REVIEWED
By Sarah Collins at 12:28 pm, Nov 22, 2022

Worklist: 6172

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
C2022-2469	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2487		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2489		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2492		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2514		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2022-2517		AVK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2526	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2022-2528		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 11/18/22 Analyst: Anne Nord
Plate lot#: 220802 Plate re-test: 2/2/23

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: 22B52016-1 **Urine Blank:** blood only **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: I41142J** 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 1ng/ml, 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 curve range 3-100 (1ng calibrator dropped due to ratio being out of range.)*

The internal blood control was also injected at the end of the run, it evaporated and did not inject, that injection was not evaluated.

Idaho State Police
Forensic Services

Request for Departure from an Analytical Method or Quality Standard

Deviation Number (assigned by QM): **TOX-22-02**

Date of Request:
03/02/2022

Requestor/Discipline:
Celena Shrum/Toxicology

Analytical Method/Quality Standard, Revision #:
Toxicology AM #25, AM #26, and AM #27, Revision 13

Temporary or Permanent Deviation:
Permanent

Scope of Deviation (record specific information, e.g. affected programs, evidence types, expected end date; etc):

Deviation will remain in place until the change is made in the next method revision.

Deviation Request (Describe detailed instructions of the changes being made; include reference to specific section number(s) in the method manual):

Toxicology AM #25 3.3.1.1 Internal standards are prepared by the ToxBox plate manufacturer and contained on the 96 well plate. If the run contains urine samples, a positive external urine
control must also be run.

Toxicology AM #26 3.3.2 A negative control will be run with each extraction. If the run contains urine samples, a negative urine control and external positive urine control must also be included.

Toxicology AM #27 3.3.2 A negative control will be run with each extraction. If the run contains urine samples, a negative urine control and positive external urine control will also be included in the run.

The deviation is to include the option of using an internal urine control in lieu of an external urine control.

Technical Justification for Analytical Method Deviations:

Internal controls serve the same purpose as external controls but also helps to avoid the possible issues that can occur with using external controls (incorrect spiking, incorrect preparation, evaporation of compounds, etc.). If these errors occur, runs need to be repeated and this wastes time, sample, and supplies.

Technical Review

☒ Departure approved
Comments:

☐ Departure Not Approved
Comments:

Approver: Rachel Cutler Date: 3/2/22
Title: Lab Manager

Quality Review

Quality Approver: Jason Crowe
Title: Quality Manager
Date: 3/2/2022

	1	2	3	4	5	6
a	cal 1	Internal control urine	2528-1			
b	cal 2	negative blood	negative urine			
c	cal 3	2469-2	2514-1			
d	cal 4	2487-1				
e	Cal 5	2489-1				
f	cal 6	2492-1				
g	cal 7	2517-1				
h	Internal control (blood)	2526-2				

Plate position 3

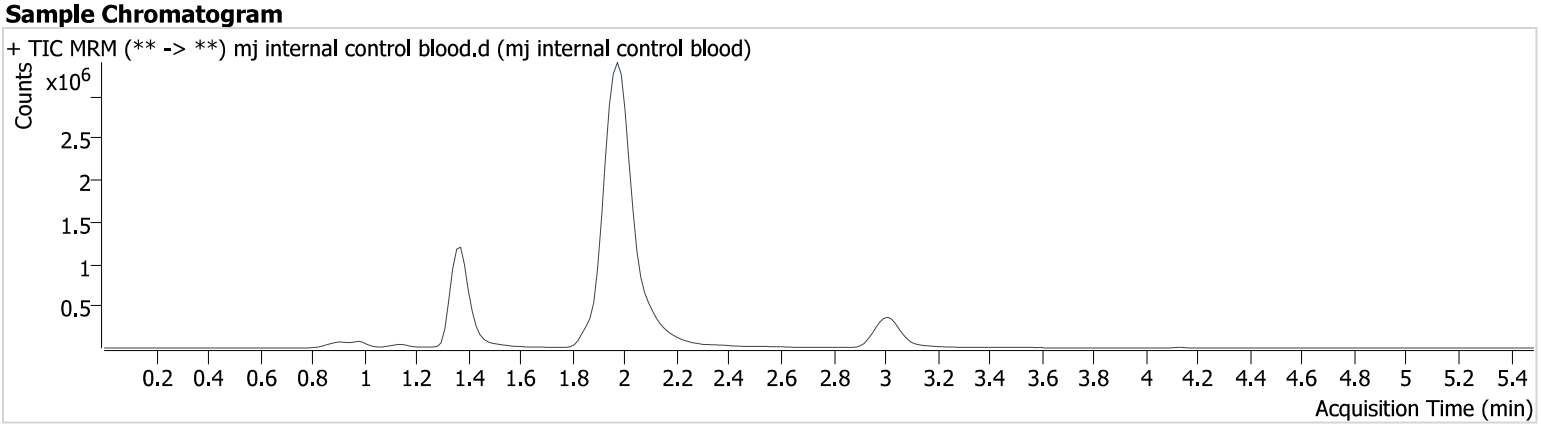
c2022-____-_

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

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	11/18/2022 5:47:42 PM		
Sample Info.			

