3/17/2022

REVIEWED
By Britany Wylie at 10:26 am, Mar 18, 2022

Worklist: 5686

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2022-0397	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0407	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0412	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0458	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-0467	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-0487	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-0496	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2022-0512	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0528	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 3/16/22 Analyst: Anne Nord
Plate lot#: 211018 Plate re-test: 4/18/22

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: 22B52020 **Urine Blank:** 21522 **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 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:

	1	2	3	4	5	6
a	cal 1	Internal control (urine)	467-1			
b	cal 2	negative blood	487-1			
c	cal 3	397-1	496-2			
d	cal 4	407-1		negative urine		
e	Cal 5	412-1				
f	cal 6	512-1				
g	cal 7	528-1				
h	Internal control (blood)	458-1				

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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

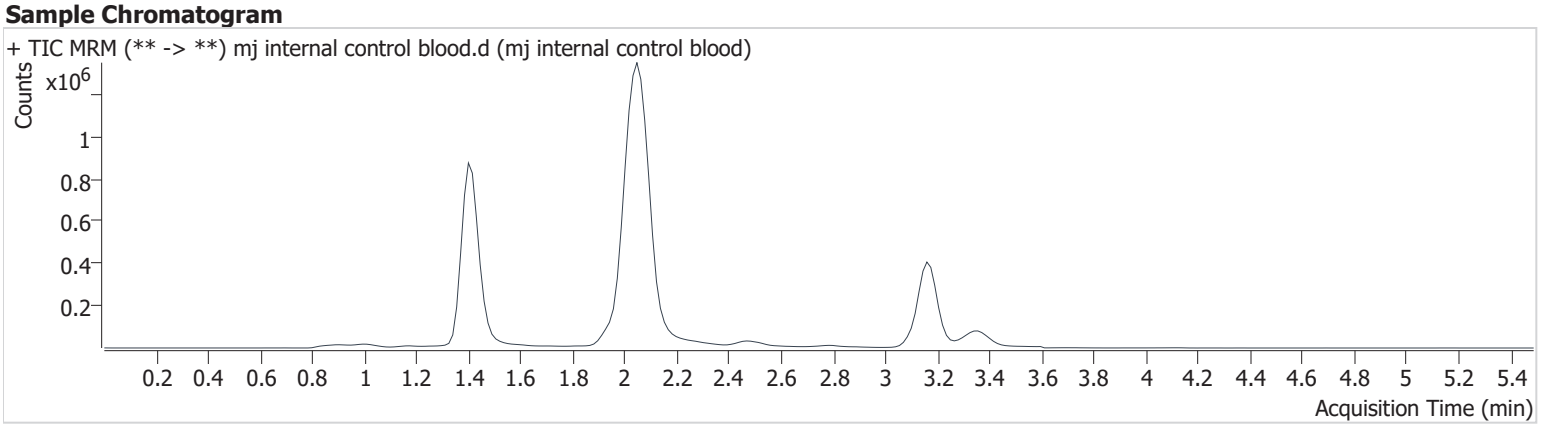
Quality Approver: Jason Crowe
Title: Quality Manager
Date: 3/2/2022

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 3:04:13 PM		
Sample Info.			

Sample Chromatogram



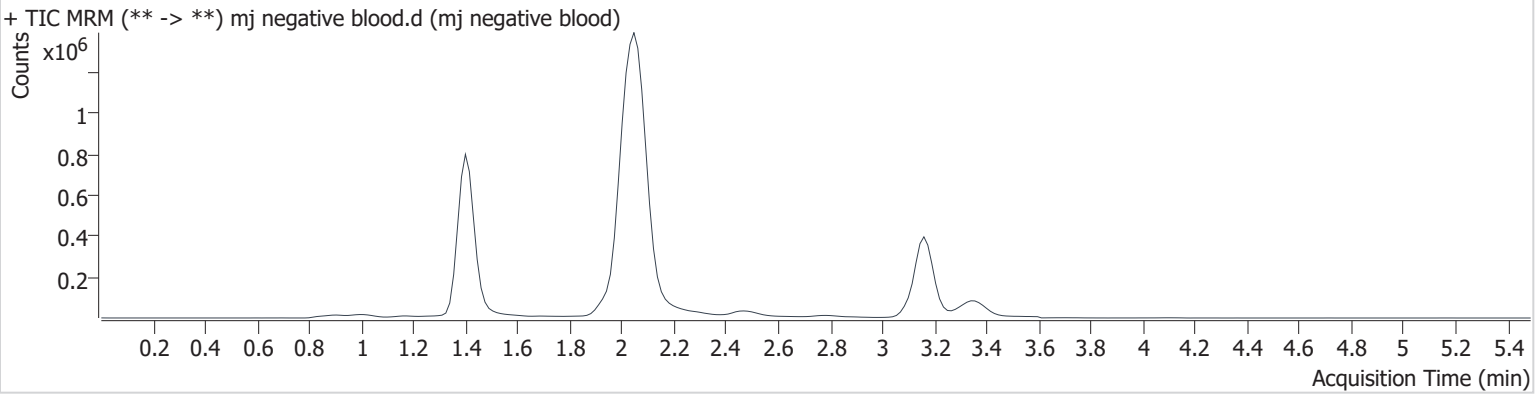
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	46436	1897.2	793.2	∞	2595597	5.247 ng/ml
THC-COOH	1.431	143632	372.9	39.6	2661.6	693514	14.797 ng/ml
THC	3.182	208557	4359.7	24.8	466.1	1707163	4.654 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update	3/17/2022 8:14:17 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	3/16/2022 3:10:57 PM		
Sample Info.			

