REVIEWED
By Tamara Salazar at 11:56 am, Aug 14, 2020

8/12/2020 BW

Worklist: 4429

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2020-1484	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1492	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1495	10	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1507	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1533	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1543	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1553	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1560	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1572	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1574	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-2504	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-2597	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-2601	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1679	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1919	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1935	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1961	2	BCK	AM 27 Blood THC Quant by LC-QQQ

BW

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

Extraction Date: 8/12/20 Analyst: Britany Wylie
Plate lot#: 200303 Plate Expiration: 9-3-2020
Mobile phase A: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
MTBE LCMS Methanol Hexane
Blank Blood Lot: 20G20792 **Urine Blank:** 73020 **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: ~~10~~ 5 BW 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: Curves limited: THC-OH 3-100

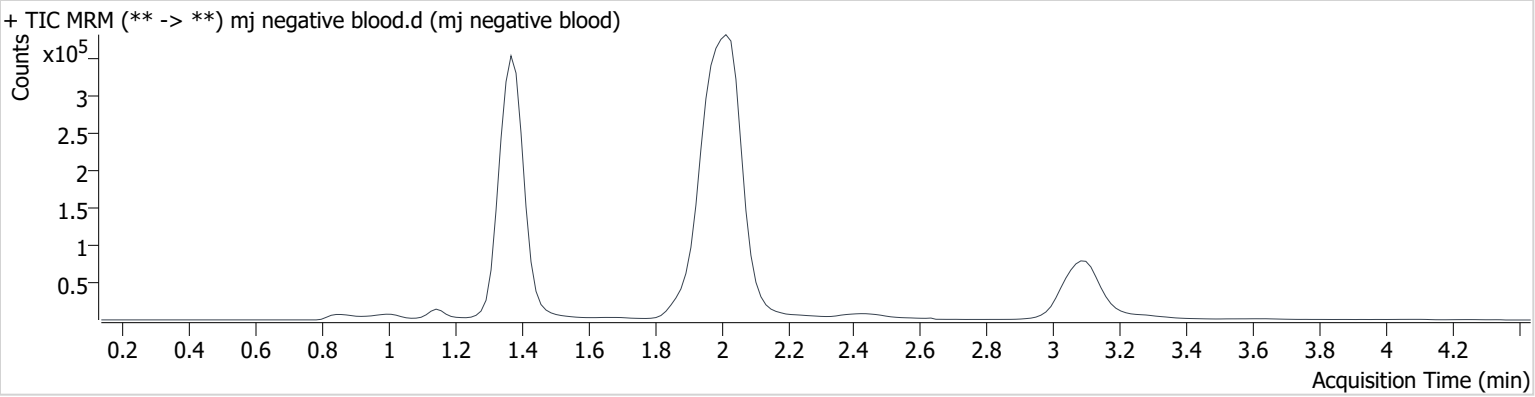
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 PM

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-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/12/2020 1:13:33 PM		
Sample Info.			

Sample Chromatogram



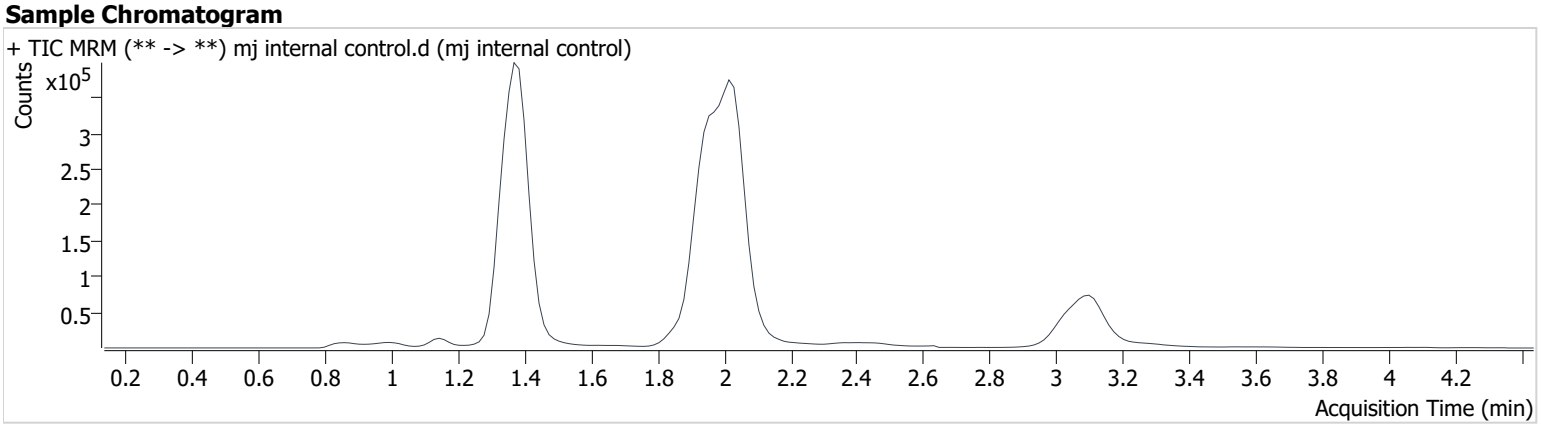
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 PM

