

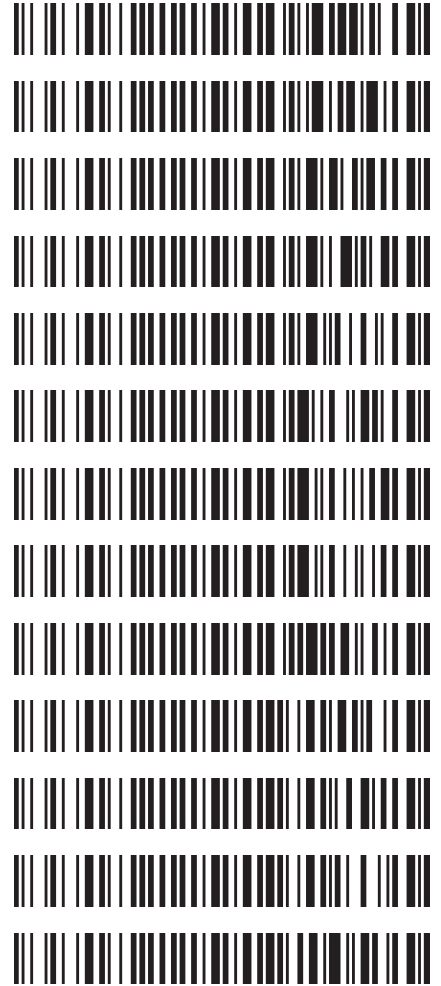
9/28/2020

**REVIEWED**

By Britany Wylie at 8:17 am, Sep 29, 2020

**Worklist: 4541**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2020-1803	1	UCK	AM 30 Urine THC Screen by LC-QTOF
C2020-1805	1	UCK	AM 30 Urine THC Screen by LC-QTOF
C2020-1823	2	UCK	AM 30 Urine THC Screen by LC-QTOF
C2020-1828	1	BCK	AM 30 Blood THC Screen by LC-QTOF
C2020-1830	1	UCK	AM 30 Urine THC Screen by LC-QTOF
C2020-1837	1	BCK	AM 30 Blood THC Screen by LC-QTOF
C2020-1838	1	BCK	AM 30 Blood THC Screen by LC-QTOF
C2020-1840	1	BCK	AM 30 Blood THC Screen by LC-QTOF
C2020-1851	1	BCK	AM 30 Blood THC Screen by LC-QTOF
C2020-1860	1	BCK	AM 30 Blood THC Screen by LC-QTOF
C2020-1863	1	AVK	AM 30 Blood THC Screen by LC-QTOF
C2020-1871	1	BCK	AM 30 Blood THC Screen by LC-QTOF
C2020-1883	1	UCK	AM 30 Urine THC Screen by LC-QTOF



## AM# 30: Screening of THC and Metabolites in Blood and Urine by LC-QTOF

Extraction Date: 9/24/20

Analyst: Anne Nord

Plate lot#: 200723

Plate Expiration: 1-23-2021

**Mobile phase A:** 0.1% formic acid in water

**Mobile phase B:** 0.1% Formic acid in Methanol

**Blank Blood Lot:** 20G20792

**Column:** Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

**LCMS-QQ ID:** 70044

**Blank Urine Lot:** 73020

### 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 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.  
Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: K52558G**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500µL 0.1% formic acid in water blood sample, 500 µL 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,  $r^2$  values  $\geq 0.98$  for each analyte
- 3. Evaluate samples: RT within +/- 2% or 0.1 min of administrative control, Mass Accuracy of 0 (+/- 10), and/or Mass Abundance Score of 40 or greater.
- 4. Did all QCs pass for each analyte? (if not, describe in comments section)
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Evaluated C-THC only, response on THC and THC-OH was too low and variable at the lower end of the curves.

