Worklist: 6248

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
C2023-0084	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-0168	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-0212	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-0268	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-0293	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-0317	3	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-0352	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
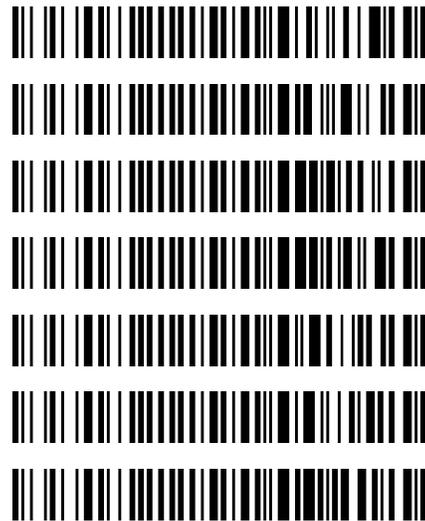
AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date 2/14/23 Analyst: Anne Nord
Plate lot#: 230113 Plate re-test: 7/13/23

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 run **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. SN > 10
☒ 4. Case sample response for THC 1ng/ml LOD 3ng/ml LOQ, OH-THC 3ng/mL LOD and LOQ, Carboxy-THC: 5 ng/mL (qualitative only). 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. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *autosampler failed prior to injecting any samples for this run. It was reset and samples were injected 2/15/23.*
Curve limits THC-OH 3-100 dropped cal 1 due to poor response for qualifier ion.

|	1	2	3	4	5	6
a	cal 1	Internal blood control	0352-1 mixing plate			
b	cal 2	negative blood	0352-1 SLE and Injection plate			
c	cal 3	0084-1				
d	cal 4	0168-2				
e	Cal 5	0212-2				
f	cal 6	0268-1				
g	cal 7	0293-1				
h	Internal control (blood)	0317-3				

Plate position 3

c2023-_____-_

AM #27 Cannabinoids

Batch results   D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update   2/15/2023 12:17:38 PM

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	2/15/2023 10:04:29 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj internal control blood.d (mj internal control blood)
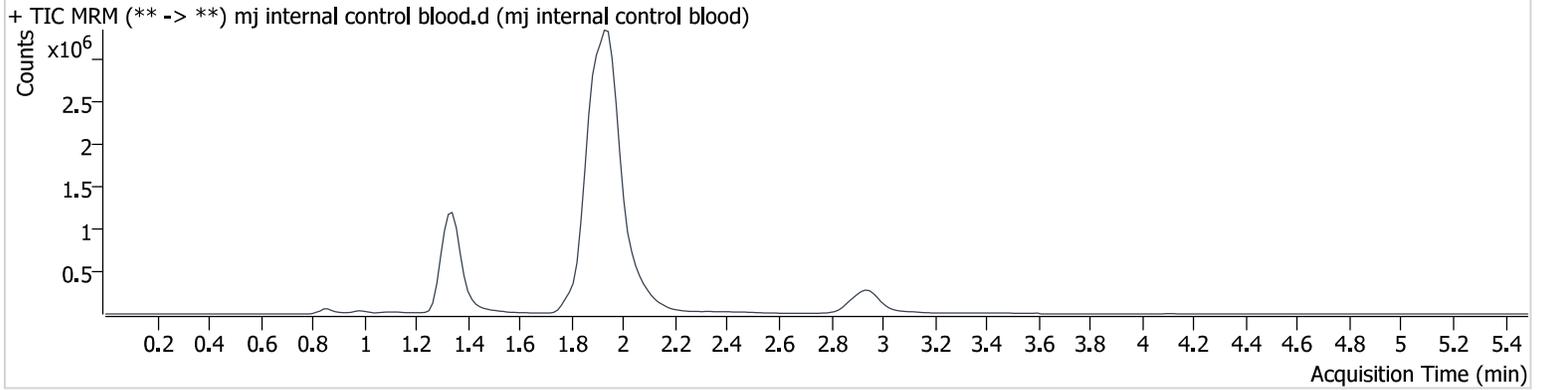
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.334	70074	∞	1005.25	∞	3620141	4.635 ng/ml
THC-COOH	1.358	97745	1847.4	246.03	∞	1266291	15.332 ng/ml
THC	2.956	231143	∞	24.39	470.6	1793212	4.904 ng/ml

AM #27 Cannabinoids

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	2/15/2023 10:11:05 AM		
Sample Info.			

