REVIEWED
By Celena Shrum at 11:24 am, Mar 28, 2020

3/26/2020 BW

Worklist: 4114

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2020-0455	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0459	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-0489	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0506	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0525	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0537	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-0554	3	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0562	1	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: 3/25/20 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: 20A52255 **Urine Blank:** 32420 **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. *Shaker ID: 66759*
☒ 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 cases only evaluated for Carboxy-THC
Curves limited: 3-100- THC and Hydroxy-THC; 5-250 Carboxy-THC

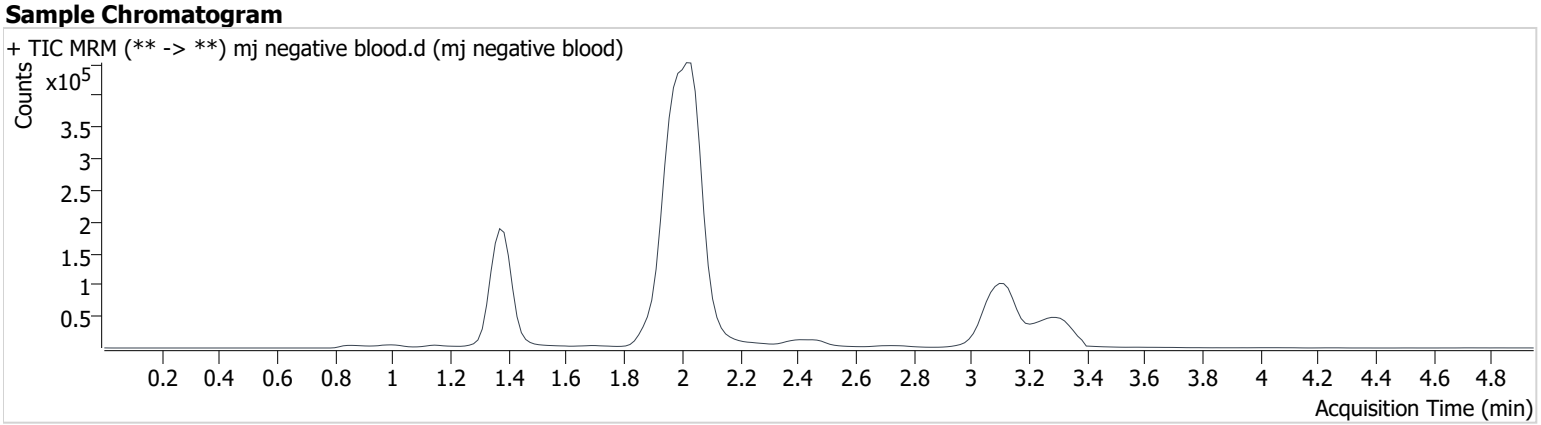
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 1:42:28 PM		
Sample Info.			

Sample Chromatogram



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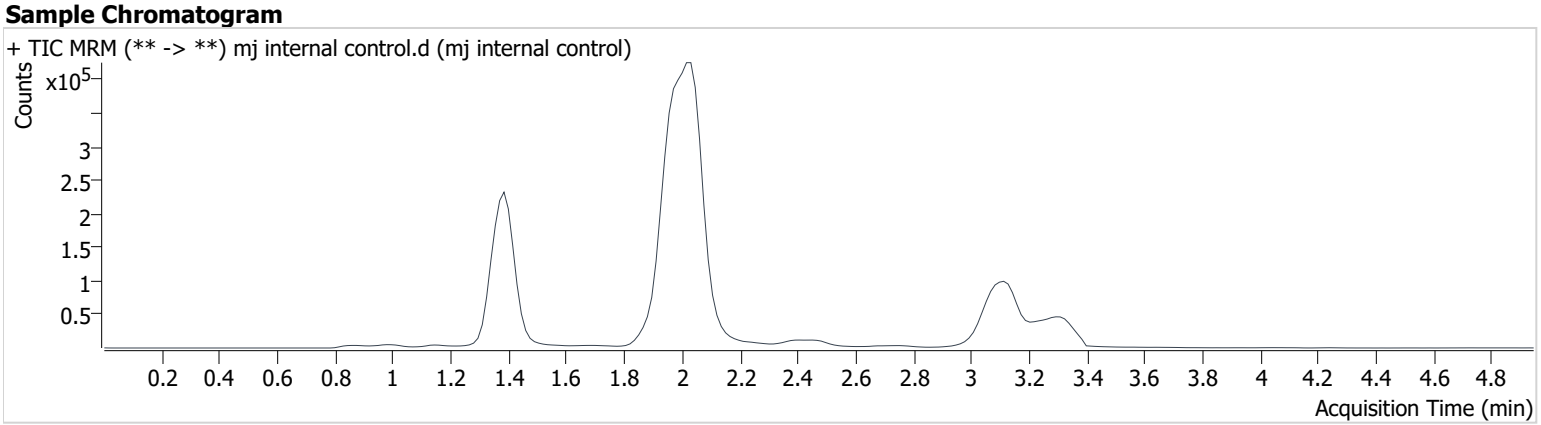
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 1:34:44 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.375	79863	∞	11.2	∞	553546	4.999	ng/ml
THC-COOH	1.400	64671	347.2	256.3	1384.6	413384	15.664	ng/ml
THC	3.138	25934	∞	25.9	∞	655646	4.663	ng/ml

