

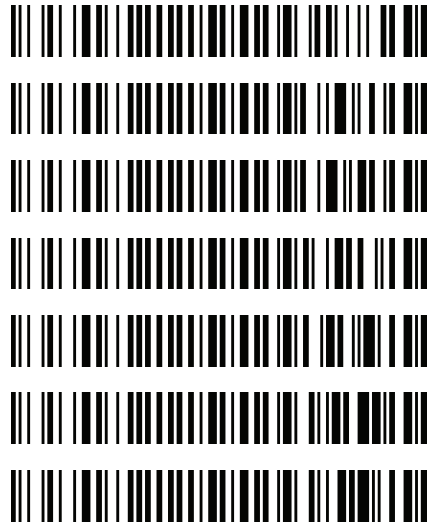
**REVIEWED**  
By Tamara Salazar at 11:42 am, Oct 24, 2019

10/23/2019

*Bylee*

**Worklist: 3771**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2019-1958	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2019-1968	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2019-1969	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2019-1970	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2019-1976	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2019-1979	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2019-1980	1	BCK	AM 27 Blood THC Quant by LC-QQQ



BWylee

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

Extraction Date: 10/22/19  
Plate lot#: 190716

Analyst: Britany Wylie  
Plate Expiration: 1-16-2020

**Mobile phase A:** 0.1% Formic Acid in LCMS Water MTBE  
**Mobile phase B:** 0.1% Formic acid in Acetonitrile Hexane  
**Blank Blood Lot:** 19H52275-3  
**LCMS-QQQ ID:** 69679  
**Column:** UCT Selectra DA 100 x 2.1mm 3um  
**LCMS Methanol**

### 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. *Shaker ID: 66759*
- 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 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: *Blood samples only in this run. Curve ranges: THC-OH: 5-100; carboxy-THC: 10-250, THC 3-100: THC-OH reported qualitatively*

# AM #27 Cannabinoids

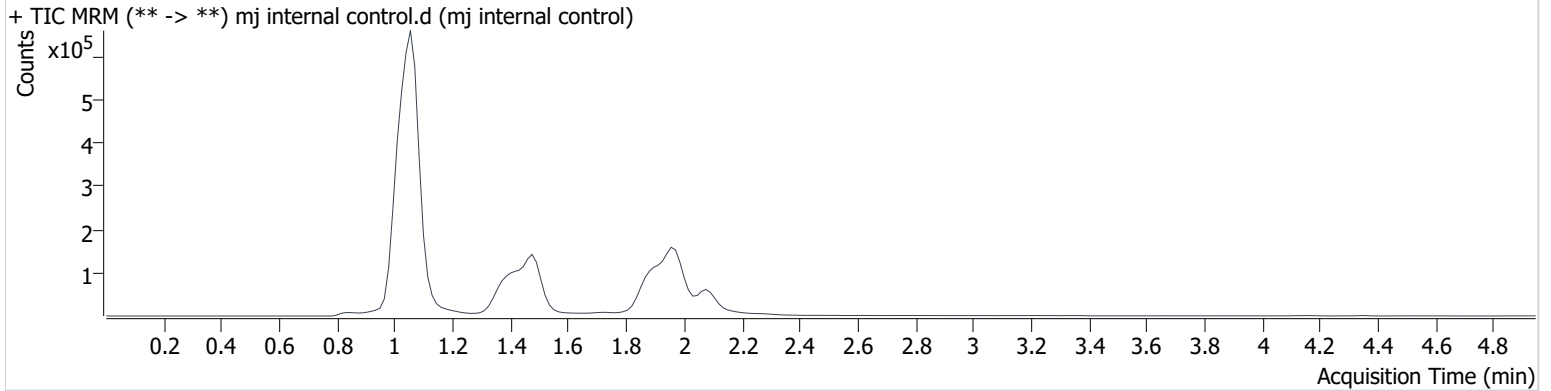
*BWylee*

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 12:39:03 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.055	134748	40634.8	35.7	684.2	824161	14.438 ng/ml
THC-OH	1.059	188549	∞	8.8	∞	2111634	4.559 ng/ml
THC	1.980	27170	∞	24.1	99.6	876243	4.651 ng/ml

