









GA

4/1/2021

REVIEWED
By Brittany Wylie at 10:01 am, Apr 02, 2021

Worklist: 4873

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2021-0537	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0582	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0605	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-0632	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-0634	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0652	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-0668	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-0678	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

~~A~~

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

Extraction Date 3/30/21
Plate lot#: 201206

Analyst: Anne Nord
Plate Expiration: 06/06/2021

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: 20J20793 **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 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:

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	1	2	3	4	5	6
a	cal 1 cal 100 ng	neg blood	605-1			QC 1
b	cal 2 cal 50 ng	537-1	632-1			cal 100 ng
c	cal 3 cal 25 ng	582-1	668-1			cal 50 ng
d	cal 4 cal 10 ng	634-1				cal 25 ng
e	cal 5 cal 5 ng	652-1				cal 10 ng
f	cal 6 cal 3 ng	678-1				cal 5 ng
g	cal 7 cal 1 ng	neg urine				cal 3 ng
h	QC 1	excon urine				cal 1 ng

C2021-0__-__

Toxicology AM method 27/26 external prep information



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	

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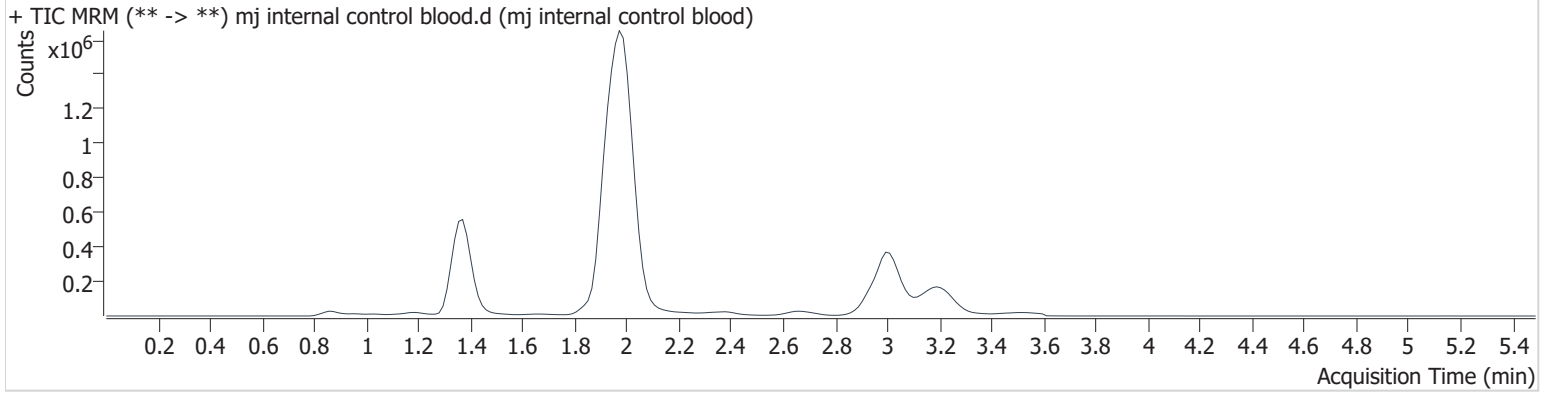
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 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	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 11:28:07 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	235117	∞	8.8	∞	1658653	4.554 ng/ml
THC-COOH	1.386	133148	453.1	34.9	10464 7.6	684135	14.401 ng/ml
THC	3.016	100414	∞	23.4	710.2	1059657	3.899 ng/ml

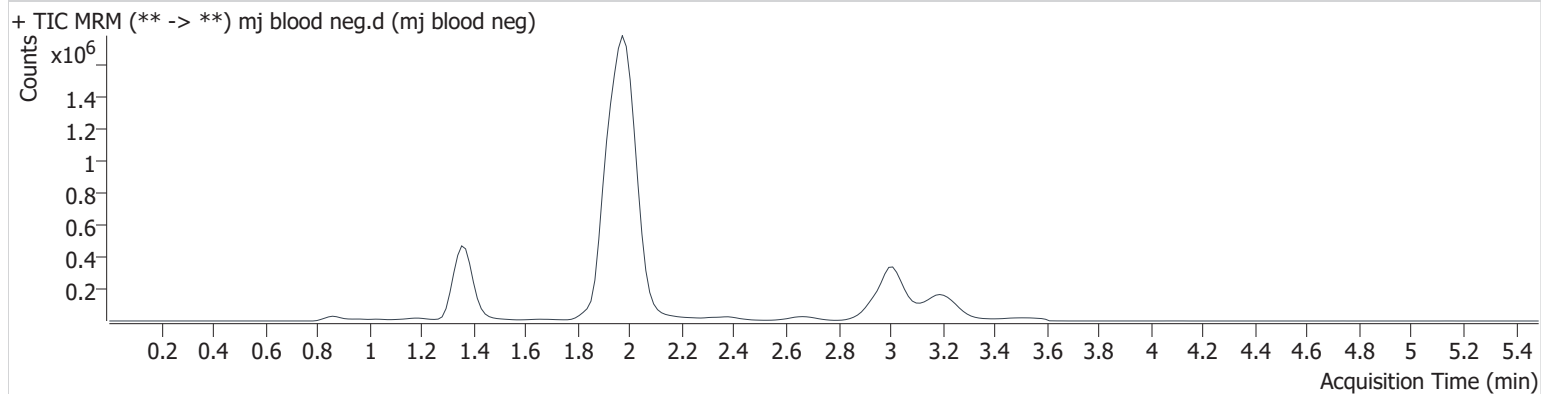
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj blood neg.d
Type	Sample	Sample	mj blood neg
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 11:34:51 PM		
Sample Info.			

