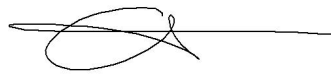


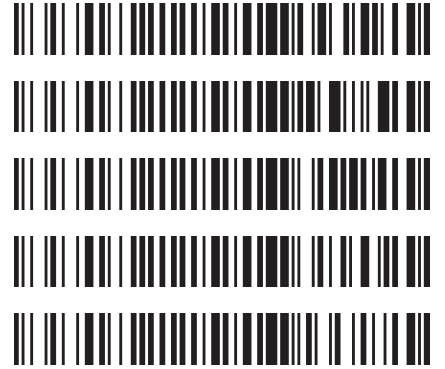
REVIEWED

By Tamara Salazar at 3:21 pm, Sep 09, 2022



Worklist: 6095

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2022-1913	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-1922	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-1924	3	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-1991	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-2014	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 9/8/22
Plate lot#: 220309

Analyst: Anne Nord
Plate re-test: 9/9/2022

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE LCMS Methanol

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 22B52016-1 **Urine Blank:** 7722

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.

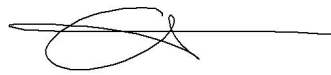
Analytic:

- 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 not evaluated due ratio ^{lowering} ~~lowing~~ as concentration increased. ^{9/9/22}



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 ToxBBox 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:



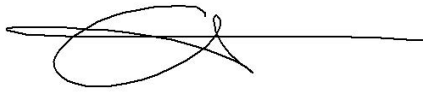
Approver: Rachel Cutler
Title: Lab Manager

Date: 3/2/22

Quality Review

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





	1	2	3	4	5	6
a	cal 1	Internal urine				
b	cal 2	negative blood				
c	cal 3	1922-1				
d	cal 4	1924-3				
e	Cal 5	1991-1				
f	cal 6	2014-1				
g	cal 7	negative urine				
h	Internal control (blood)	1913-2				

Plate position 3

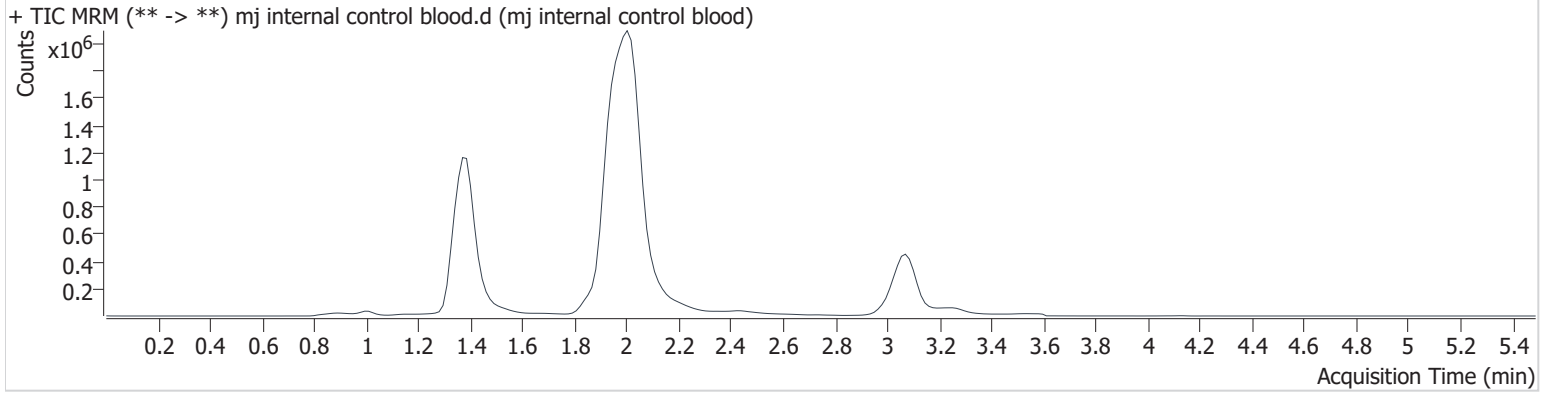
c2022-____-__

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 5:10:41 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	86625	∞	280.1	2005.2	1246294	14.326 ng/ml
THC	3.092	285526	∞	24.9	∞	2513304	4.614 ng/ml

