REVIEWED
By Anne Nord at 9:43 am, Jul 21, 2020

7/16/2020 BW

Worklist: 4367

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2020-1207	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1244	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1254	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1256	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1282	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1292	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1297	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1298	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1323	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1352	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1356	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1357	5	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1358	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1359	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1373	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1390	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: 7/17/20 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: 71520 AMN_ **Urine Blank:** 6920 **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 cases only evaluated for Carboxy-THC
2 cases set to inject using incorrect method- additional blank added to restabilize and the samples were reinjected.

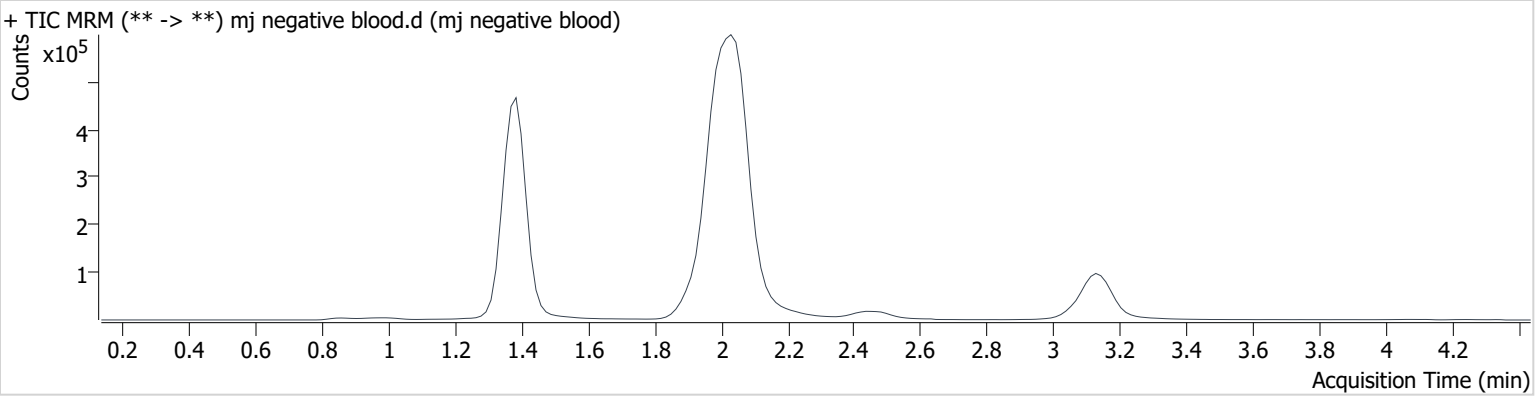
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 8:14:24 PM		
Sample Info.			

Sample Chromatogram



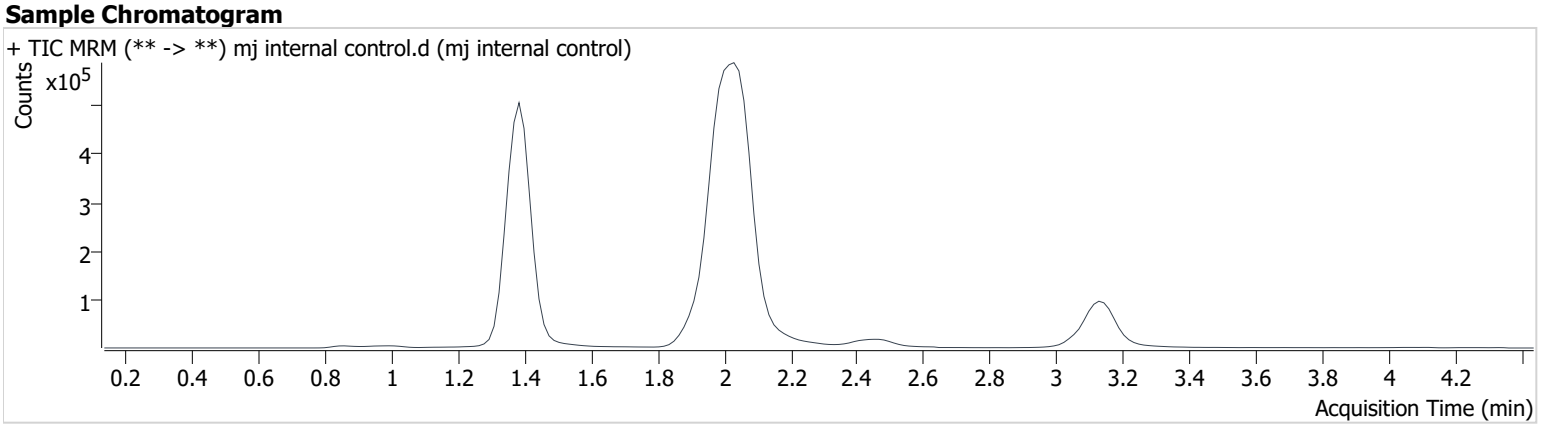
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 8:06:42 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	115994	∞	11.3	∞	1463299	4.773	ng/ml
THC-COOH	1.416	125849	404.4	36.2	286.5	698002	15.001	ng/ml
THC	3.149	48240	∞	24.3	16689460688850.1	624972	4.332	ng/ml