Sample Chromatogram



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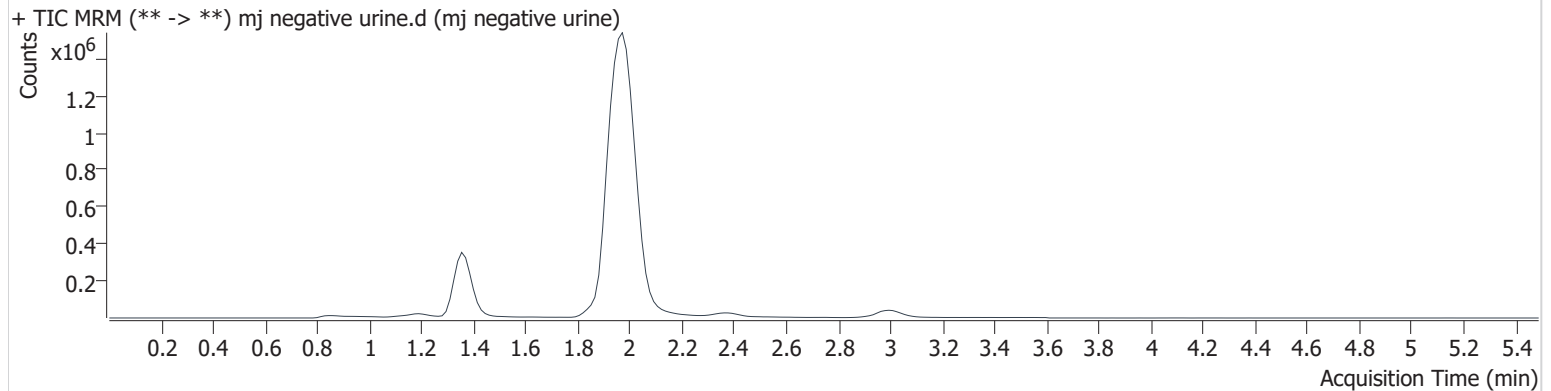
AM #27 Cannabinoids

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Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G2	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2021 12:48:37 AM		

Sample Info.

Sample Chromatogram



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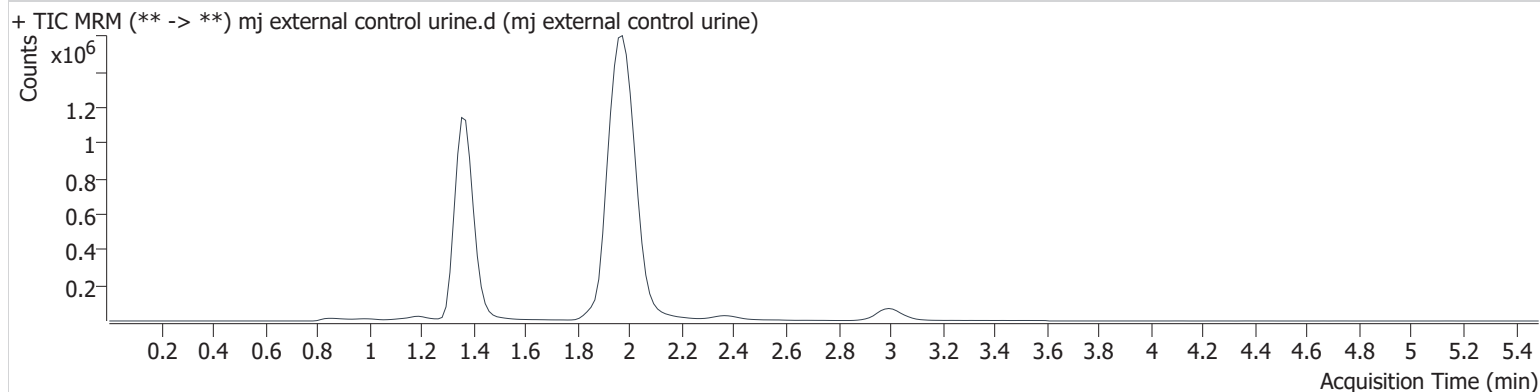
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj external control urine.d
Type	Sample	Sample	mj external control urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H2	Comment	
Injection Volume	10		
Acq. Date-Time	3/31/2021 12:55:21 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.361	2517810	∞	12.1	∞	1659725	53.290 ng/ml
THC-COOH	1.386	302573	470724.4	36.0	478.3	494659	43.967 ng/ml
THC	3.001	126129	∞	24.1	609.8	325381	14.781 ng/ml

