REVIEWED
By Celena Shrum at 3:02 pm, Oct 01, 2019

REVIEWED
By Sarah Pickle at 9:49 am, Oct 15, 2019

9/24/2019

Worklist: 3719

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-1741	1	165064	AM 27 Blood THC Quant by LC-QQQ
C2019-1744	1	165065	AM 27 Blood THC Quant by LC-QQQ
C2019-1754	1	165066	AM 27 Blood THC Quant by LC-QQQ
C2019-1772	1	165067	AM 27 Blood THC Quant by LC-QQQ
C2019-1790	1	165070	AM 27 Blood THC Quant by LC-QQQ
C2019-1792	4	165068	AM 27 Blood THC Quant by LC-QQQ
C2019-1830	1	165069	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: 09-24-19 Analyst: Anne Nord
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: 19H52275-1 **Urine Blank:** 8919 **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. *Shaker ID: 66759*
☒ 4. Pipette **500µL 0.1% formic acid in water** 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 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? Y / N
☒ 6 Enter QCs into control charting.
☒ 7 Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

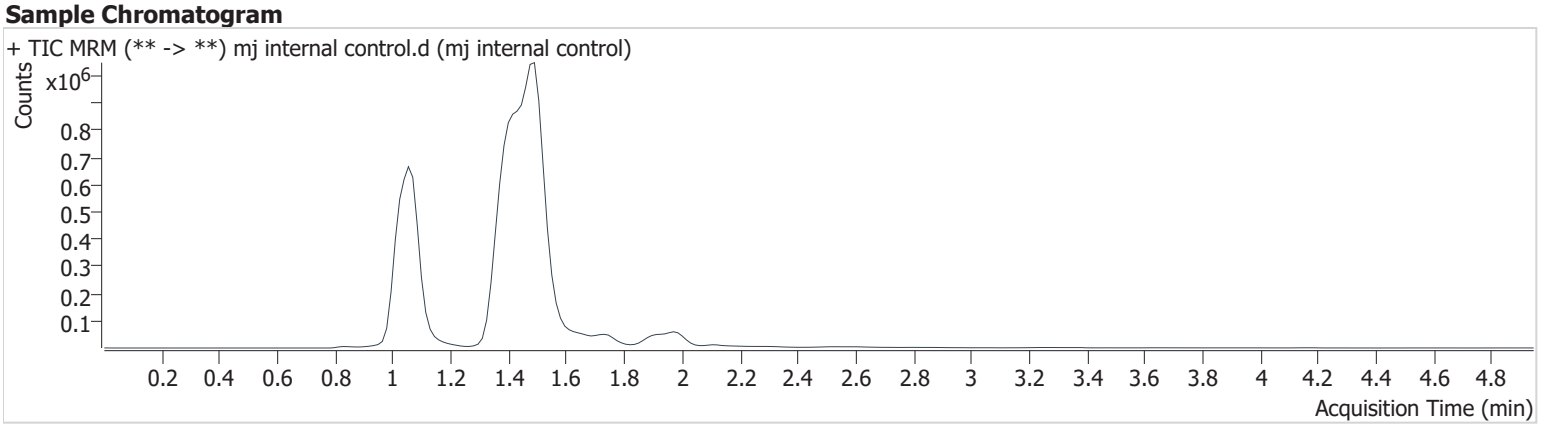
COMMENTS: *Calibrator 7 did not inject on the first injection, data from the failed injection was not used. It was re-injected.*

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 PM

Instrument	69679	**Data File**	mj internal control.d
Type	QC	**Sample**	mj internal control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/24/2019 1:10:16 PM		
Sample Info.			

