


REVIEWED

By Sarah Collins at 12:31 pm, Mar 15, 2021

3/11/2021

Byylee

Worklist: 4837

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2021-0345	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-0360	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0398	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0418	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-0428	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0430	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0431	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0489	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0491	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-0503	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0505	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0528	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

Bylye

AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 3/9/20

Analyst: Britany Wylie

Plate lot#: 201206

Plate Expiration: 6-6-2021

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: 2020J20793 **Urine Blank:** 2121

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: urine samples evaluated for THC-COOH only;
Curve limitation: THC-OH 3-100

AM #27 Cannabinoids

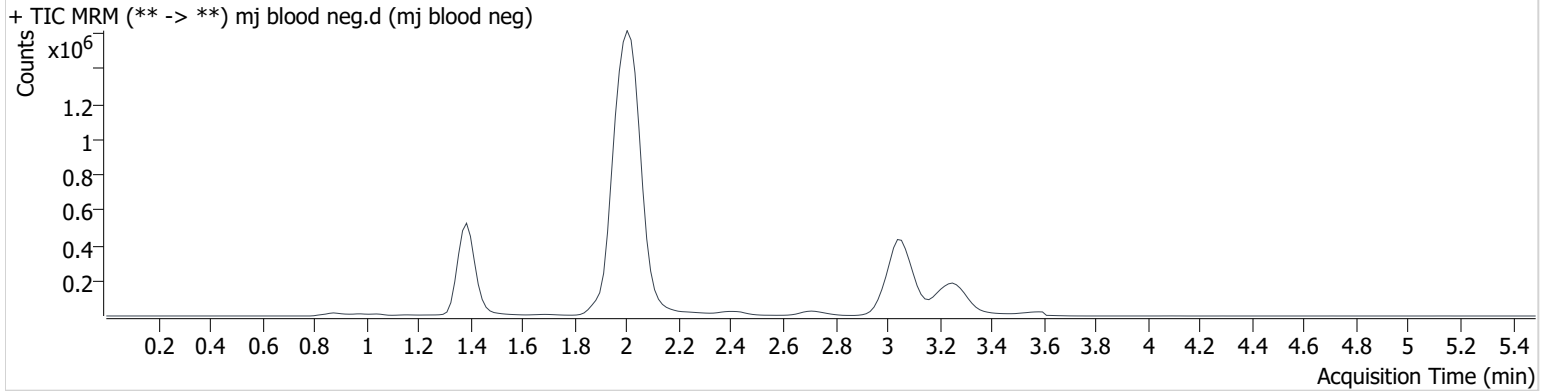
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 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	3/9/2021 8:11:43 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.324	10877	∞			634079	1.656 ng/ml Low

AM #27 Cannabinoids

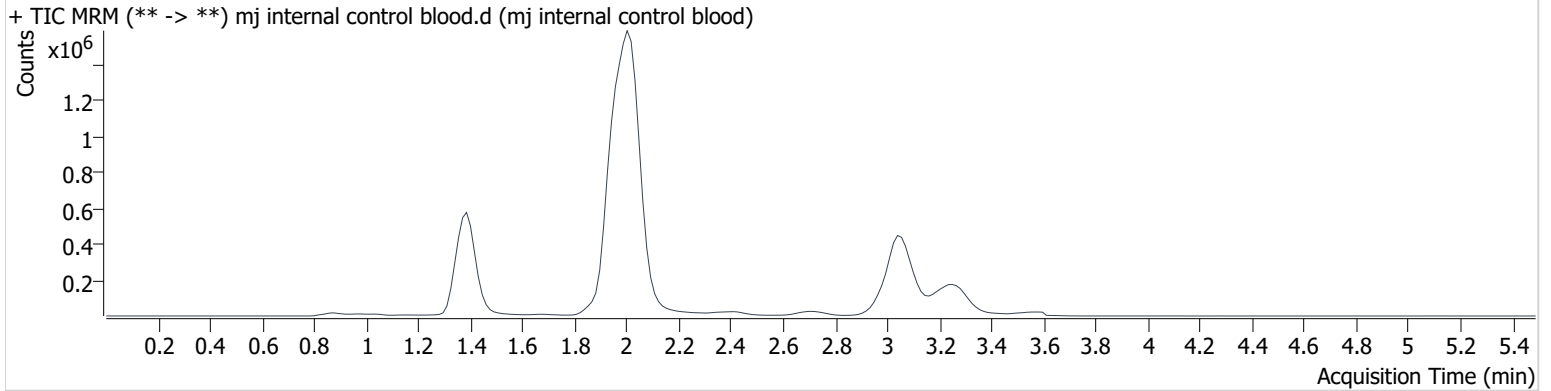
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 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	3/9/2021 8:04:59 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	23528	∞	1081.5	∞	1818839	4.462 ng/ml
THC-COOH	1.414	166811	∞	22.6	∞	632248	13.958 ng/ml
THC	3.077	128131	∞	25.0	∞	1386879	3.835 ng/ml

