4/19/2021

REVIEWED
By Britany Wylie at 9:11 am, Apr 20, 2021

Worklist: 4914

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2021-0613	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0711	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-0719	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0734	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-0748	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-0792	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0793	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0795	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-0844	3	URINE	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-0851	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0858	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ

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

Extraction Date 4/15/21 Analyst: Anne Nord
Plate lot#: 201206 Plate Expiration: 06/06/2021

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: 20K20702 **Urine Blank: 2121** **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: *Run started on 4/15/21 part way into the run the pressure maxed out and the instrument shut down. 4/16/21 I reconstituted the samples that had run; I changed the column and needle seat. The samples that ran before were re-injected for analysis. Curve limits 3-100 THC, THC-OH evaluated qualitatively due to control accuracy being low.*

	1	2	3	4	5	6
a	cal 1	neg blood	844-3			QC 1
b	cal 2	613-1	795-3			cal 100 ng
c	cal 3	792-1	858-1			cal 50 ng
d	cal 4	851-1	734-1			cal 25 ng
e	Cal 5	719-1 moved to G3 SLE well plugged	711-2			cal 10ng
f	cal 6	793-1	748-1			cal 5 ng
g	cal 7	negative urine	719-1			cal 3 ng
h	Internal control	external control urine				cal 1ng

C2021-0___-_

Toxicology AM method 27/26 external prep information

working solution 15 ug/ml in meoh C-THC, THC-OH, 7.5 ug/ml THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH
Ppd 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

AM 27/26 blood control 100 ul working solution lot () in 9900 ul blood lot ()

		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	

AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine **out of use**

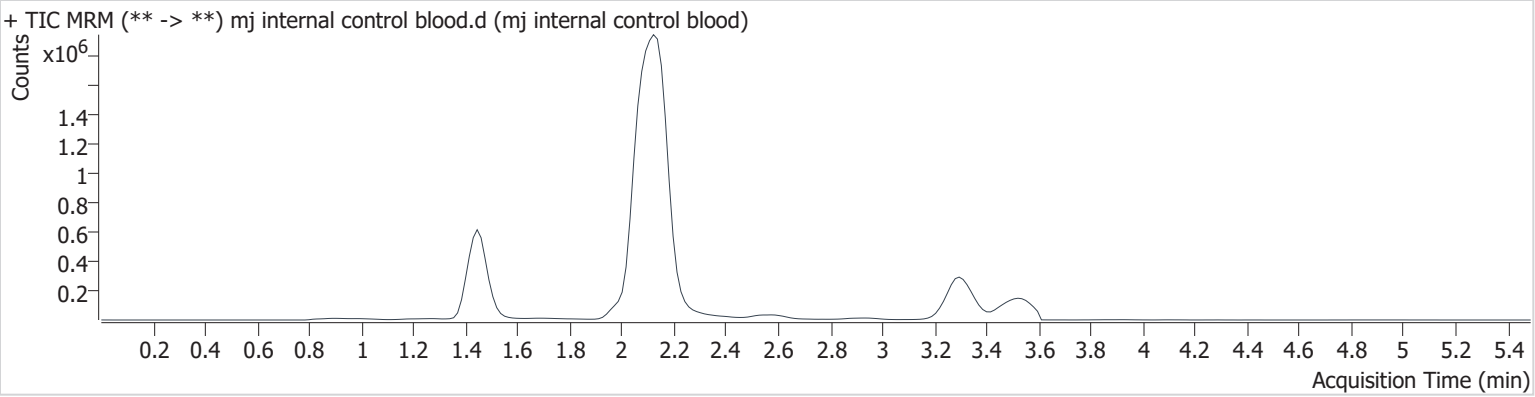
ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	1/12/2021
ppd 1/13/21 Exp 7/1/21 neg urine lot 10120	lot 11321	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	3/28/2021
ppd 3/29/21 Exp 7/1/21 neg urine lot 2121	lot 32921	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 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	4/16/2021 11:49:46 AM		
Sample Info.			

Sample Chromatogram



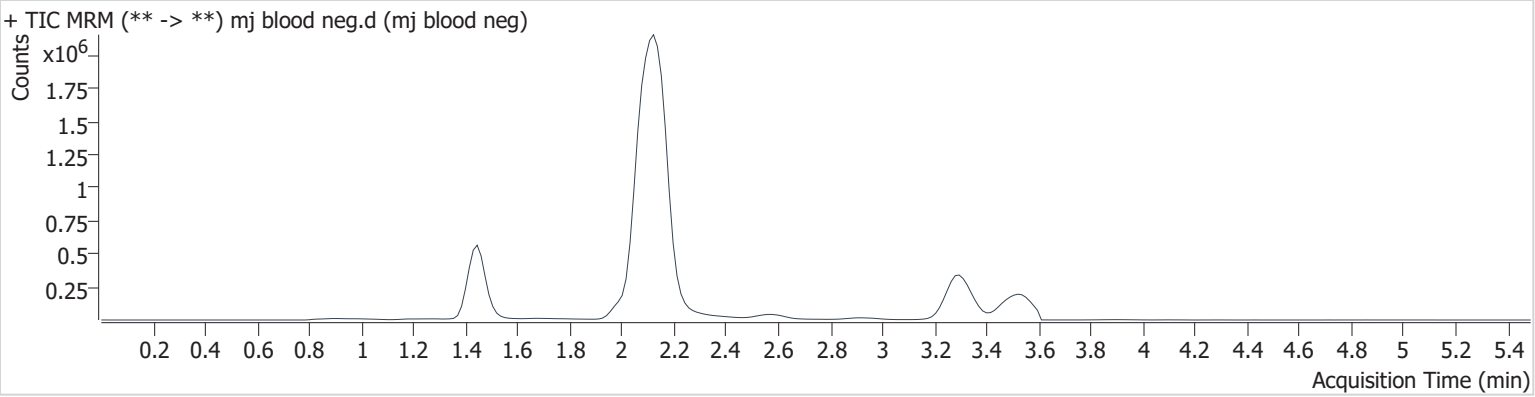
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	205652	∞	10.8	∞	1913598	3.378 ng/ml
THC-COOH	1.476	130149	734.0	36.4	257.5	737094	14.723 ng/ml
THC	3.332	97016	970.6	25.0	105.9	1051751	4.140 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj blood neg.d
Type	Sample	**Sample**	mj blood neg
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/16/2021 11:56:28 AM		
Sample Info.			