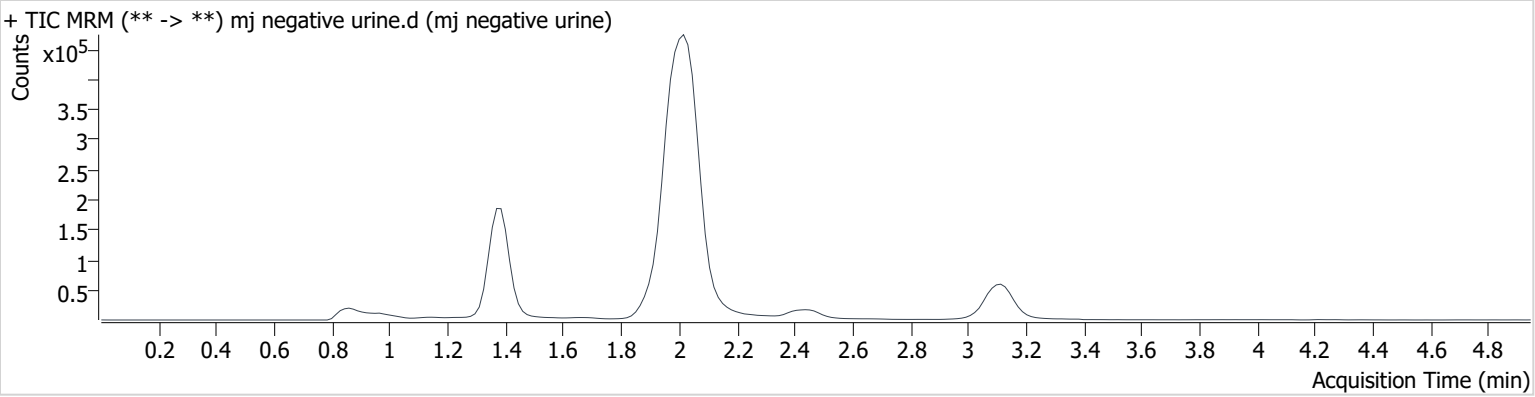
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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-H2	**Comment**	
Injection Volume	10		
Acq. Date-Time	3/25/2020 3:30:03 PM		
Sample Info.			

Sample Chromatogram



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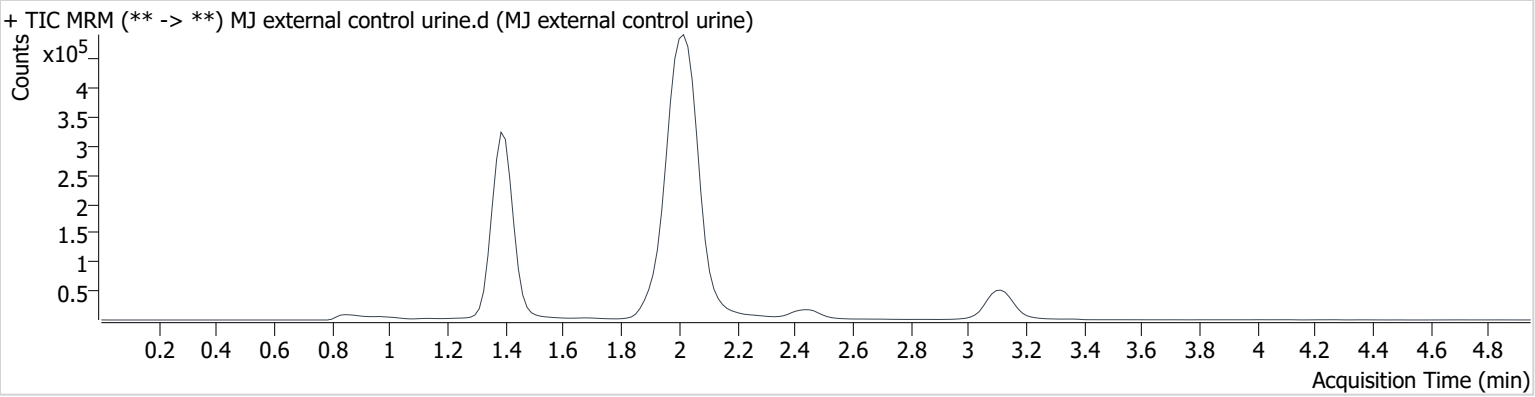
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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**	Britany Wylie
Sample Position	P3-A3	**Comment**	
Injection Volume	10		
Acq. Date-Time	3/25/2020 3:37:48 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.375	184463	∞	12.6	∞	551257	11.314	ng/ml
THC-COOH	1.415	145815	1688.9	233.6	595.0	356689	38.034	ng/ml
THC	3.138	8719	∞	29.3	4256607 436253.1	345868	3.229	ng/ml

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Toxicology AM method 27/26 external prep information