Sample Chromatogram
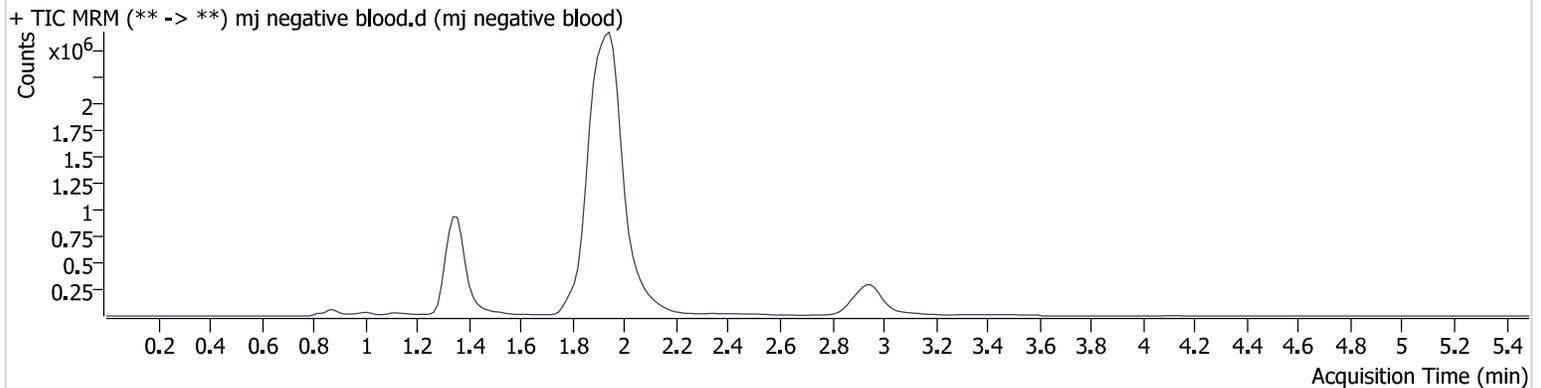
+ TIC MRM (** -> **) mj negative blood.d (mj negative blood)

AM #27 Cannabinoids

Batch results   D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update   2/15/2023 12:17:38 PM

Instrument	69679	**Data File**	end of run blood control blood A2.d
Type	Sample	**Sample**	end of run blood control blood A2
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/15/2023 11:49:59 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) end of run blood control blood A2.d (end of run blood control blood A2)
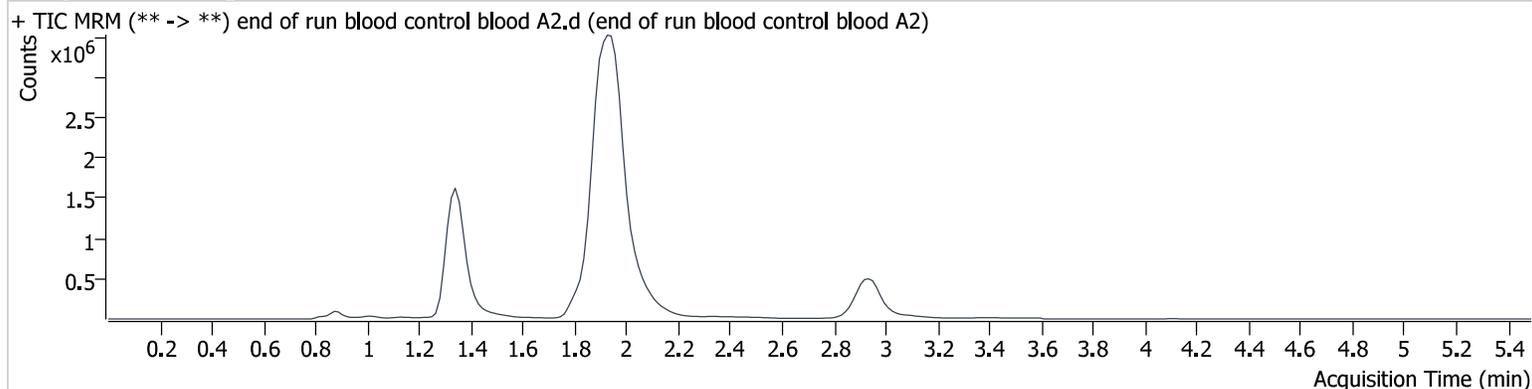
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.334	92432	440.7	996.36	∞	4646959	4.737 ng/ml
THC-COOH	1.373	129906	854.0	263.01	∞	1680758	15.350 ng/ml
THC	2.956	373878	3654.0	24.01	11804.2	2887125	4.925 ng/ml

