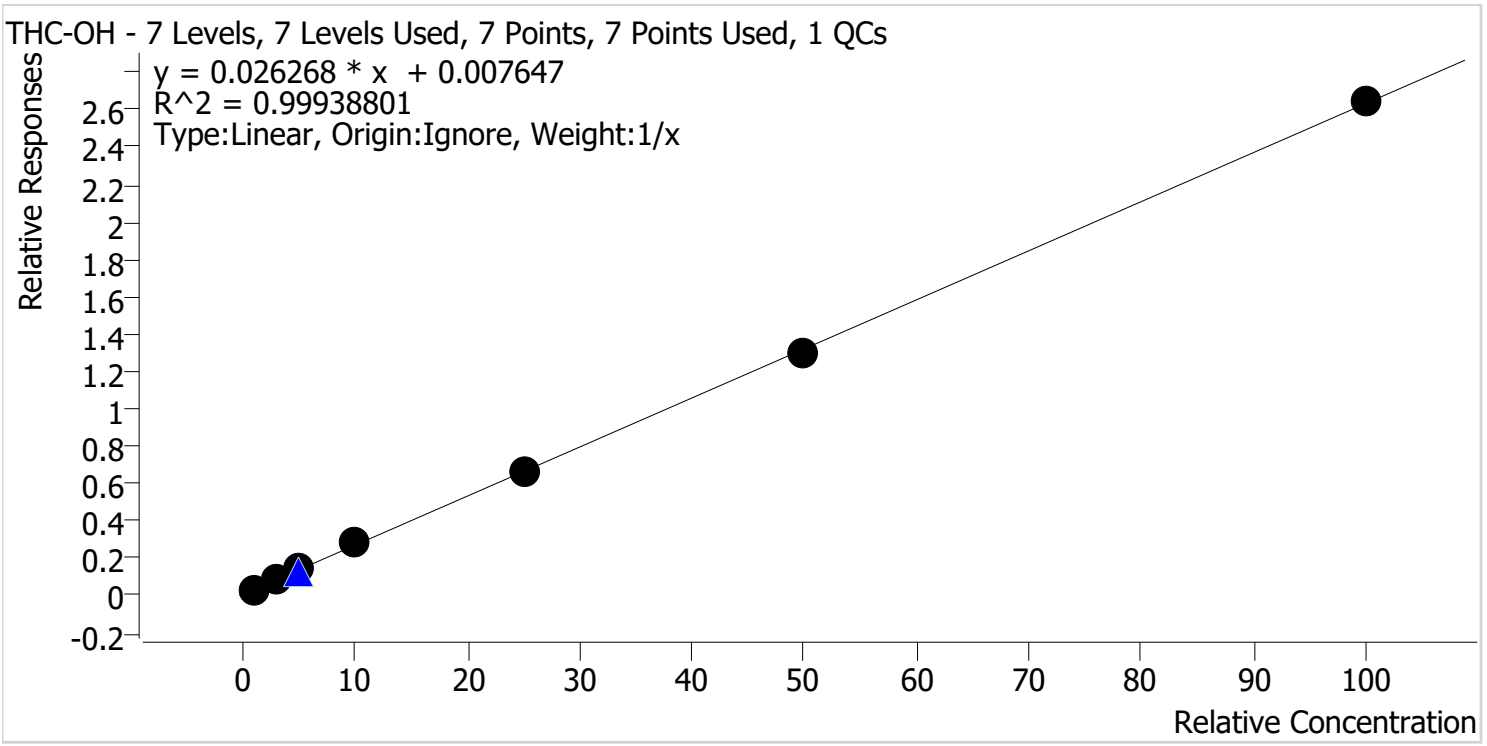
Batch results	D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Last Cal. Update	2/4/2021 12:06 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	5.0	5.4	108.1
mj cal2	2	✔	10.0	9.4	94.2
mj cal 3	3	✔	20.0	20.1	100.7
mj cal 4	4	✔	50.0	49.4	98.7
mj cal 5	5	✔	75.0	74.0	98.7
mj cal 6	6	✔	100.0	98.1	98.1
mj cal 7	7	✔	250.0	253.5	101.4

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Last Cal. Update	2/4/2021 12:06 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



