










1/14/2021

REVIEWED

By Brittany Wylie at 11:33 am, Jan 14, 2021

**Worklist: 4736**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2020-2566	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2609	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2615	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0009	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0023	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0037	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-0047	3	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0056	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0070	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 1/13/21  
Plate lot#: 200723

Analyst: Anne Nord  
Plate Expiration: 01/23/2021

**Mobile phase A:** 0.1% Formic Acid in LCMS Water MTBE  
**Mobile phase B:** 0.1% Formic acid in Acetonitrile Hexane  
LCMS Methanol

**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  $\geq 0.98$  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.*

A

	1	2	3	4	5	6
a	cal 1ng	neg blood	negative urine			QC 1
b	cal 3 ng	20-2615-1	urine control		cal 100 ng	
c	cal 5 ng	21-0009-1	20-2566-2		cal 50 ng	
d	cal 10ng	21-0023-1	20-2609-1		cal 25 ng	
e	25	21-0047-3	21-0037-1		cal 10ng	
f	50	21-0056-1			cal 5 ng	
g	100	21-0070-1			cal 3 ng	
h	QC 1				cal 1ng	

C20 - - - - -

# 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	
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## AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine

out of use

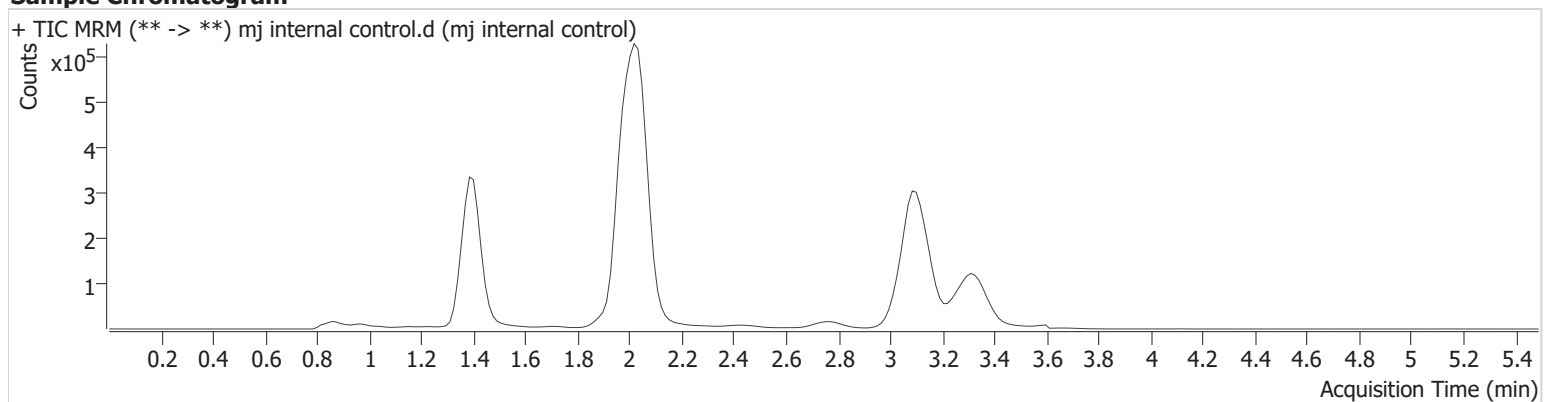
ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	1/12/2021
ppd 1/13/21 Exp 7/1/21 neg urine lot 10120	lot 11321	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\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj internal control.d
<b>Type</b>	QC	<b>Sample</b>	mj internal control
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 1:38:19 PM		