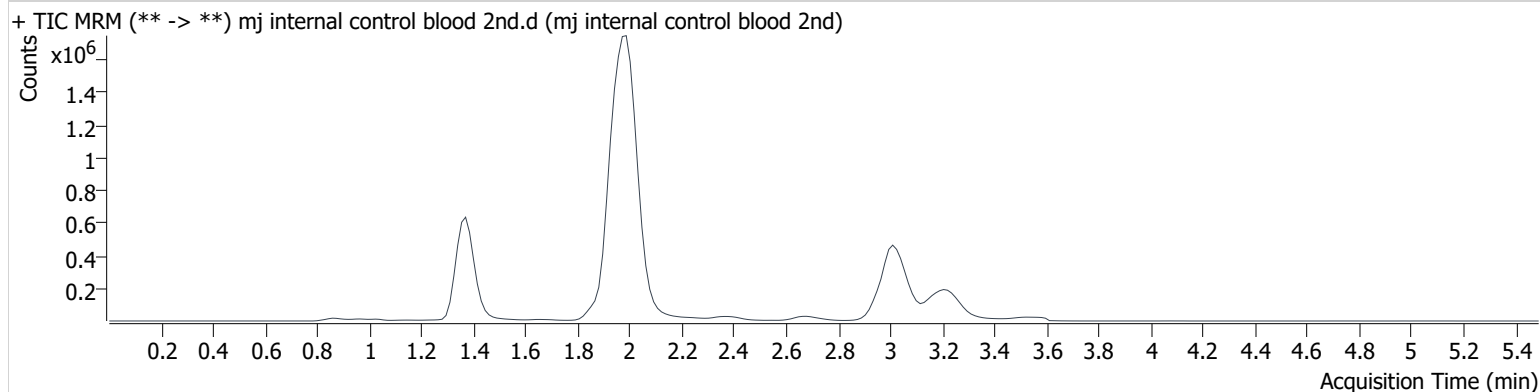
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 PM

Instrument	69679	Data File	mj internal control blood 2nd.d
Type	QC	Sample	mj internal control blood 2nd
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	3/9/2021 10:19:02 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	23682	∞	1086.6	∞	1880172	4.361 ng/ml
THC-COOH	1.399	167259	∞	22.5	406.4	660829	13.423 ng/ml
THC	3.046	138560	∞	25.3	∞	1497504	3.840 ng/ml

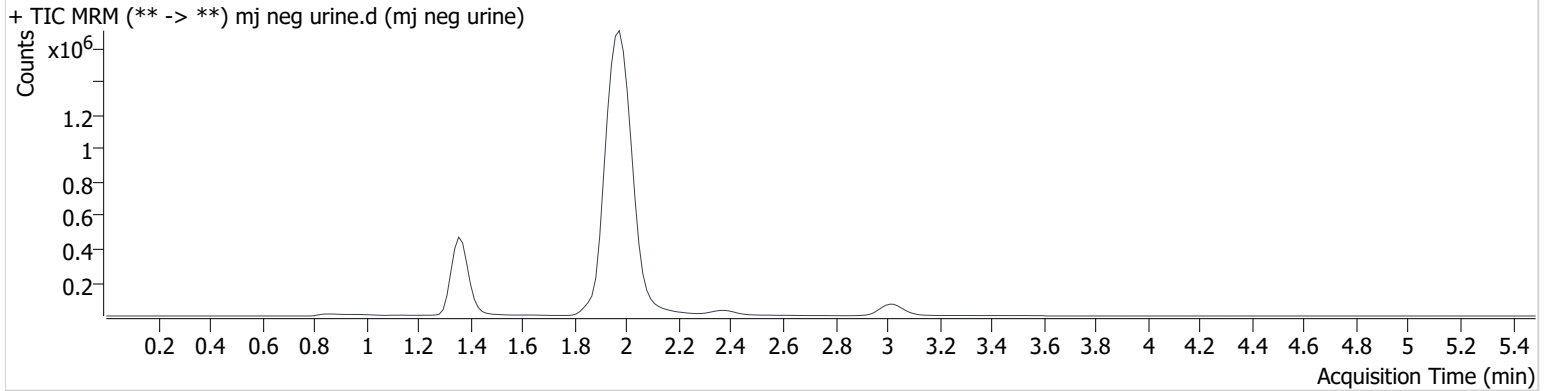
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 PM

Instrument	69679	Data File	mj neg urine.d
Type	Sample	Sample	mj neg urine
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-C3	Comment	
Injection Volume	10		
Acq. Date-Time	3/9/2021 10:32:24 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.304	1506	∞	1393.3 High	∞	1722063	0.875 ng/ml Low
THC-COOH	1.414	3292	57.9			491712	1.135 ng/ml Low

