12/10/2019

REVIEWED
By Sarah Pickle at 9:23 am, Dec 12, 2019

BW

Worklist: 3874

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
C2019-2197	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2254	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2256	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2289	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2302	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2334	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

BW

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

Extraction Date: 12/9/19 Analyst: Britany Wylie
Plate lot#: 190716 Plate Expiration: 1-16-2020

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: 19H52275 **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. 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** in wells of analytical plate.
☒ 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 6. Transfer **800µL of blood+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: *Blood samples only in this run. Curve ranges: carboxy-THC: 10-250, THC 3-100; Not Evaluated: THC-OH*

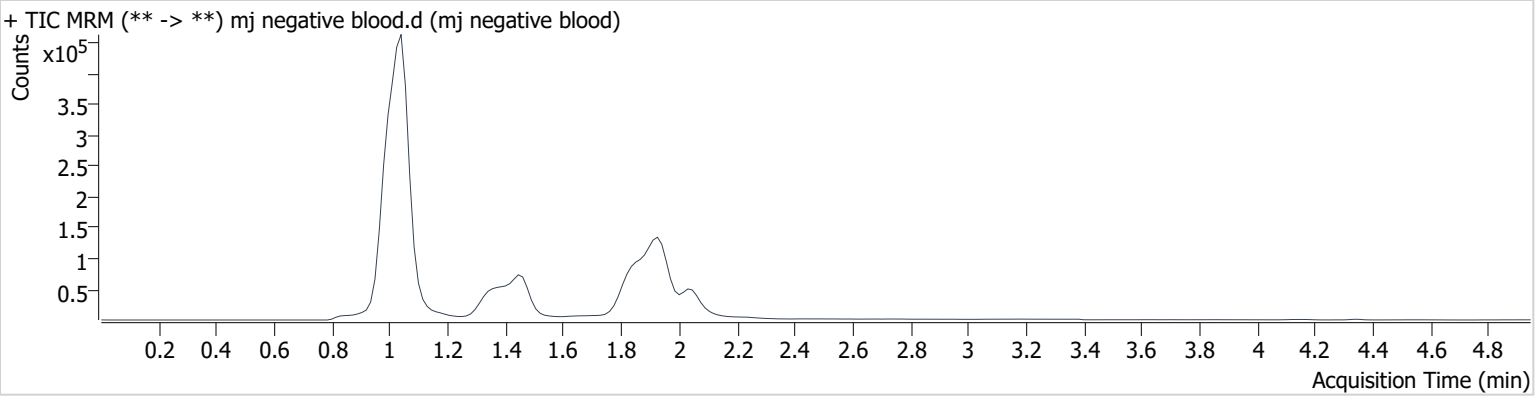
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj negative blood.d
Type	Sample	**Sample**	mj negative blood
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 12:47:07 PM		
Sample Info.			

Sample Chromatogram



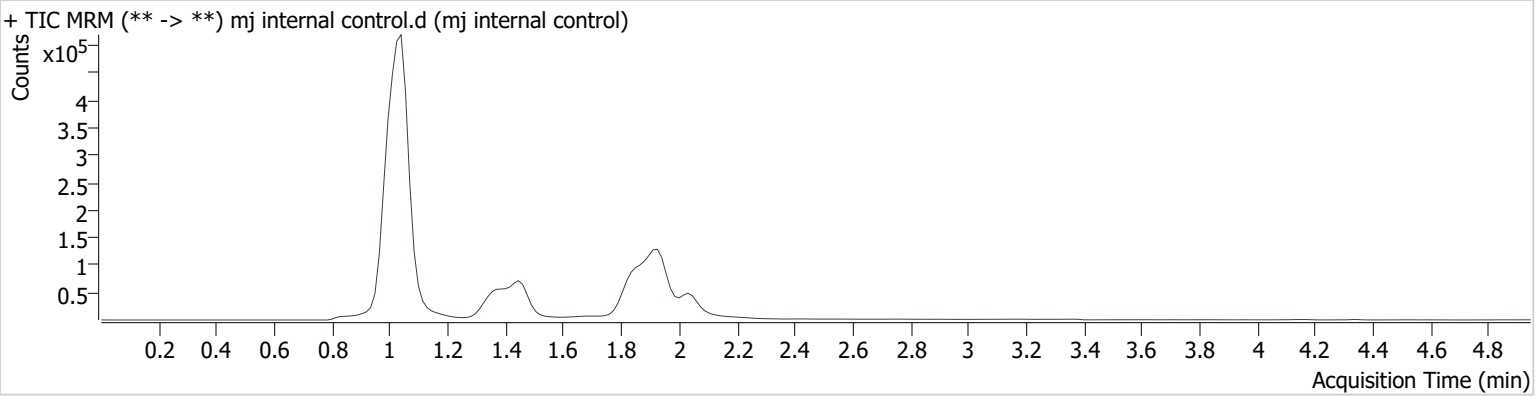
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj internal control.d
Type	QC	**Sample**	mj internal control
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 12:39:25 PM		
Sample Info.			