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

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

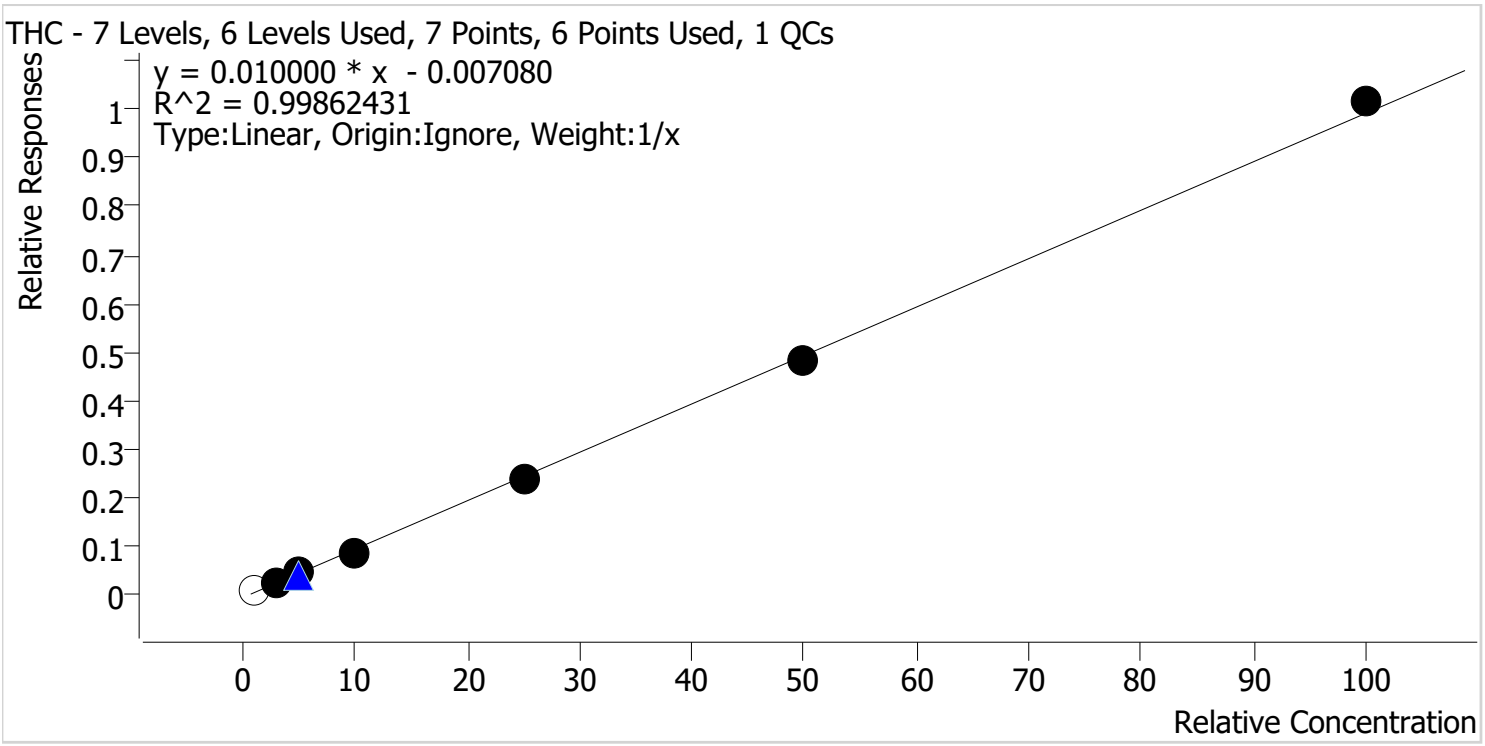
ppd 02/13/20 Exp 08/13/20 lot b81320 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-THC by AMN

AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (11420)

ppd 02/13/20 Exp 08/13/20 lot u81320 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC by AMN

