














Worklist: 4407

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2020-1362	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1366	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1367	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1368	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1376	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1391	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1409	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-1420	1	AVK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1432	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1441	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-1464	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

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

Extraction Date: 7/31/20
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:

THC-OH not evaluated for urine samples.

Toxicology AM method 27/26 external prep information ~~GA~~

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: 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 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (73020)

out of use

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	
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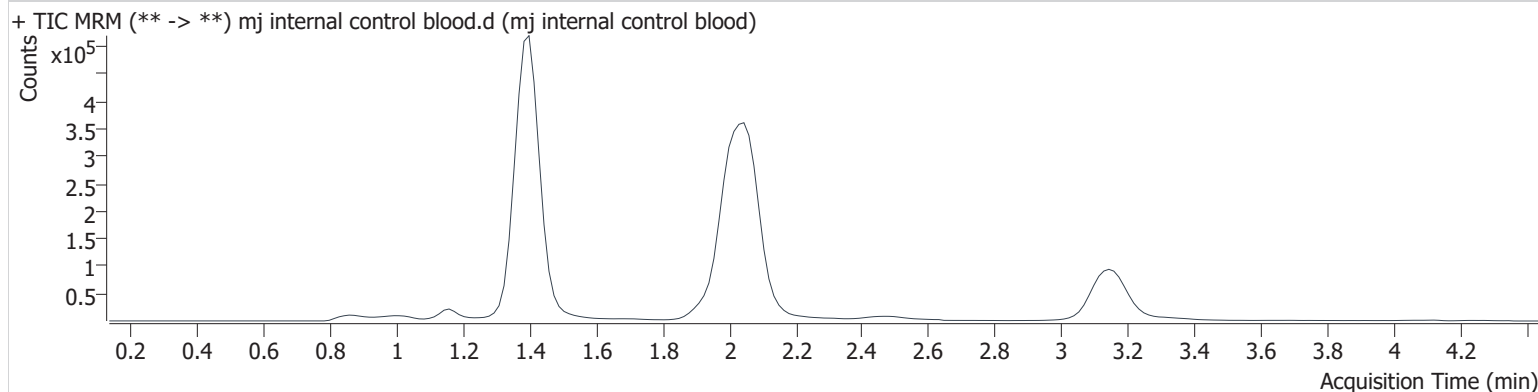
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 PM

Instrument	69679	Data File	mj internal control blood.d
Type	QC	Sample	mj internal control blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	7/31/2020 10:47:45 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	81869	7197.9	11.1	77.0	1314421	4.196 ng/ml
THC-COOH	1.416	159106	317.3	35.2	610.0	844267	15.771 ng/ml
THC	3.149	46521	∞	25.0	77798 92355 288.7	625070	4.125 ng/ml

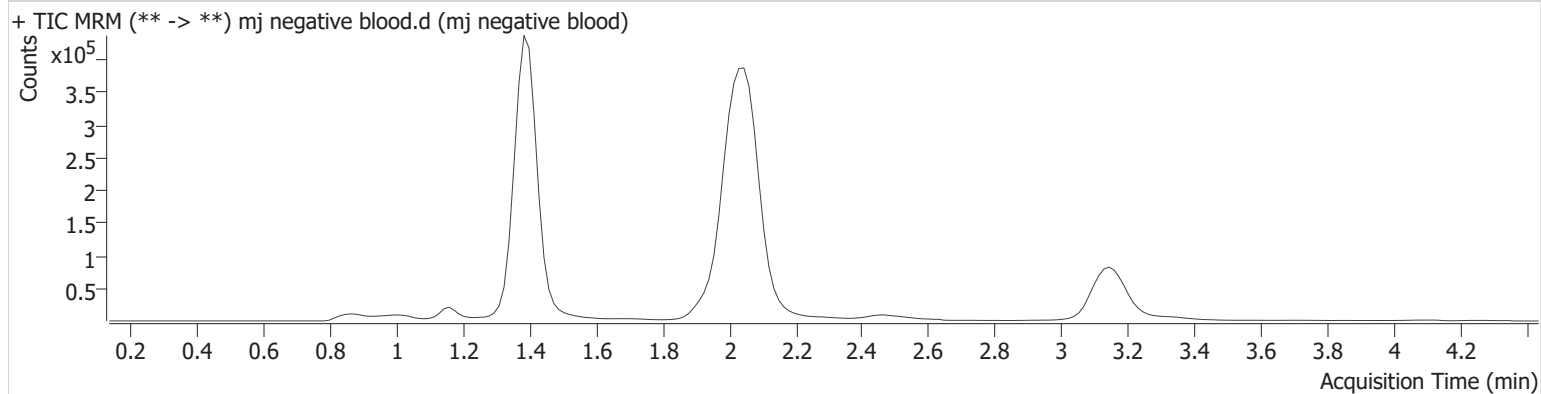
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 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	7/31/2020 10:55:27 AM		
Sample Info.			