Sample Chromatogram



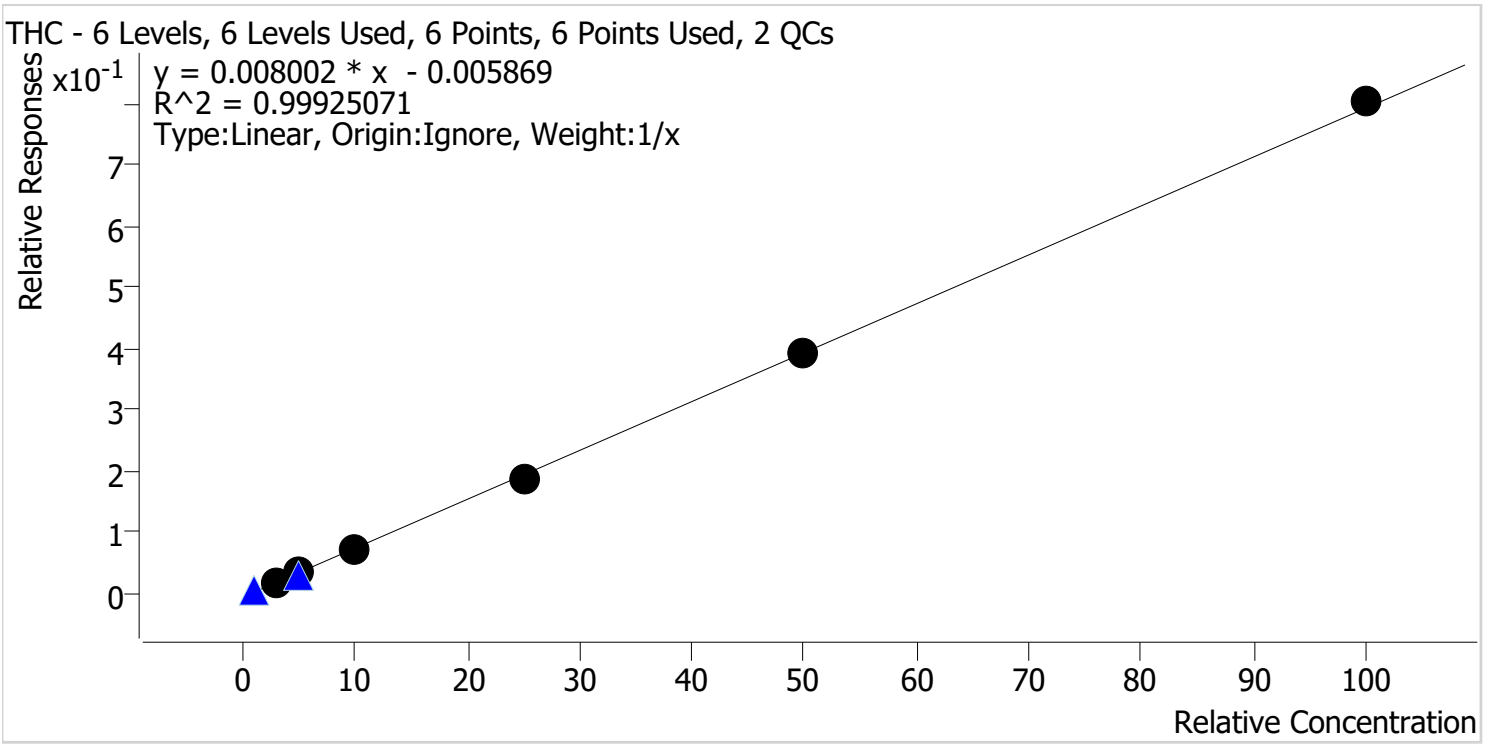
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.040	103027	455.3	36.6	1581.1	621884	14.439	ng/ml	
~~THC-OH~~	~~1.044~~	~~173010~~	~~∞~~	~~7.4 Low~~	~~∞~~	~~1741032~~	~~4.420~~	~~ng/ml~~	NE
THC	1.935	19825	467.1	25.8	∞	691200	4.318	ng/ml	