## Sample Chromatogram



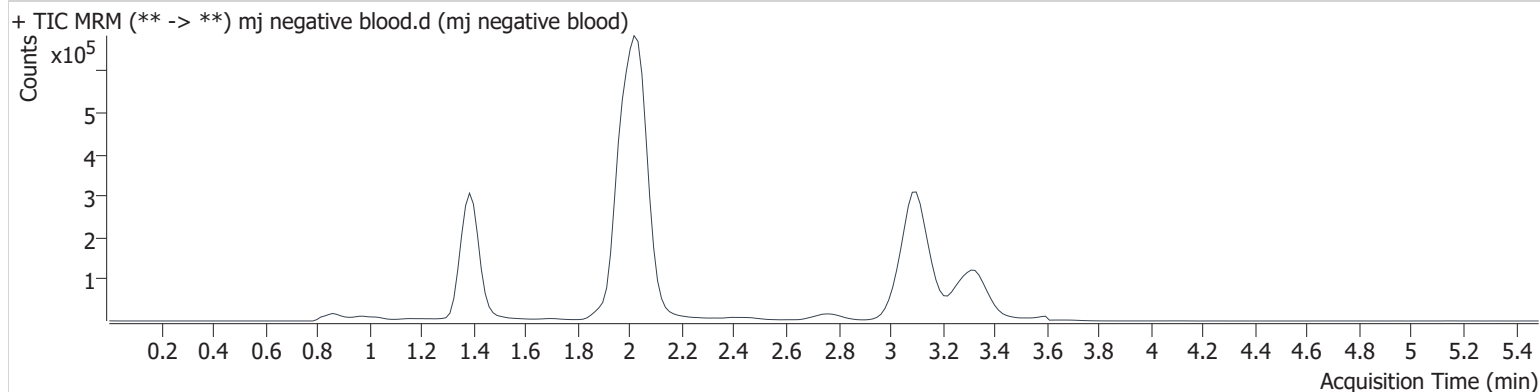
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	160585	654.6	9.9	∞	897451	4.876 ng/ml
THC-COOH	1.416	82447	147.8	36.5	39258.6	441594	14.866 ng/ml
THC	3.137	71617	1336.2	26.5	426.0	697475	4.360 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj negative blood.d
<b>Type</b>	Sample	<b>Sample</b>	mj negative blood
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-A2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 1:45:03 PM		

## Sample Chromatogram



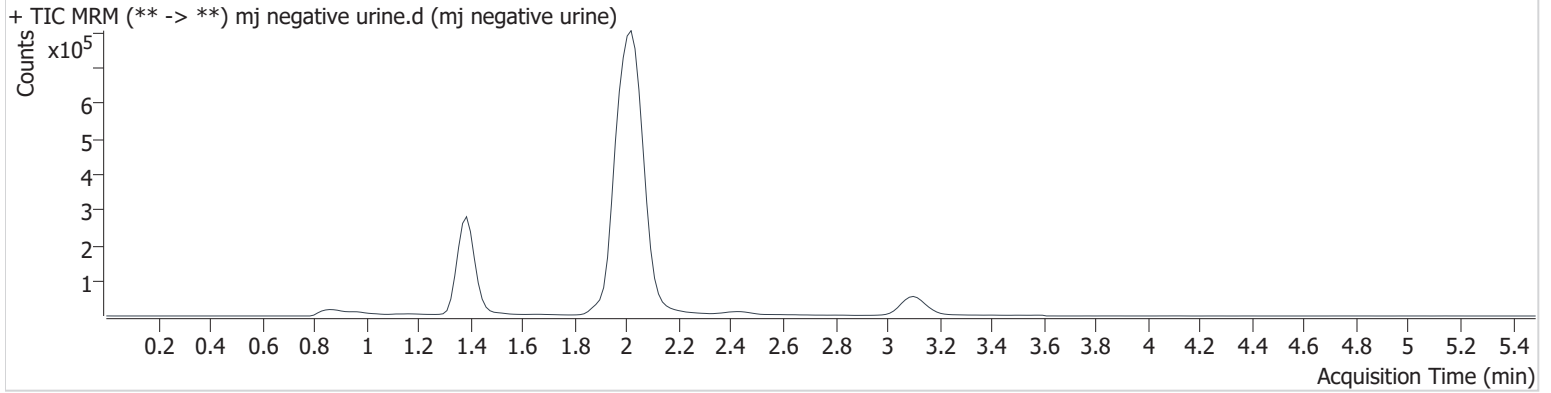
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	26365	∞			1058326	0.478 ng/ml <b>Low</b>

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj negative urine.d
<b>Type</b>	Sample	<b>Sample</b>	mj negative urine
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-A3	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 3:12:10 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



