

REVIEWED

By Anne Nord at 11:02 am, Dec 24, 2020

12/24/2020

BWylee

Worklist: 4694

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2020-2395	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-2410	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-2460	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-2478	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-2539	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

BWylee

Extraction Date: 12/22/20

Analyst: Britany Wylie

Plate lot#: 200723

Plate Expiration: 1-23-2021

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE LCMS Methanol

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 20G20792 **Urine Blank:** _____

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 250ul 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** 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 ^{BW}THC and OH-THC 3ng/mL (quantitative blood), Carboxy-THC: ^{5 BW}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. *THC sample response > 1ng/mL (quantitative blood) BW*
- 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: No urine samples in this batch

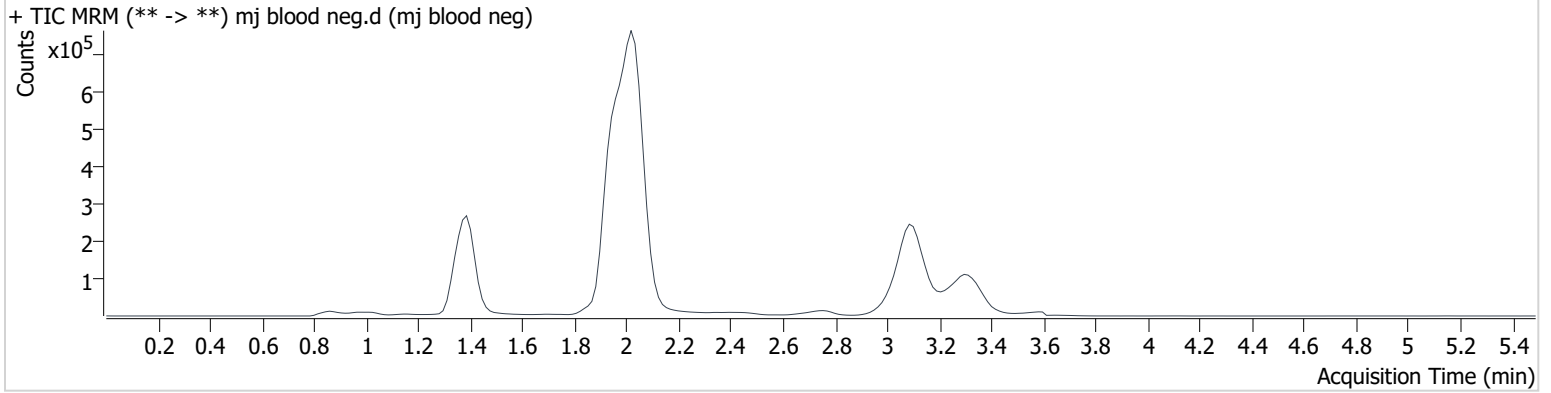
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

Instrument	69679	Data File	mj blood neg.d
Type	Sample	Sample	mj blood neg
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	12/22/2020 12:25:06 PM		
Sample Info.			

Sample Chromatogram



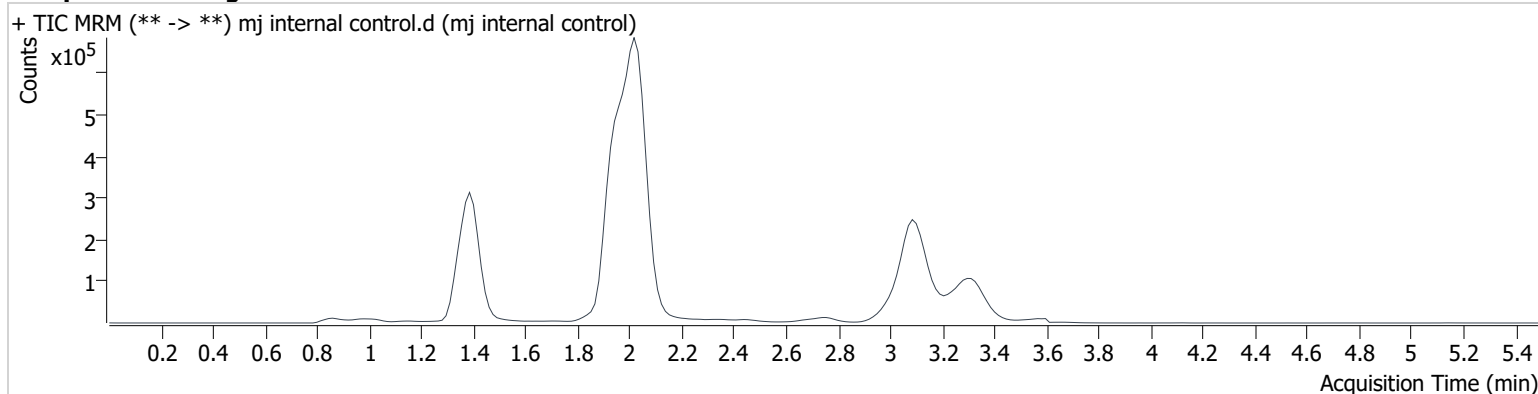
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