Compound Calibration Report

Agilent | Trusted Answers

Batch results	D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Last Cal. Update	12/11/2019 11:49 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3

BW



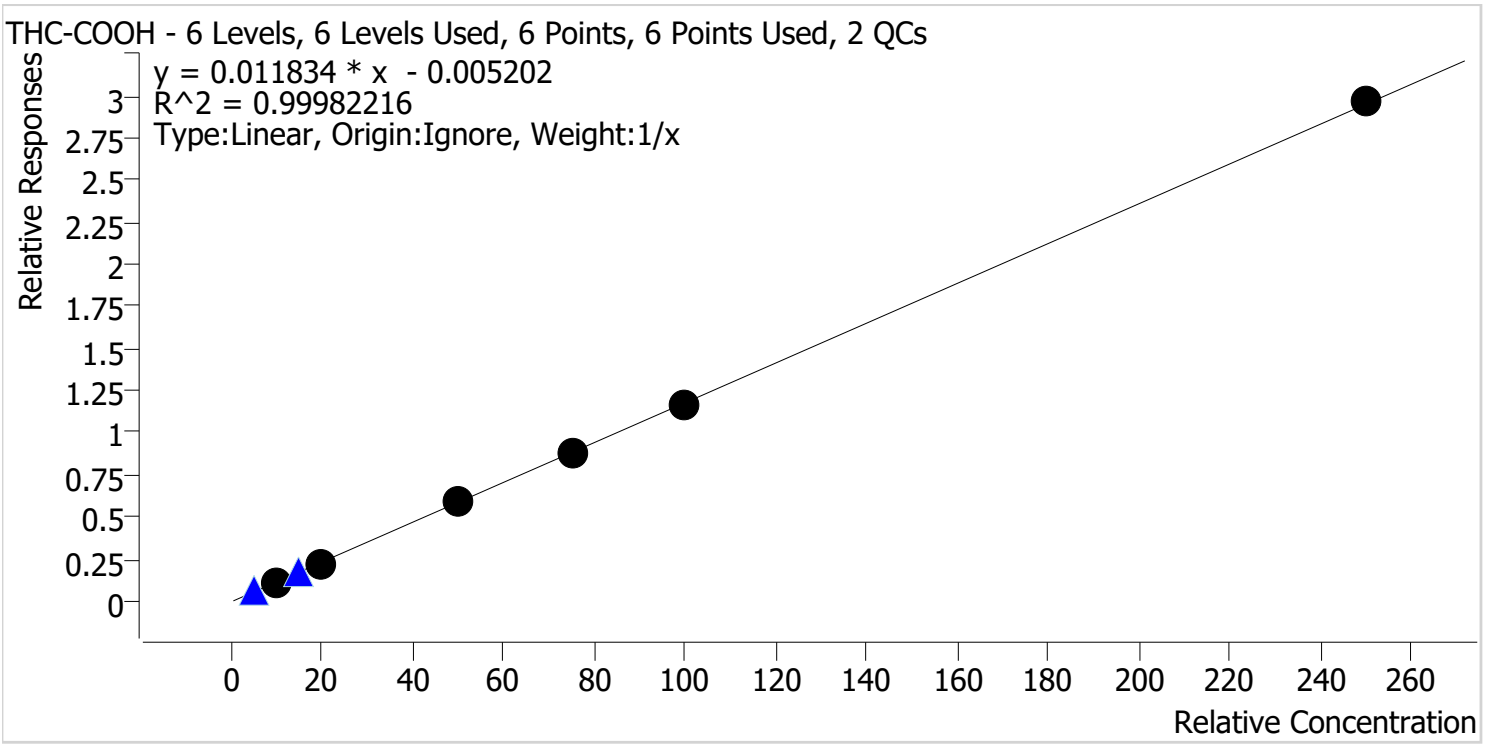
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 6 cal 2	2	✔	3.0	3.2	106.7
mj cal 5 cal 3	3	✔	5.0	5.0	100.5
mj cal 4	4	✔	10.0	9.6	96.3
mj cal 3 cal 5	5	✔	25.0	23.8	95.0
mj cal 2 cal 6	6	✔	50.0	50.1	100.2
mj qc1 cal 7	7	✔	100.0	101.3	101.3

BW 12/11/19

Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Last Cal. Update	12/11/2019 11:49 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9