Sample Chromatogram



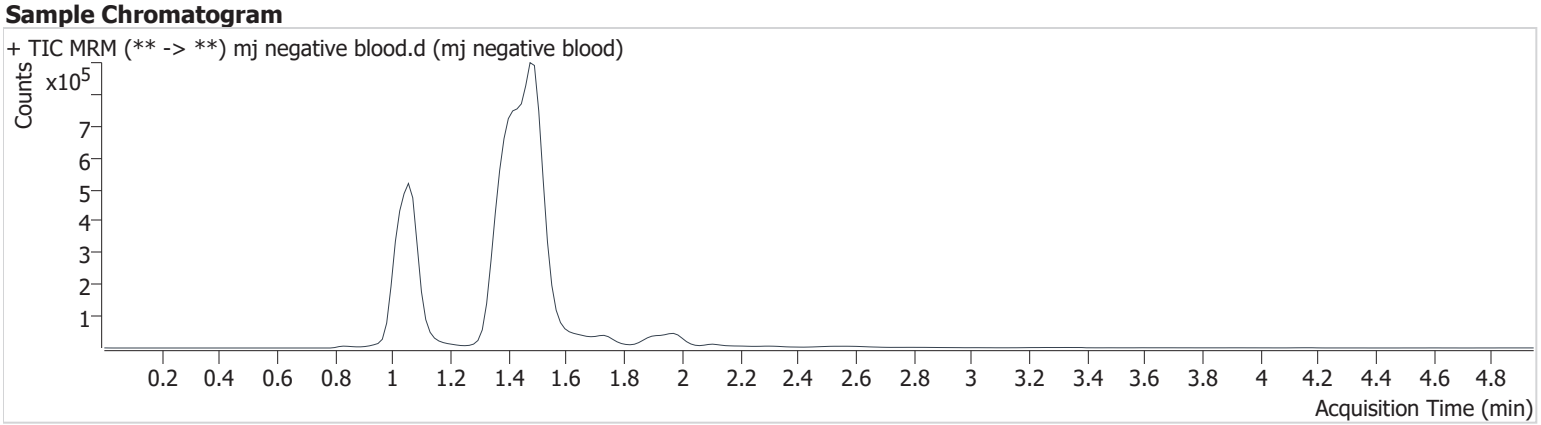
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.070	161095	253.08	35.1	1906.2	932988	14.637 ng/ml
THC-OH	1.059	174406	∞	8.0	∞	2084168	5.303 ng/ml
THC	1.980	13114	∞	21.9	21.331	445524	4.372 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 PM

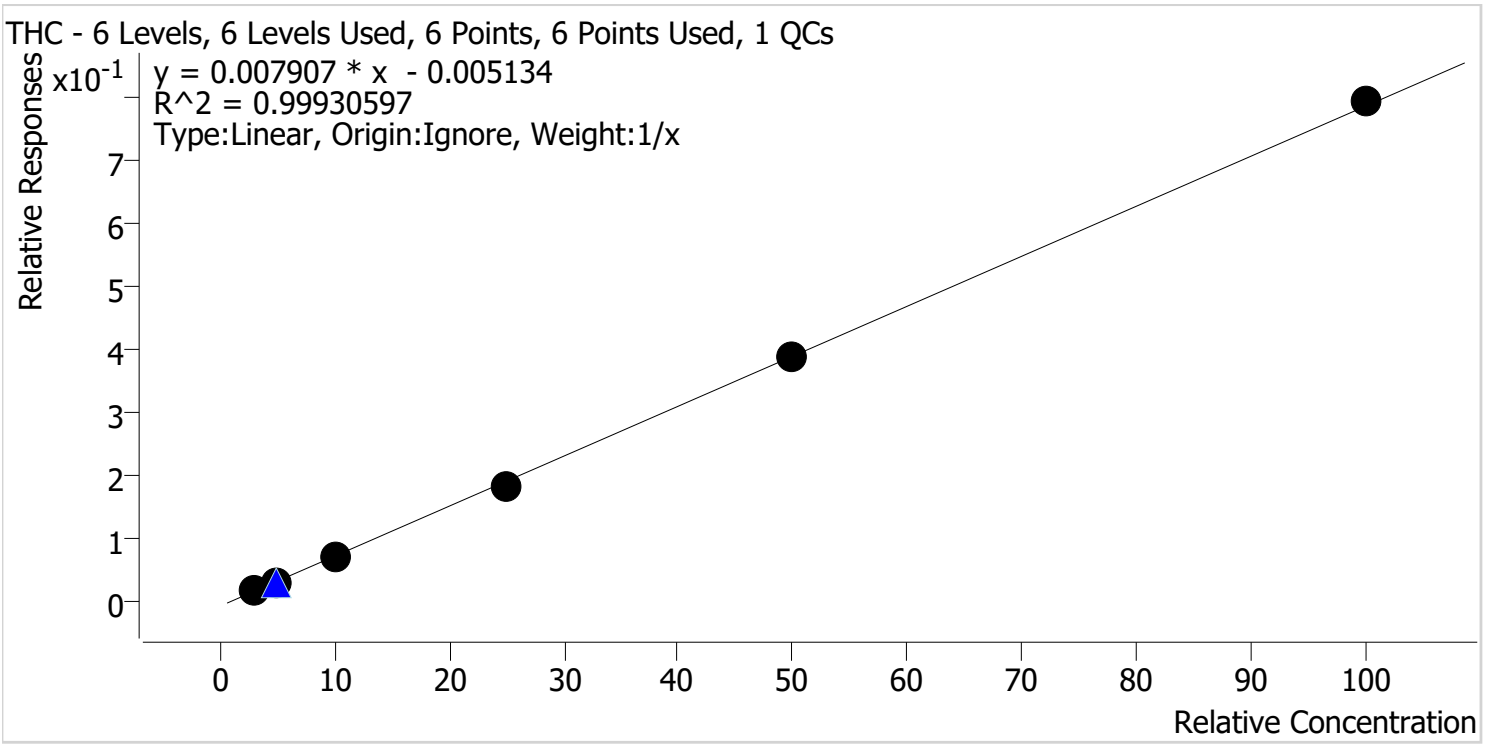
Instrument	69679	**Data File**	mj negative blood.d
Type	Sample	**Sample**	mj negative blood
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/24/2019 1:18:00 PM		
Sample Info.			

