

APPROVED

By Anne Nord at 4:08 pm, May 05, 2022

5/3/2022

BWylee

Worklist: 5850

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2022-0625	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-0637	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-0645	1	CBUK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-0734	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ



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

LC-MS/MS

Extraction Date 5/2/22 Analyst: Britany Wylie
Plate lot#: 211018 Plate re-test: 4/18/22

Mobile phase A: 0.1% Formic Acid in LCMS Water MTBE
Mobile phase B: 0.1% Formic acid in Acetonitrile Hexane
LCMS Methanol

Blank Blood Lot: 22B52020 **Urine Blank:** 21522 **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.
- 4. Pipette 500µL 0.1% formic acid in water blood sample, 500 ul saturated phosphate buffer in urine 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 1ng/ml, OH-THC 3ng/mL (quantitative blood), Carboxy-THC: 5 ng/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: urine batch only; oh-thc 3-100 due to ratio on cal 1 being outside acceptance criteria

BW 5-5-22

C2022-0625-2 did not have a blank run prior to sample sample was reconstituted and reinjected-however wrong plate position was used, plate position corrected and reinjected data evaluated *BW*

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	1	2	3	4	5	6
a	cal 1	Internal control urine ^{BW} 5-5-22	external blood control			
b	cal 2	negative urine				
c	cal 3	external urine control				
d	cal 4	625-2				
e	Cal 5	645-1				
f	cal 6	637-1				
g	cal 7	734-1				
h	Internal control (blood)	neg blood				

c2022-0____-__

P2A2- not used as blood was used in well plate and not urine, external urine control used

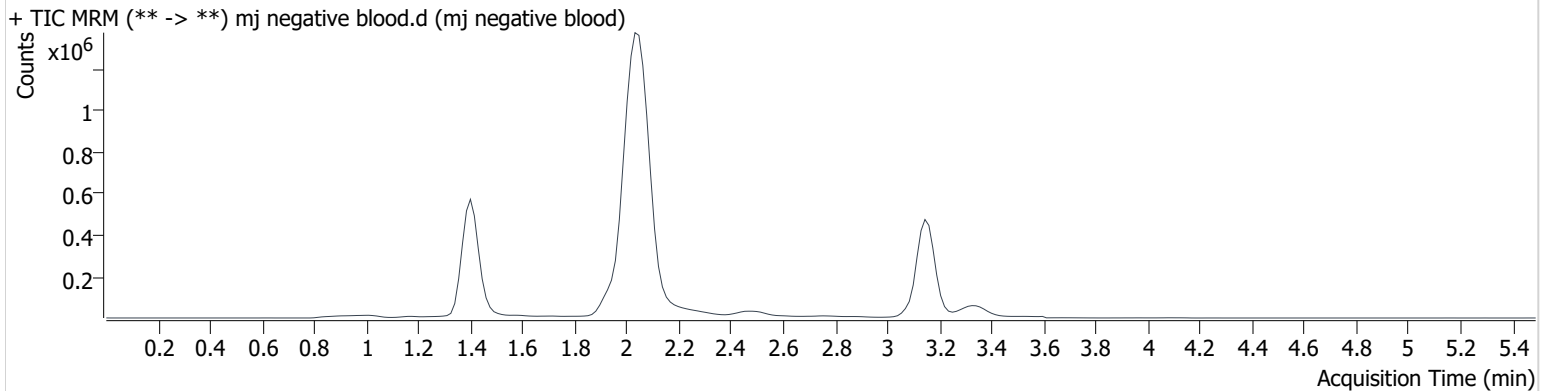
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 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-H2	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2022 1:17:04 PM		
Sample Info.			

Sample Chromatogram



AM #27 Cannabinoids

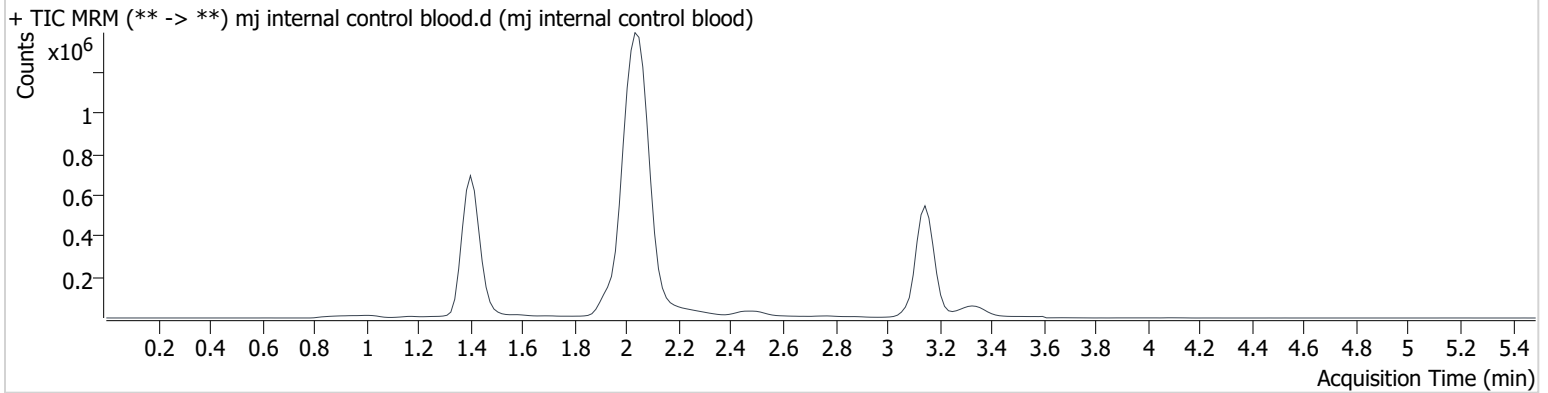
BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 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	5/2/2022 1:10:20 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	293551	∞	12.4	∞	2096508	5.429 ng/ml
THC-COOH	1.433	44481	∞	255.8	∞	506022	15.083 ng/ml
THC	3.152	275184	∞	25.6	∞	2198864	4.631 ng/ml