Compound Calibration Report

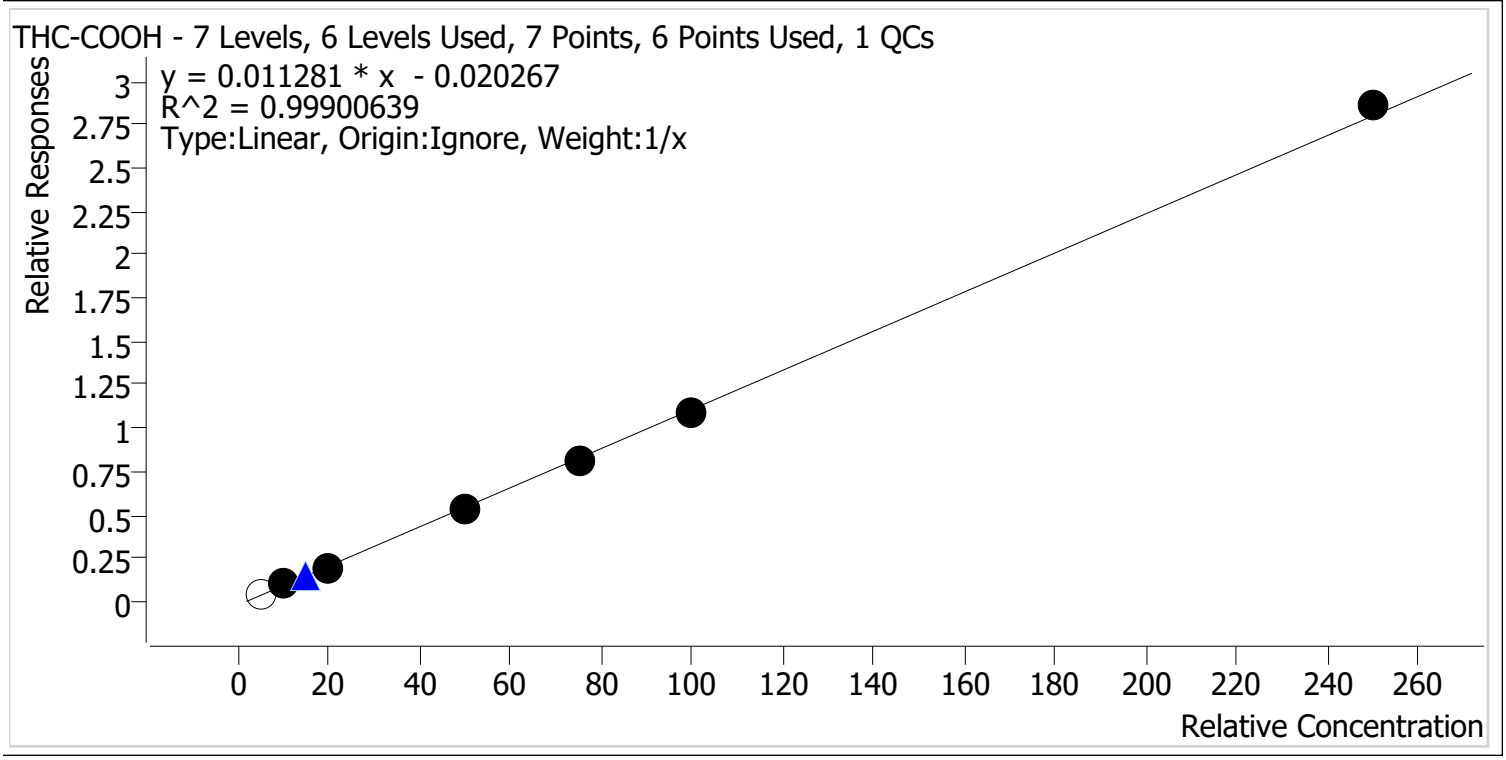
Batch results	D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Last Cal. Update	3/25/2020 10:12 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✗	1.0	1.6	162.1
mj cal2	2	✔	3.0	3.3	110.3
mj cal 3	3	✔	5.0	5.0	100.2
mj cal 4	4	✔	10.0	9.3	93.0
mj cal 5	5	✔	25.0	24.1	96.3
mj cal 6	6	✔	50.0	48.9	97.8
mj cal 7	7	✔	100.0	102.4	102.4

