4/2/21- reviewed corrections to control/calibration positions on am 27 plate map BW

2/17/2021

REVIEWED
By Britany Wylie at 2:29 pm, Feb 17, 2021

Worklist: 4789

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2021-0225	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0262	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0281	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-0289	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 02/11/21 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: 20J20793 **Urine Blank:** **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: *Blood only in this run*
Samples originally injected on 2/11/21, response for lower level cals was poor, samples reconstituted on 2/12/21 and reinjected 2/16/21. The injections on 2/16/21 were evaluated.

	1	2	3	4	5	6
a	cal 1 ~~cal 100 ng~~	neg blood				QC 1
b	cal 2 ~~cal 50 ng~~	225-1				cal 100 ng
c	cal 3 ~~cal 25 ng~~	262-1				cal 50 ng
d	cal 4 ~~cal 10ng~~	281-1				cal 25 ng
e	cal 5 ~~cal 5 ng~~	289-1				cal 10ng
f	cal 6 ~~cal 3 ng~~					cal 5 ng
g	cal 7 ~~cal 1ng~~					cal 3 ng
h	QC 1					cal 1ng

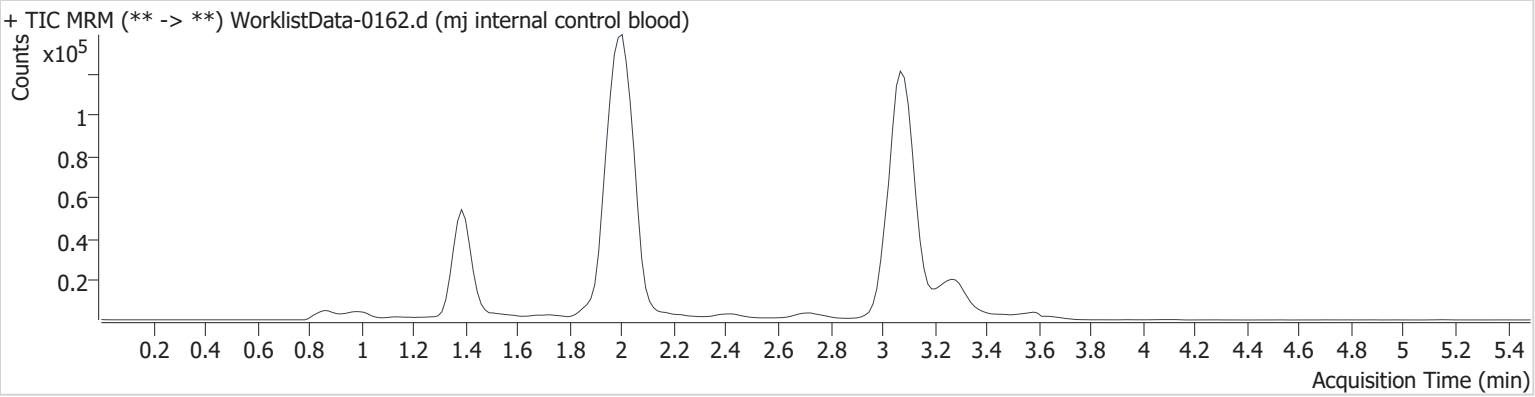
C2021-0___-_

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0162.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	2/16/2021 4:15:37 PM		
Sample Info.			

