







REVIEWED

By Anne Nord at 12:14 pm, Sep 21, 2021

9/16/2021

Byylee

Worklist: 5240

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2021-1923	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1981	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2003	1	UCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2004	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2014	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-2071	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

AM# 27: Quantitation of THC and Metabolites in Blood and Urine *by Wyllie*

LC-MS/MS

Extraction Date: 9/16/21

Analyst: Britany Wyllie

Plate lot#: 210412

Plate Expiration: 10-12-2021

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 21D52496

Neg Urine Lot: 83121

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 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:

AM 27 Extraction 9-16-21

BWylee

	1	2	3	4	5	6
a	cal 1 ng	NEG	2014			QC 1
b	cal 3 ng	1923				cal 100 ng
c	cal 5 ng	1981				cal 50 ng
d	cal 10ng	2004				cal 25 ng
e	cal 25 ng	2071				cal 10ng
f	cal 50 ng	neg urine				cal 5 ng
g	cal 100 ng	urine ctrl				cal 3 ng
h	QC 1	2003			NEG	cal 1ng

Lab case C2021-____-1

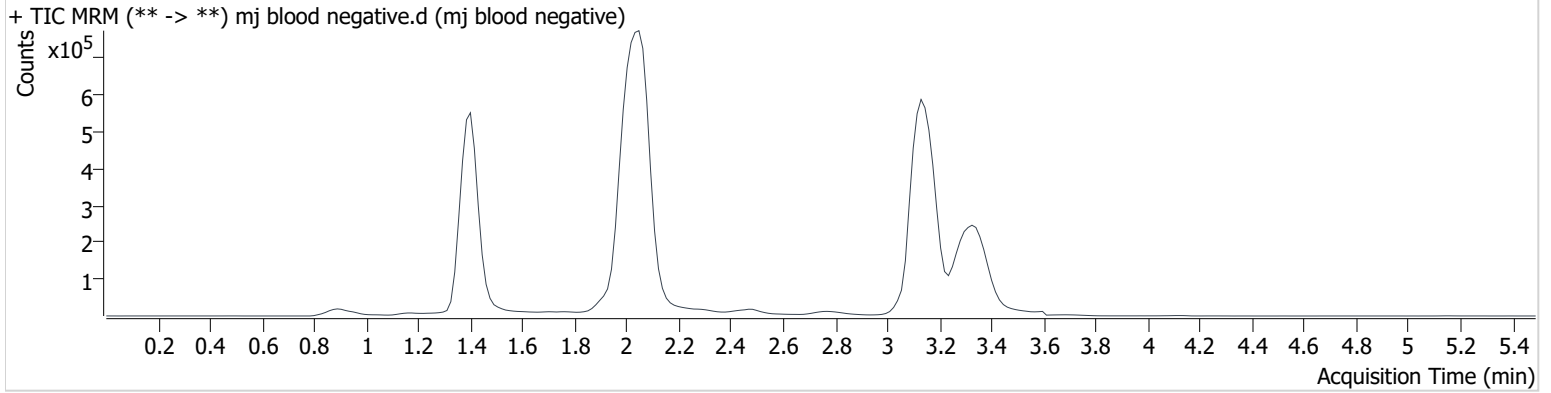
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Calibration Last Update 9/21/2021 7:03:57 AM

Instrument	69679	Data File	mj blood negative.d
Type	Sample	Sample	mj blood negative
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	9/16/2021 10:52:59 AM		
Sample Info.			

Sample Chromatogram



AM #27 Cannabinoids

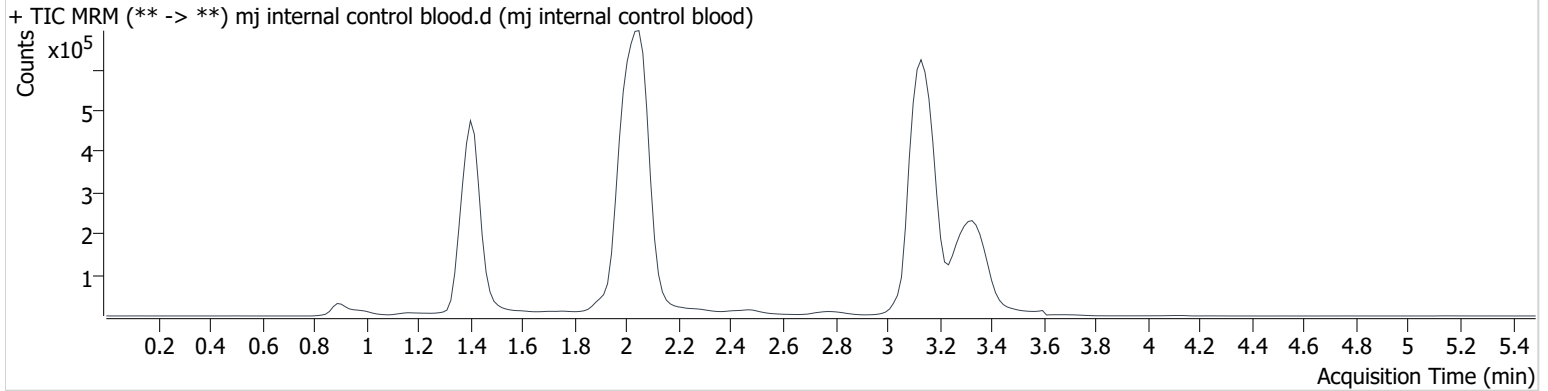
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Calibration Last Update 9/21/2021 7:03:57 AM