Compound Calibration Report

Batch results	D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin	
Last Cal. Update	2/15/2023 12:17 PM	
Analyst Name	ISP\datastor	
Analyte	THC	**Internal Standard** THC-d3

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

$y = 0.028990 * x - 0.013280$
$R^2 = 0.99873527$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	121.0
mj cal 2	2	✓	3.0	2.9	96.2
mj cal 3	3	✓	5.0	4.6	92.1
mj cal 4	4	✓	10.0	9.2	92.4
mj cal 5	5	✓	25.0	24.1	96.5
mj cal 6	6	✓	50.0	49.9	99.9
mj cal 7	7	✓	100.0	102.0	102.0

Compound Calibration Report

|	
Batch results	D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Last Cal. Update	2/15/2023 12:17 PM
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

$y = 0.005580 * x - 0.008353$
$R^2 = 0.99979959$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.4	107.5
mj cal 2	2	✓	10.0	9.6	96.3
mj cal 3	3	✓	20.0	19.3	96.7
mj cal 4	4	✓	50.0	49.7	99.5
mj cal 5	5	✓	75.0	75.0	100.0
mj cal 6	6	✓	100.0	99.5	99.5
mj cal 7	7	✓	250.0	251.5	100.6

Batch results D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin

Last Cal. Update 2/15/2023 12:17 PM

Analyst Name ISP\datastor

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

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs

$y = 0.005215 * x - 0.004812$

$R^2 = 0.99889253$

Type:Linear, Origin:Ignore, Weight:1/x
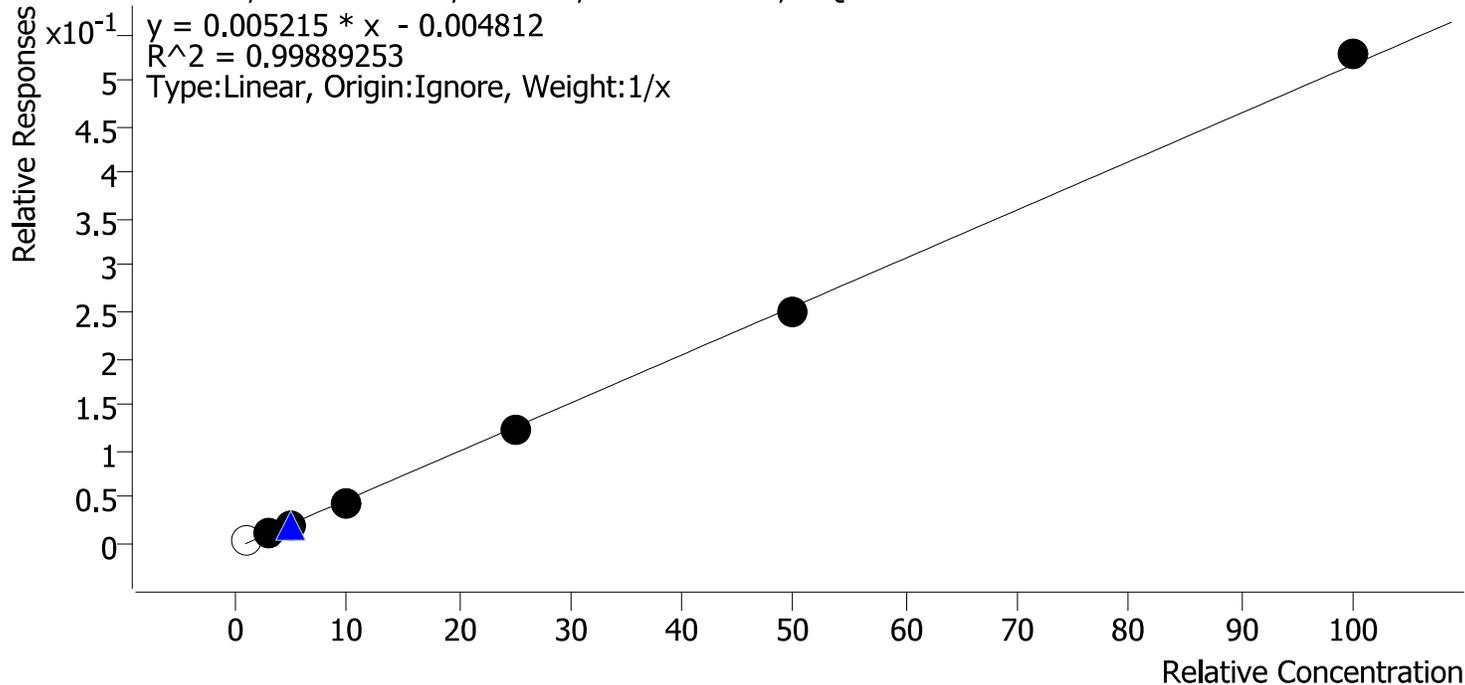
| Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✗	1.0	1.7	171.1
mj cal 2	2	✓	3.0	3.3	110.0
mj cal 3	3	✓	5.0	5.0	99.2
mj cal 4	4	✓	10.0	9.4	93.8
mj cal 5	5	✓	25.0	24.2	96.8
mj cal 6	6	✓	50.0	49.0	98.1
mj cal 7	7	✓	100.0	102.1	102.1

AM #27 Cannabinoids

Batch results   D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update   2/15/2023 12:17:38 PM

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	2/15/2023 9:18:14 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj cal 1.d (mj cal 1)
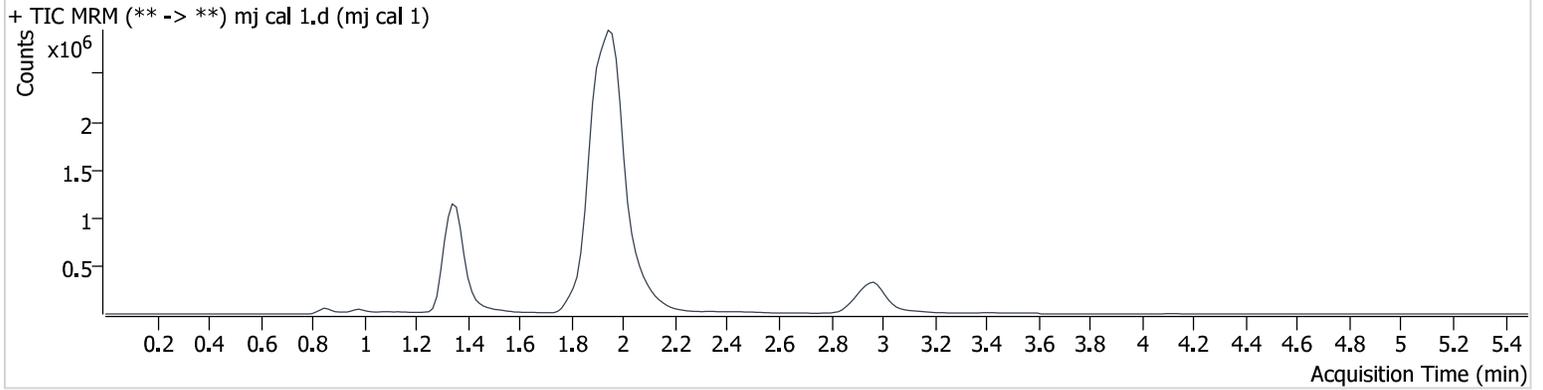
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.364	17583	575.9	857.30	∞	4277666	1.711 ng/ml	Low
THC-COOH	1.373	31527	∞	270.89	∞	1456206	5.377 ng/ml	
THC	2.986	51869	736.5	23.87	47.5	2378987	1.210 ng/ml	