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	12/22/2020 12:18:22 PM		
Sample Info.			

Sample Chromatogram



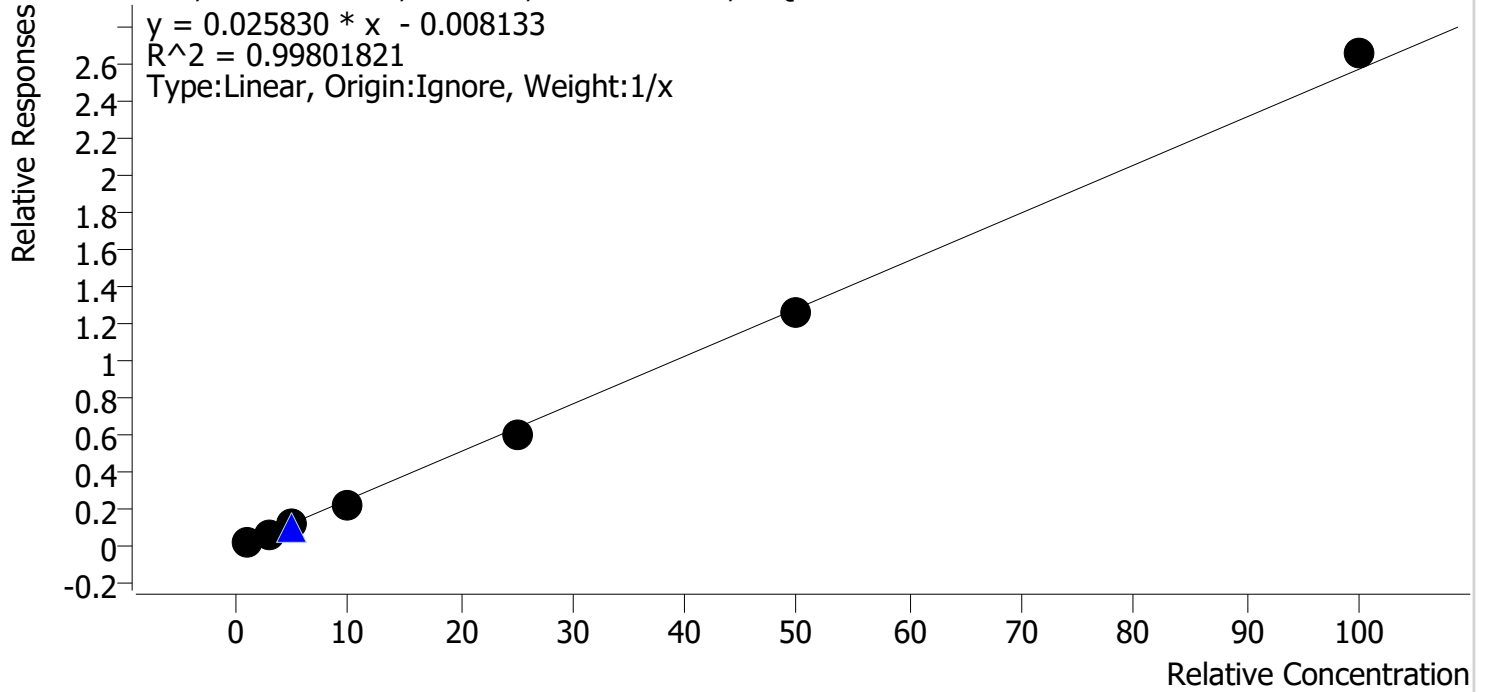
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	148743	∞	10.5	∞	893352	4.914 ng/ml
THC-COOH	1.416	84165	3246.6	34.6	63.9	454907	14.509 ng/ml
THC	3.137	58725	∞	26.2	∞	575392	4.266 ng/ml

