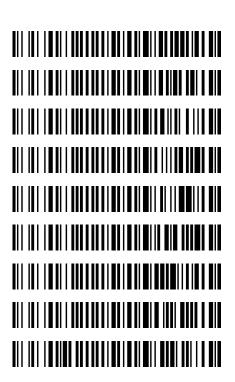
REVIEWED

By Tamara Salazar at 1:48 pm, Aug 08, 2023

Worklist: 6456

LAB CASE	<u>ITEM</u>	ITEM TYPE	DESCRIPTION
C2023-1429	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2023-1444	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-1449	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-1496	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2023-1546	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2023-1613	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
C2023-1657	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
C2023-1687	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
M2023-2977	3	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 7/31/23 Analyst: Anne Nord Plate lot#: 230627 Plate re-test: 12/27/2023

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: 23C57106 Urine Blank: 61423 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 (calibrated pipette) blood or 1000μL hydrolyzed urine Pipette ID: K52558G in wells of analytical (standards) plate.

- ☑ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- Δ 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.
- ✓ 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
- \boxtimes 8. Wait 5 minutes.
- □ Solution
 □ Solution
 □ Add 2.25mL MTBE. (Add in 3 increments of 750uL)
- \boxtimes 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)
- \boxtimes 13. Wait 5 minutes.
- ☑ 14. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- ☑ 16. Reconstitute in 100µL 100% MeOH and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- \boxtimes 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥ 0.98 for each analyte
- \boxtimes 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. SN > 10
- ☑ 5. Did all QCs pass for each analyte? (if not is it describe in comments section)
- ☑ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:

C2023-1546-1 and the end of the run blood control dried out and did not inject. Both samples were reconstituted and reinjected the next day.

	1	2	3	4	5	6
а	cal 1	Internal control urine	negative urine			
b	cal 2	negative blood	1429-2			
С	cal 3	1444-1	1496-1			
d	cal 4	1449-1	1546-1			
е	cal 5	1613-1	1687-1			
f	cal 6	1657-1				
g	cal 7					
h	Internal control (blood)	M2023-2977-3				

Plate position 3

c2023-___--



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/1/2023 12:35:18 PM

Instrument **Type** Acq. Method **Sample Position** 69679 Sample AM 27 THC quant.m

P3-B2 **Injection Volume** 10 7/31/2023 8:48:57 PM

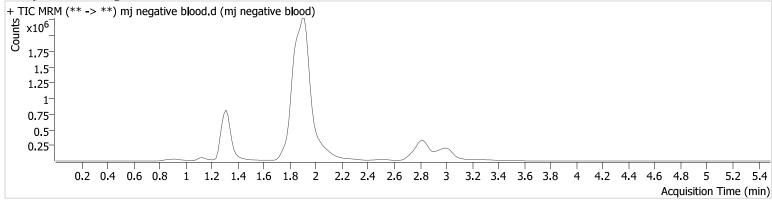
Acq. Date-Time Sample Info.

Data File Sample

Operator Comment

mj negative blood.d mj negative blood Anne Nord

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Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument
Type
Acq. Method
Sample Position

Injection Volume

Acq. Date-Time

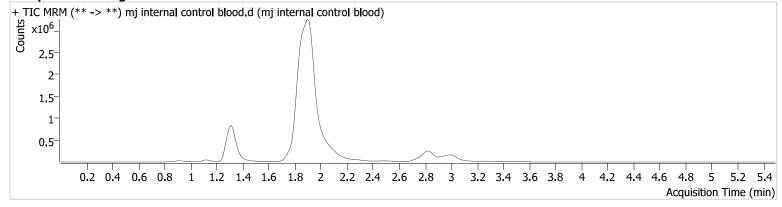
Sample Info.