Instrument	69679	**Data File**	mj internal control.d
Type	QC	**Sample**	mj internal control
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/12/2020 1:05:51 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	72797	∞	12.2	52.8	1178094	4.455	ng/ml
THC-COOH	1.401	123757	875.9	36.1	321.6	685671	15.138	ng/ml
THC	3.104	41695	∞	27.3	∞	564786	4.265	ng/ml

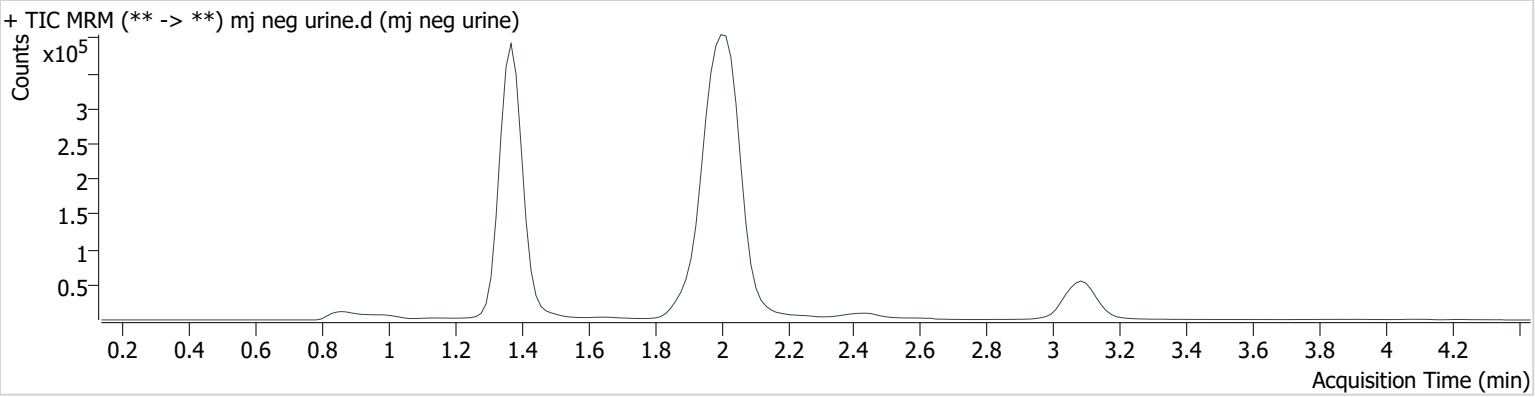
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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-A4	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/12/2020 5:11:44 PM		
Sample Info.			

Sample Chromatogram



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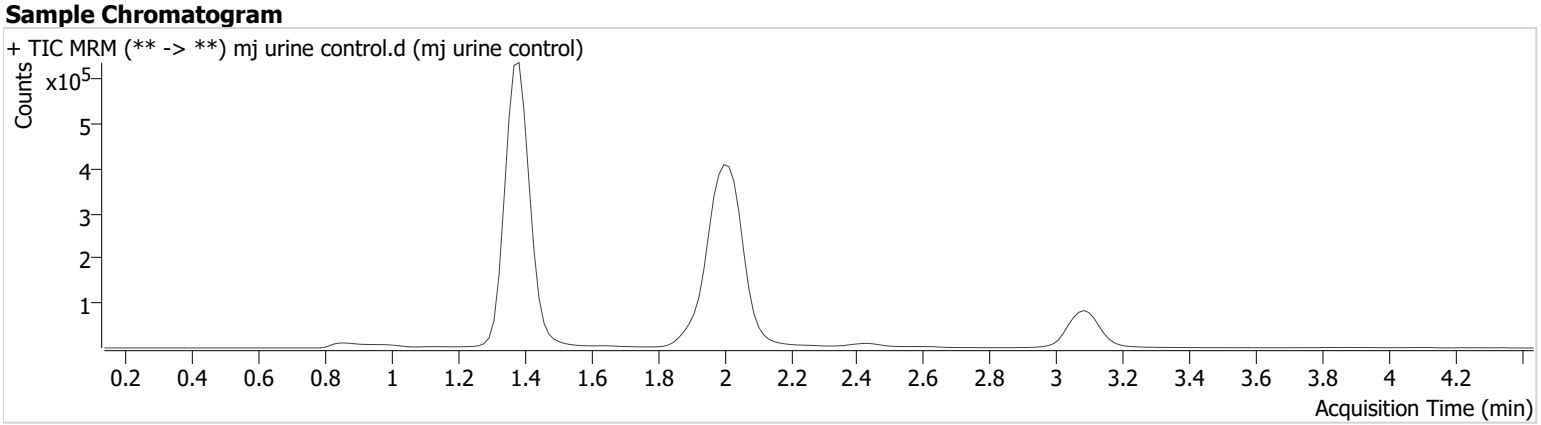
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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-B4	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/12/2020 5:19:28 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	341898	565.3	12.9	655.6	1408581	15.378	ng/ml
THC-COOH	1.401	309596	1252.1	36.2	208.9	616886	39.727	ng/ml
THC	3.104	76099	∞	23.8	∞	478992	8.758	ng/ml

Toxicology AM method 27/26 external prep information

BW

working solution 1 ug/ml in meoh C-THC, THC-OH, THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 75 ulTHC-OH in 9767.5 ul meOH
Ppd 2/13/20 Exp: 8/13/20 lot 21320 by AMN

