APPROVED
By Anne Nord at 8:56 am, Apr 21, 2020

4/20/2020 BW

Worklist: 4176

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2020-0606	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0607	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0622	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-0637	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0639	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-0661	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-0665	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0676	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ

BW

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

Extraction Date: 4/17/20 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.
☒ 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- Hydroxy-THC; 10-250 Carboxy-THC

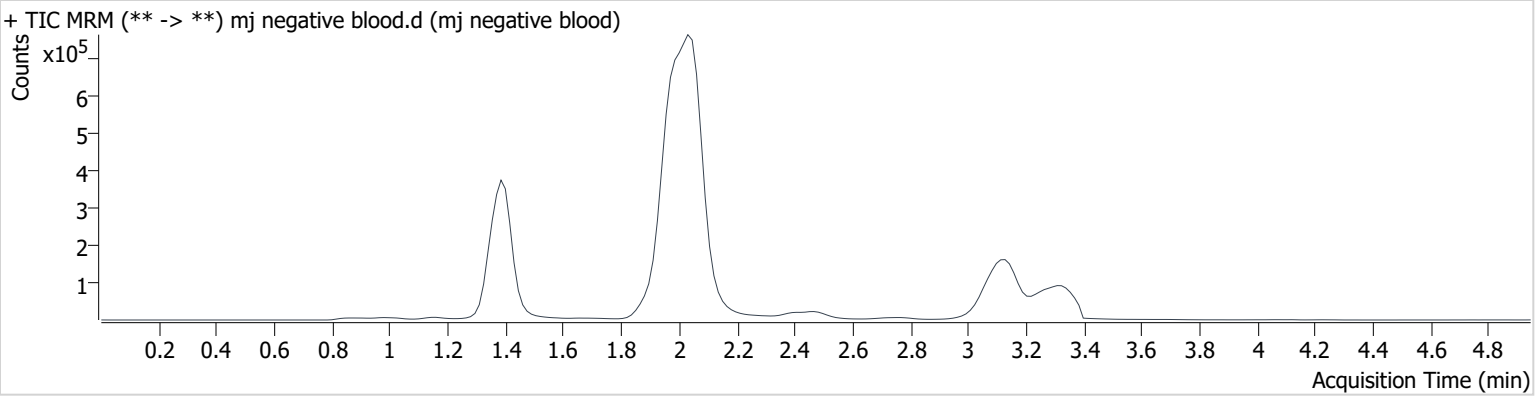
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 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-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/17/2020 2:50:06 PM		
Sample Info.			

Sample Chromatogram



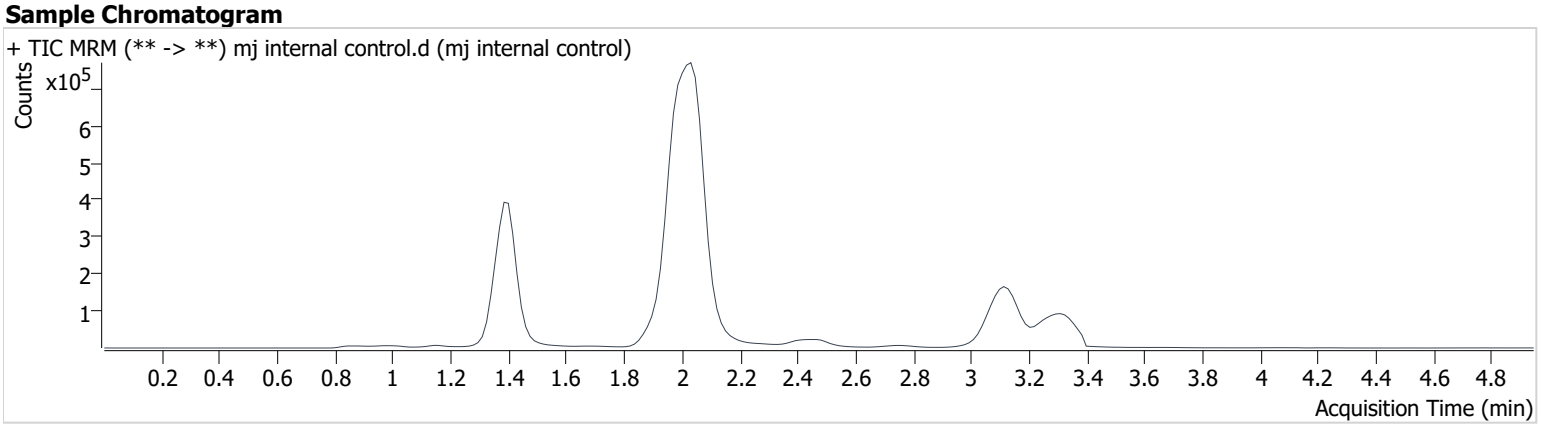
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 AM

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	4/17/2020 2:42:23 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	93608	∞	9.7	∞	1013531	4.900	ng/ml
THC-COOH	1.415	115769	148.1	201.8	150.9	634011	15.619	ng/ml
THC	3.138	34203	∞	26.7	∞	923211	4.216	ng/ml