AM #27 Cannabinoids

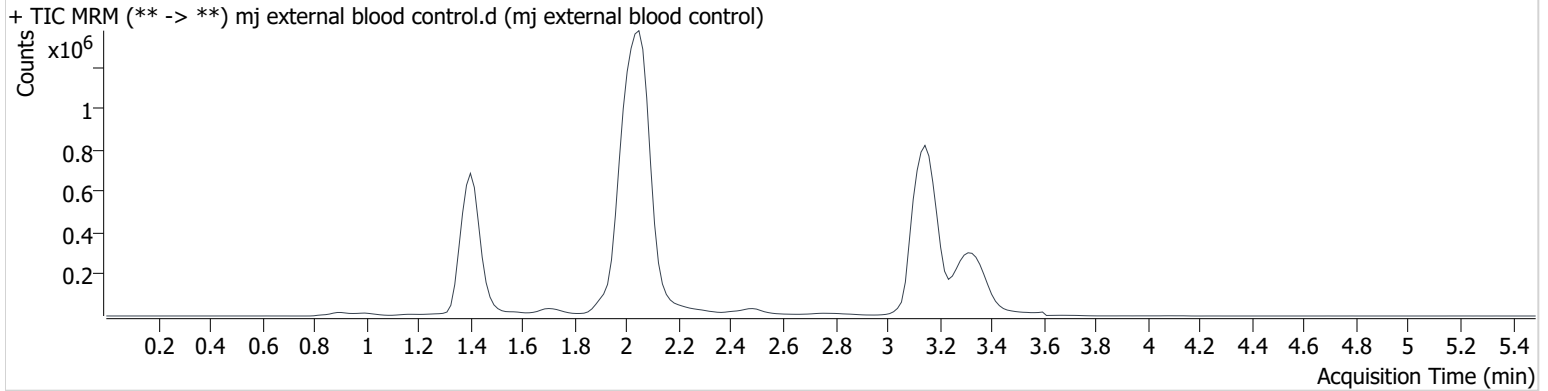
BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 AM

Instrument	69679	Data File	mj external blood control.d
Type	Sample	Sample	mj external blood control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A3	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2022 2:30:34 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	794600	∞	10.1	∞	1867778	15.293 ng/ml
THC-COOH	1.433	34236	∞	277.2	69.6	477764	12.614 ng/ml
THC	3.167	713752	∞	24.8	∞	4003223	6.428 ng/ml

AM #27 Cannabinoids

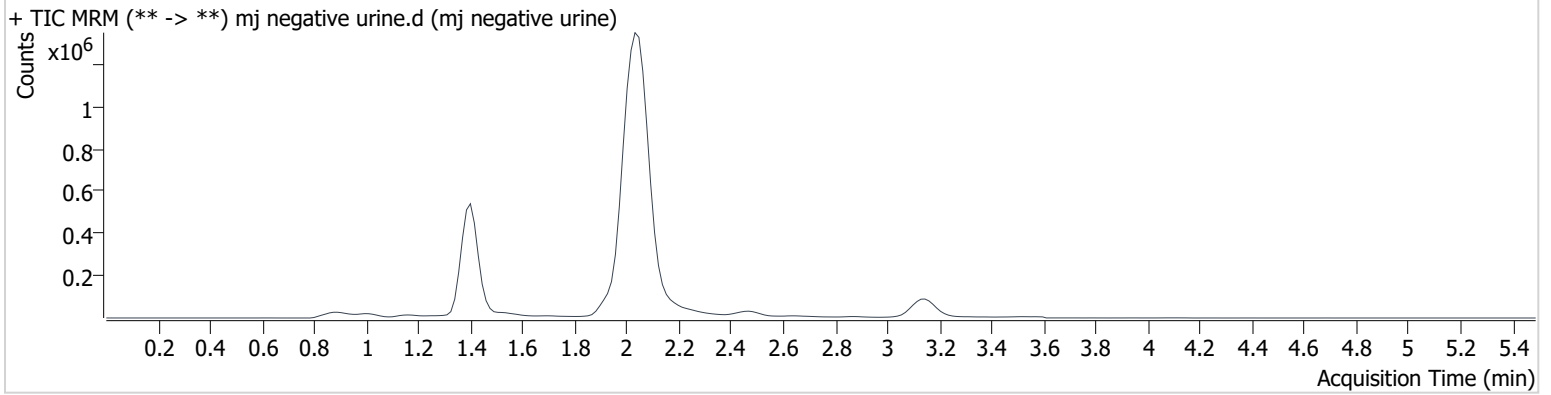
BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 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-B2	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2022 1:23:46 PM		

Sample Info.