Drug	lot	expiration
C-THC	FE07171501	9/1/2020
THC-OH	~~FE07721601~~	7/1/2021
THC	FE001041701	3/1/2022

THC-OH Lot # FE07221601
BW 8-14-20

AM 27/26 blood control 100 ul working solution lot (91319) in 9900 ul blood lot (20A52255) Out of use

ppd 02/13/20 Exp 08/13/20	lot b81320	Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-THC	by AMN	

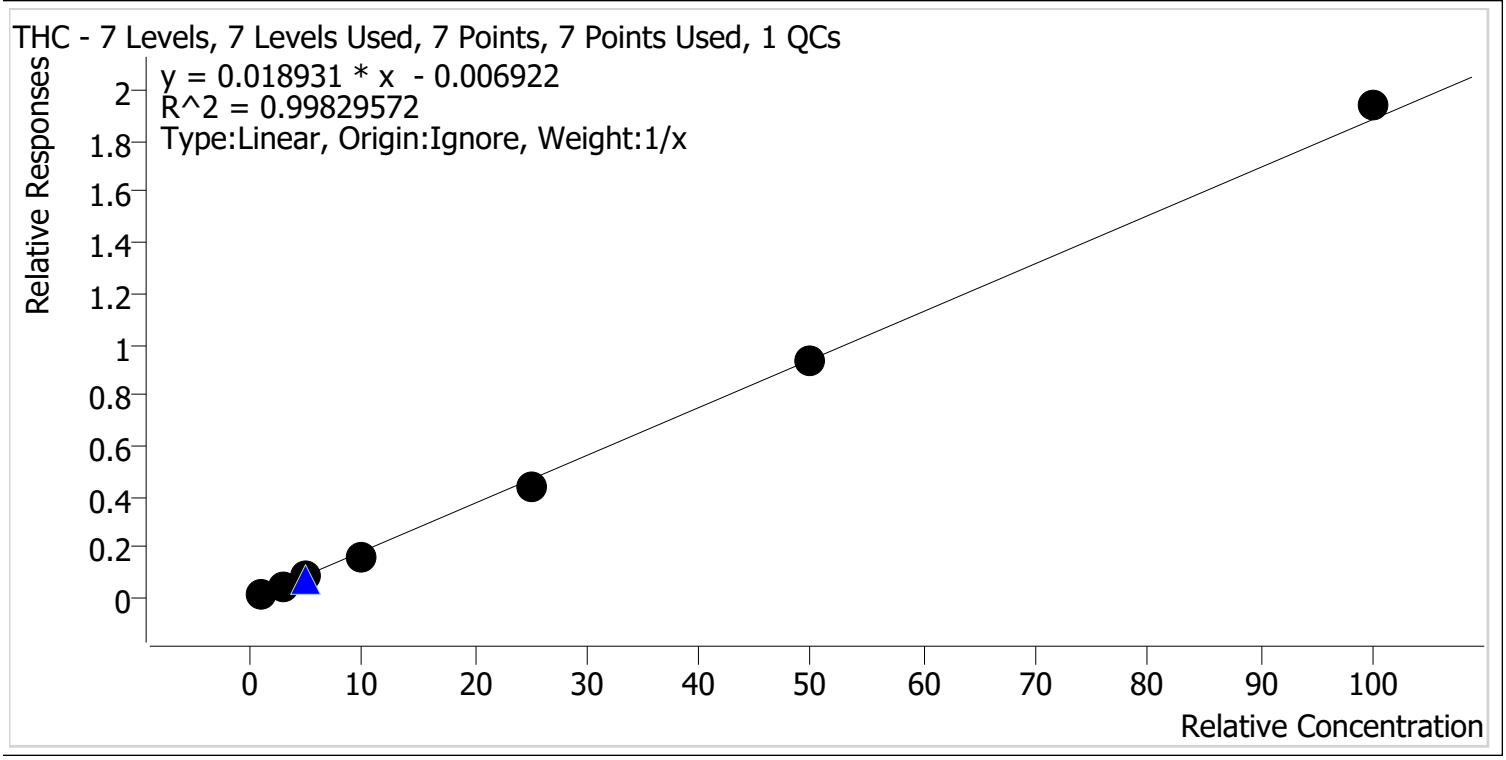
AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (6920) out of use

ppd 4/17/20 Exp 9120	lot u101720	Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC	by BAW	6/8/2020
ppd 6/9/20 exp 8/13/20	lot 6920	Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC	by amn	7/15/2020
ppd 2.5mL 7/17/20 one time use	lot 71720	Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC	by baw	7/17/2020
ppd 7/30/20 exp 8/13/20 (urine lot 73020)	lot u81320	Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC	by amn	

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Last Cal. Update	8/12/2020 9:32 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3