Compound Calibration Report

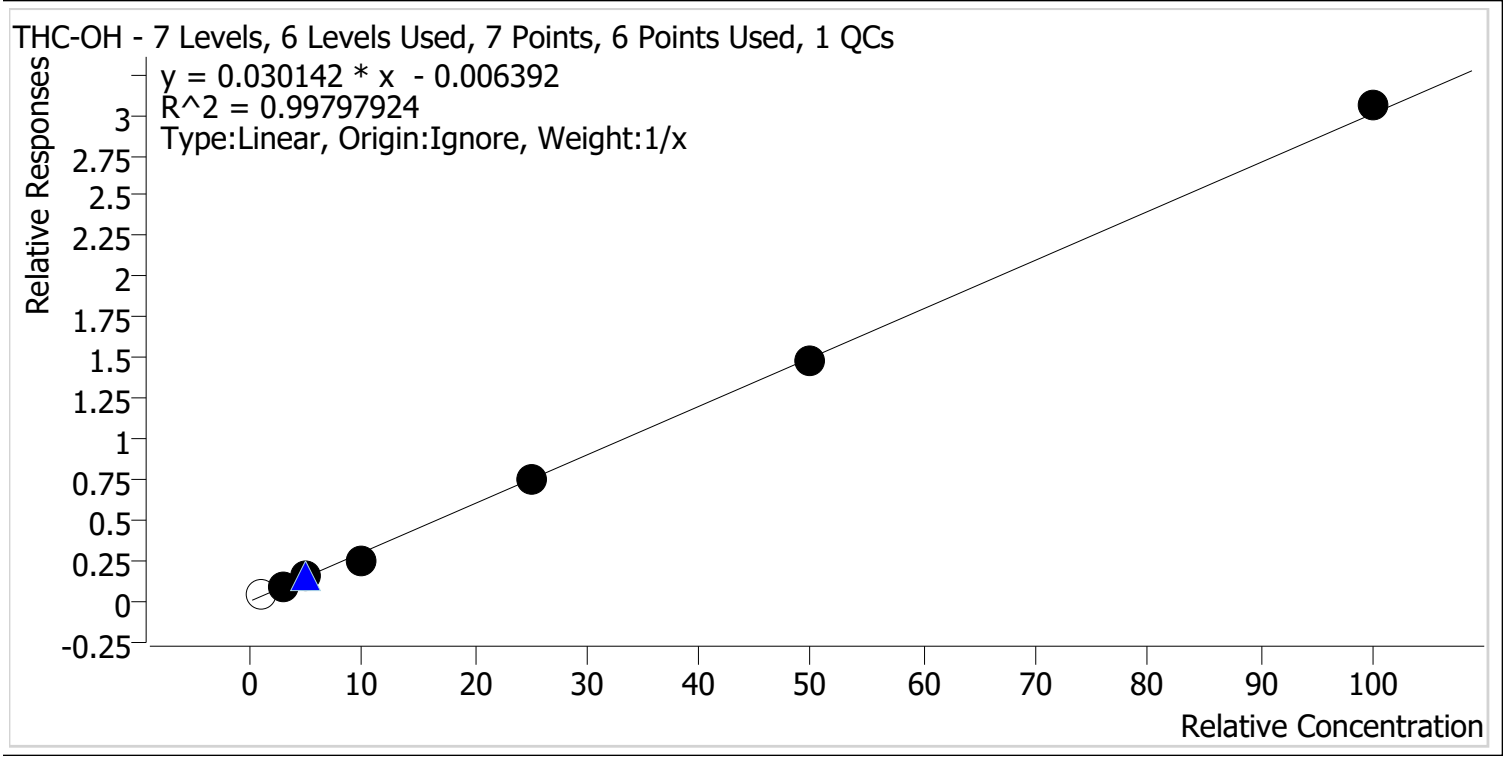
Batch results	D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Last Cal. Update	3/25/2020 10:12 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✗	5.0	5.8	115.3
mj cal2	2	✔	10.0	10.8	107.8
mj cal 3	3	✔	20.0	19.6	98.0
mj cal 4	4	✔	50.0	48.4	96.7
mj cal 5	5	✔	75.0	73.1	97.5
mj cal 6	6	✔	100.0	97.9	97.9
mj cal 7	7	✔	250.0	255.2	102.1

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Last Cal. Update	3/25/2020 10:12 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✗	1.0	1.6	155.8
mj cal2	2	✔	3.0	3.3	109.5
mj cal 3	3	✔	5.0	5.3	105.2
mj cal 4	4	✔	10.0	8.5	85.4
mj cal 5	5	✔	25.0	24.8	99.4
mj cal 6	6	✔	50.0	49.4	98.8
mj cal 7	7	✔	100.0	101.6	101.6

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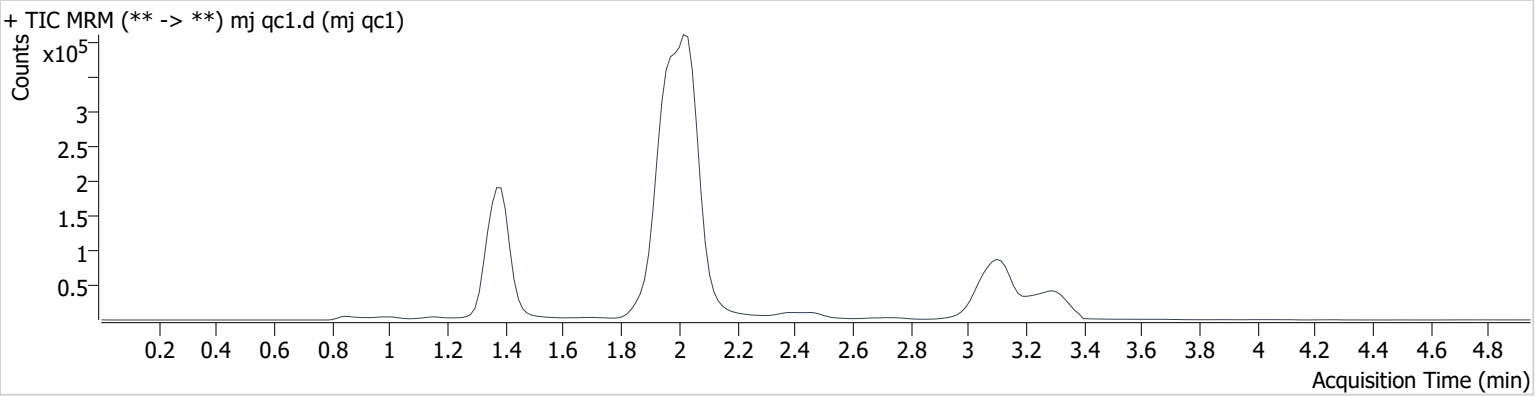
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 12:40:41 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.375	23349	∞	8.1 **Low**	∞	575645	1.558	ng/ml **Low**
THC-COOH	1.415	17962	37.2	343.3 **High**	27.2	401087	5.766	ng/ml **Low**
THC	3.138	5525	∞	25.1	∞	605310	1.621	ng/ml **Low**