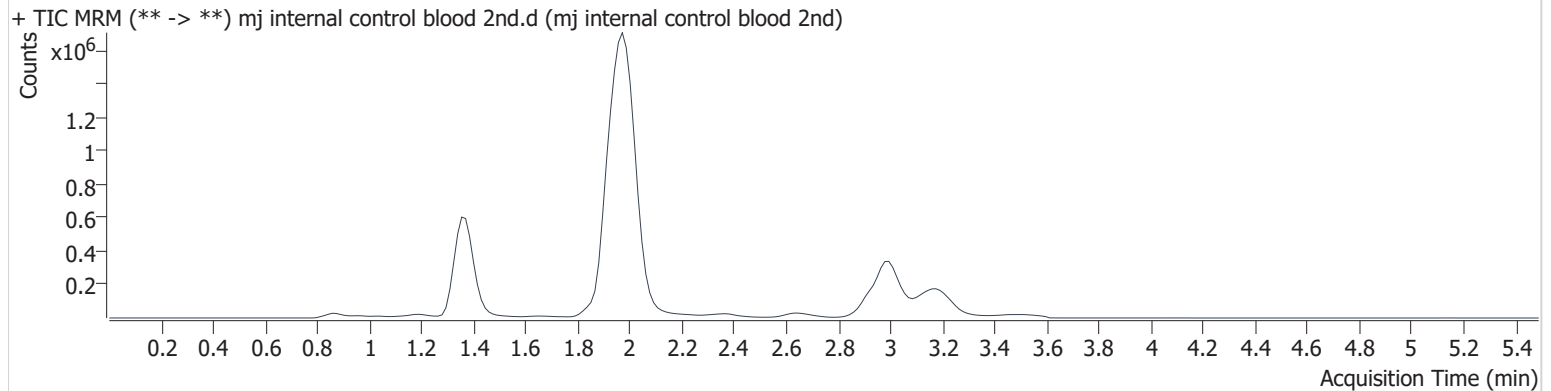
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 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** Anne Nord
Sample Position P3-H1 **Comment**
Injection Volume 10
Acq. Date-Time 3/31/2021 1:42:17 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.361	248222	∞	8.6	∞	1780195	4.472 ng/ml
THC-COOH	1.386	136839	621.0	36.4	272.0	735470	13.794 ng/ml
THC	3.001	101391	∞	25.5	87925 05579 656.5	1045283	3.983 ng/ml

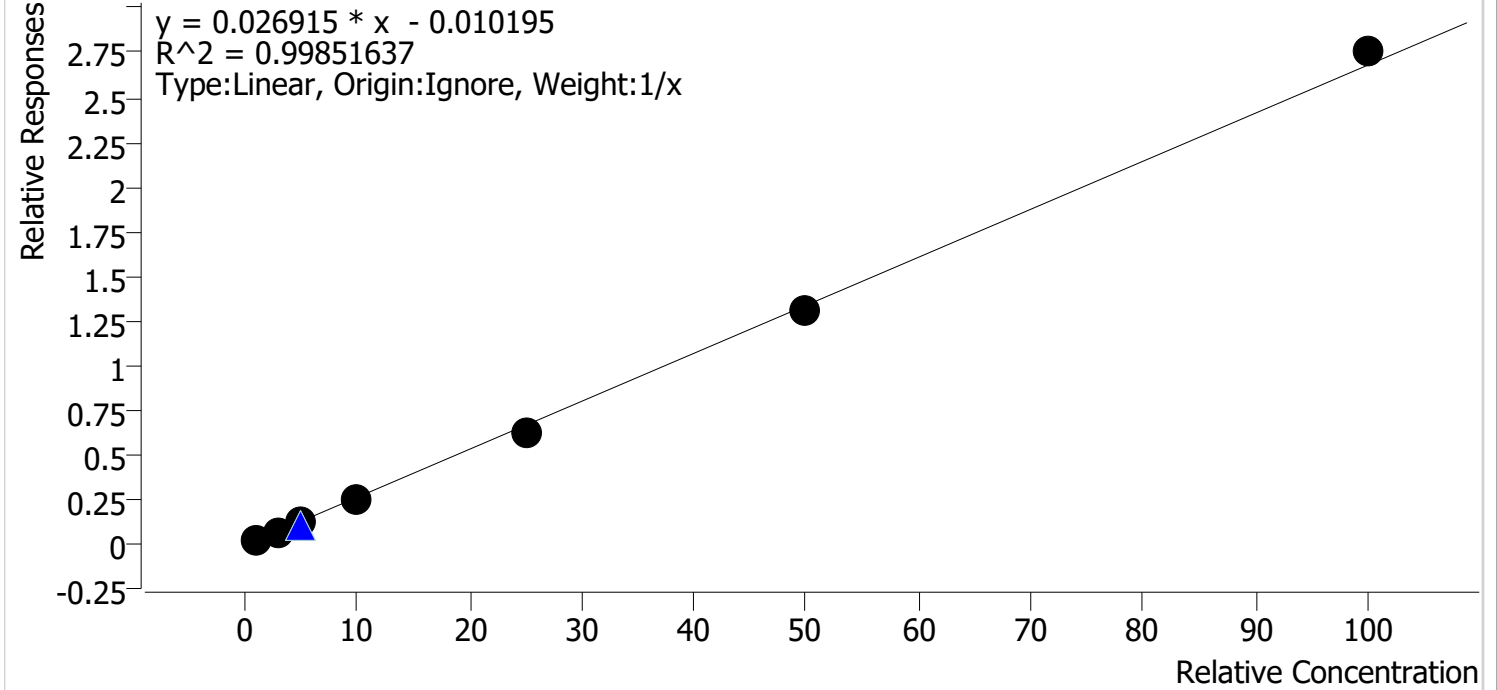
Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Last Cal. Update 3/31/2021 11:46 AM
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	117.7
mj cal2	2	✓	3.0	2.8	92.6
mj cal 3	3	✓	5.0	5.0	99.2
mj cal 4	4	✓	10.0	9.5	95.0
mj cal 5	5	✓	25.0	23.6	94.2
mj cal 6	6	✓	50.0	49.2	98.5
mj cal 7	7	✓	100.0	102.8	102.8