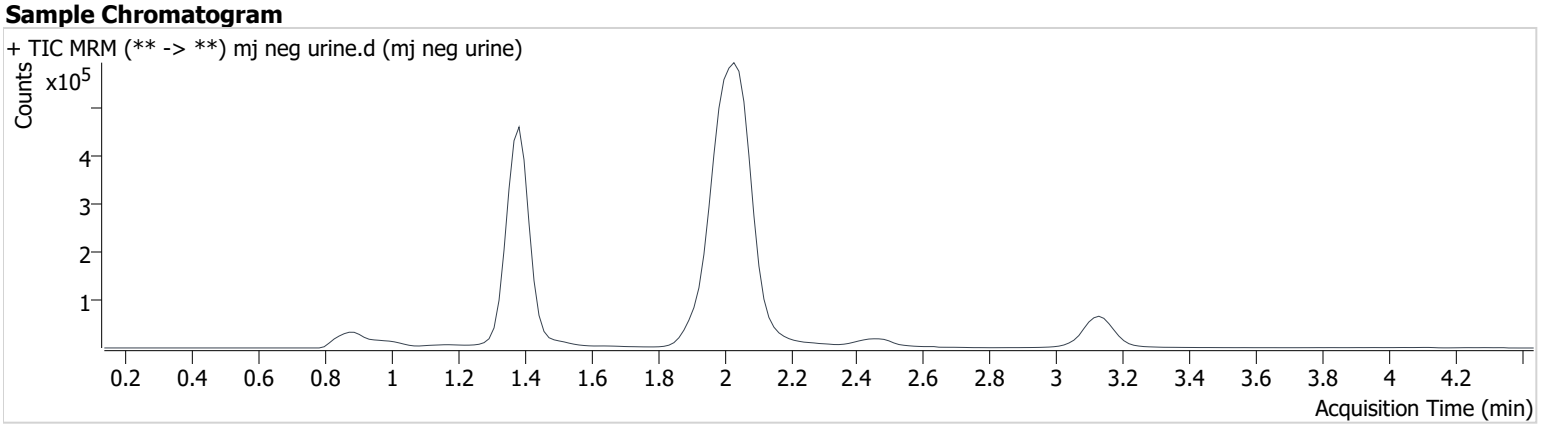
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 AM

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-H3	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/18/2020 12:27:22 AM		
Sample Info.			

Sample Chromatogram



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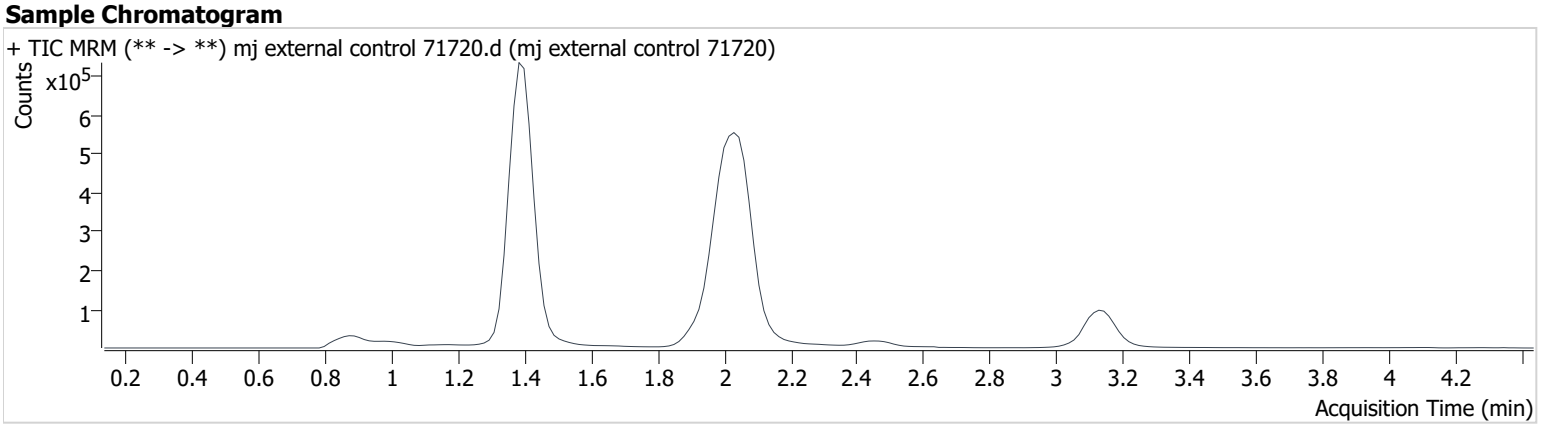
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 AM

Instrument	69679	**Data File**	mj external control 71720.d
Type	Sample	**Sample**	mj external control 71720 urine
Acq. Method	AM 27 THC quant.m	**Operator**	Britany Wylie
Sample Position	P3-A4	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/18/2020 12:42:42 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	475817	∞	51.2 **High**	∞	1615499	17.159	ng/ml
THC-COOH	1.416	338118	1193.4	34.7	1388.9	634397	42.181	ng/ml
THC	3.149	143812	∞	24.9	∞	504343	14.827	ng/ml