Sample Chromatogram



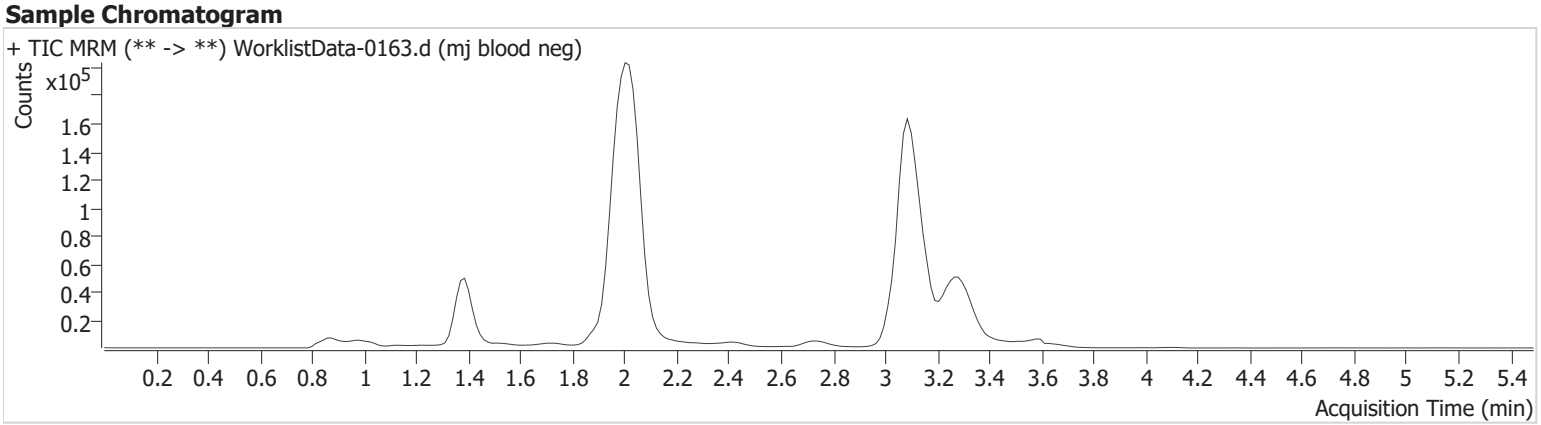
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	6015	∞	776.5	∞	78537	4.381 ng/ml
THC-COOH	1.399	37434	∞	23.3	∞	66827	14.614 ng/ml
THC	3.107	58876	4570.4	35.1	195.6	575348	3.817 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0163.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	2/16/2021 4:22:21 PM		
Sample Info.			

Sample Chromatogram



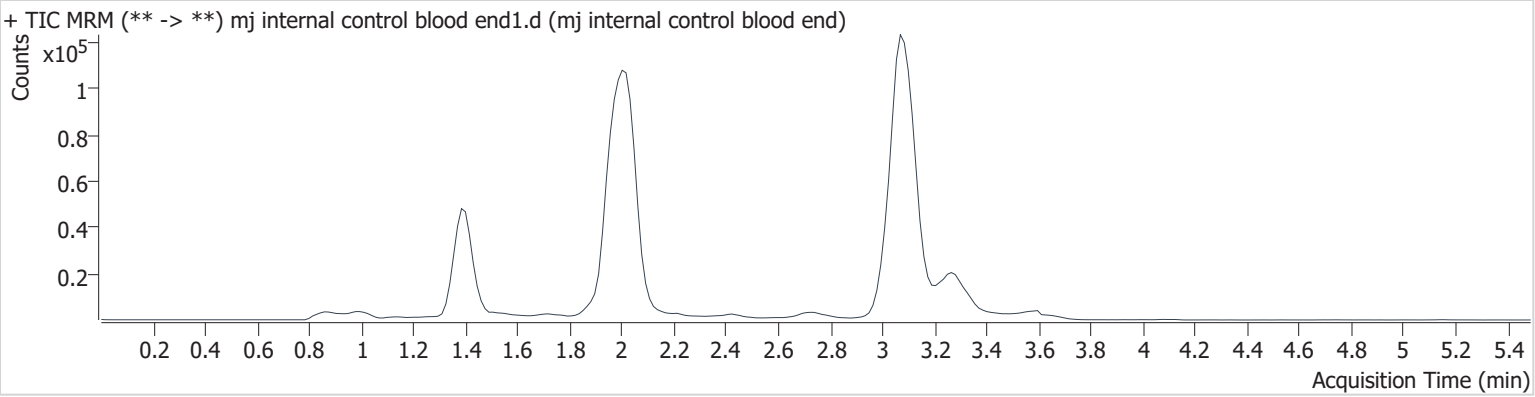
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.409	823	∞	1678.5 **High**	∞	130744	0.618 ng/ml	**Low**

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	mj internal control blood end1.d
Type	QC	**Sample**	mj internal control blood end
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/16/2021 5:22:33 PM		
Sample Info.			