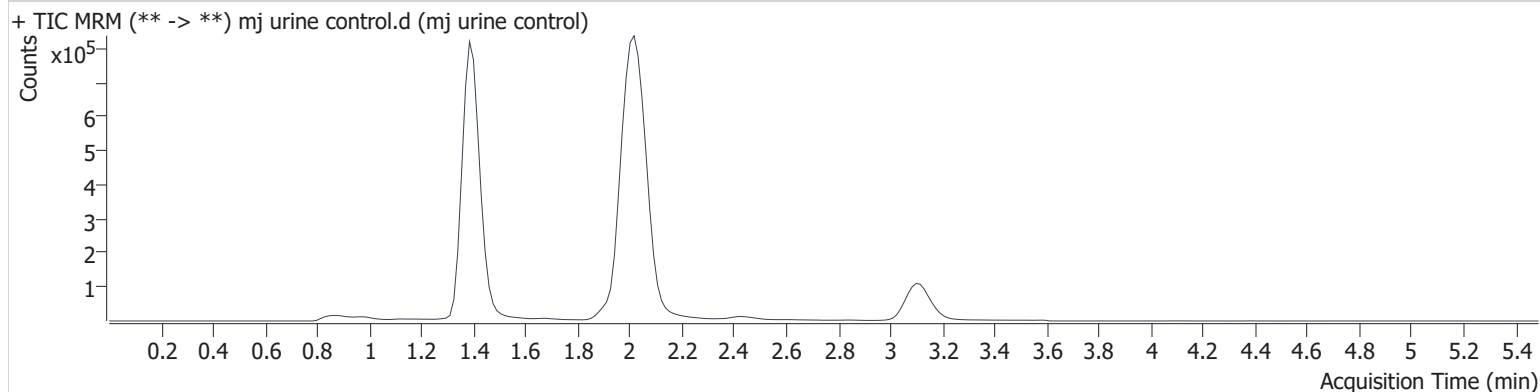
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.316	13165	∞			973590	0.152 ng/ml <b>Low</b>

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj urine control.d
<b>Type</b>	Sample	<b>Sample</b>	mj urine control
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-B3	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 3:18:54 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	1710746	∞	15.1 <b>High</b>	∞	955368	50.905 ng/ml
THC-COOH	1.416	223693	7435.7	35.0	716.5	362892	46.620 ng/ml
THC	3.122	241252	1095.1	22.9	1034.8	439568	21.663 ng/ml