AM #27 Cannabinoids

Batch results   D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update 2/15/2023 12:17:38 PM

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	2/15/2023 9:24:58 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj cal 2.d (mj cal 2)
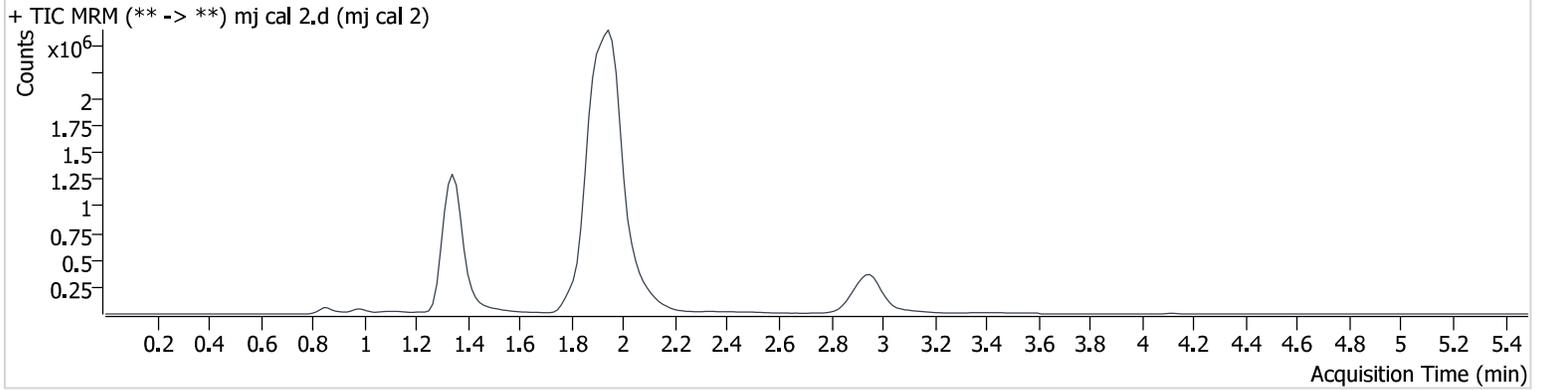
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	54257	717.9	1035.50	∞	4377907	3.299 ng/ml
THC-COOH	1.373	68510	∞	261.21	702.3	1510496	9.626 ng/ml
THC	2.971	177180	3471.7	24.31	99.9	2518068	2.885 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update 2/15/2023 12:17:38 PM