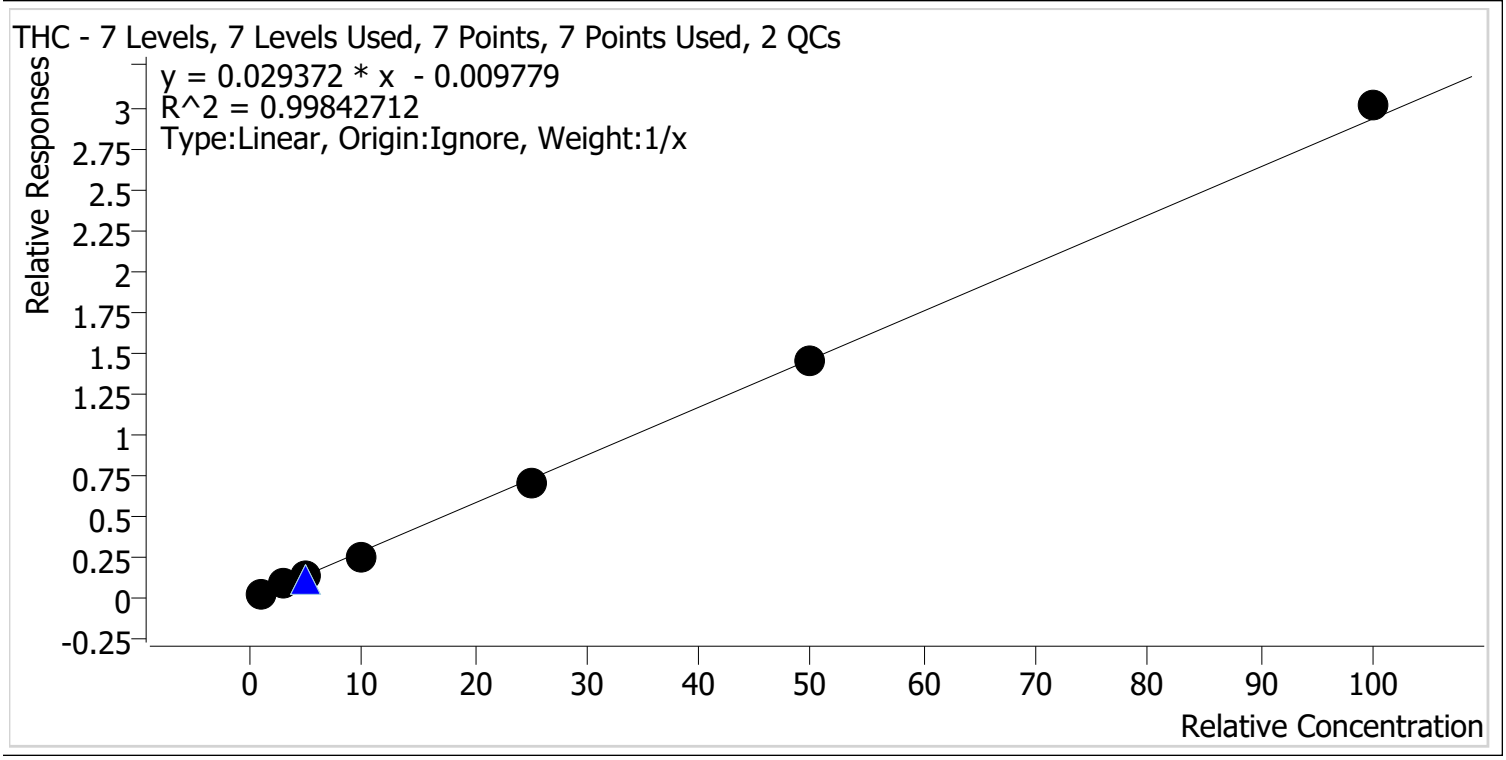
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	5123	∞	802.9	∞	66818	4.386 ng/ml
THC-COOH	1.414	33337	∞	22.9	1854.2	59142	14.693 ng/ml
THC	3.107	61964	∞	37.6	∞	578940	3.977 ng/ml