Sample Chromatogram



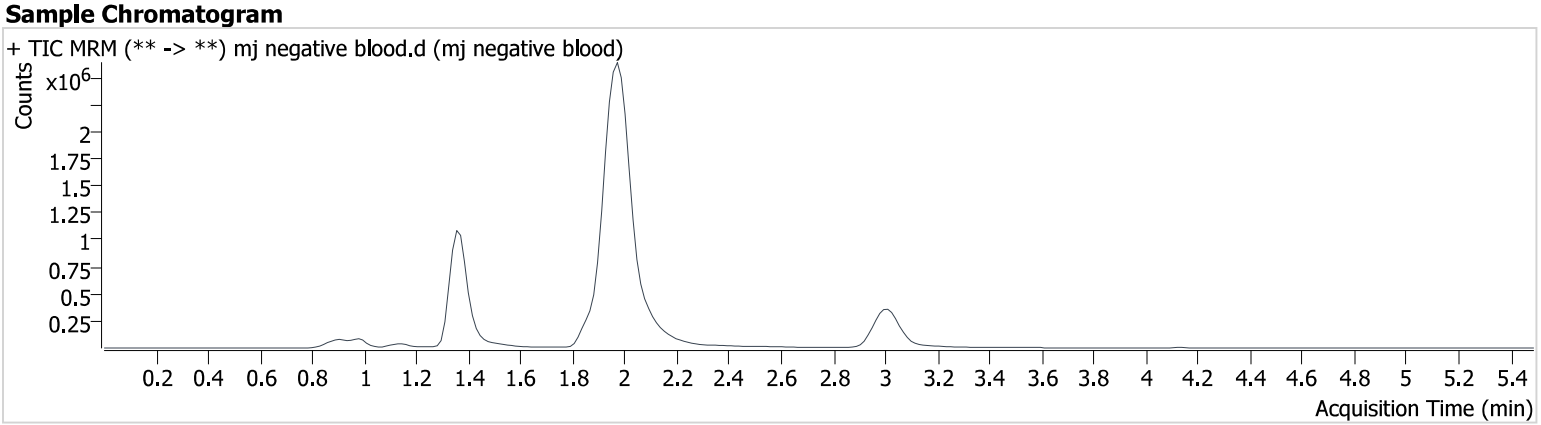
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	72084	290.7	750.83	∞	3496883	5.042 ng/ml
THC-COOH	1.388	91234	527.3	201.26	∞	1178228	14.503 ng/ml
THC	3.031	271100	1352.5	26.70	∞	2228867	4.728 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update	11/21/2022 9:29:00 AM

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-B2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/18/2022 5:54:26 PM		
Sample Info.			

Sample Chromatogram

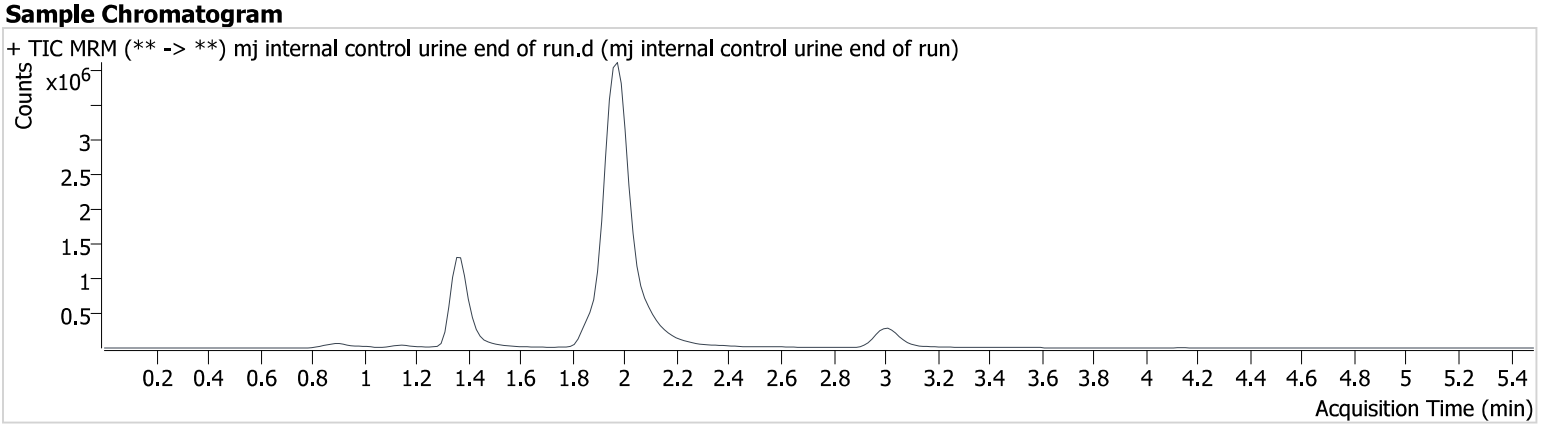


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

Instrument	69679	**Data File**	mj internal control urine end of run.d
Type	Sample	**Sample**	mj internal control urine end of run
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/18/2022 7:55:07 PM		
Sample Info.			