Sample Chromatogram

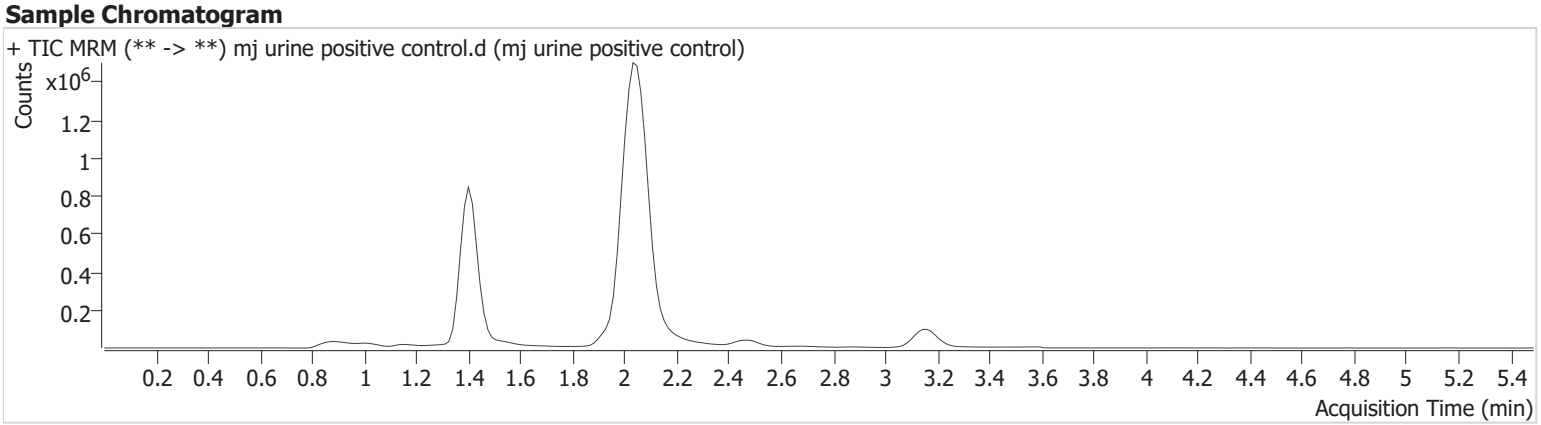


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 AM

Instrument	69679	**Data File**	mj urine positive control.d
Type	Sample	**Sample**	mj urine positive control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	3/16/2022 5:24:33 PM		
Sample Info.			

Sample Chromatogram



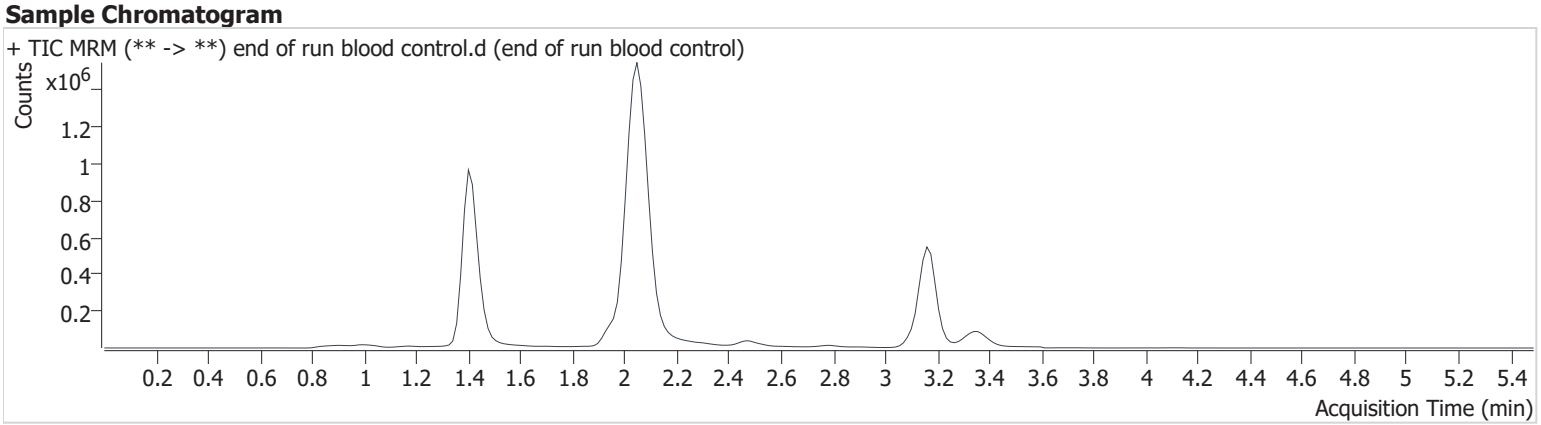
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	48927	6097.5	795.5	∞	2676005	5.361 ng/ml
THC-COOH	1.431	116021	243.4	41.4	773.1	567876	14.612 ng/ml
THC	3.167	66277	492.8	27.7	105.6	554175	4.564 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 5:31:17 PM		
Sample Info.			