Sample Chromatogram

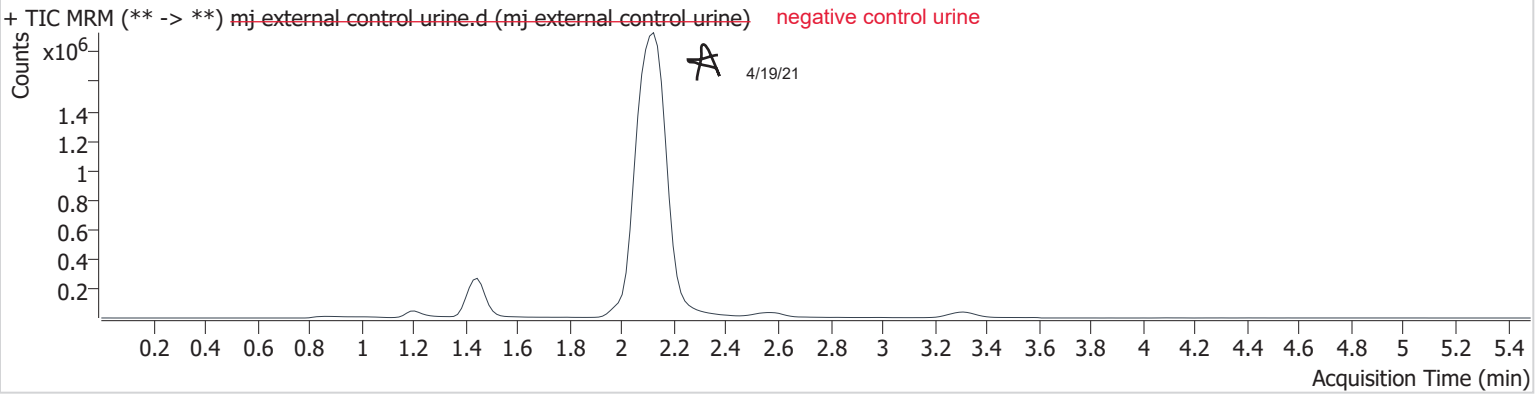


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update	4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj external control urine.d
Type	Sample	**Sample**	~~mj external control urine~~ negative control urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord 4/19/21
Sample Position	P3-G2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/16/2021 1:23:17 PM		
Sample Info.			

Sample Chromatogram

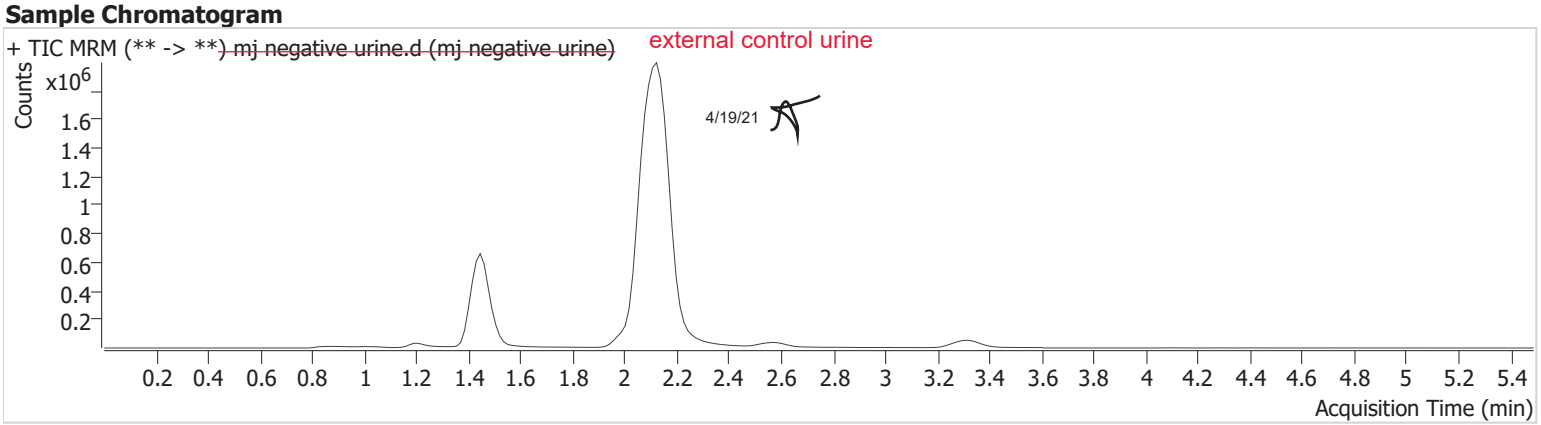


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj negative urine.d
Type	Sample	**Sample**	mj ~~negative urine~~ external control urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord 4/19/21
Sample Position	P3-H2	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/16/2021 1:29:59 PM		
Sample Info.			

Sample Chromatogram



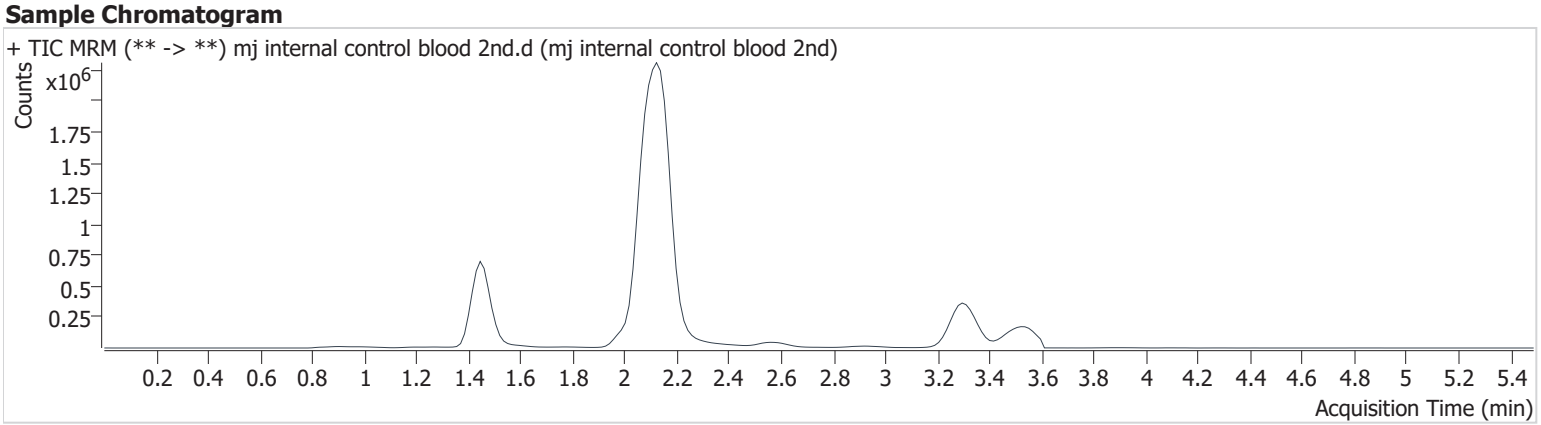
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	1400555	∞	11.9	∞	995181	51.188 ng/ml
THC-COOH	1.476	169696	2422.7	36.5	95508.0	322477	41.076 ng/ml
THC	3.332	78595	1724.5	23.6	93.5	283963	11.014 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj internal control blood 2nd.d
Type	QC	**Sample**	mj internal control blood 2nd
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/16/2021 3:03:27 PM		
Sample Info.			