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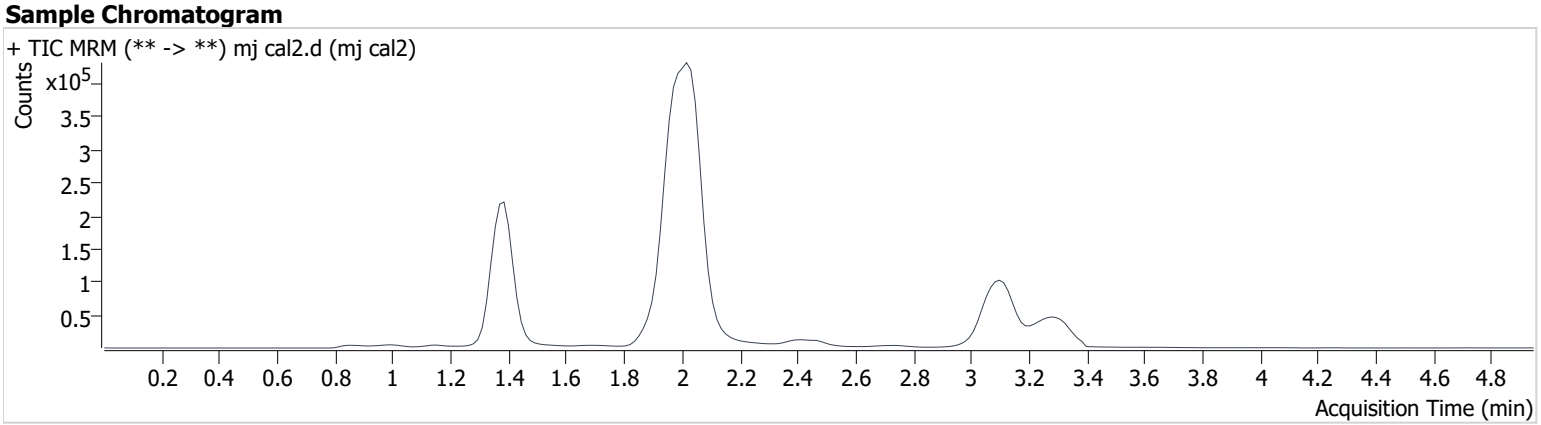
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 12:48:25 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.375	53560	∞	10.8	∞	578399	3.284	ng/ml
THC-COOH	1.400	40846	106.9	262.7	64.5	403066	10.780	ng/ml
THC	3.123	17054	∞	24.4	∞	655839	3.308	ng/ml

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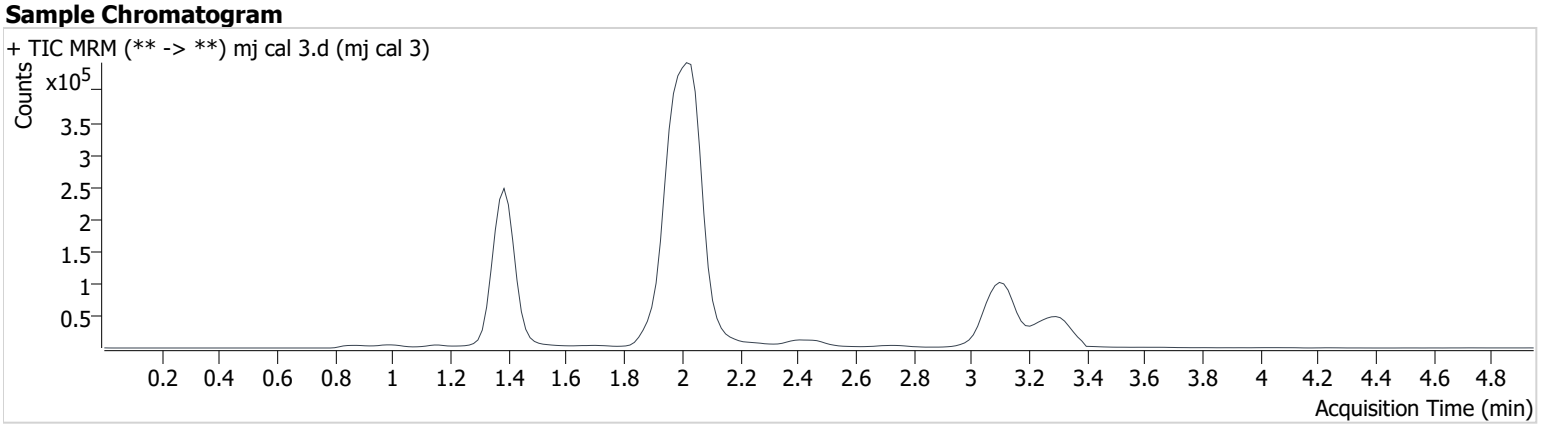
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 12:56:09 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	83412	∞	10.6	∞	548080	5.261	ng/ml
THC-COOH	1.400	79093	145.9	249.3	240.3	394004	19.591	ng/ml
THC	3.138	26633	∞	28.3	∞	618873	5.011	ng/ml