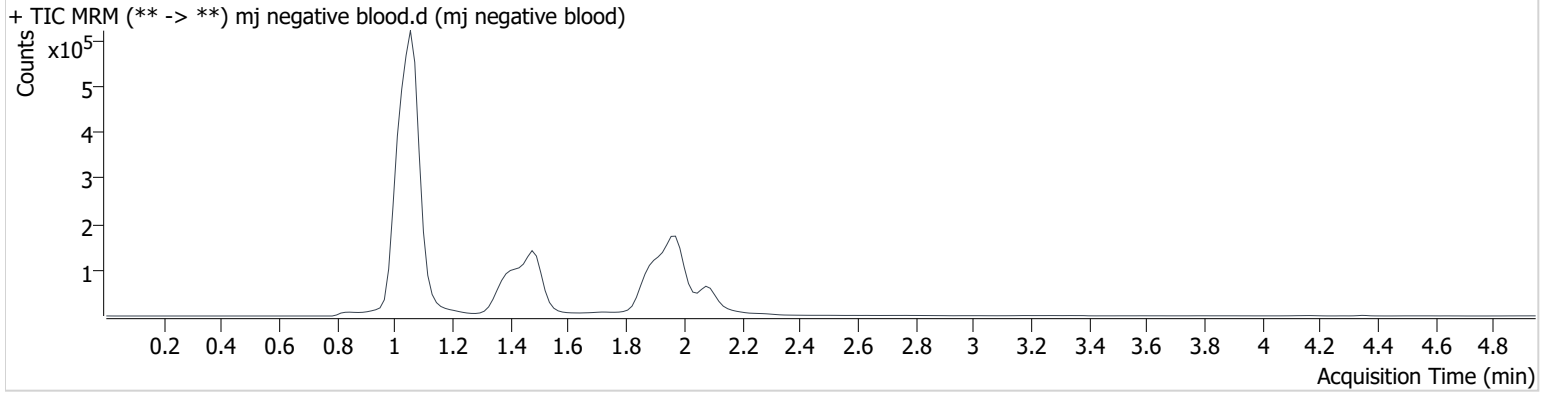
# AM #27 Cannabinoids

*BWylie*

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-A2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 12:46:44 PM		
<b>Sample Info.</b>			