Sample Chromatogram



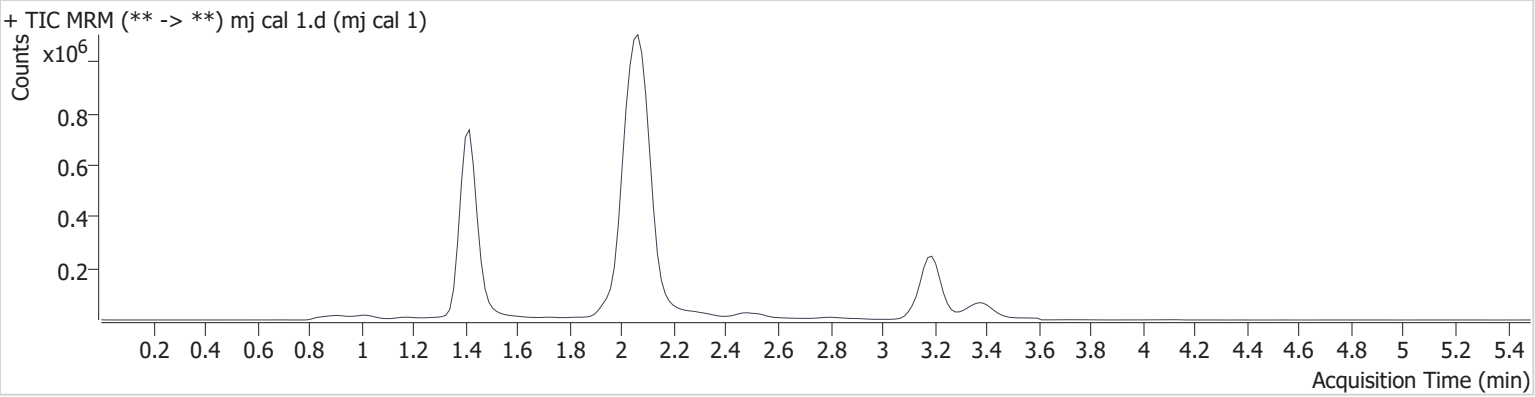
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	47860	187.3	769.5	1214.7	2532436	5.538 ng/ml
THC-COOH	1.431	159145	2325.2	36.3	14508	709693	15.933 ng/ml
					0.0		
THC	3.182	254753	3594.3	25.0	270.7	2124341	4.576 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 2:10:37 PM		
Sample Info.			

Sample Chromatogram



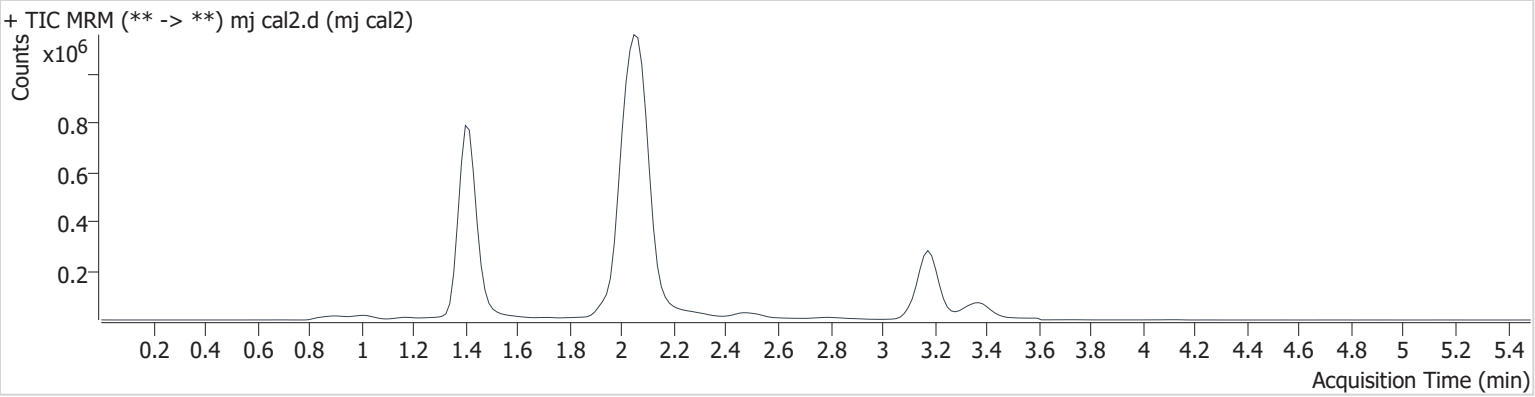
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	10118	37.1	719.0	∞	2669002	1.181 ng/ml	Low
THC-COOH	1.446	40491	157.2	41.9	192.7	636860	5.288 ng/ml	
THC	3.212	27626	450.0	29.8	114.2	1215579	1.191 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 AM

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	3/16/2022 2:17:22 PM		
Sample Info.			