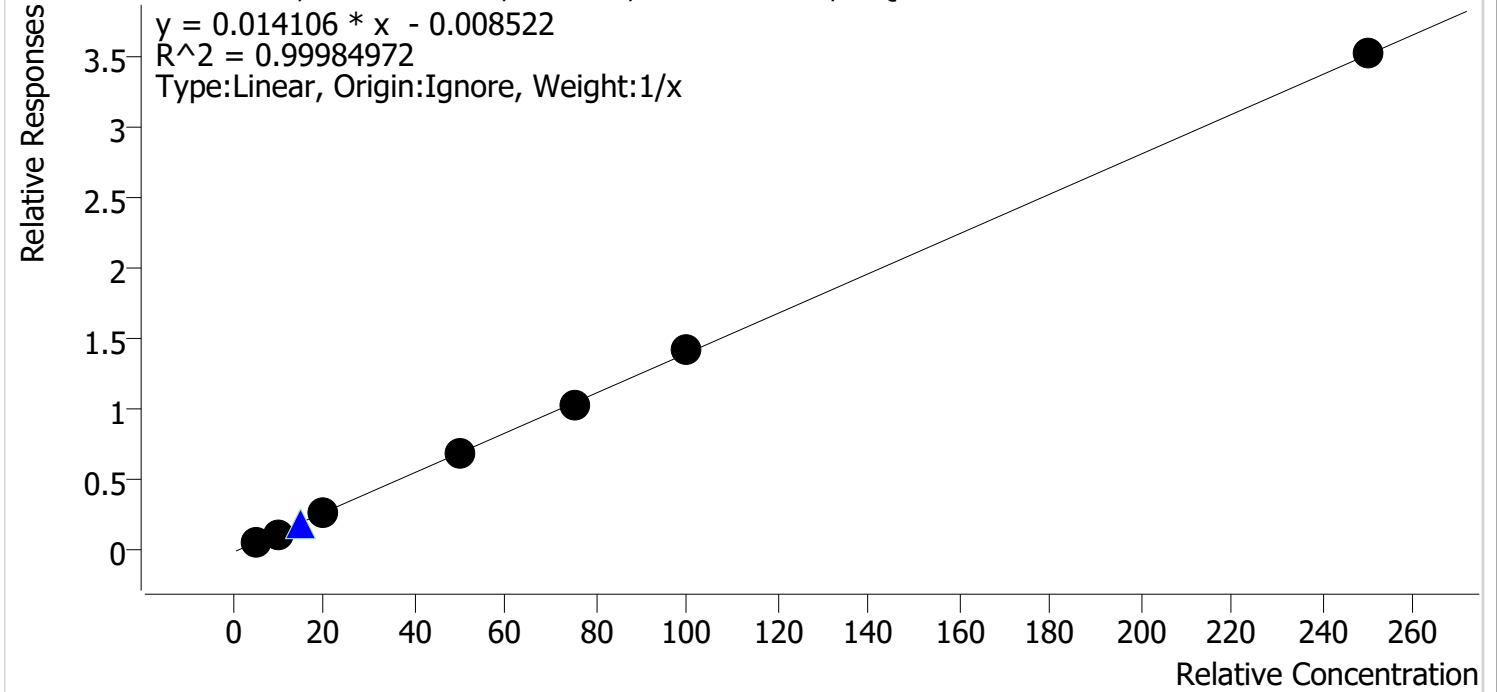
Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Last Cal. Update 3/31/2021 11:46 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, 2 QCs