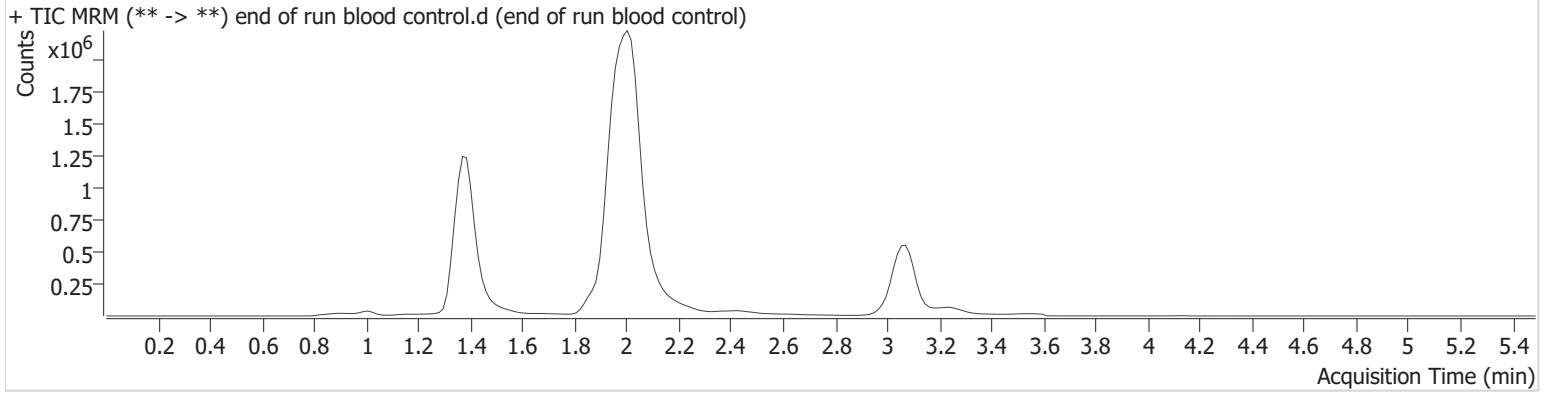
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 AM

Instrument	69679	Data File	end of run blood control.d
Type	Sample	Sample	end of run blood control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 6:51:10 PM		

Sample Info.

Sample Chromatogram



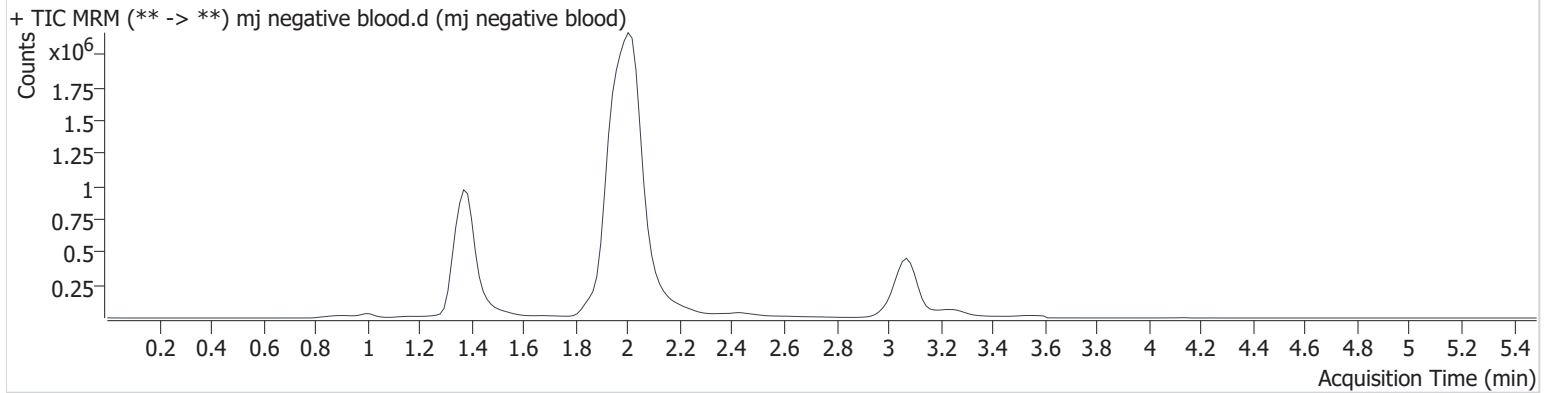
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	91801	∞	279.6	∞	1269133	14.849 ng/ml
THC	3.077	346873	2240.5	23.4	∞	3029797	4.647 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 5:24:10 PM		
Sample Info.			