Instrument	69679	Data File	mj internal control blood.d
Type	QC	Sample	mj internal control blood
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	9/16/2021 10:46:17 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	27154	∞	741.5	∞	1508178	4.802 ng/ml
THC-COOH	1.431	81933	293.5	38.9	177.1	514326	17.438 ng/ml
THC	3.152	345848	∞	27.4	1660.1	3221603	4.416 ng/ml

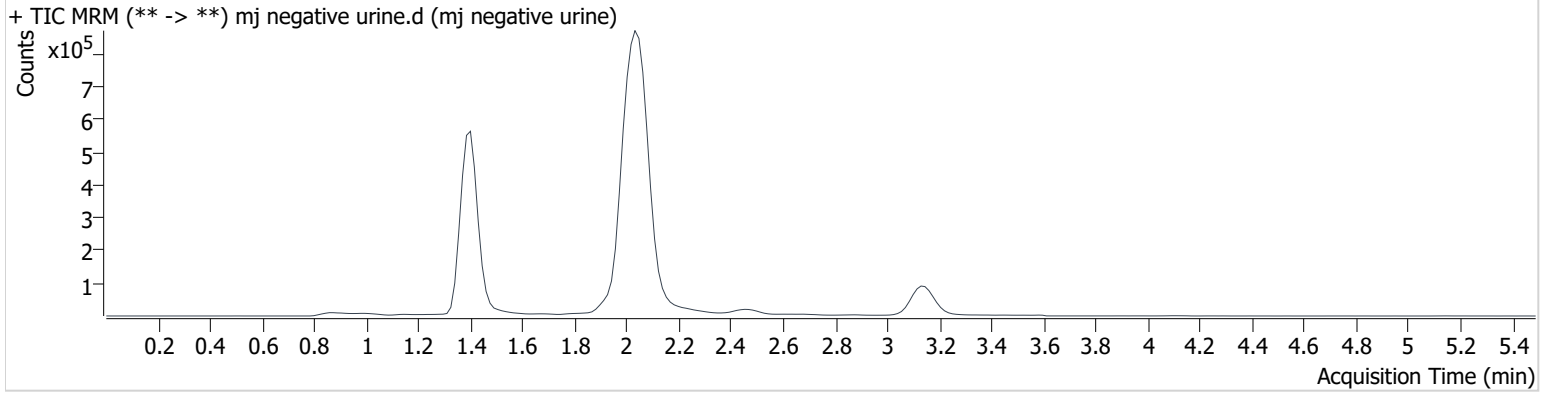
AM #27 Cannabinoids

B. Wylie

Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Calibration Last Update 9/21/2021 7:03:57 AM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-F2	Comment	
Injection Volume	10		
Acq. Date-Time	9/16/2021 11:59:37 AM		
Sample Info.			

Sample Chromatogram



AM #27 Cannabinoids

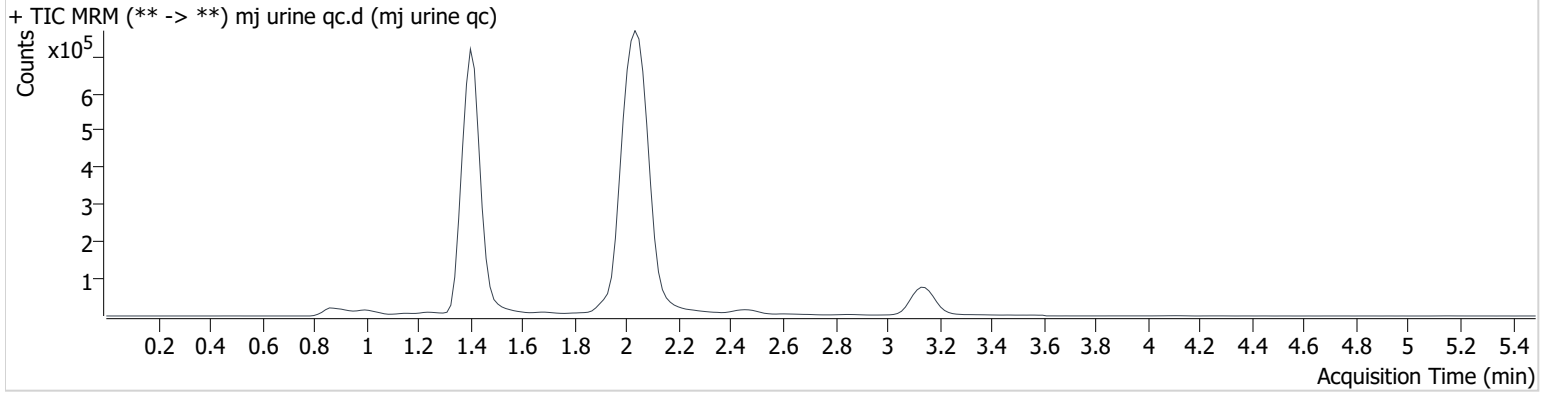
BWylie

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Calibration Last Update 9/21/2021 7:03:57 AM