Sample Chromatogram



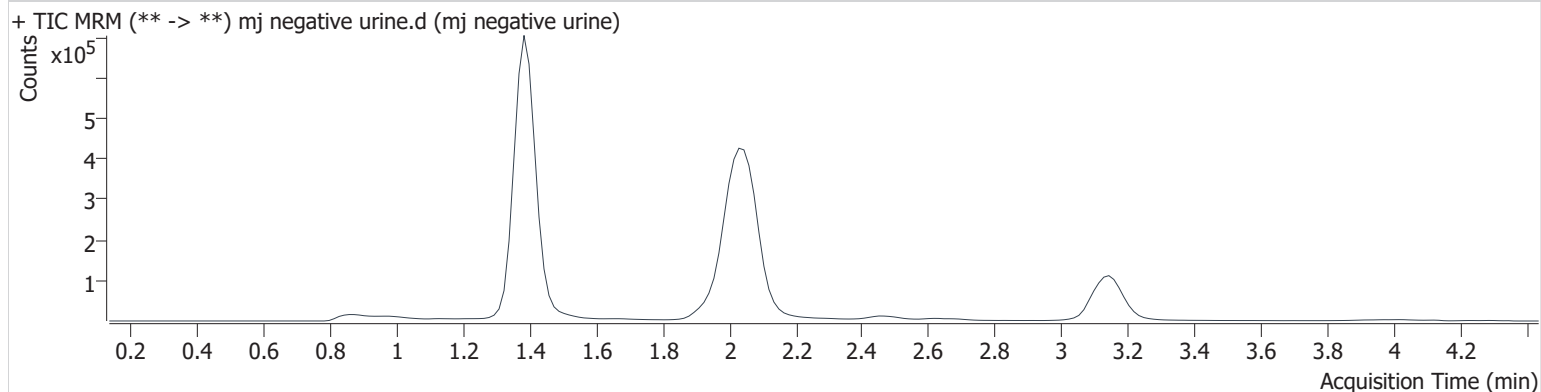
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 PM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D3	Comment	
Injection Volume	10		
Acq. Date-Time	7/31/2020 1:44:21 PM		
Sample Info.			

Sample Chromatogram



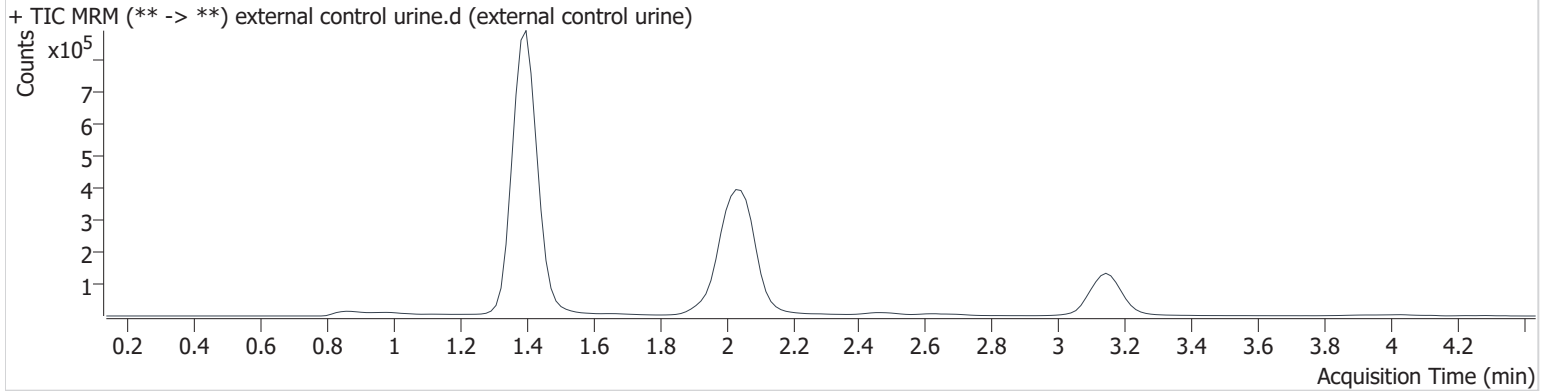
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 PM