# Toxicology AM method 27/26 external prep information



working solution 1 ug/ml in meoh C-THC, THC-OH, 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 lot (73020)

out of use

ppd 8/26/20 Exp 7/1/21	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

AM 26 and AM 30 plate map extracted 9/24/20

	1	2	3	4	5	6
a	cal 100 ng	neg blood	1871-1			QC 1
b	cal 50 ng	1828-1	neg urine			cal 100 ng
c	cal 25 ng	1837-1	urine control			cal 50 ng
d	cal 10ng	1838-1	1803-1			cal 25 ng
e	cal 5 ng	1851-1	1805-1			cal 10ng
f	cal 3 ng	1860-1	1823-2			cal 5 ng
g	cal 1ng	1863-1	1830-1			cal 3 ng
h	QC 1	1840-1	1883-1			cal 1ng

c2020-

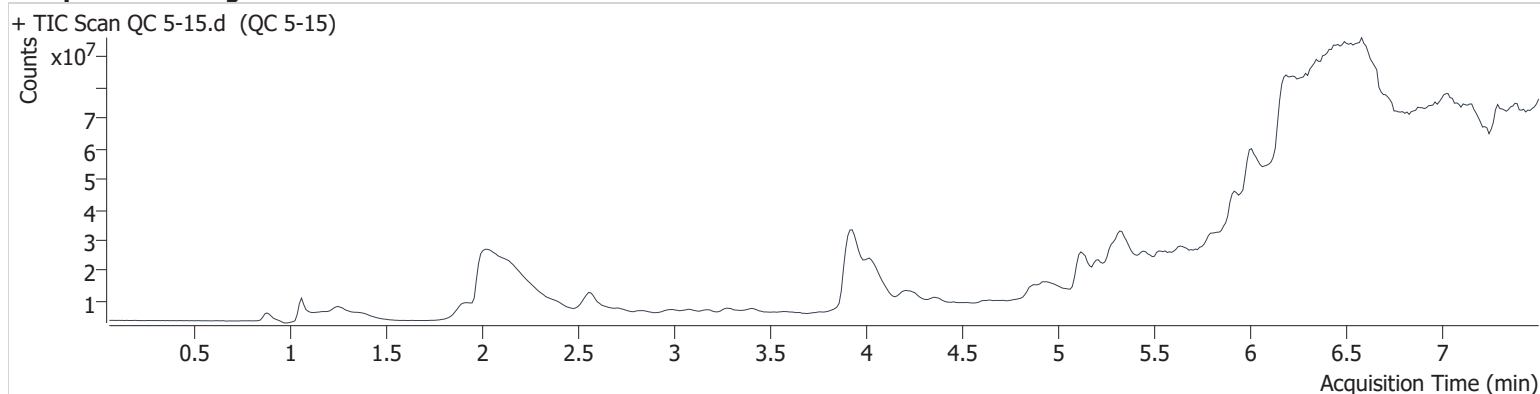
GA

# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	QC 5-15.d
<b>Type</b>	QC	<b>Sample</b>	QC 5-15
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-H1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 2:11:44 PM		

## Sample Chromatogram



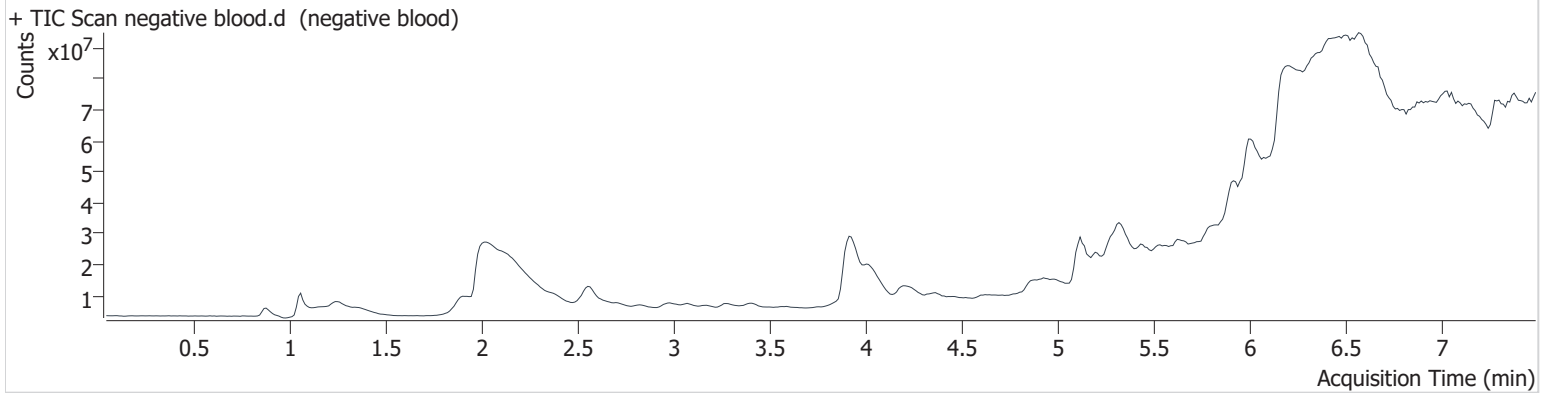
Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.666	502855	1.27	83.4	2407456	16.8958 ng/ml

# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	negative blood.d
<b>Type</b>	Sample	<b>Sample</b>	negative blood
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-A2	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 2:21:16 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



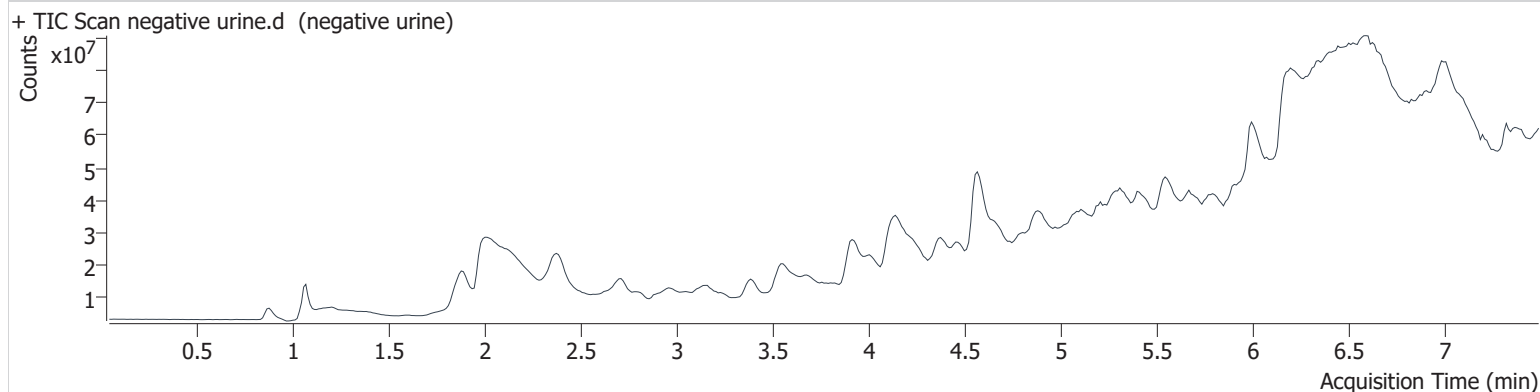
OA

# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	negative urine.d
<b>Type</b>	Sample	<b>Sample</b>	negative urine
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-B3	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 3:47:04 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



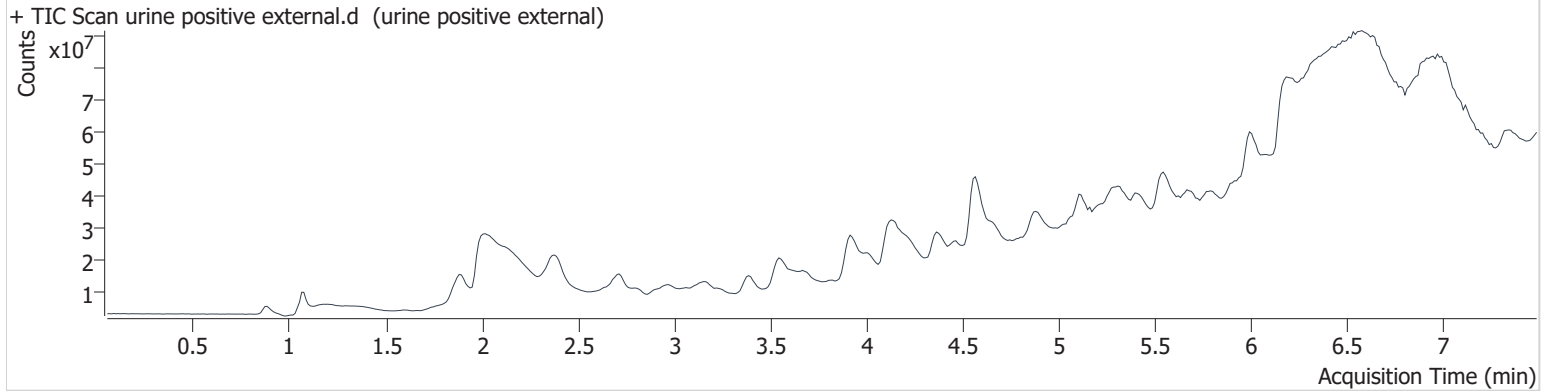
# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	urine positive external.d
<b>Type</b>	Sample	<b>Sample</b>	urine positive external
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-C3	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 3:56:36 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.659	1052157	1.42	89.0	2508510	36.8711 ng/ml