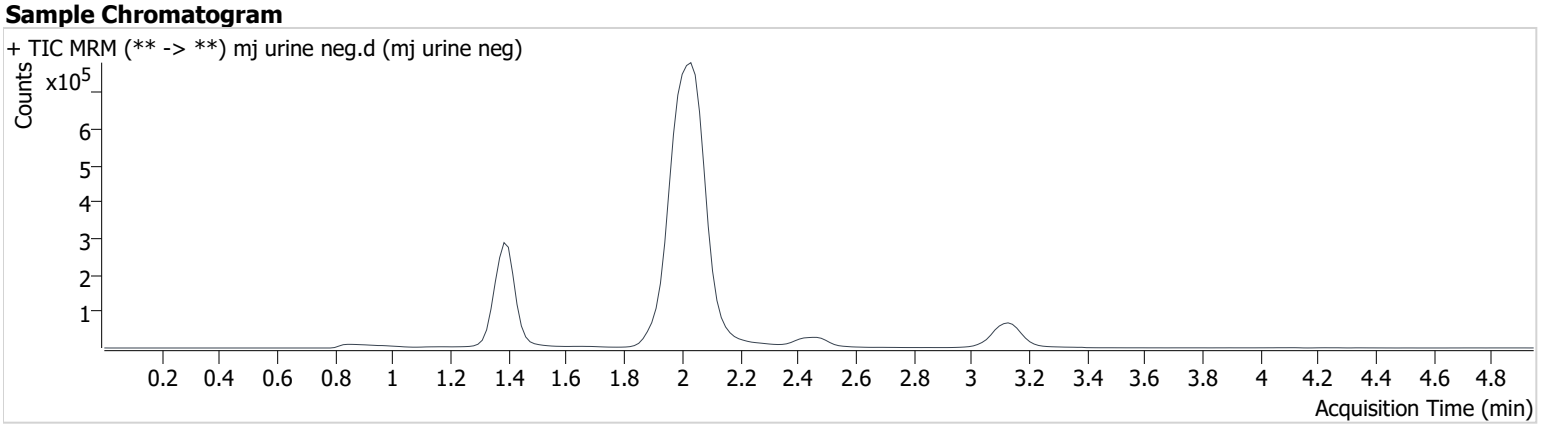
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 AM

Instrument	69679	**Data File**	mj urine neg.d
Type	Sample	**Sample**	mj urine neg
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-F2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/17/2020 4:06:57 PM		
Sample Info.			

Sample Chromatogram



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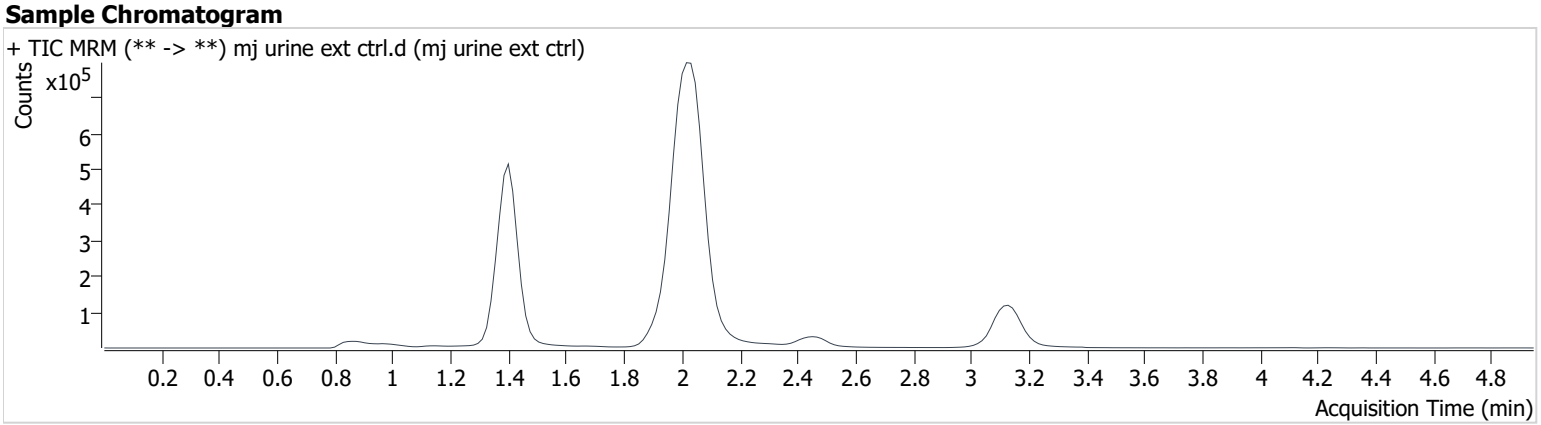
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 AM

Instrument	69679	**Data File**	mj urine ext ctrl.d
Type	Sample	**Sample**	mj urine ext ctrl
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-G2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/17/2020 4:14:41 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	316651	∞	10.5	∞	1006449	16.354	ng/ml
THC-COOH	1.415	258449	4819.4	178.2	5471.9	512776	40.441	ng/ml
THC	3.138	123798	∞	24.5	∞	697126	18.986	ng/ml