Instrument	69679	Data File	mj urine qc.d
Type	Sample	Sample	mj urine qc
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-G2	Comment	
Injection Volume	10		
Acq. Date-Time	9/16/2021 12:12:57 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	181884	∞	676.1	60155.4	1403497	33.748 ng/ml
THC-COOH	1.431	136128	421.5	43.6	84770.9	339205	42.953 ng/ml
THC	3.152	29584	∞	28.0	53.1	463159	2.753 ng/ml

Toxicology AM method 27/26 external prep information

BWylee

working solution 15 ug/ml in meoh C-THC, THC-OH, 7.5 ug/ml 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 8/26/21 Exp: 8/26/22 lot 82621 by AMN

Drug	lot	expiration
C-THC	FE04151901	6/1/2024
THC-OH	FE06152002	6/1/2025
THC	FE04222001	5/1/2025

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

out of use

ppd 8/26/21 Exp 8/26/22 neg urine lot 5621	lot u82621	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

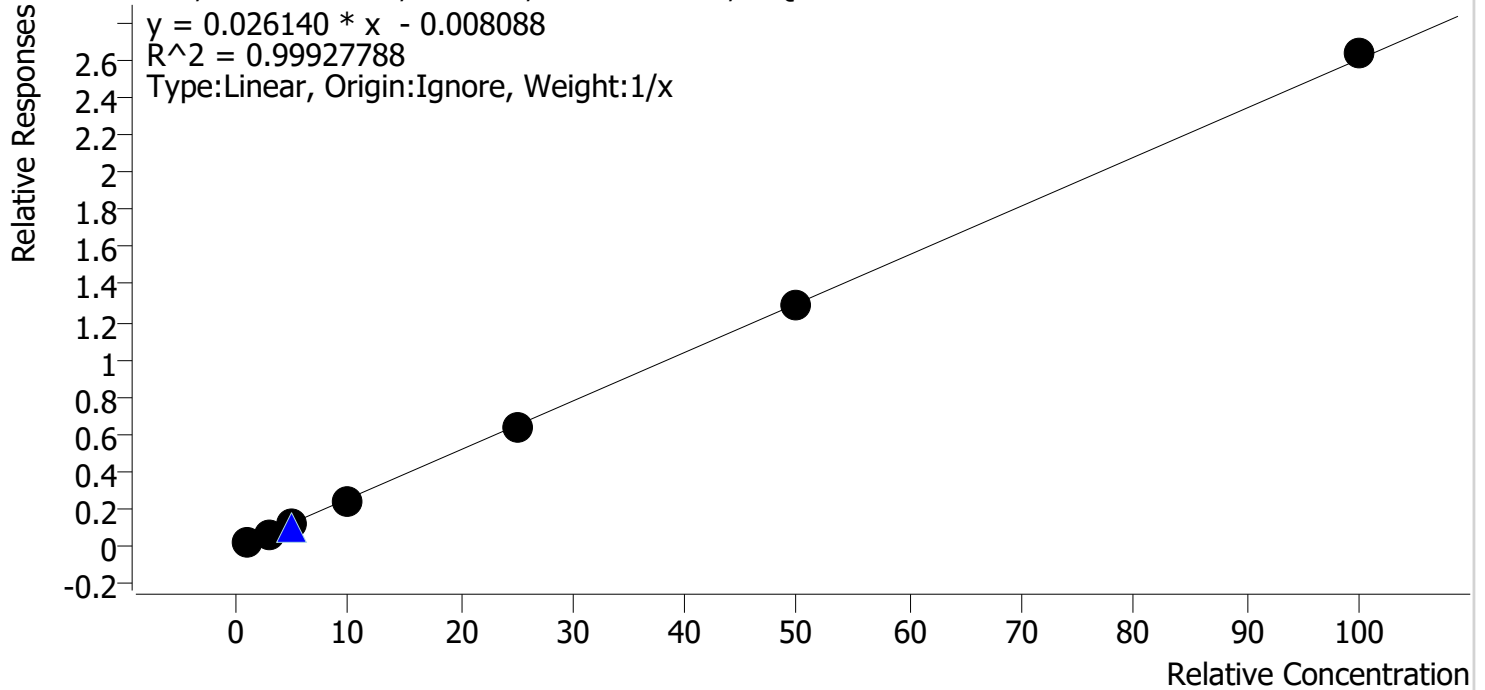
Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Last Cal. Update 9/21/2021 7:03 AM
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 cal1	1	✓	1.0	1.2	119.3
mj cal2	2	✓	3.0	2.8	92.2
mj cal 3	3	✓	5.0	4.7	93.5
mj cal 4	4	✓	10.0	9.5	94.8
mj cal 5	5	✓	25.0	24.8	99.2
mj cal 6	6	✓	50.0	49.9	99.9
mj cal 7	7	✓	100.0	101.2	101.2