Sample Chromatogram



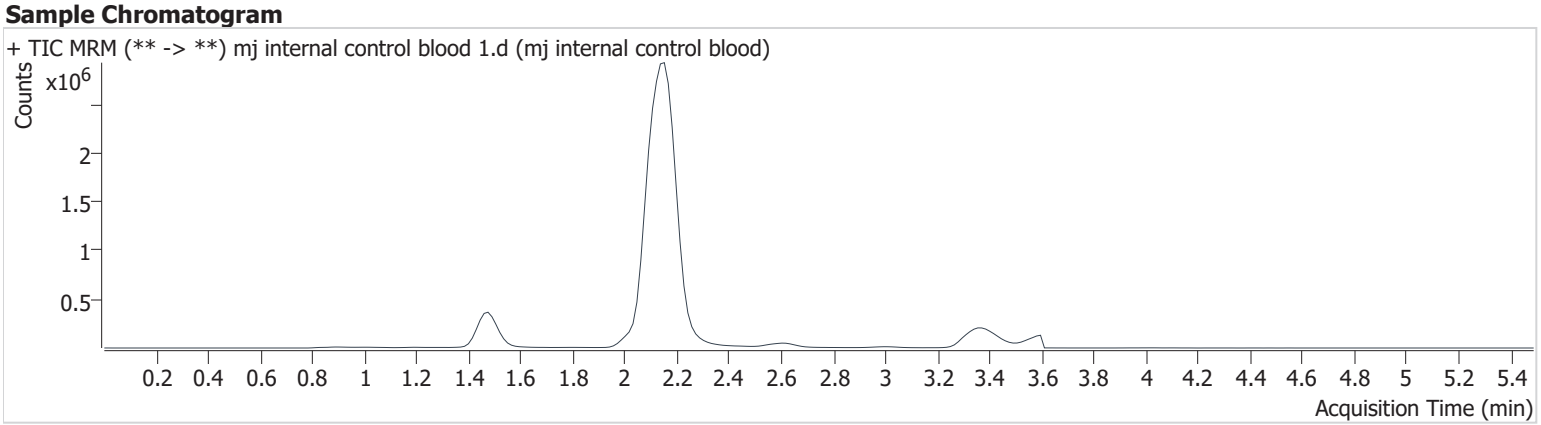
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	261000	∞	9.3	∞	2103747	3.989 ng/ml
THC-COOH	1.476	147644	163.6	36.0	12004 2.5	759723	16.063 ng/ml
THC	3.317	126268	1377.9	24.9	241.0	1374364	4.126 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj internal control blood 1.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	4/19/2021 10:03:32 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.466	135774	∞	10.5	∞	1061319	4.131 ng/ml
THC-COOH	1.505	89299	149283.6	37.1	6773.2	504608	14.753 ng/ml
THC	3.422	62718	488.7	25.6	119.6	703786	4.023 ng/ml

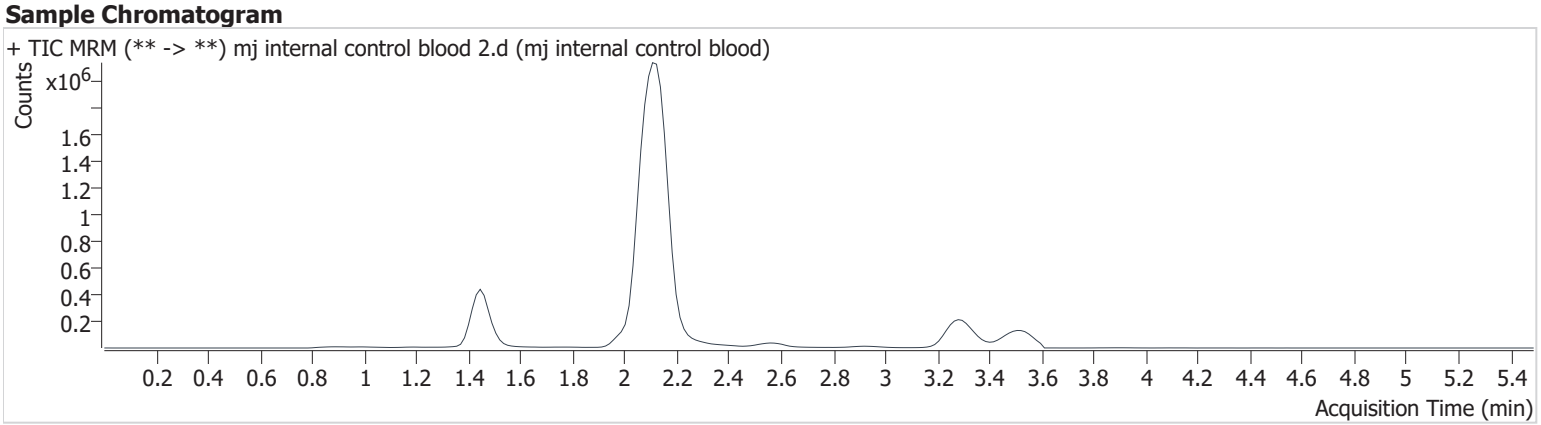
Two samples the samples and this control were reconstituted on 4/19/21 and run, this control was run before the samples.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 3:32:34 PM

Instrument	69679	**Data File**	mj internal control blood 2.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	4/19/2021 10:36:56 AM		
Sample Info.			