69679 QC AM 27 THC quant.m P3-H1

10 7/31/2023 8:42:23 PM Data File Sample Operator Comment mj internal control blood.d mj internal control blood

Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	34881	∞	841.67	∞	2595345	4.409 ng/ml
THC-COOH	1.327	52433	∞	288.30	∞	704234	14.177 ng/ml
THC	2.839	29416	∞	373.83	∞	1060754	4.173 ng/ml



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument **Type** Acq. Method **Sample Position Injection Volume** 69679 Sample AM 27 THC quant.m

P3-A2 10

Acq. Date-Time 7/31/2023 11:07:24 PM

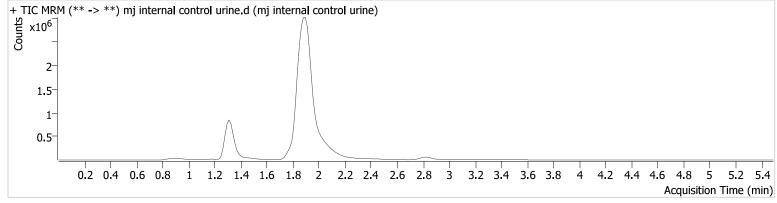
Sample Info.

Data File Sample Operator Comment

mj internal control urine.d mj internal control urine

Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	32079	278.8	785.11	∞	2533644	4.171 ng/ml
THC-COOH	1.342	44476	∞	313.62	∞	633981	13.441 ng/ml
THC	2.839	9873	492.8	363.99	2806.9	344626	4.296 ng/ml



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/1/2023 12:35:18 PM

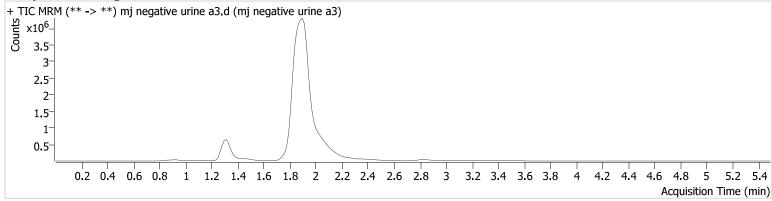
Instrument
Type
Acq. Method
Sample Position

69679 Sample AM 27 THC quant.m

P3-A3 10 7/31/2023 10:08:03 PM

Injection Volume Acq. Date-Time Sample Info. Data File Sample Operator Comment mj negative urine a3.d mj negative urine a3 Anne Nord

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Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument **Type** Acq. Method **Sample Position**

Injection Volume

69679 Sample AM 27 THC quant.m

P3-H1 10

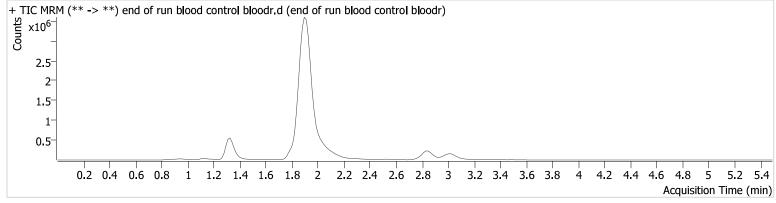
Acq. Date-Time 8/1/2023 11:36:17 AM

Sample Info.

Data File Sample Operator Comment end of run blood control bloodr.d end of run blood control bloodr

Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	18721	173.5	873.00	177014 074175 818.0	1405968	4.371 ng/ml
THC-COOH	1.342	31479	∞	286.52	∞	434549	13.833 ng/ml
THC	2.869	20334	174.0	377.93	1431.2	731528	4.181 ng/ml

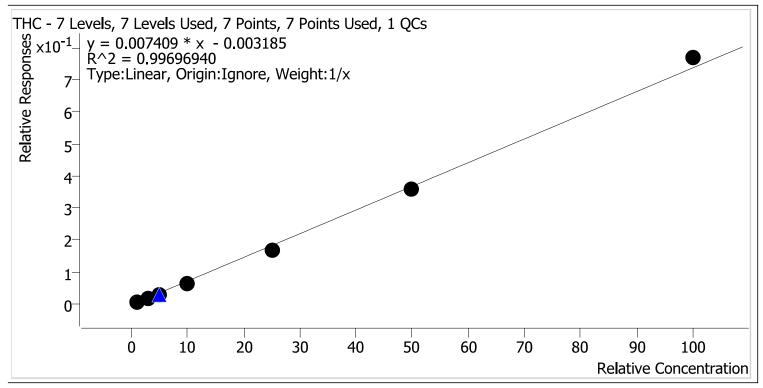
Compound Calibration Report



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Last Cal. Update8/4/2023 8:06 AMAnalyst NameISP\datastor