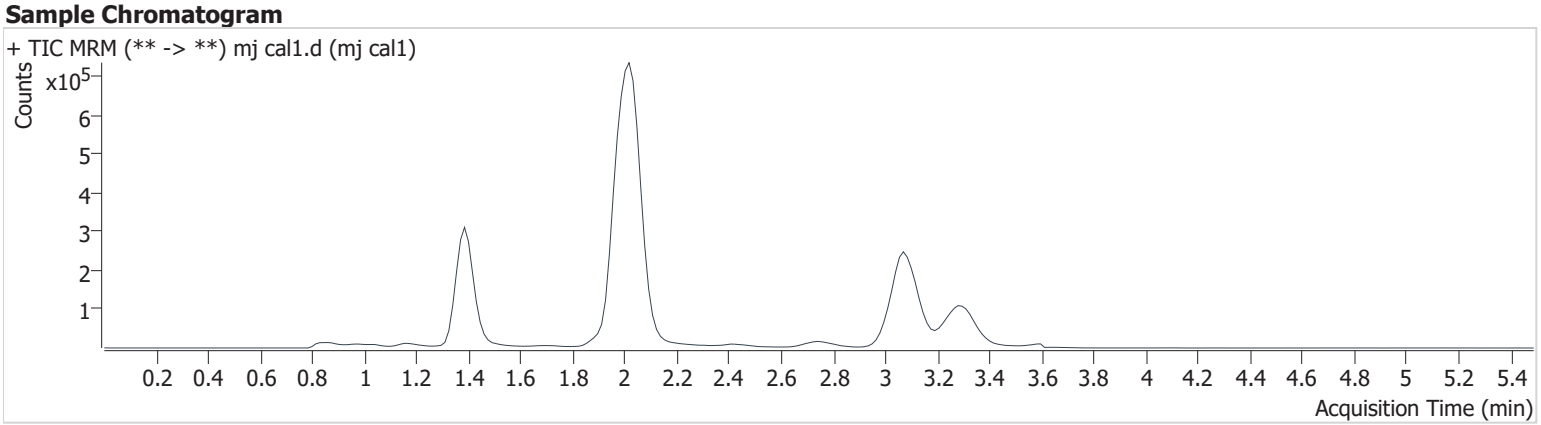
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	1.0	0.9	92.3
mj cal2	2	✔	3.0	2.9	95.7
mj cal 3	3	✔	5.0	5.5	110.8
mj cal 4	4	✔	10.0	10.4	103.8
mj cal 5	5	✔	25.0	24.6	98.4
mj cal 6	6	✔	50.0	49.3	98.6
mj cal 7	7	✔	100.0	100.4	100.4

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 PM

Instrument	69679	**Data File**	mj cal1.d
Type	Cal	**Sample**	mj cal1
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/2/2021 11:14:32 AM		
Sample Info.			

Sample Chromatogram



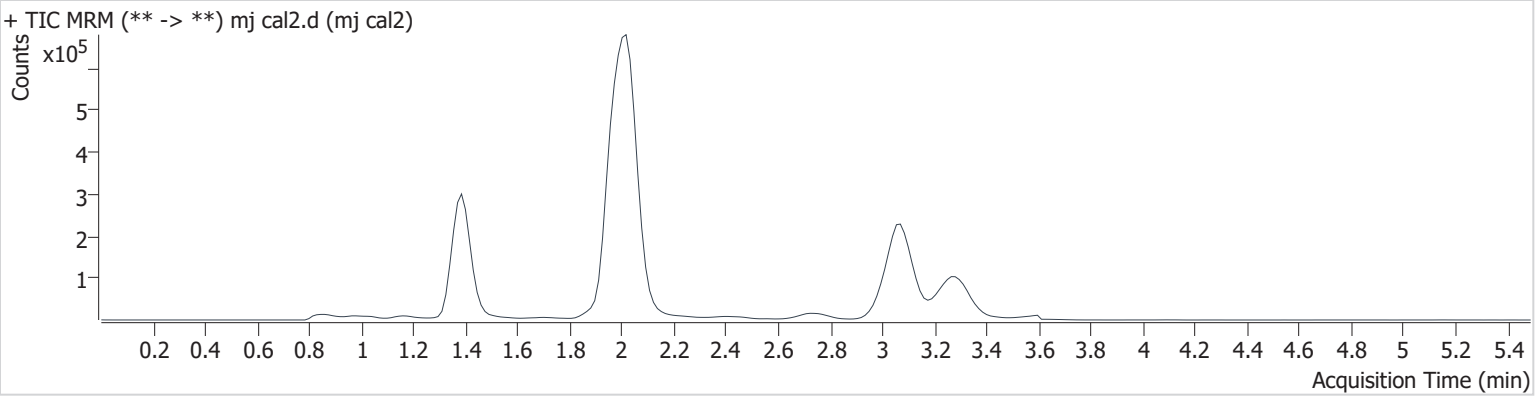
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.391	31089	∞	10.2	∞	974959	0.923 ng/ml	Low
THC-COOH	1.416	23132	30.8	37.7	42.8	400604	5.406 ng/ml	
THC	3.107	11049	133.4	24.6	29.5	507266	1.165 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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	2/2/2021 11:21:18 AM		
Sample Info.			