Sample Chromatogram



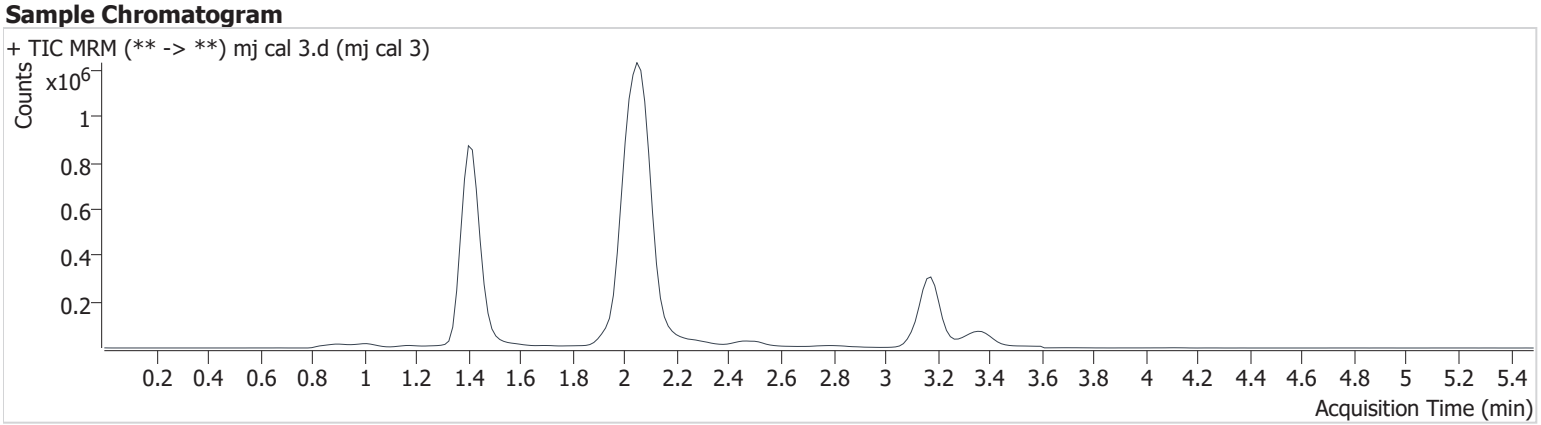
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.409	26710	2365.6	772.9	∞	2723853	2.916 ng/ml	Low
THC-COOH	1.446	87233	141.1	39.5	53.1	663900	9.781 ng/ml	
THC	3.197	94590	1180.5	26.0	167.5	1315843	2.903 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 2:24:06 PM		
Sample Info.			

Sample Chromatogram



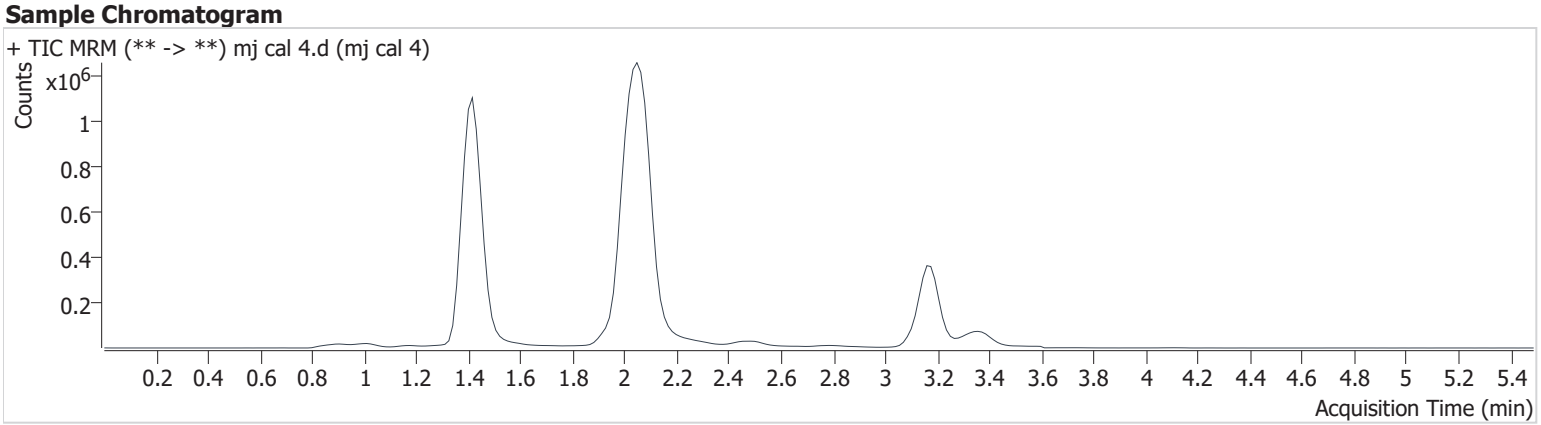
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	45126	319.6	818.7	∞	2828472	4.689 ng/ml
THC-COOH	1.446	195678	548.7	37.3	217.1	708726	19.368 ng/ml
THC	3.182	164786	2566.4	25.1	772.0	1392454	4.521 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 2:30:48 PM		
Sample Info.			