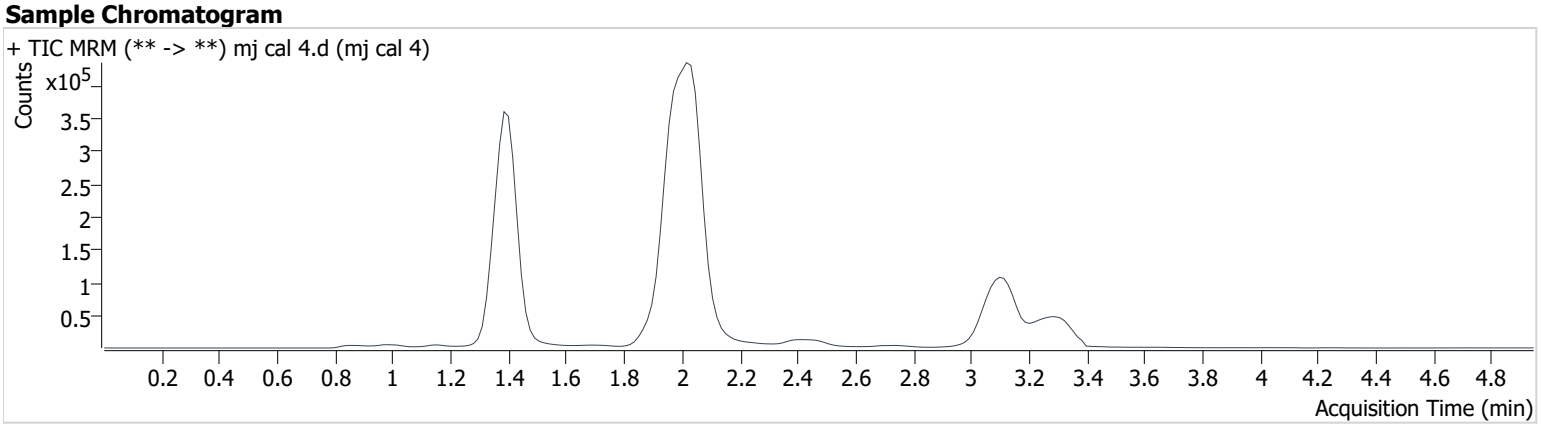
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 1:03:54 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.375	144063	∞	14.2	∞	573581	8.545	ng/ml
THC-COOH	1.400	217301	3180.4	227.7	1892.8	413640	48.364	ng/ml
THC	3.138	56245	∞	27.1	∞	654893	9.296	ng/ml

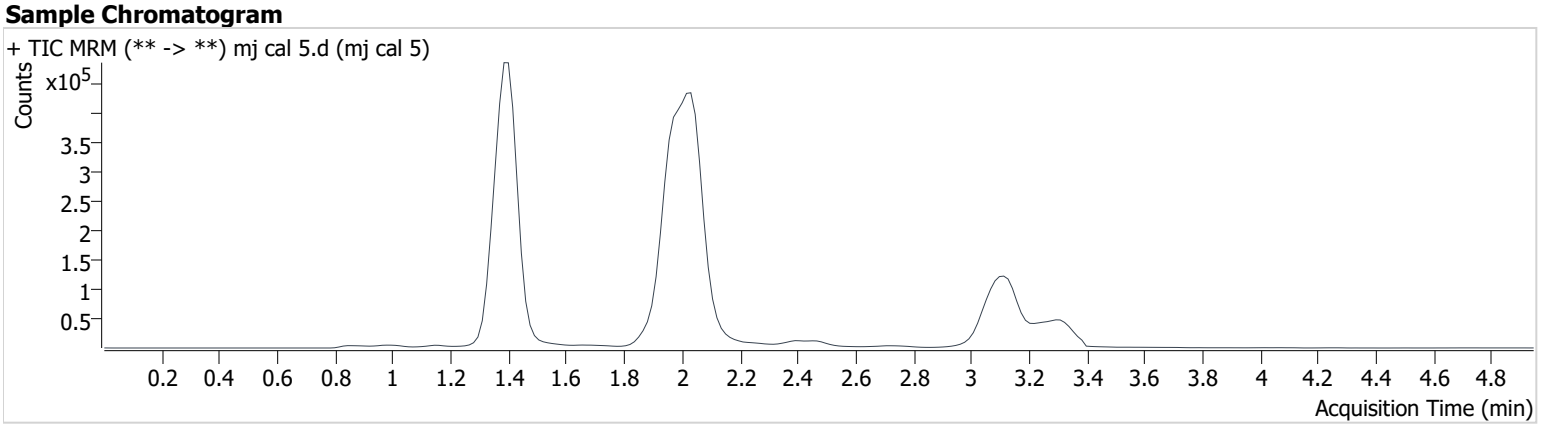
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 1:11:36 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.375	428948	∞	12.4	∞	577772	24.843	ng/ml
THC-COOH	1.400	336994	1127.3	229.1	973518.4	418663	73.148	ng/ml
THC	3.138	158437	∞	26.7	∞	677953	24.077	ng/ml

