






REVIEWED

By Anne Nord at 11:39 am, Jun 16, 2021

6/11/2021

Boylee

Worklist: 5046

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2021-1243	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1291	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1331	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-1334	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1337	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	

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

LC-MS/MS

Extraction Date: 06/09/21

Analyst: Britany Wylie

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: 21D52496-5

Neg Urine Lot: 5621

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: curves limitations: THC-OH 3-100

am 27
6/9/21 WORKLIST 5046

BWylee

	1	2	3	4	5	6
A	IS + Cal. 1	neg blood				IS + QC_1
B	IS + Cal. 2	1243-1				IS + Cal. 7
C	IS + Cal. 3	1291-1				IS + Cal. 6
D	IS + Cal. 4	1334-1				IS + Cal. 5
E	IS + Cal. 5	neg urine				IS + Cal. 4
F	IS + Cal. 6	urine control				IS + Cal. 3
G	IS + Cal. 7	1331-1				IS + Cal. 2
H	IS + QC_1	1337-1				IS + Cal. 1

All wells to contain 100 µl of residual DMSO

Case #: C2021-____-__

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 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

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 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	1/12/2021
ppd 1/13/21 Exp 7/1/21 neg urine lot 10120	lot 11321	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	3/28/2021
ppd 3/29/21 Exp 7/1/21 neg urine lot 2121	lot 32921	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	5/27/2021
ppd 5/28/21 Exp 7/1/21 neg urine lot 5621	lot 52821	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

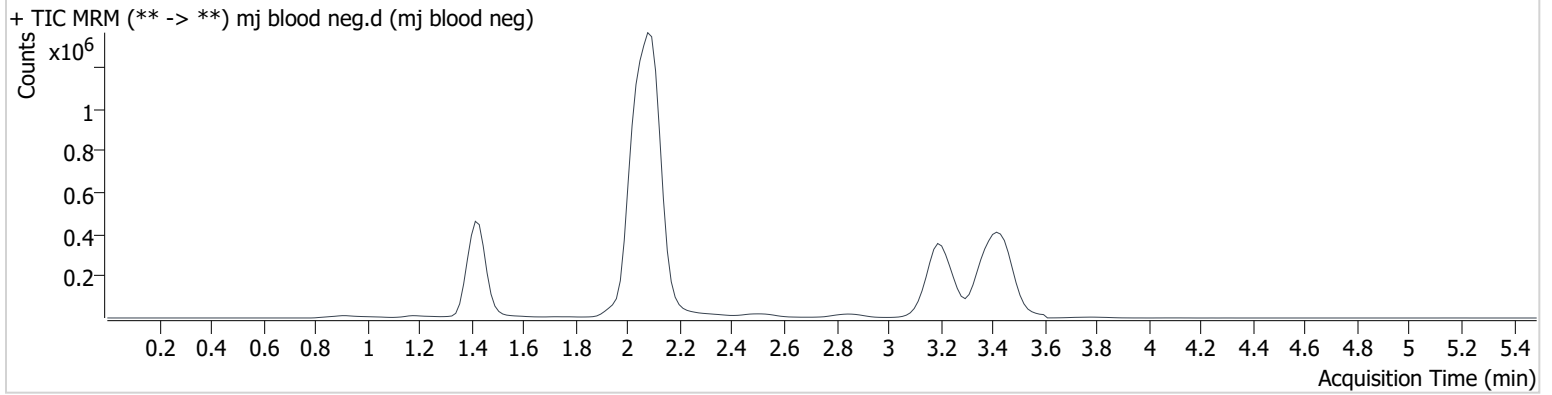
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 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	6/9/2021 10:29:46 AM		
Sample Info.			

Sample Chromatogram



AM #27 Cannabinoids

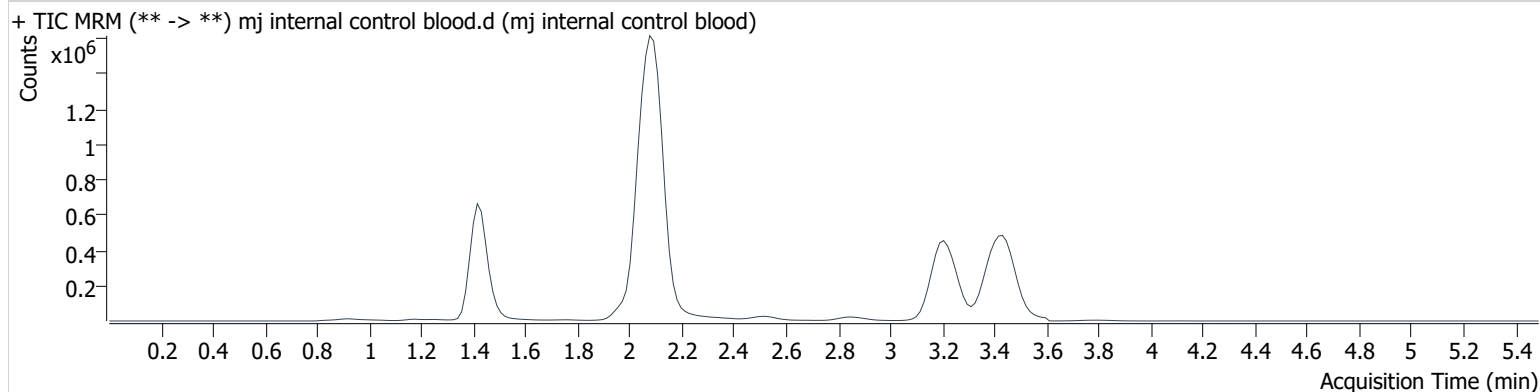
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 PM