Sample Chromatogram



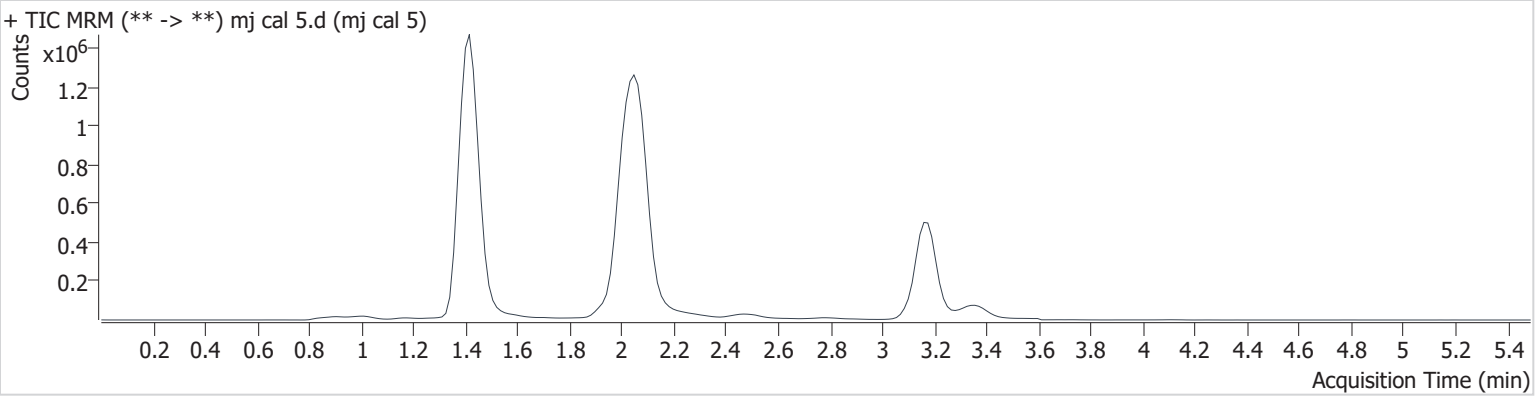
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	89274	321.2	839.7	918.8	2829783	9.186 ng/ml
THC-COOH	1.431	523276	9249.1	38.2	999.4	712375	49.742 ng/ml
THC	3.182	370051	13250.5	24.5	748.8	1443344	9.327 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 2:37:30 PM		
Sample Info.			

Sample Chromatogram



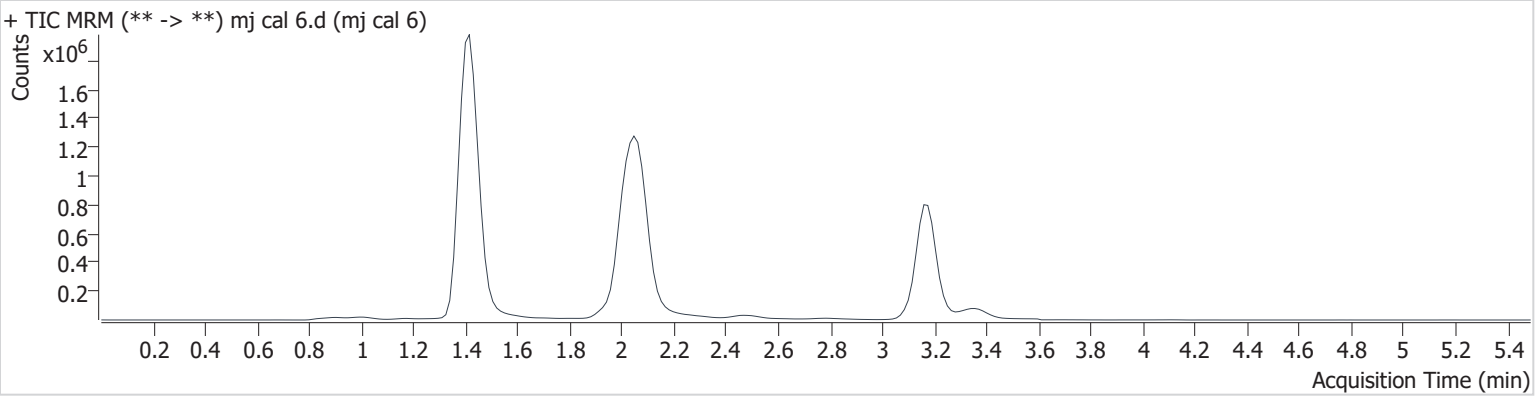
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	231560	1186.0	832.2	∞	2767584	24.216 ng/ml
THC-COOH	1.431	771885	2041.3	37.6	35650	687081	75.505 ng/ml
THC	3.182	1030752	14555.2	24.5	2.2 1878.8	1469981	24.815 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 2:44:13 PM		
Sample Info.			