AM #27 Cannabinoids

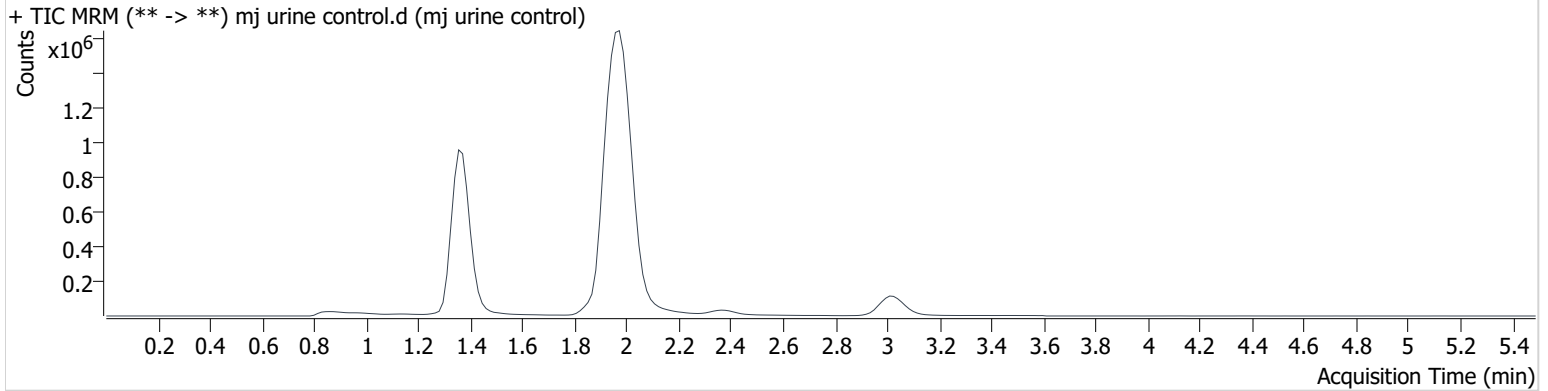
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 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-D3	Comment	
Injection Volume	10		
Acq. Date-Time	3/9/2021 10:45:48 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	245179	∞	733.8 Low	∞	1761804	42.012 ng/ml
THC-COOH	1.384	250810	3840.4	21.8	∞	503108	25.662 ng/ml
THC	3.031	152884	∞	24.3	∞	583807	10.202 ng/ml

AM #27 Cannabinoids

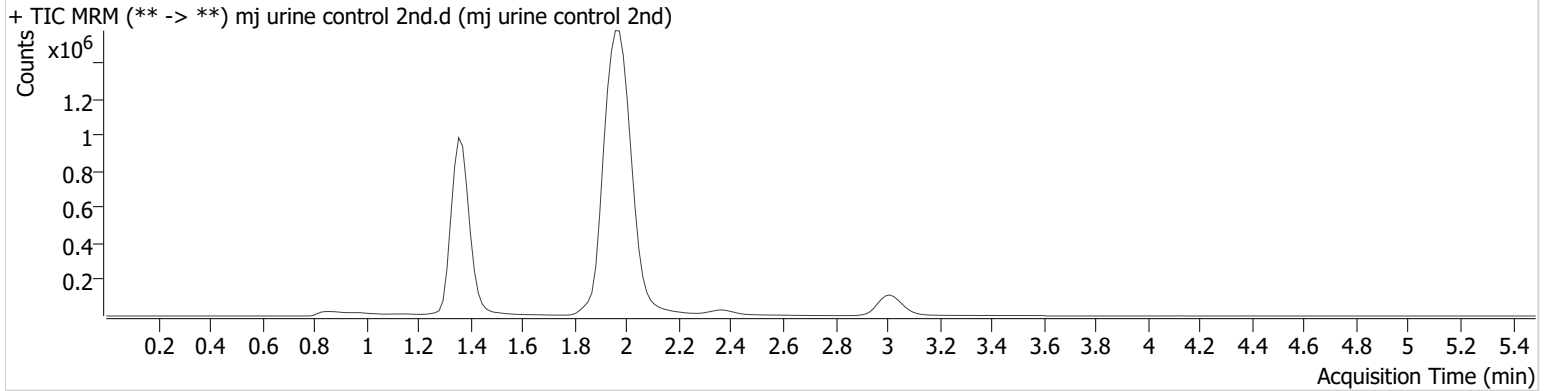
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Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 PM

Instrument	69679	Data File	mj urine control 2nd.d
Type	Sample	Sample	mj urine control 2nd
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-D3	Comment	
Injection Volume	10		
Acq. Date-Time	3/9/2021 11:32:44 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	246414	∞	726.3 Low	∞	1784731	41.686 ng/ml
THC-COOH	1.384	255401	∞	21.1	1411.1	491716	26.704 ng/ml
THC	3.016	159011	∞	23.5	∞	575857	10.737 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 8/26/20 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

