

Worklist: 4446

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2020-1579	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1581	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-1584	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1588	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1595	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1617	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
M2020-2742	4	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2020-2854	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2164	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2165	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2192	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2193	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2198	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2375	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: 8/19/20
Plate lot#: 200303

Analyst: Anne Nord
Plate Expiration: 09-03-2020

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: 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 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 and 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:

Needle seat plugged after cal 6 ran. The Needle seat was replaced. Cal 7 and the internal control ran before the system could equilibrate, those two samples were reinjected; the reinjections were evaluated.

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 ul THC-OH in 9767.5 ul meOH

Ppd 2/13/20 Exp: 9/1/20 lot 21320 by AMN

Drug	lot	expiration
C-THC	FE07171501	9/1/2020
THC-OH	FE07221601	7/1/2021
THC	FE01041701	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
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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 9/1/20 (urine lot 73020)	lot u81320	Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC	by amn	

CA

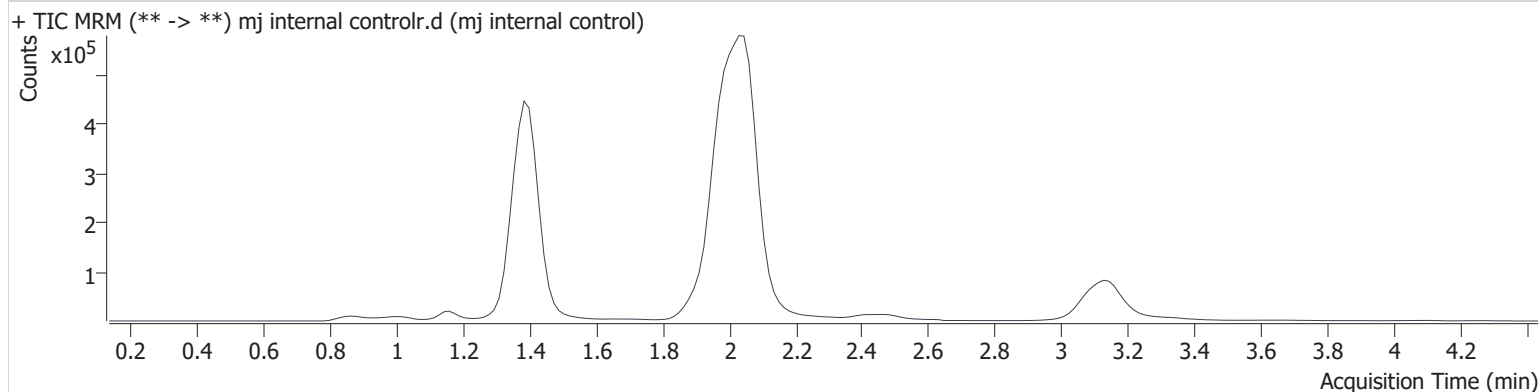
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 PM

Instrument	69679	Data File	mj internal control.r.d
Type	QC	Sample	mj internal control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	8/19/2020 5:02:32 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	97850	∞	9.4	∞	1210188	4.658 ng/ml
THC-COOH	1.416	132555	422.3	36.9	433.9	764919	14.504 ng/ml
THC	3.149	43847	∞	24.9	21161 68714 0428.6	591667	4.193 ng/ml