BW



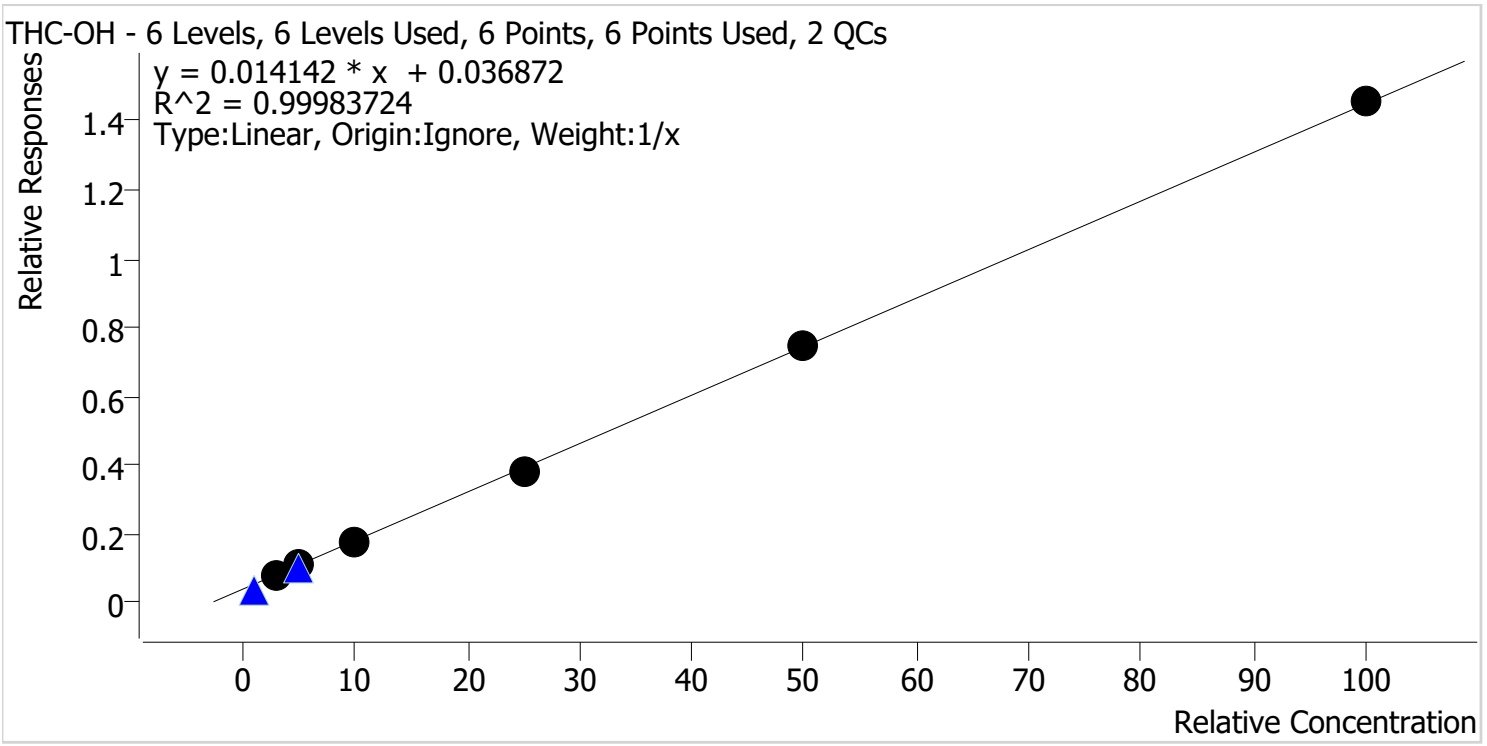
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj ~~cal 6~~ cal 2	2	✔	10.0	10.3	103.4
mj ~~cal 5~~ cal 3	3	✔	20.0	19.4	97.2
mj cal 4	4	✔	50.0	49.8	99.6
mj ~~cal 3~~ cal 5	5	✔	75.0	75.5	100.7
mj ~~cal 2~~ cal 6	6	✔	100.0	98.6	98.6
mj ~~qc1~~ cal 7	7	✔	250.0	251.3	100.5

BW 12/11/19

Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Last Cal. Update	12/11/2019 11:49 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3

BW



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 6	2	✔	3.0	3.1	104.1
mj cal 5	3	✔	5.0	4.9	98.5
mj cal 4	4	✔	10.0	9.9	98.7
mj cal 3	5	✔	25.0	24.4	97.8
mj cal 2	6	✔	50.0	50.2	100.4
mj qc1	7	✔	100.0	100.4	100.4

* compound not evaluated in this run, ratios were out of acceptable range and poor peak shape