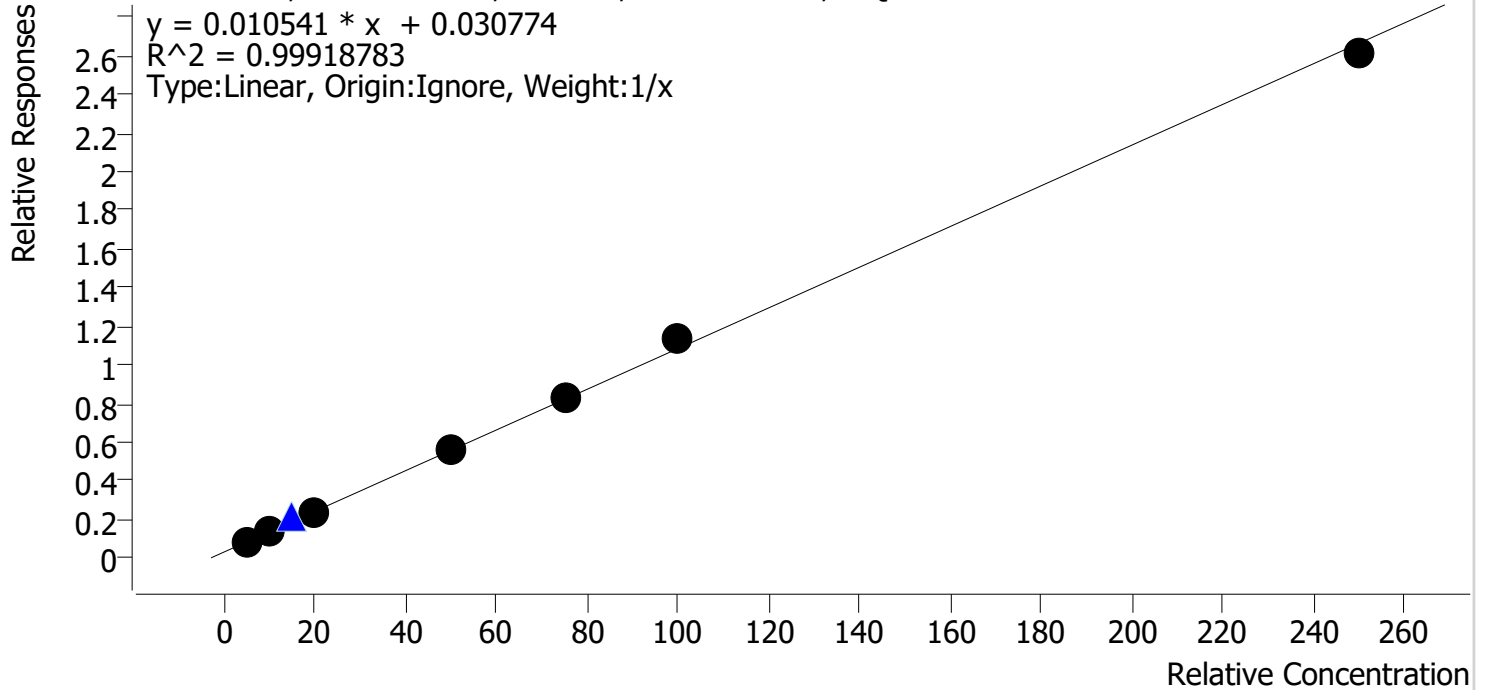


# Compound Calibration Report



<b>Batch results</b>	D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin		
<b>Last Cal. Update</b>	9/24/2020 5:03 PM		
<b>Analyst Name</b>	ISP\datastor		
<b>Analyte</b>	THC-COOH	<b>Internal Standard</b>	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
CAL 1	1	✓	5.0	5.0	100.2
CAL 2	2	✓	10.0	9.6	96.1
CAL 3	3	✓	20.0	19.9	99.3
CAL 4	4	✓	50.0	50.5	100.9
CAL 5	5	✓	75.0	75.9	101.2
CAL 6	6	✓	100.0	104.2	104.2
CAL 7	7	✓	250.0	244.9	98.0