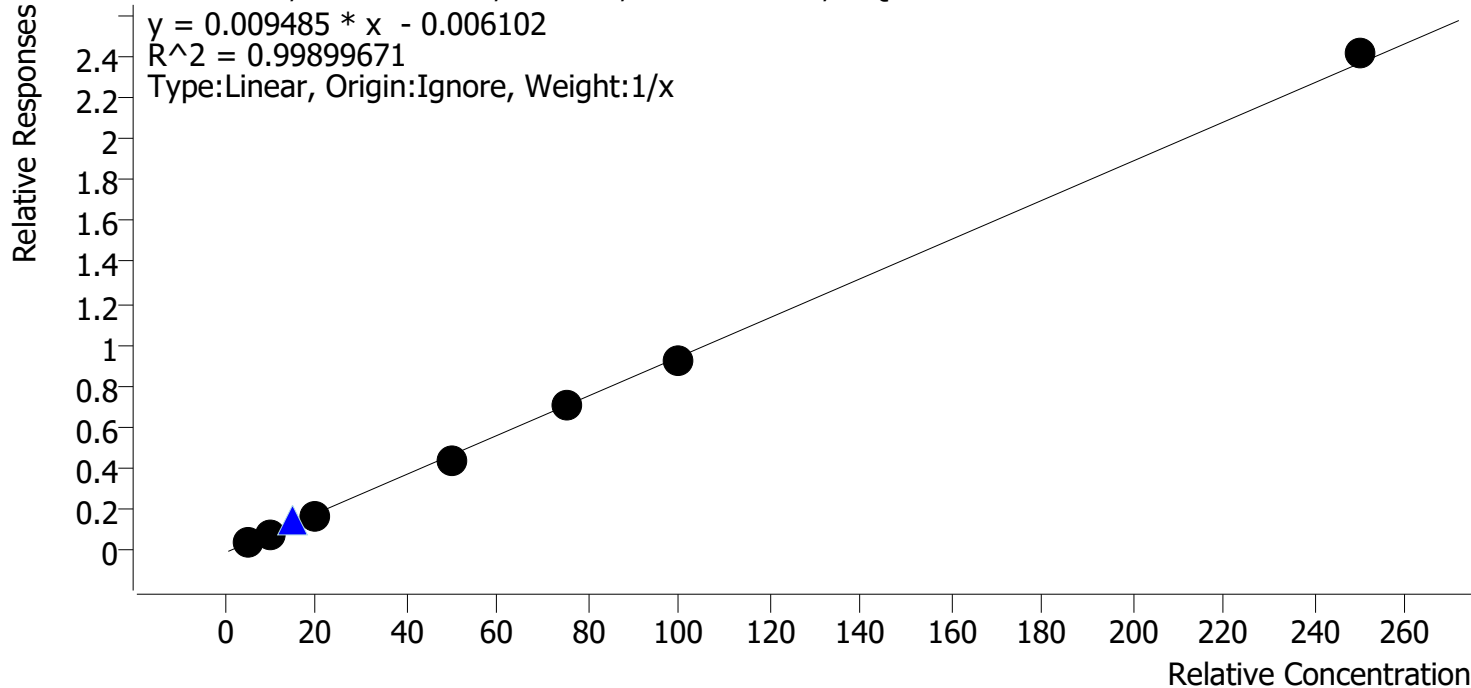
Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Last Cal. Update 9/21/2021 7:03 AM
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 cal1	1	✓	5.0	5.6	111.6
mj cal2	2	✓	10.0	9.7	96.9
mj cal 3	3	✓	20.0	19.4	96.9
mj cal 4	4	✓	50.0	47.1	94.3
mj cal 5	5	✓	75.0	75.2	100.3
mj cal 6	6	✓	100.0	98.2	98.2
mj cal 7	7	✓	250.0	254.8	101.9