Sample Chromatogram



AM #27 Cannabinoids

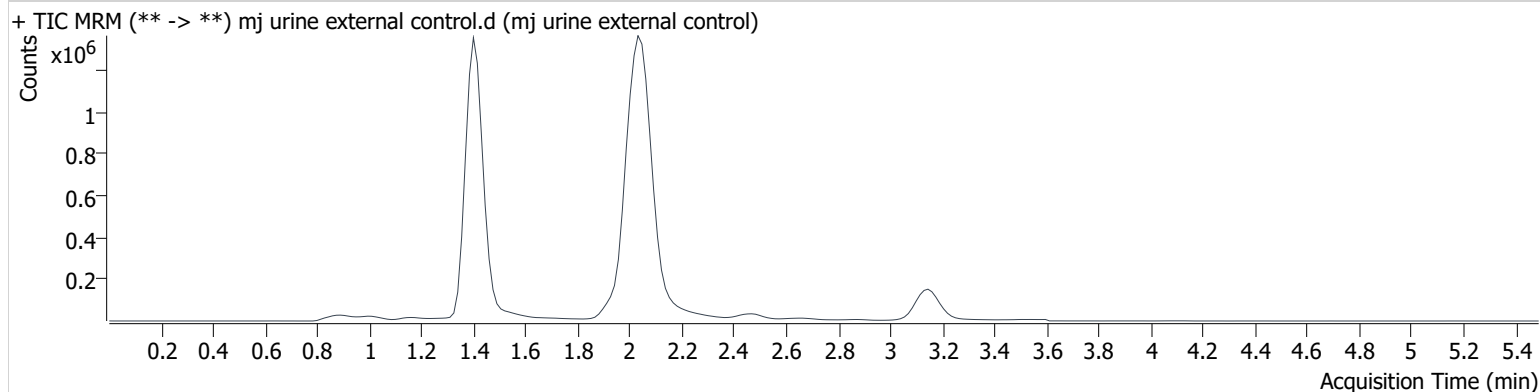
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Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 AM

Instrument	69679	Data File	mj urine external control.d
Type	Sample	Sample	mj urine external control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-C2	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2022 1:30:30 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	2844332	∞	13.7	∞	1846173	53.838 ng/ml
THC-COOH	1.433	111464	∞	289.9	∞	379734	46.334 ng/ml
THC	3.152	326367	∞	25.0	∞	547705	20.550 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 in 9900 ul blood

ppd 12/27/21 exp 8/26/22 blood lot 21D52496	lot b122721	Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	by amn
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AM 27/26 urine control 400 ul working solution in 9600 ul urine

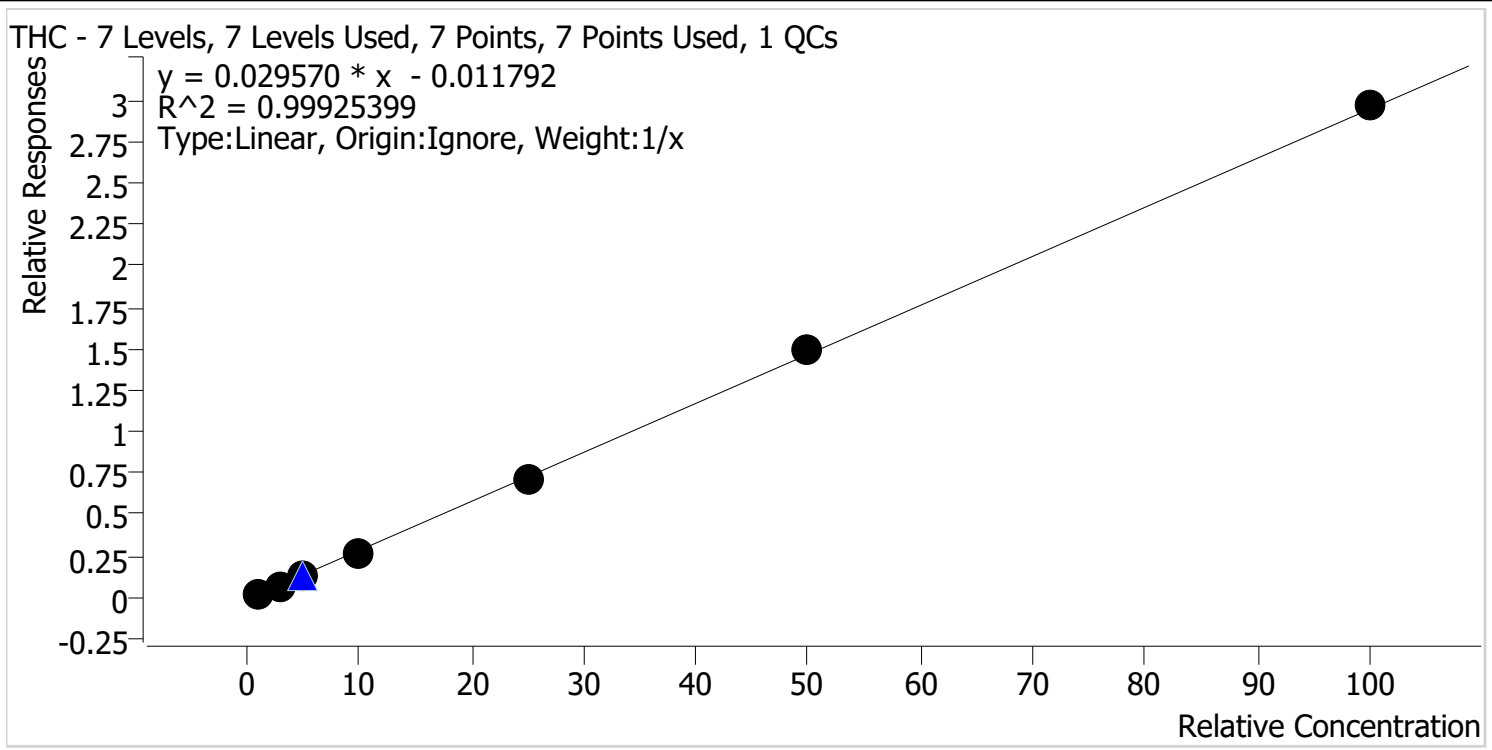
out of use

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	last used 11/1/21
ppd 11/2/21 Exp 8/26/22 neg urine lot 83121	lot u11221	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	last used prior to 4/26/22
ppd 4/26/2022 exp 8/26/22 neg urine lot 21522	lot u42622	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by baw	