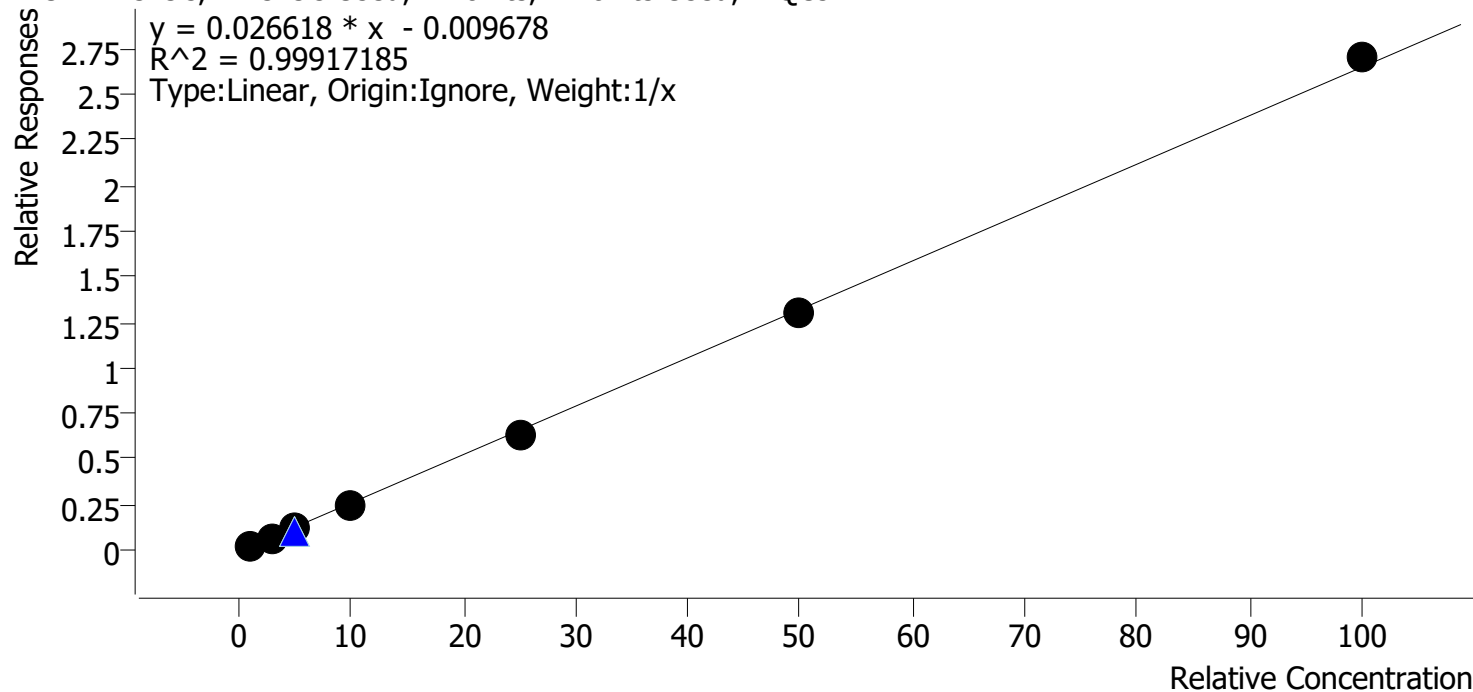
ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	1/12/2021
ppd 1/13/21 Exp 7/1/21 neg urine lot 10120	lot 11321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

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Last Cal. Update 3/10/2021 7:17 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** **THC-d3**

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs



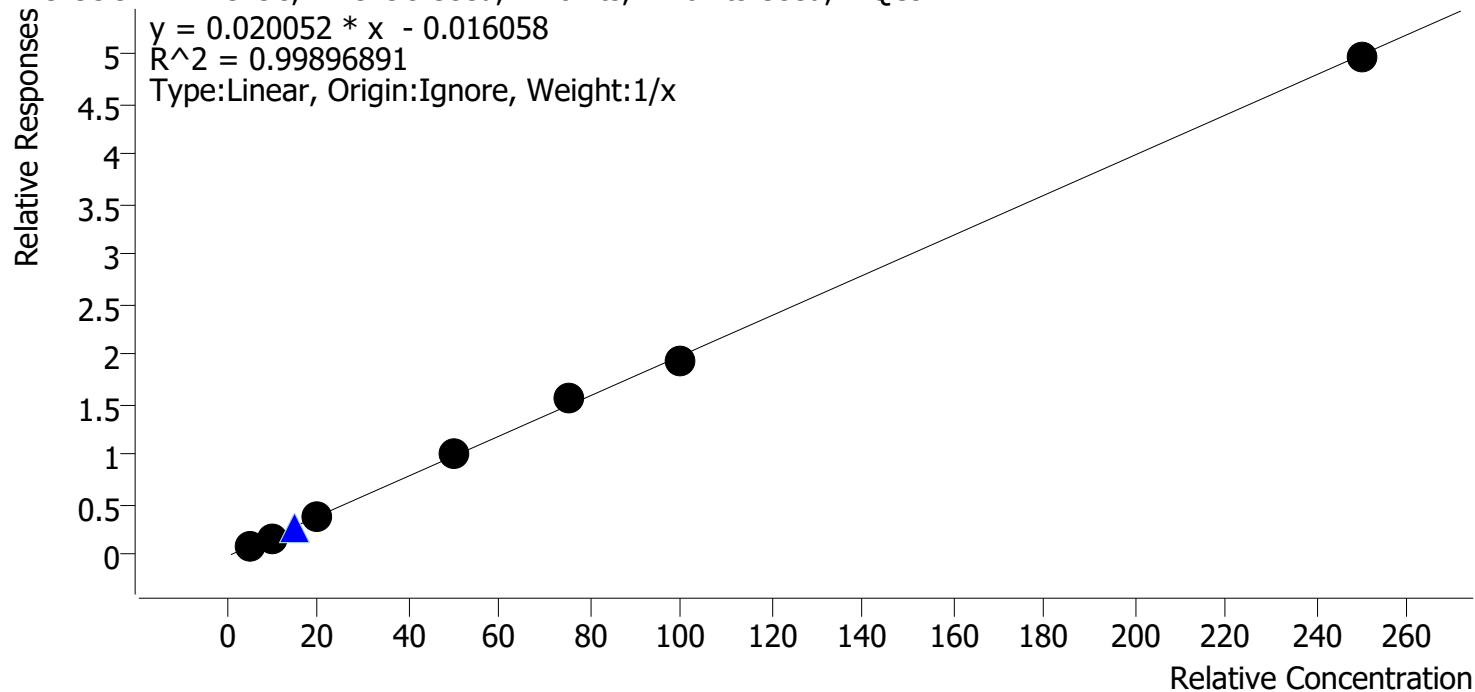
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	1.2	117.0
mj cal2	2	✓	3.0	2.8	94.7
mj cal 3	3	✓	5.0	4.7	94.3
mj cal 4	4	✓	10.0	9.6	96.3
mj cal 5	5	✓	25.0	24.2	96.8
mj cal 6	6	✓	50.0	49.5	99.0
mj cal 7	7	✓	100.0	102.0	102.0