BW

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

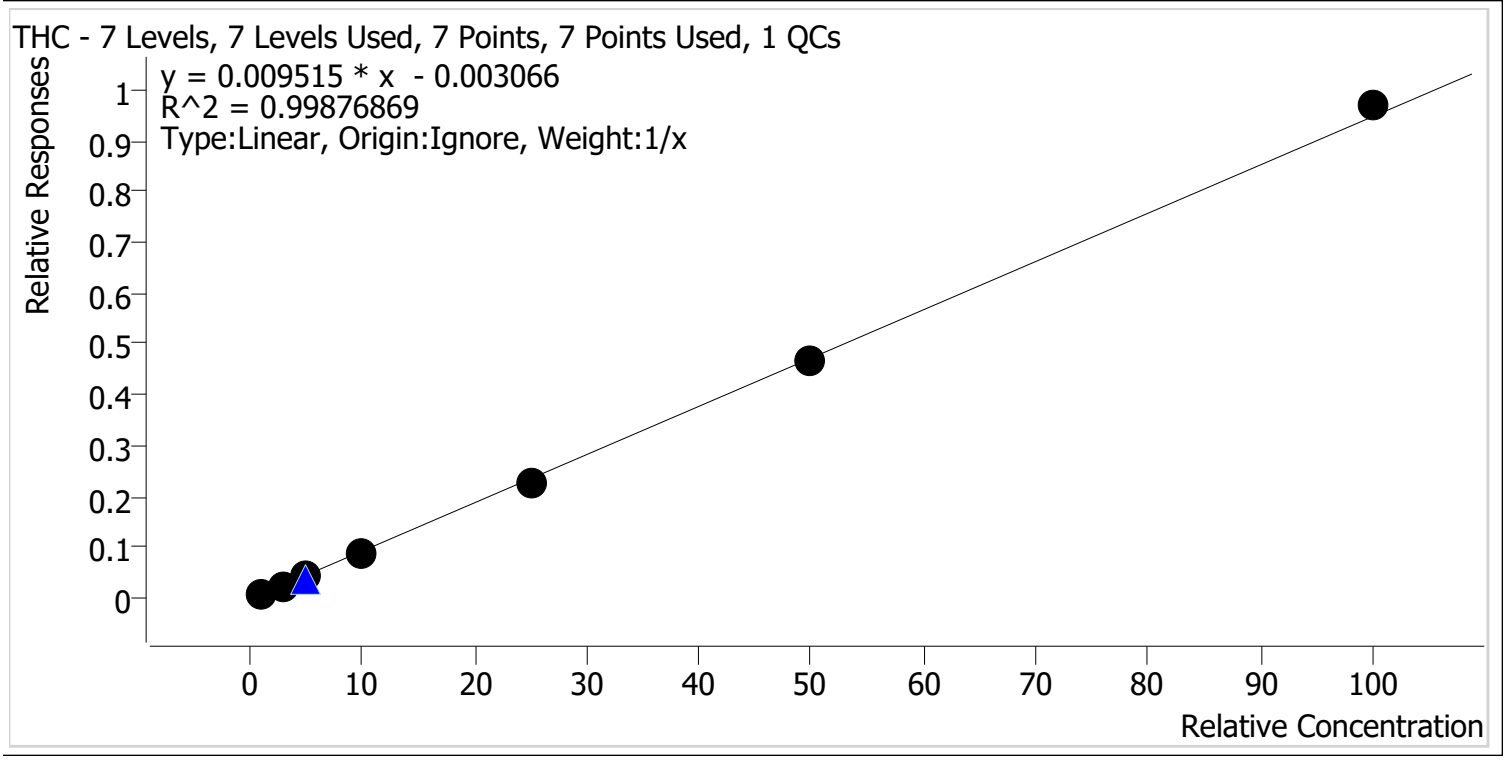
AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (32420)

ppd 4/17/20 Exp 9120 lot u101720 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC by BAW

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Last Cal. Update	4/18/2020 10:24 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3