BW 12/11/19

AM #27 Cannabinoids

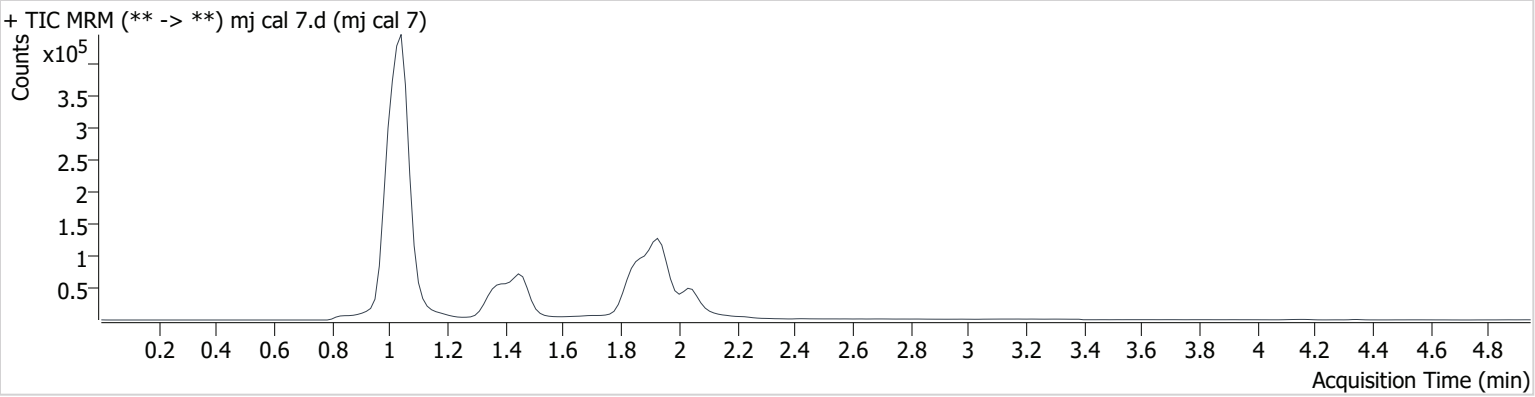
BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj cal 7.d
Type	QC	**Sample**	mj ~~cal 7~~ qc1
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 11:45:27 AM		
Sample Info.			

BW 12/11/19

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.040	39250	169.3	31.1	947.7	590868	6.053	ng/ml Low
THC	1.935	4179	191.5	45.7 High	∞	673895	1.508	ng/ml Low

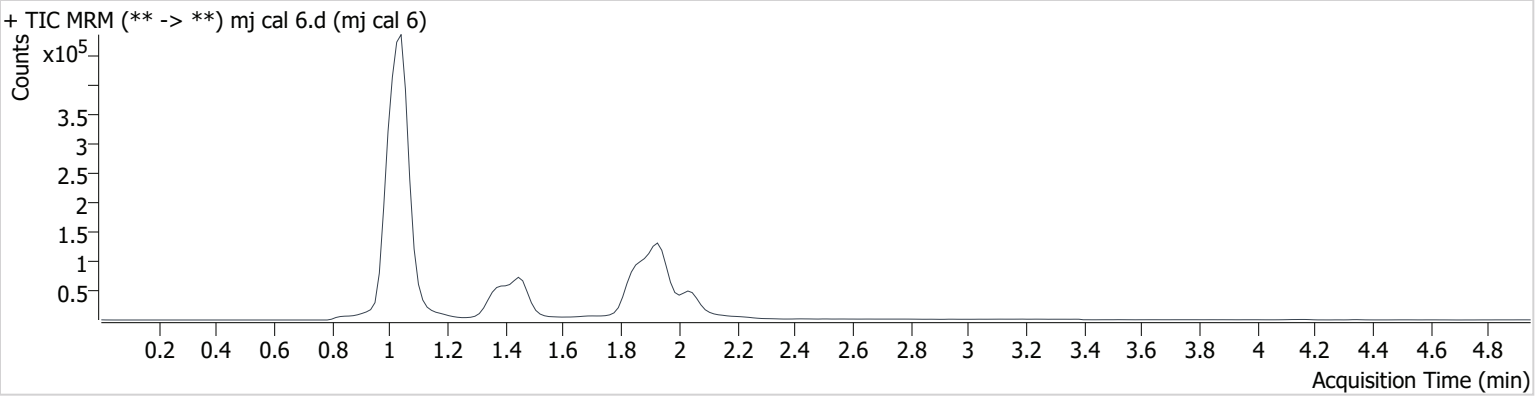
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj ~~cal 6~~ cal 2 BW
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie 12/11/19
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 11:53:11 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.040	69947	223.2	34.8	591.9	597212	10.337	ng/ml	
~~THC-OH~~	~~1.044~~	~~134797~~	~~∞~~	~~6.3 Low~~	~~∞~~	~~1662982~~	~~3.124~~	~~ng/ml~~	NE
THC	1.935	13465	649.1	28.1	240.8	681864	3.201	ng/ml	