Sample Chromatogram

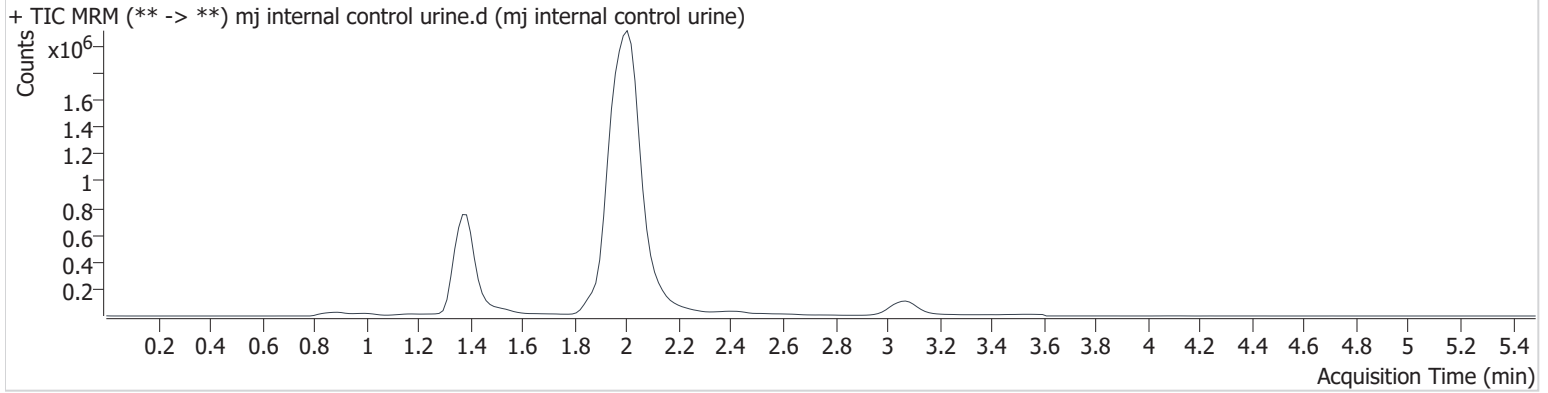


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 AM

Instrument	69679	Data File	mj internal control urine.d
Type	Sample	Sample	mj internal control urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 5:17:25 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	43956	∞	285.0	∞	638605	14.201 ng/ml
THC	3.092	80168	∞	25.0	193.7	702344	4.634 ng/ml

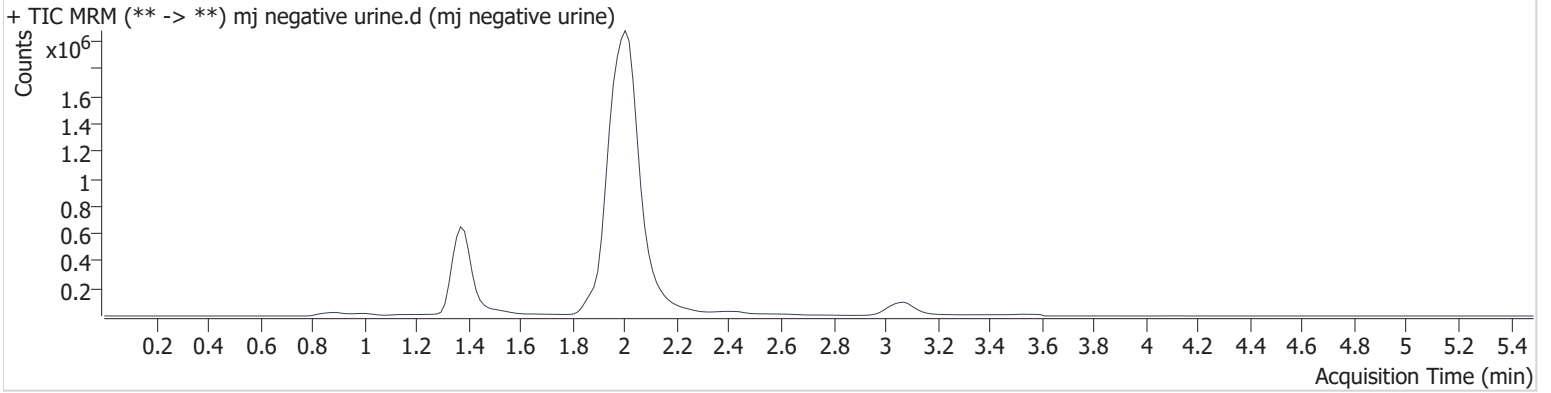
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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-G2	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 6:31:04 PM		

Sample Info.