BW



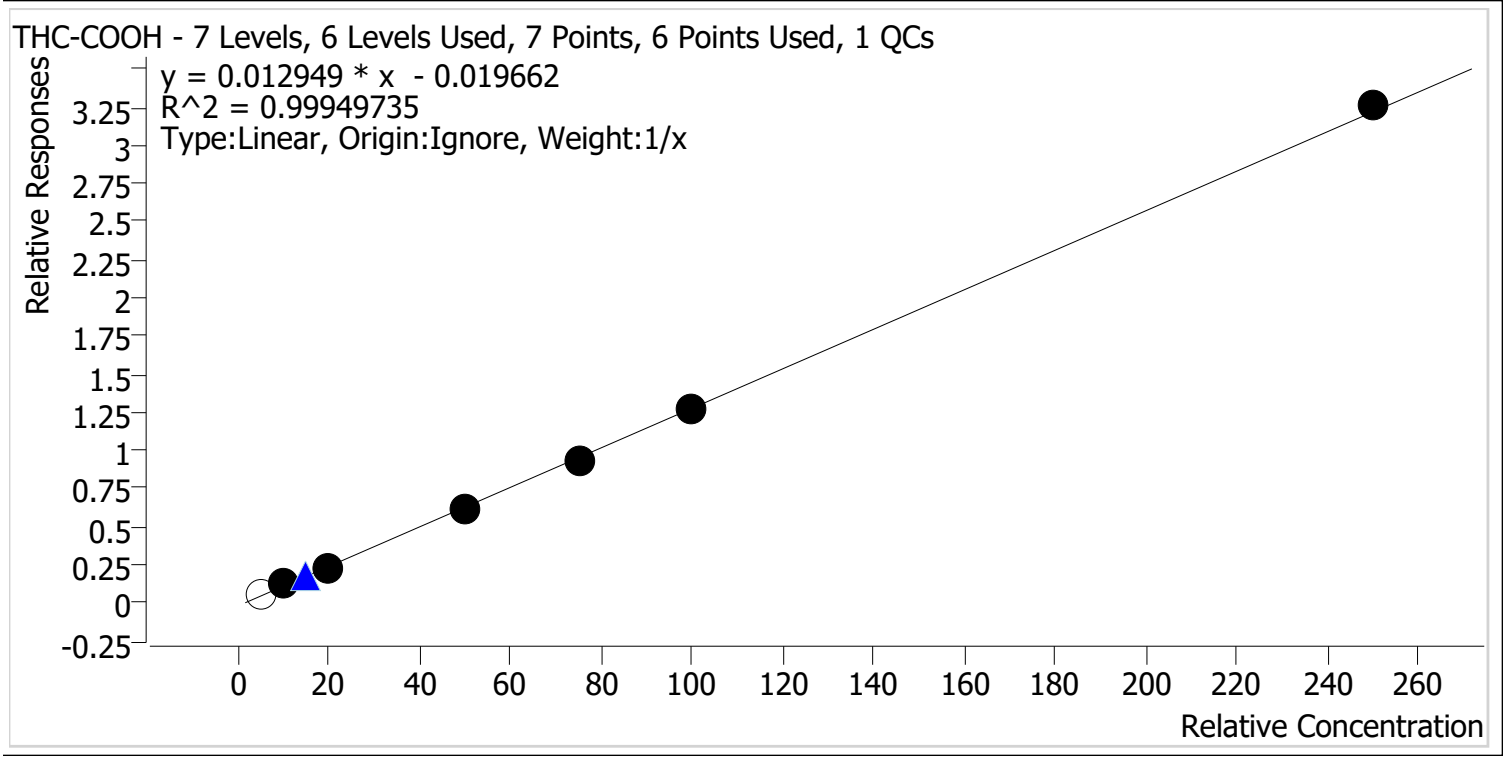
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✔	1.0	1.2	118.3
mj cal2	2	✔	3.0	2.9	97.9
mj cal 3	3	✔	5.0	4.7	93.9
mj cal 4	4	✔	10.0	9.2	92.0
mj cal 5	5	✔	25.0	24.1	96.3
mj cal 6	6	✔	50.0	49.7	99.3
mj cal 7	7	✔	100.0	102.2	102.2

Compound Calibration Report

Agilent | Trusted Answers

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Last Cal. Update	4/18/2020 10:24 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9