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2020 Data\lam 27-28 122220\QuantResults\cannq.batch.bin
Last Cal. Update 12/22/2020 2:07 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** **THC-d3**

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



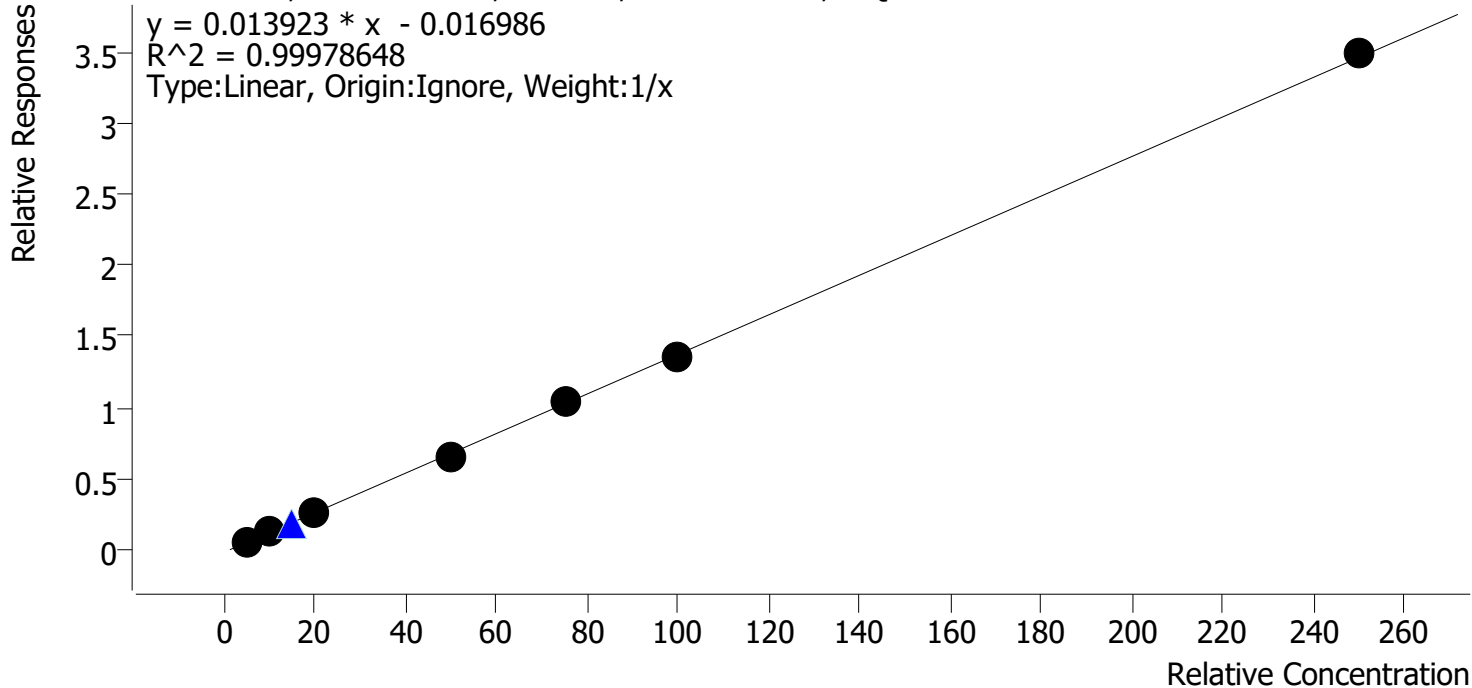
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	119.3
mj cal 2	2	✓	3.0	2.9	97.5
mj cal 3	3	✓	5.0	4.8	96.8
mj cal 4	4	✓	10.0	9.0	90.3
mj cal 5	5	✓	25.0	23.7	94.8
mj cal 6	6	✓	50.0	49.1	98.1
mj cal 7	7	✓	100.0	103.2	103.2

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Last Cal. Update 12/22/2020 2:07 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