Sample Chromatogram



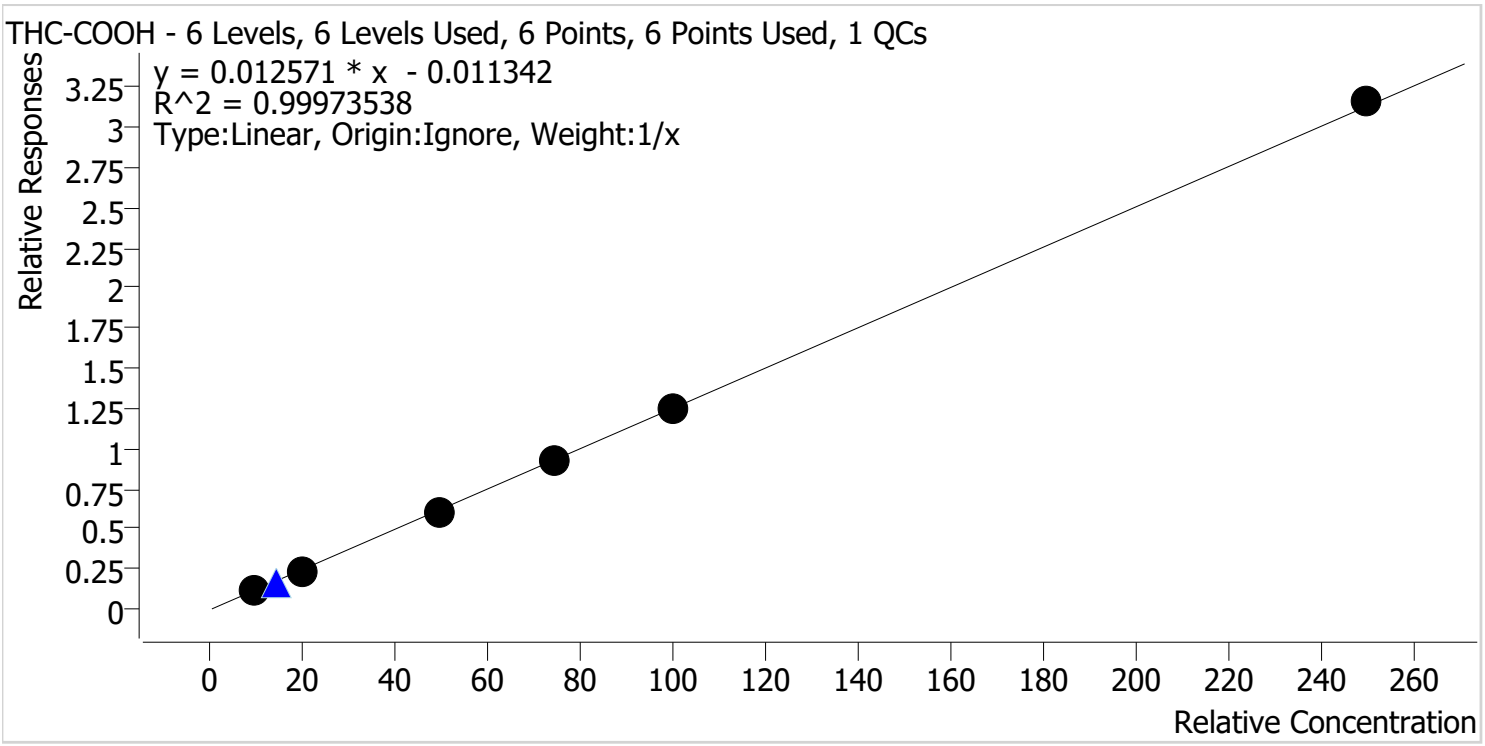
Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Last Cal. Update	9/24/2019 4:23 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✔	3.0	3.3	111.0
mj cal 3	3	✔	5.0	4.8	95.3
mj cal 4	4	✔	10.0	9.5	95.1
mj cal 5	5	✔	25.0	24.3	97.3
mj cal 6	6	✔	50.0	50.2	100.4
mj cal 7	7	✔	100.0	100.9	100.9