Sample Chromatogram

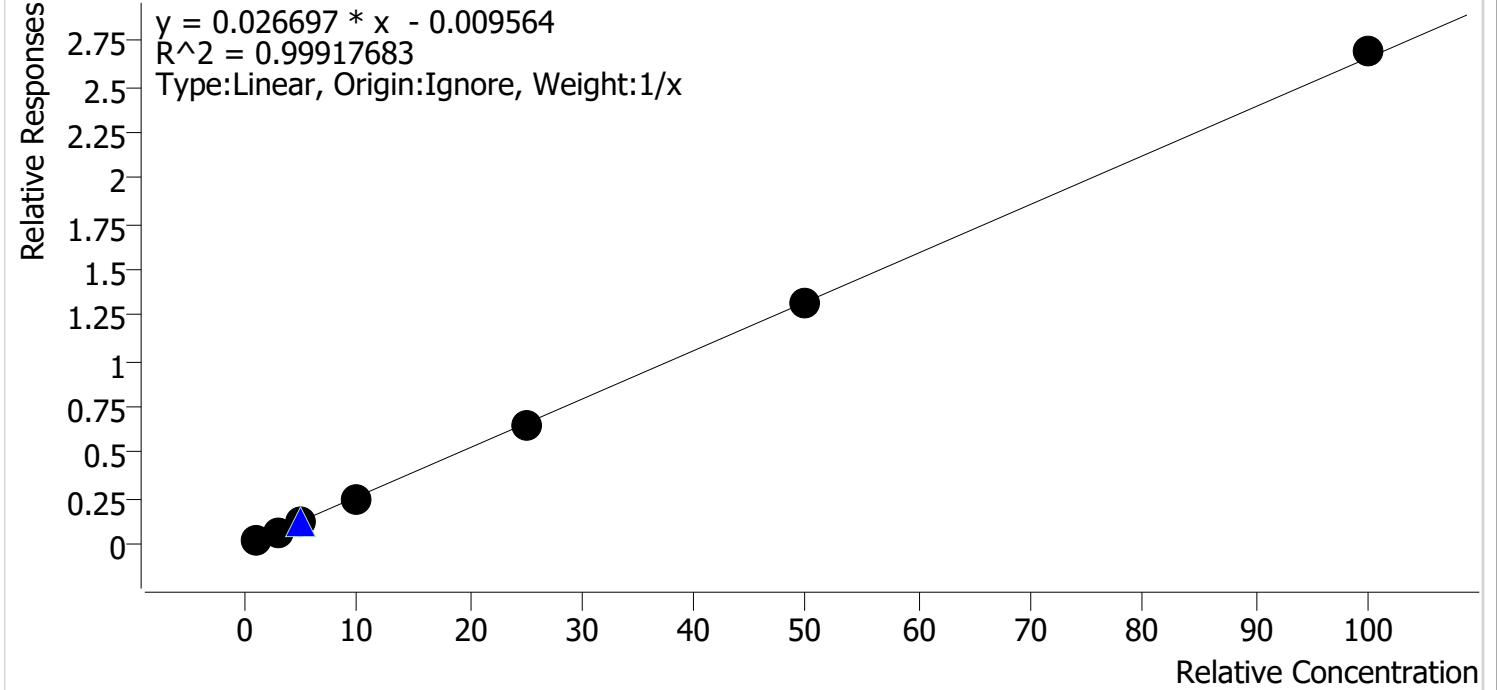


Compound Calibration Report



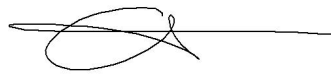
Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Last Cal. Update 9/9/2022 8:32 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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