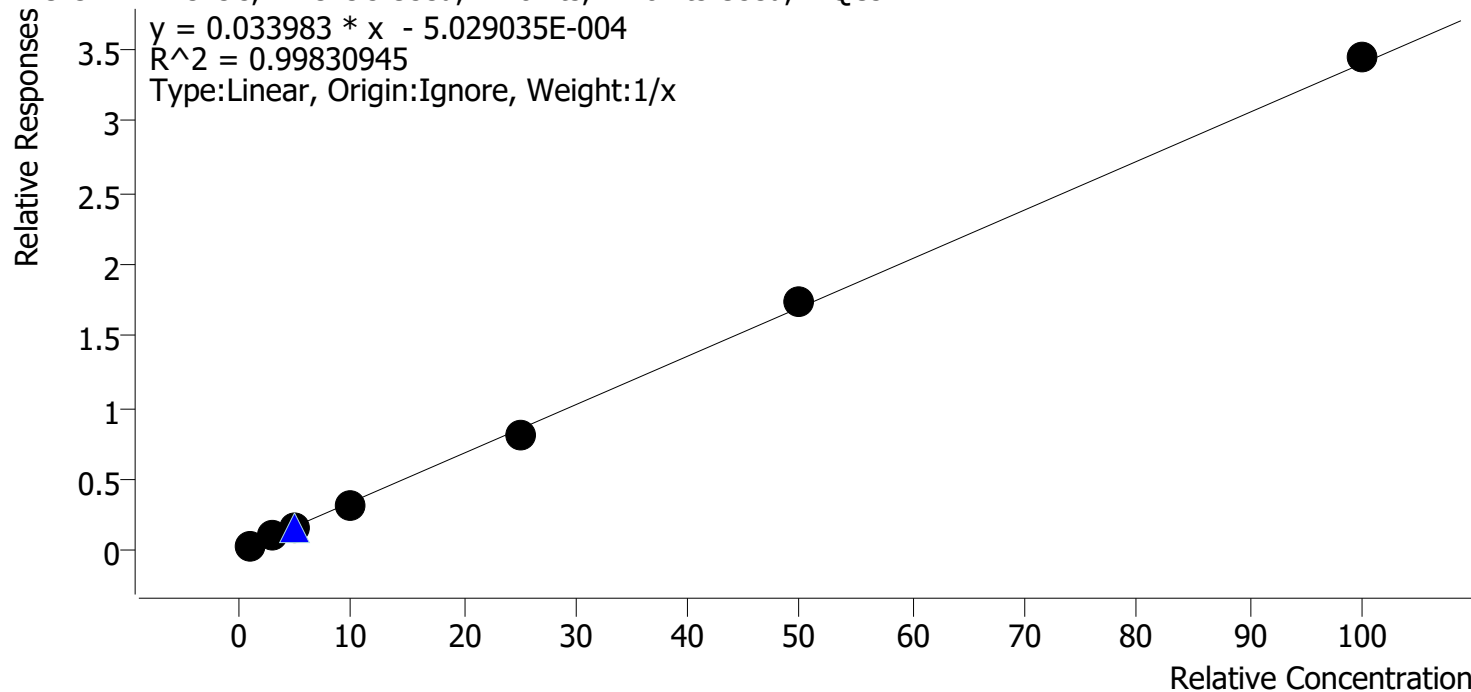
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.3	105.7
mj cal 2	2	✓	10.0	9.7	96.9
mj cal 3	3	✓	20.0	19.9	99.4
mj cal 4	4	✓	50.0	48.9	97.7
mj cal 5	5	✓	75.0	75.5	100.6
mj cal 6	6	✓	100.0	98.9	98.9
mj cal 7	7	✓	250.0	251.9	100.8

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Last Cal. Update 12/22/2020 2:07 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 cal 1	1	✓	1.0	1.1	106.3
mj cal 2	2	✓	3.0	3.4	114.0
mj cal 3	3	✓	5.0	4.5	90.7
mj cal 4	4	✓	10.0	9.1	91.1
mj cal 5	5	✓	25.0	23.7	95.0
mj cal 6	6	✓	50.0	50.9	101.8
mj cal 7	7	✓	100.0	101.2	101.2