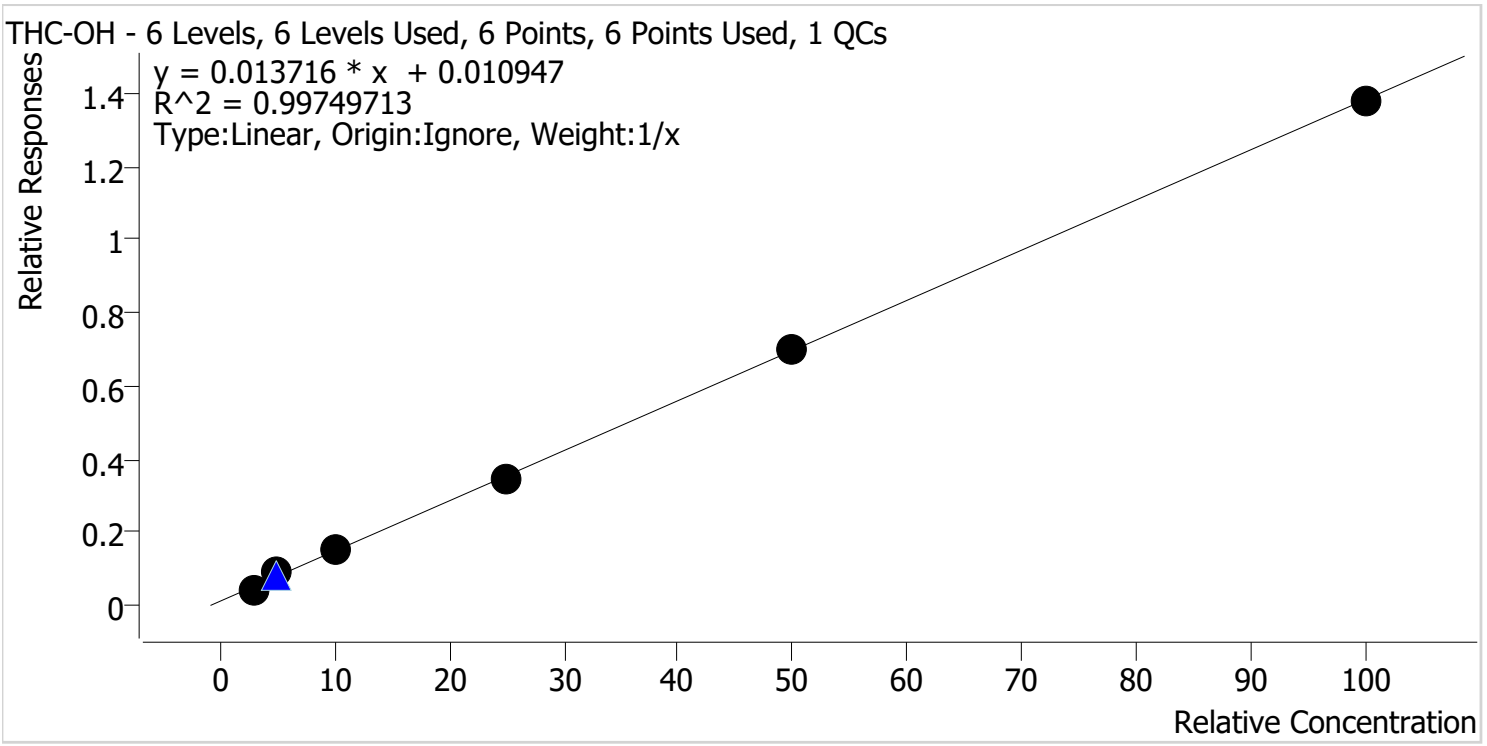
Batch results	D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Last Cal. Update	9/24/2019 4:23 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✔	10.0	10.4	104.4
mj cal 3	3	✔	20.0	19.7	98.4
mj cal 4	4	✔	50.0	49.2	98.5
mj cal 5	5	✔	75.0	73.8	98.4
mj cal 6	6	✔	100.0	99.3	99.3
mj cal 7	7	✔	250.0	252.5	101.0

Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Last Cal. Update	9/24/2019 4:23 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



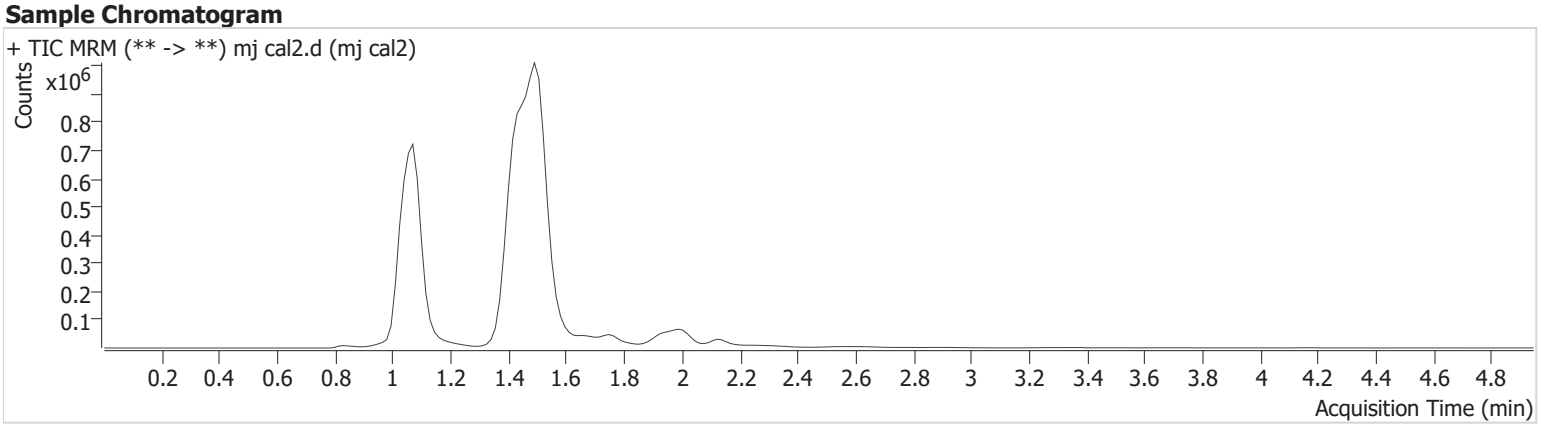
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✔	3.0	2.3	77.8
mj cal 3	3	✔	5.0	5.9	118.5
mj cal 4	4	✔	10.0	10.6	105.5
mj cal 5	5	✔	25.0	24.7	98.7
mj cal 6	6	✔	50.0	50.0	100.0
mj cal 7	7	✔	100.0	99.5	99.5

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 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	9/24/2019 12:23:51 PM		
Sample Info.			