BW



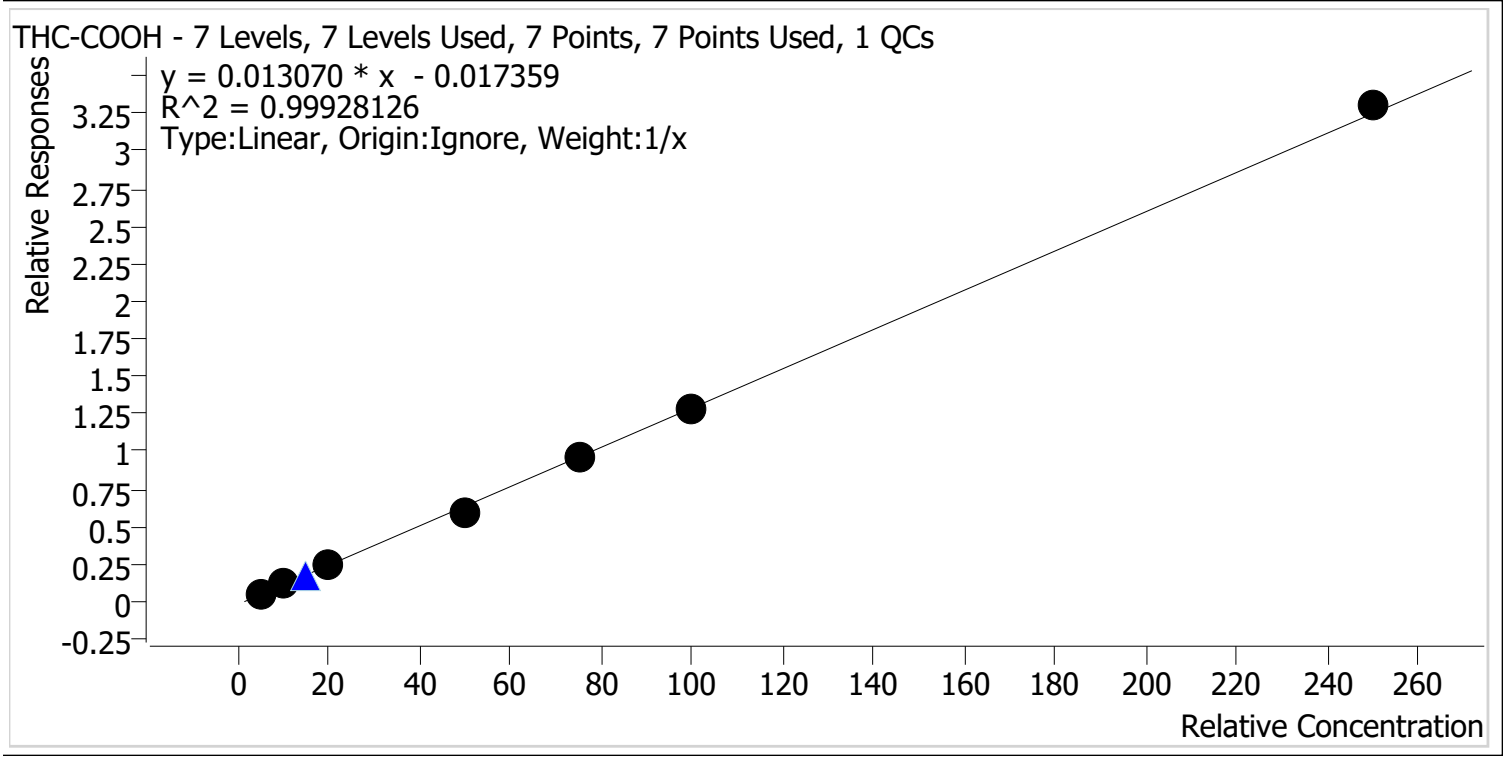
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✔	1.0	1.2	120.3
mj cal2	2	✔	3.0	2.9	96.9
mj cal 3	3	✔	5.0	4.7	94.4
mj cal 4	4	✔	10.0	9.2	91.8
mj cal 5	5	✔	25.0	23.7	94.8
mj cal 6	6	✔	50.0	49.4	98.8
mj cal 7	7	✔	100.0	102.9	102.9

Compound Calibration Report

Agilent | Trusted Answers

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Last Cal. Update	8/12/2020 9:32 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9