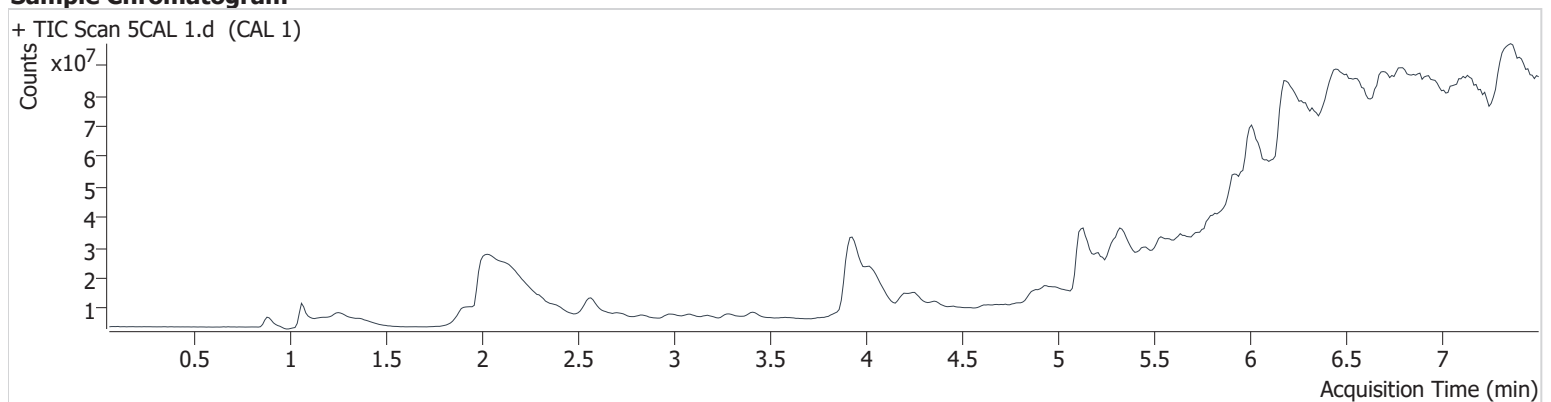
CA

# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	5CAL 1.d
<b>Type</b>	Cal	<b>Sample</b>	CAL 1
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-A1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 1:04:51 PM		

## Sample Chromatogram



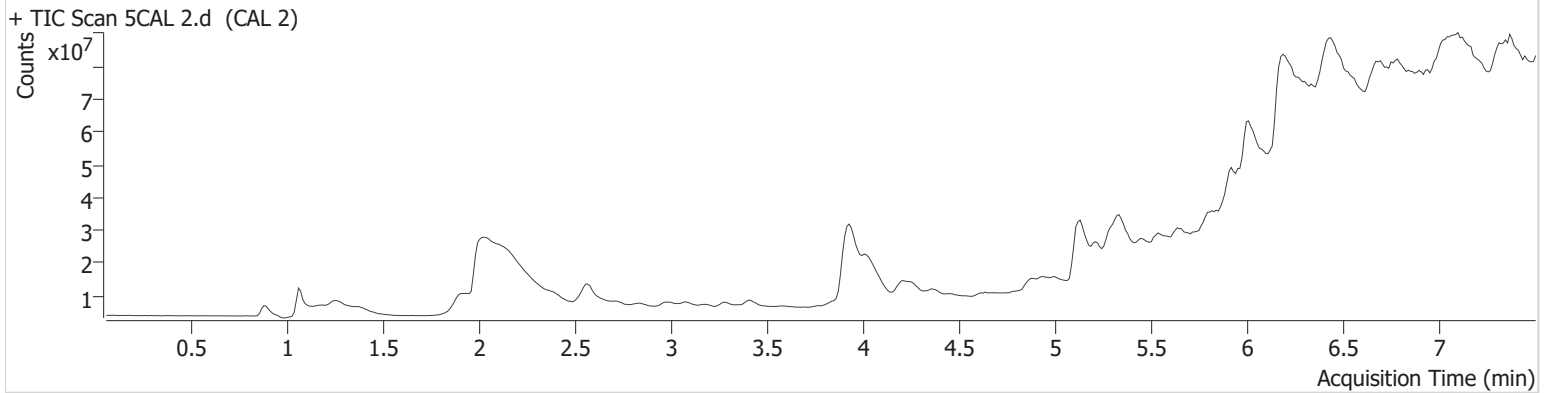
Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.662	144581	1.61	87.0	1729303	5.0121 ng/ml

# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	5CAL 2.d
<b>Type</b>	Cal	<b>Sample</b>	CAL 2
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-B1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 1:14:33 PM		

## Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.662	280369	1.59	84.3	2122406	9.6125 ng/ml

GA

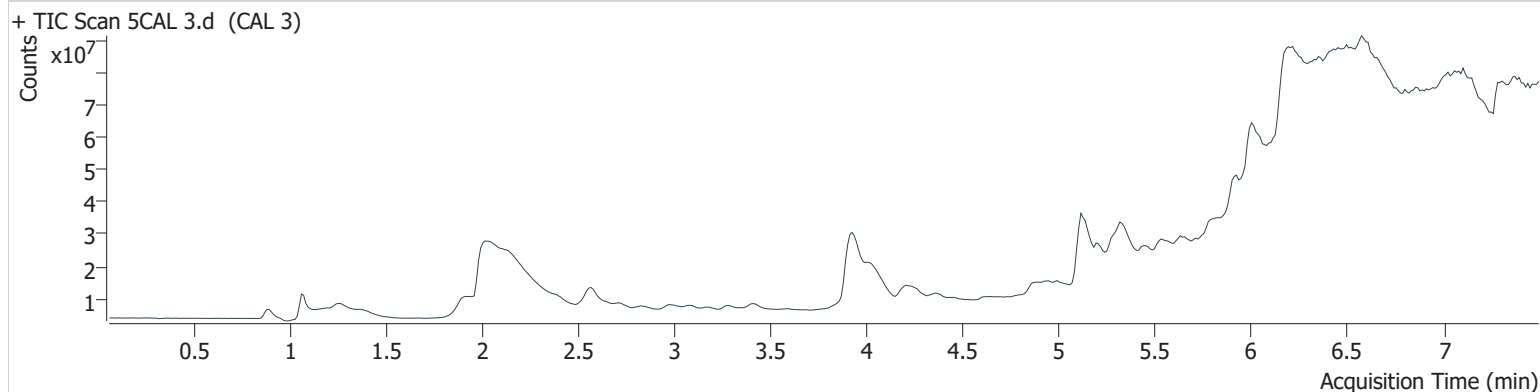
# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	5CAL 3.d
<b>Type</b>	Cal	<b>Sample</b>	CAL 3
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-C1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 1:24:05 PM		