Sample Chromatogram



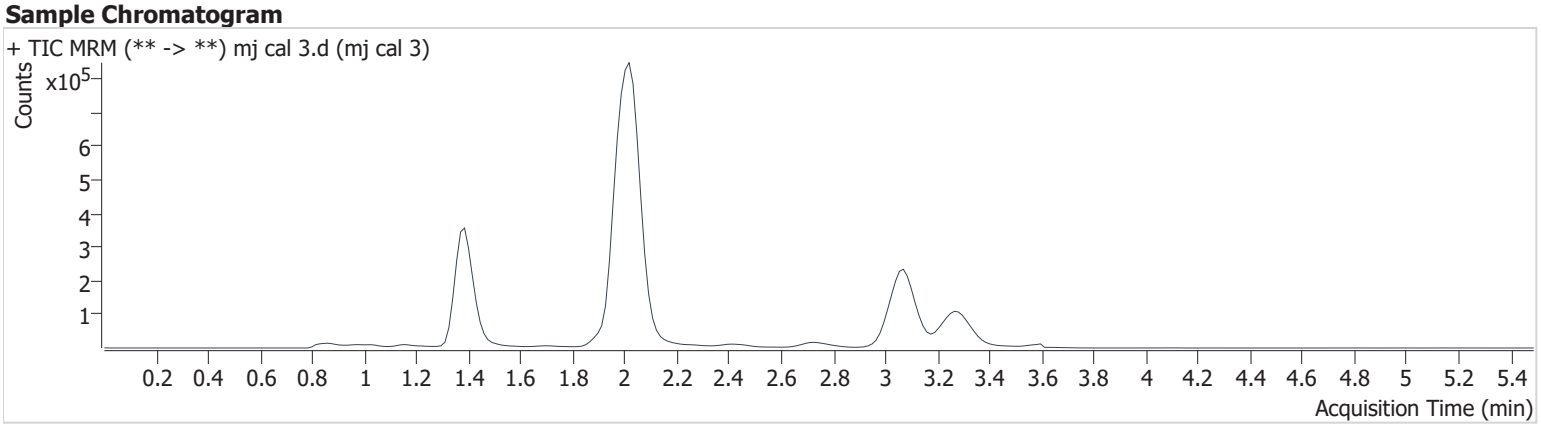
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.391	75814	∞	11.3	∞	913043	2.870 ng/ml	Low
THC-COOH	1.416	44053	56.6	38.0	18087.6	390845	9.422 ng/ml	
THC	3.107	29406	175.0	27.2	78.3	445342	2.890 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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	2/2/2021 11:28:02 AM		
Sample Info.			