GA

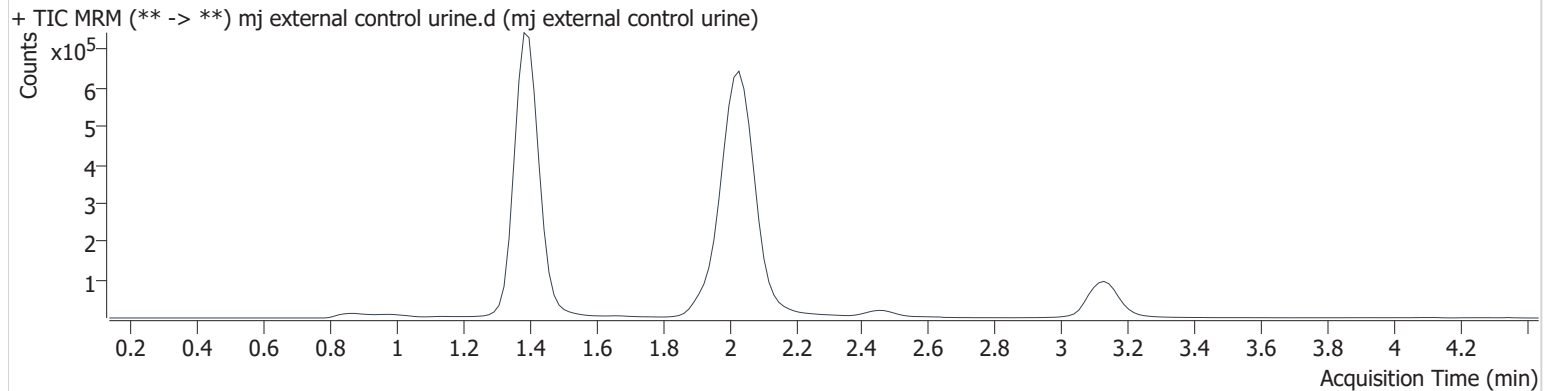
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 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-F3	Comment	
Injection Volume	10		
Acq. Date-Time	8/19/2020 7:59:13 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	378232	∞	12.2	∞	1734370	12.359 ng/ml
THC-COOH	1.416	340512	1071.7	35.6	4316.3	757869	36.927 ng/ml
THC	3.149	119905	∞	24.8	271.5	502702	12.844 ng/ml

EA

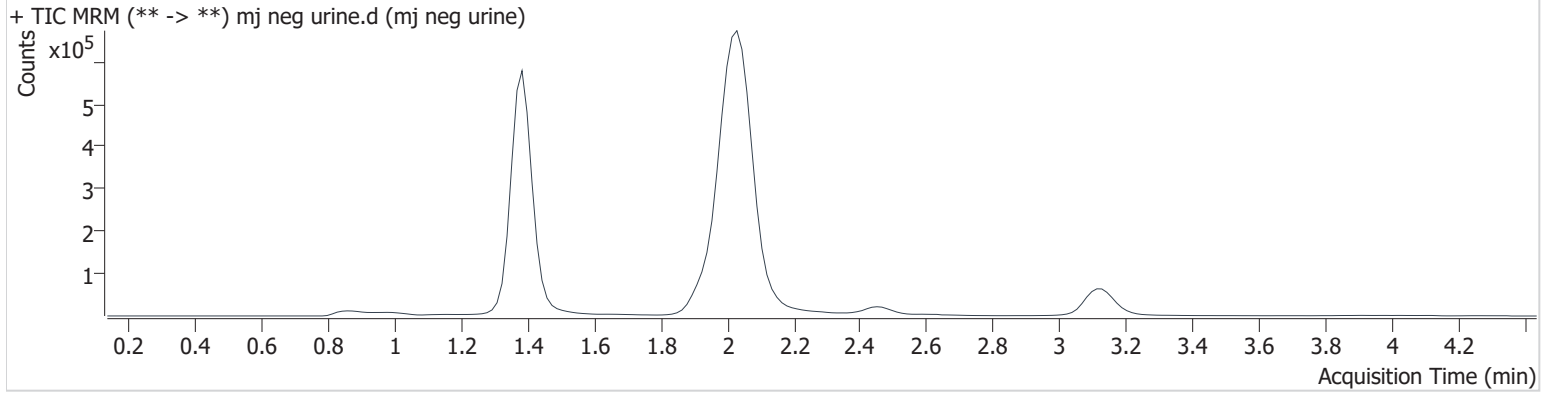
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 PM

Instrument	69679	Data File	mj neg urine.d
Type	Sample	Sample	mj neg urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A4	Comment	
Injection Volume	10		
Acq. Date-Time	8/19/2020 8:52:53 PM		

Sample Info.