Sample Chromatogram



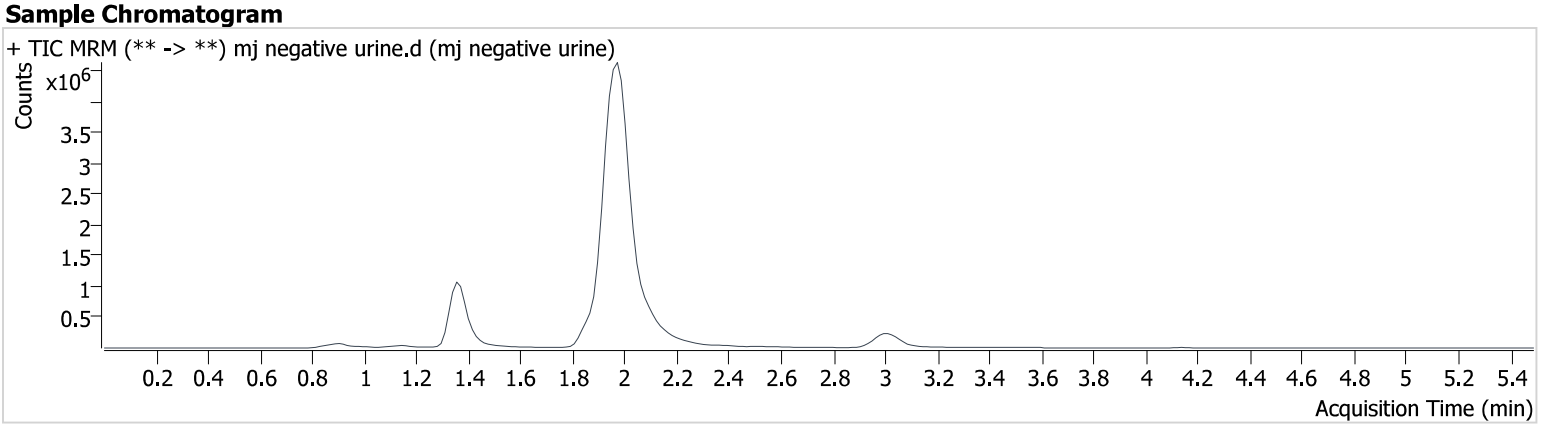
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	68683	239.6	904.35	∞	3637368	4.698 ng/ml
THC-COOH	1.388	100241	475.5	240.33	∞	1178589	15.781 ng/ml
THC	3.016	205469	∞	23.28	∞	1698504	4.704 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update	11/21/2022 9:29:00 AM

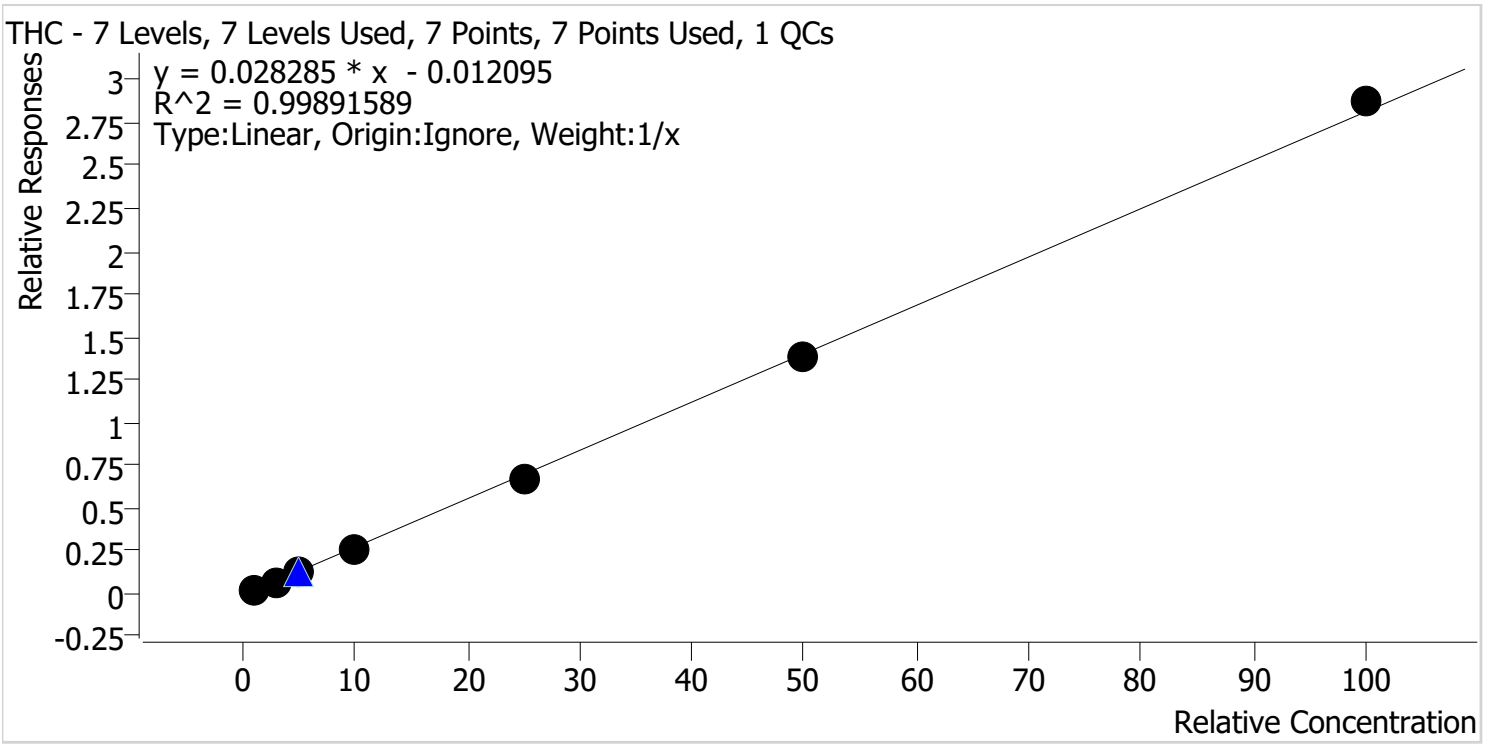
Instrument	69679	**Data File**	mj negative urine.d
Type	Sample	**Sample**	mj negative urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B3	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/18/2022 7:35:00 PM		
Sample Info.			