BW

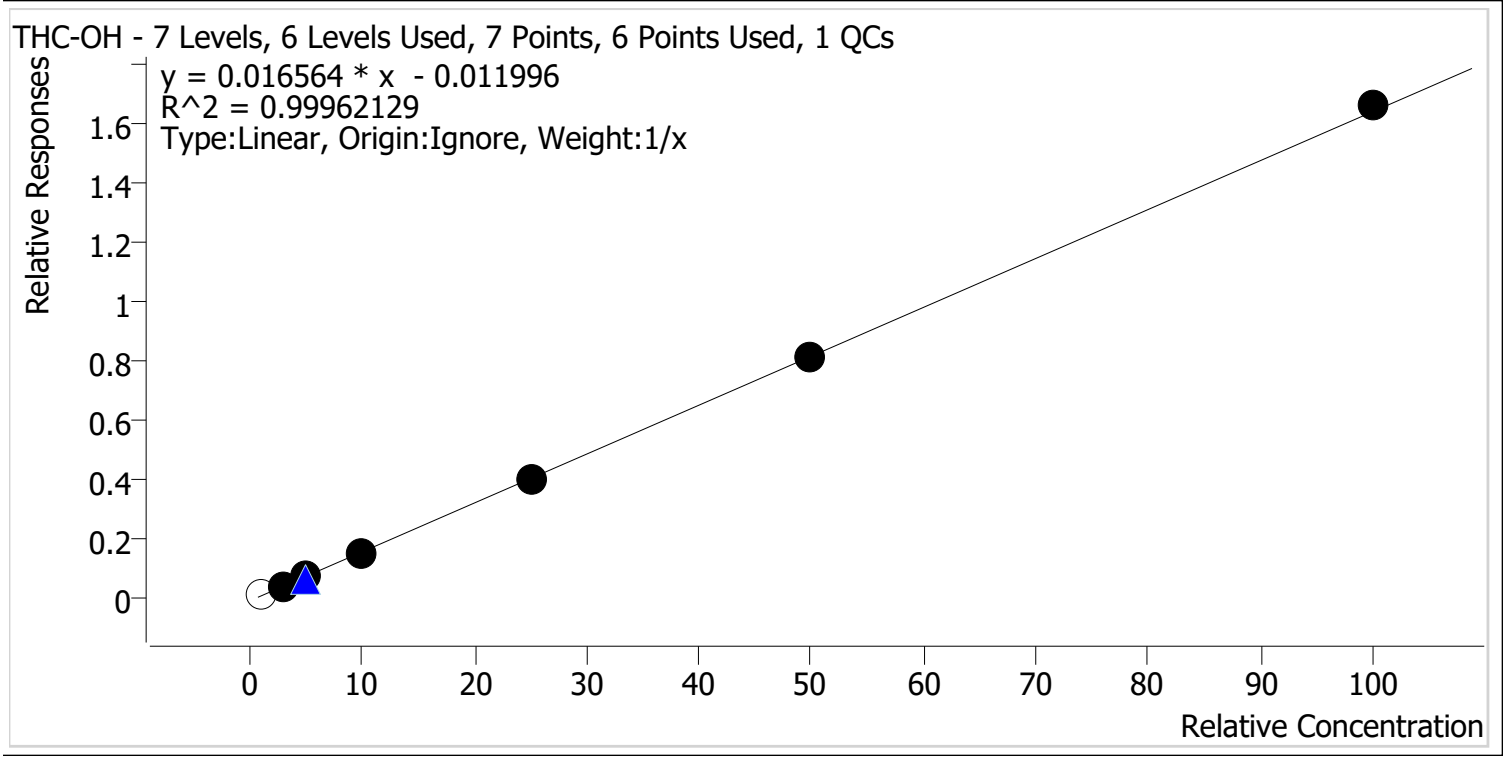


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✔	5.0	5.4	107.7
mj cal2	2	✔	10.0	9.9	99.4
mj cal 3	3	✔	20.0	19.7	98.5
mj cal 4	4	✔	50.0	47.1	94.1
mj cal 5	5	✔	75.0	74.2	99.0
mj cal 6	6	✔	100.0	99.7	99.7
mj cal 7	7	✔	250.0	254.0	101.6

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Last Cal. Update	8/12/2020 9:32 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3

BW



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✗	1.0	1.5	145.6
mj cal2	2	✔	3.0	3.2	106.1
mj cal 3	3	✔	5.0	5.0	99.2
mj cal 4	4	✔	10.0	9.6	96.5
mj cal 5	5	✔	25.0	24.5	98.0
mj cal 6	6	✔	50.0	49.5	99.0
mj cal 7	7	✔	100.0	101.2	101.2

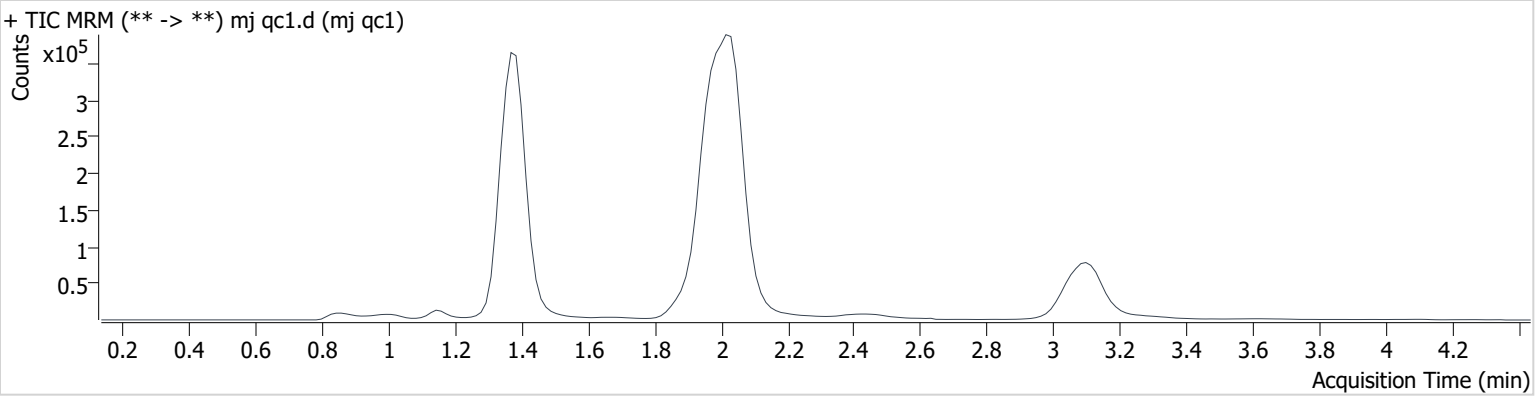
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 PM