**Sample Info.**

## Sample Chromatogram



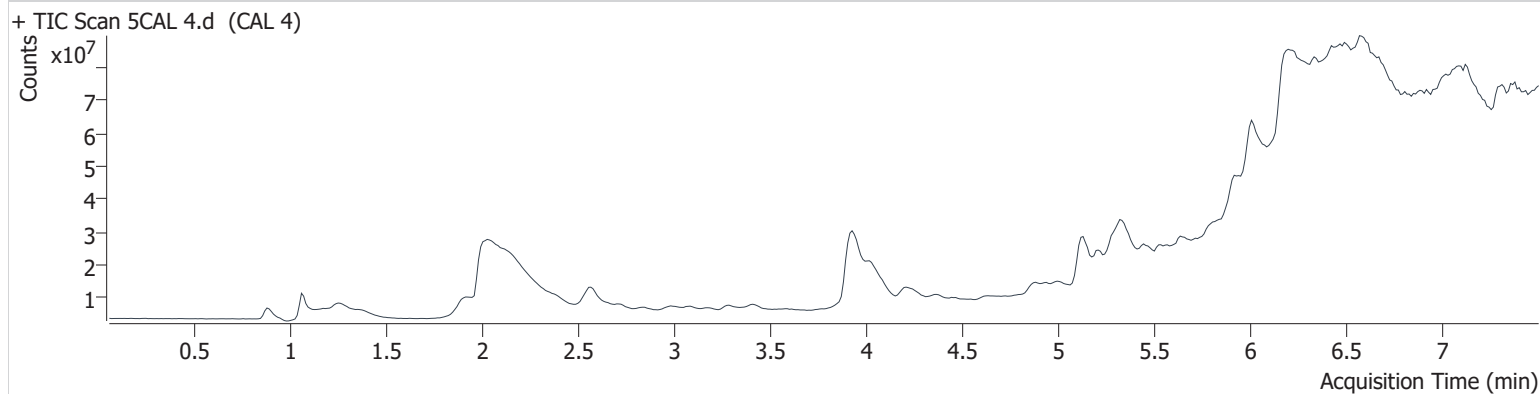
Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.672	612047	1.16	87.2	2548816	19.8610 ng/ml

# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	5CAL 4.d
<b>Type</b>	Cal	<b>Sample</b>	CAL 4
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-D1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 1:33:37 PM		

## Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.662	1474130	1.19	88.7	2620005	50.4569 ng/ml

EA

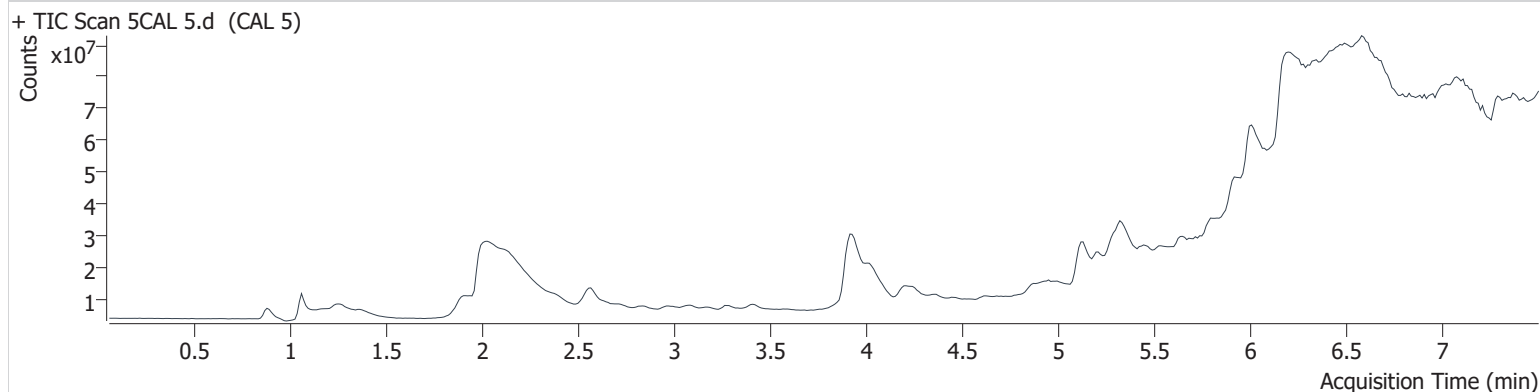
# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	5CAL 5.d
<b>Type</b>	Cal	<b>Sample</b>	CAL 5
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-E1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 1:43:09 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.664	2115661	2.25	95.9	2546030	75.9117 ng/ml