AM #27 Cannabinoids

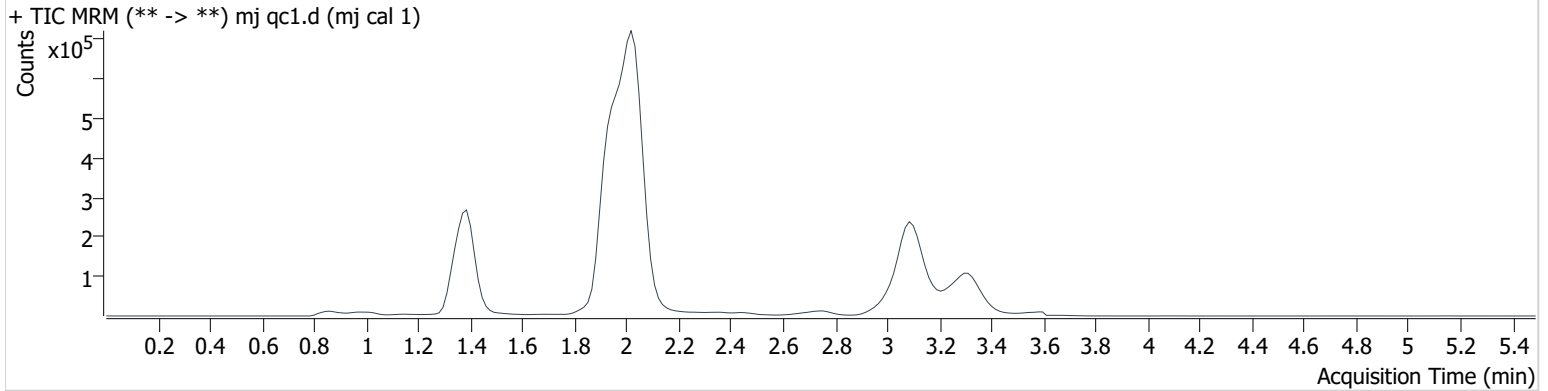
BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

Instrument	69679	Data File	mj qc1.d
Type	Cal	Sample	mj cal 1
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	12/22/2020 11:31:14 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	33162	∞	9.4	∞	931358	1.063 ng/ml Low
THC-COOH	1.401	25388	46.6	37.6	∞	448520	5.286 ng/ml
THC	3.137	13534	∞	29.2	∞	596875	1.193 ng/ml

AM #27 Cannabinoids

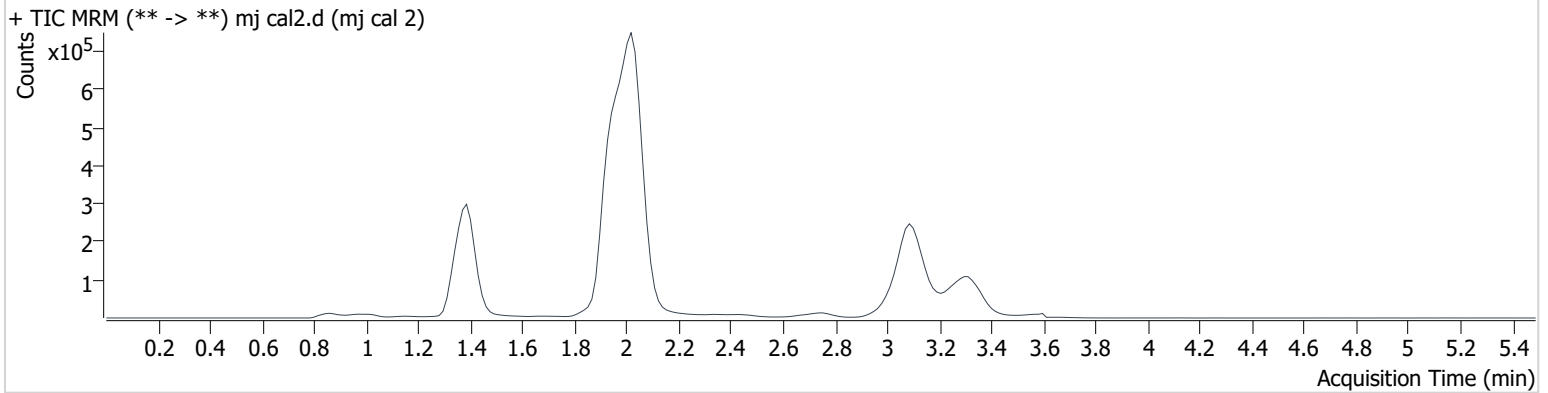
BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal 2
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	12/22/2020 11:37:59 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	108311	∞	9.2	∞	936170	3.419 ng/ml
THC-COOH	1.416	53329	1451.9	34.2	73.3	452404	9.687 ng/ml
THC	3.122	41302	∞	26.8	∞	612858	2.924 ng/ml