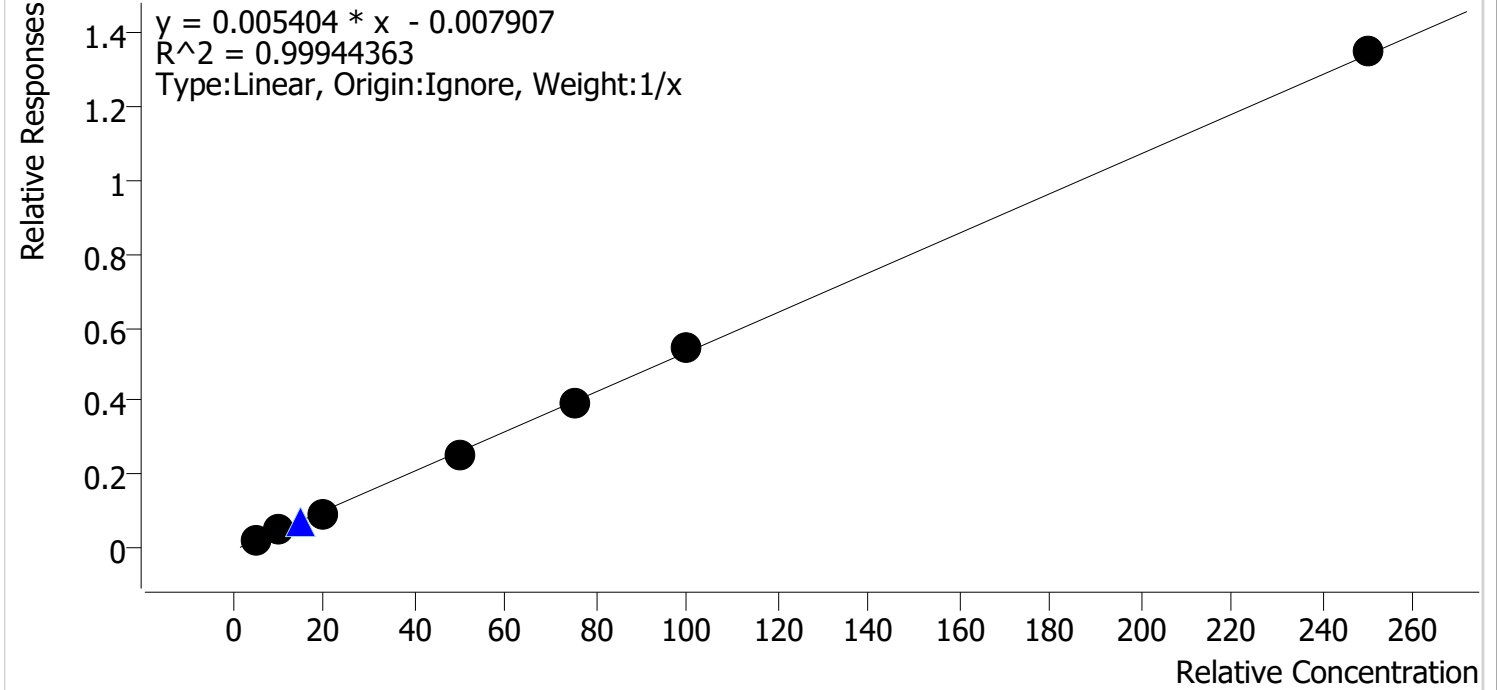
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	118.1
mj cal 2	2	✓	3.0	2.9	95.6
mj cal 3	3	✓	5.0	4.7	93.4
mj cal 4	4	✓	10.0	9.2	92.4
mj cal 5	5	✓	25.0	24.8	99.2
mj cal 6	6	✓	50.0	49.9	99.9
mj cal 7	7	✓	100.0	101.3	101.3

Compound Calibration Report



Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Last Cal. Update 9/9/2022 8:32 AM
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 cal 1	1	✓	5.0	5.3	106.9
mj cal 2	2	✓	10.0	10.1	100.9
mj cal 3	3	✓	20.0	18.9	94.5
mj cal 4	4	✓	50.0	48.5	97.0
mj cal 5	5	✓	75.0	73.5	98.0
mj cal 6	6	✓	100.0	102.1	102.1
mj cal 7	7	✓	250.0	251.6	100.6

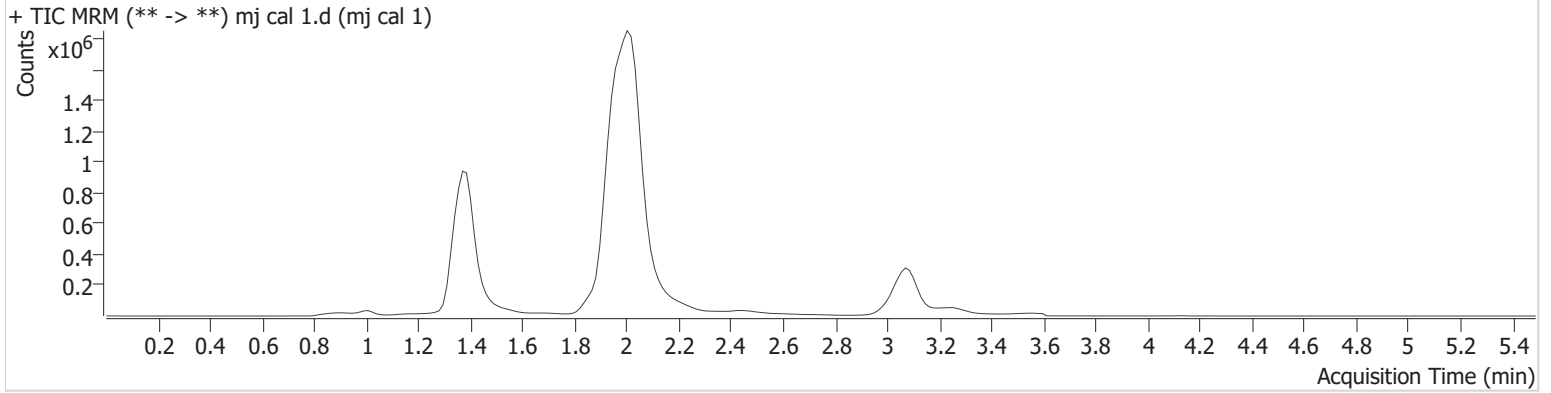
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 4:16:52 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	22515	∞	265.7	137.9	1073193	5.346 ng/ml
THC	3.092	43041	454.2	28.0	163.2	1958633	1.181 ng/ml