BW

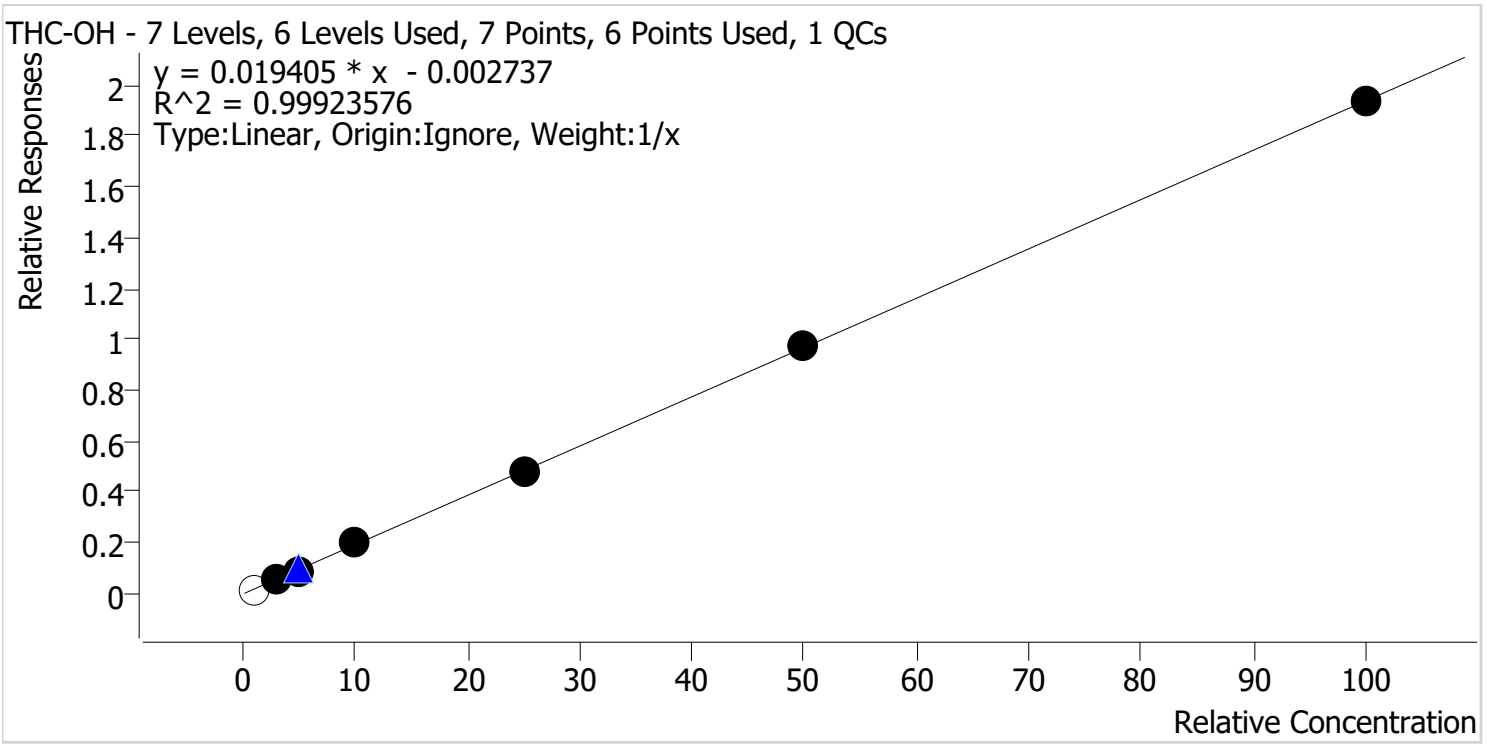


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✗	5.0	6.1	122.3
mj cal2	2	✔	10.0	10.6	106.1
mj cal 3	3	✔	20.0	19.6	97.9
mj cal 4	4	✔	50.0	48.7	97.5
mj cal 5	5	✔	75.0	73.2	97.6
mj cal 6	6	✔	100.0	99.8	99.8
mj cal 7	7	✔	250.0	253.1	101.3

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Last Cal. Update	4/18/2020 10:24 AM
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	0.9	88.3
mj cal2	2	✔	3.0	3.3	109.3
mj cal 3	3	✔	5.0	4.4	88.0
mj cal 4	4	✔	10.0	10.3	102.7
mj cal 5	5	✔	25.0	24.9	99.5
mj cal 6	6	✔	50.0	50.4	100.7
mj cal 7	7	✔	100.0	99.8	99.8

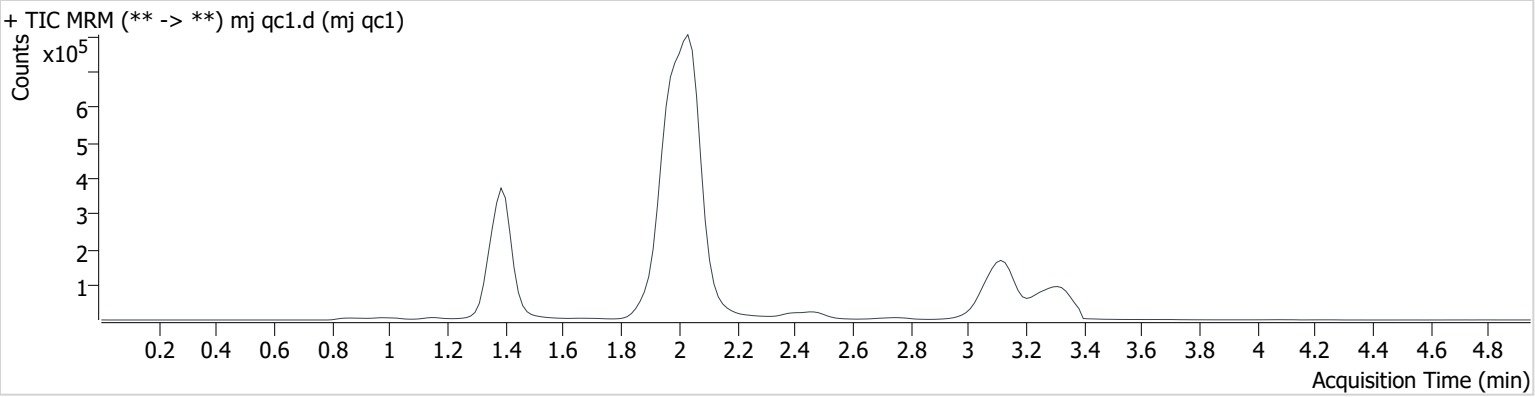
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 AM

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	4/17/2020 1:48:19 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	16442	∞	15.8 **High**	∞	1141617	0.883	ng/ml **Low**
THC-COOH	1.415	42673	579.6	236.0 **High**	377.9	716686	6.116	ng/ml **Low**
THC	3.138	8267	∞	23.9	∞	1009604	1.183	ng/ml **Low**