AM #27 Cannabinoids

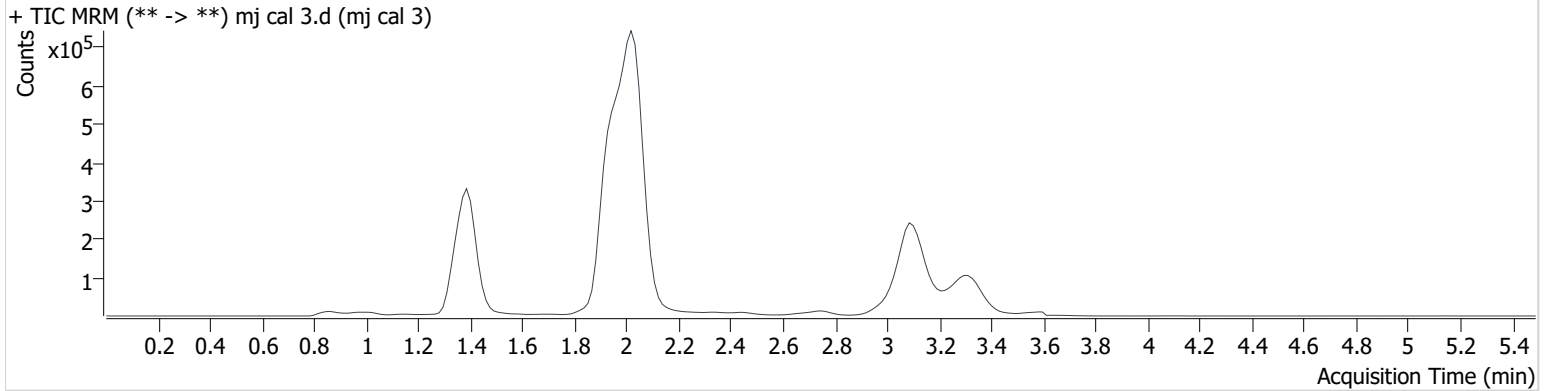
BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-C1	Comment	
Injection Volume	10		
Acq. Date-Time	12/22/2020 11:44:43 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	142851	∞	12.2	∞	930309	4.533 ng/ml
THC-COOH	1.416	116384	833.7	34.9	74.9	447863	19.885 ng/ml
THC	3.122	68260	∞	25.4	∞	584276	4.838 ng/ml

AM #27 Cannabinoids

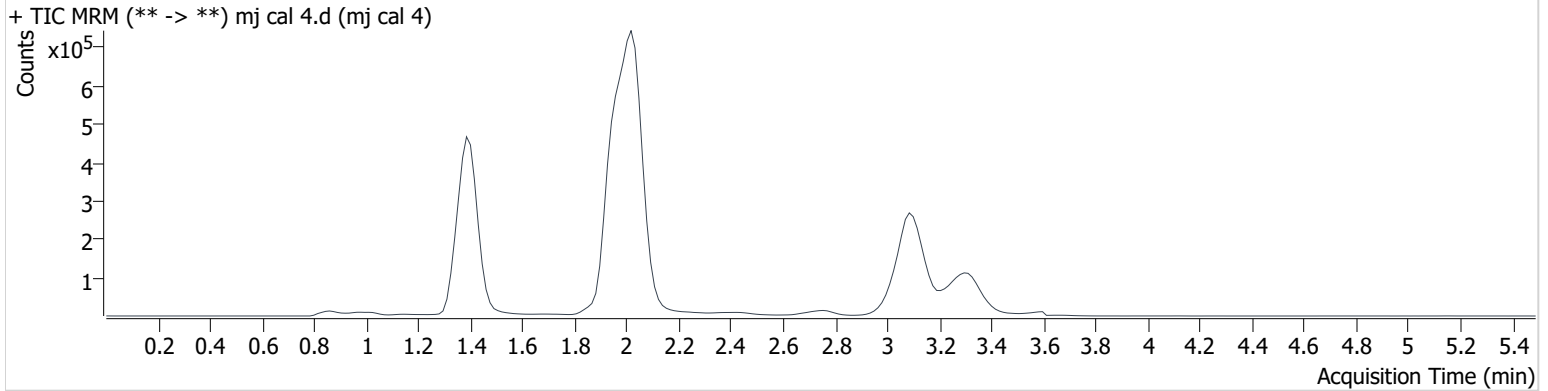
BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