Sample Chromatogram

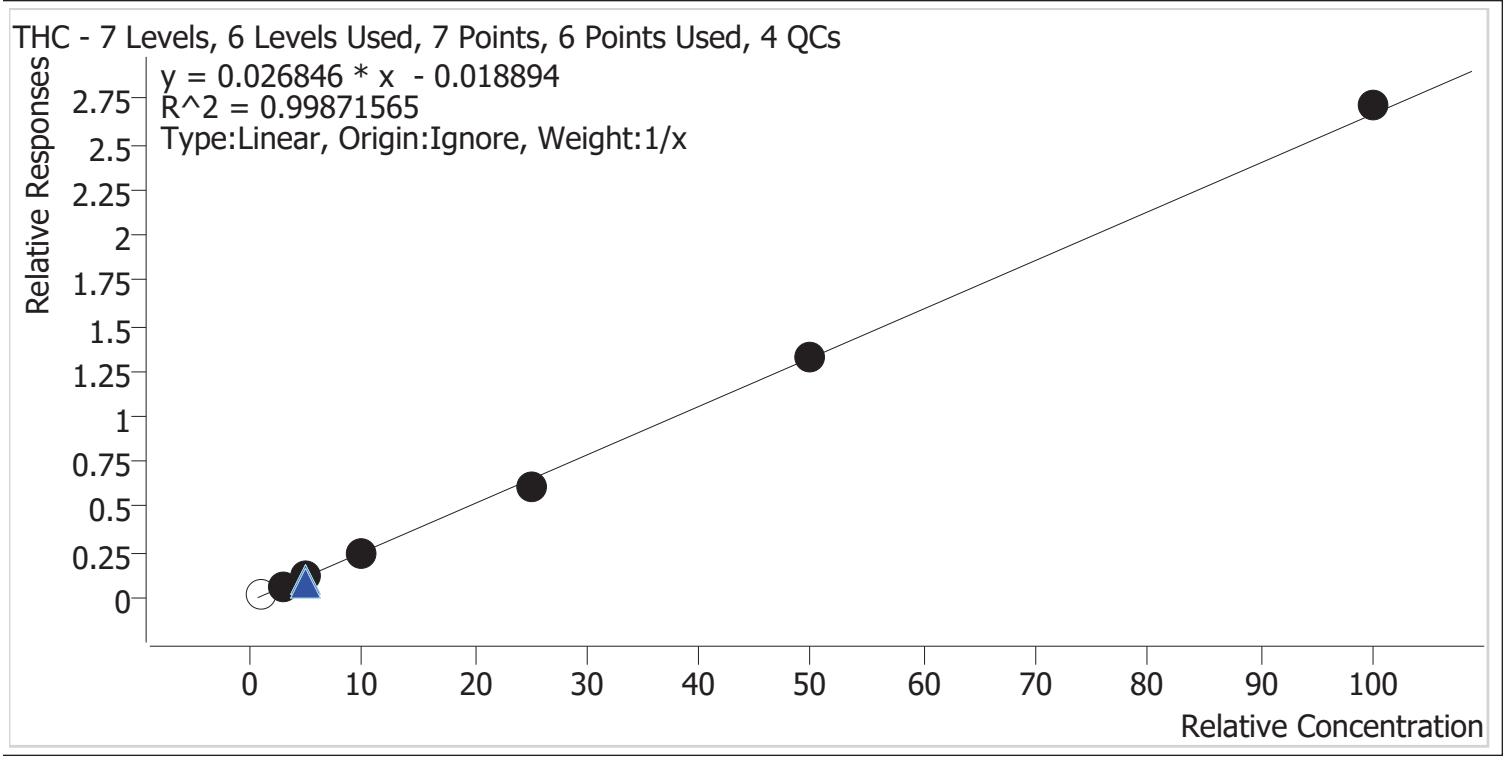


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	156921	∞	10.0	∞	1243492	4.067 ng/ml
THC-COOH	1.476	94233	286.6	36.8	27.7	529781	14.821 ng/ml
THC	3.332	61643	598.1	27.3	75.0	685367	4.054 ng/ml

Two samples the samples and this control were reconstituted on 4/19/21 and run, this control was run after the samples.

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Last Cal. Update	4/19/2021 11:19 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3