Instrument	69679	**Data File**	mj qc1.d
Type	Cal	**Sample**	mj qc1
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/12/2020 12:11:47 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	14431	1.3 **Low**	14.6 **High**	20.5	1189841	1.456	ng/ml **Low**
THC-COOH	1.401	34600	41.8	39.9	318.5	652387	5.386	ng/ml
THC	3.119	9496	∞	24.5	422.7	598789	1.203	ng/ml **Low**

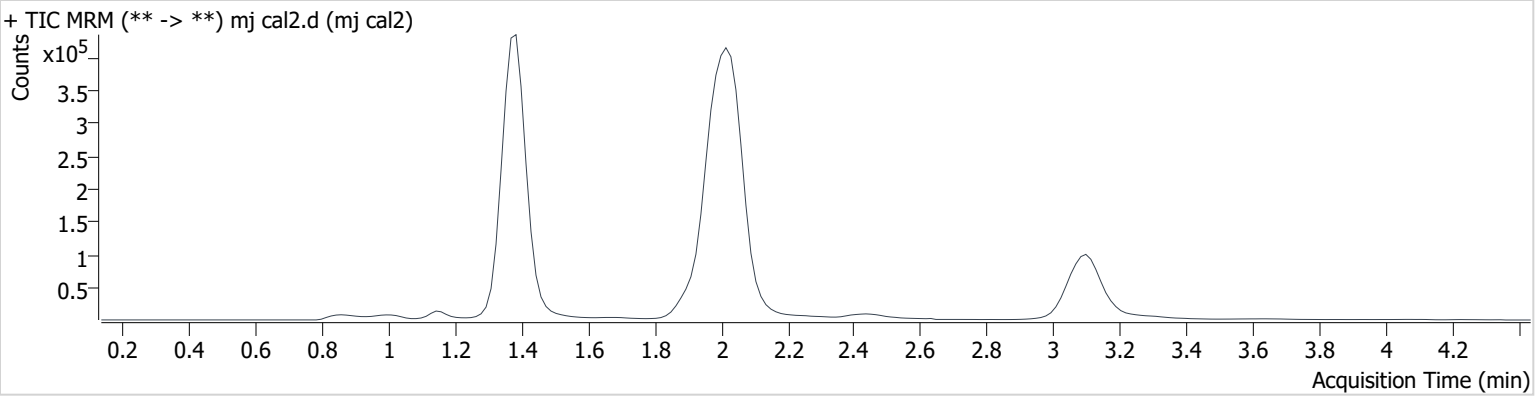
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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	8/12/2020 12:19:30 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
THC-OH	1.378	50618	∞	12.8	∞	1242461	3.184	ng/ml	
THC-COOH	1.401	77270	60175.2	37.6	148.8	686531	9.940	ng/ml	
THC	3.104	31930	∞	26.3	5869954 3063733 .3	663745	2.907	ng/ml	Low

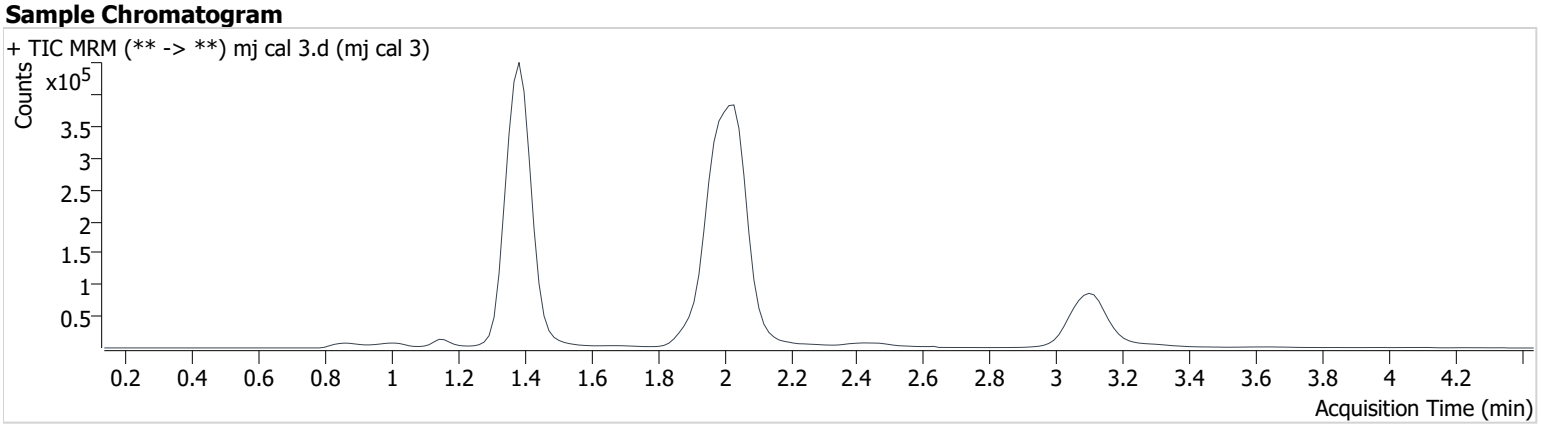
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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	8/12/2020 12:27:13 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	83679	∞	12.5	195.1	1192471	4.961	ng/ml
THC-COOH	1.401	158901	735.8	36.2	52388.9	662038	19.693	ng/ml
THC	3.119	48893	∞	25.5	3761810 2687841 .6	592866	4.722	ng/ml