Evaluated carboxy-THC only
BW

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

AM 27/26 blood control 100 ul working solution lot (91319) in 9900 ul blood lot (20A522

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

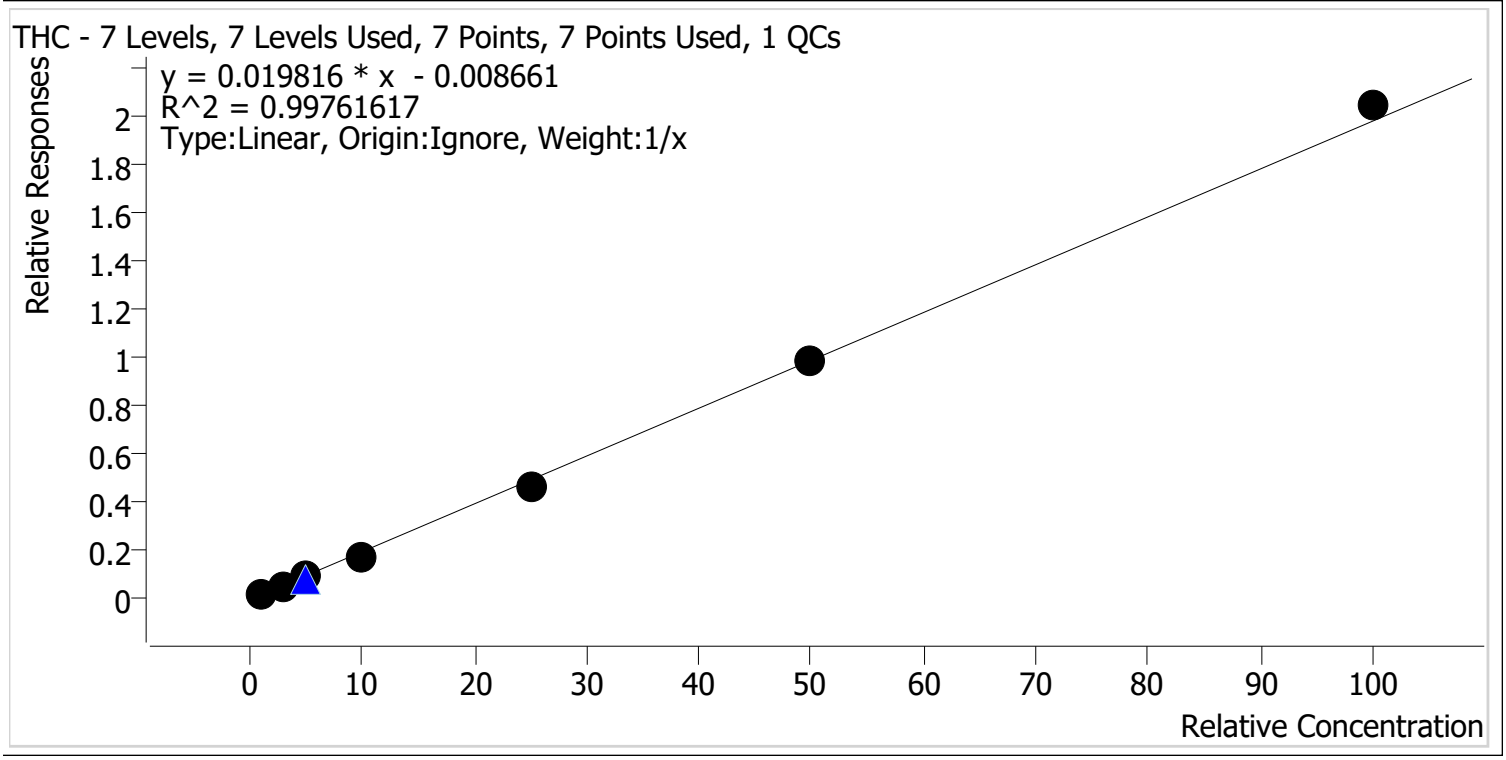
AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (6 out of use

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

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Last Cal. Update	7/18/2020 1:55 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3