Analyte THC Internal Standard THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	123.2
mj cal 2	2	✓	3.0	3.0	99.6
mj cal 3	3	✓	5.0	4.6	91.7
mj cal 4	4	✓	10.0	9.1	90.9
mj cal 5	5	✓	25.0	23.3	93.3
mj cal 6	6	✓	50.0	48.6	97.1
mj cal 7	7	✓	100.0	104.2	104.2

Compound Calibration Report

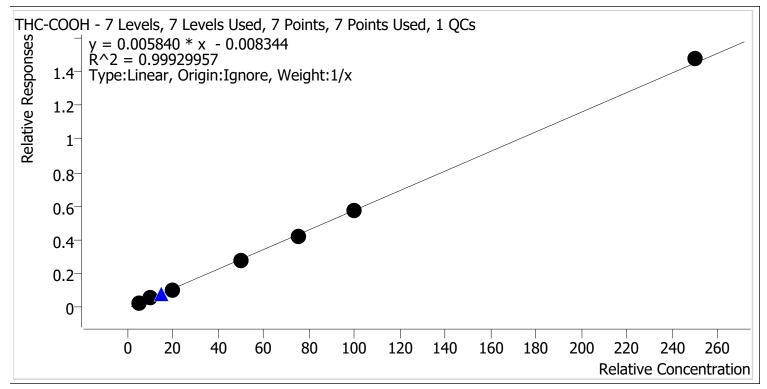


D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin **Batch results**

Last Cal. Update 8/1/2023 12:35 PM

Analyst Name ISP\datastor

Analyte THC-COOH Internal Standard THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	~	5.0	5.5	109.6
mj cal 2	2	~	10.0	10.0	100.2
mj cal 3	3	~	20.0	18.8	93.9
mj cal 4	4	~	50.0	48.7	97.4
mj cal 5	5	~	75.0	73.3	97.7
mj cal 6	6	~	100.0	99.3	99.3
mj cal 7	7	~	250.0	254.4	101.7

Compound Calibration Report

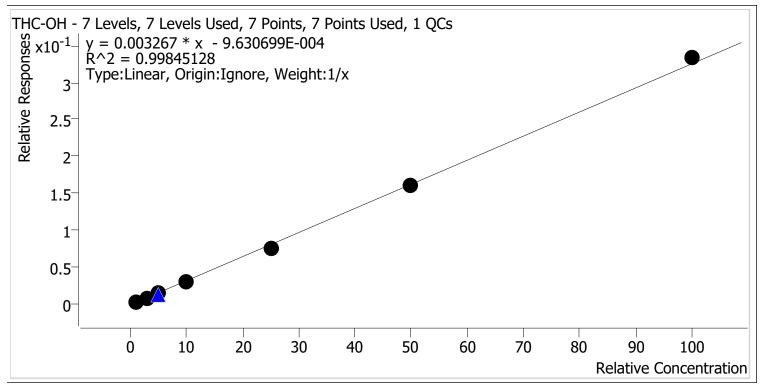


D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin **Batch results**

Last Cal. Update 8/1/2023 12:35 PM

Analyst Name ISP\datastor

Analyte THC-OH THC-OH-d3 **Internal Standard**



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	~	1.0	1.2	121.2
mj cal 2	2	~	3.0	2.9	96.7
mj cal 3	3	~	5.0	4.6	92.2
mj cal 4	4	~	10.0	9.3	93.1
mj cal 5	5	~	25.0	23.7	94.8
mj cal 6	6	~	50.0	49.8	99.6
mj cal 7	7	~	100.0	102.5	102.5



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument
Type
Acq. Method
Sample Position
Injection Volume