Sample Chromatogram

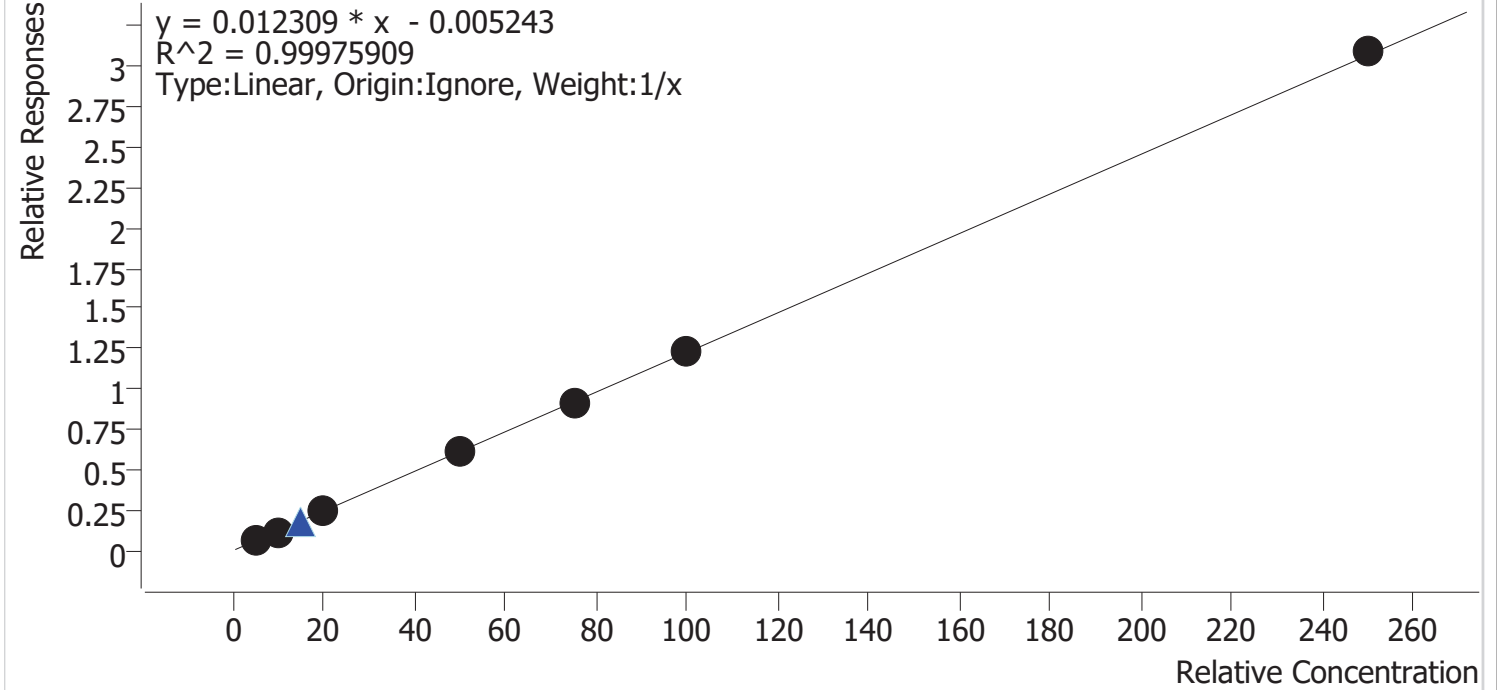


Compound Calibration Report



Batch results	D:\MassHunter\Data\2020 Data\lam 27-28 081920\QuantResults\cann.batch.bin		
Last Cal. Update	8/20/2020 1:01 PM		
Analyst Name	ISP\datastor		
Analyte	THC-COOH	Internal Standard	THC-COOH-d9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.4	108.4
mj cal2	2	✓	10.0	9.5	95.3
mj cal 3	3	✓	20.0	19.5	97.6
mj cal 4	4	✓	50.0	49.3	98.7
mj cal 5	5	✓	75.0	74.7	99.6
mj cal 6	6	✓	100.0	99.7	99.7
mj cal 7	7	✓	250.0	251.8	100.7