BW



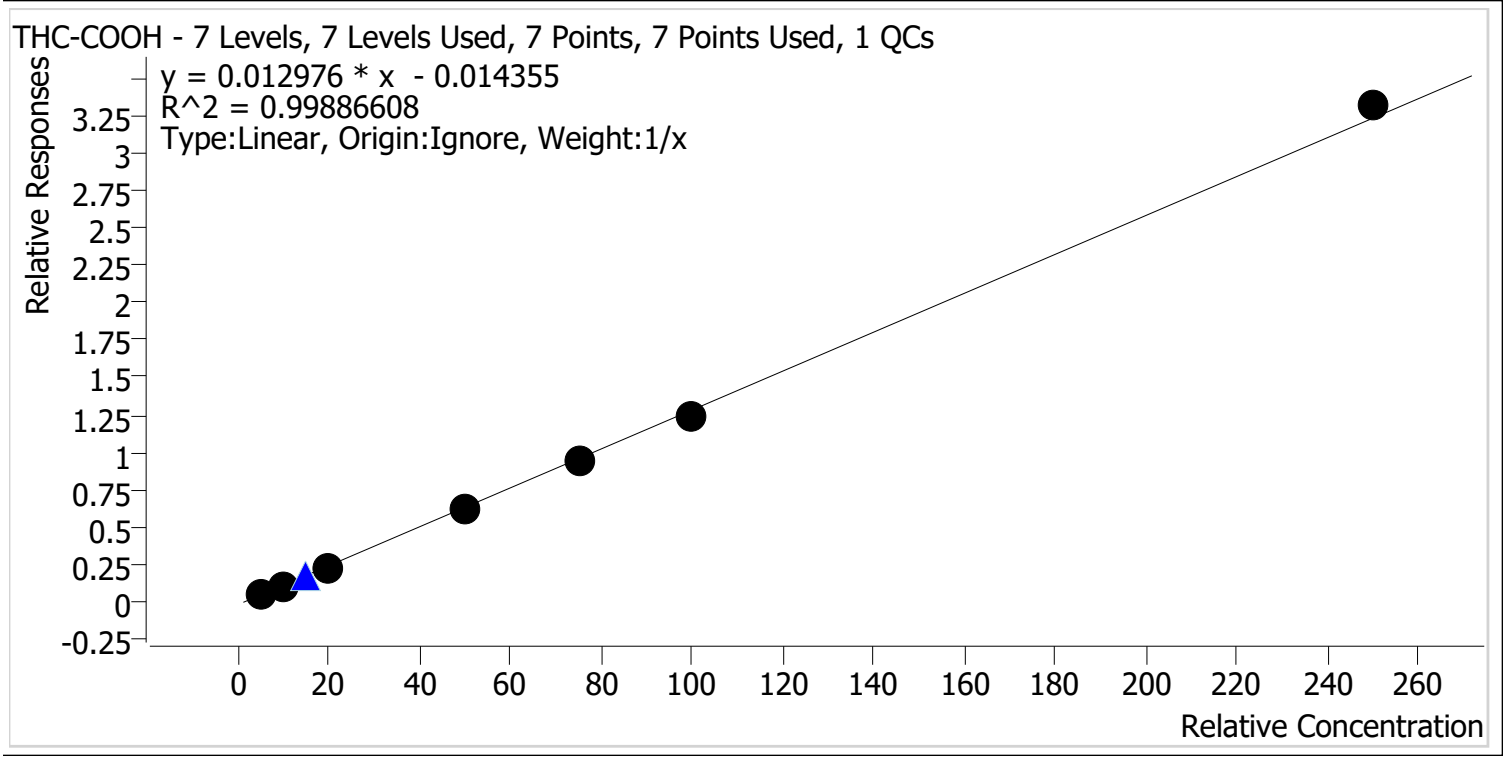
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✔	1.0	1.2	122.3
mj cal2	2	✔	3.0	2.9	97.1
mj cal 3	3	✔	5.0	4.8	96.0
mj cal 4	4	✔	10.0	8.8	88.3
mj cal 5	5	✔	25.0	23.5	94.0
mj cal 6	6	✔	50.0	49.5	99.1
mj cal 7	7	✔	100.0	103.2	103.2

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Last Cal. Update 7/18/2020 1:55 AM
Analyst Name ISP\datastor