Sample Chromatogram



Compound Calibration Report

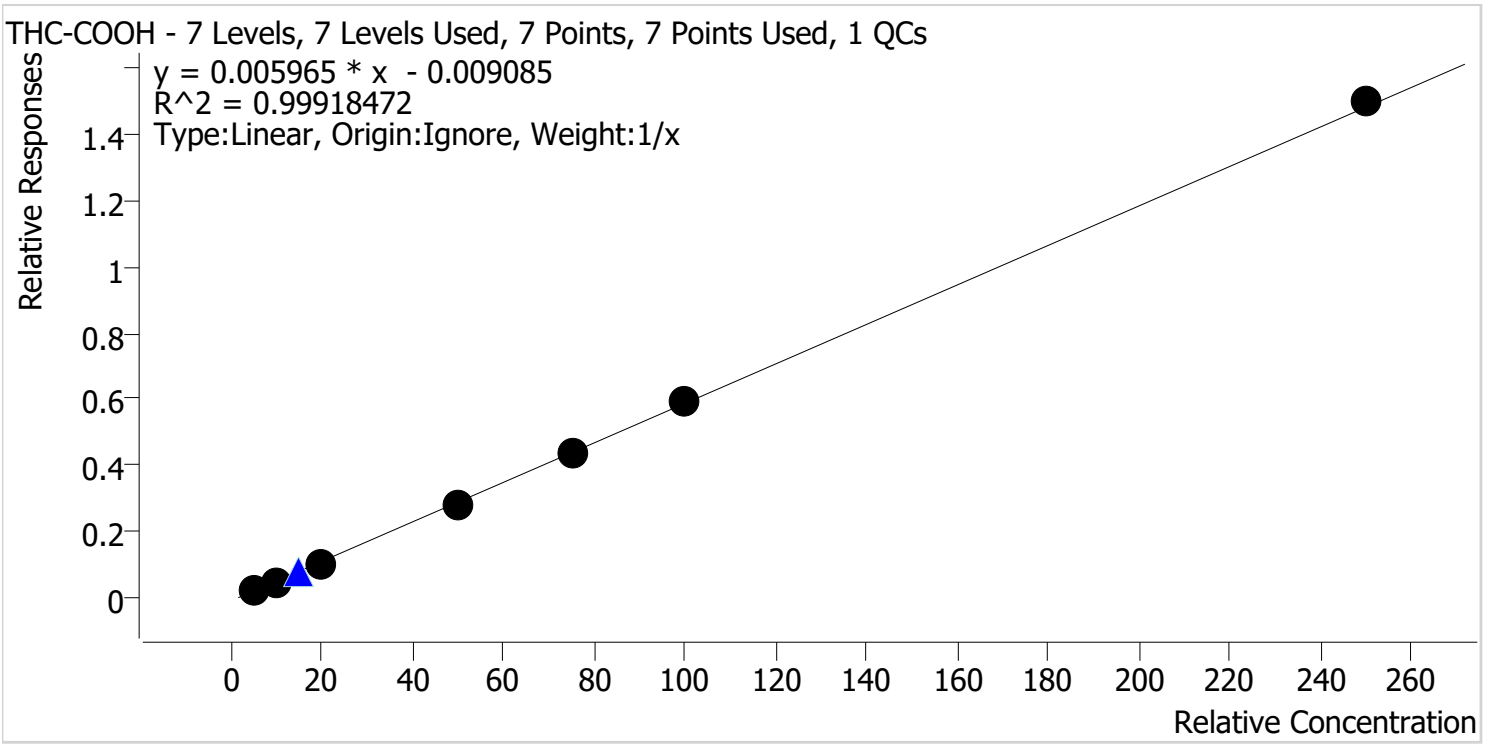
Batch results	D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Last Cal. Update	11/21/2022 9:29 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	119.7
mj cal 2	2	✔	3.0	2.9	95.1
mj cal 3	3	✔	5.0	4.7	93.0
mj cal 4	4	✔	10.0	9.4	94.3
mj cal 5	5	✔	25.0	24.1	96.5
mj cal 6	6	✔	50.0	49.7	99.4
mj cal 7	7	✔	100.0	102.1	102.1