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	2/15/2023 9:31:34 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj cal 3.d (mj cal 3)
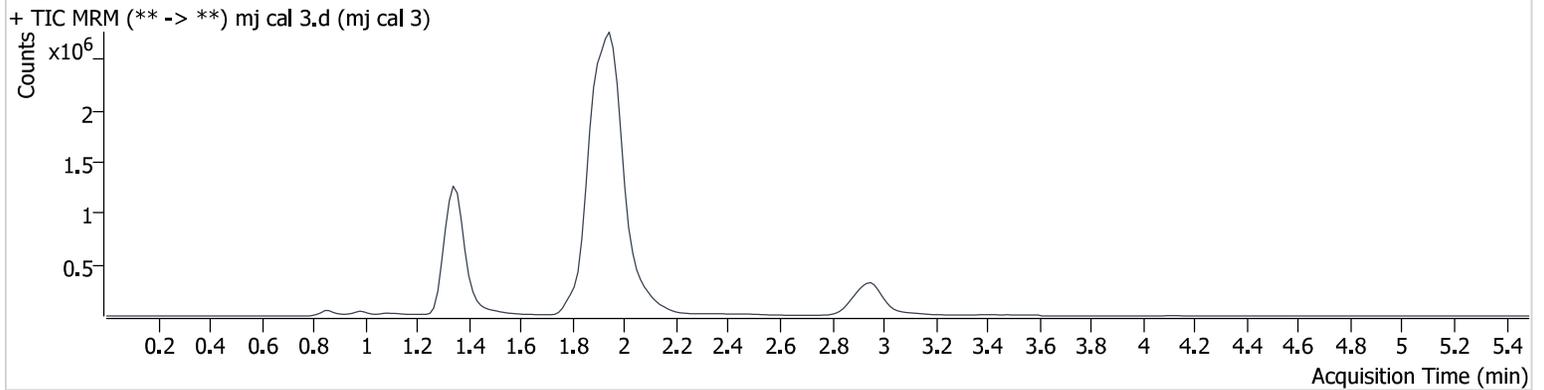
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	76576	149104.6	996.55	∞	3637777	4.959 ng/ml
THC-COOH	1.373	134017	859.6	258.18	2882.1	1346197	19.340 ng/ml
THC	2.956	254385	3461.4	25.24	141.8	2116680	4.604 ng/ml

AM #27 Cannabinoids

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	2/15/2023 9:38:08 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj cal 4.d (mj cal 4)
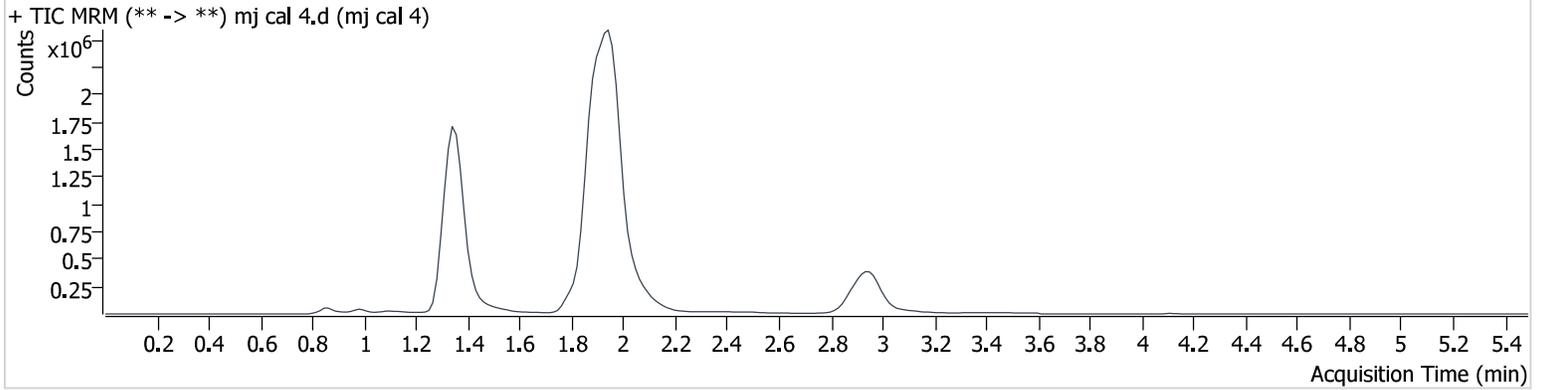
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	167287	2023.8	932.66	∞	3792243	9.382 ng/ml
THC-COOH	1.373	359796	∞	249.43	∞	1336548	49.745 ng/ml
THC	2.956	572710	2049.5	24.27	1029.4	2250792	9.235 ng/ml