Sample Chromatogram



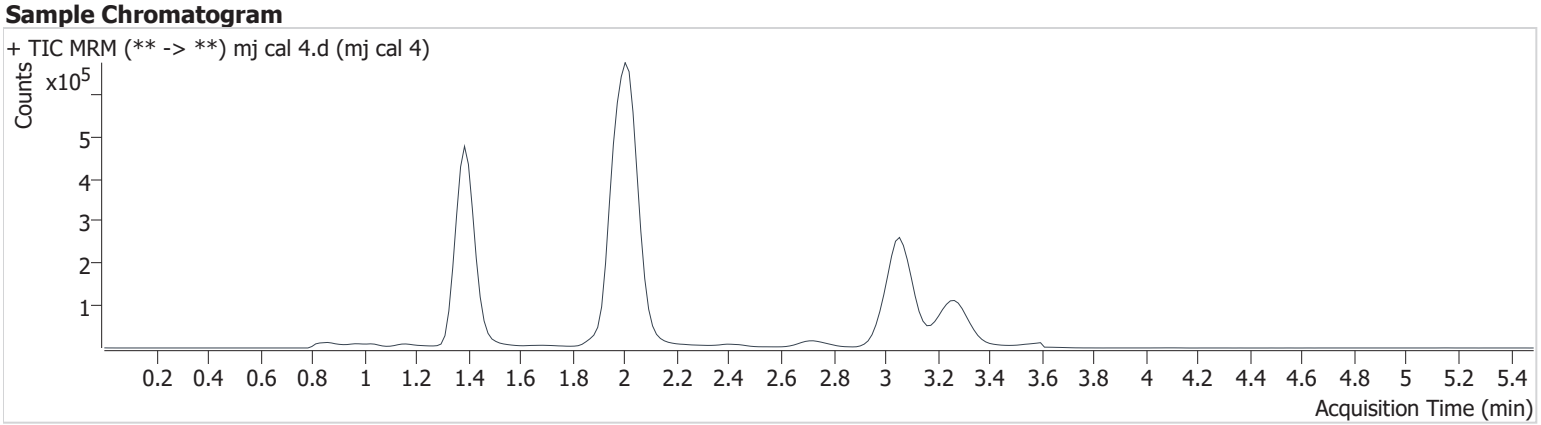
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	142206	∞	9.7	∞	928228	5.541 ng/ml
THC-COOH	1.401	104668	192.2	35.6	56383.7	403508	20.138 ng/ml
THC	3.092	54943	543.2	26.4	402.9	480126	4.777 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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	2/2/2021 11:34:46 AM		
Sample Info.			

Sample Chromatogram



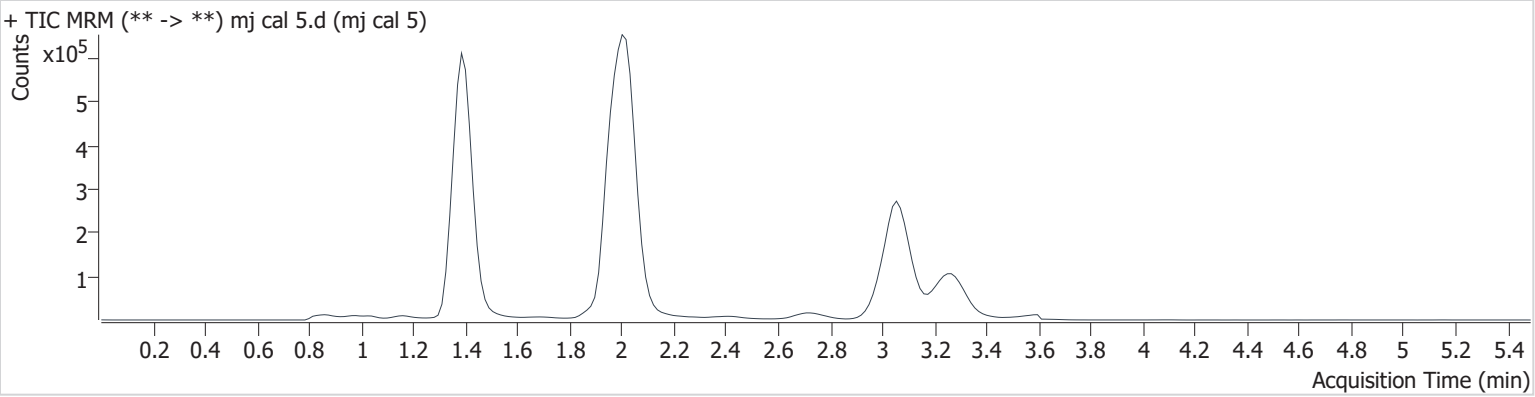
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	267297	∞	10.5	∞	953622	10.380 ng/ml
THC-COOH	1.401	274367	547.2	35.9	1674.3	415966	49.375 ng/ml
THC	3.092	121803	1236.8	25.7	513.7	513671	9.560 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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	2/2/2021 11:41:29 AM		
Sample Info.			