Compound Calibration Report

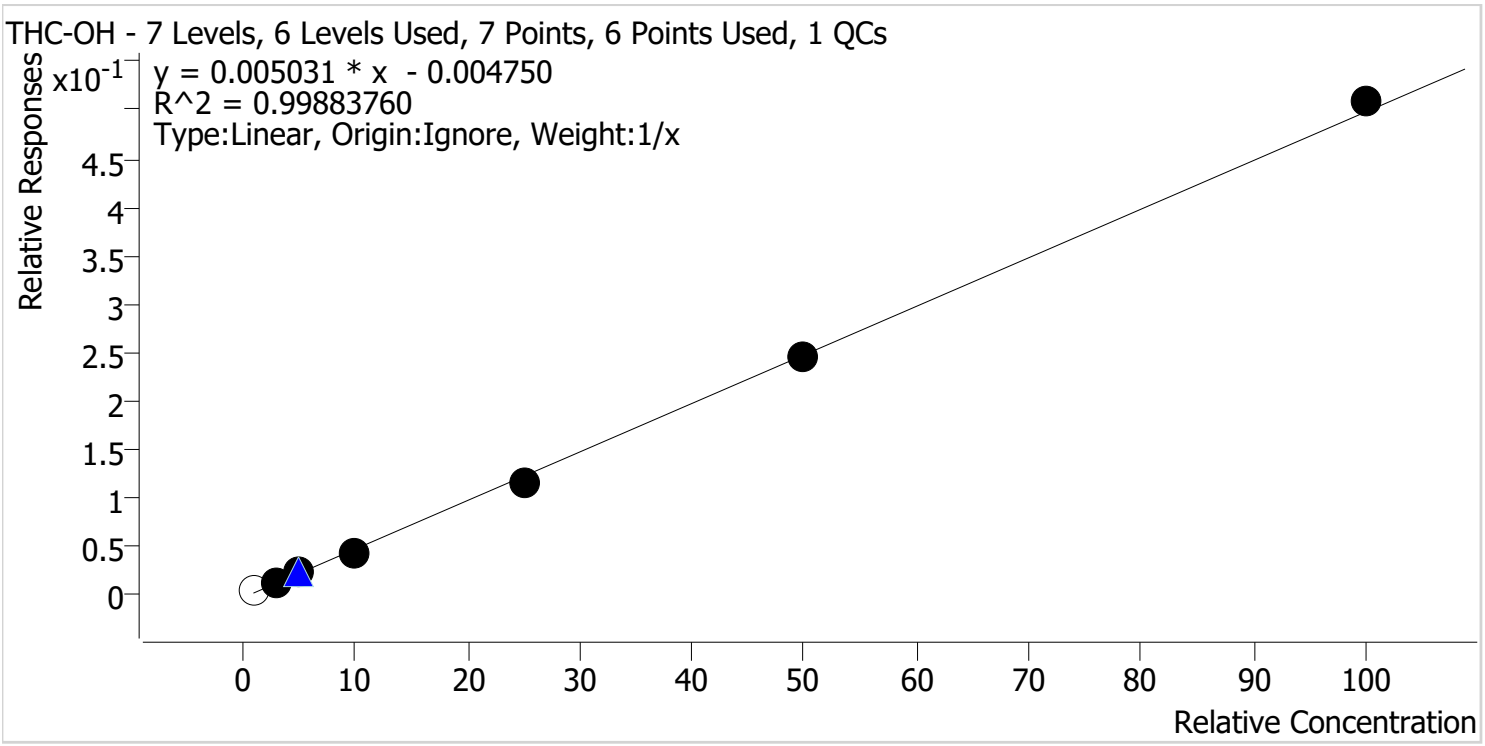
Batch results	D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Last Cal. Update	11/21/2022 9:29 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.6	111.3
mj cal 2	2	✔	10.0	9.9	98.7
mj cal 3	3	✔	20.0	18.6	93.1
mj cal 4	4	✔	50.0	47.9	95.9
mj cal 5	5	✔	75.0	73.8	98.4
mj cal 6	6	✔	100.0	101.4	101.4
mj cal 7	7	✔	250.0	252.8	101.1

Compound Calibration Report

Batch results	D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Last Cal. Update	11/21/2022 9:29 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



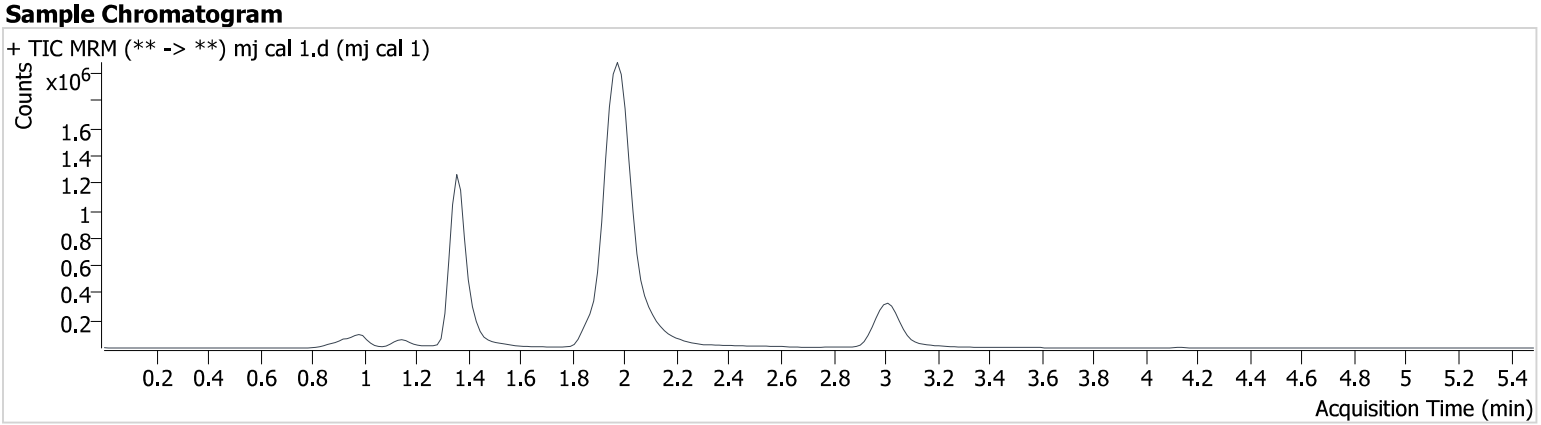
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✗	1.0	1.7	165.3
mj cal 2	2	✔	3.0	3.3	109.3
mj cal 3	3	✔	5.0	5.0	100.5
mj cal 4	4	✔	10.0	9.4	93.8
mj cal 5	5	✔	25.0	23.9	95.7
mj cal 6	6	✔	50.0	49.3	98.6
mj cal 7	7	✔	100.0	102.1	102.1

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

Instrument	69679	**Data File**	mj cal 1.d
Type	Cal	**Sample**	mj cal 1
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/18/2022 5:00:32 PM		
Sample Info.			