AM #27 Cannabinoids

Batch results   D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update   2/15/2023 12:17:38 PM

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	2/15/2023 9:44:44 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj cal 5.d (mj cal 5)
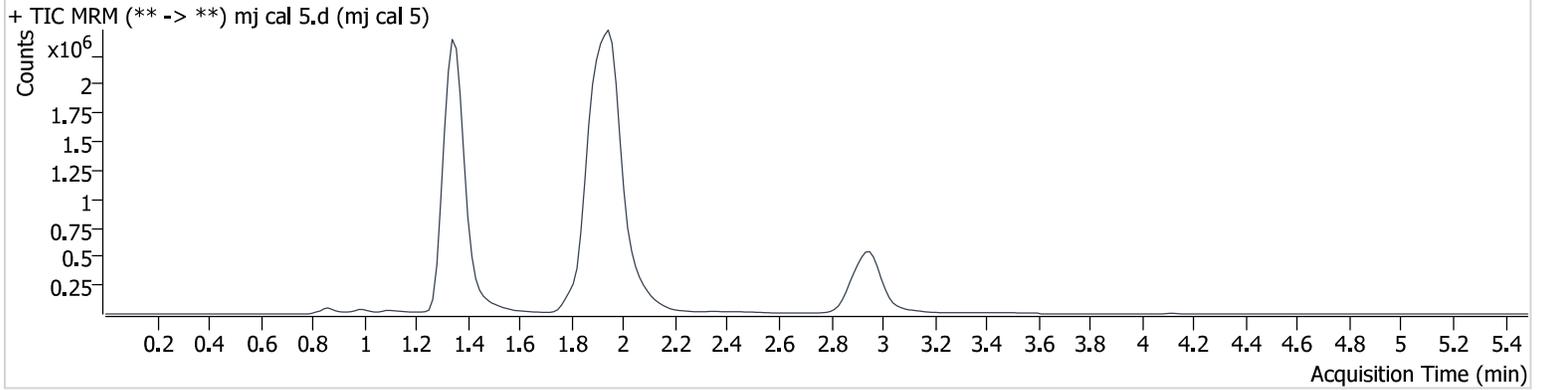
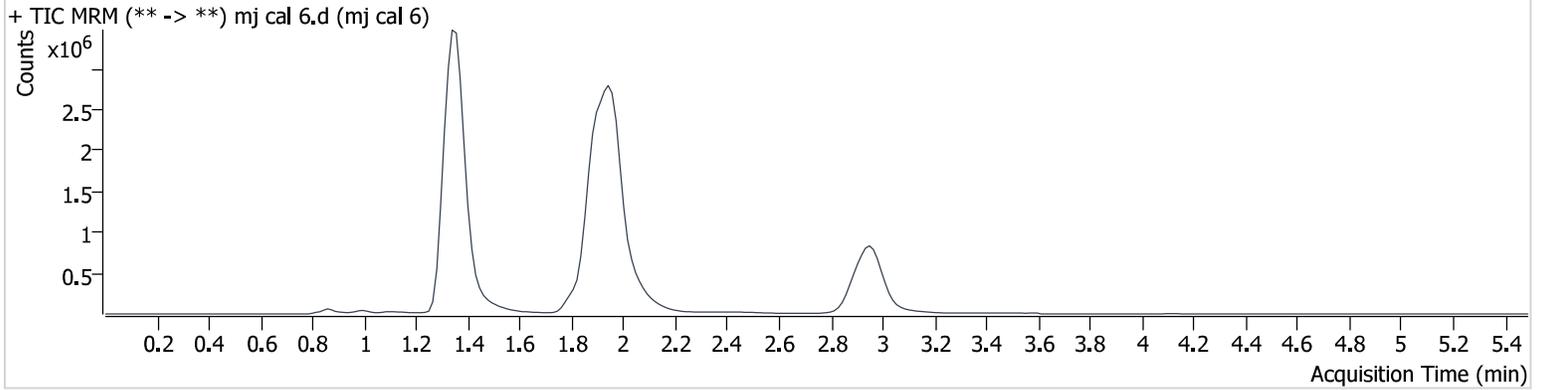| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	467480	∞	844.86	∞	3850022	24.207 ng/ml
THC-COOH	1.373	528635	∞	254.12	∞	1289620	74.965 ng/ml
THC	2.956	1558558	9615.2	23.89	3486.3	2272017	24.121 ng/ml