Sample Chromatogram



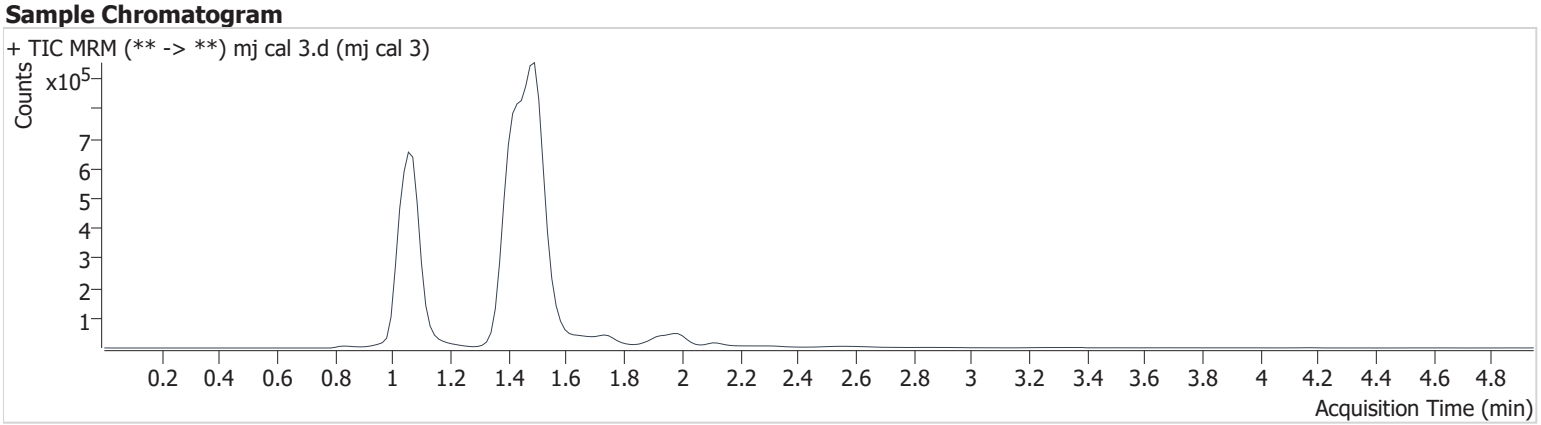
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-COOH	1.070	103302	134.16	36.3	60.11	861672	10.439 ng/ml	
THC-OH	1.074	101543	∞	8.5	∞	2363354	2.334 ng/ml	Low
THC	2.010	9420	68.591	19.7	15.986	444320	3.330 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 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	9/24/2019 12:31:36 PM		
Sample Info.			

Sample Chromatogram



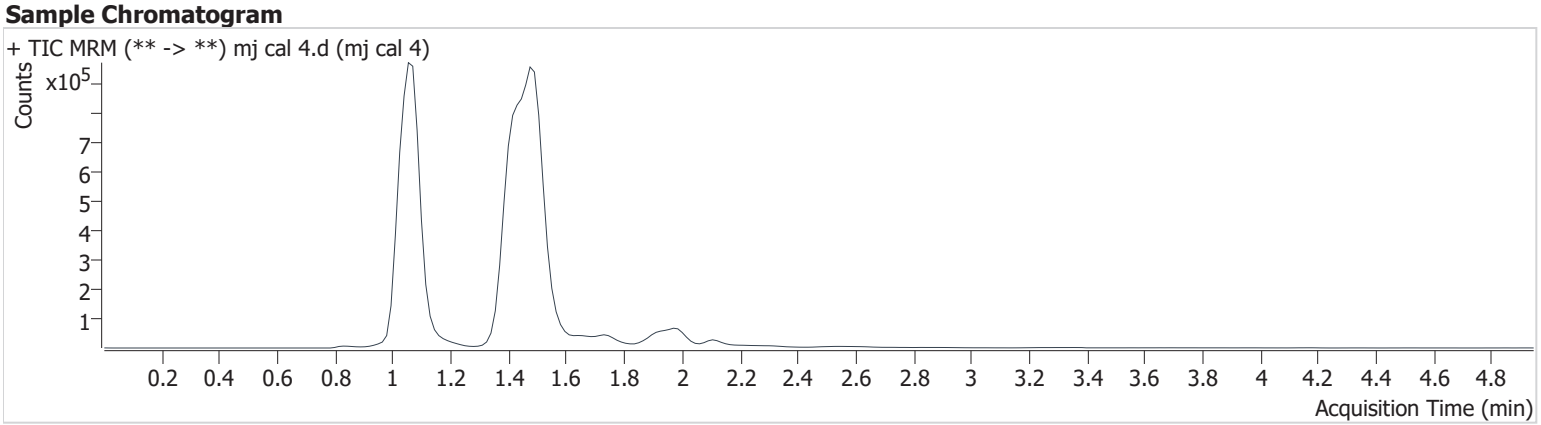
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.070	191542	167.23	36.7	1180.8	811376	19.681 ng/ml
THC-OH	1.074	167104	∞	8.7	∞	1812259	5.924 ng/ml
THC	1.980	10873	185.76	22.7	109.82	333952	4.767 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 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	9/24/2019 12:39:20 PM		
Sample Info.			