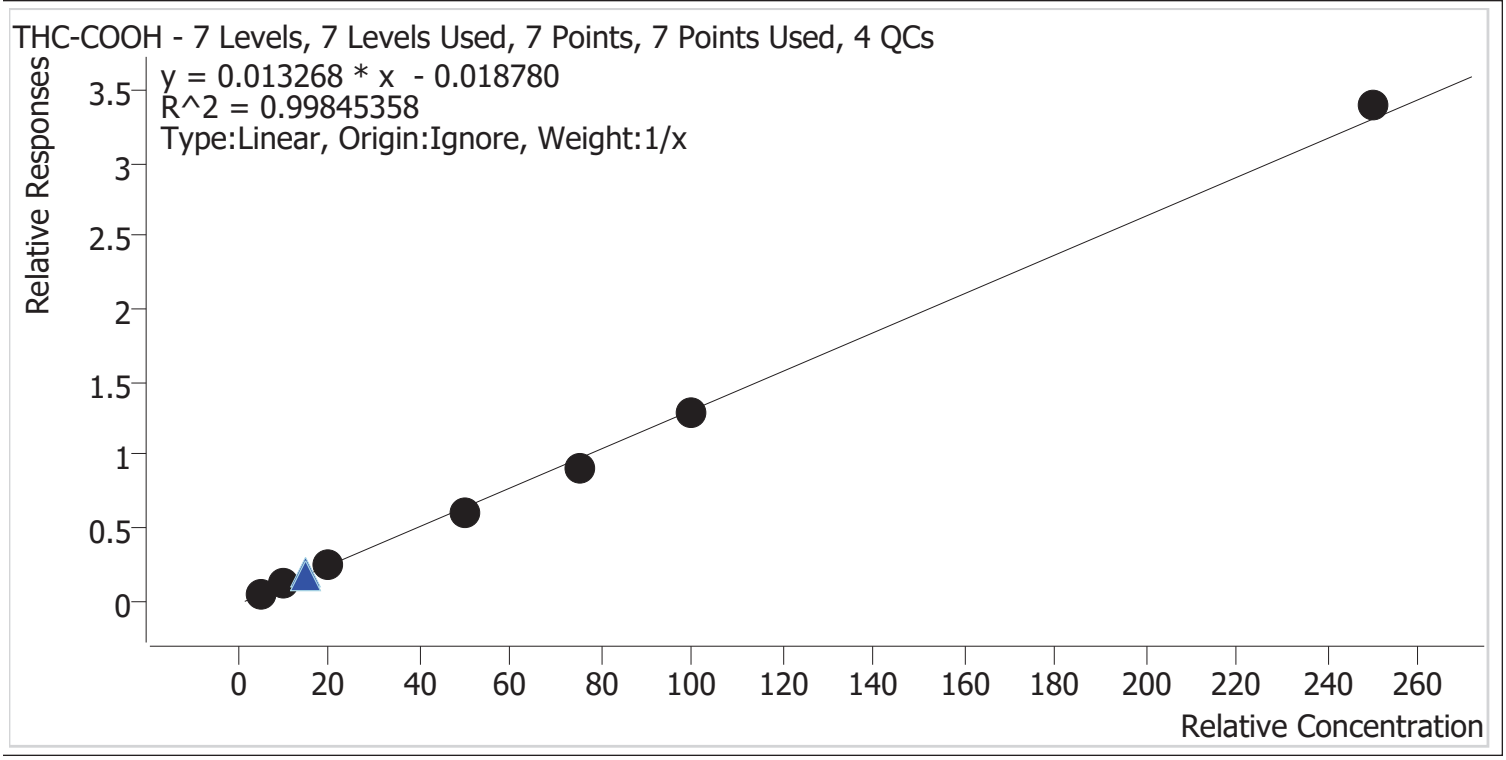


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	×	1.0	1.5	151.8
mj cal2	2	✓	3.0	3.2	105.6
mj cal 3	3	✓	5.0	5.1	102.4
mj cal 4	4	✓	10.0	9.7	97.4
mj cal 5	5	✓	25.0	23.2	92.7
mj cal 6	6	✓	50.0	50.0	100.1
mj cal 7	7	✓	100.0	101.7	101.7

Calibrator 1 dropped due to not meeting ratio requirement.

Compound Calibration Report

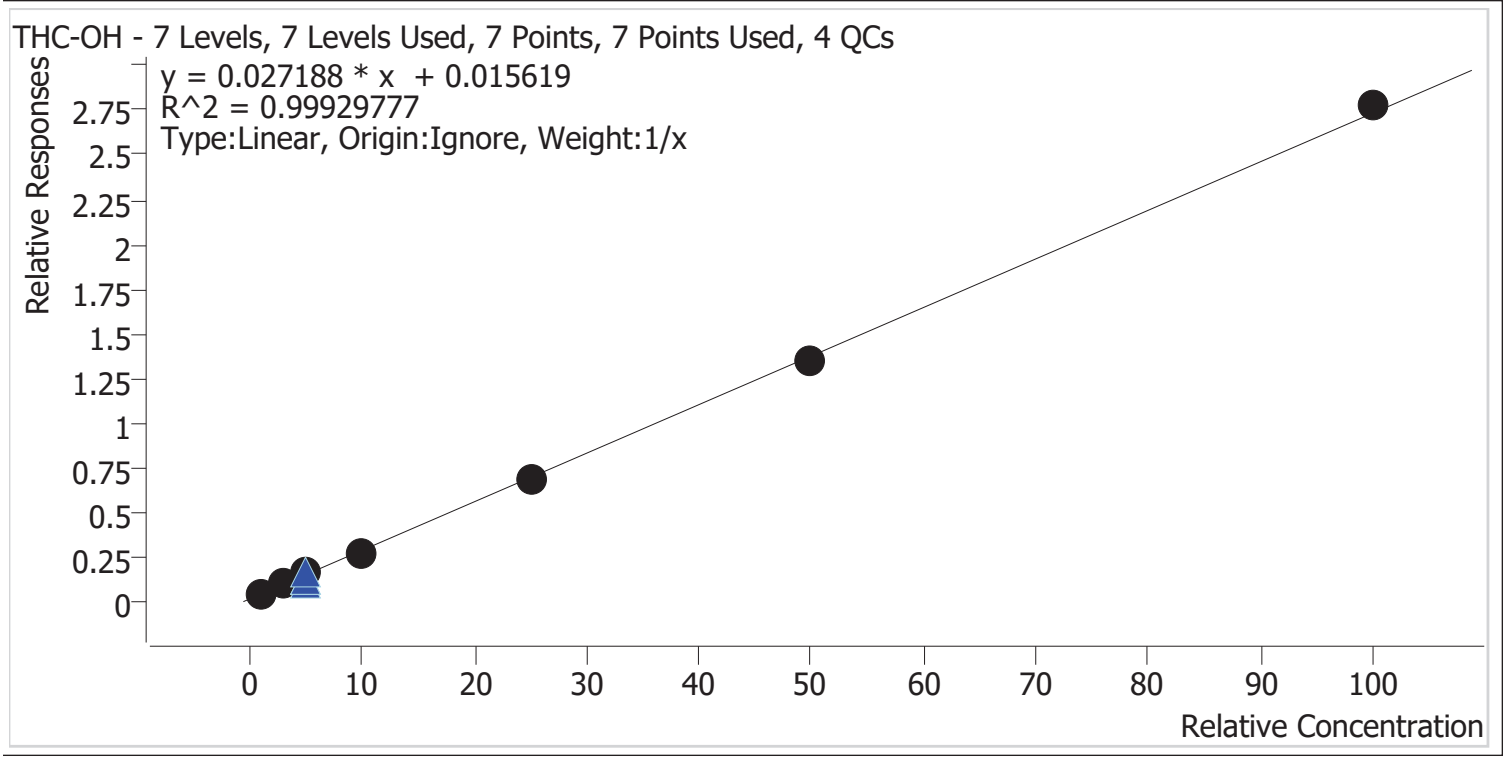
Batch results	D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Last Cal. Update	4/19/2021 11:19 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.4	107.8
mj cal2	2	✓	10.0	10.1	100.6
mj cal 3	3	✓	20.0	20.0	100.2
mj cal 4	4	✓	50.0	47.5	95.1
mj cal 5	5	✓	75.0	70.6	94.2
mj cal 6	6	✓	100.0	99.5	99.5
mj cal 7	7	✓	250.0	256.9	102.8