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	6/9/2021 10:23:04 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	293743	2953.1	9.5	∞	1745260	4.910 ng/ml
THC-COOH	1.446	128177	221.5	41.0	99245.1	682892	14.659 ng/ml
THC	3.242	154459	∞	25.1	7325539 2457691 .5	1341798	4.387 ng/ml

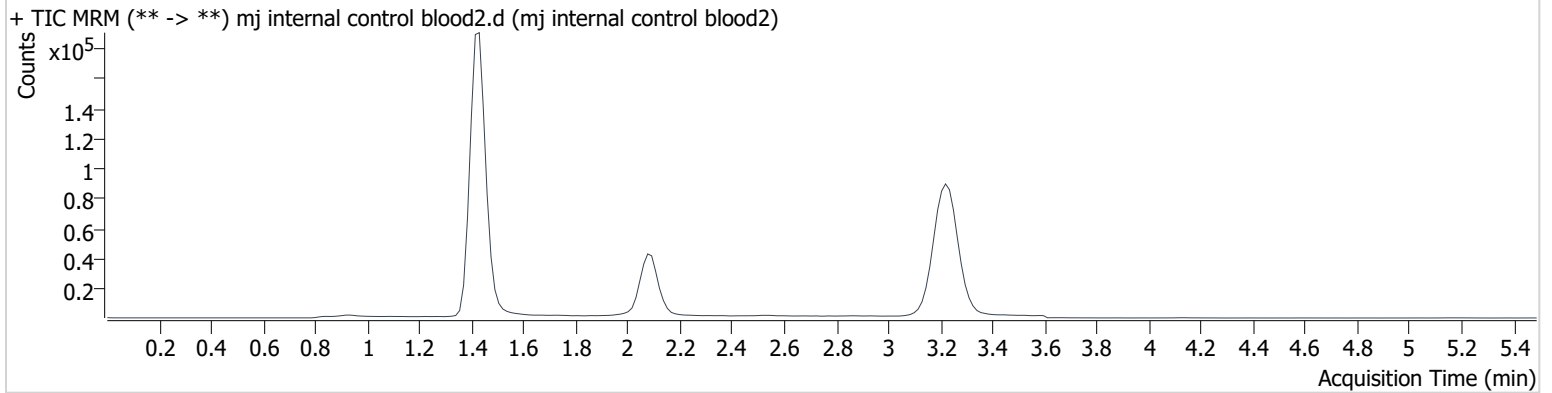
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 PM

Instrument	69679	Data File	mj internal control blood2.d
Type	Sample	Sample	mj internal control blood2
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	Vial 4	Comment	
Injection Volume	10		
Acq. Date-Time	6/9/2021 11:23:04 AM		
Sample Info.			

Sample Chromatogram



wrong plate position injected- 2nd control not evaluated
BW

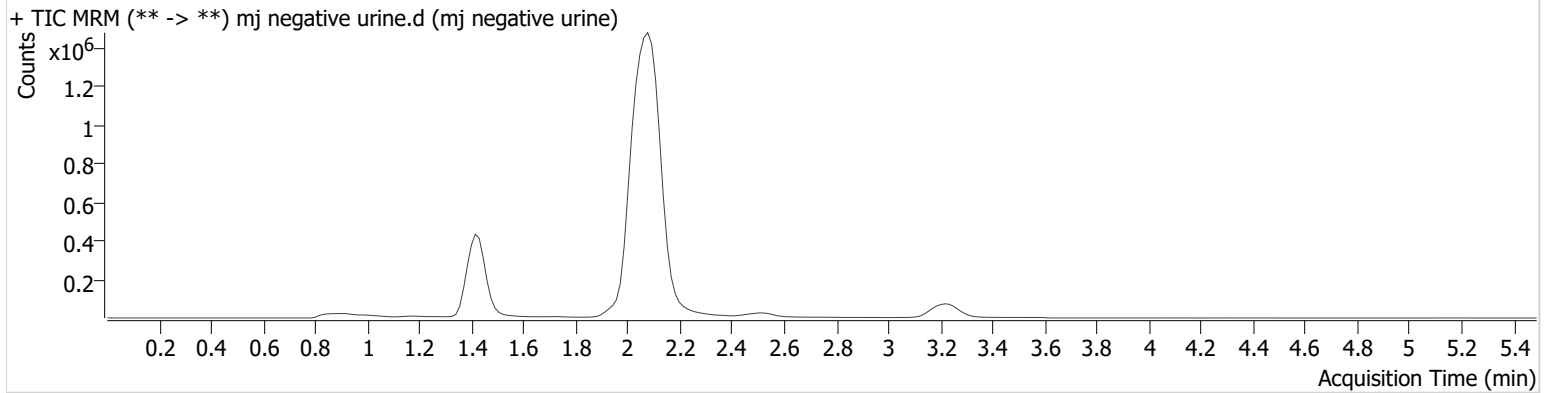
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 PM

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-E2	Comment	
Injection Volume	10		
Acq. Date-Time	6/9/2021 11:36:18 AM		
Sample Info.			