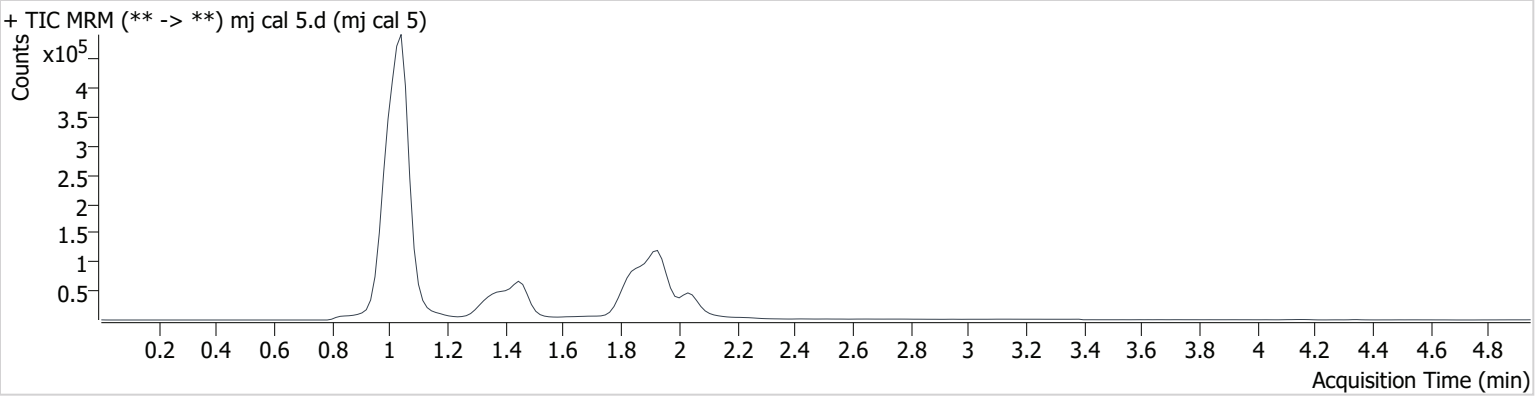
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj ~~cal 5~~ cal 3 BW
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie 12/11/19
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 12:00:54 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.040	133176	701.5	37.7	1872.9	592577	19.431	ng/ml	
~~THC-OH~~	~~1.044~~	~~173162~~	~~∞~~	~~8.1~~	~~∞~~	~~1625445~~	~~4.926~~	~~ng/ml~~	NE
THC	1.935	22200	1015.8	25.7	169.0	646396	5.025	ng/ml	

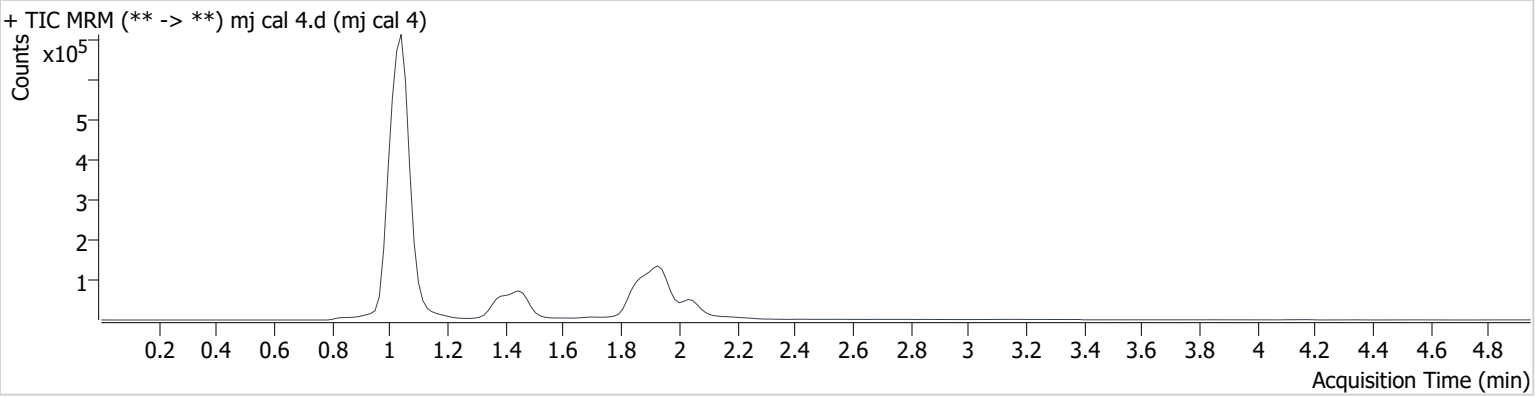
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 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	12/9/2019 12:08:36 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.040	335928	1470.8	37.9	3739.0	574812	49.824	ng/ml	
~~THC-OH~~	~~1.044~~	~~286608~~	~~∞~~	~~8.4~~	~~∞~~	~~1623713~~	~~9.874~~	~~ng/ml~~	NE
THC	1.950	47466	1308.9	26.9	∞	666798	9.629	ng/ml	