BW

Analyte THC-COOH **Internal Standard** THC-COOH-d9

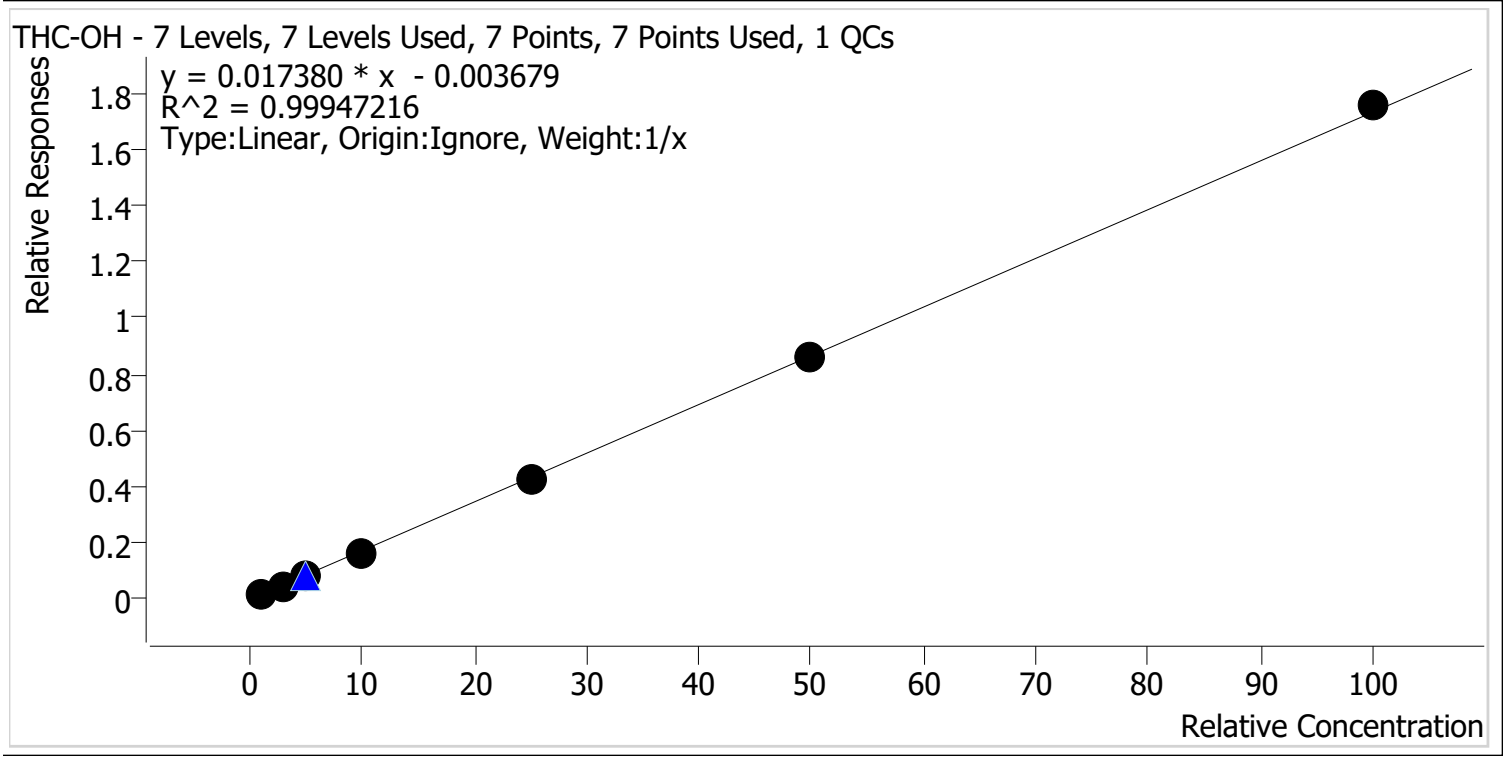


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✔	5.0	5.5	110.7
mj cal2	2	✔	10.0	9.9	98.9
mj cal 3	3	✔	20.0	19.2	95.9
mj cal 4	4	✔	50.0	48.3	96.6
mj cal 5	5	✔	75.0	74.1	98.7
mj cal 6	6	✔	100.0	96.6	96.6
mj cal 7	7	✔	250.0	256.4	102.6

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Last Cal. Update	7/18/2020 1:55 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	1.2	115.4
mj cal2	2	✔	3.0	2.9	95.1
mj cal 3	3	✔	5.0	4.7	93.3
mj cal 4	4	✔	10.0	9.7	97.1
mj cal 5	5	✔	25.0	24.6	98.4
mj cal 6	6	✔	50.0	49.7	99.4
mj cal 7	7	✔	100.0	101.3	101.3

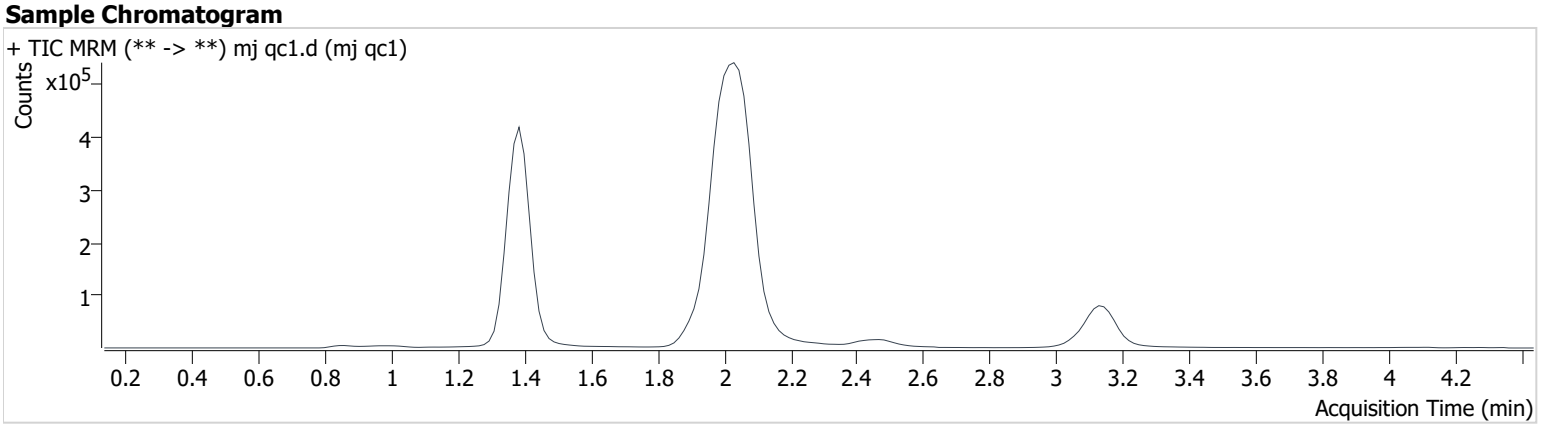
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 7:12:45 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	21900	∞	12.2	∞	1337386	1.154	ng/ml Low
THC-COOH	1.416	35852	54694.9	35.0	374.7	624184	5.533	ng/ml Low
THC	3.149	8717	∞	26.1	43.1	559456	1.223	ng/ml Low