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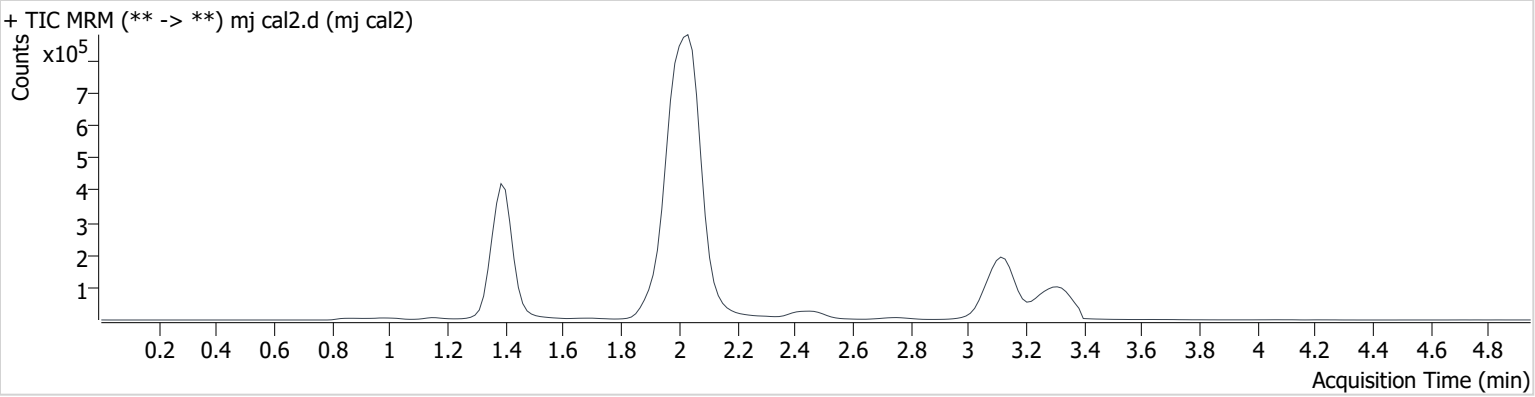
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 AM

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	4/17/2020 1:56:04 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	69404	∞	9.8	∞	1140018	3.278	ng/ml
THC-COOH	1.415	83005	287.8	209.9	1088.7	705203	10.608	ng/ml
THC	3.138	26724	∞	24.8	∞	1074261	2.937	ng/ml Low

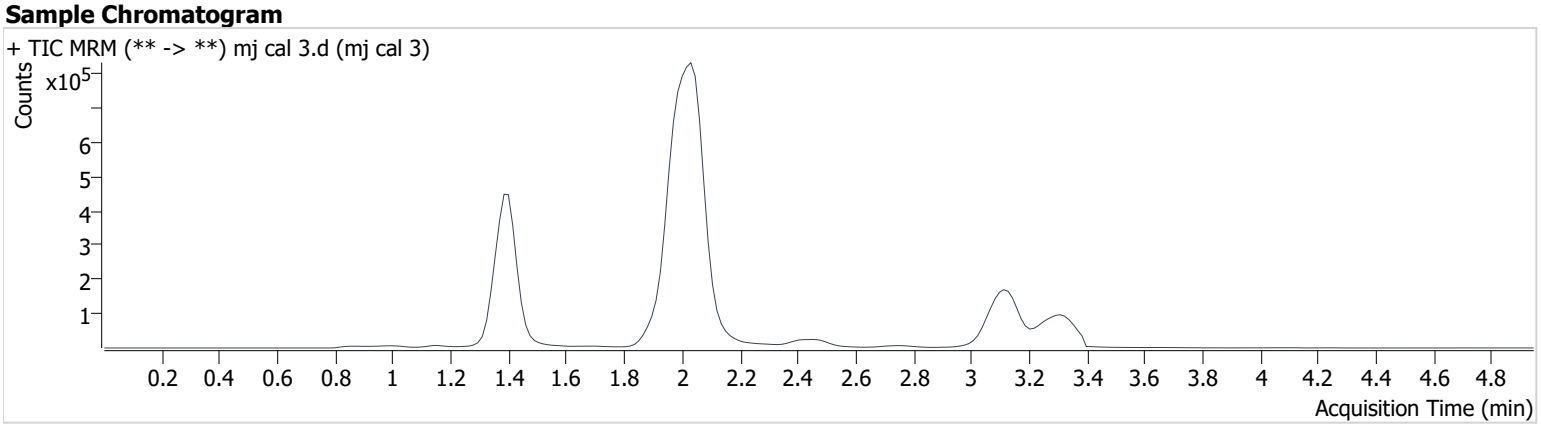
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 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	4/17/2020 2:03:47 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	91444	∞	13.7	∞	1106161	4.401	ng/ml
THC-COOH	1.415	162997	488.6	194.5	678040.7	697197	19.572	ng/ml
THC	3.138	39465	∞	25.1	∞	948191	4.697	ng/ml