AM #27 Cannabinoids

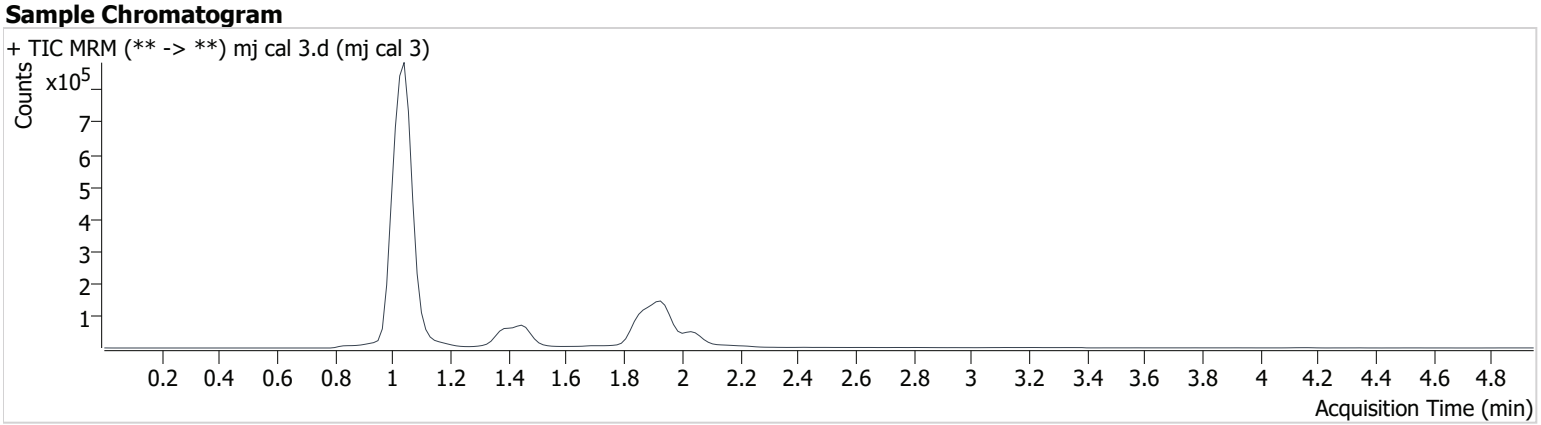
BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj ~~cal 3~~ cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 12:16:19 PM		
Sample Info.			

BW 12/11/19

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.040	491192	4682.3	37.9	1888.8	552728	75.534	ng/ml	
~~THC-OH~~	~~1.044~~	~~601987~~	~~∞~~	~~11.3~~	~~∞~~	~~1573842~~	~~24.440~~	~~ng/ml~~	NE
THC	1.935	122265	887.3	26.7	6125.7	663620	23.757	ng/ml	