Sample Chromatogram



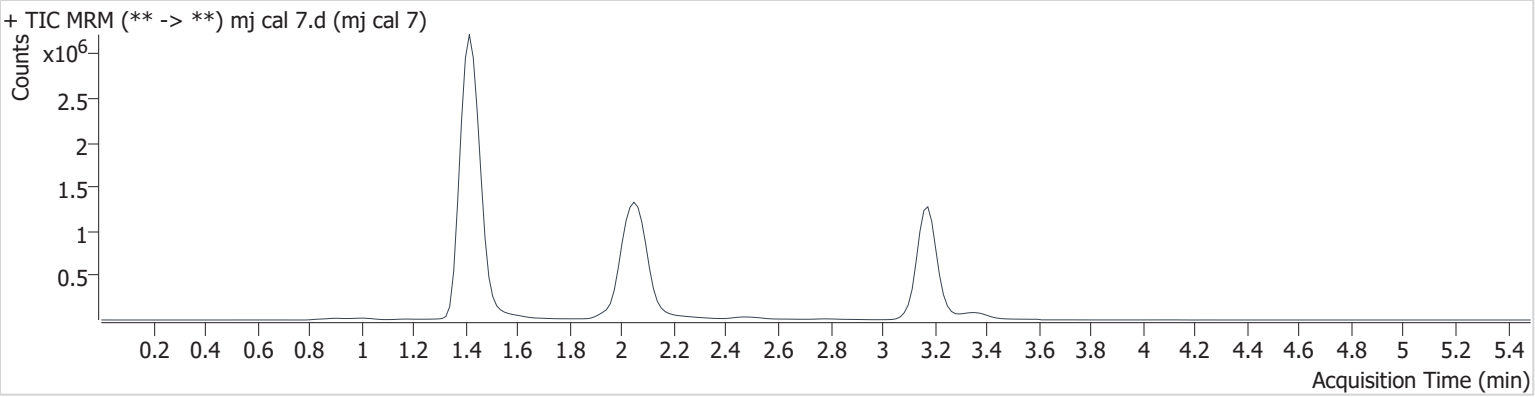
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	467037	18406.6	799.3	∞	2678456	50.371 ng/ml
THC-COOH	1.431	993646	2809.9	39.0	969.7	673394	98.836 ng/ml
THC	3.182	2220267	37474.2	24.3	∞	1561595	49.904 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Calibration Last Update 3/17/2022 8:14:17 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	3/16/2022 2:50:55 PM		
Sample Info.			

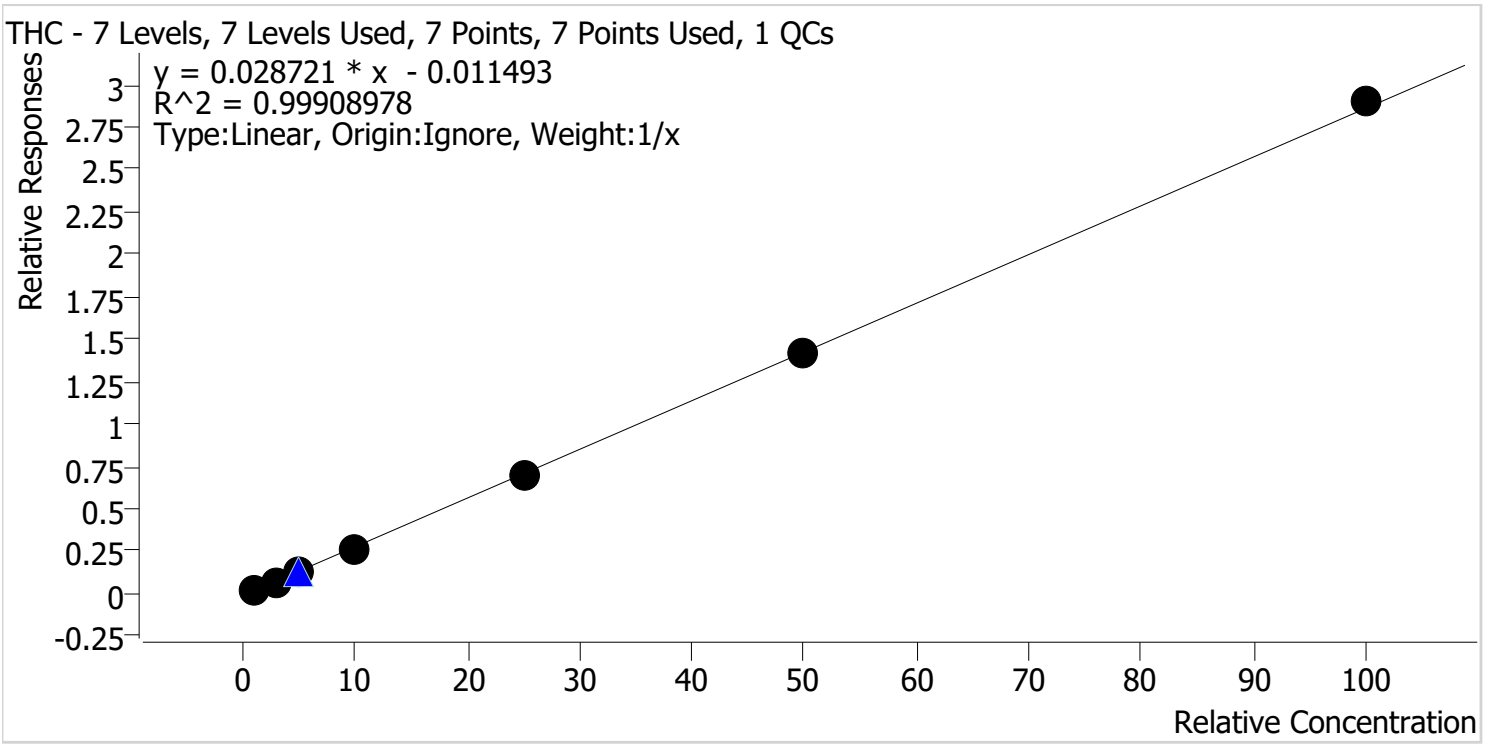
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	863776	∞	801.9	∞	2457616	101.442 ng/ml
THC-COOH	1.446	2233822	3970.9	38.8	4970.2	591029	251.479 ng/ml
THC	3.182	4312611	27672.7	24.4	7602.9	1487598	101.339 ng/ml