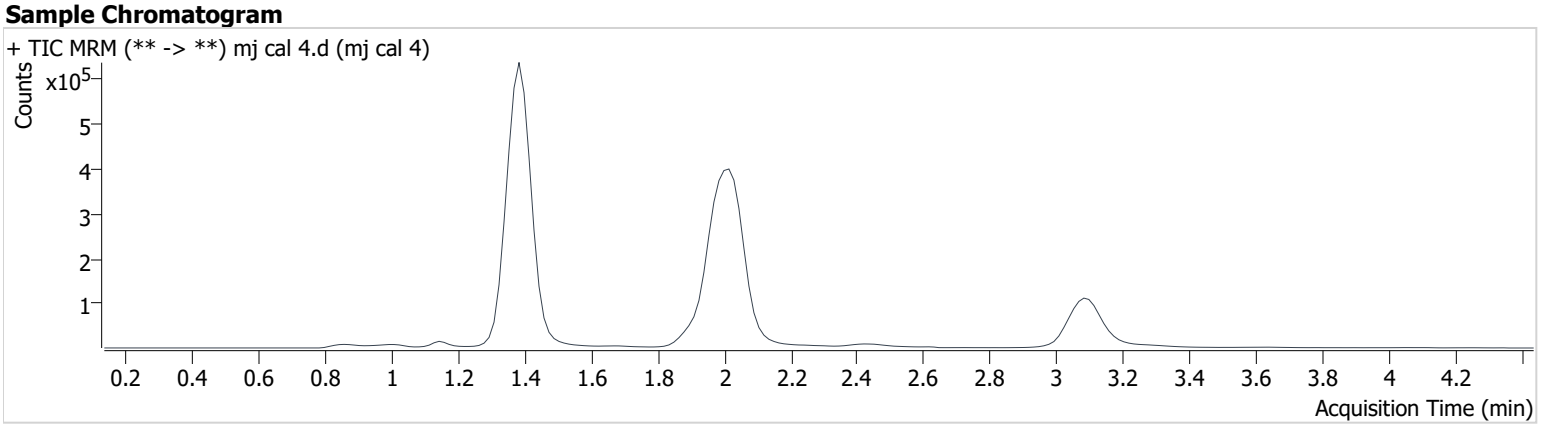
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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	8/12/2020 12:34:57 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	179097	∞	11.4	∞	1211822	9.647	ng/ml
THC-COOH	1.401	397085	2806.3	38.1	1512.6	664173	47.072	ng/ml
THC	3.104	106252	∞	24.3	∞	636790	9.180	ng/ml

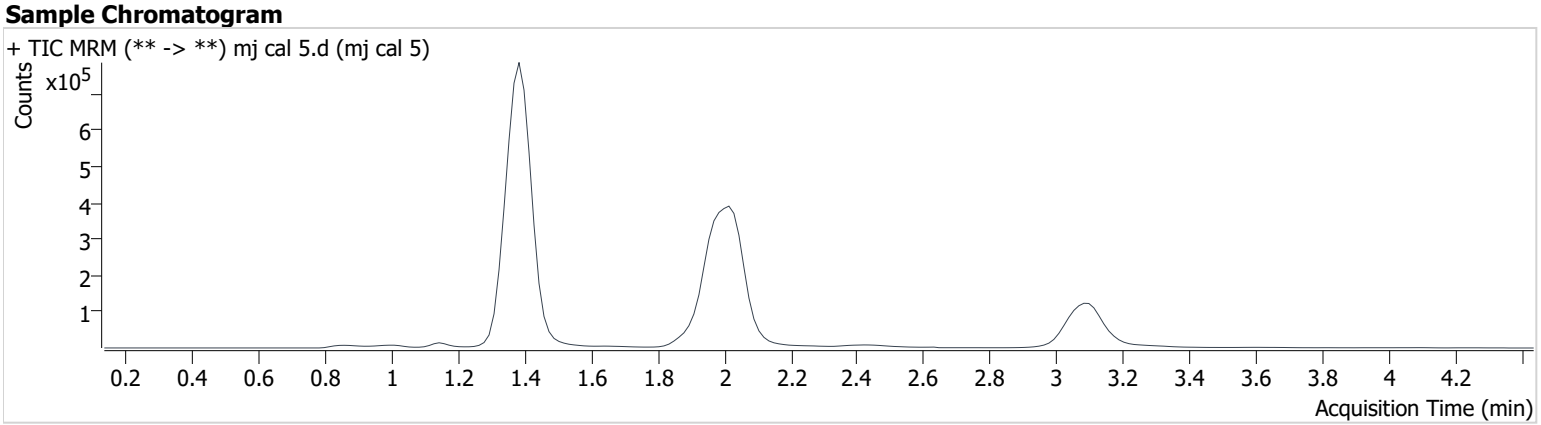
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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	8/12/2020 12:42:41 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	467937	∞	11.8	435.5	1188730	24.489	ng/ml
THC-COOH	1.401	616963	593.2	37.6	3504.1	647489	74.234	ng/ml
THC	3.104	268204	∞	24.2	∞	606877	23.710	ng/ml