69679 Cal AM 27 THC c

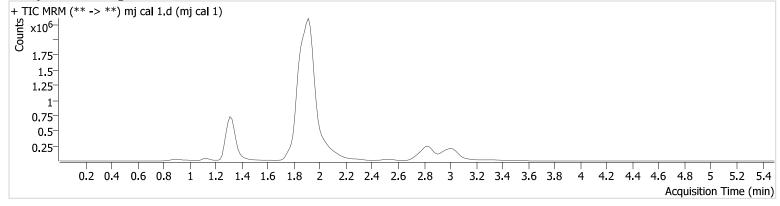
AM 27 THC quant.m P3-A1 10

Acq. Date-Time 7/31/2023 7:49:35 PM **Sample Info.**

Data File Sample Operator Comment

mj cal 1.d mj cal 1 Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Con	C.
THC-OH	1.334	8054	96.3	753.19	∞	2688062	1.212 ng/ml	Low
THC-COOH	1.342	14666	65.5	285.45	∞	619819	5.480 ng/ml	
THC	2.854	5865	51.6	390.17	∞	986792	1.232 ng/ml	



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument **Type** Acq. Method **Sample Position** 69679 Cal AM 27 THC quant.m P3-B1

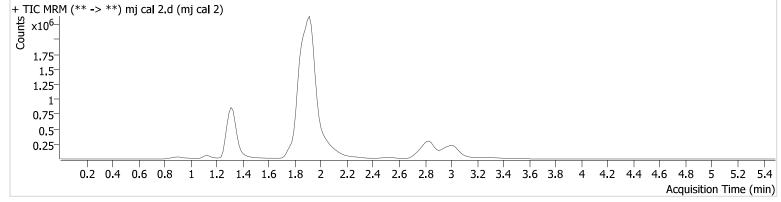
Injection Volume 10 Acq. Date-Time 7/31/2023 7:56:19 PM

Sample Info.

Data File Sample

Operator Comment mj cal 2.d mj cal 2 Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Con	C.
THC-OH	1.319	25386	705.3	824.19	∞	2982449	2.900 ng/ml	Low
THC-COOH	1.342	36026	∞	279.98	∞	718021	10.020 ng/ml	
THC	2.854	24345	213.3	381.73	∞	1284354	2.988 ng/ml	



D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin **Batch results**

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument **Type** Acq. Method **Sample Position Injection Volume** 69679 Cal AM 27 THC quant.m

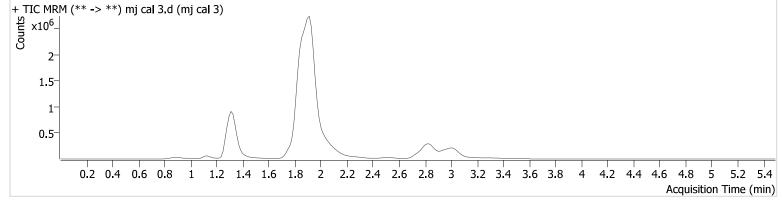
P3-C1 10

Acq. Date-Time 7/31/2023 8:02:53 PM Sample Info.

Data File Sample Operator

mj cal 3.d mj cal 3 Anne Nord Comment

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	40193	∞	880.93	∞	2852678	4.608 ng/ml
THC-COOH	1.342	72656	∞	283.61	∞	716625	18.789 ng/ml
THC	2.839	37279	685.6	401.80	∞	1211339	4.583 ng/ml



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument
Type
Acq. Method
Sample Position

Injection Volume

Acq. Date-Time

Sample Info.