Sample Chromatogram



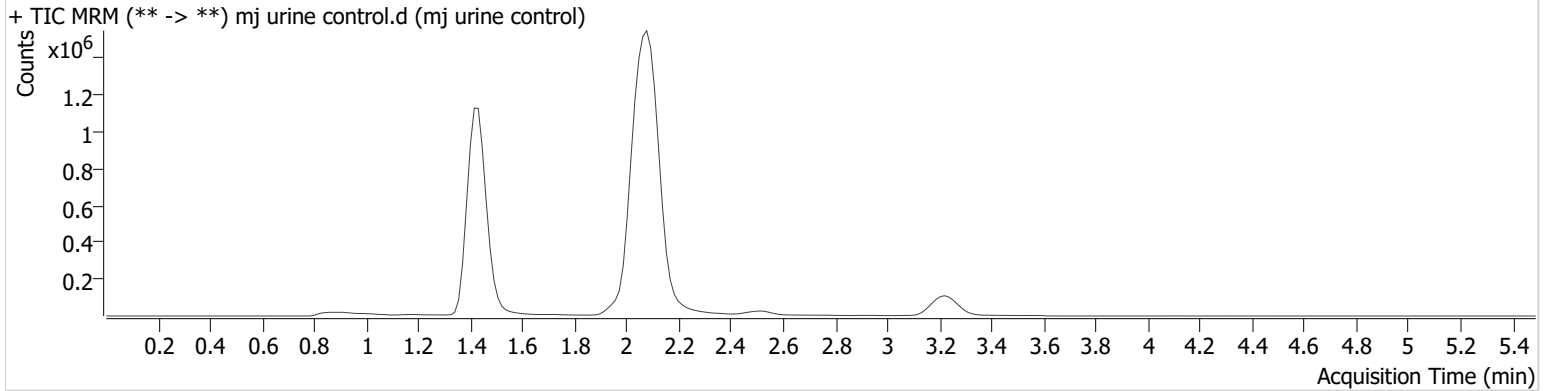
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 PM

Instrument	69679	Data File	mj urine control.d
Type	Sample	Sample	mj urine control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-F2	Comment	
Injection Volume	10		
Acq. Date-Time	6/9/2021 11:49:39 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	2514242	∞	12.8	∞	1593789	45.950 ng/ml
THC-COOH	1.461	295036	534071.1	41.2	202615.4	496880	43.916 ng/ml
THC	3.242	189649	∞	22.8	∞	539950	12.688 ng/ml

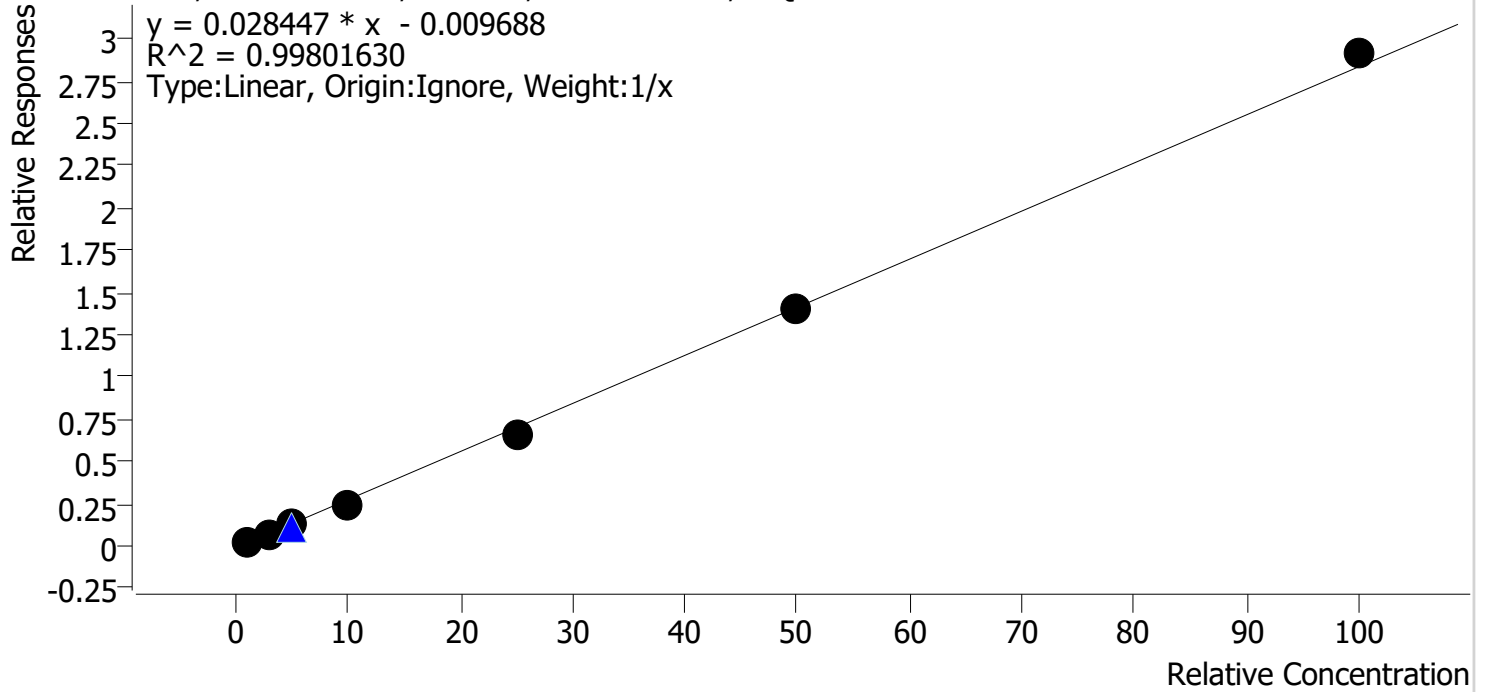
Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Last Cal. Update 6/9/2021 2:59 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 cal1	1	✓	1.0	1.2	124.6
mj cal2	2	✓	3.0	2.9	95.4
mj cal 3	3	✓	5.0	4.6	92.5
mj cal 4	4	✓	10.0	9.0	90.3
mj cal 5	5	✓	25.0	23.7	94.9
mj cal 6	6	✓	50.0	49.8	99.6
mj cal 7	7	✓	100.0	102.7	102.7