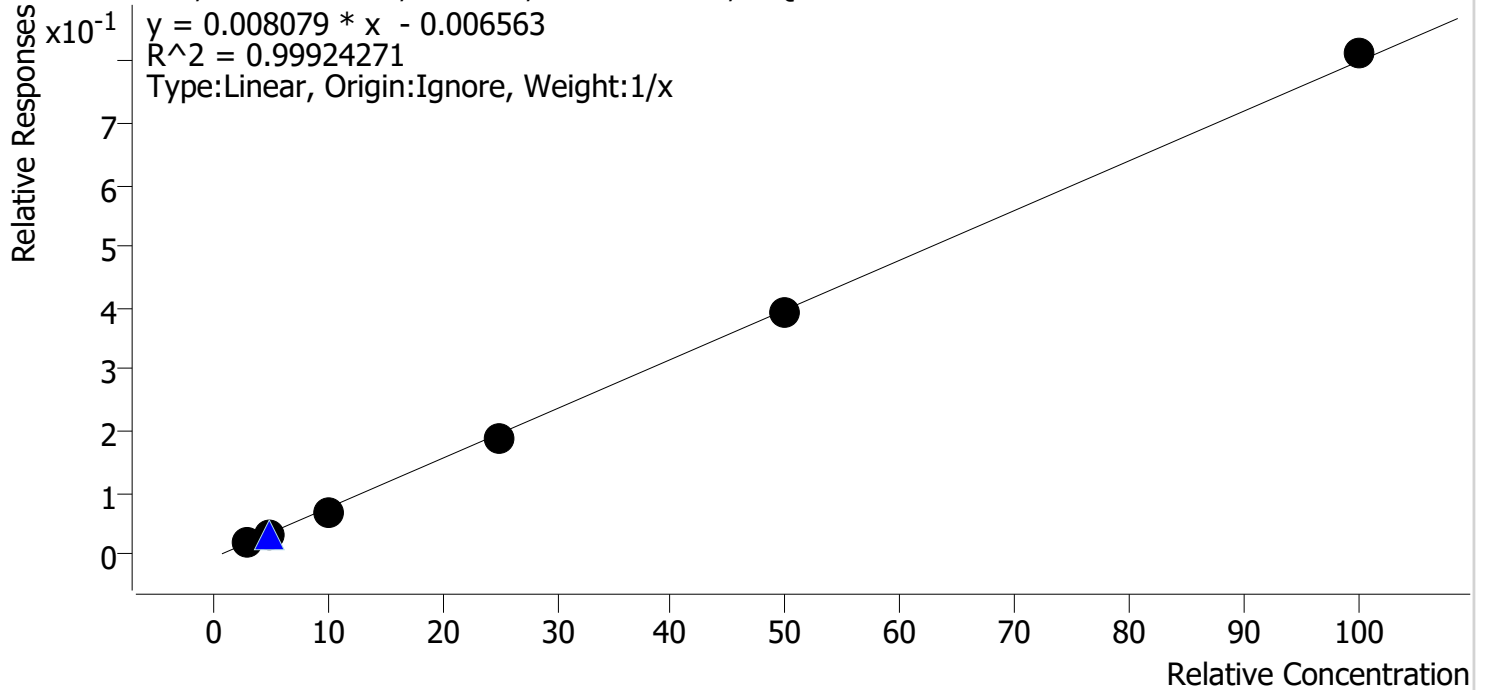
## Sample Chromatogram



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Last Cal. Update** 10/24/2019 8:50 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-d3 *Bylee*

THC - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✓	3.0	3.3	108.5
mj cal 3	3	✓	5.0	5.0	99.7
mj cal 4	4	✓	10.0	9.4	94.1
mj cal 5	5	✓	25.0	24.2	96.9
mj cal 6	6	✓	50.0	49.6	99.2
mj cal 7	7	✓	100.0	101.5	101.5

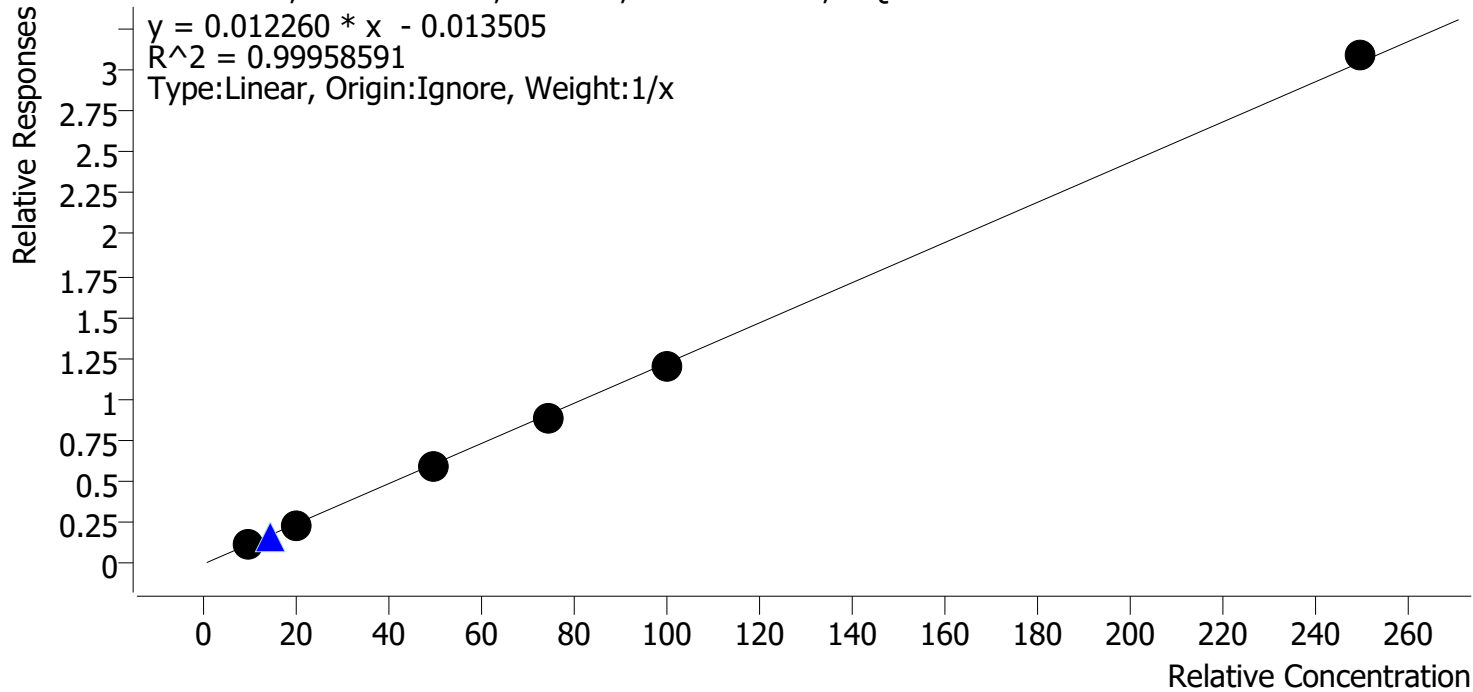
# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Last Cal. Update** 10/24/2019 8:50 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH *BWylee*