Instrument	69679	Data File	external control urine.d
Type	Sample	Sample	external control urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E3	Comment	
Injection Volume	10		
Acq. Date-Time	7/31/2020 1:52:05 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	561522	648.6	15.0 High	1191.6	2005035	17.545 ng/ml
THC-COOH	1.416	423052	2016.5	36.0	380.6	795596	42.278 ng/ml
THC	3.164	209418	∞	23.6	∞	661893	16.697 ng/ml

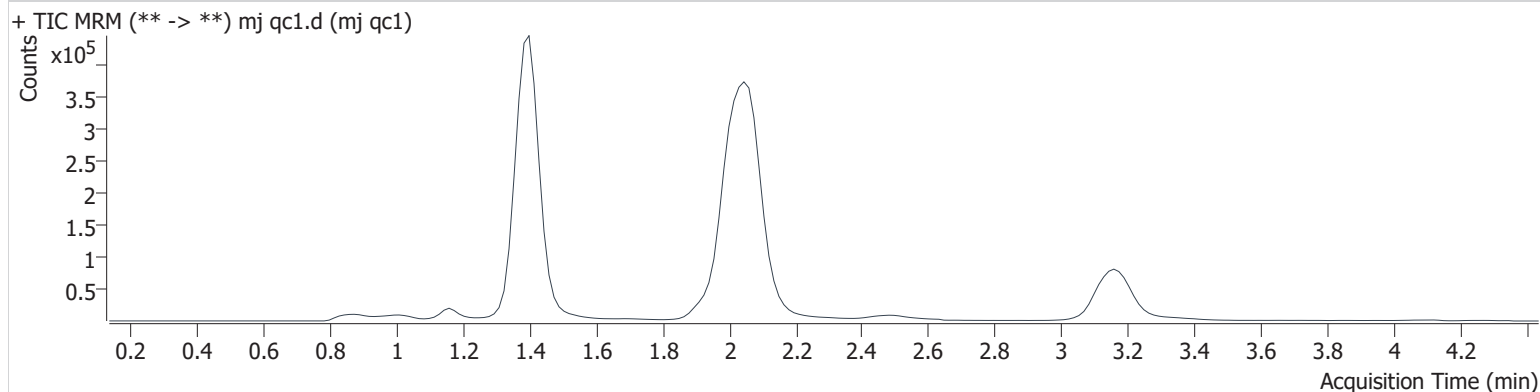
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 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	7/31/2020 9:53:44 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	17088	∞	12.0	310.5	1320882	1.171 ng/ml	Low
THC-COOH	1.431	43808	114.9	37.1	36.5	793444	5.483 ng/ml	Low
THC	3.179	9987	∞	23.7	48.8	572626	1.164 ng/ml	Low