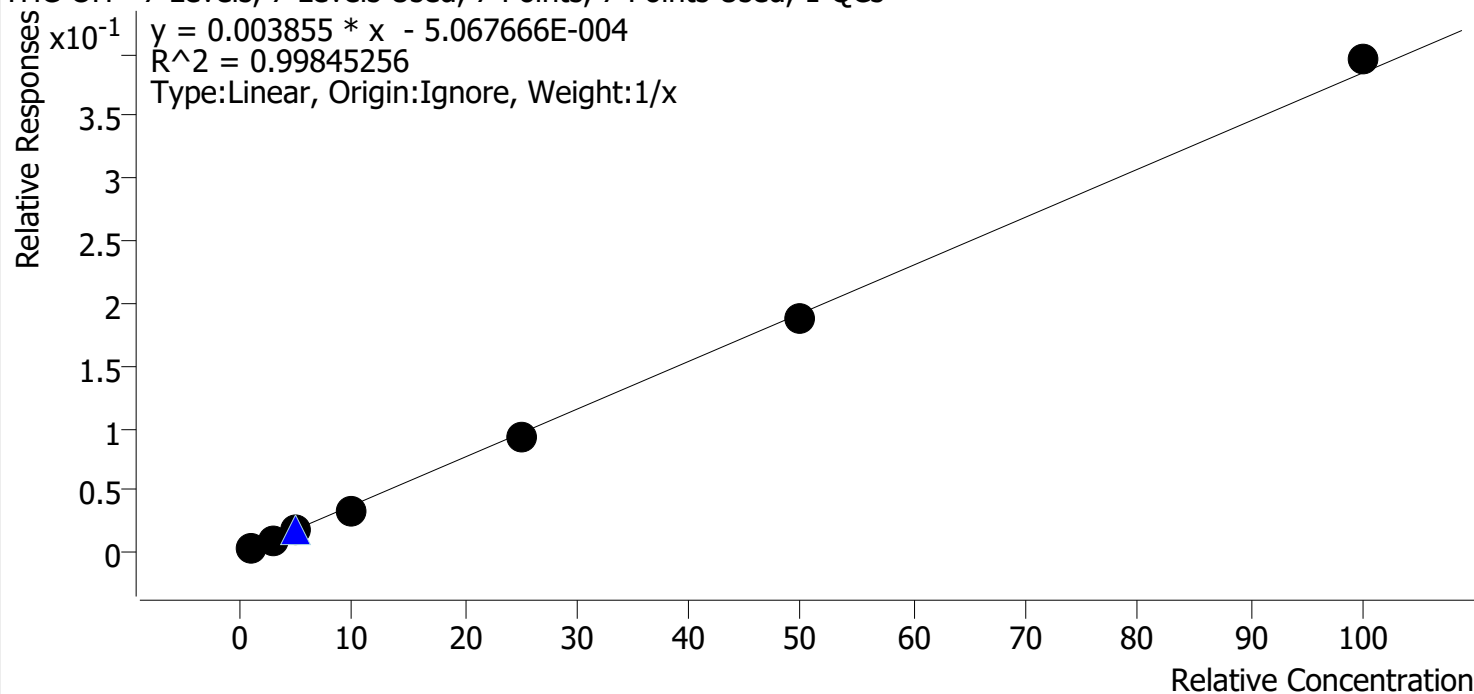
Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Last Cal. Update 9/21/2021 7:03 AM
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 cal1	1	✓	1.0	1.2	120.2
mj cal2	2	✓	3.0	2.9	98.3
mj cal 3	3	✓	5.0	4.6	91.6
mj cal 4	4	✓	10.0	9.2	91.6
mj cal 5	5	✓	25.0	24.4	97.7
mj cal 6	6	✓	50.0	48.9	97.9
mj cal 7	7	✓	100.0	102.7	102.7

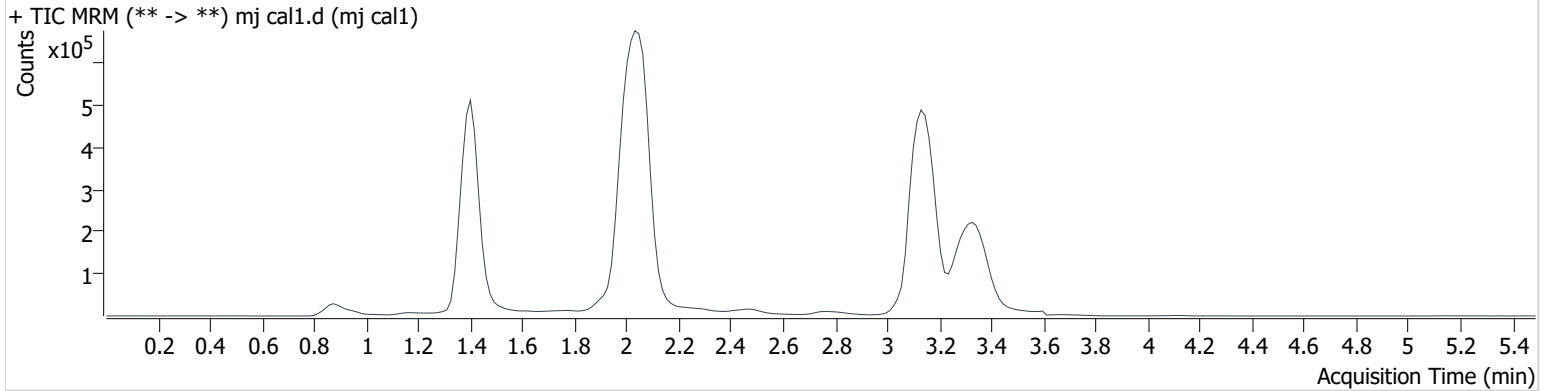
AM #27 Cannabinoids

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Calibration Last Update 9/21/2021 7:03:57 AM