**Internal Standard** THC-COOH-d9

THC-COOH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 1 QCs



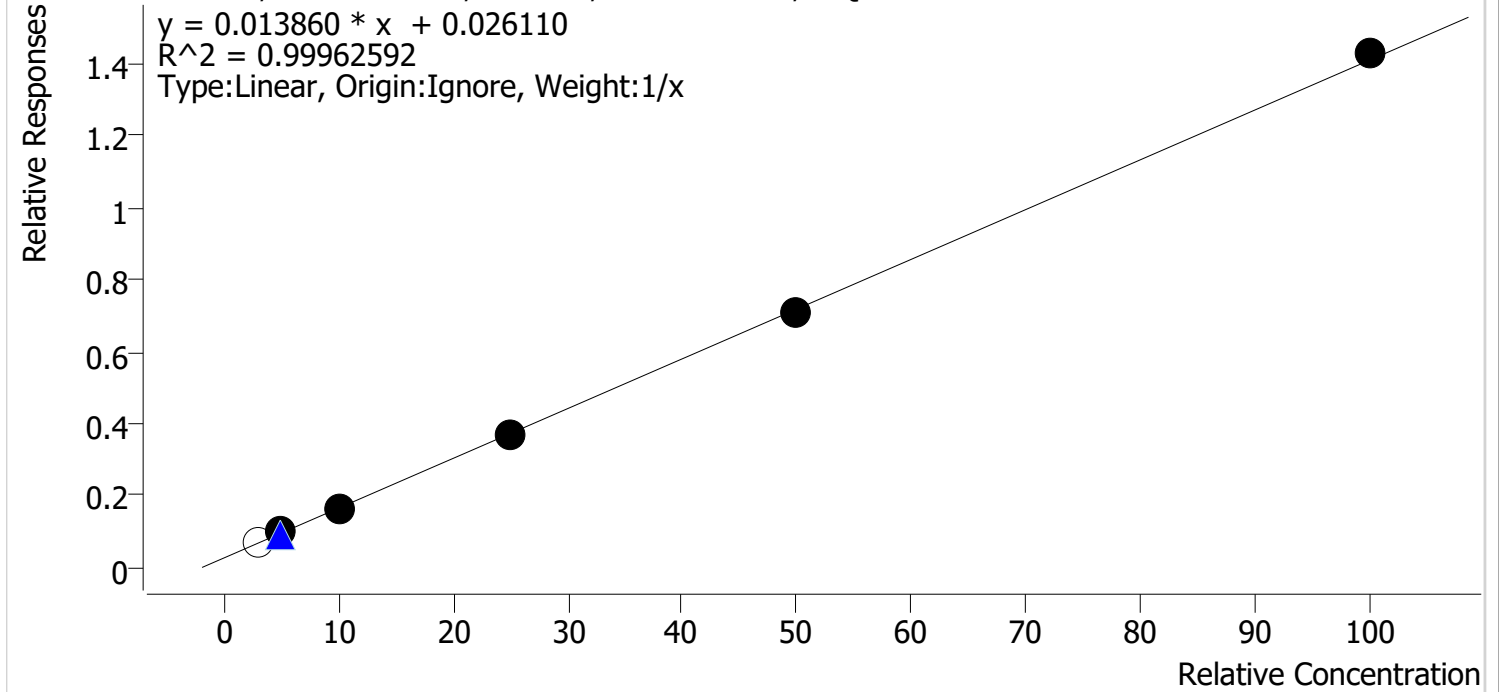
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✓	10.0	10.5	104.9
mj cal 3	3	✓	20.0	19.8	98.9
mj cal 4	4	✓	50.0	48.9	97.9
mj cal 5	5	✓	75.0	73.7	98.2
mj cal 6	6	✓	100.0	98.7	98.7
mj cal 7	7	✓	250.0	253.4	101.4

# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Last Cal. Update** 10/24/2019 8:50 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH *BWylee*

**Internal Standard** THC-OH-d3

THC-OH - 6 Levels, 5 Levels Used, 6 Points, 5 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	x	3.0	3.3	109.6
mj cal 3	3	✓	5.0	5.1	102.6
mj cal 4	4	✓	10.0	10.0	100.0
mj cal 5	5	✓	25.0	24.5	97.9
mj cal 6	6	✓	50.0	49.1	98.2
mj cal 7	7	✓	100.0	101.3	101.3

\*THC-OH evaluated qualitatively in this run, cal curve range 5-100

*BW*

# AM #27 Cannabinoids

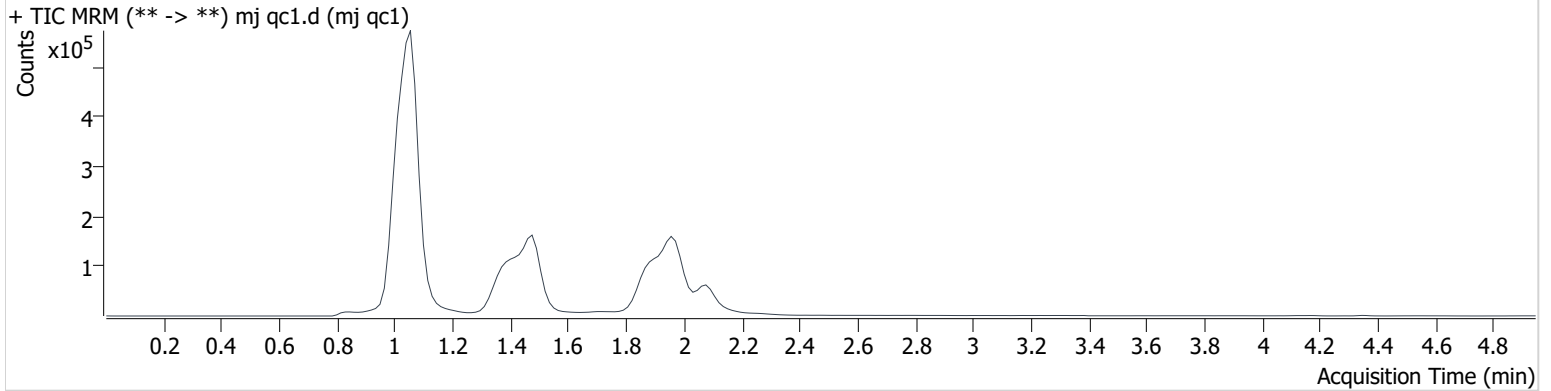
*BWylie*

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<b>Instrument</b>	69679	<b>Data File</b>	mj qc1.d
<b>Type</b>	Sample	<b>Sample</b>	mj qc1
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Britany Wylie
<b>Sample Position</b>	P3-A1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 11:45:04 AM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.055	49152	285.8	31.9	115.6	836876	5.892 ng/ml <b>Low</b>
THC	1.965	6201	∞	16.6 <b>Low</b>	11.8	904024	1.661 ng/ml <b>Low</b>