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Last Cal. Update 3/10/2021 7:17 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, 2 QCs



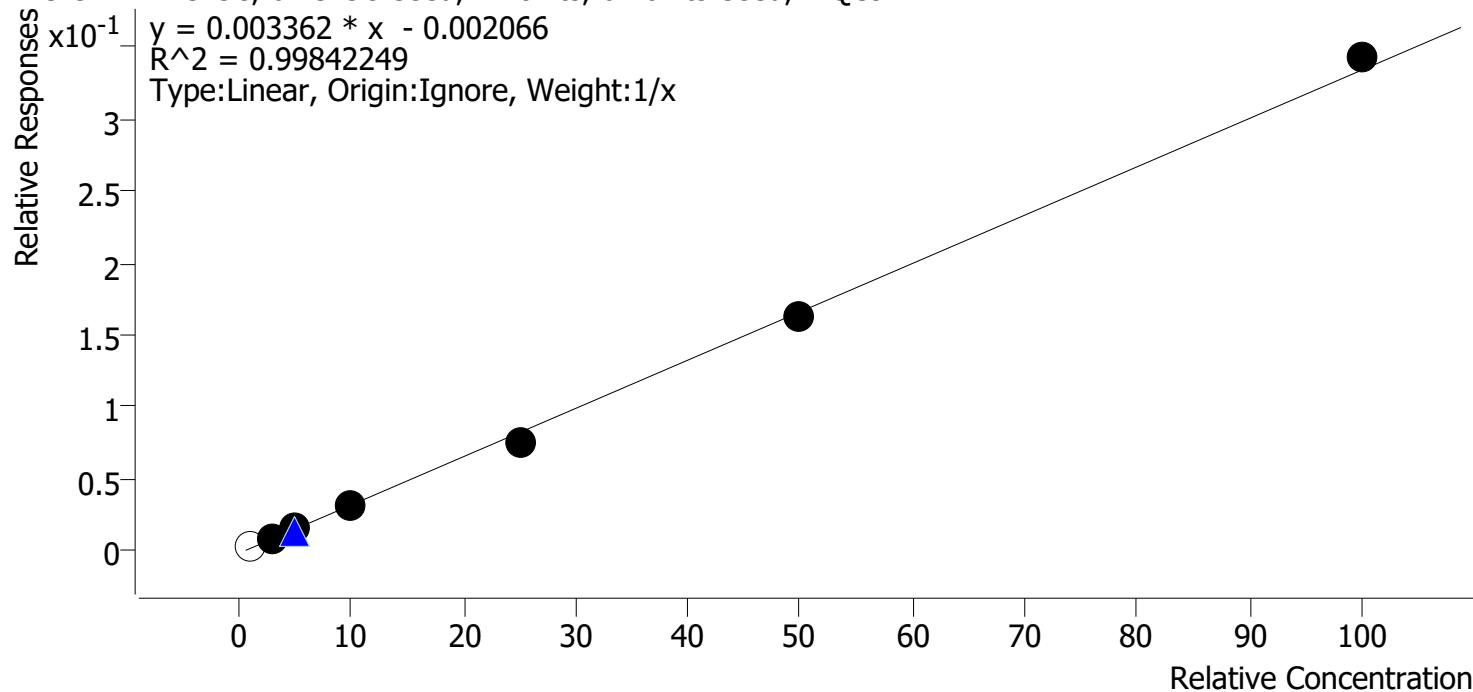
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.2	104.9
mj cal2	2	✓	10.0	9.2	92.3
mj cal 3	3	✓	20.0	19.9	99.7
mj cal 4	4	✓	50.0	50.2	100.4
mj cal 5	5	✓	75.0	79.4	105.8
mj cal 6	6	✓	100.0	97.5	97.5
mj cal 7	7	✓	250.0	248.5	99.4

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Last Cal. Update 3/10/2021 7:17 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, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	x	1.0	1.6	158.8
mj cal2	2	✓	3.0	3.2	106.3
mj cal 3	3	✓	5.0	5.2	103.2
mj cal 4	4	✓	10.0	9.6	96.4
mj cal 5	5	✓	25.0	23.3	93.2
mj cal 6	6	✓	50.0	49.1	98.3
mj cal 7	7	✓	100.0	102.6	102.6