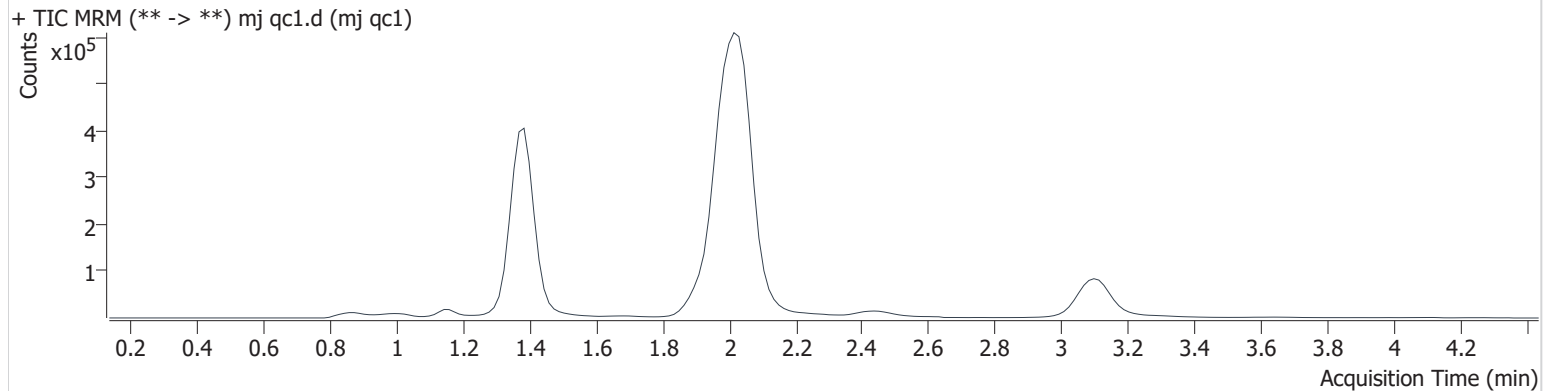
EA

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 PM

Instrument	69679	Data File	mj qc1.d
Type	Cal	Sample	mj qc1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	8/19/2020 2:31:16 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.378	14432	∞	11.6	∞	1197363	0.797 ng/ml	Low
THC-COOH	1.416	42949	89.5	33.6	41.1	698410	5.422 ng/ml	
THC	3.119	10211	∞	29.5	24.8	579223	1.222 ng/ml	Low

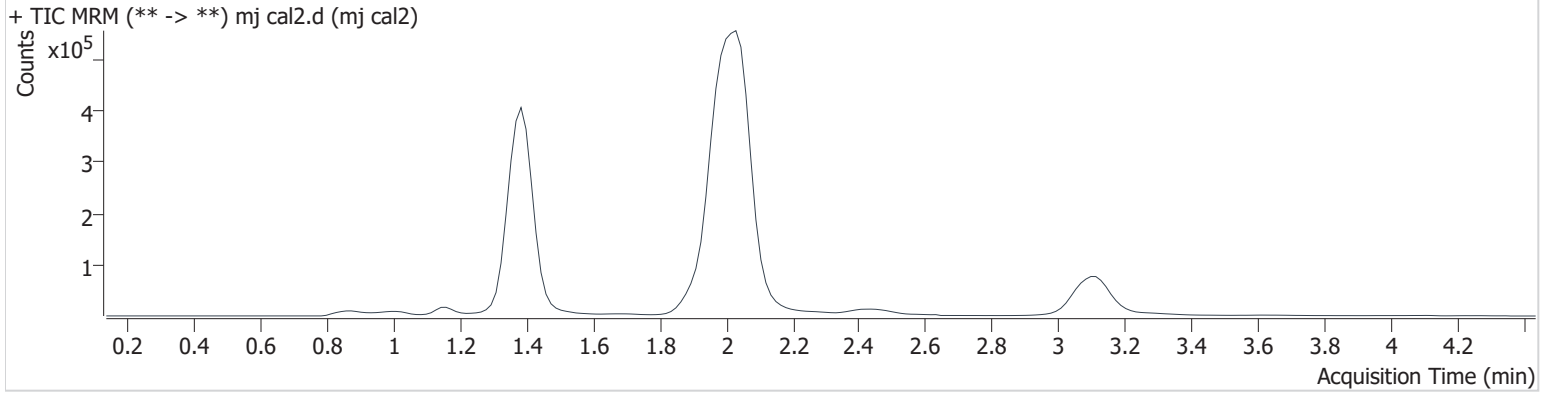
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 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	8/19/2020 2:39:00 PM		

Sample Info.