# AM #27 Cannabinoids

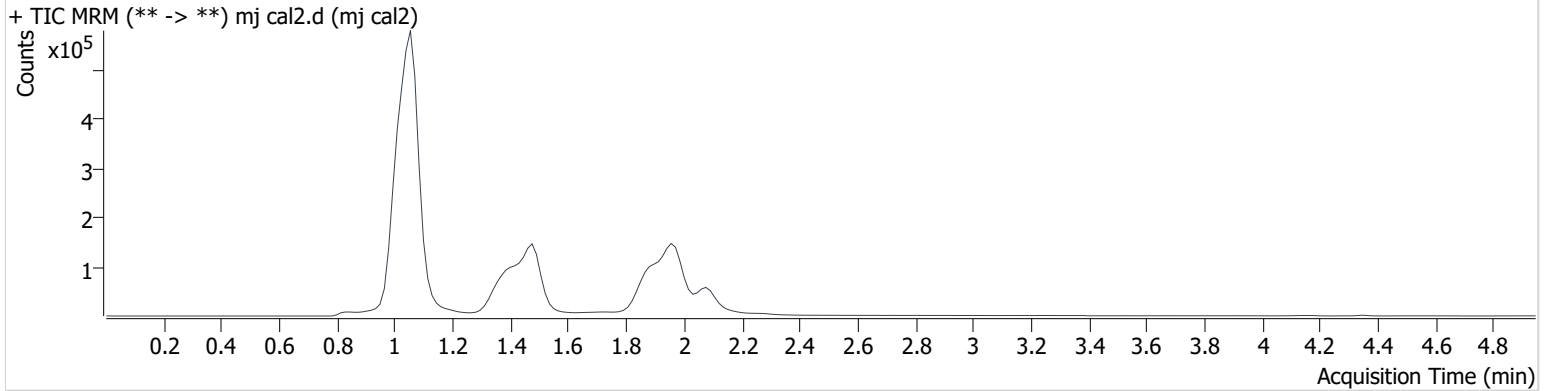
*BWylie*

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-B1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 11:52:48 AM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.055	90472	556.0	35.9	26607.1	786265	10.487 ng/ml
THC-OH	1.059	142064	∞	6.9 <b>Low</b>	∞	1981602	3.289 ng/ml
THC	1.965	16314 529957447255	355.0	31.7	∞	826364	3.256 ng/ml

# AM #27 Cannabinoids

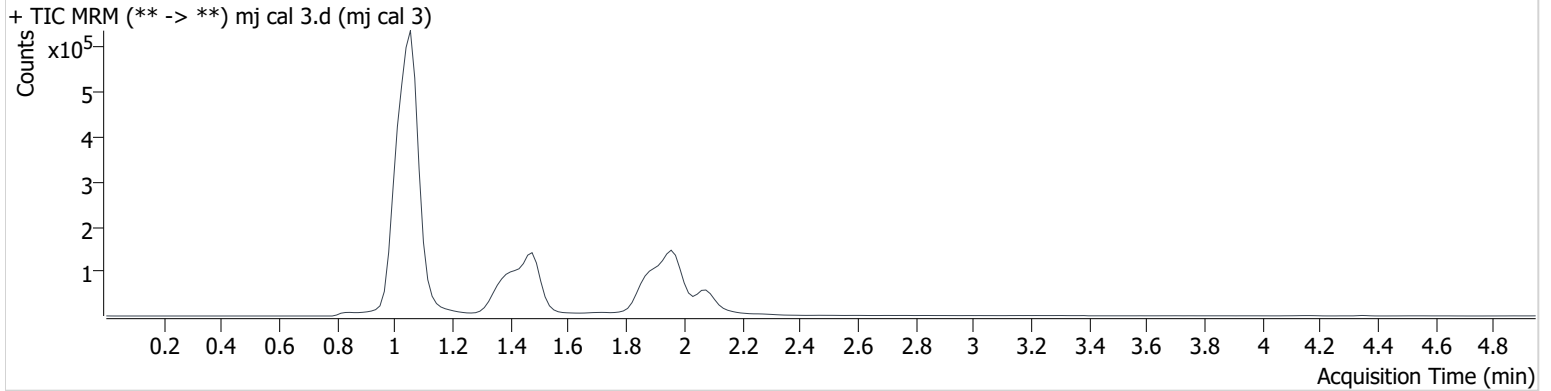
BWylie

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-C1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 12:00:32 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.055	176404	1359.0	38.6	1188.8	770043	19.787 ng/ml
THC-OH	1.059	192251	∞	8.4	∞	1978013	5.129 ng/ml
THC	1.965	26957	∞	28.9	∞	799315	4.987 ng/ml