Instrument	69679	Data File	mj cal1.d
Type	Cal	Sample	mj cal1
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	9/16/2021 9:59:21 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	7493	∞	875.7	∞	1816440	1.202 ng/ml Low
THC-COOH	1.431	26310	23.2	41.6	40.7	561980	5.579 ng/ml
THC	3.152	62567	∞	28.1	2418.2	2708109	1.193 ng/ml

AM #27 Cannabinoids

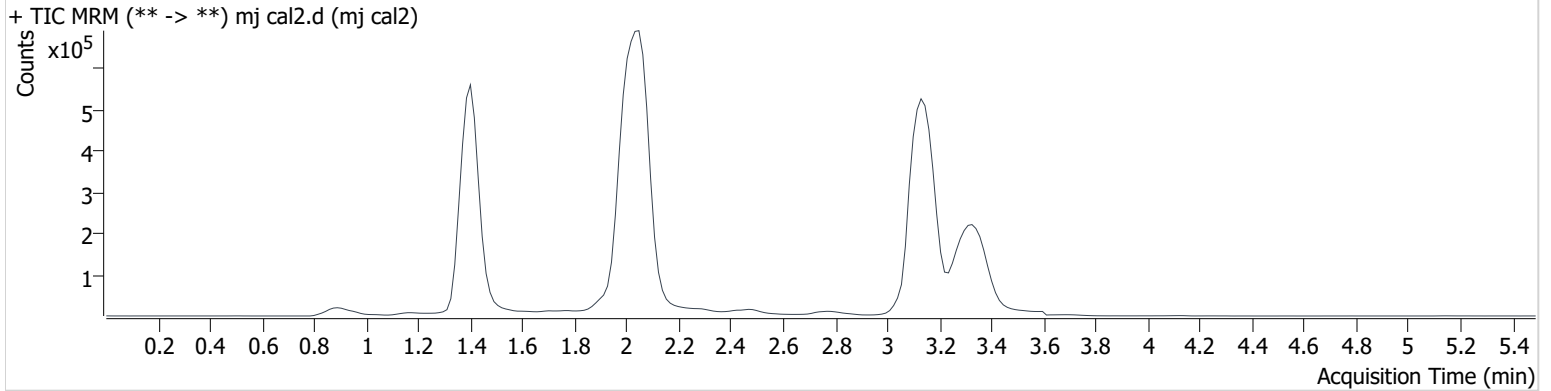
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Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Calibration Last Update 9/21/2021 7:03:57 AM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	9/16/2021 10:06:05 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	20520	∞	854.2	∞	1890104	2.948 ng/ml Low
THC-COOH	1.431	50726	36.5	42.9	21.6	591094	9.691 ng/ml
THC	3.152	179137	∞	27.0	380.7	2788371	2.767 ng/ml

AM #27 Cannabinoids

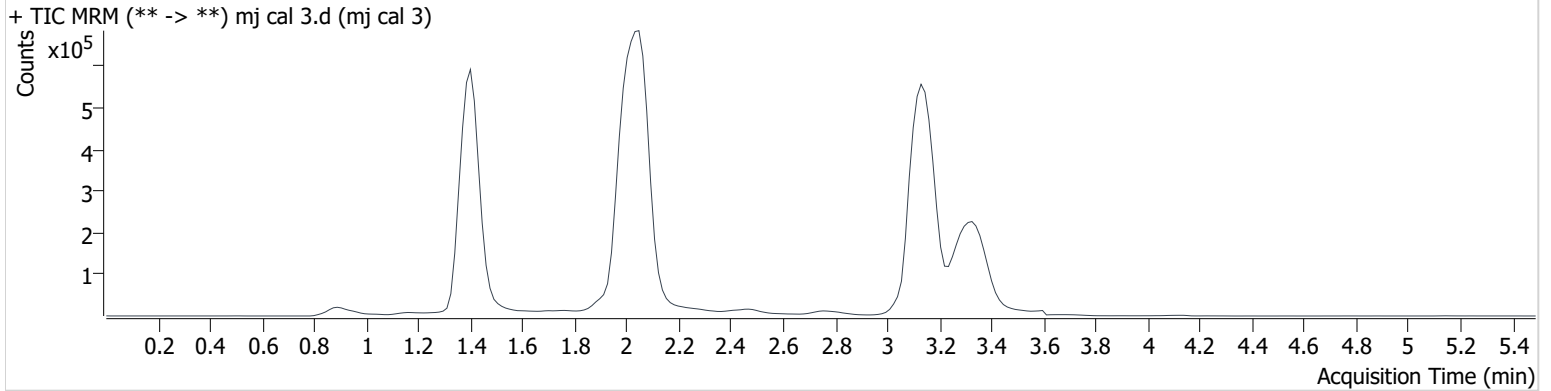
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Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Calibration Last Update 9/21/2021 7:03:57 AM