AM #27 Cannabinoids

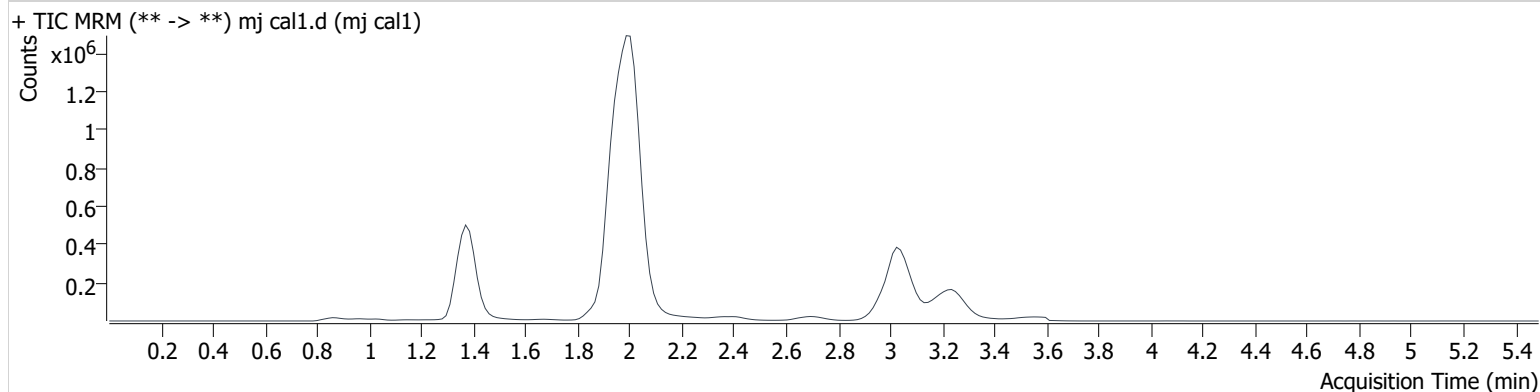
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 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	3/9/2021 7:17:49 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	6071	∞	1662.8 High	∞	1855671	1.588 ng/ml Low
THC-COOH	1.399	50718	713.5	21.0	240.8	569141	5.245 ng/ml
THC	3.061	26083	∞	24.9	∞	1214681	1.170 ng/ml

AM #27 Cannabinoids

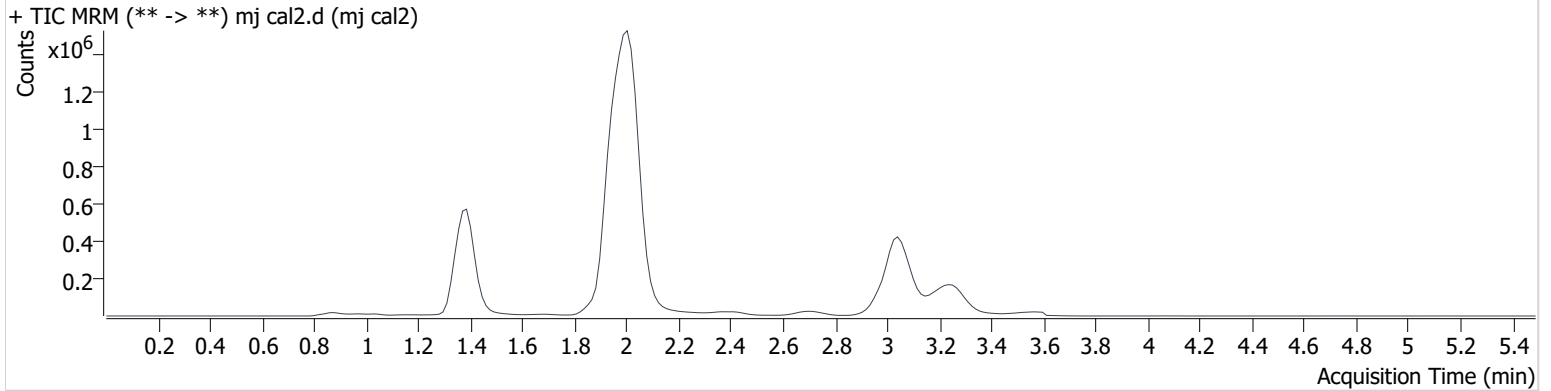
BWylie

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Calibration Last Update 3/10/2021 7:17:03 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	3/9/2021 7:24:33 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	17420	∞	924.2	∞	2012624	3.189 ng/ml
THC-COOH	1.399	105598	∞	23.6	∞	625000	9.227 ng/ml
THC	3.061	92035	∞	26.1	∞	1396293	2.840 ng/ml

AM #27 Cannabinoids

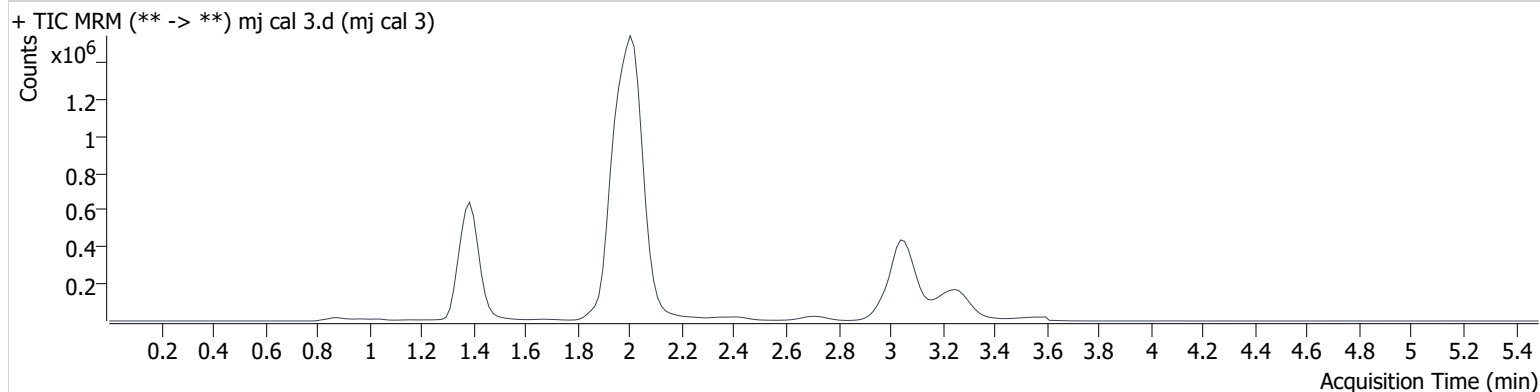
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Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 PM