AM #27 Cannabinoids

Batch results   D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update 2/15/2023 12:17:38 PM

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	2/15/2023 9:51:18 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj cal 6.d (mj cal 6)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	1002690	3717.5	832.05	∞	3996696	49.032 ng/ml
THC-COOH	1.373	723525	∞	250.70	14214.7	1323802	99.454 ng/ml
THC	2.956	3338557	3101017656 989630.0	24.04	∞	2327010	49.948 ng/ml

AM #27 Cannabinoids

Batch results   D:\MassHunter\Data\2023\am 27-28\021423\QuantResults\cann.batch.bin
Calibration Last Update   2/15/2023 12:17:38 PM

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	2/15/2023 9:57:54 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) mj cal 7.d (mj cal 7)
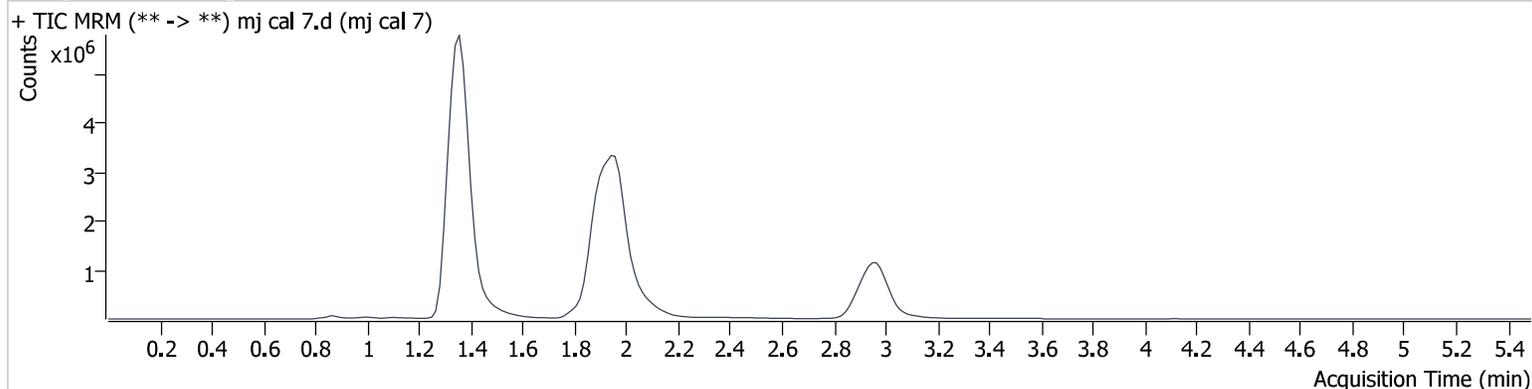
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	1908758	87863.1	806.67	∞	3616979	102.120 ng/ml
THC-COOH	1.373	1595889	25629.9	241.82	∞	1144117	251.494 ng/ml
THC	2.971	5791492	∞	24.30	12883.8	1967523	101.996 ng/ml