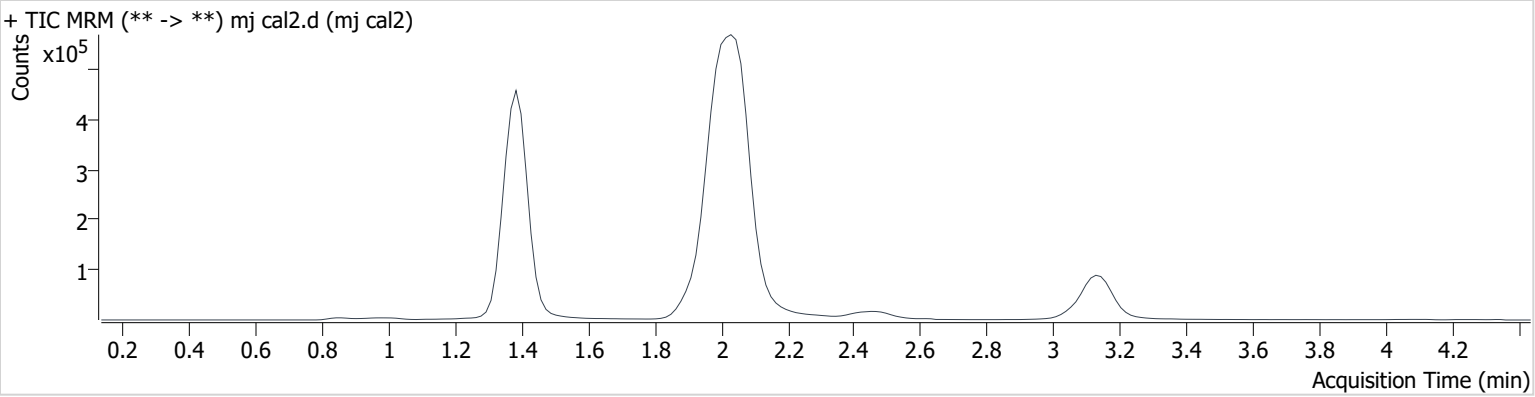
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 7:20:28 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	64352	∞	11.0	∞	1401787	2.853	ng/ml Low
THC-COOH	1.416	74849	76.8	37.3	289.0	656620	9.891	ng/ml Low
THC	3.149	29386	∞	24.3	6703796 516684.3	598909	2.913	ng/ml Low

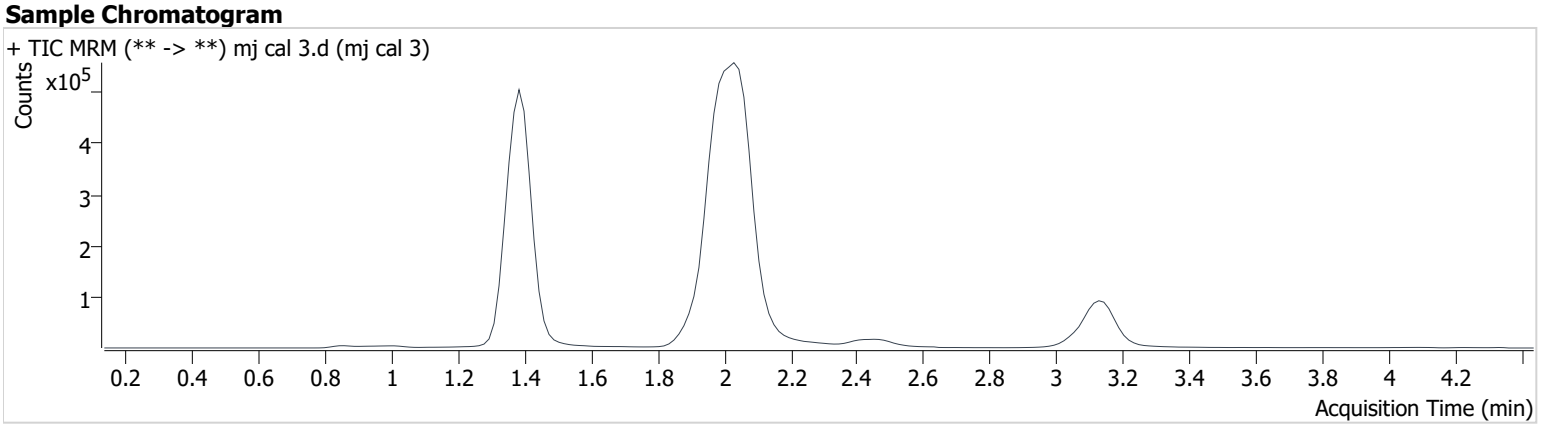
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 7:28:12 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	110052	∞	11.8	∞	1421404	4.667	ng/ml
THC-COOH	1.416	159948	395.7	36.5	1796.6	681991	19.181	ng/ml
THC	3.134	52870	∞	24.1	∞	611510	4.800	ng/ml