Compound Calibration Report

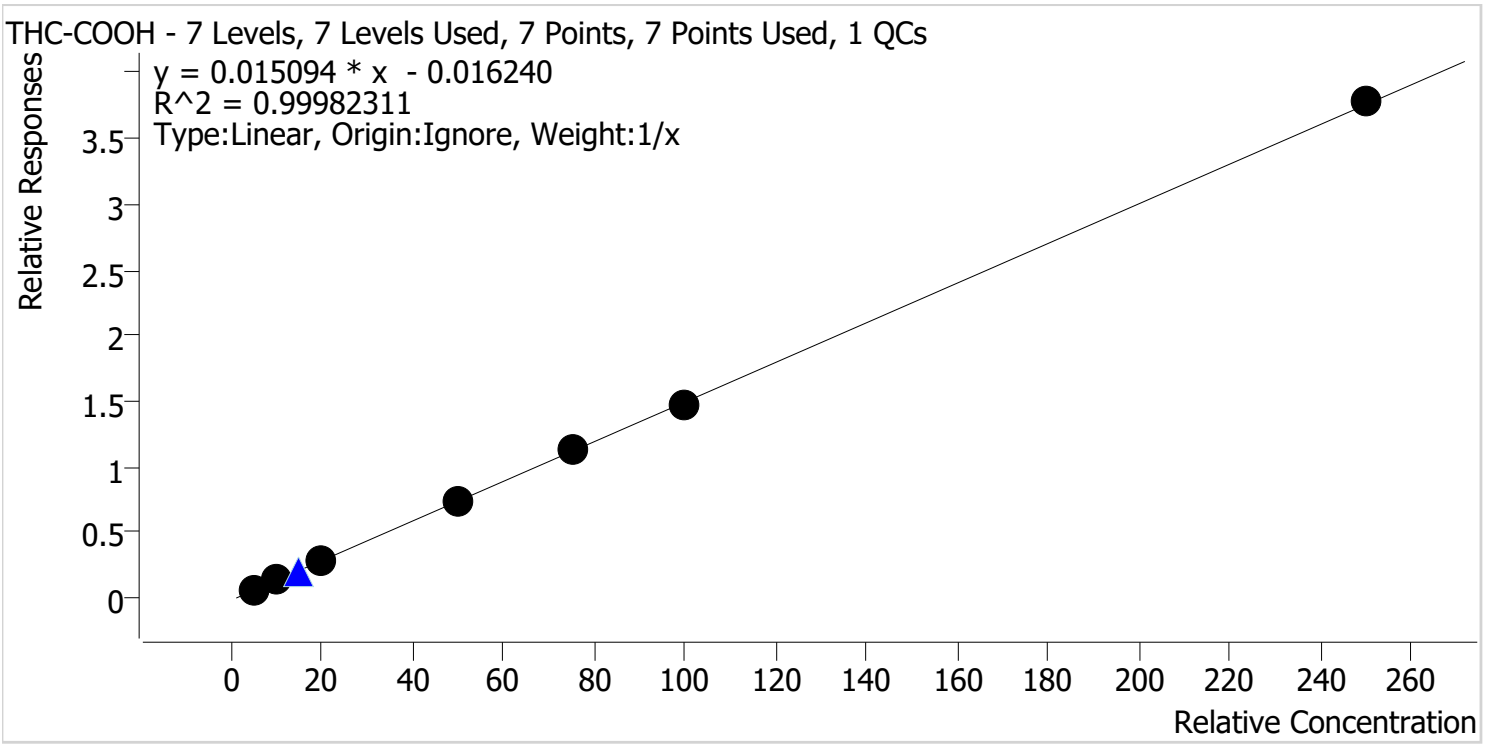
Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Last Cal. Update 3/17/2022 8:14 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	119.1
mj cal2	2	✔	3.0	2.9	96.8
mj cal 3	3	✔	5.0	4.5	90.4
mj cal 4	4	✔	10.0	9.3	93.3
mj cal 5	5	✔	25.0	24.8	99.3
mj cal 6	6	✔	50.0	49.9	99.8
mj cal 7	7	✔	100.0	101.3	101.3