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	9/16/2021 10:12:47 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	32514	∞	789.9	∞	1896118	4.579 ng/ml
THC-COOH	1.431	106154	177.5	43.2	3221.6	597632	19.370 ng/ml
THC	3.152	319447	4279.0	26.8	671.7	2800075	4.674 ng/ml

AM #27 Cannabinoids

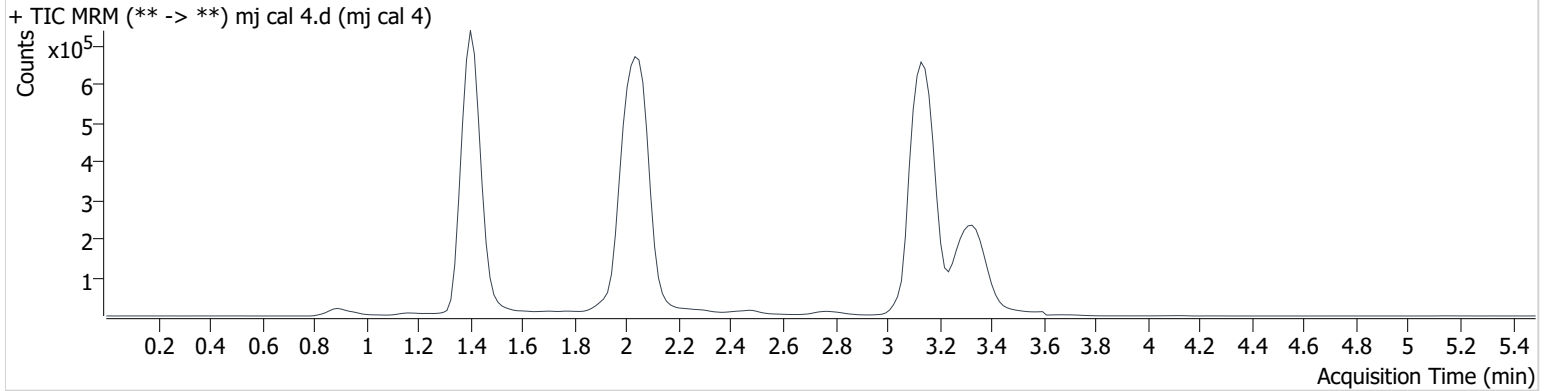
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Calibration Last Update 9/21/2021 7:03:57 AM

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	9/16/2021 10:19:30 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	65354	302.8	839.2	∞	1876716	9.165 ng/ml
THC-COOH	1.431	266491	1428.5	43.7	200.9	604383	47.130 ng/ml
THC	3.152	703625	∞	26.5	∞	2936638	9.475 ng/ml

AM #27 Cannabinoids

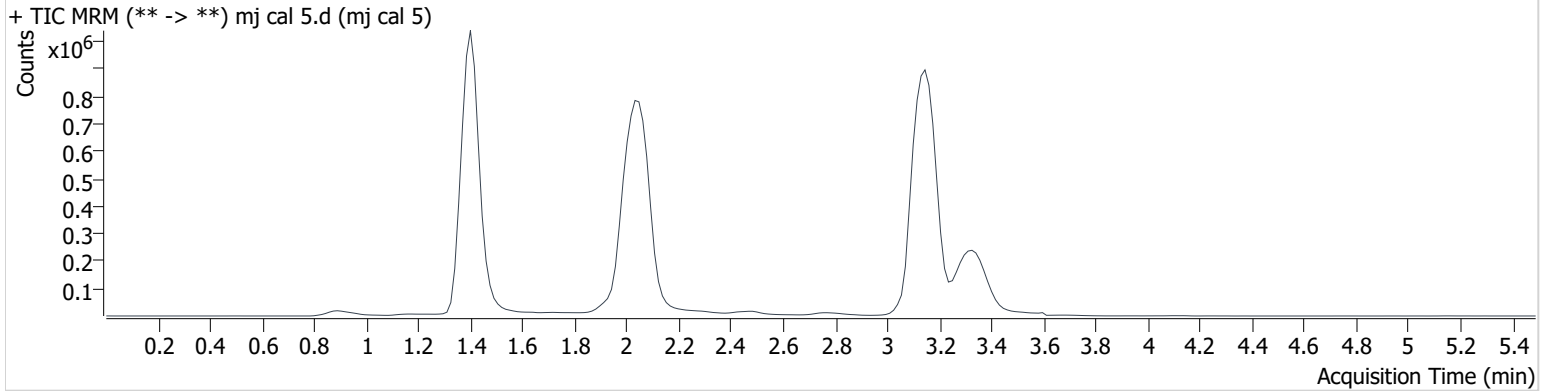
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Calibration Last Update 9/21/2021 7:03:57 AM