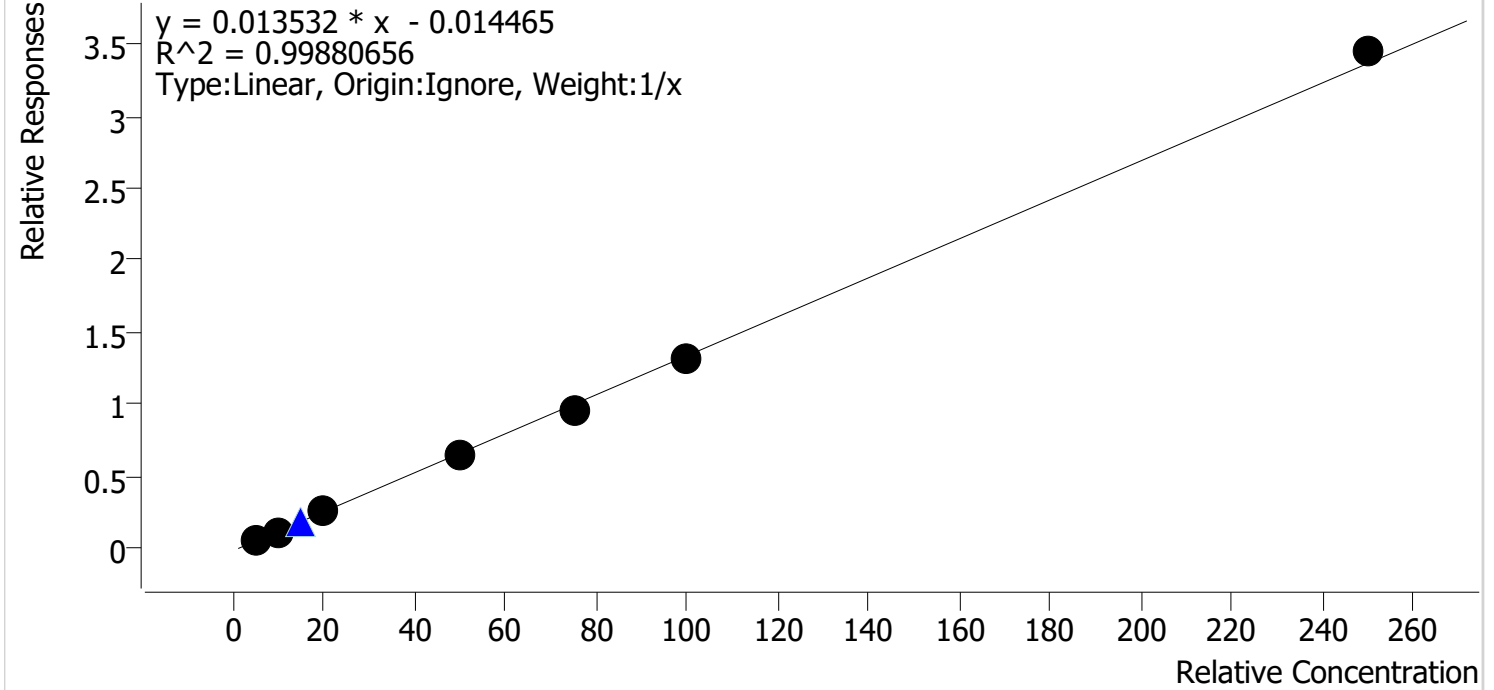


# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Last Cal. Update** 1/13/2021 4:08 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-d9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



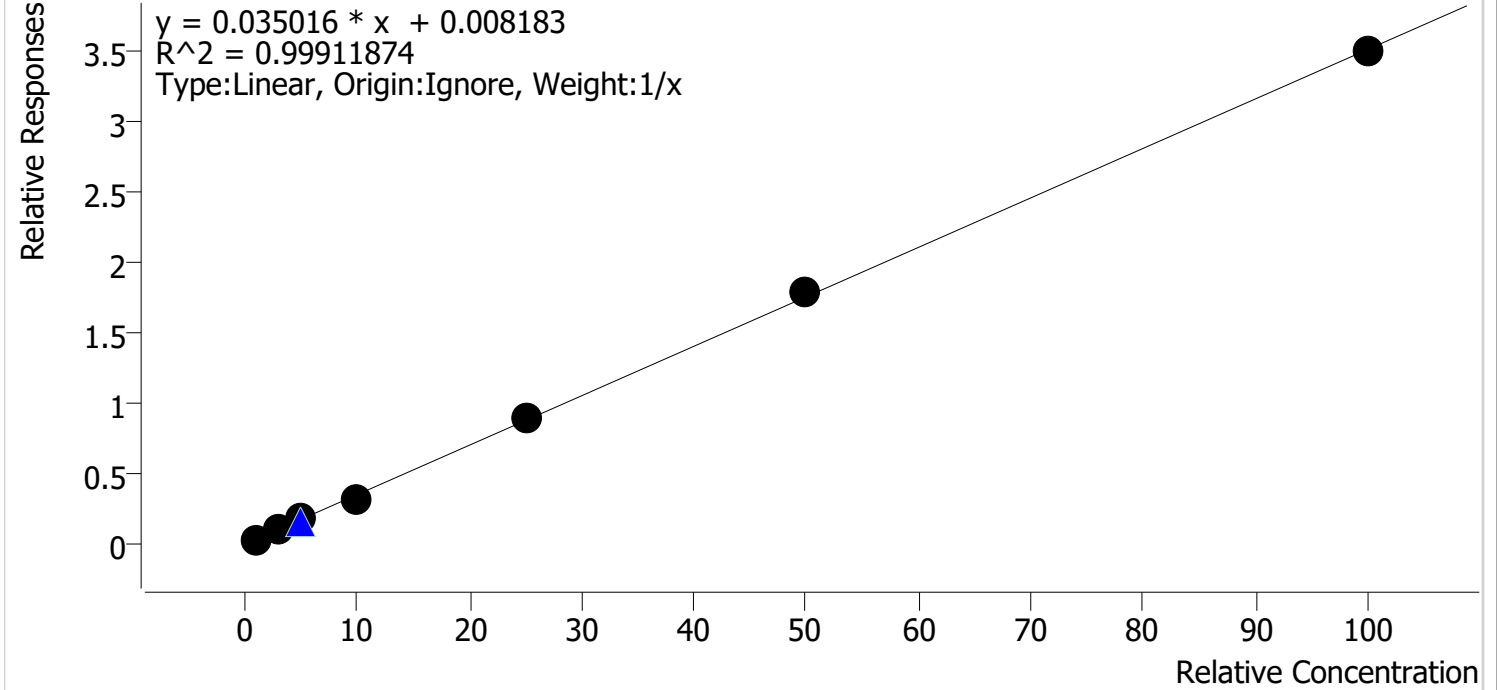
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.6	111.6
mj cal2	2	✓	10.0	9.7	96.7
mj cal 3	3	✓	20.0	19.6	97.8
mj cal 4	4	✓	50.0	48.6	97.1
mj cal 5	5	✓	75.0	71.3	95.0
mj cal 6	6	✓	100.0	99.4	99.4
mj cal 7	7	✓	250.0	256.0	102.4

# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Last Cal. Update** 1/13/2021 4:08 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	0.9	89.6
mj cal2	2	✓	3.0	3.3	111.6
mj cal 3	3	✓	5.0	5.3	105.7
mj cal 4	4	✓	10.0	9.2	91.8
mj cal 5	5	✓	25.0	25.1	100.5
mj cal 6	6	✓	50.0	50.6	101.3
mj cal 7	7	✓	100.0	99.5	99.5

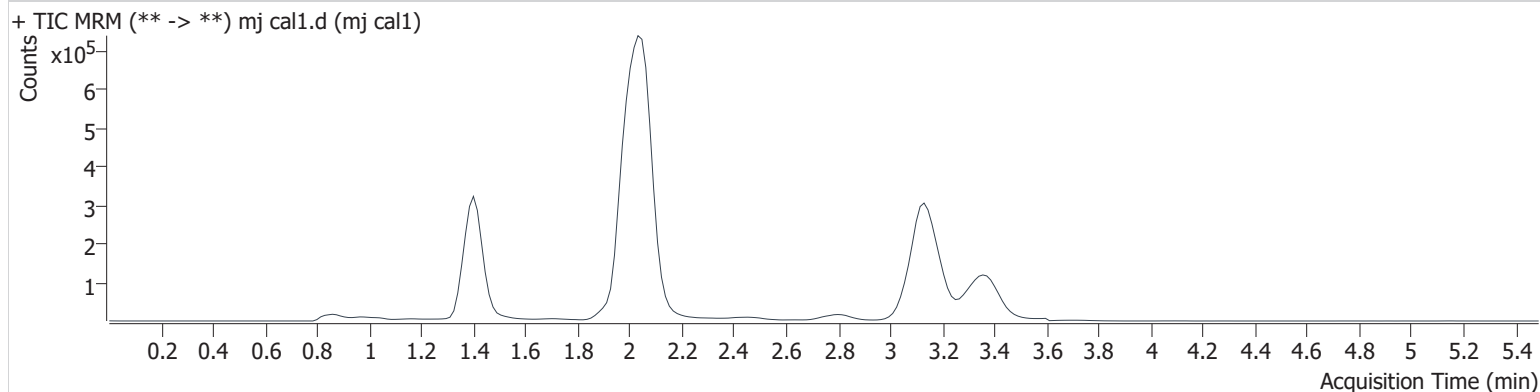
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal1.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal1
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-A1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 12:51:09 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	41281	∞	12.8	∞	1043277	0.896 ng/ml <b>Low</b>
THC-COOH	1.431	27277	19.1	36.2	101.7	446811	5.580 ng/ml
THC	3.167	14082	102.2	29.2	∞	707646	1.150 ng/ml

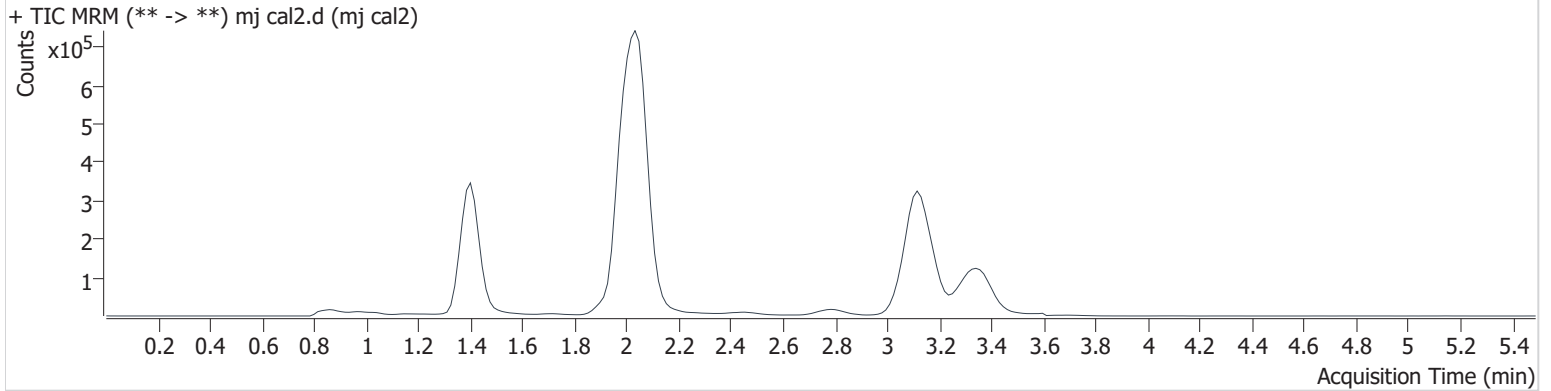
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal2.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal2
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-B1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 12:57:55 PM		