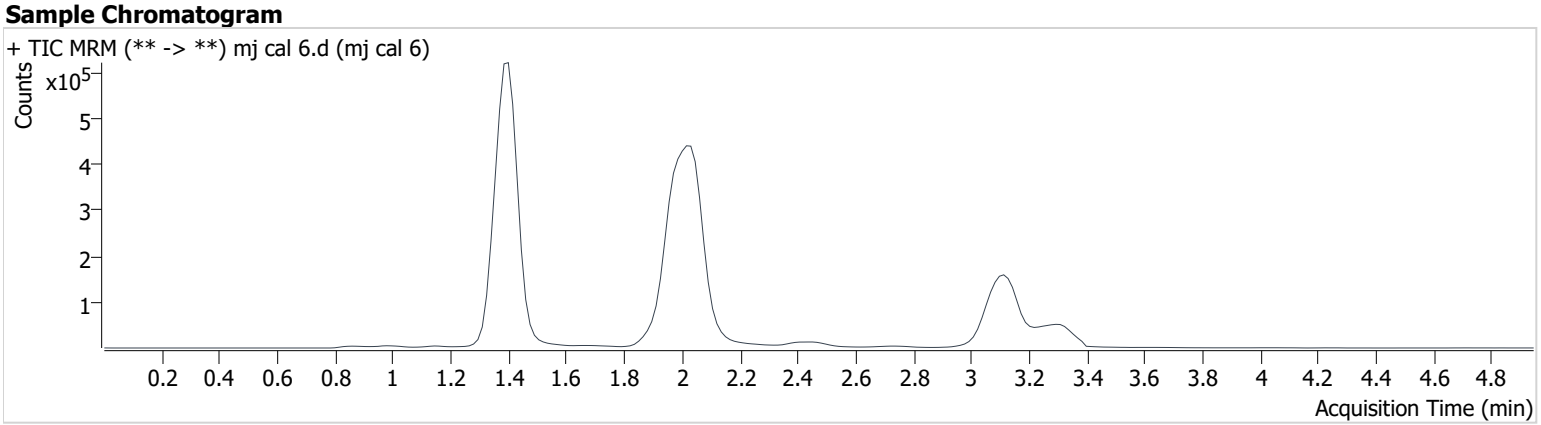
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 1:19:20 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	809233	∞	13.0	∞	545580	49.421	ng/ml
THC-COOH	1.415	420614	3753.7	228.4	2272.1	387939	97.906	ng/ml
THC	3.138	332332	∞	25.6	∞	689253	48.923	ng/ml

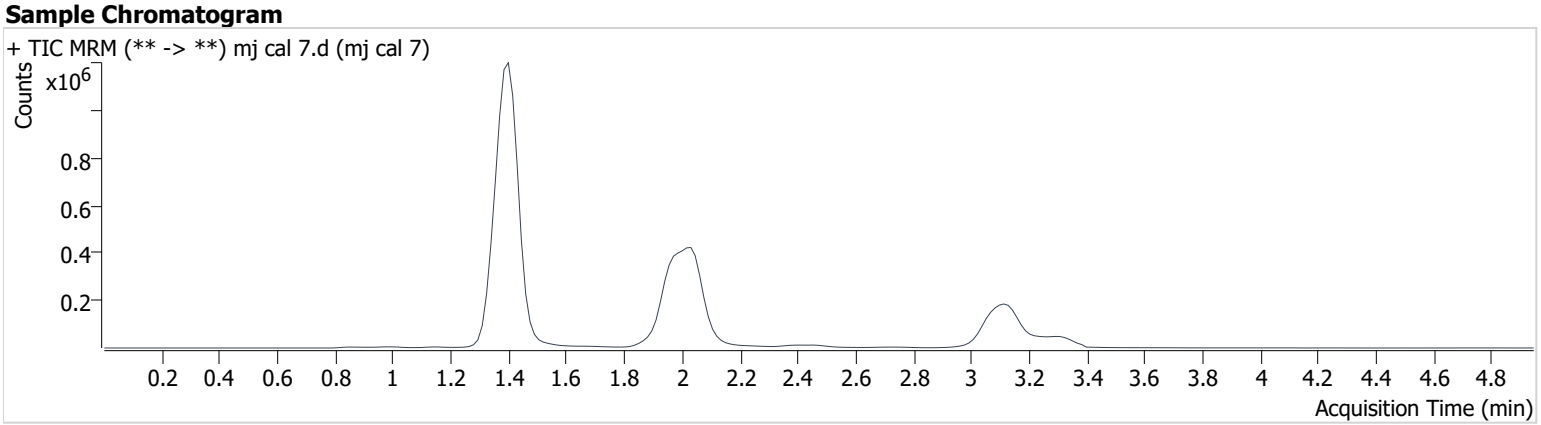
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 3-25-2020\QuantResults\thcq.batch.bin
Calibration Last Update 3/25/2020 10:12:04 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	3/25/2020 1:27:02 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.375	1666115	∞	13.4	∞	544937	101.647	ng/ml
THC-COOH	1.400	1049964	19892.0	224.4	7134.4	367274	255.211	ng/ml
THC	3.138	637549	∞	26.8	∞	627017	102.384	ng/ml