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Last Cal. Update	4/19/2021 11:19 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



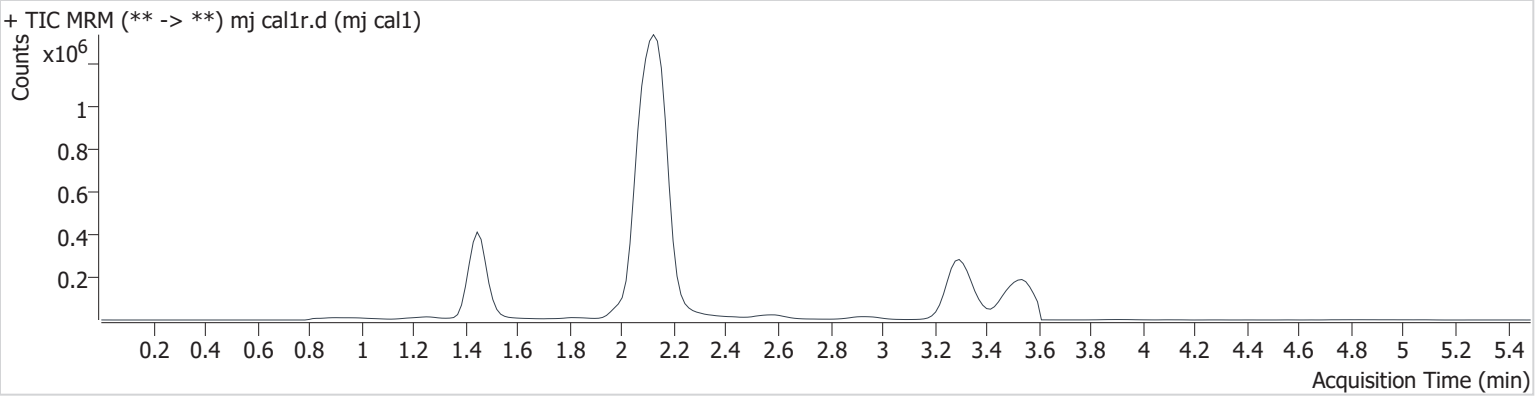
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	1.1	106.7
mj cal2	2	✓	3.0	3.0	98.5
mj cal 3	3	✓	5.0	5.2	103.9
mj cal 4	4	✓	10.0	9.2	91.7
mj cal 5	5	✓	25.0	24.7	99.0
mj cal 6	6	✓	50.0	49.4	98.7
mj cal 7	7	✓	100.0	101.5	101.5

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj cal1r.d
Type	Cal	**Sample**	mj cal1
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/16/2021 11:02:51 AM		
Sample Info.			

Sample Chromatogram



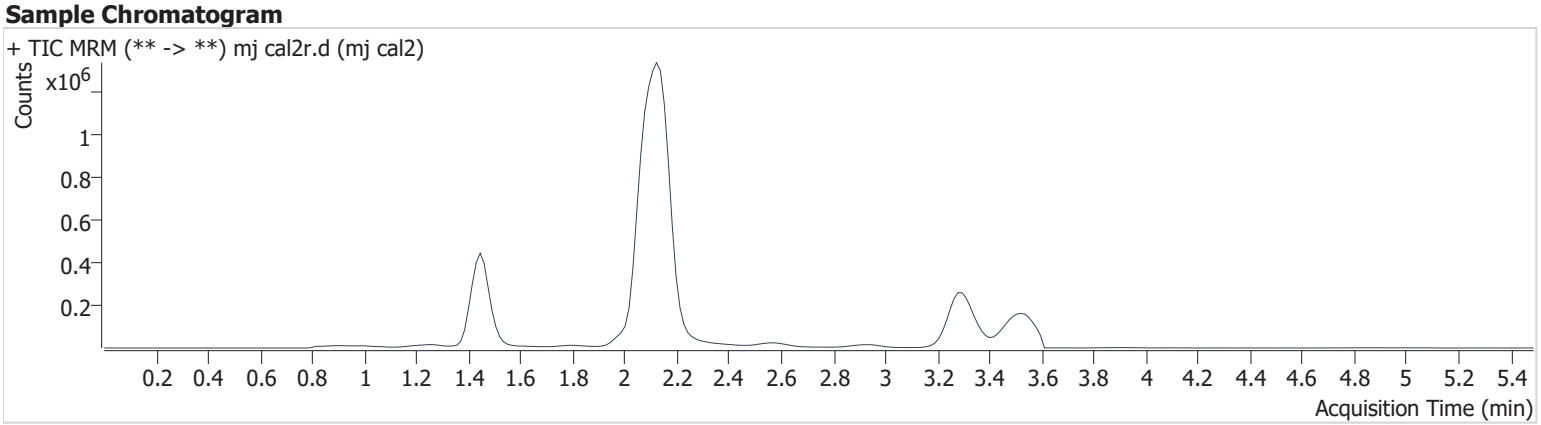
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	63488	∞	8.2	∞	1422478	1.067 ng/ml	Low
THC-COOH	1.476	27141	21.4	32.1	16888.7	514967	5.388 ng/ml	
THC	3.332	14122	38.1	35.4 High	12.8	645819	1.518 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj cal2r.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	4/16/2021 11:09:35 AM		
Sample Info.			