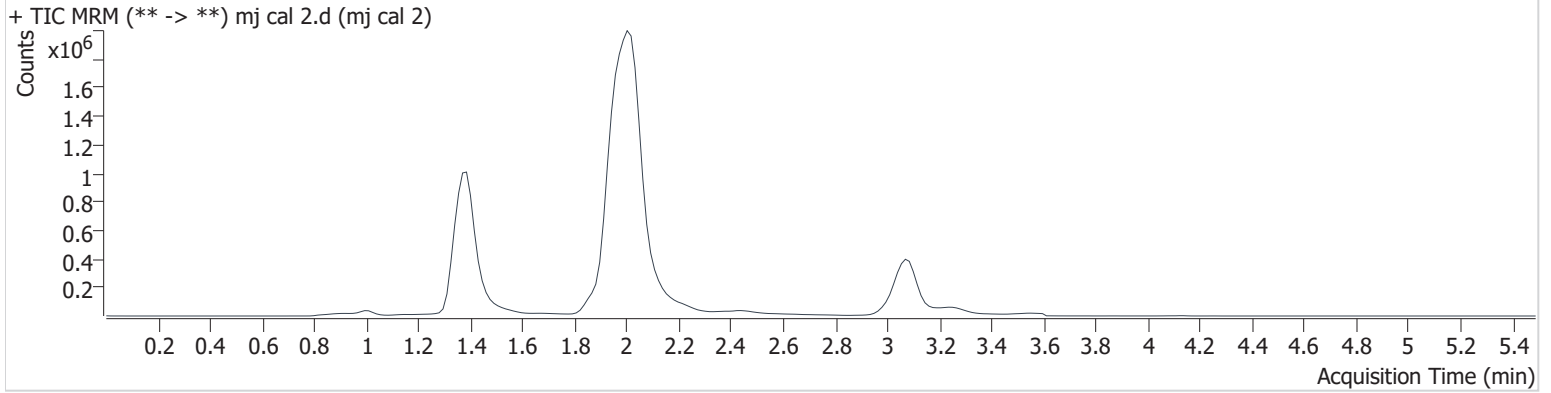
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 4:23:37 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	50078	531.5	267.2	∞	1074241	10.090 ng/ml
THC	3.092	153846	2342.0	24.2	161.2	2294826	2.869 ng/ml

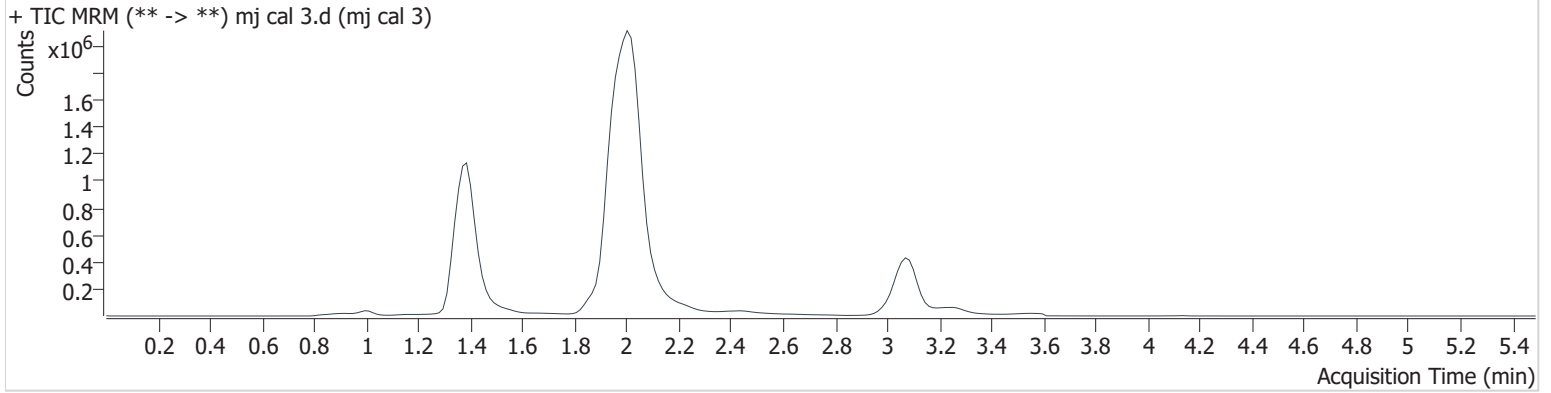
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 4:30:21 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.418	104617	618.4	270.5	∞	1110861	18.891 ng/ml
THC	3.092	269474	∞	24.1	635.2	2340861	4.670 ng/ml