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	3/9/2021 7:31:17 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	30434	∞	1042.9	∞	1991135	5.161 ng/ml
THC-COOH	1.414	238468	∞	22.8	∞	621536	19.935 ng/ml
THC	3.077	165526	∞	24.0	5744803 9827106 .5	1428695	4.716 ng/ml

AM #27 Cannabinoids

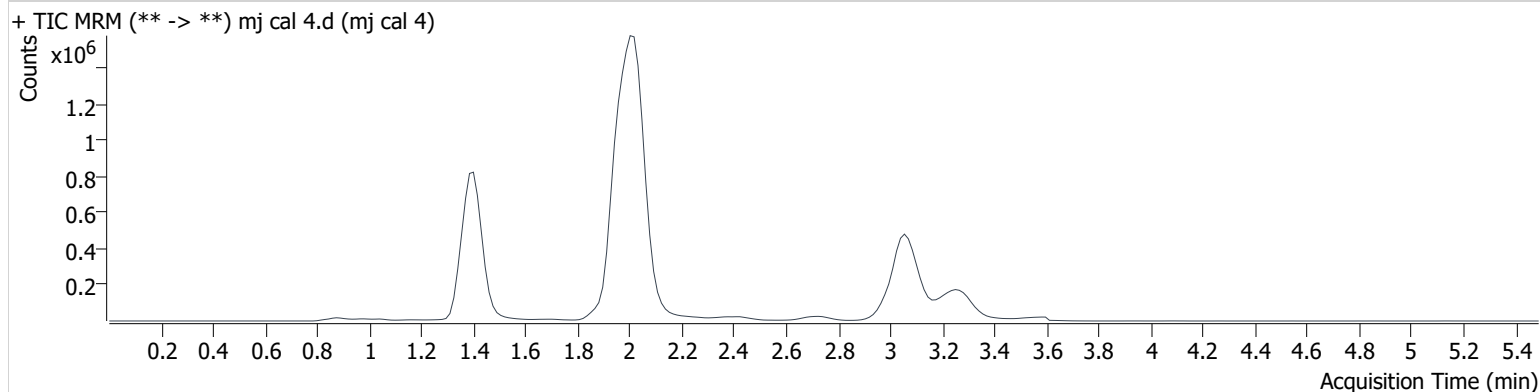
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Calibration Last Update 3/10/2021 7:17:03 PM

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	3/9/2021 7:38:02 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	59353	∞	993.7	∞	1956772	9.638 ng/ml
THC-COOH	1.414	632813	∞	23.7	∞	638700	50.211 ng/ml
THC	3.092	356026	∞	23.9	∞	1444199	9.625 ng/ml

AM #27 Cannabinoids

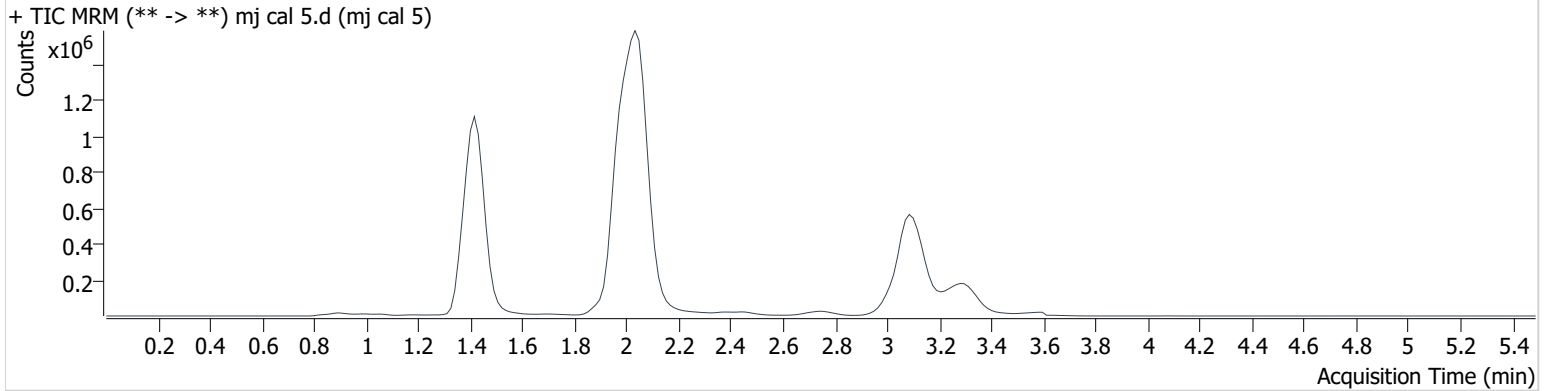
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Calibration Last Update 3/10/2021 7:17:03 PM

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	3/9/2021 7:44:45 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	150394	∞	921.2	∞	1971137	23.311 ng/ml
THC-COOH	1.444	1010648	2188.4	22.4	3171.1	641456	79.374 ng/ml
THC	3.122	918531	∞	23.2	∞	1448017	24.195 ng/ml