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	12/22/2020 11:51:27 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	285508	∞	11.4	∞	924099	9.106 ng/ml
THC-COOH	1.416	292757	2245.6	36.1	101873.3	441341	48.864 ng/ml
THC	3.122	140932	∞	25.2	∞	626019	9.031 ng/ml

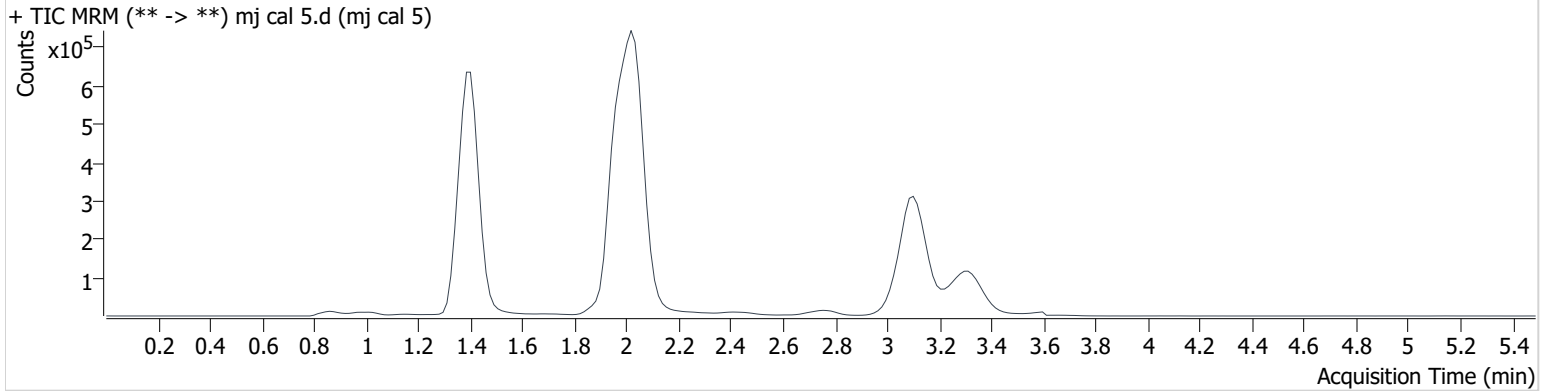
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	12/22/2020 11:58:11 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	737487	∞	11.7	∞	914346	23.750 ng/ml
THC-COOH	1.416	442027	788.7	37.0	1229.6	427696	75.452 ng/ml
THC	3.122	381039	∞	25.0	∞	630619	23.708 ng/ml

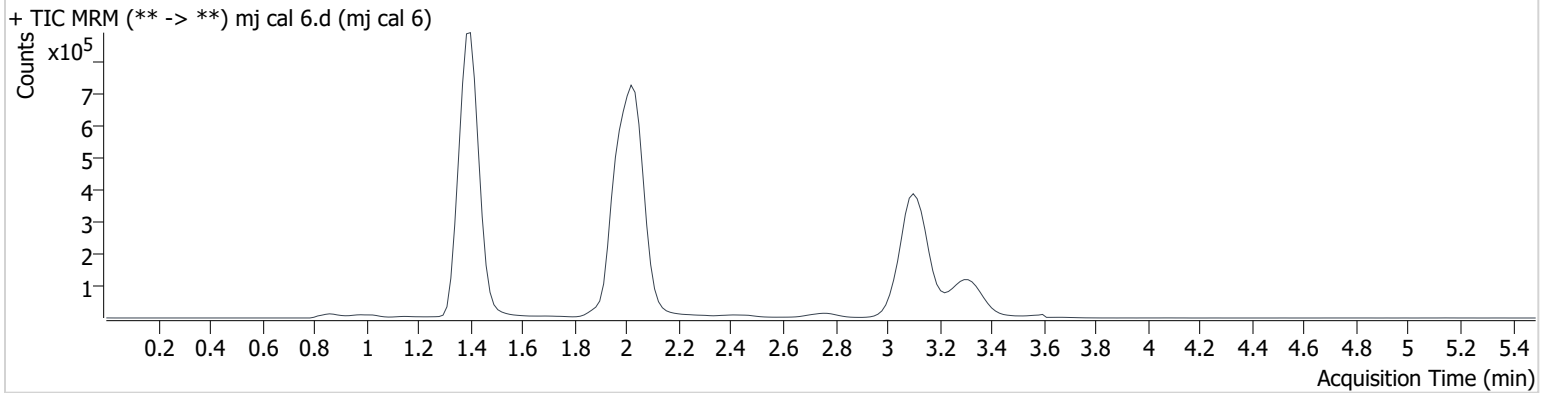
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