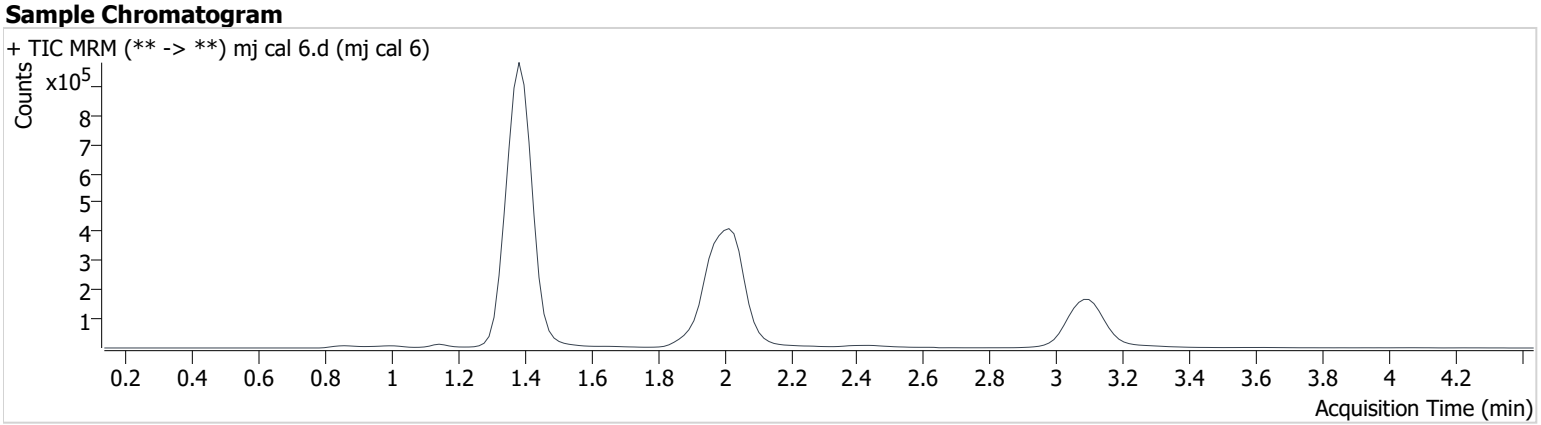
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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	8/12/2020 12:50:25 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	933227	858.9	11.8	3493.0	1154769	49.514	ng/ml
THC-COOH	1.401	817954	2825.1	37.5	1215.2	636170	99.704	ng/ml
THC	3.104	562320	∞	24.2	∞	605541	49.419	ng/ml

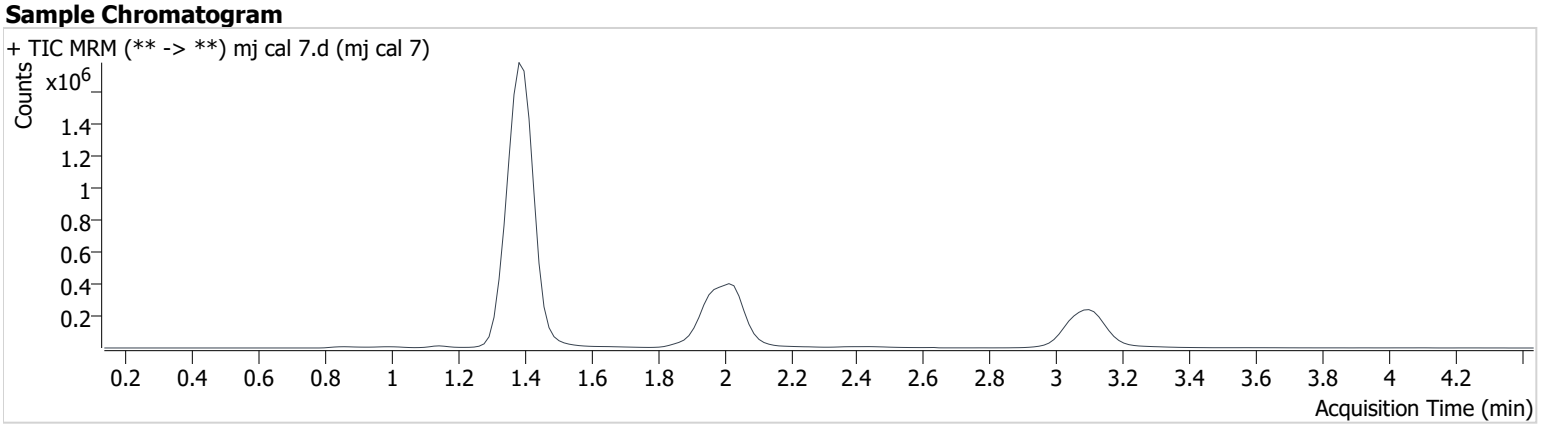
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 8-12-20\QuantResults\thcq.batch.bin
Calibration Last Update 8/12/2020 9:32:56 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	8/12/2020 12:58:09 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	1903550	∞	12.1	∞	1143713	101.205	ng/ml
THC-COOH	1.401	2036592	5838.4	37.9	893264.6	616780	253.971	ng/ml
THC	3.104	1146542	∞	24.0	∞	590905	102.859	ng/ml