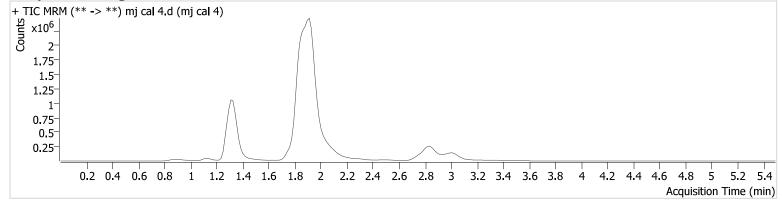
69679 Cal AM 27 THC qua

AM 27 THC quant.m P3-D1 10

7/31/2023 8:09:27 PM

Data File Sample Operator Comment mj cal 4.d mj cal 4 Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	77408	∞	856.00	∞	2628687	9.309 ng/ml
THC-COOH	1.342	194815	∞	273.61	∞	705547	48.709 ng/ml
THC	2.854	66255	∞	403.93	∞	1032706	9.089 ng/ml



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument **Type** Acq. Method **Sample Position** 69679 Cal AM 27 THC quant.m P3-E1

Injection Volume 10 7/31/2023 8:16:03 PM

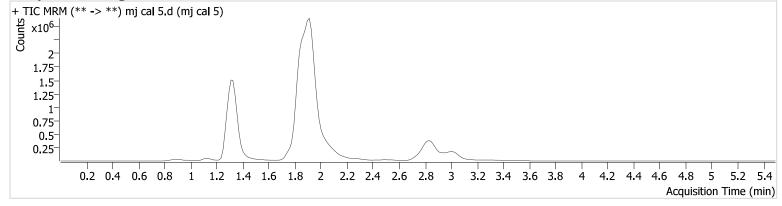
Acq. Date-Time Sample Info.

Data File Sample Operator

Comment

mj cal 5.d mj cal 5 Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	219554	∞	860.16	∞	2872853	23.690 ng/ml
THC-COOH	1.342	315308	∞	271.35	∞	751113	73.310 ng/ml
THC	2.854	205165	∞	402.74	∞	1209344	23.326 ng/ml



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument
Type
Acq. Method
Sample Position
Injection Volume

69679 Cal AM 27 THC quant.m

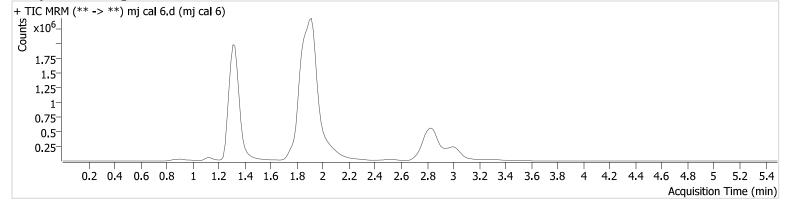
AM 27 THC quant P3-F1 10

7/31/2023 8:22:39 PM

Acq. Date-Time Sample Info.

Data File Sample Operator Comment mj cal 6.d mj cal 6 Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	458758	∞	832.06	∞	2835574	49.822 ng/ml
THC-COOH	1.342	390113	∞	270.68	∞	682249	99.340 ng/ml
THC	2.839	464073	∞	395.77	∞	1301134	48.567 ng/ml



Batch results D:\MassHunter\Data\2023\am 27-28\073123\QuantResults\cann.batch.bin

Calibration Last Update 8/4/2023 8:06:44 AM

Instrument
Type
Acq. Method
Sample Position
Injection Volume

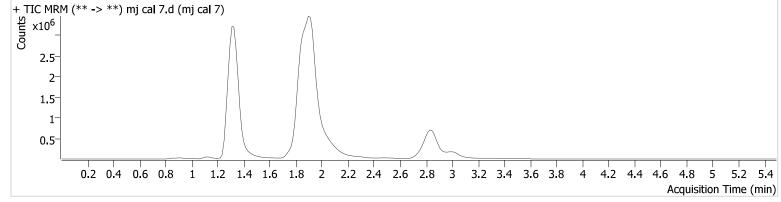
69679 Cal AM 27 THC quant.m

P3-G1 10

Acq. Date-Time 7/31/2023 8:29:13 PM **Sample Info.**

Data File Sample Operator Comment mj cal 7.d mj cal 7 Anne Nord

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Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	856601	16882.0	824.48	∞	2566784	102.458 ng/ml
THC-COOH	1.342	929718	∞	266.42	∞	629429	254.352 ng/ml
THC	2.839	758086	∞	392.40	∞	985838	104.214 ng/ml