Sample Chromatogram



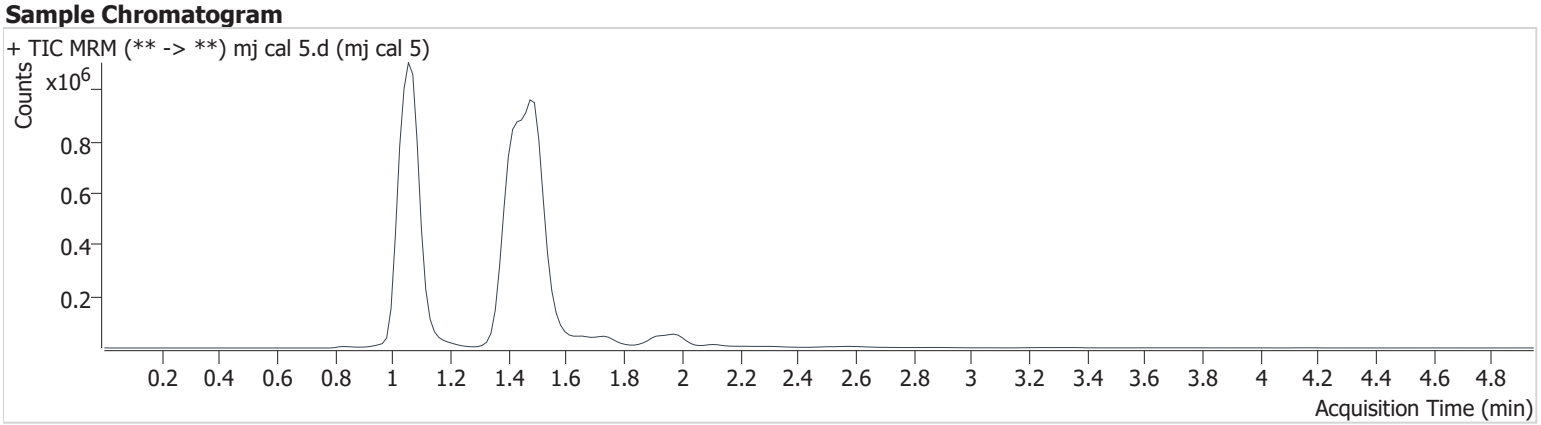
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.070	499596	4403.2	38.6	2449.4	822085	49.245 ng/ml
THC-OH	1.074	358460	∞	9.2	∞	2302444	10.552 ng/ml
THC	1.980	30836	2270	23.8	203.84	440131	9.510 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 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	9/24/2019 12:47:04 PM		
Sample Info.			

Sample Chromatogram



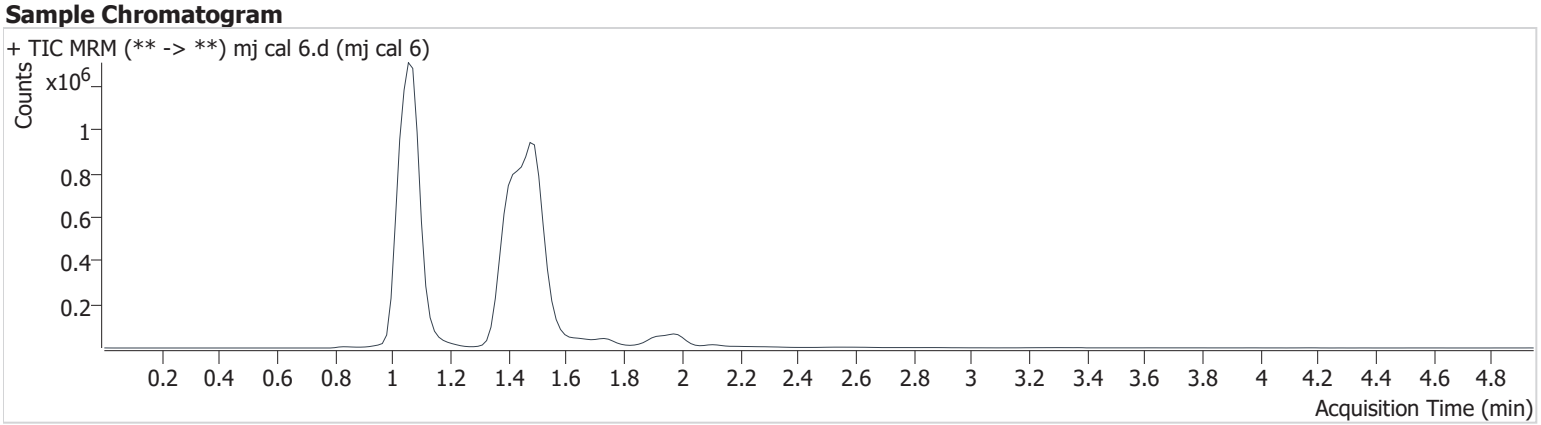
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.070	719665	1502.8	37.9	3813.9	785403	73.792 ng/ml
THC-OH	1.059	670268	∞	10.5	∞	1919175	24.664 ng/ml
THC	1.980	61235	225.03	23.0	149.93	327234	24.315 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 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	9/24/2019 12:54:48 PM		
Sample Info.			