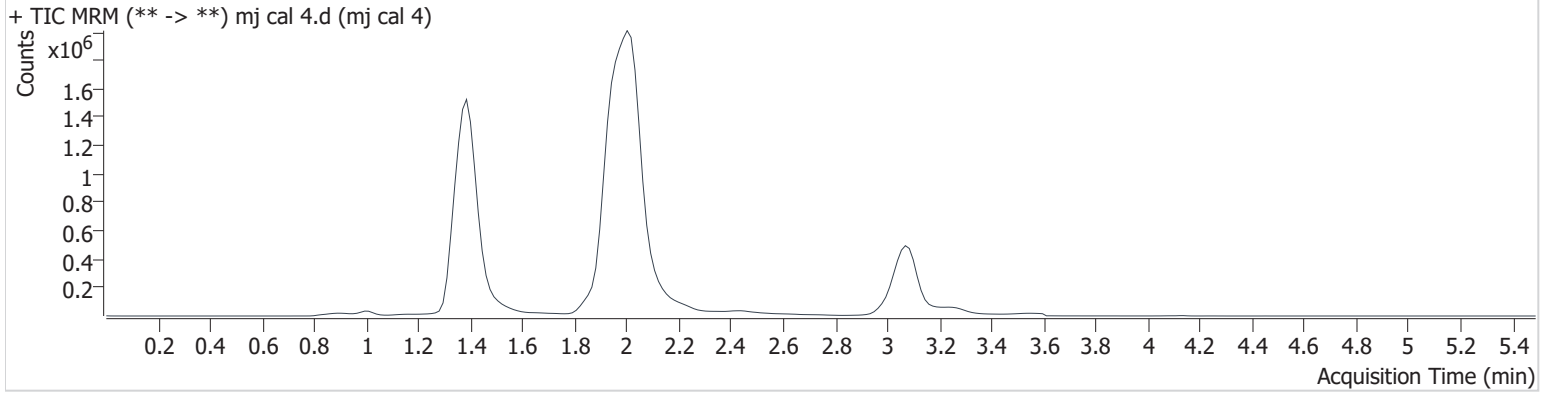
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 4:37:05 PM		

Sample Info.

Sample Chromatogram



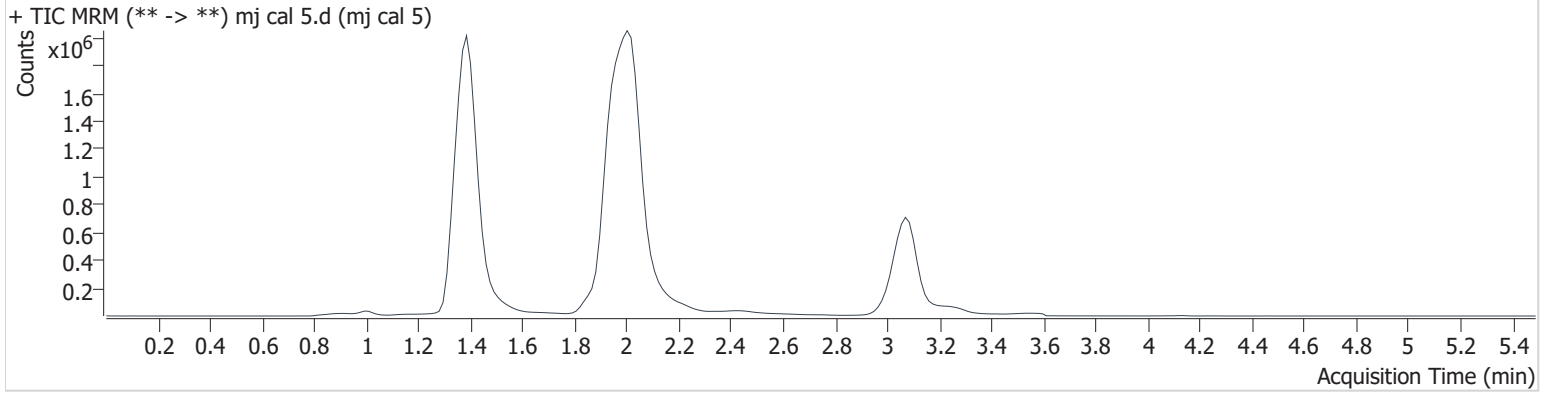
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	305010	∞	273.7	∞	1200204	48.492 ng/ml
THC	3.092	585441	∞	23.8	∞	2468795	9.241 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 4:43:50 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	451624	2017.2	266.2	∞	1159943	73.514 ng/ml
THC	3.077	1647544	∞	24.2	∞	2524377	24.805 ng/ml