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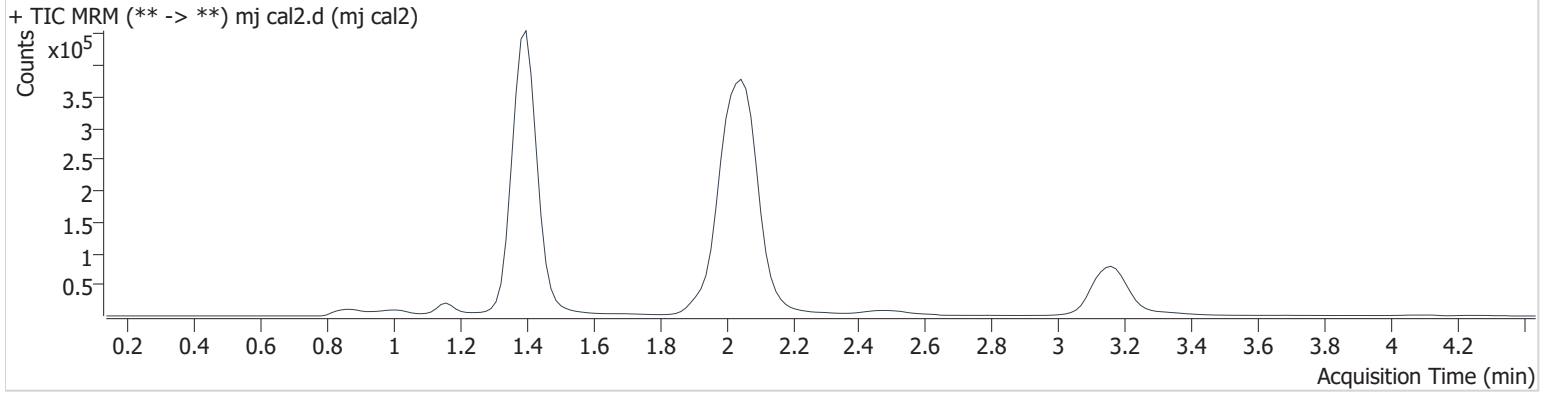
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 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	7/31/2020 10:01:28 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.393	48234	563.3	11.1	41.2	1258719	2.727 ng/ml	Low
THC-COOH	1.416	88990	349.6	36.7	106.9	798473	9.825 ng/ml	Low
THC	3.179	27637	∞	24.3	127.2	544950	2.893 ng/ml	Low

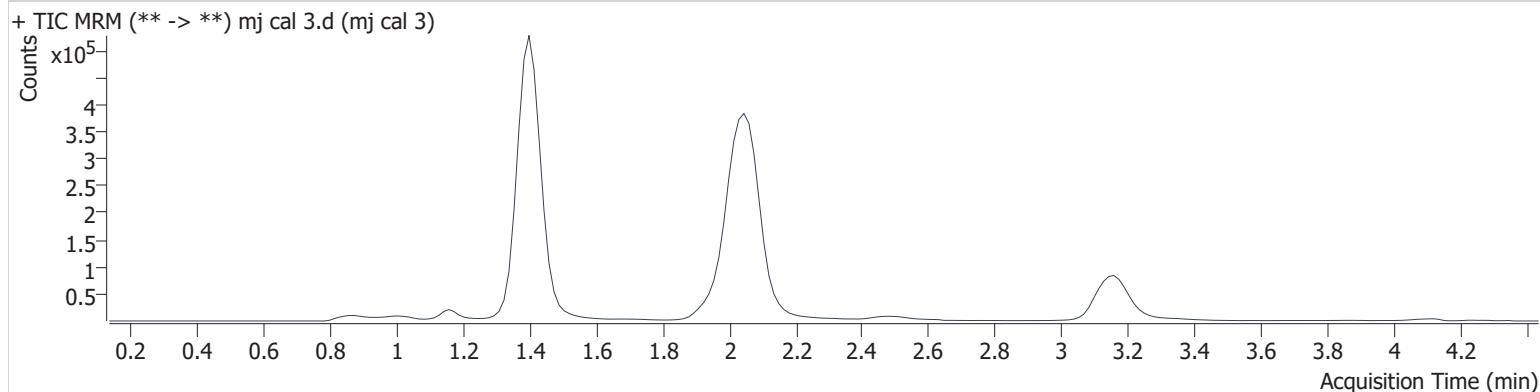
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 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	7/31/2020 10:09:10 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	88685	∞	11.7	63.0	1210174	4.870 ng/ml
THC-COOH	1.416	184786	1004.2	36.4	369.7	762472	19.933 ng/ml
THC	3.179	42514	∞	28.0	184.6	516837	4.532 ng/ml