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2022\lam 27-28\050222\QuantResults\lam 27.batch.bin
Last Cal. Update 5/3/2022 10:41 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** **THC-d3**



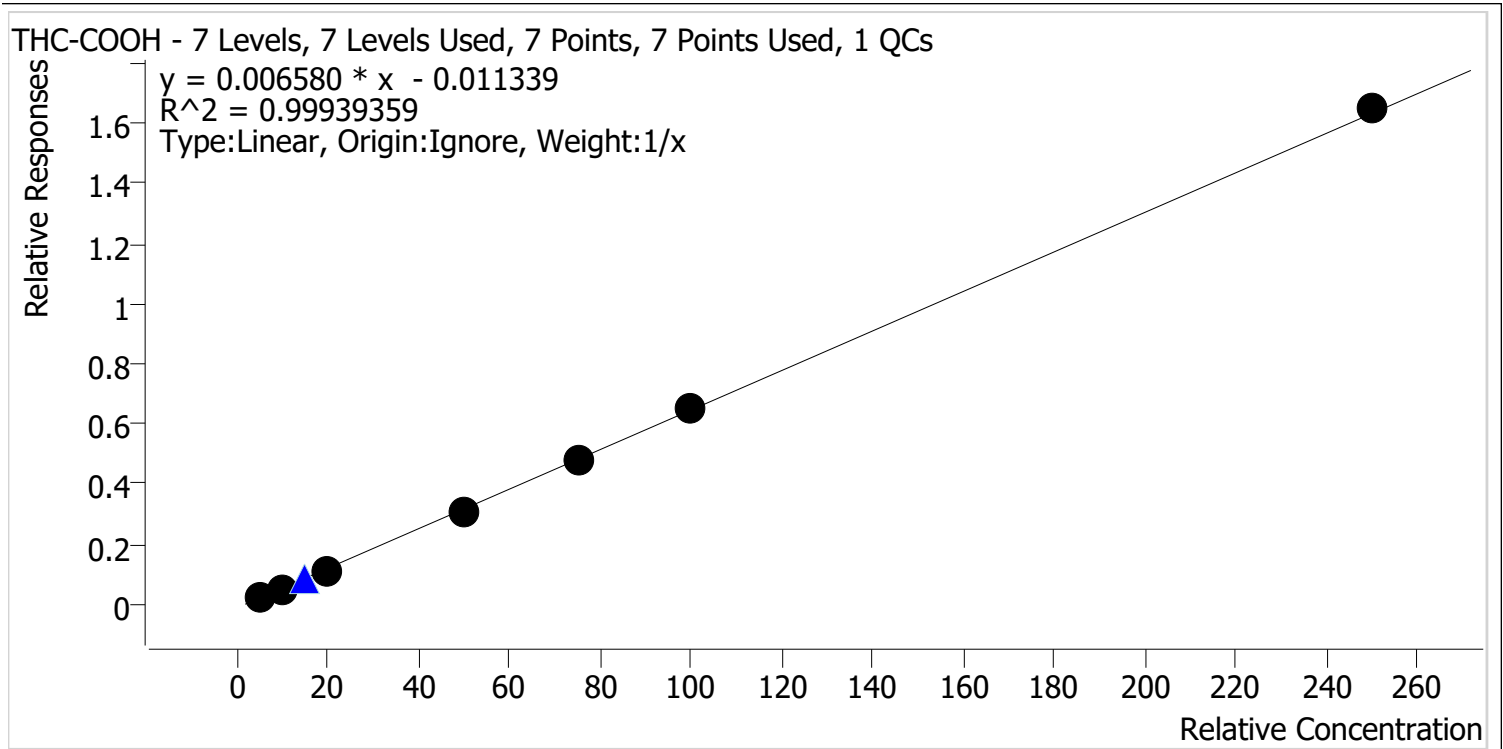
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	117.3
mj cal 2	2	✓	3.0	2.8	94.6
mj cal 3	3	✓	5.0	4.7	94.5
mj cal 4	4	✓	10.0	9.4	93.8
mj cal 5	5	✓	25.0	24.4	97.7
mj cal 6	6	✓	50.0	50.8	101.6
mj cal 7	7	✓	100.0	100.7	100.7

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2022\lam 27-28\050222\QuantResults\lam 27.batch.bin
Last Cal. Update 5/3/2022 10:41 AM
Analyst Name ISP\datastor
Analyte THC-COOH