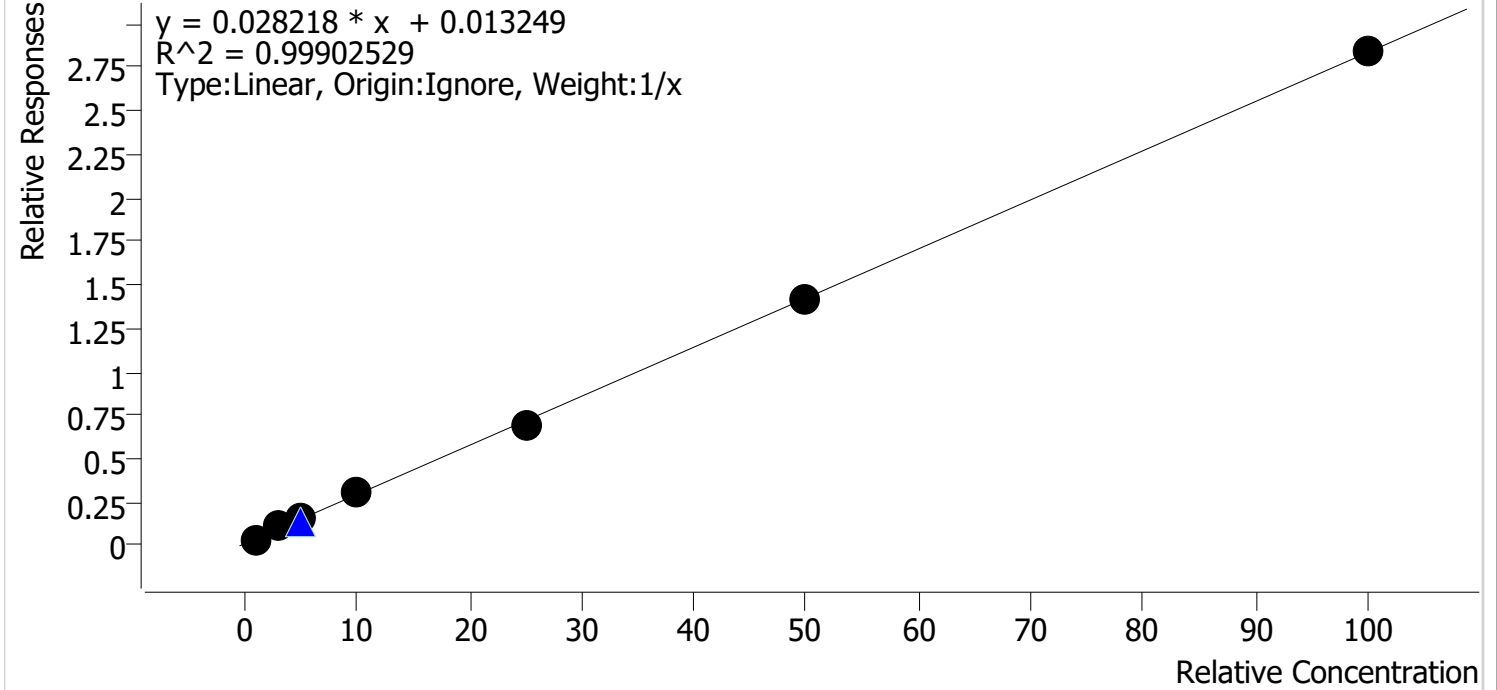
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.3	105.5
mj cal2	2	✓	10.0	9.6	95.6
mj cal 3	3	✓	20.0	20.0	99.9
mj cal 4	4	✓	50.0	49.3	98.6
mj cal 5	5	✓	75.0	74.4	99.2
mj cal 6	6	✓	100.0	101.0	101.0
mj cal 7	7	✓	250.0	250.5	100.2

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Last Cal. Update 3/31/2021 11:46 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, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	0.8	78.7
mj cal2	2	✓	3.0	3.4	114.4
mj cal 3	3	✓	5.0	5.4	107.1
mj cal 4	4	✓	10.0	10.3	103.0
mj cal 5	5	✓	25.0	24.3	97.4
mj cal 6	6	✓	50.0	49.7	99.3
mj cal 7	7	✓	100.0	100.1	100.1

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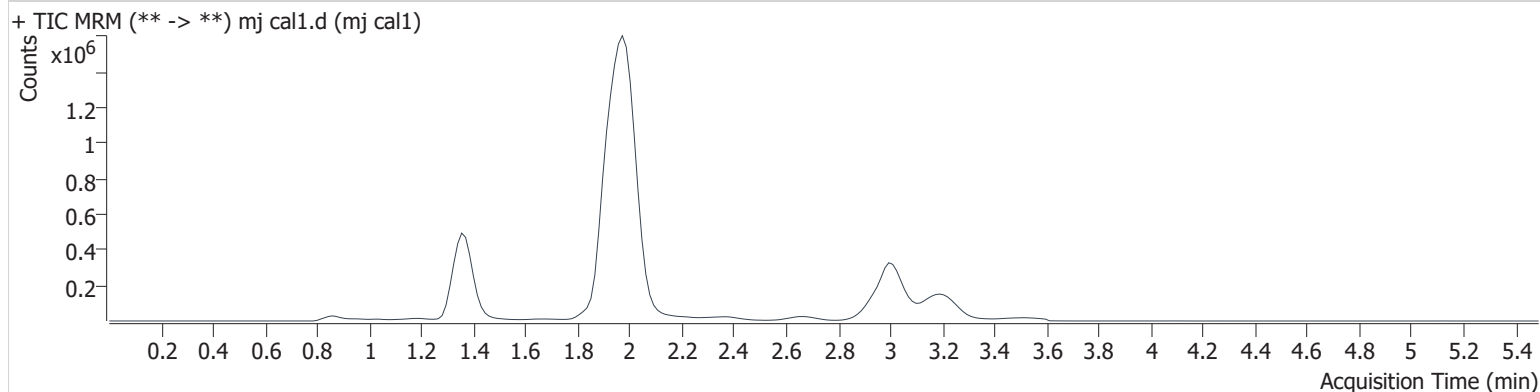
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj cal1.d
Type	Cal	Sample	mj cal1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 10:41:00 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	62661	∞	9.7	∞	1766654	0.787 ng/ml Low
THC-COOH	1.386	45419	55.7	32.0	64.0	689487	5.274 ng/ml
THC	3.016	21188	378.6	26.1	∞	986578	1.177 ng/ml