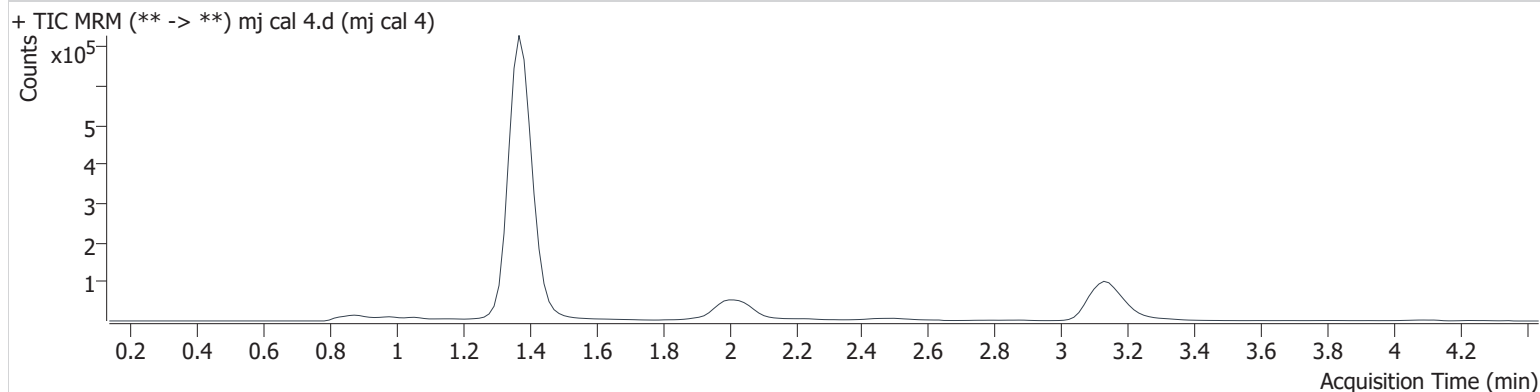
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 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	7/31/2020 10:16:54 AM		

Sample Chromatogram



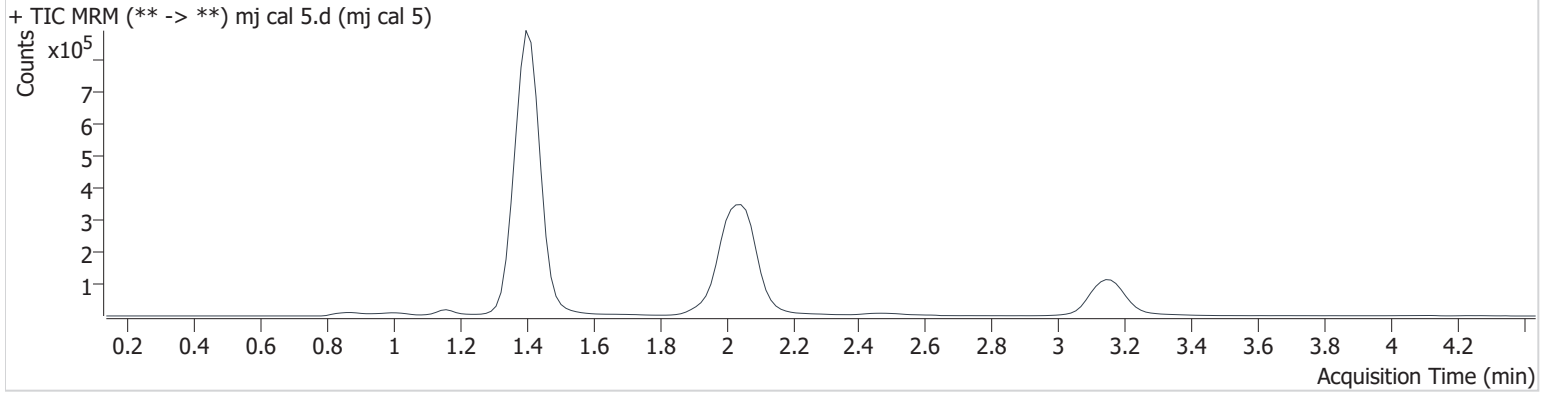
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.378	334249	1616.4	11.4	522.0	2249273	9.487 ng/ml
THC-COOH	1.401	215736	1111.7	35.9	471.6	370350	46.199 ng/ml
THC	3.149	117996	∞	25.2	14390 85716 4683.7	622622	10.105 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 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	7/31/2020 10:24:38 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	535183	∞	11.3	∞	1344481	24.779 ng/ml
THC-COOH	1.416	699417	2235.9	37.3	1814.5	738282	74.369 ng/ml
THC	3.164	245863	∞	24.5	∞	544999	23.698 ng/ml