Internal Standard THC-COOH-d9



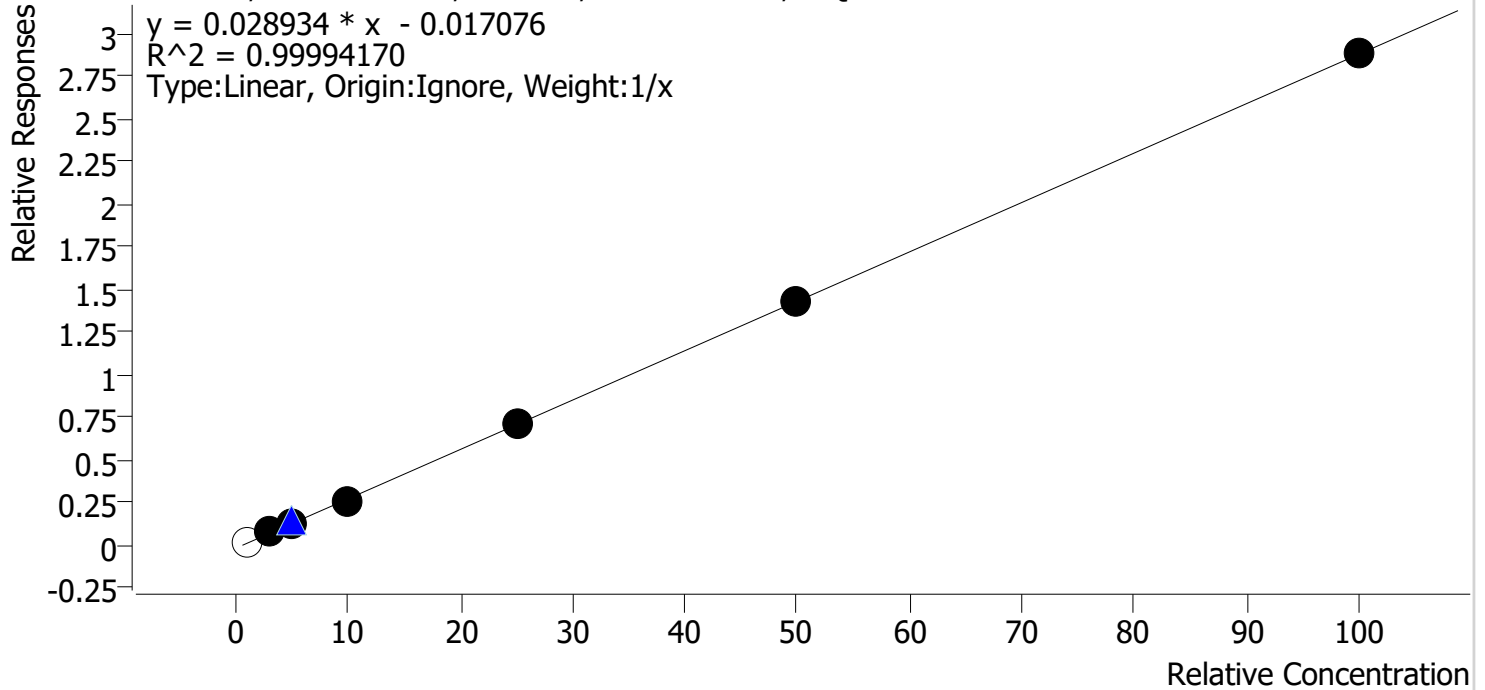
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.5	110.5
mj cal 2	2	✓	10.0	9.9	98.8
mj cal 3	3	✓	20.0	18.6	92.8
mj cal 4	4	✓	50.0	48.4	96.9
mj cal 5	5	✓	75.0	74.7	99.6
mj cal 6	6	✓	100.0	100.4	100.4
mj cal 7	7	✓	250.0	252.4	101.0

Compound Calibration Report

Boyle

Batch results D:\MassHunter\Data\2022\lam 27-28\050222\QuantResults\lam 27.batch.bin
Last Cal. Update 5/3/2022 10:41 AM
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 cal 1	1	x	1.0	1.3	131.9
mj cal 2	2	✓	3.0	3.1	102.9
mj cal 3	3	✓	5.0	5.0	99.2
mj cal 4	4	✓	10.0	9.8	97.8
mj cal 5	5	✓	25.0	24.9	99.7
mj cal 6	6	✓	50.0	50.1	100.3
mj cal 7	7	✓	100.0	100.1	100.1

Cal 1 dropped due to ratio being outside acceptance

5-5-22

~~Cal 7 dropped due to accuracy~~

5-2-22

5-5-22

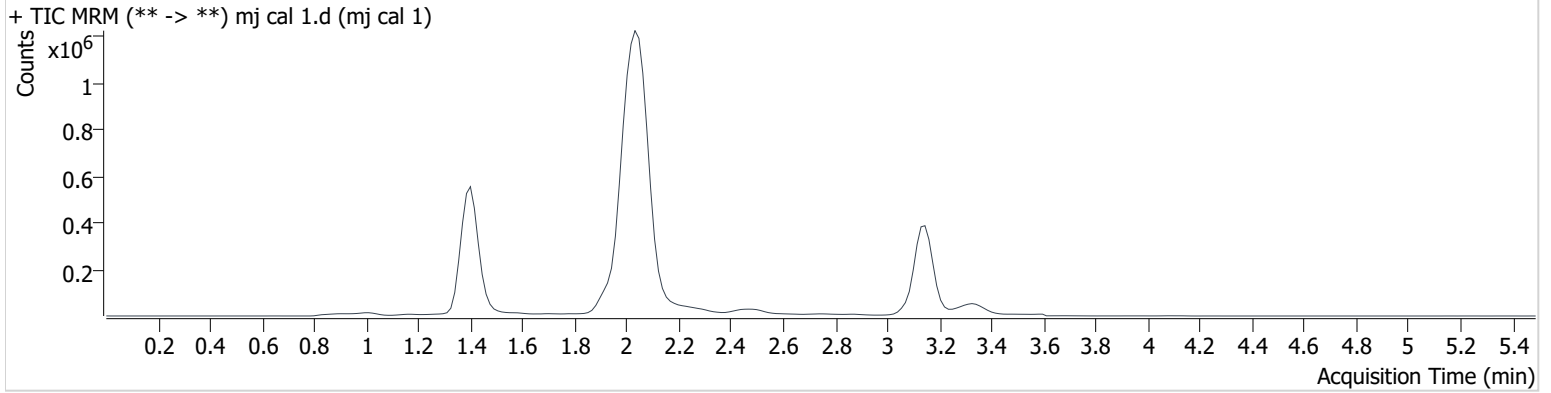
AM #27 Cannabinoids

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Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 AM