Compound Calibration Report

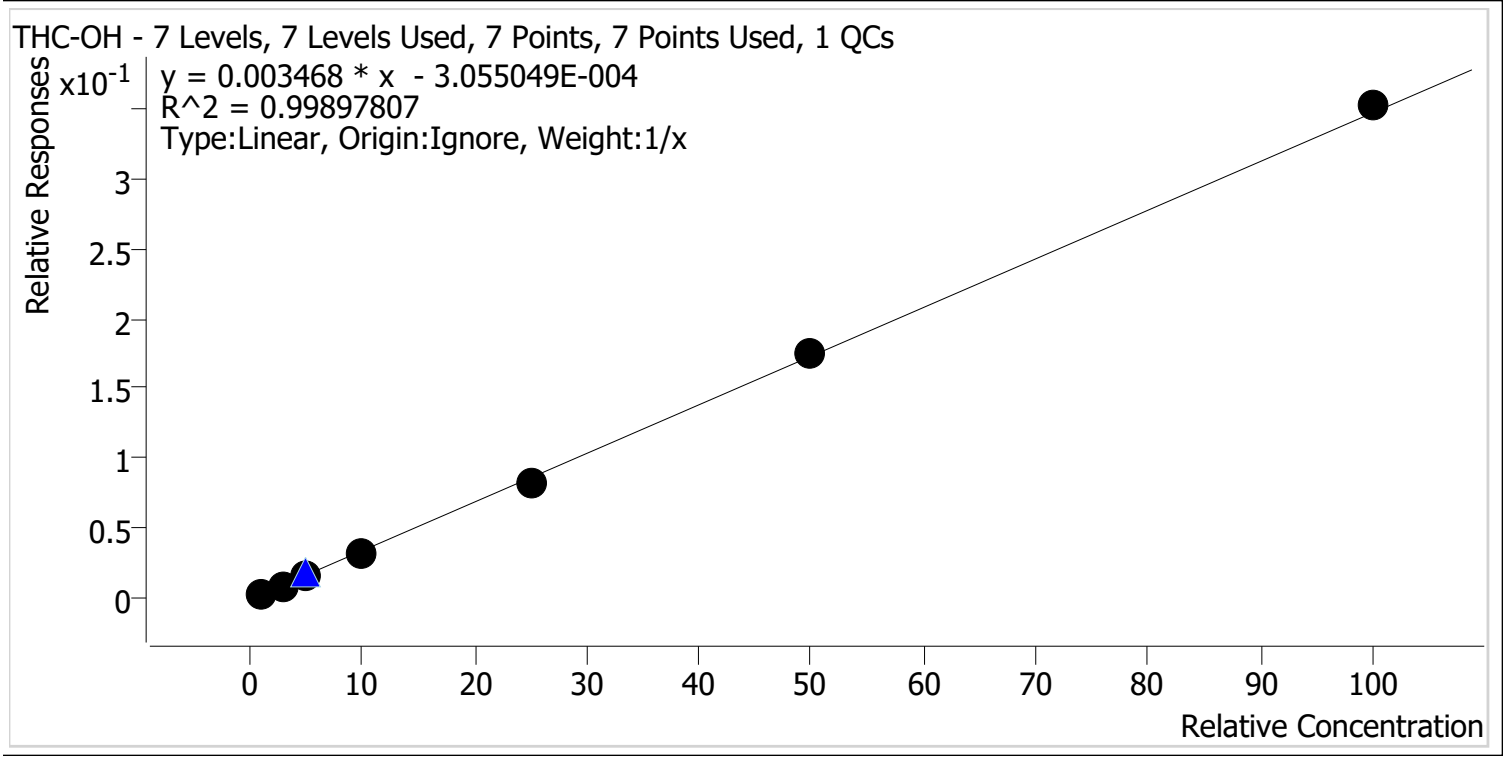
Batch results	D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Last Cal. Update	3/17/2022 8:14 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.3	105.8
mj cal2	2	✔	10.0	9.8	97.8
mj cal 3	3	✔	20.0	19.4	96.8
mj cal 4	4	✔	50.0	49.7	99.5
mj cal 5	5	✔	75.0	75.5	100.7
mj cal 6	6	✔	100.0	98.8	98.8
mj cal 7	7	✔	250.0	251.5	100.6

Compound Calibration Report

Batch results D:\MassHunter\Data\2022\am 27-28\031622\QuantResults\cann.batch.bin
Last Cal. Update 3/17/2022 8:14 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	1.2	118.1
mj cal2	2	✔	3.0	2.9	97.2
mj cal 3	3	✔	5.0	4.7	93.8
mj cal 4	4	✔	10.0	9.2	91.9
mj cal 5	5	✔	25.0	24.2	96.9
mj cal 6	6	✔	50.0	50.4	100.7
mj cal 7	7	✔	100.0	101.4	101.4