Sample Chromatogram



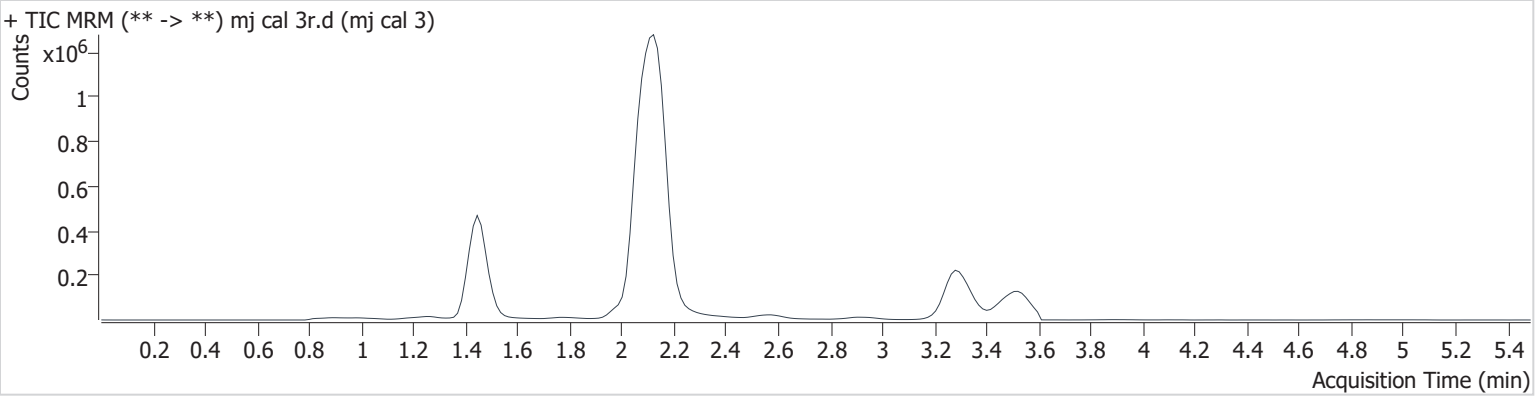
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	134110	∞	8.5	∞	1397480	2.955 ng/ml	Low
THC-COOH	1.476	57269	56.1	34.8	52.7	499556	10.056 ng/ml	
THC	3.332	41912	92.4	27.3	25.4	633303	3.169 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj cal 3r.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	4/16/2021 11:16:17 AM		
Sample Info.			

Sample Chromatogram



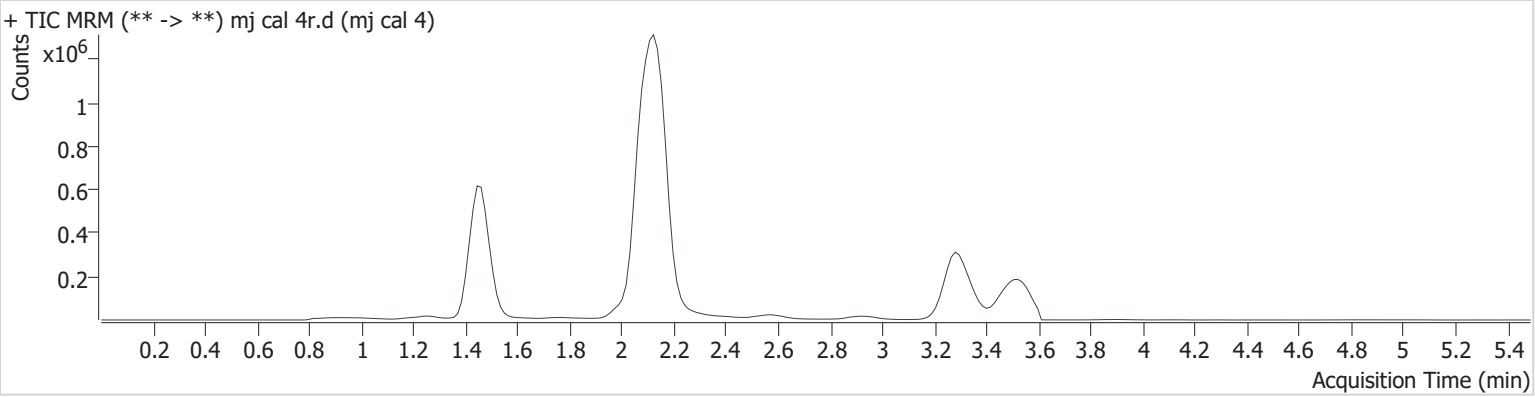
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	208226	∞	8.9	∞	1327359	5.195 ng/ml
THC-COOH	1.476	125407	265839.2	34.2	18.9	507362	20.045 ng/ml
THC	3.332	73131	288.0	25.4	79.8	616729	5.121 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj cal 4r.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	4/16/2021 11:22:59 AM		
Sample Info.			

Sample Chromatogram



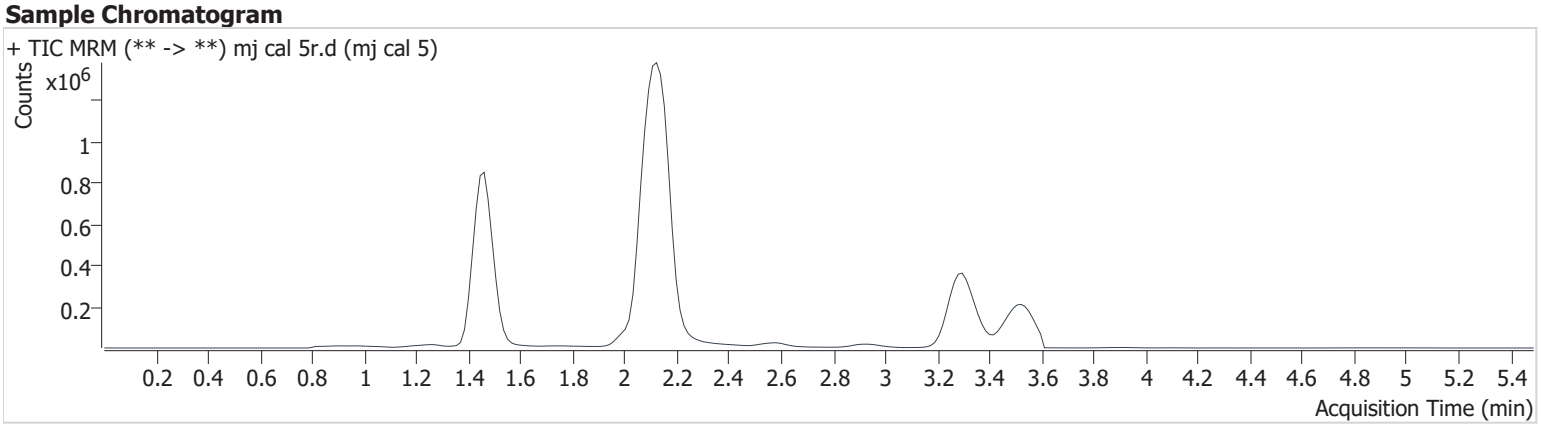
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	378675	∞	10.5	∞	1429382	9.169 ng/ml
THC-COOH	1.476	315765	348.5	36.2	725.8	515925	47.544 ng/ml
THC	3.332	164377	1478.4	24.8	298.1	677553	9.741 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj cal 5r.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	4/16/2021 11:29:40 AM		
Sample Info.			