Instrument	69679	Data File	mj cal 6.d
Type	Cal	Sample	mj cal 6
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-F1	Comment	
Injection Volume	10		
Acq. Date-Time	12/22/2020 12:04:54 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	1563605	∞	11.6	∞	904130	50.906 ng/ml
THC-COOH	1.416	584208	1563.7	37.0	906.8	429613	98.891 ng/ml
THC	3.137	823795	∞	23.7	∞	654266	49.061 ng/ml

AM #27 Cannabinoids

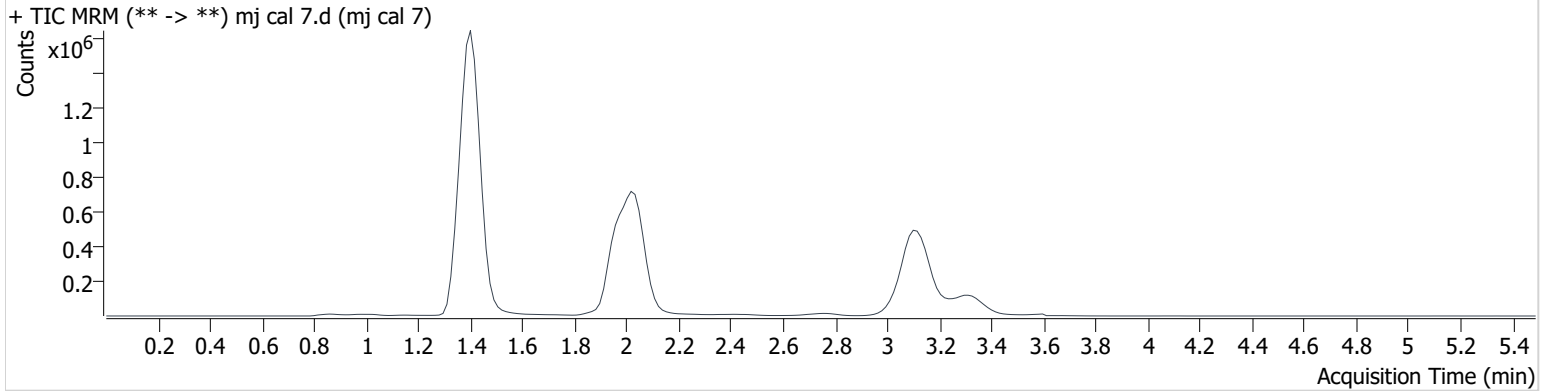
BWylie

Batch results D:\MassHunter\Data\2020 Data\am 27-28 122220\QuantResults\cannq.batch.bin
Calibration Last Update 12/22/2020 2:07:32 PM

Instrument	69679	Data File	mj cal 7.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	12/22/2020 12:11:38 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	3142145	∞	12.0	∞	913599	101.223 ng/ml
THC-COOH	1.416	1478440	4649.3	36.7	2535.9	423546	251.935 ng/ml
THC	3.137	1642355	∞	24.2	∞	617729	103.246 ng/ml

	1	2	3	4	5	6
A	IS + Cal. 1	neg blood				IS + QC_1
B	IS + Cal. 2	c2395-1				IS + Cal. 7
C	IS + Cal. 3	c2410-1				IS + Cal. 6
D	IS + Cal. 4	c2460-1				IS + Cal. 5
E	IS + Cal. 5	c2478-1				IS + Cal. 4
F	IS + Cal. 6	c2539-1				IS + Cal. 3
G	IS + Cal. 7					IS + Cal. 2
H	IS + QC_1					IS + Cal. 1

All wells to contain 100 µl of residual DMSO

Case #: 2020- -