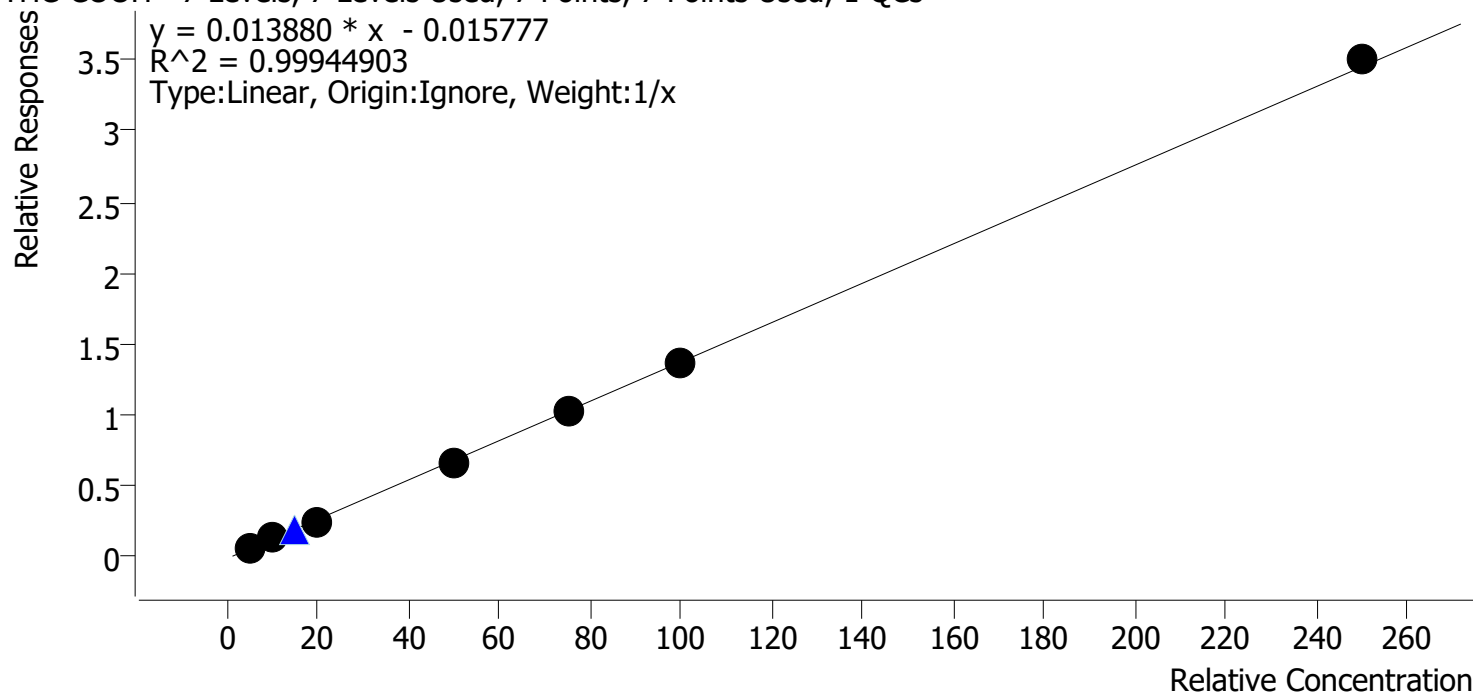
Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Last Cal. Update 6/9/2021 2:59 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