AM #27 Cannabinoids

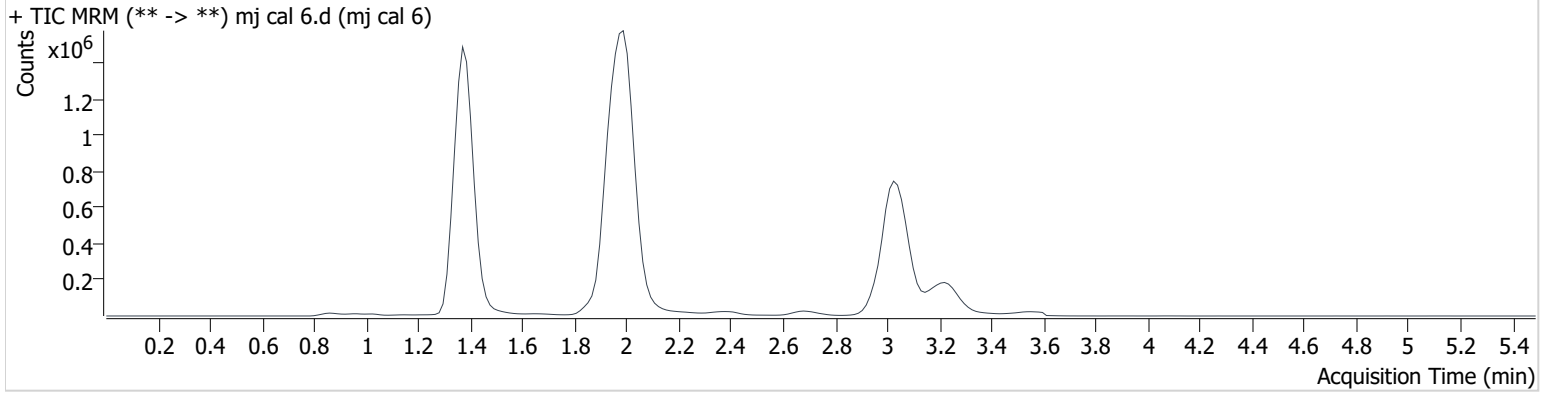
BWylie

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Calibration Last Update 3/10/2021 7:17:03 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	3/9/2021 7:51:29 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	294976	∞	864.5	∞	1807967	49.148 ng/ml
THC-COOH	1.399	1155827	∞	22.5	∞	596048	97.506 ng/ml
THC	3.046	1840530	∞	23.4	∞	1407338	49.497 ng/ml

AM #27 Cannabinoids

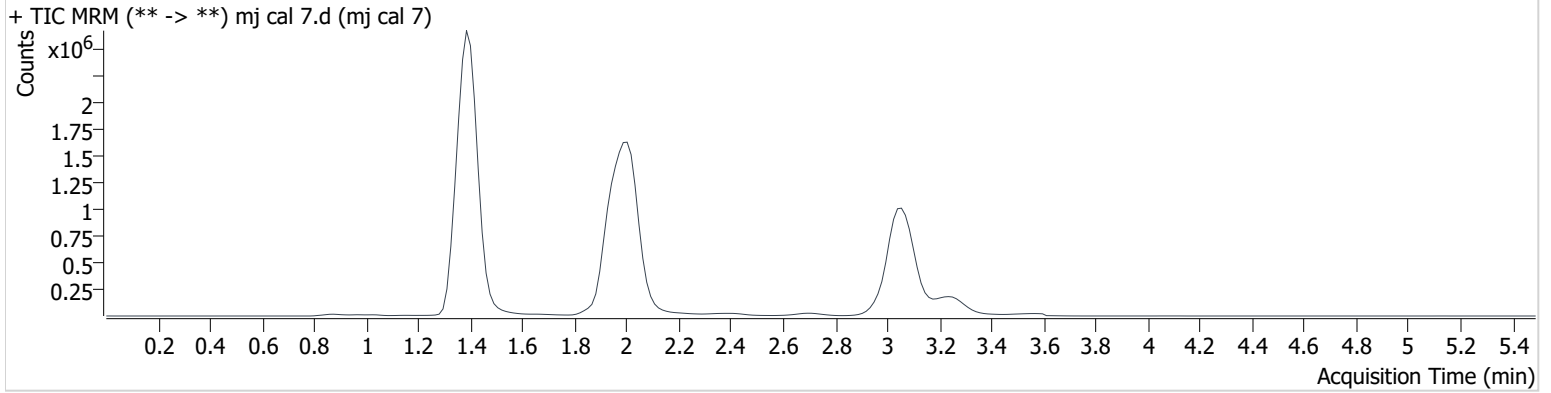
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\030921\QuantResults\thcq.batch.bin
Calibration Last Update 3/10/2021 7:17:03 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	3/9/2021 7:58:15 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	644192	∞	833.9	∞	1879852	102.553 ng/ml
THC-COOH	1.399	3014799	∞	23.6	∞	606975	248.502 ng/ml
THC	3.061	3685361	∞	23.9	∞	1362850	101.956 ng/ml

am 27
3/9/21 worklist 4837

BWylee

	1	2	3	4	5	6
A	IS + Cal. 1	neg blood	505			IS + QC_1
B	IS + Cal. 2	360	528			IS + Cal. 7
C	IS + Cal. 3	398	neg urine			IS + Cal. 6
D	IS + Cal. 4	428	urine ctrl			IS + Cal. 5
E	IS + Cal. 5	430	345			IS + Cal. 4
F	IS + Cal. 6	431	418			IS + Cal. 3
G	IS + Cal. 7	489	491			IS + Cal. 2
H	IS + QC_1	503				IS + Cal. 1

All wells to contain 100 µl of residual DMSO

Case #: C2021-0 -1