Compound Calibration Report

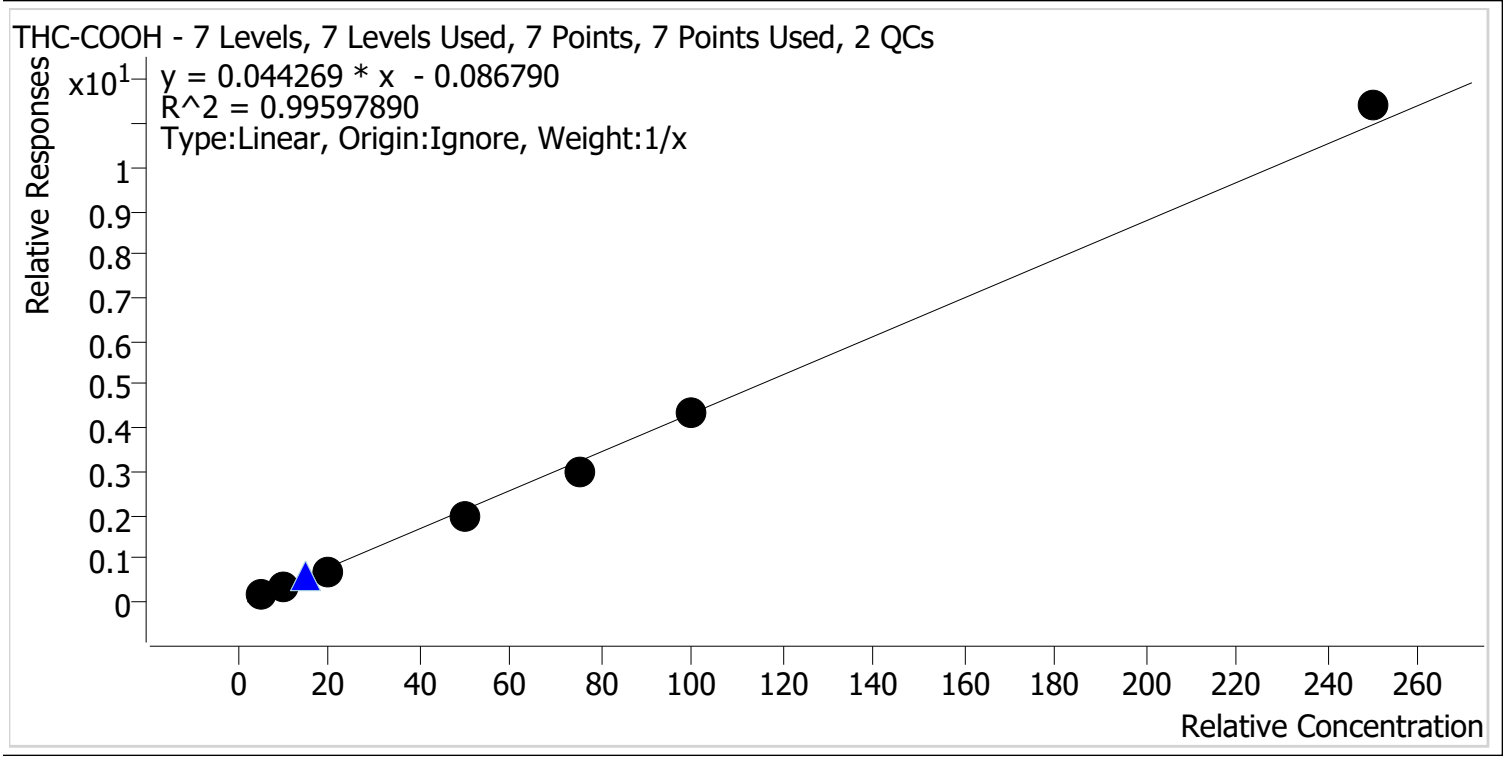
Batch results	D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Last Cal. Update	2/17/2021 7:46 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	1.0	1.2	115.9
mj cal2	2	✔	3.0	3.1	102.1
mj cal 3	3	✔	5.0	4.7	94.0
mj cal 4	4	✔	10.0	9.1	91.3
mj cal 5	5	✔	25.0	23.8	95.2
mj cal 6	6	✔	50.0	49.4	98.8
mj cal 7	7	✔	100.0	102.7	102.7