Instrument	69679	Data File	mj cal 1.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	5/2/2022 12:16:46 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	42091	∞	16.0 High	∞	1996587	1.319 ng/ml Low
THC-COOH	1.433	11236	∞	269.8	∞	449371	5.523 ng/ml
THC	3.152	40947	∞	27.8	∞	1789244	1.173 ng/ml

AM #27 Cannabinoids

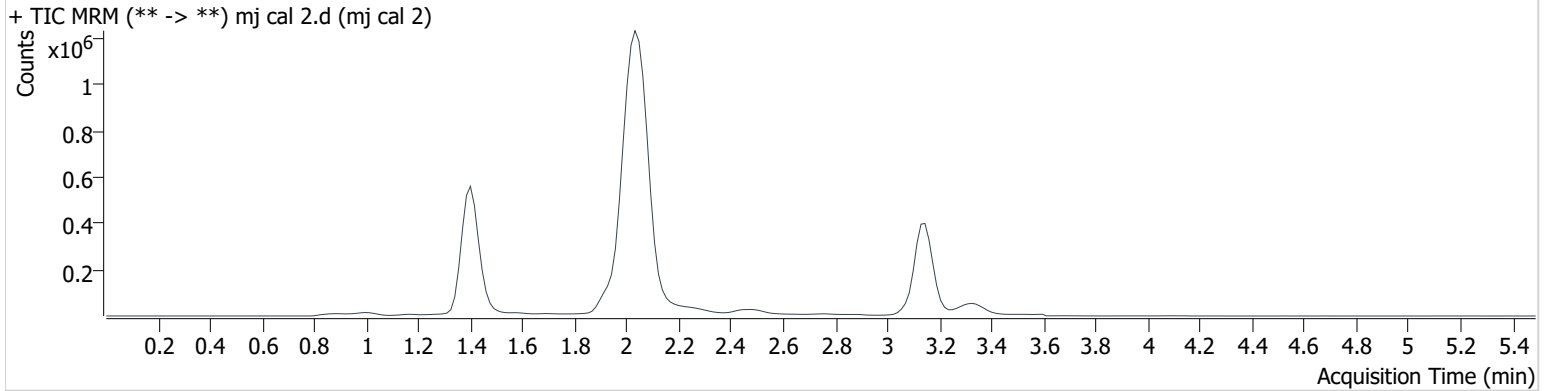
BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 AM

Instrument	69679	Data File	mj cal 2.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	5/2/2022 12:23:30 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	131617	∞	13.5	∞	1822556	3.086 ng/ml
THC-COOH	1.433	22385	∞	275.2	∞	417185	9.878 ng/ml
THC	3.152	123284	∞	28.3	∞	1709791	2.837 ng/ml

AM #27 Cannabinoids

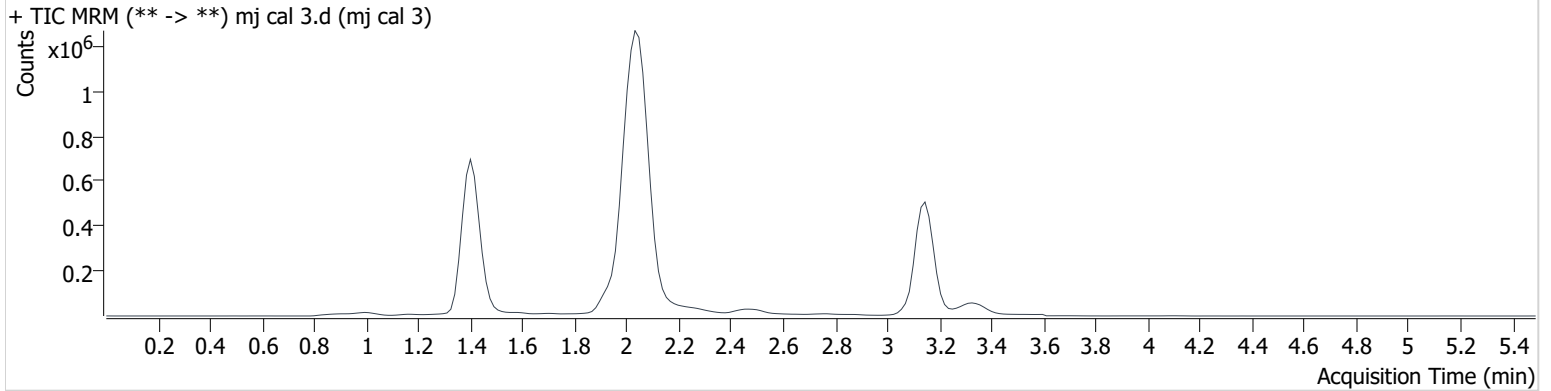
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Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 AM

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	5/2/2022 12:30:12 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	263449	∞	12.8	∞	2083187	4.961 ng/ml
THC-COOH	1.433	53541	∞	267.1	∞	483110	18.566 ng/ml
THC	3.152	264614	∞	25.6	∞	2069227	4.723 ng/ml