Sample Chromatogram



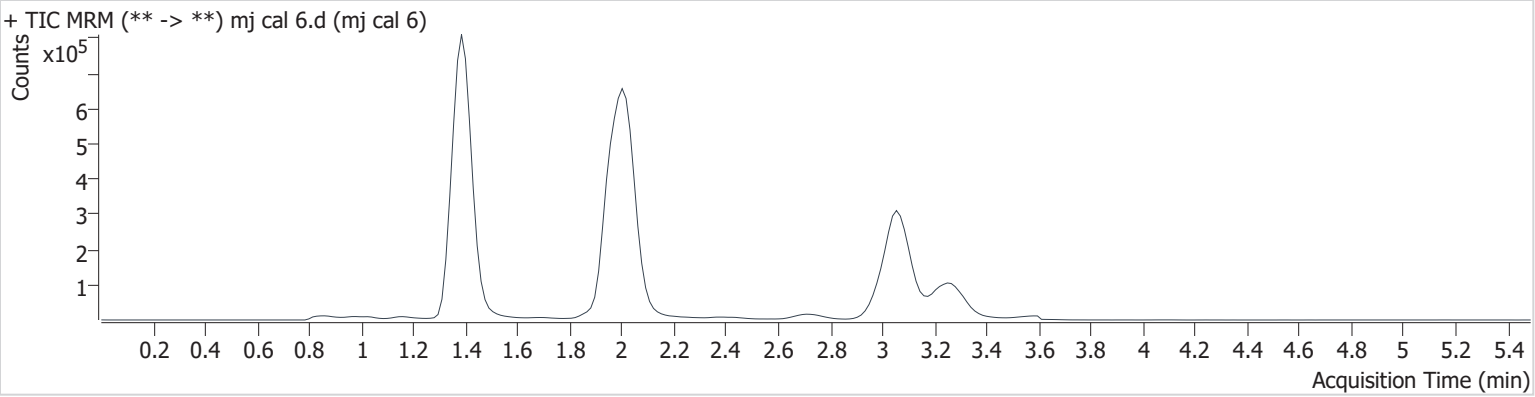
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	617471	∞	10.7	∞	943973	24.611 ng/ml
THC-COOH	1.401	407725	525.8	36.7	31127 8.4	409010	74.015 ng/ml
THC	3.092	282745	1489.6	24.4	596.8	473958	23.572 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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	2/2/2021 11:48:13 AM		
Sample Info.			

Sample Chromatogram



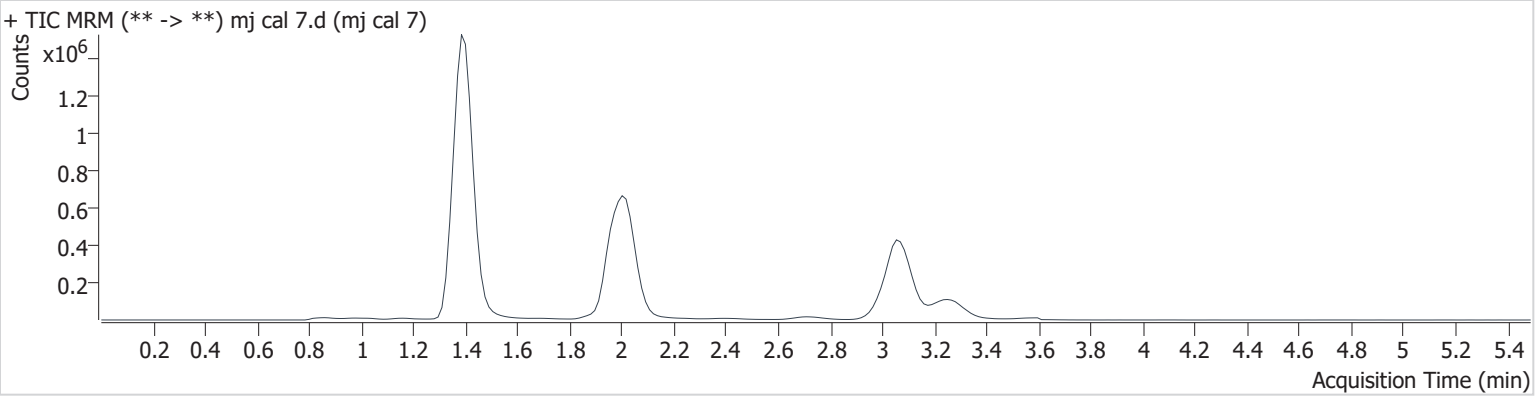
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	1227620	∞	11.6	∞	942036	49.319 ng/ml
THC-COOH	1.401	543034	5072.3	37.2	771.2	409188	98.141 ng/ml
THC	3.077	576069	1669.5	24.0	3587.4	454441	49.733 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\020221\QuantResults\mj.batch.bin
Calibration Last Update 2/4/2021 12:06:29 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	2/2/2021 11:54:57 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	2476538	∞	11.8	∞	936734	100.357 ng/ml
THC-COOH	1.401	1339998	2591.8	37.8	1801.3	387991	253.503 ng/ml
THC	3.077	1226881	6523.8	23.8	3760.2	468955	102.304 ng/ml