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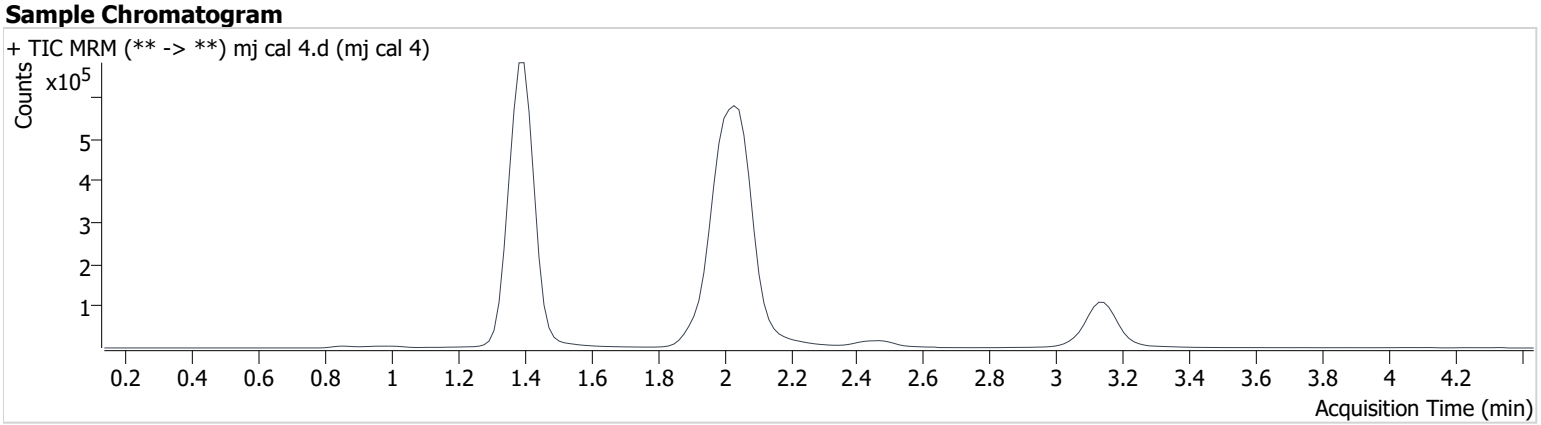
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 7:35:54 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	245285	∞	11.2	∞	1486266	9.707	ng/ml
THC-COOH	1.416	429159	1695.8	36.4	1706.7	700995	48.288	ng/ml
THC	3.149	107071	∞	25.6	∞	643951	8.828	ng/ml

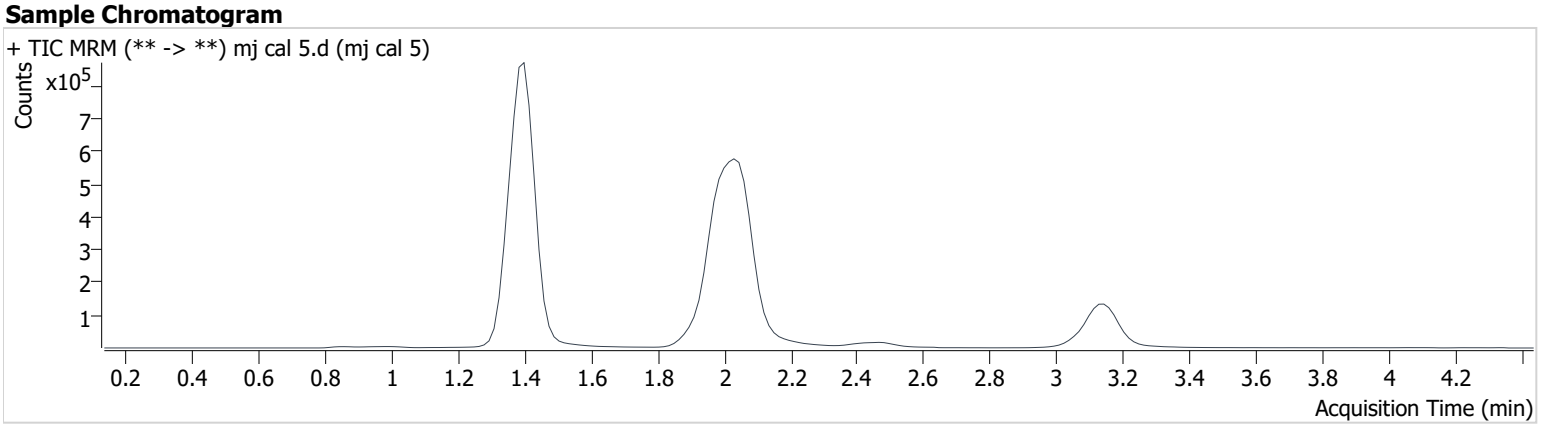
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 7:43:36 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	631303	∞	11.6	∞	1489983	24.590	ng/ml
THC-COOH	1.416	653195	1783.2	37.3	1249.8	690069	74.055	ng/ml
THC	3.149	291256	∞	24.7	∞	637410	23.497	ng/ml