Sample Chromatogram



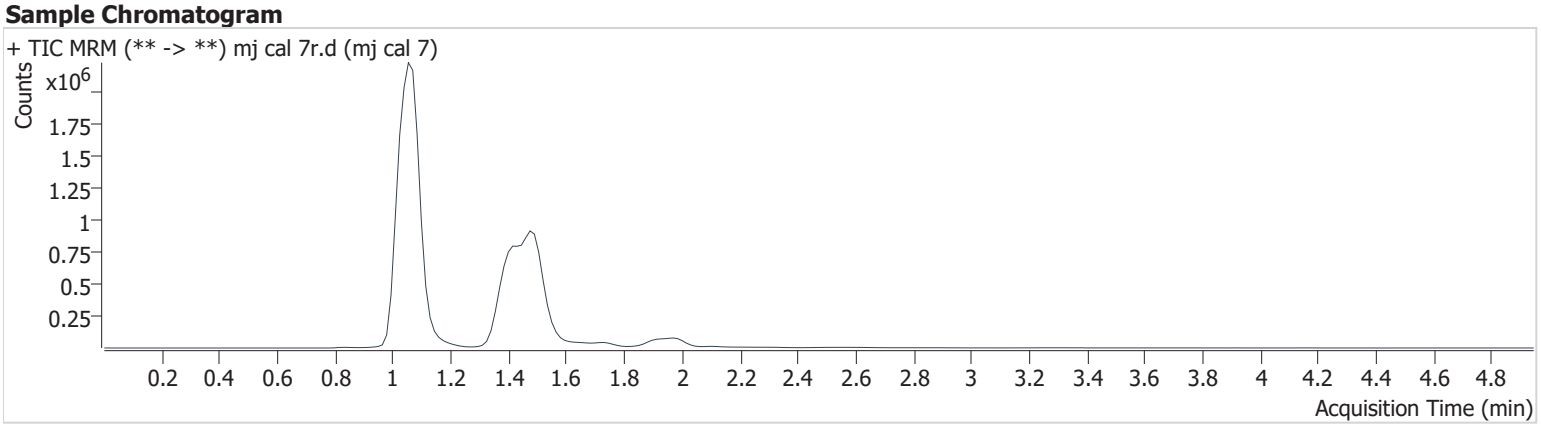
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.070	961053	2108.4	37.8	20069	776800	99.318 ng/ml
THC-OH	1.059	1268150	∞	11.1	∞	1820286	49.994 ng/ml
THC	1.980	130548	3273.5	24.5	∞	333093	50.215 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am28-27 092419\QuantResults\cann quant.batch.bin
Calibration Last Update 9/24/2019 4:23:00 PM

Instrument	69679	**Data File**	mj cal 7r.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	9/24/2019 1:25:45 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.070	2258067	3.0112E+06	38.6	1.2642 E+06	713860	252.526 ng/ml
THC-OH	1.059	2355329	∞	11.5	∞	1711568	99.530 ng/ml
THC	1.980	253102	∞	25.4	851.32	319408	100.863 ng/ml