Sample Chromatogram



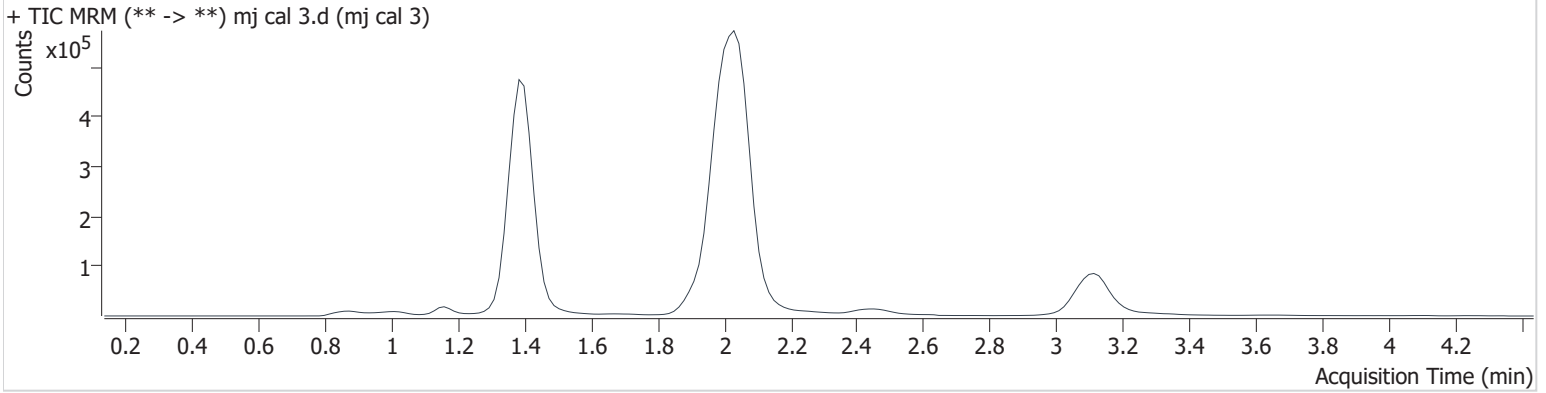
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	70283	∞	9.2	∞	1159745	3.522 ng/ml	
THC-COOH	1.416	78821	137.6	35.3	66.5	703237	9.531 ng/ml	
THC	3.119	27041	∞	27.2	50891	554928	2.858 ng/ml	Low
					56989			
					0419.9			

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 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	8/19/2020 2:46:44 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	108415	∞	10.2	∞	1179119	5.281 ng/ml
THC-COOH	1.416	168108	384.3	36.1	96186.2	715538	19.512 ng/ml
THC	3.119	46668	∞	25.2	86772 66342 4533.3	560283	4.677 ng/ml

EA

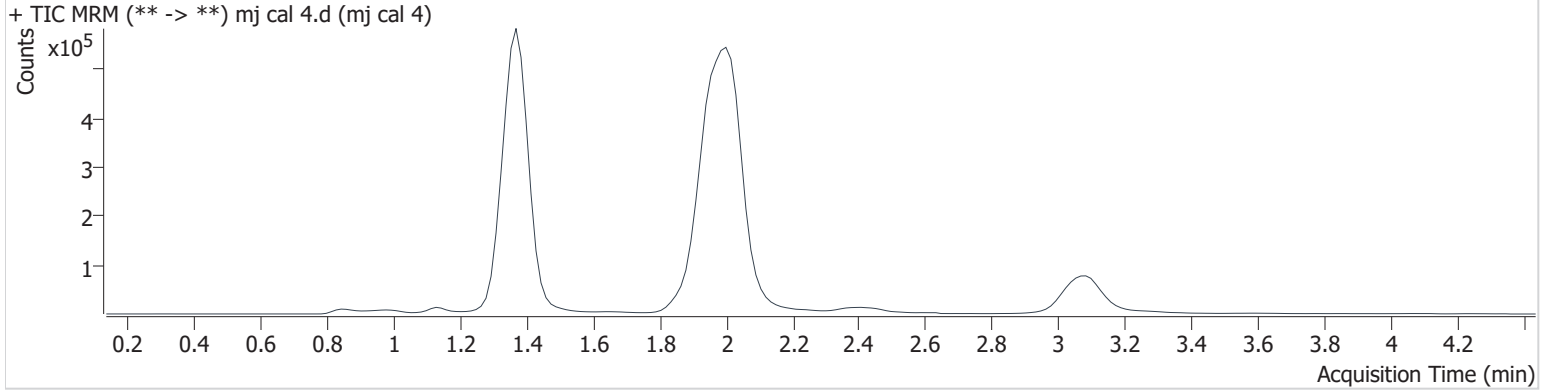
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 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	8/19/2020 2:54:28 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.363	182995	∞	10.9	∞	1034084	10.052 ng/ml
THC-COOH	1.386	418510	1302.0	36.5	1109.7	695028	49.344 ng/ml
THC	3.089	87084	∞	25.6	∞	508590	9.303 ng/ml