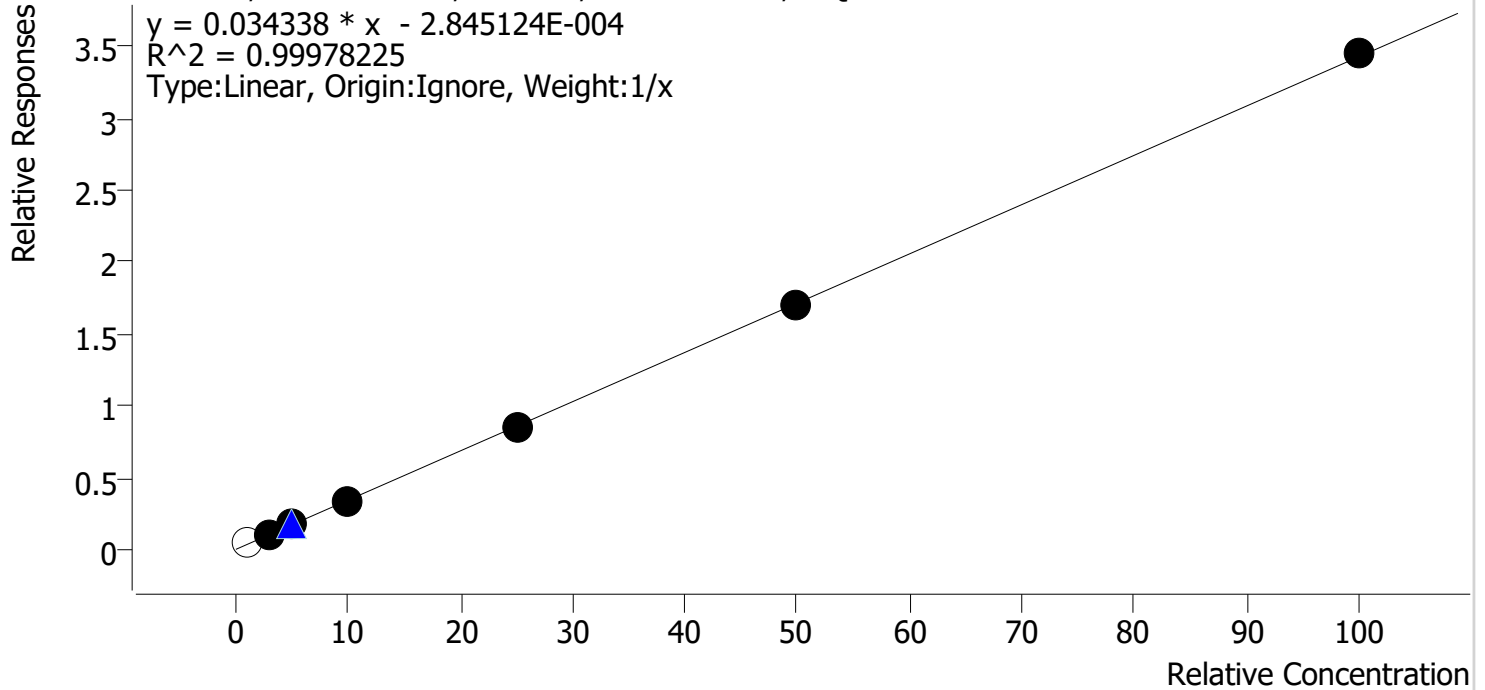
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.5	109.3
mj cal2	2	✓	10.0	9.9	98.8
mj cal 3	3	✓	20.0	19.0	95.2
mj cal 4	4	✓	50.0	48.4	96.8
mj cal 5	5	✓	75.0	74.9	99.8
mj cal 6	6	✓	100.0	98.7	98.7
mj cal 7	7	✓	250.0	253.6	101.5

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Last Cal. Update 6/9/2021 2:59 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	x	1.0	1.6	156.0
mj cal2	2	✓	3.0	3.2	106.4
mj cal 3	3	✓	5.0	4.8	96.8
mj cal 4	4	✓	10.0	9.7	97.4
mj cal 5	5	✓	25.0	24.8	99.0
mj cal 6	6	✓	50.0	49.9	99.7
mj cal 7	7	✓	100.0	100.6	100.6

cal 1 ratio out

AM #27 Cannabinoids

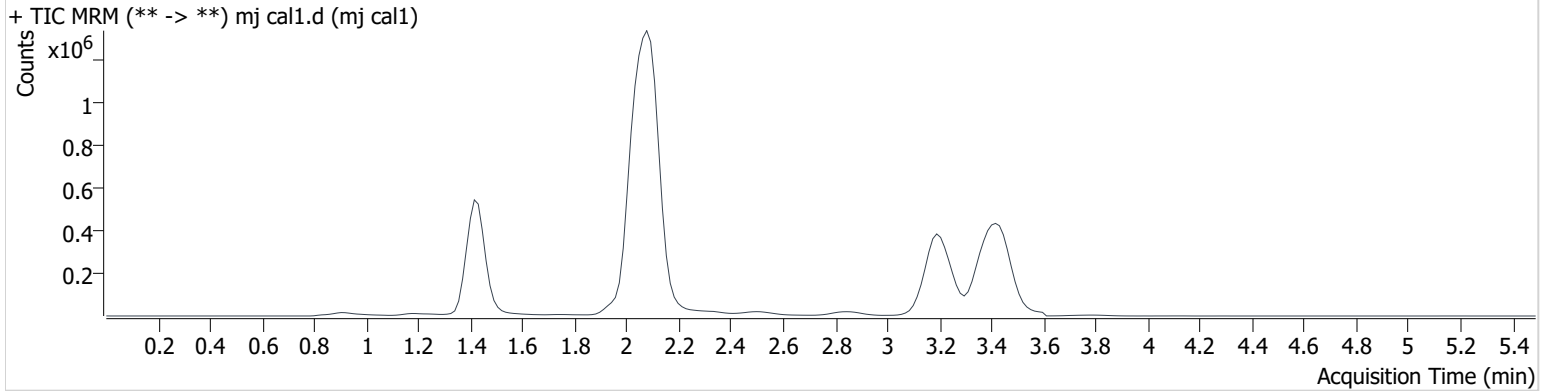
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 PM