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	9/16/2021 10:26:11 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	171593	∞	745.9	2933.9	1831481	24.435 ng/ml
THC-COOH	1.416	395513	270.7	44.5	541.6	559197	75.212 ng/ml
THC	3.152	1837978	∞	26.5	∞	2871659	24.794 ng/ml

AM #27 Cannabinoids

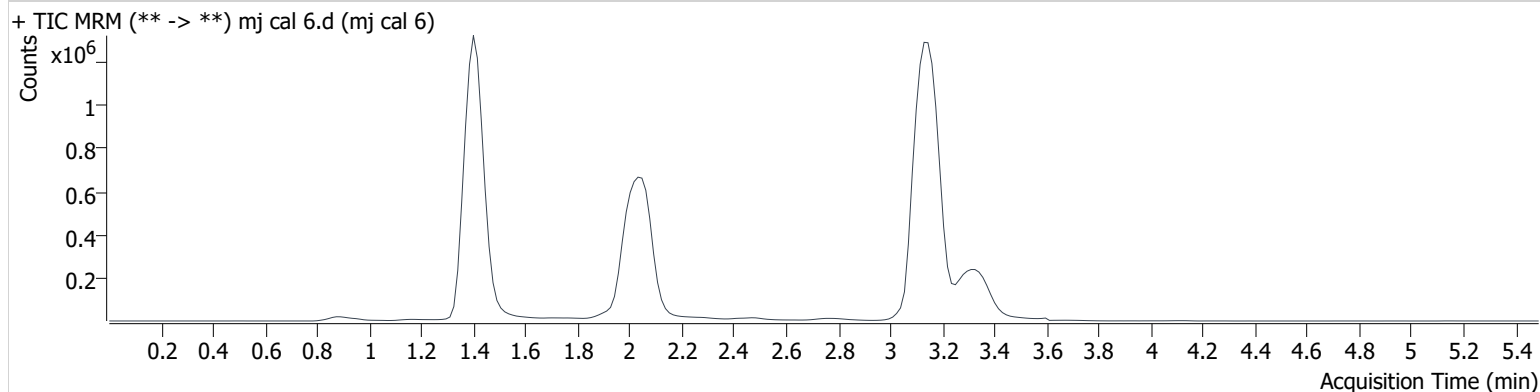
BWylie

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Calibration Last Update 9/21/2021 7:03:57 AM

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	9/16/2021 10:32:53 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	351333	940786666994	727.8	21333.3	1867025	48.945 ng/ml
THC-COOH	1.431	535828	643699.0	44.3	3775.4	579169	98.182 ng/ml
THC	3.152	3883905	∞	27.2	∞	2993723	49.939 ng/ml

AM #27 Cannabinoids

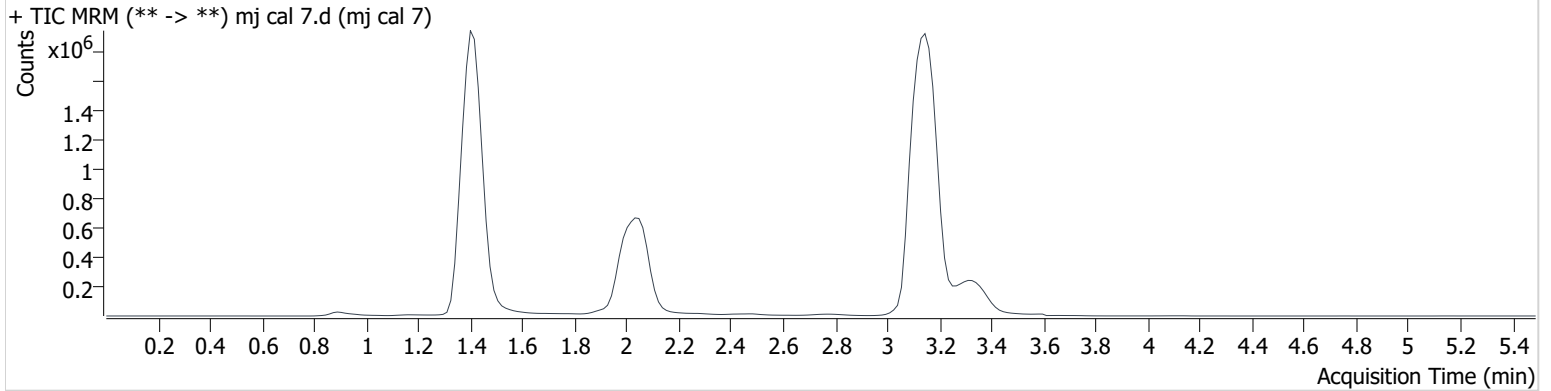
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\091621\QuantResults\cannq.batch.bin
Calibration Last Update 9/21/2021 7:03:57 AM

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	9/16/2021 10:39:35 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	638245	∞	716.5	∞	1613712	102.727 ng/ml
THC-COOH	1.431	1207726	2475.8	44.8	1038.2	500916	254.836 ng/ml
THC	3.152	7394640	∞	27.5	∞	2805030	101.157 ng/ml