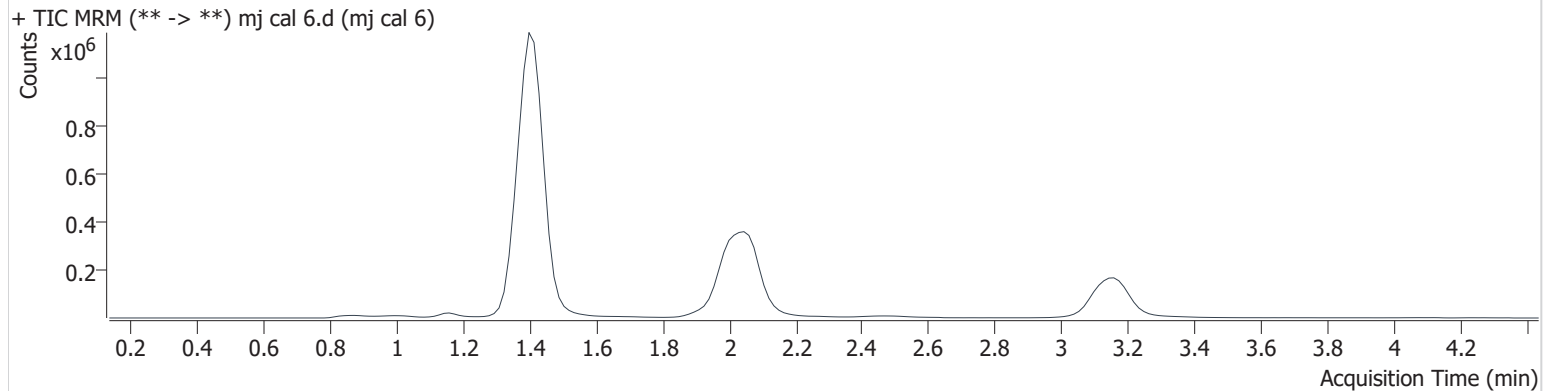
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 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	7/31/2020 10:32:21 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	1065741	∞	11.6	1398.1	1326675	49.621 ng/ml
THC-COOH	1.416	1034168	233.5	38.0	4129.6	819480	98.663 ng/ml
THC	3.164	561168	∞	23.9	∞	597296	49.073 ng/ml

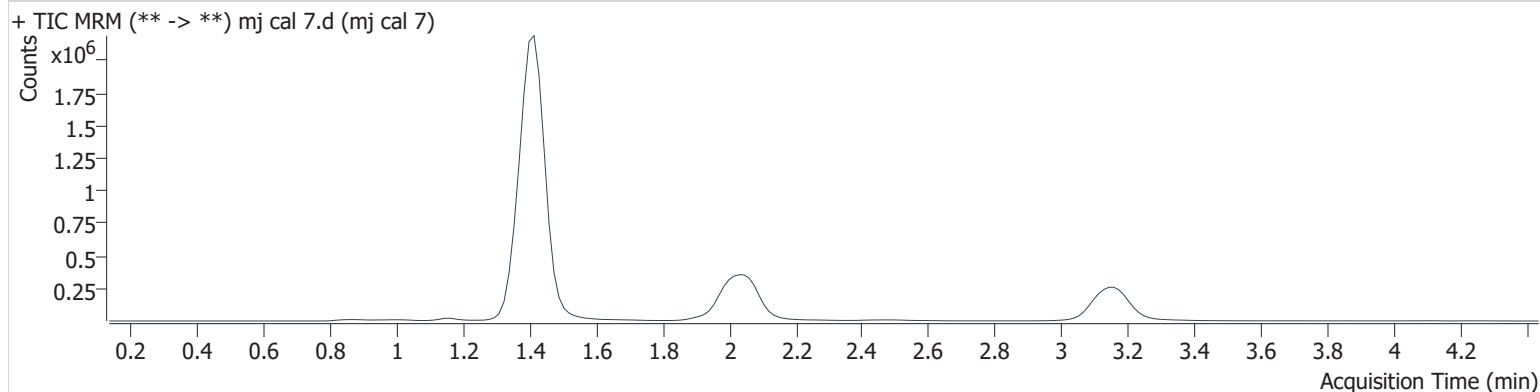
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 7-29-20\QuantResults\cann.batch.bin
Calibration Last Update 7/31/2020 2:54:11 PM

Instrument	69679	Data File	mj cal 7.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	7/31/2020 10:40:03 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.393	2137449	∞	11.9	∞	1297694	101.345 ng/ml
THC-COOH	1.416	2480295	6259.0	37.8	3965.5	753079	255.529 ng/ml
THC	3.164	1149888	∞	24.2	∞	584160	102.535 ng/ml