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	6/9/2021 9:36:09 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	98116	∞	6.5 Low	∞	1841156	1.560 ng/ml Low
THC-COOH	1.446	40680	133.6	39.5	24.6	677124	5.465 ng/ml
THC	3.227	30949	∞	26.8	1163770 2773964 .4	1201247	1.246 ng/ml

AM #27 Cannabinoids

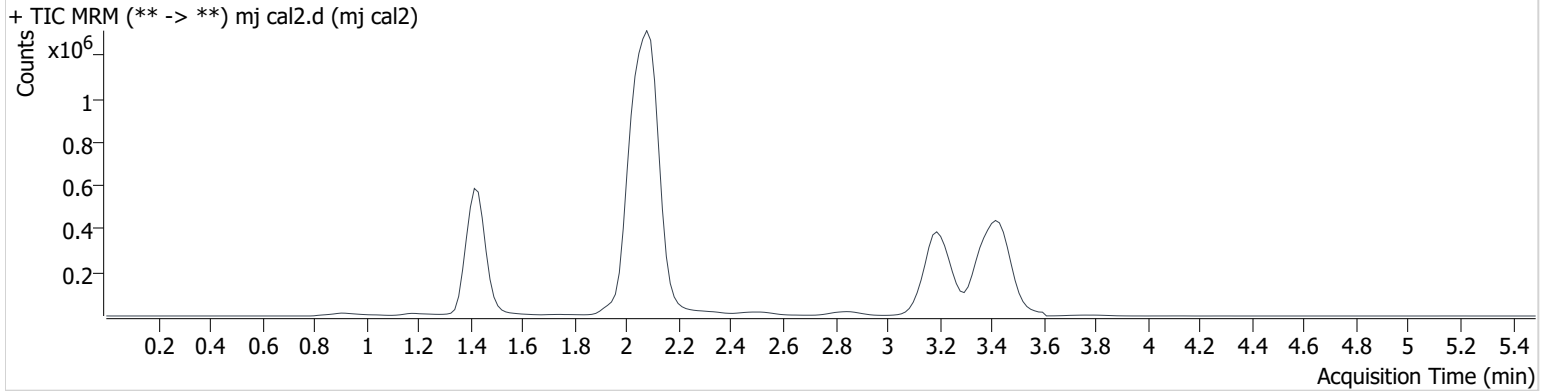
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 PM

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	6/9/2021 9:42:53 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	208853	∞	9.4	∞	1909799	3.193 ng/ml
THC-COOH	1.461	83360	205.6	42.0	118.0	687260	9.875 ng/ml
THC	3.227	83576	∞	25.4	∞	1165118	2.862 ng/ml

AM #27 Cannabinoids

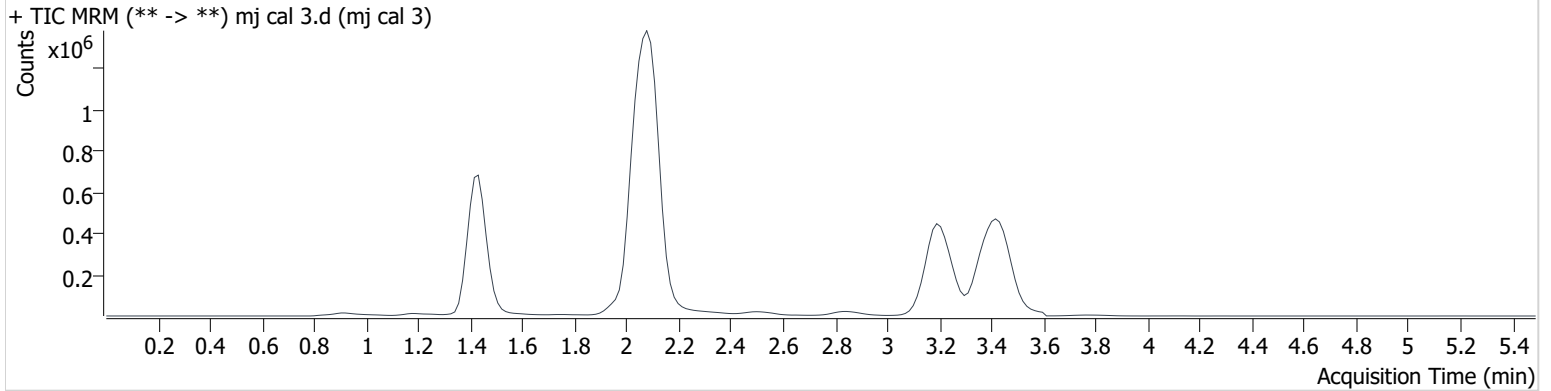
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 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	6/9/2021 9:49:35 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	312205	∞	10.5	∞	1881102	4.842 ng/ml
THC-COOH	1.446	176274	662.0	41.0	2426.1	709697	19.031 ng/ml
THC	3.227	164316	∞	25.4	∞	1348693	4.623 ng/ml