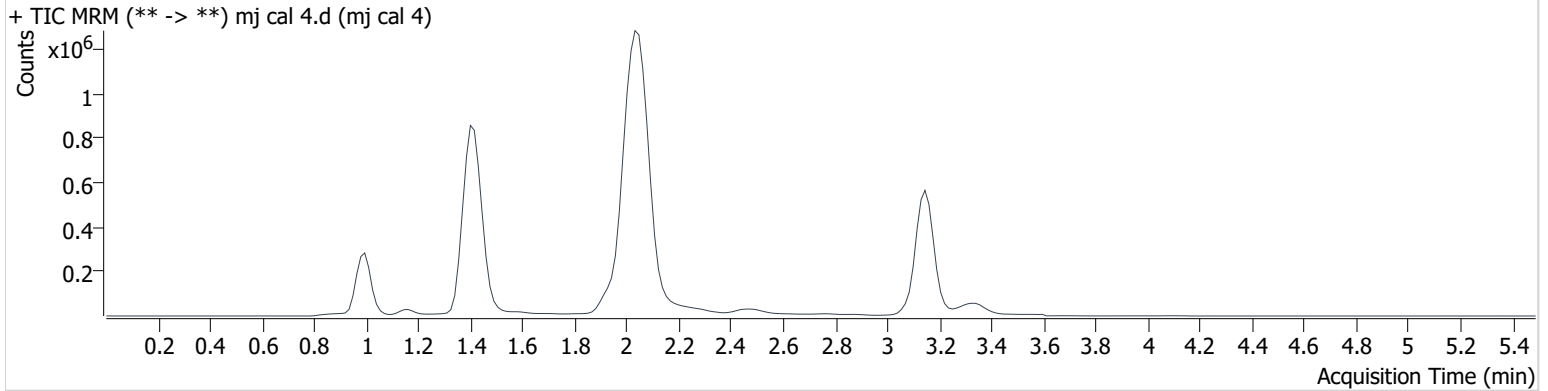
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 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	5/2/2022 12:36:55 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	545948	∞	12.1	∞	2053339	9.779 ng/ml
THC-COOH	1.433	146799	∞	264.9	∞	477626	48.434 ng/ml
THC	3.152	523695	∞	24.6	∞	1972619	9.377 ng/ml

AM #27 Cannabinoids

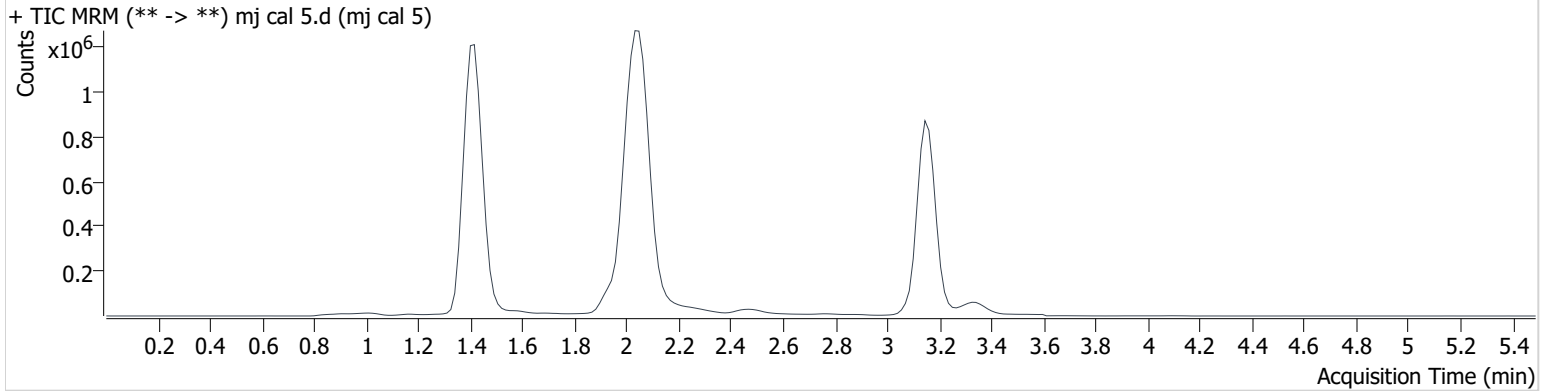
BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 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	5/2/2022 12:43:37 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1514953	∞	11.9	∞	2150611	24.936 ng/ml
THC-COOH	1.433	235625	∞	250.5	∞	490526	74.726 ng/ml
THC	3.167	1564583	∞	24.8	∞	2203000	24.417 ng/ml

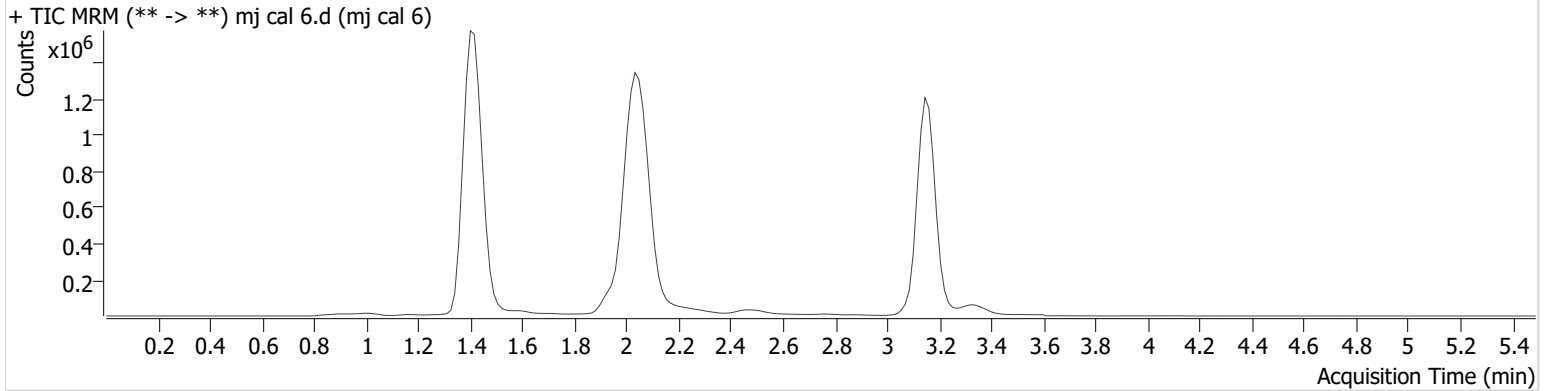
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 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	5/2/2022 12:50:19 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	2882497	∞	12.4	∞	2010769	50.135 ng/ml
THC-COOH	1.433	293434	∞	254.6	∞	451719	100.447 ng/ml
THC	3.152	2997007	∞	24.9	∞	2011772	50.779 ng/ml