**Sample Info.**

## Sample Chromatogram



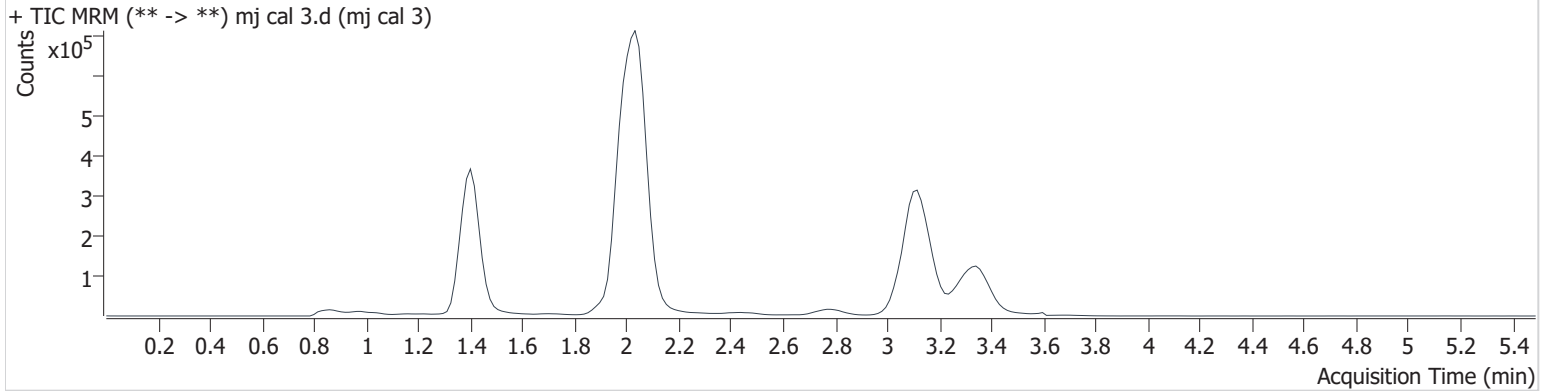
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	126177	∞	9.9	∞	1006205	3.347 ng/ml
THC-COOH	1.431	55960	66.0	35.8	74.9	480993	9.666 ng/ml
THC	3.167	49108	365.3	25.6	369.2	754986	2.900 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 3.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 3
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-C1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 1:04:40 PM		