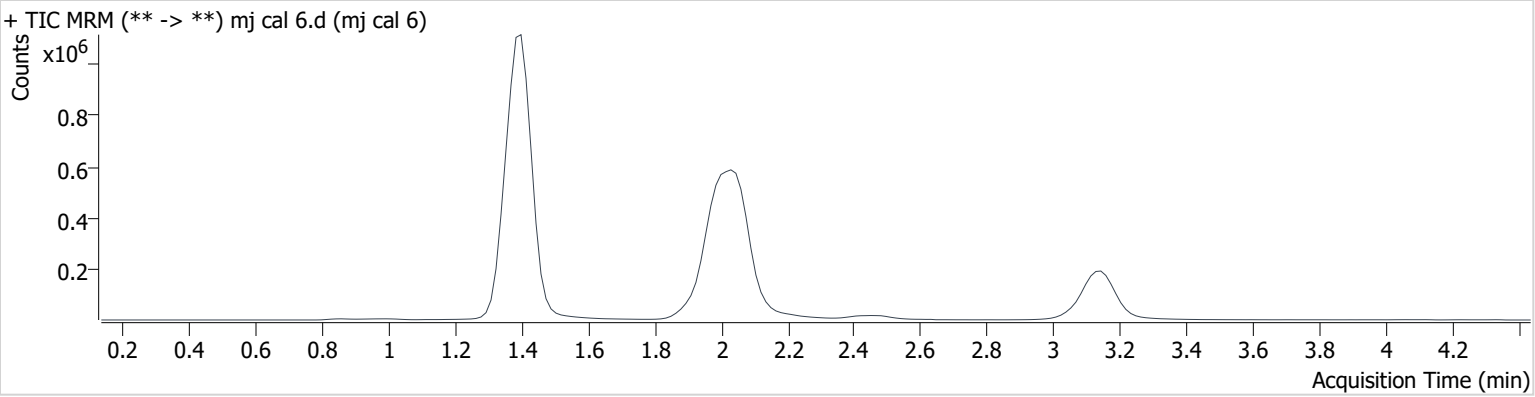
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 7:51:18 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	1303395	∞	11.8	∞	1514952	49.715	ng/ml
THC-COOH	1.416	857036	4375.5	37.5	6729.9	691347	96.644	ng/ml
THC	3.149	626512	∞	23.1	∞	643923	49.538	ng/ml

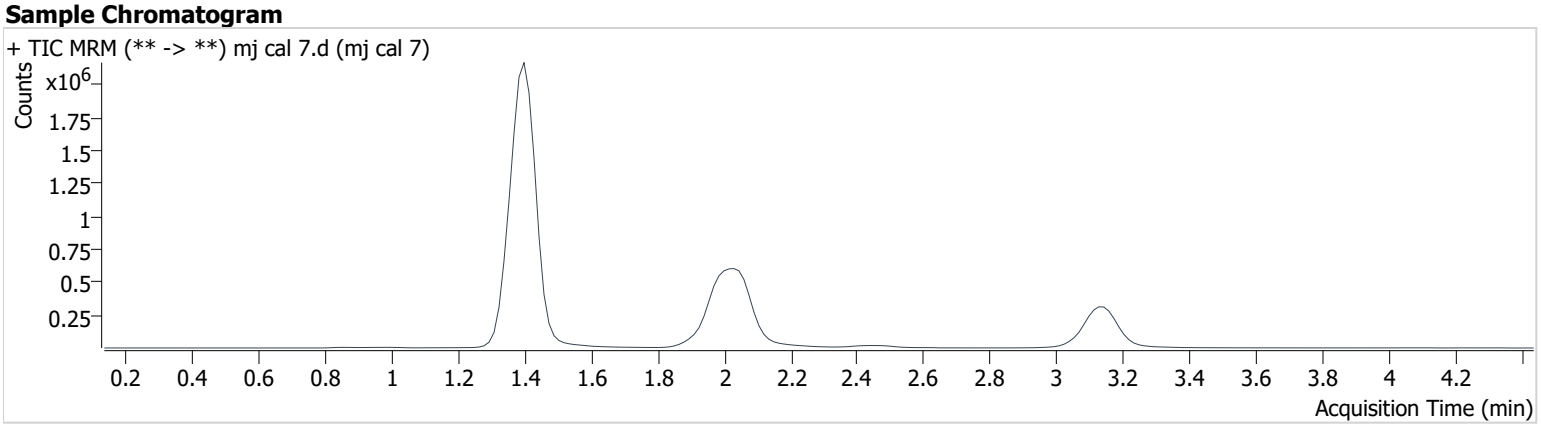
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27 7-17-20\QuantResults\cann.batch.bin
Calibration Last Update 7/18/2020 1:55:05 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	7/17/2020 7:59:00 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	2805970	∞	12.1	∞	1596902	101.314	ng/ml
THC-COOH	1.416	2173091	3806730.6	37.7	10036.0	655985	256.408	ng/ml
THC	3.149	1342131	∞	24.1	∞	659102	103.200	ng/ml