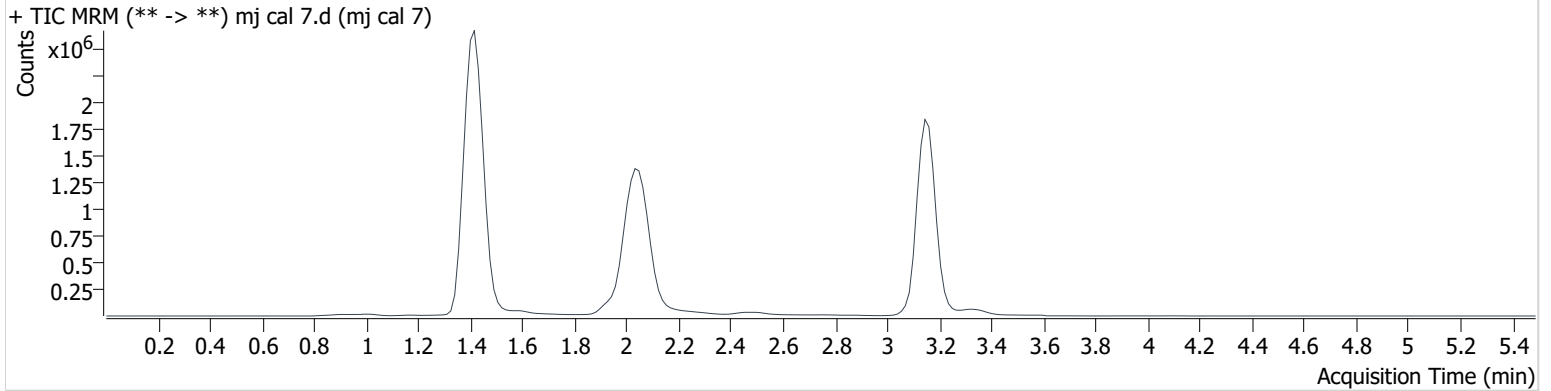
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 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	5/2/2022 12:57:01 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	5575825	∞	12.5	∞	1936534	100.102 ng/ml
THC-COOH	1.433	701100	∞	247.2	∞	425014	252.425 ng/ml
THC	3.152	5733803	∞	25.1	∞	1933340	100.695 ng/ml

AM #27 Cannabinoids

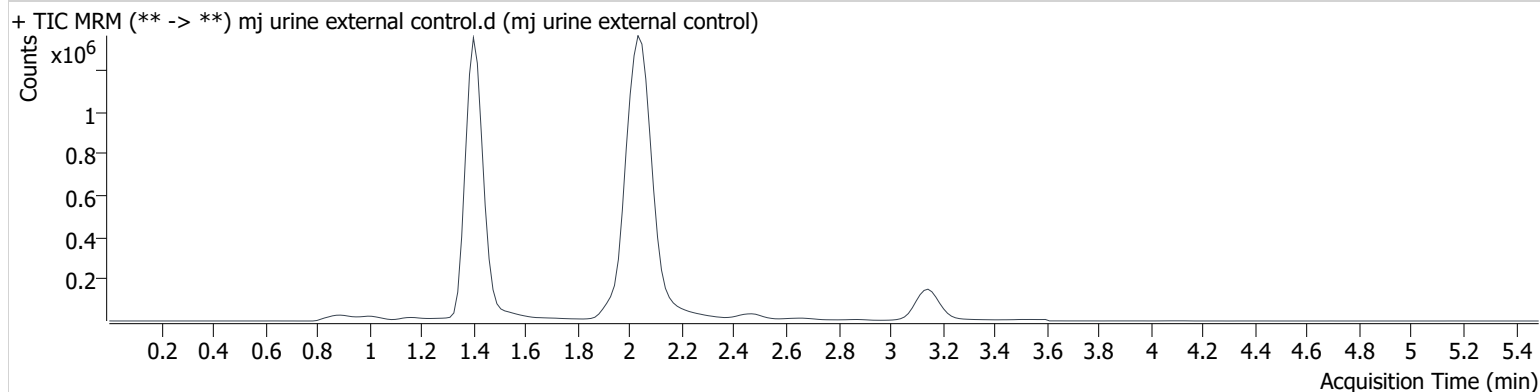
BWylie

Batch results D:\MassHunter\Data\2022\am 27-28\050222\QuantResults\am 27.batch.bin
Calibration Last Update 5/3/2022 10:41:08 AM

Instrument	69679	Data File	mj urine external control.d
Type	Sample	Sample	mj urine external control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-C2	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2022 1:30:30 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	2844332	∞	13.7	∞	1846173	53.838 ng/ml
THC-COOH	1.433	111464	∞	289.9	∞	379734	46.334 ng/ml
THC	3.152	326367	∞	25.0	∞	547705	20.550 ng/ml