GA

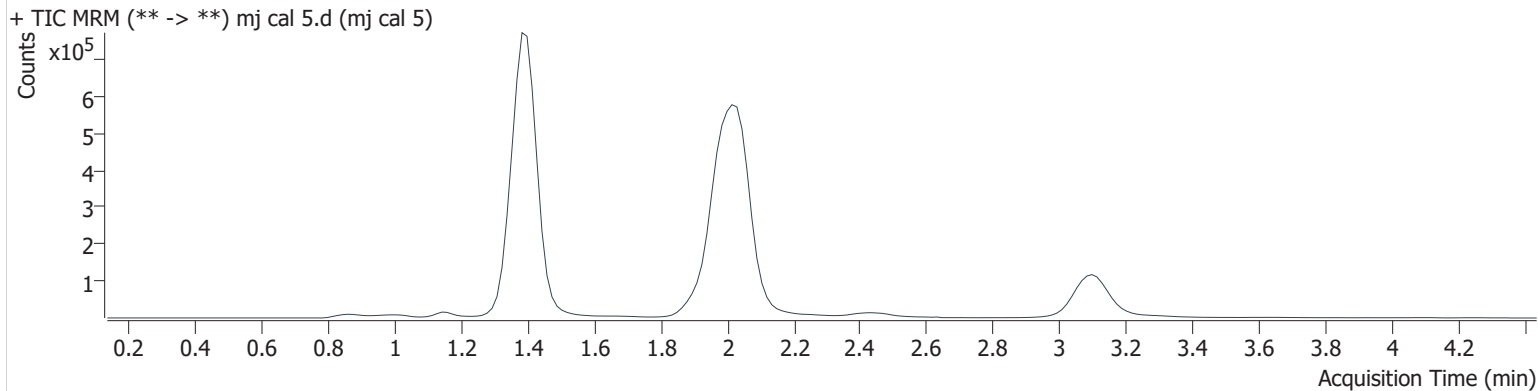
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 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	8/19/2020 3:02:12 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.378	464420	∞	11.0	∞	1070379	24.469 ng/ml
THC-COOH	1.401	629783	3341.2	37.3	3797.4	689181	74.663 ng/ml
THC	3.104	244350	∞	24.1	∞	554263	23.489 ng/ml