Sample Chromatogram



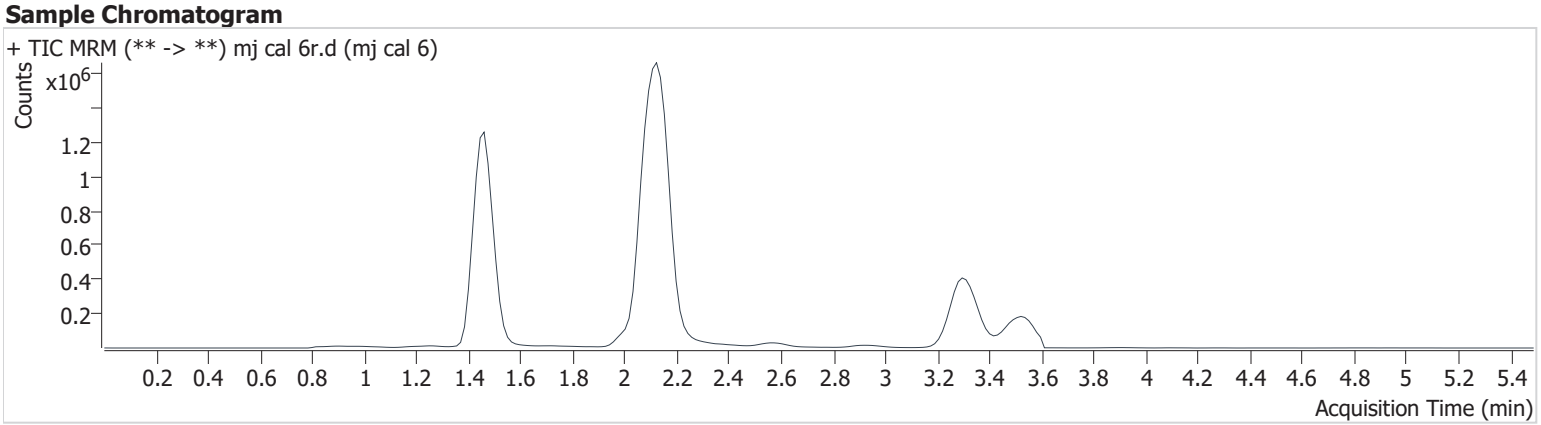
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	1006876	∞	11.0	∞	1462990	24.739 ng/ml
THC-COOH	1.476	482622	2222.5	36.8	776.0	525572	70.625 ng/ml
THC	3.332	415168	2113.4	24.2	530.5	688058	23.180 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj cal 6r.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	4/16/2021 11:36:22 AM		
Sample Info.			

Sample Chromatogram



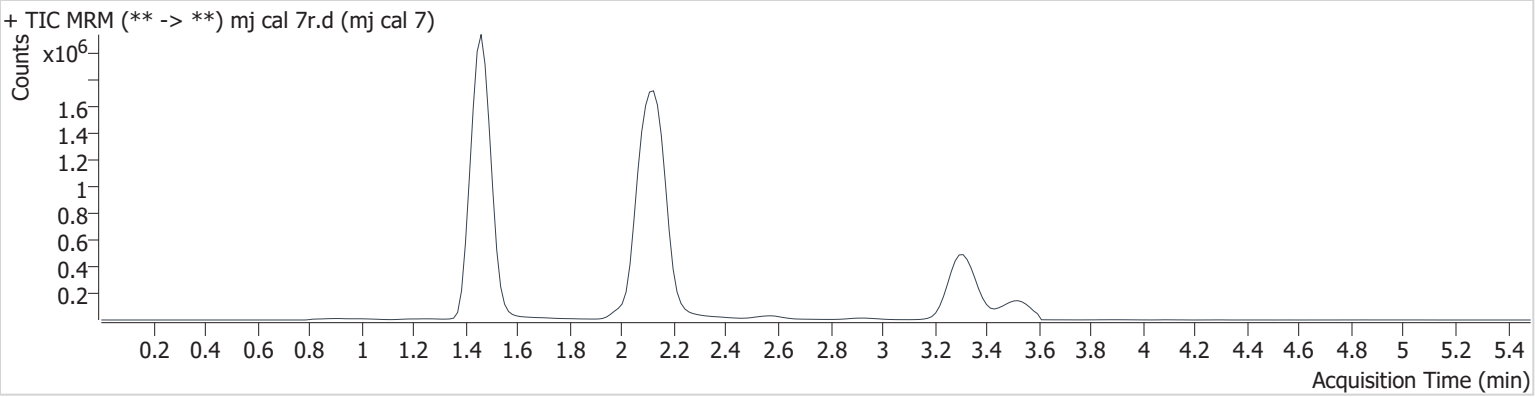
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	2069077	∞	11.5	∞	1524304	49.351 ng/ml
THC-COOH	1.476	761168	464.2	37.0	115.1	585148	99.456 ng/ml
THC	3.332	941777	8794.5	23.1	2318.4	711038	50.041 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\041521\QuantResults\cann quant.batch.bin
Calibration Last Update 4/19/2021 11:19:49 AM

Instrument	69679	**Data File**	mj cal 7r.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	4/16/2021 11:43:04 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	3929830	∞	11.8	∞	1415722	101.523 ng/ml
THC-COOH	1.476	1781492	3667.5	37.3	10305	525577	256.886 ng/ml
					73.6		
THC	3.332	1807174	8863.0	23.1	1622.2	666187	101.750 ng/ml

Idaho State Police
Forensic Services
Toxicology Discipline

Request for Departure from an Analytical Method

Date of Request
02/24/2021

Forensic Scientist
Anne Nord

Analytical Method
Toxicology AM #27: Quantitative Analysis of THC and Metabolites in Blood and Urine by LCMS-QQQ

Request

The method currently reads:
4.3.2.5 If any points are dropped from the approved quantitative range of the curve, the compound will be reported qualitatively. For calibrators and controls 10 ng and below, the accuracy must be within 30%, for calibrators and controls greater than 10 ng/mL the accuracy must be within 20%. If a control falls outside the accuracy range, at the analyst's discretion, the compound may be reported qualitatively.

I would like to add in the following exception:

If the 1ng/ml point is dropped for THC, the quantitative range will be 3-50 ng/ml.

Discipline Leader Review

☒ Departure approved
Comments: This deviation is approved and will remain in effect until it is changed in the actual method.

☐ Departure Not Approved
Comments:

Celena Shrum
Toxicology Discipline Lead
Date: 02/24/2021