AM #27 Cannabinoids

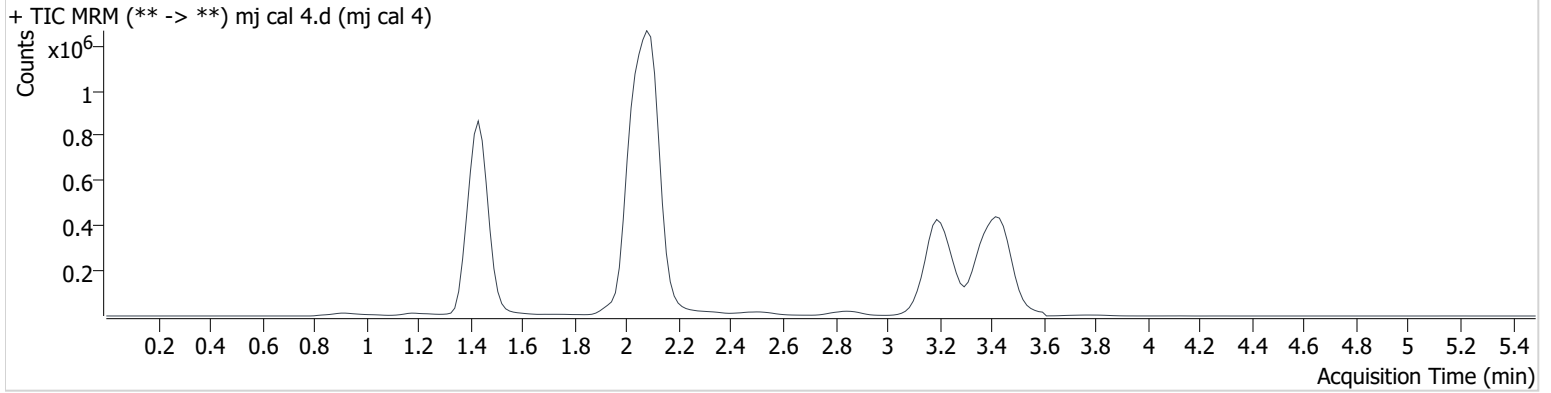
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 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	6/9/2021 9:56:16 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	631097	∞	10.4	∞	1889224	9.737 ng/ml
THC-COOH	1.446	466322	2291.7	41.1	14893.3	711041	48.386 ng/ml
THC	3.227	300585	∞	24.5	∞	1216609	9.026 ng/ml

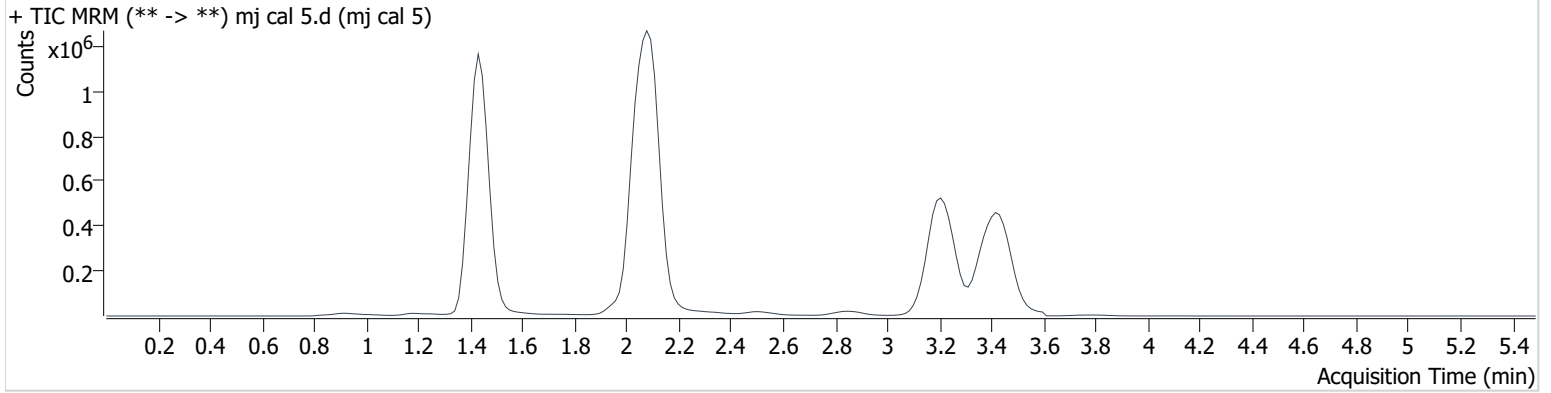
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 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	6/9/2021 10:02:58 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	1459902	∞	11.1	∞	1717651	24.761 ng/ml
THC-COOH	1.446	682037	1008.2	41.6	470554.2	666370	74.876 ng/ml
THC	3.242	843503	∞	23.2	∞	1267646	23.732 ng/ml