# AM #27 Cannabinoids

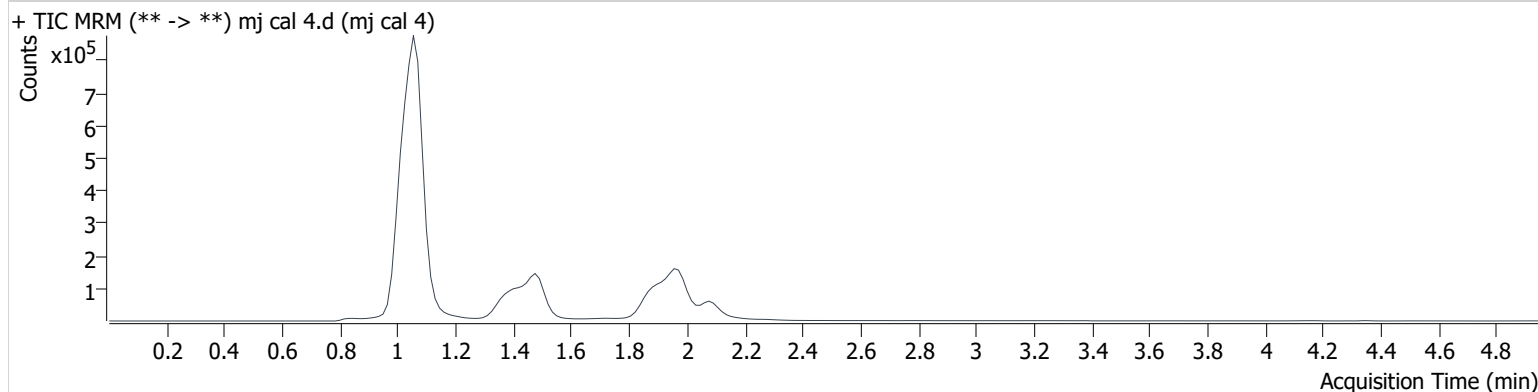
*BWylie*

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-D1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 12:08:14 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.055	472980	3114.9	38.8	2943.1	806525	48.936 ng/ml
THC-OH	1.059	344159	∞	9.6	∞	2089555	10.000 ng/ml
THC	1.980	60098	∞	24.5	∞	864873	9.414 ng/ml

# AM #27 Cannabinoids

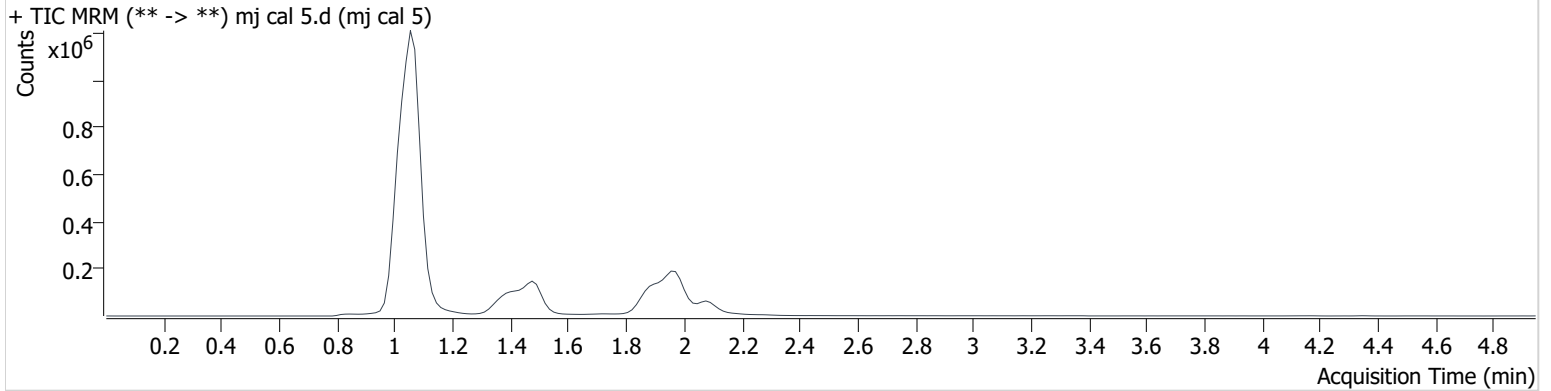
*Bylye*

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-E1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 12:15:56 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.070	778304	1532.5	39.0	4136.2	874790	73.672 ng/ml
THC-OH	1.059	858844	∞	10.7	∞	2350864	24.476 ng/ml
THC	1.980	180807	∞	25.6	∞	956288	24.216 ng/ml