## Sample Chromatogram



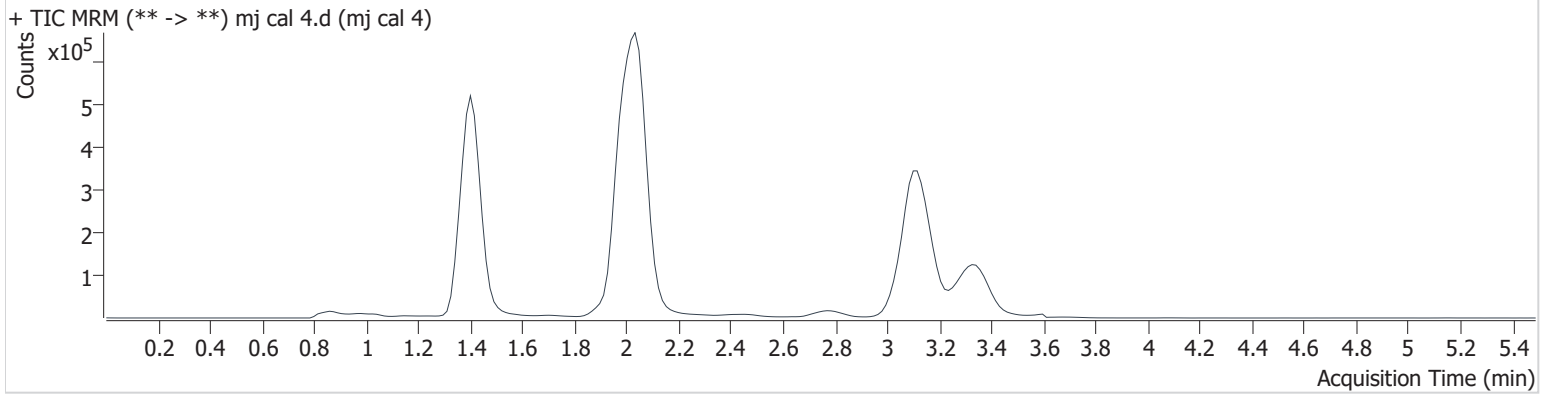
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	181108	∞	9.7	840.7	937359	5.284 ng/ml
THC-COOH	1.416	113681	180.7	35.2	569.7	454188	19.565 ng/ml
THC	3.167	79969	937.0	24.8	458.4	675927	4.966 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 4.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 4
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-D1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 1:11:23 PM		

**Sample Chromatogram**



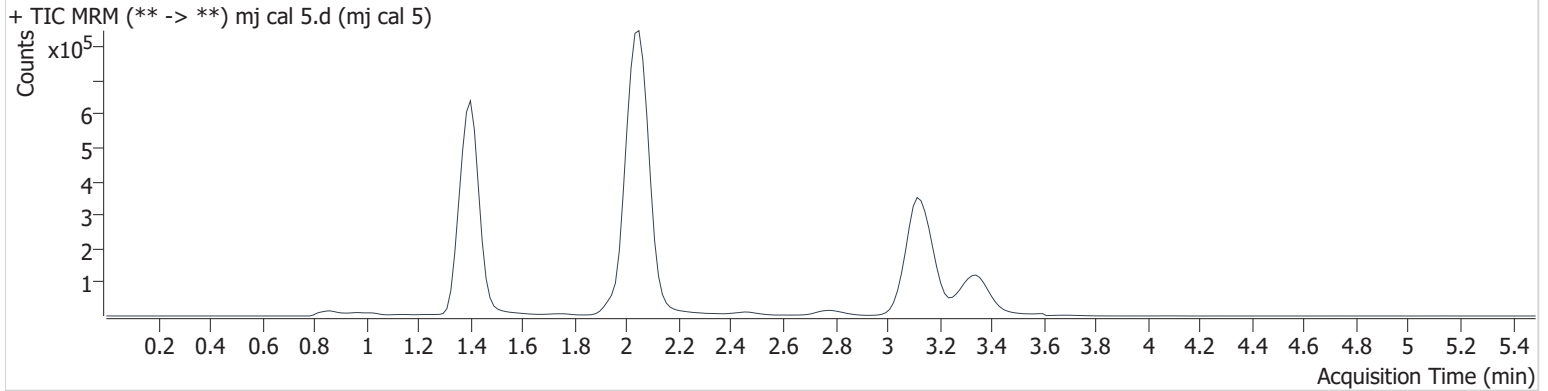
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	347493	∞	11.6	∞	1054409	9.178 ng/ml
THC-COOH	1.416	305370	506193.5	36.7	348.6	475206	48.555 ng/ml
THC	3.152	188039	2611.8	25.0	390.1	826201	9.204 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 5.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 5
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-E1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 1:18:07 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	766565	∞	11.0	∞	863037	25.132 ng/ml
THC-COOH	1.416	408791	601.2	36.7	3121.1	430288	71.274 ng/ml
THC	3.152	398923	2349.7	24.0	6458.9	657994	23.890 ng/ml