GA

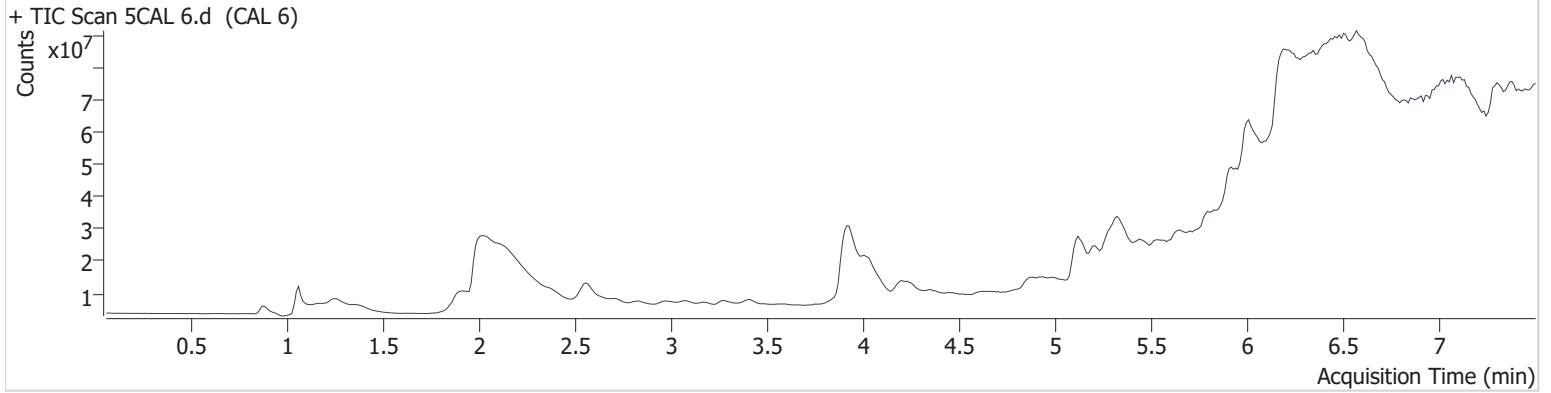
# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	5CAL 6.d
<b>Type</b>	Cal	<b>Sample</b>	CAL 6
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-F1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 1:52:41 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.667	2764461	3.74	96.0	2447443	104.2357 ng/ml

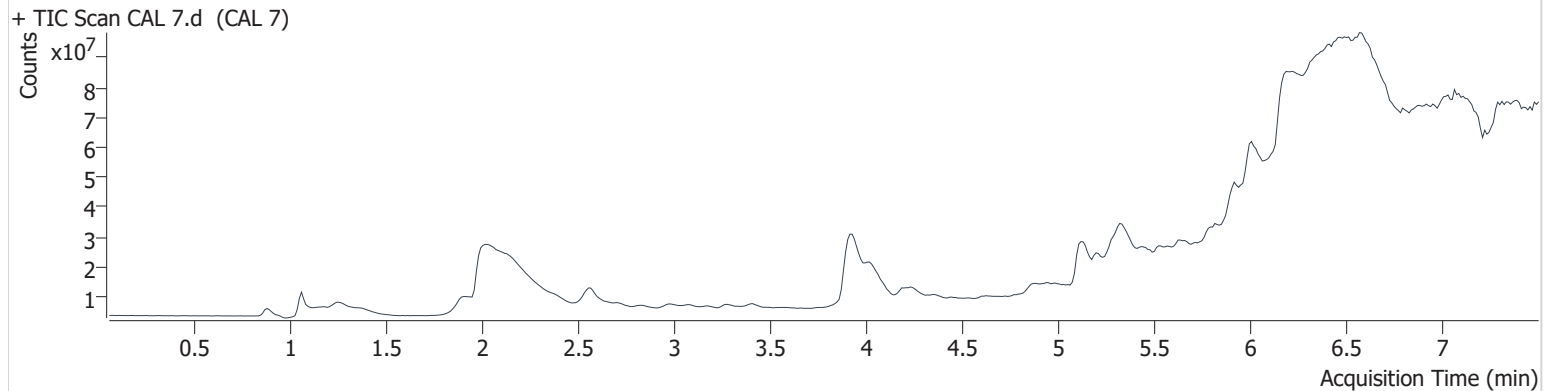
CA

# AM #30 Cannabinoids

**Batch results** D:\MassHunter\Data\2020\am 30\092420\QuantResults\cann screen.batch.bin  
**Calibration Last Update** 9/24/2020 5:03:47 PM

<b>Instrument</b>	69679	<b>Data File</b>	CAL 7.d
<b>Type</b>	Cal	<b>Sample</b>	CAL 7
<b>Acq. Method</b>	THC Screen 1122.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-G1	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	9/24/2020 2:02:12 PM		

## Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.665	6170815	3.76	98.9	2362133	244.9102 ng/ml