# AM #27 Cannabinoids

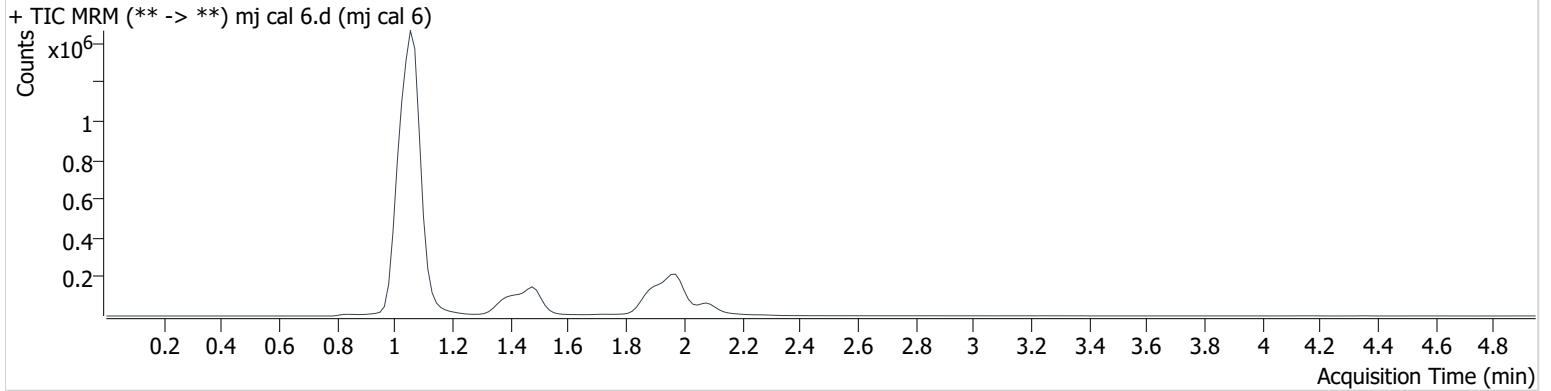
*BWylie*

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-F1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 12:23:38 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.055	974327	5841.3	39.3	8308.0	813952	98.740 ng/ml
THC-OH	1.059	1565989	∞	11.5	∞	2215127	49.124 ng/ml
THC	1.980	359384	∞	24.1	∞	911822	49.599 ng/ml

# AM #27 Cannabinoids

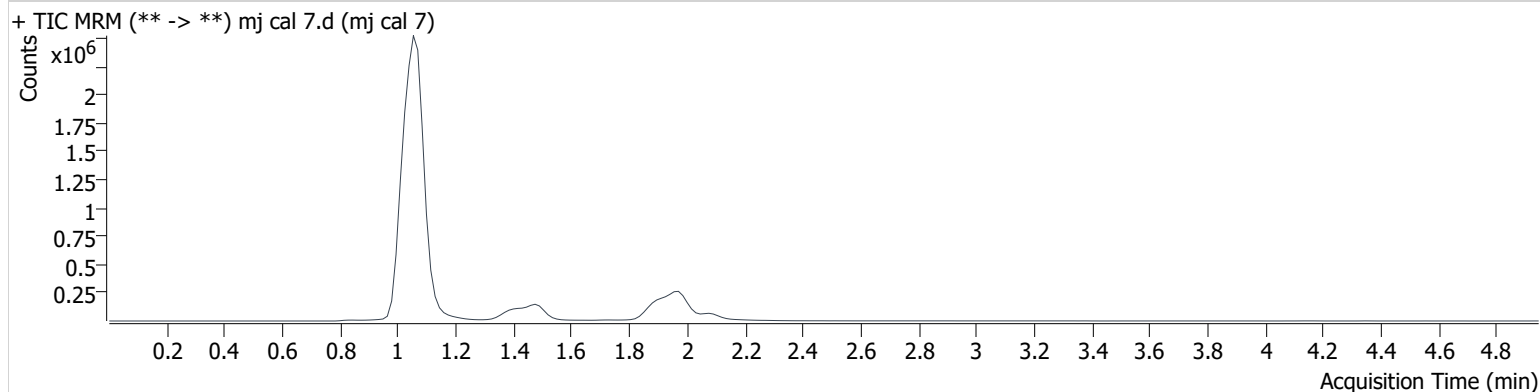
BWylee

**Batch results** D:\MassHunter\Data\2019\am28-27 102219\QuantResults\AM 27 thcq 102219.batch.bin  
**Calibration Last Update** 10/24/2019 8:50:37 AM

<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>	Britany Wylie
<b>Sample Position</b>	P3-G1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	10/22/2019 12:31:20 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.055	2268693	8507.2	39.4	921927.3	733528	253.377 ng/ml
THC-OH	1.059	3029667	∞	11.7	∞	2119116	101.271 ng/ml
THC	1.980	707531	∞	25.0	∞	869553	101.529 ng/ml