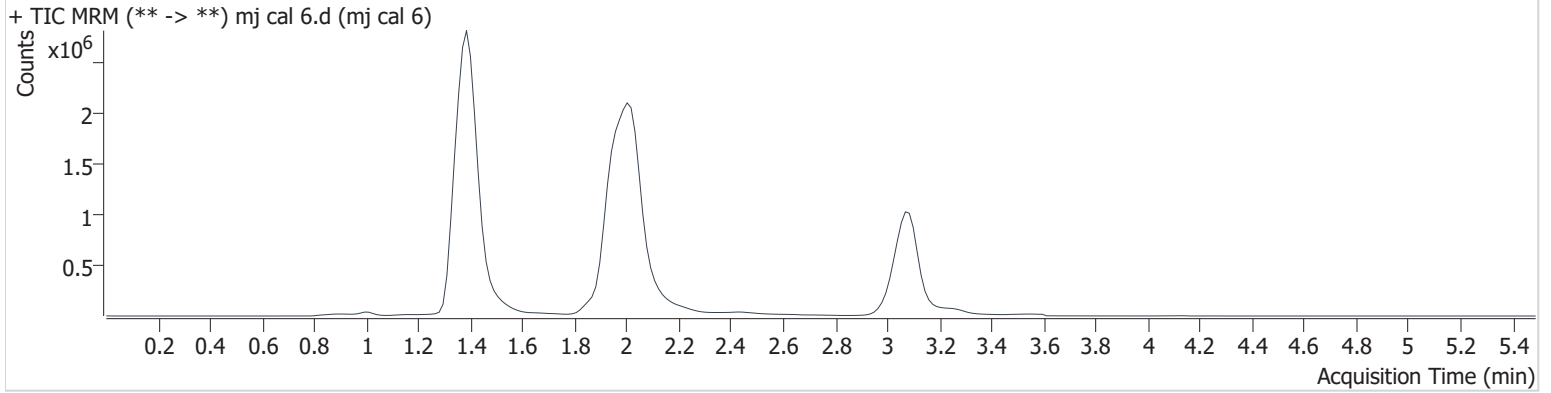
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 4:50:34 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	645389	19754.1	262.9	∞	1186683	102.107 ng/ml
THC	3.092	3356805	∞	24.8	17454 0.0	2535769	49.944 ng/ml

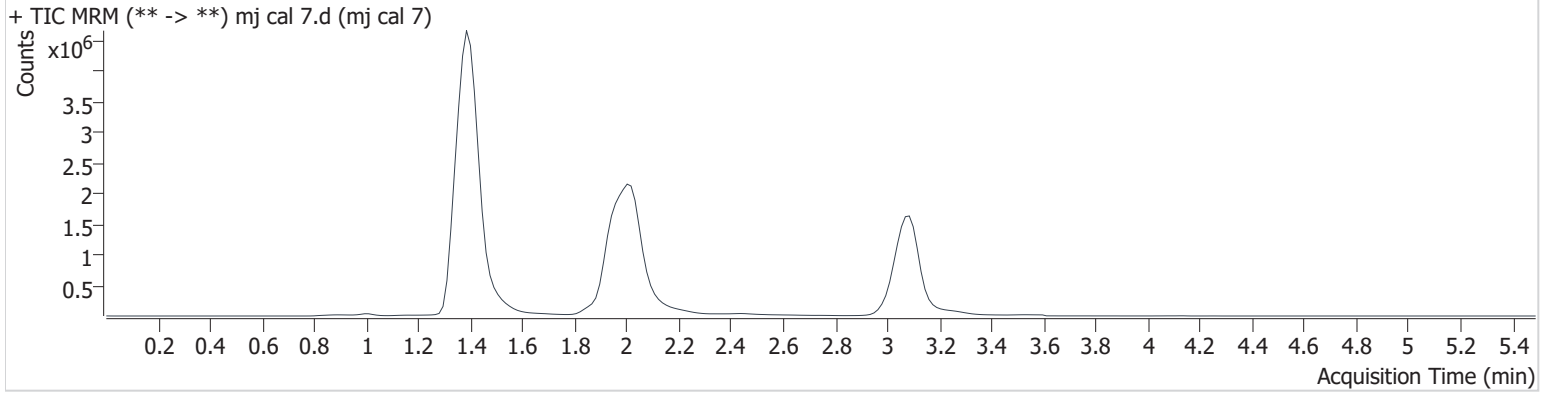
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\090822\QuantResults\cann.batch.bin
Calibration Last Update 9/9/2022 8:32:37 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	9/8/2022 4:57:18 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.403	1445643	∞	265.5	∞	1069676	251.561 ng/ml
THC	3.092	6860066	∞	24.7	∞	2545957	101.288 ng/ml