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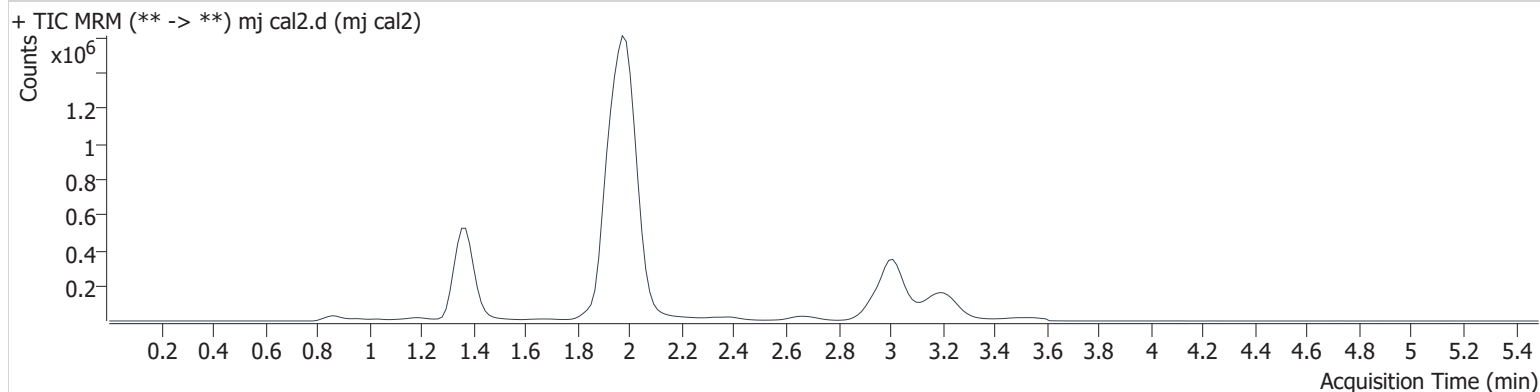
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 10:47:44 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	190732	∞	8.4	∞	1732759	3.431 ng/ml
THC-COOH	1.386	86984	115.8	36.4	118.0	688210	9.564 ng/ml
THC	3.016	67063	1585.5	26.7	188.5	1038186	2.779 ng/ml

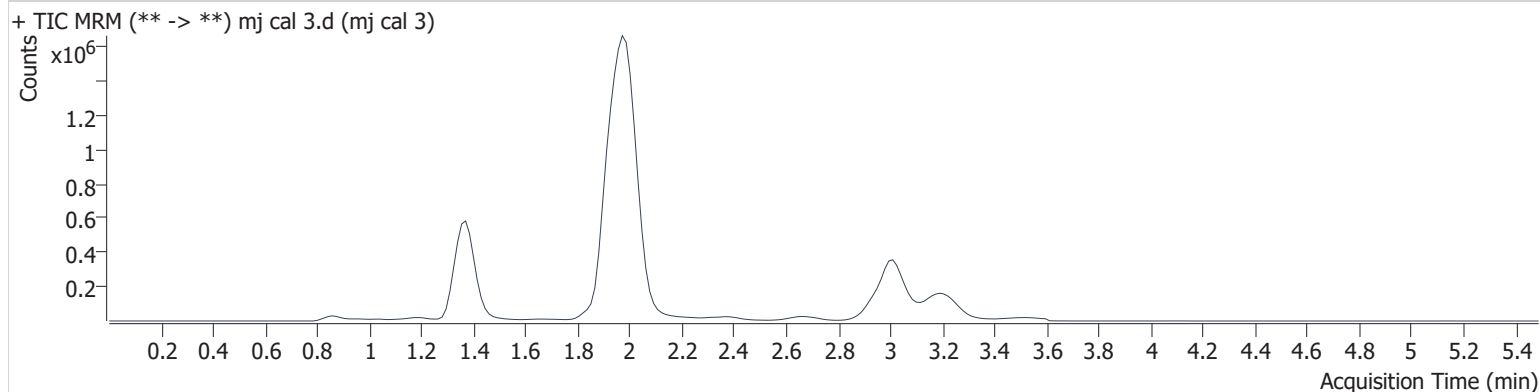
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-C1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 10:54:28 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.376	277335	∞	9.2	∞	1687309	5.355 ng/ml
THC-COOH	1.386	188501	248935.5	34.7	1903.9	690010	19.971 ng/ml
THC	3.031	125535	∞	24.5	1016.4	1018094	4.960 ng/ml