Sample Chromatogram



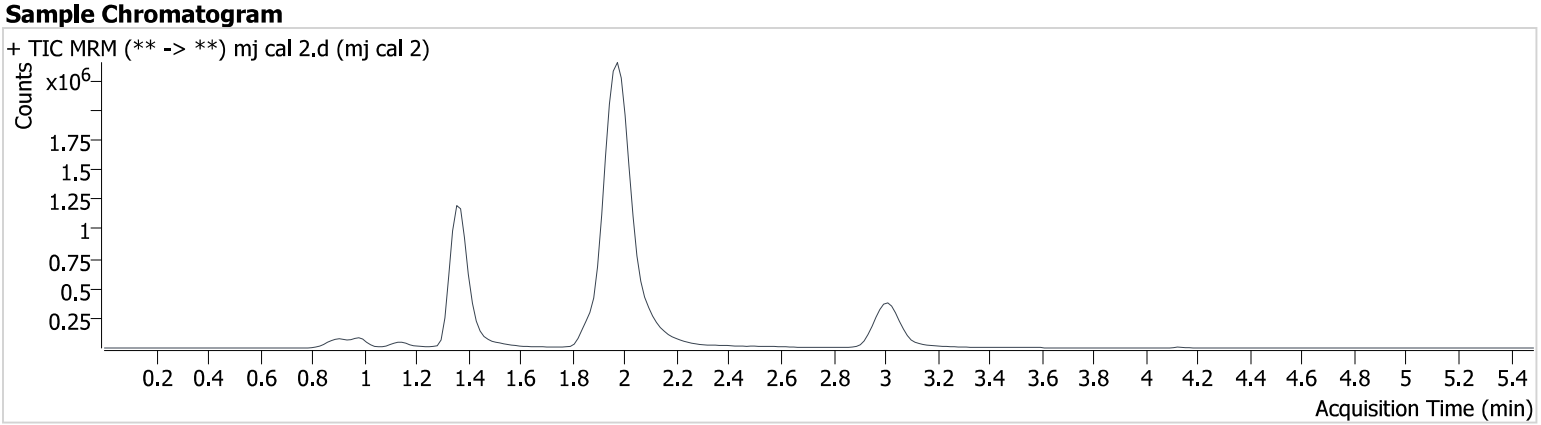
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.364	14921	310.2	964.55 **High**	∞	4185656	1.653 ng/ml	**Low**
THC-COOH	1.388	24417	∞	209.98	171.1	1012012	5.567 ng/ml	
THC	3.031	47794	246.3	25.85	60.2	2195806	1.197 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

Instrument	69679	**Data File**	mj cal 2.d
Type	Cal	**Sample**	mj cal 2
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/18/2022 5:07:16 PM		
Sample Info.			

Sample Chromatogram



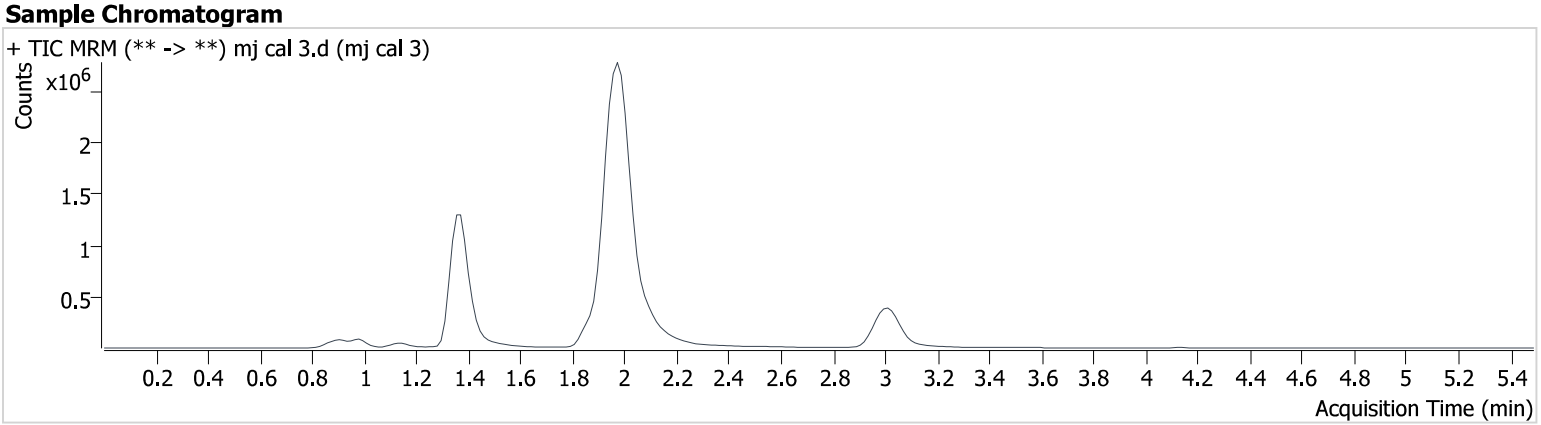
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	45453	∞	798.56	∞	3868869	3.280 ng/ml
THC-COOH	1.388	57396	136.7	202.38	197.3	1152317	9.873 ng/ml
THC	3.031	167921	∞	27.13	593.3	2448187	2.853 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

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	11/18/2022 5:14:00 PM		
Sample Info.			