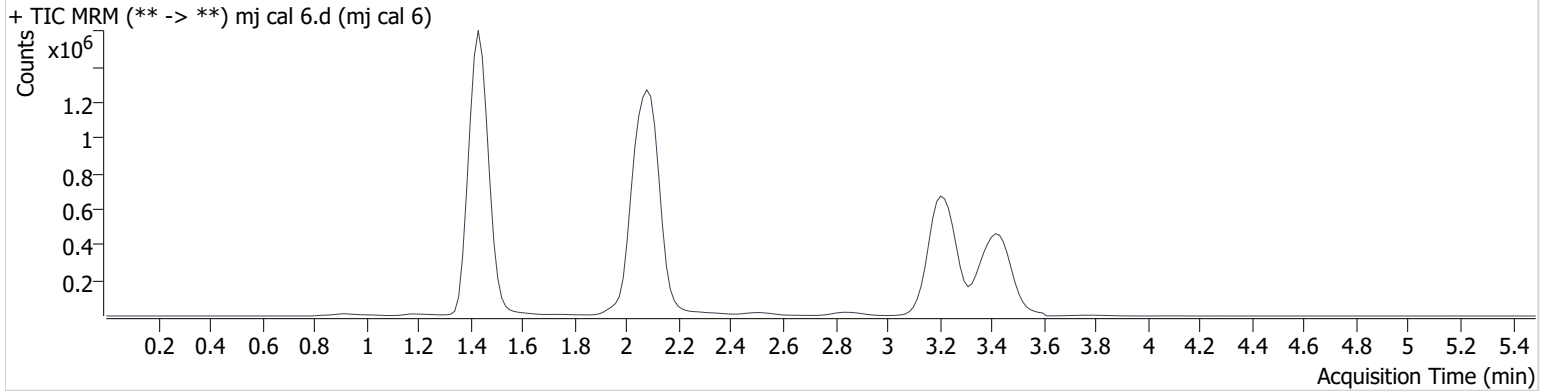
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 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	6/9/2021 10:09:40 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	2974745	∞	11.4	∞	1738057	49.852 ng/ml
THC-COOH	1.446	894385	1125.5	41.2	633077.2	660272	98.727 ng/ml
THC	3.242	1803649	∞	23.0	∞	1281767	49.807 ng/ml

AM #27 Cannabinoids

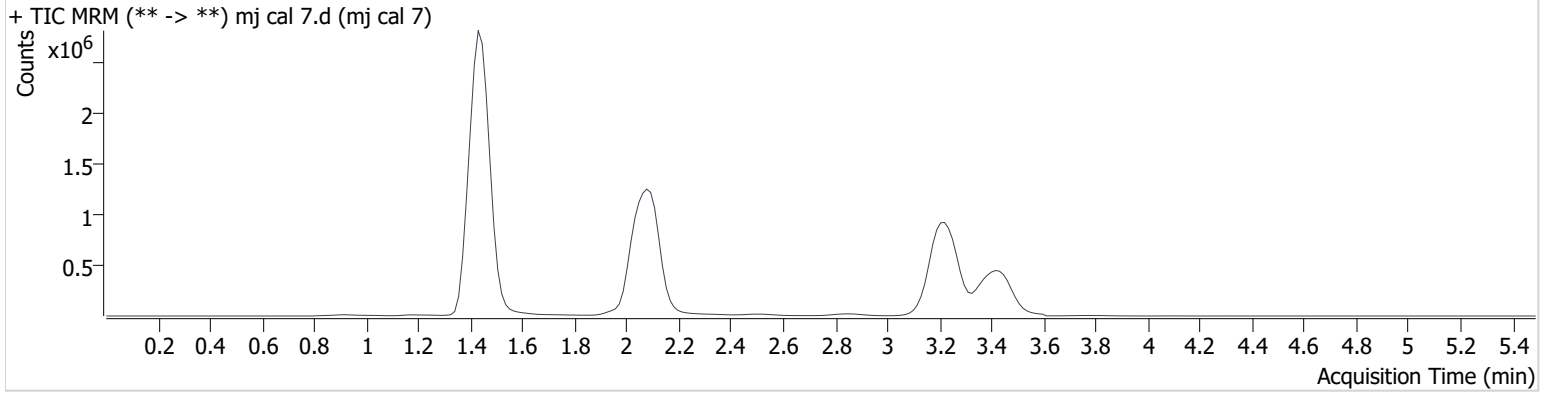
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\060921\QuantResults\cannq.batch.bin
Calibration Last Update 6/9/2021 2:59:02 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	6/9/2021 10:16:22 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	5850349	∞	11.7	∞	1693483	100.615 ng/ml
THC-COOH	1.461	2117826	1828.1	41.8	3362.2	604272	253.639 ng/ml
THC	3.242	3523604	∞	23.8	∞	1210064	102.704 ng/ml