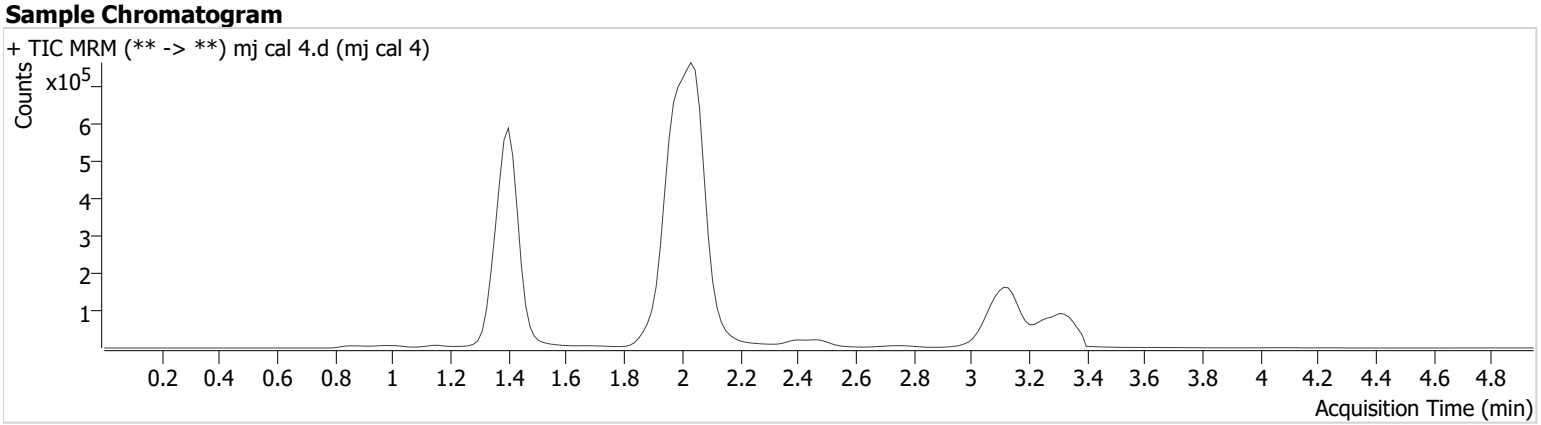
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 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	4/17/2020 2:11:30 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	212689	∞	10.6	∞	1082474	10.266	ng/ml
THC-COOH	1.415	411566	178.1	183.6	563.9	673253	48.726	ng/ml
THC	3.138	77378	∞	25.9	∞	915761	9.203	ng/ml

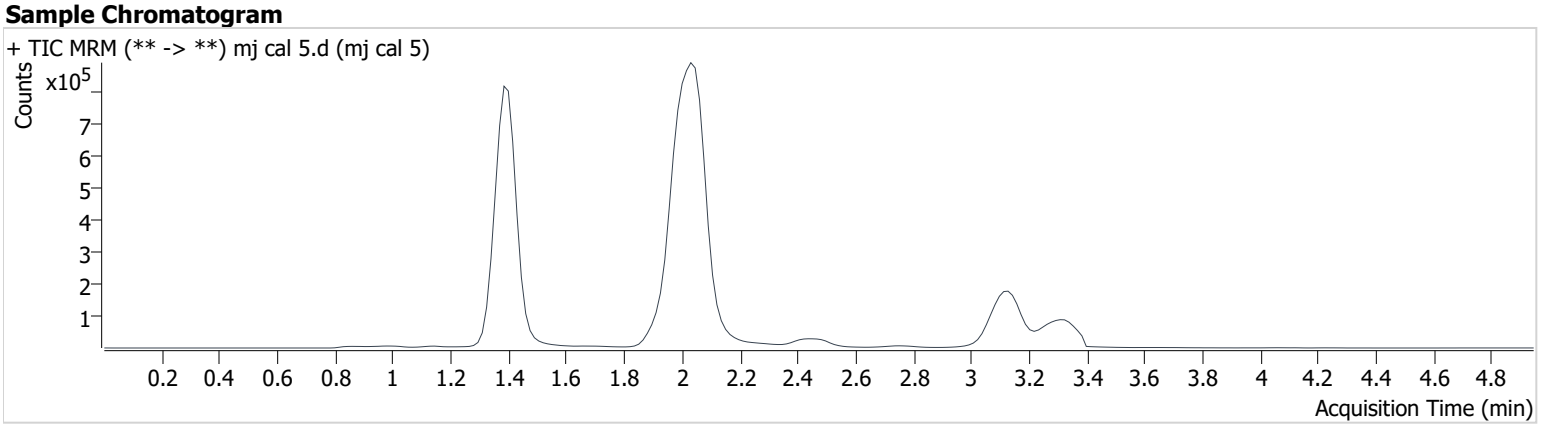
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 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	4/17/2020 2:19:14 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	554657	∞	10.9	∞	1155819	24.870	ng/ml
THC-COOH	1.400	616475	410915.3	177.3	23427.7	664240	73.189	ng/ml
THC	3.153	189145	∞	25.8	∞	837030	24.072	ng/ml