Sample Chromatogram



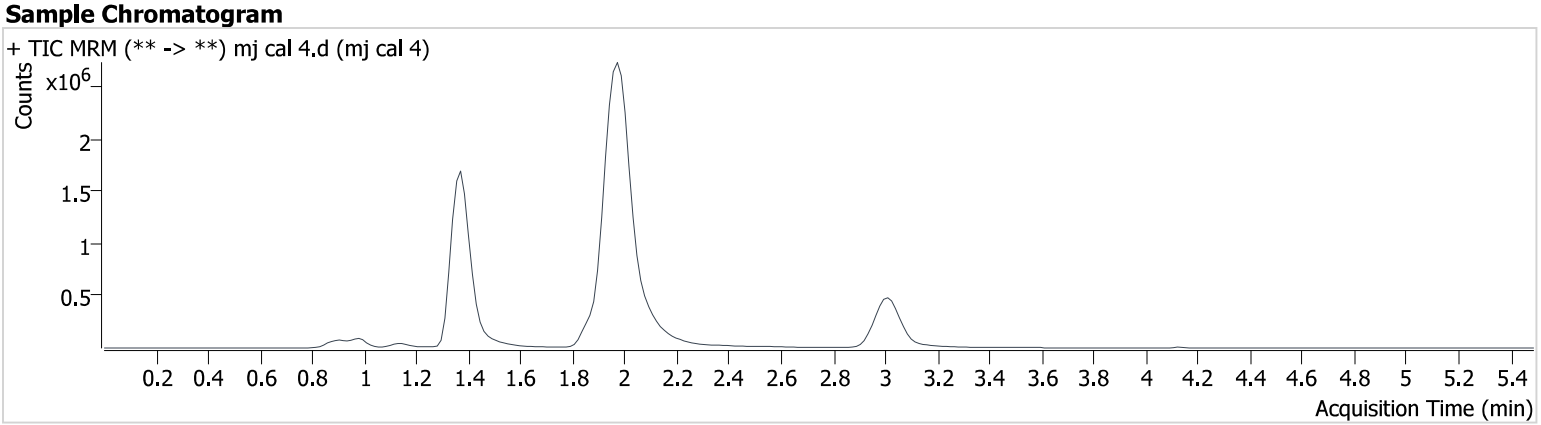
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	76959	∞	765.86	∞	3748738	5.025 ng/ml
THC-COOH	1.388	120887	∞	209.30	∞	1184874	18.626 ng/ml
THC	3.016	286405	4655.7	26.35	260.2	2397426	4.651 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

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	11/18/2022 5:20:44 PM		
Sample Info.			

Sample Chromatogram



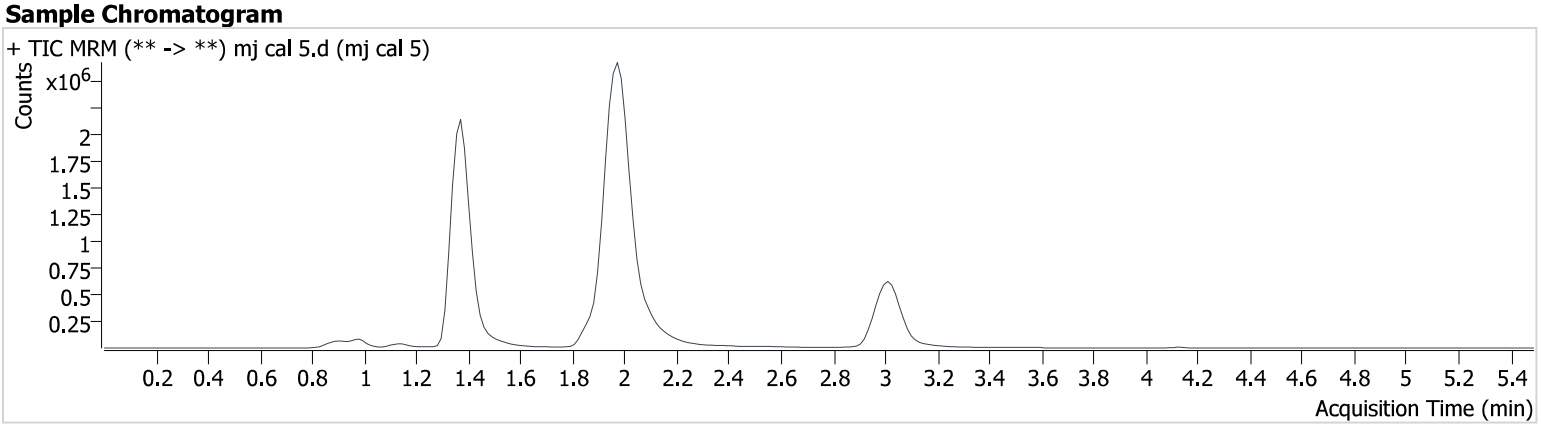
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	164119	∞	778.79	∞	3866551	9.381 ng/ml
THC-COOH	1.388	332428	1667.1	199.19	2292.1	1200886	47.927 ng/ml
THC	3.016	650568	9711.5	25.76	2630.4	2556135	9.426 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

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	11/18/2022 5:27:28 PM		
Sample Info.			