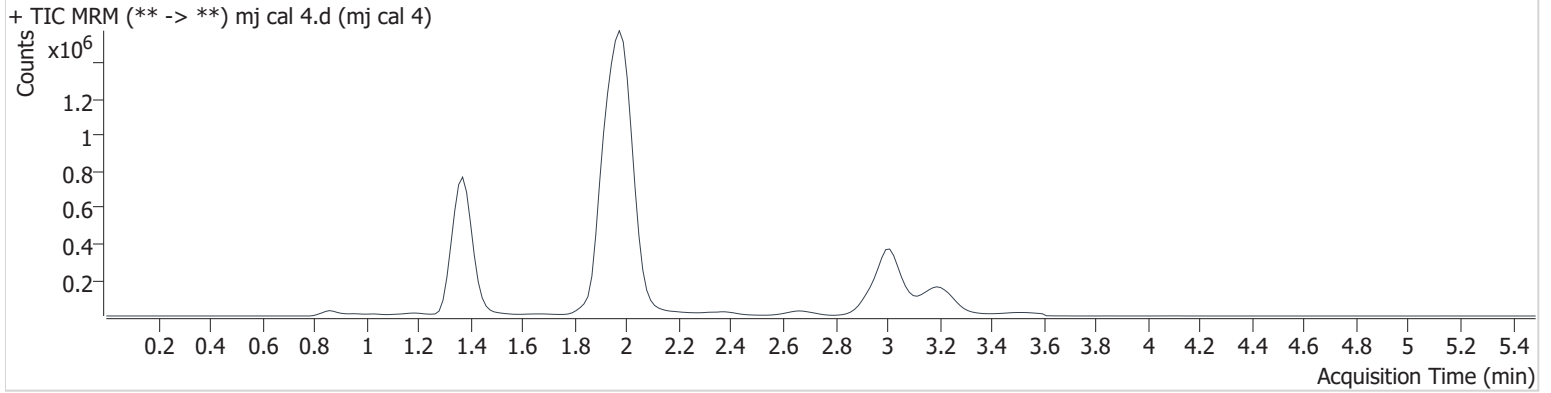
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj cal 4.d
Type	Cal	Sample	mj cal 4
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 11:01:11 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.361	503481	∞	9.9	∞	1657232	10.297 ng/ml
THC-COOH	1.386	466736	484.8	36.3	1426.5	679299	49.312 ng/ml
THC	3.016	241897	∞	24.1	∞	985109	9.502 ng/ml

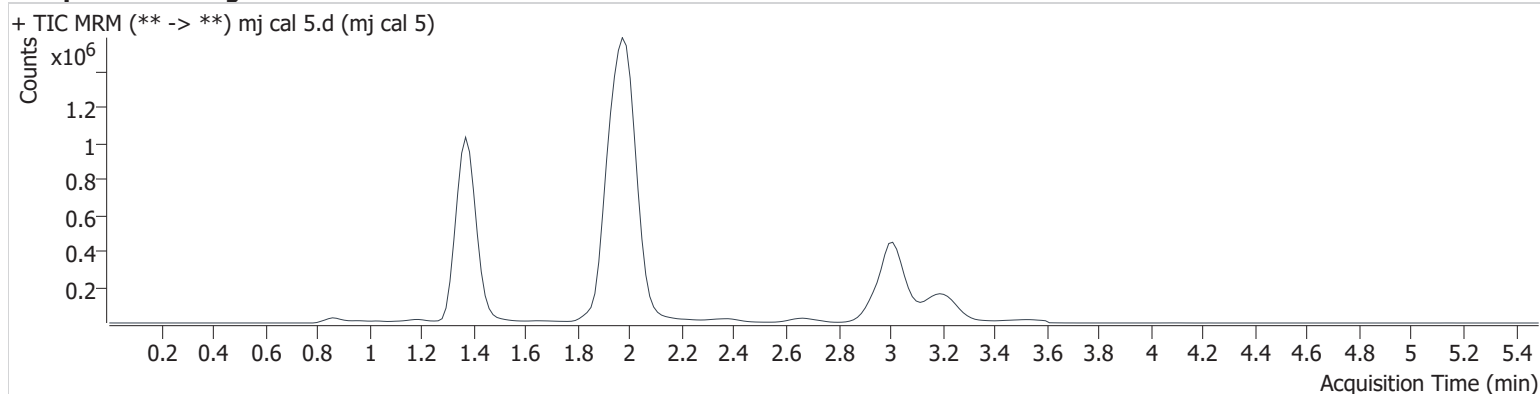
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 11:07:55 PM		

Sample Info.