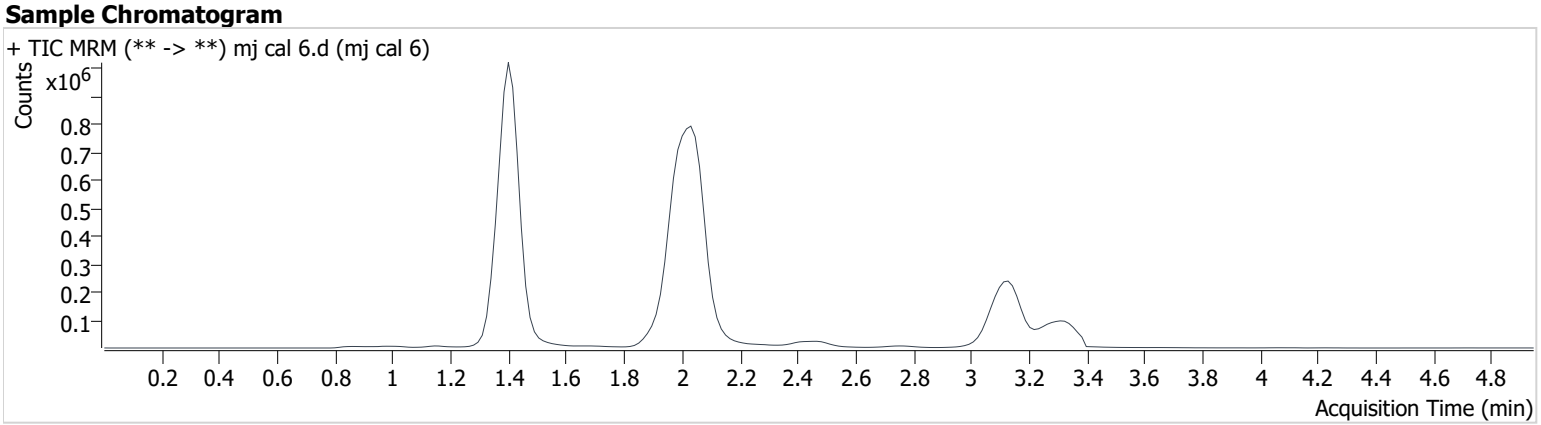
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 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	4/17/2020 2:26:57 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	1039718	∞	11.5	∞	1066627	50.373	ng/ml
THC-COOH	1.415	816620	9563.1	179.1	8485.2	641865	99.767	ng/ml
THC	3.138	449551	∞	24.9	∞	957498	49.667	ng/ml

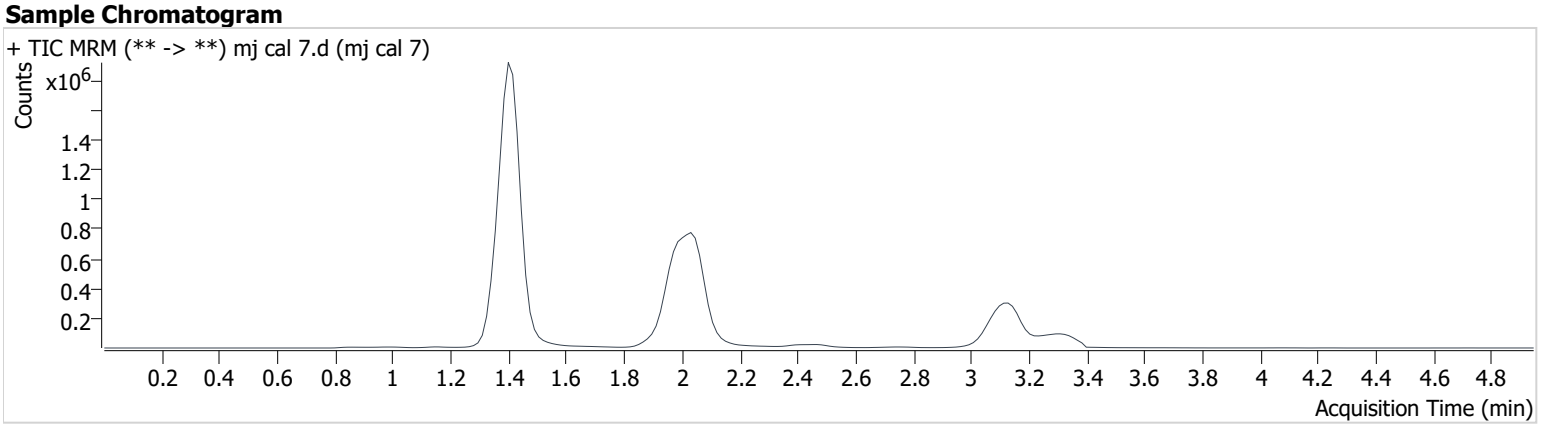
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 4-17-2020\QuantResults\thcq.batch.bin
Calibration Last Update 4/18/2020 10:24:06 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	4/17/2020 2:34:39 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	2198214	∞	12.0	∞	1136538	99.811	ng/ml
THC-COOH	1.415	2033334	6141.2	174.8	270.2	624043	253.138	ng/ml
THC	3.138	923891	∞	25.2	∞	952709	102.242	ng/ml