Sample Chromatogram



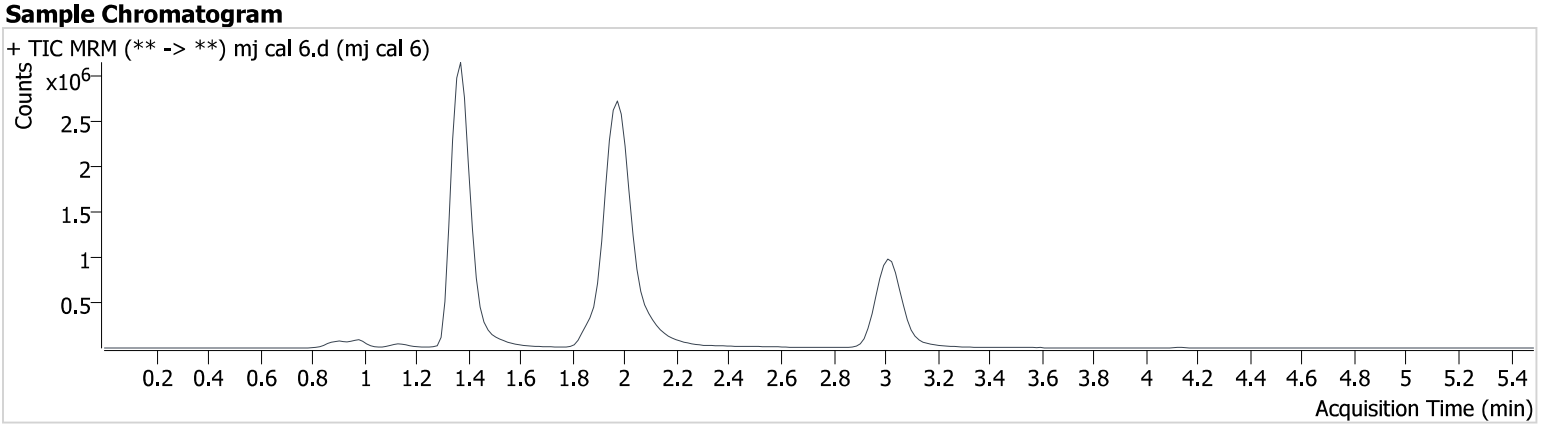
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	407761	5349.8	726.61	∞	3527658	23.920 ng/ml
THC-COOH	1.388	470229	1016.1	201.47	∞	1090317	73.820 ng/ml
THC	3.031	1598304	∞	26.09	1038.7	2385029	24.120 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

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	11/18/2022 5:34:13 PM		
Sample Info.			

Sample Chromatogram



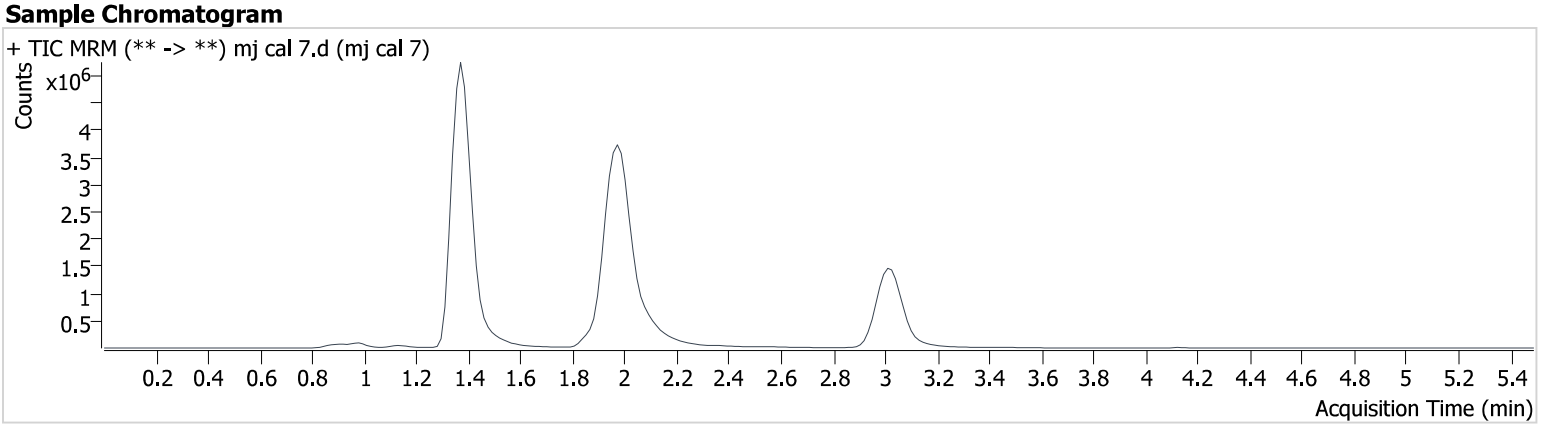
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	903835	35819.0	710.91	∞	3715698	49.295 ng/ml
THC-COOH	1.388	684876	∞	195.94	∞	1149566	101.395 ng/ml
THC	3.031	3509511	429070.9	26.84	14887.2	2519126	49.682 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\111822\QuantResults\cann.batch.bin
Calibration Last Update 11/21/2022 9:29:00 AM

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	11/18/2022 5:40:57 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.364	1754674	289586.3	702.89	∞	3448013	102.099 ng/ml
THC-COOH	1.388	1529437	10426.8	198.82	10415.0	1020371	252.791 ng/ml
THC	3.031	6496143	55119.4	27.43	3484.9	2259536	102.072 ng/ml