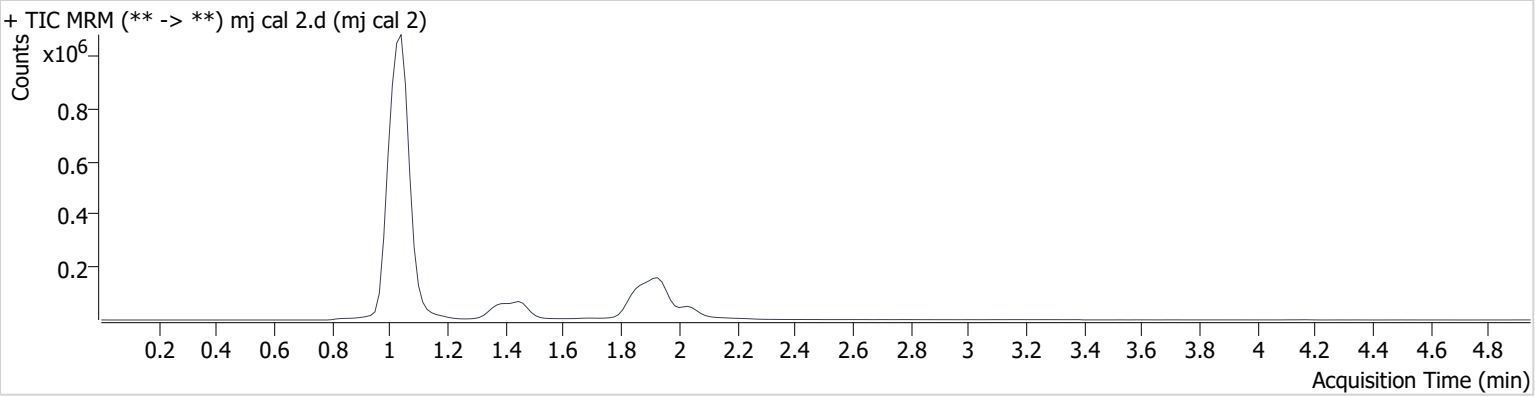
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj cal 2.d
Type	Cal	**Sample**	mj ~~cal 2~~ cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie BW 12/11/19
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 12:24:01 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.040	649850	3375.5	39.0	9488.1	559324	98.618	ng/ml	
~~THC-OH~~	~~1.044~~	~~1172252~~	~~∞~~	~~11.3~~	~~∞~~	~~1569900~~	~~50.194~~	~~ng/ml~~	NE
THC	1.935	254150	4402.9	24.6	∞	643564	50.083	ng/ml	

AM #27 Cannabinoids

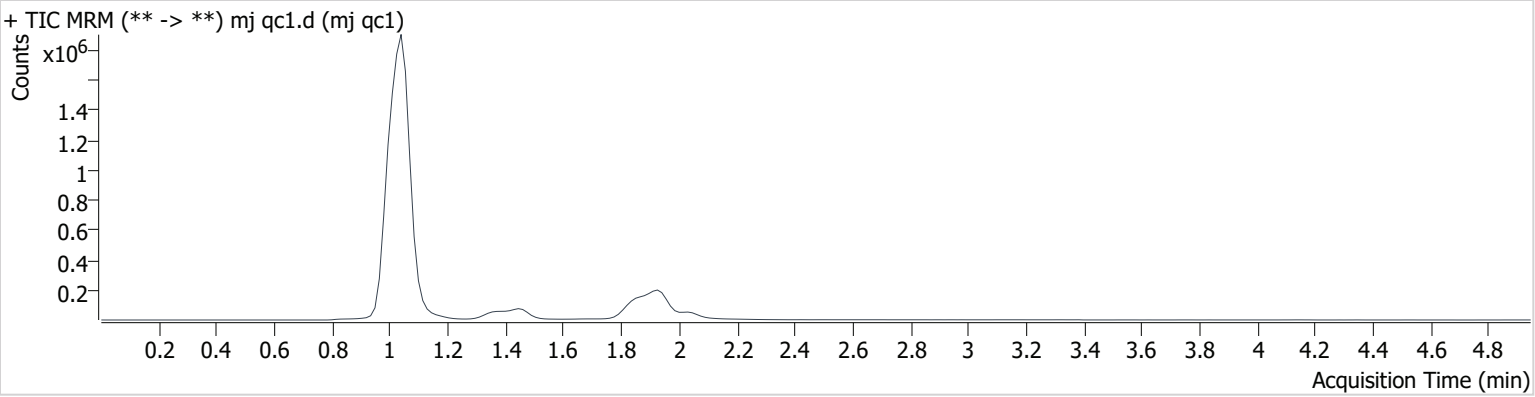
BW

Batch results D:\MassHunter\Data\2019\am27-28 120919\QuantResults\cann quant.batch.bin
Calibration Last Update 12/11/2019 11:49:39 AM

Instrument	69679	**Data File**	mj qc1.d
Type	Cal	**Sample**	mj ~~qc1~~ cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	12/9/2019 12:31:43 PM		
Sample Info.			

BW 12/11/19

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-COOH	1.040	1660714	10779.9	39.5	7663.6	559506	251.257	ng/ml	
~~THC-OH~~	~~1.044~~	~~2434297~~	~~∞~~	~~11.9~~	~~∞~~	~~1670437~~	~~100.441~~	~~ng/ml~~	NE
THC	1.935	530475	6947.3	25.3	∞	659143	101.304	ng/ml	