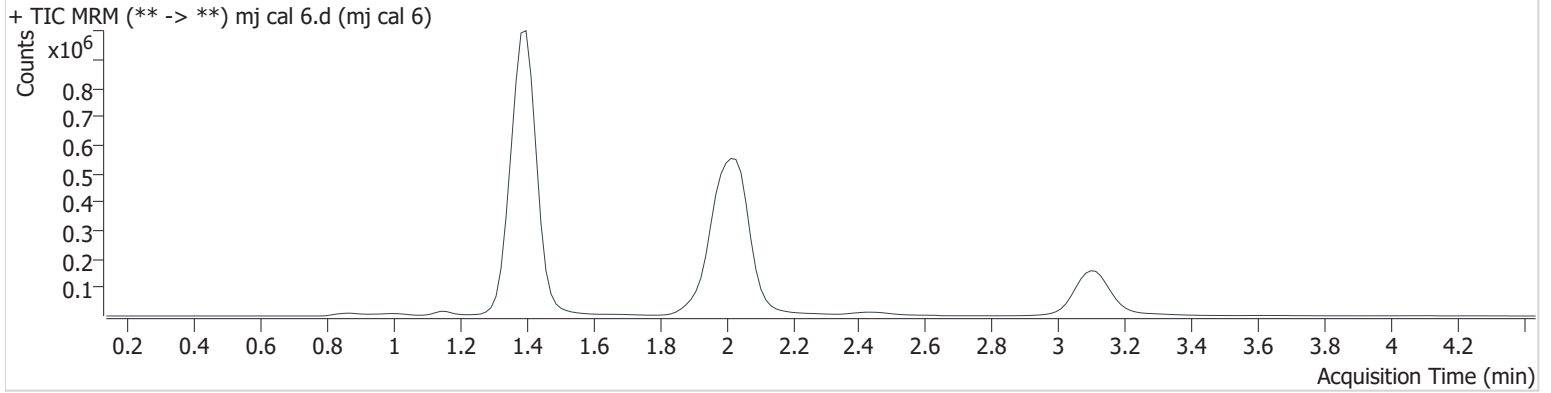
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 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	8/19/2020 3:09:55 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.378	964362	∞	11.5	∞	1107360	48.991 ng/ml
THC-COOH	1.401	858192	2455.4	37.6	7369.5	702073	99.730 ng/ml
THC	3.119	514931	∞	24.4	∞	549853	49.566 ng/ml

CA

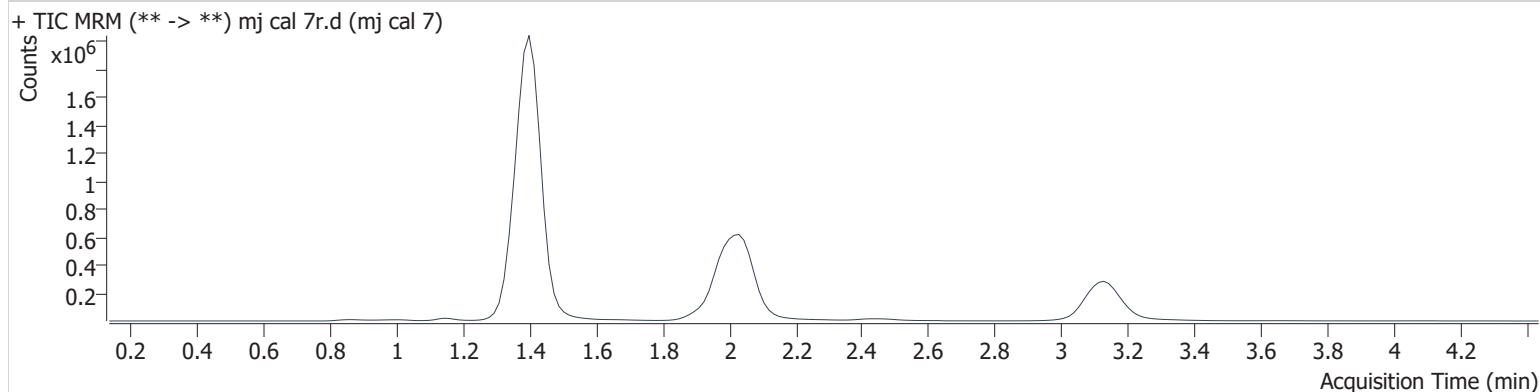
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 PM

Instrument	69679	Data File	mj cal 7r.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	8/19/2020 4:54:50 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.378	2206079	∞	11.8	∞	1228554	100.888 ng/ml
THC-COOH	1.416	2238806	5743.4	37.9	12762 22.2	723549	251.797 ng/ml
THC	3.134	1231529	∞	24.5	∞	631589	102.885 ng/ml

GA

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 081920\QuantResults\cann.batch.bin
Calibration Last Update 8/20/2020 1:01:15 PM

Instrument	69679	Data File	mj negative blood.d
Type	Sample	Sample	mj negative blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	8/19/2020 4:31:44 PM		
Sample Info.			

Sample Chromatogram