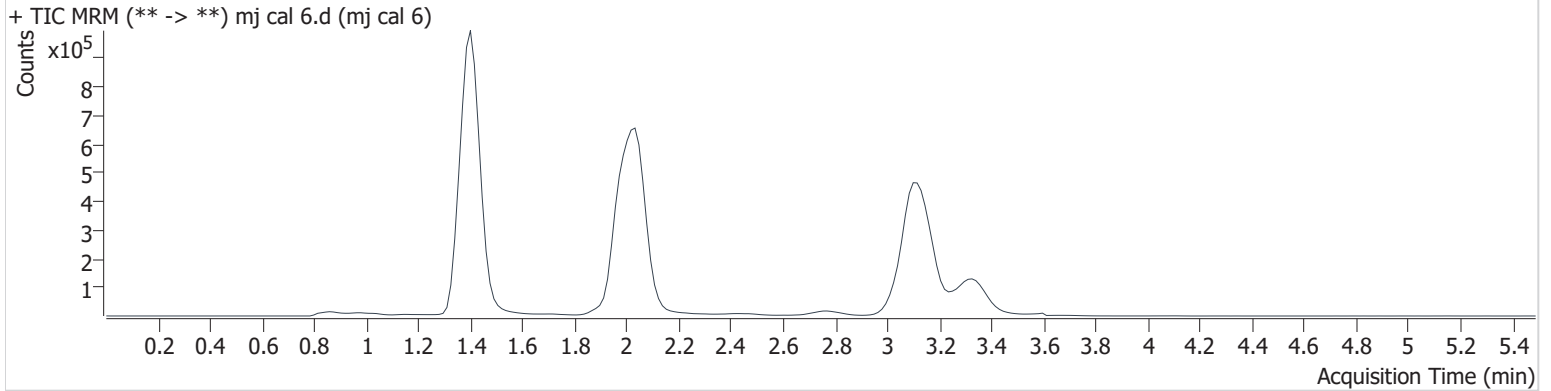
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 6.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 6
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-F1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 1:24:51 PM		

**Sample Info.**

## Sample Chromatogram



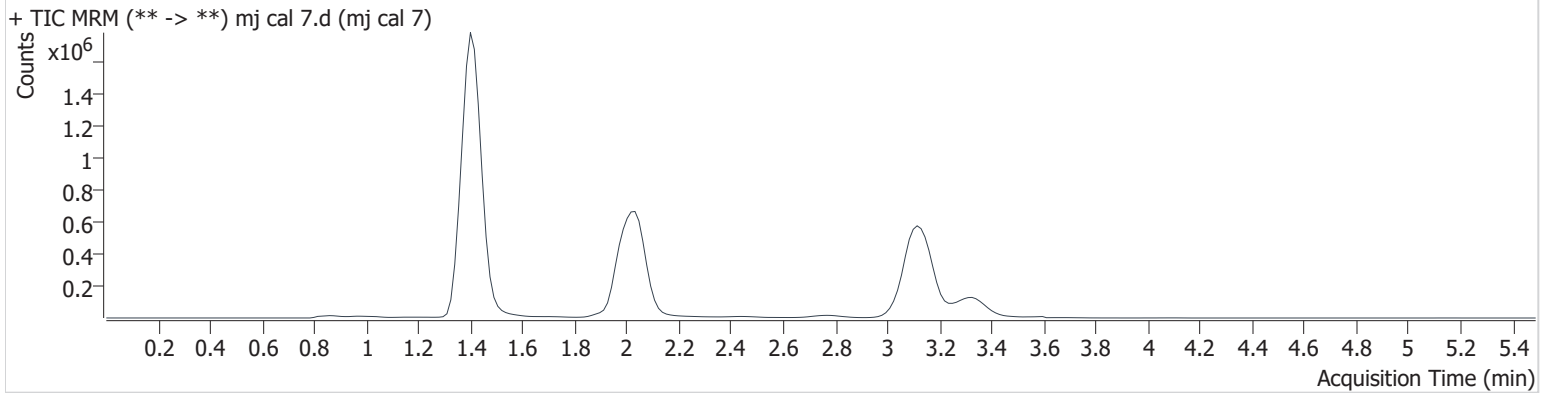
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	1818487	∞	11.6	∞	1020895	50.636 ng/ml
THC-COOH	1.416	628936	1299236.1	37.1	53791	472770	99.375 ng/ml
THC	3.137	1063470	6559.8	23.7	2307.0	839011	49.534 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2021\am 27-28\ 011321\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 1/13/2021 4:08:05 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 7.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 7
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-G1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	1/13/2021 1:31:35 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	3325189	∞	12.2	∞	951903	99.526 ng/ml
THC-COOH	1.416	1465010	2369.8	37.3	10080 55.1	424685	255.985 ng/ml
THC	3.137	1836864	5826.2	24.2	7715.5	698551	102.355 ng/ml