Sample Chromatogram



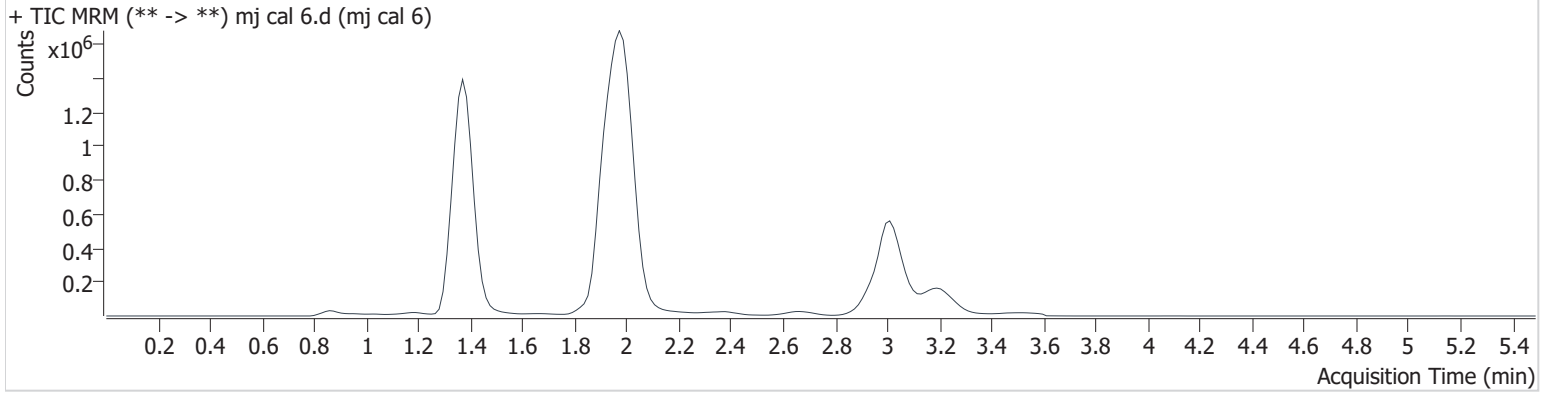
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.361	1163165	∞	11.0	∞	1661532	24.339 ng/ml
THC-COOH	1.386	700823	924458.3	36.6	602.3	672949	74.431 ng/ml
THC	3.016	626443	∞	23.4	∞	1004408	23.551 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj cal 6.d
Type	Cal	Sample	mj cal 6
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-F1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 11:14:39 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.361	2379957	∞	11.3	∞	1682474	49.660 ng/ml
THC-COOH	1.386	953885	4187.9	36.9	1981.4	673764	100.968 ng/ml
THC	3.016	1305685	∞	23.0	∞	992692	49.247 ng/ml

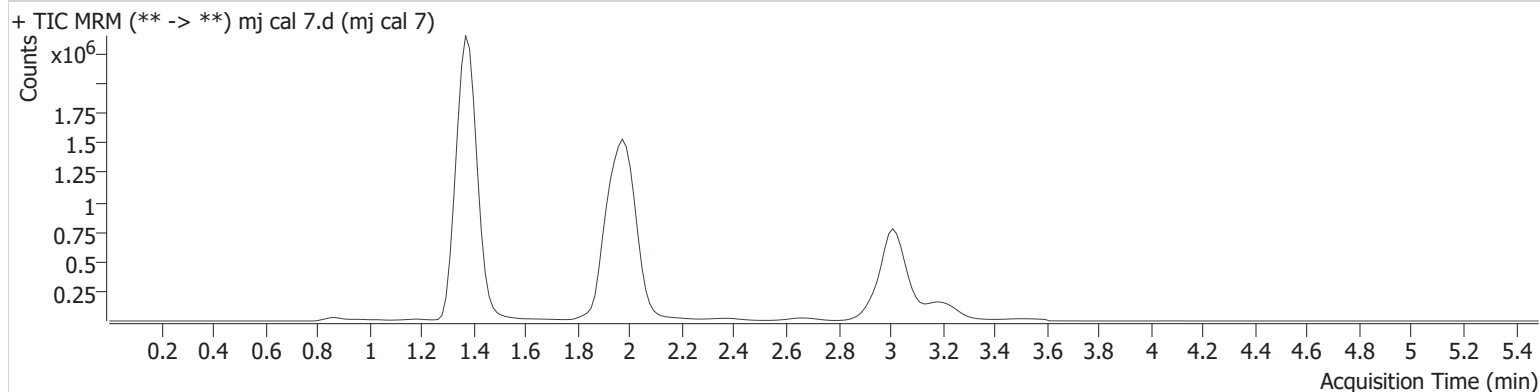
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\033021\QuantResults\thc.batch.bin
Calibration Last Update 4/1/2021 1:53:49 PM

Instrument	69679	Data File	mj cal 7.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2021 11:21:24 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.361	4430054	∞	11.8	∞	1560579	100.130 ng/ml
THC-COOH	1.386	2183462	1376.3	37.4	2192.9	619457	250.480 ng/ml
THC	3.016	2598809	∞	23.3	∞	942867	102.785 ng/ml