Compound Calibration Report

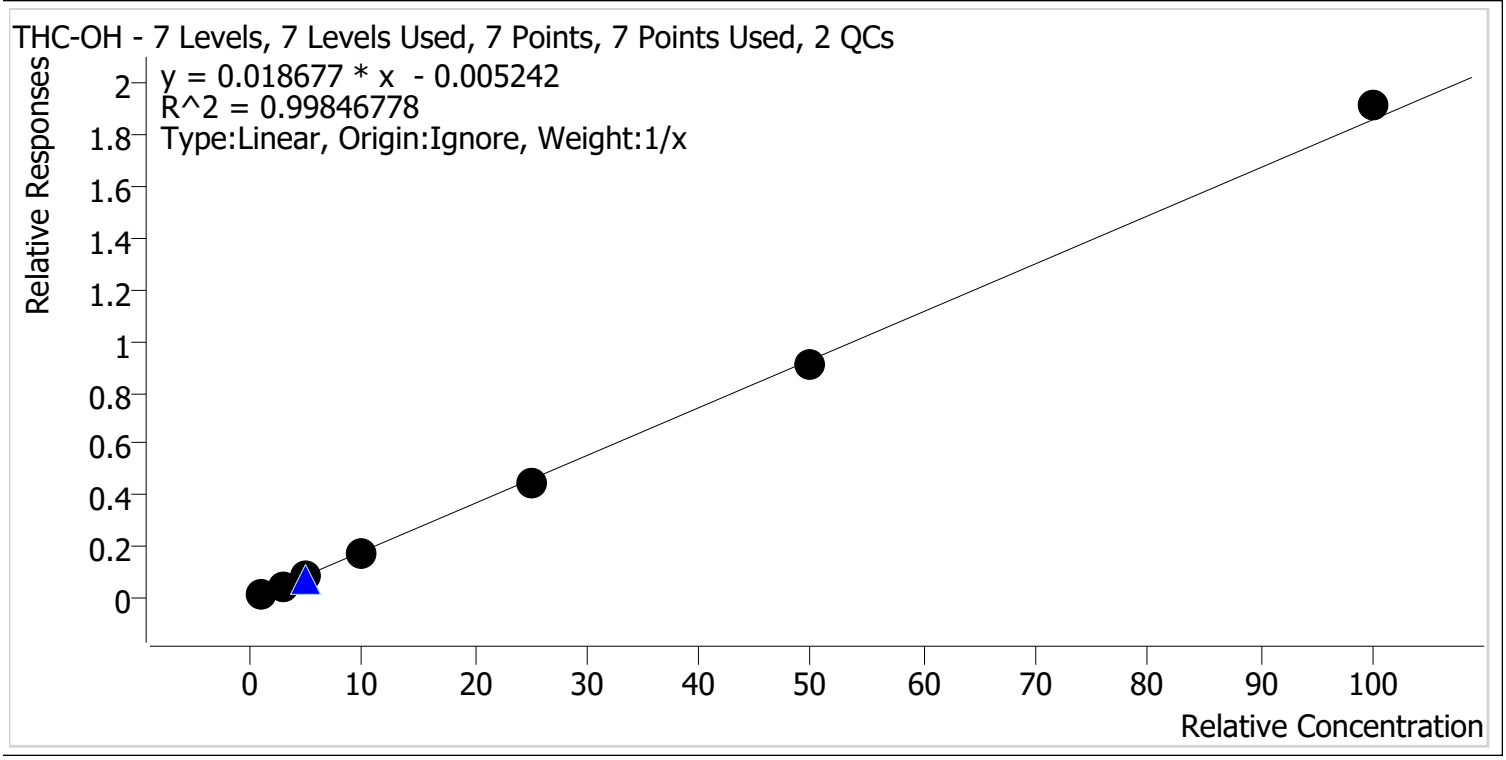
Batch results	D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Last Cal. Update	2/17/2021 7:46 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	5.0	6.2	124.7
mj cal2	2	✔	10.0	9.4	94.4
mj cal 3	3	✔	20.0	18.4	91.9
mj cal 4	4	✔	50.0	45.6	91.2
mj cal 5	5	✔	75.0	70.1	93.5
mj cal 6	6	✔	100.0	100.4	100.4
mj cal 7	7	✔	250.0	259.9	103.9

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Last Cal. Update	2/17/2021 7:46 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



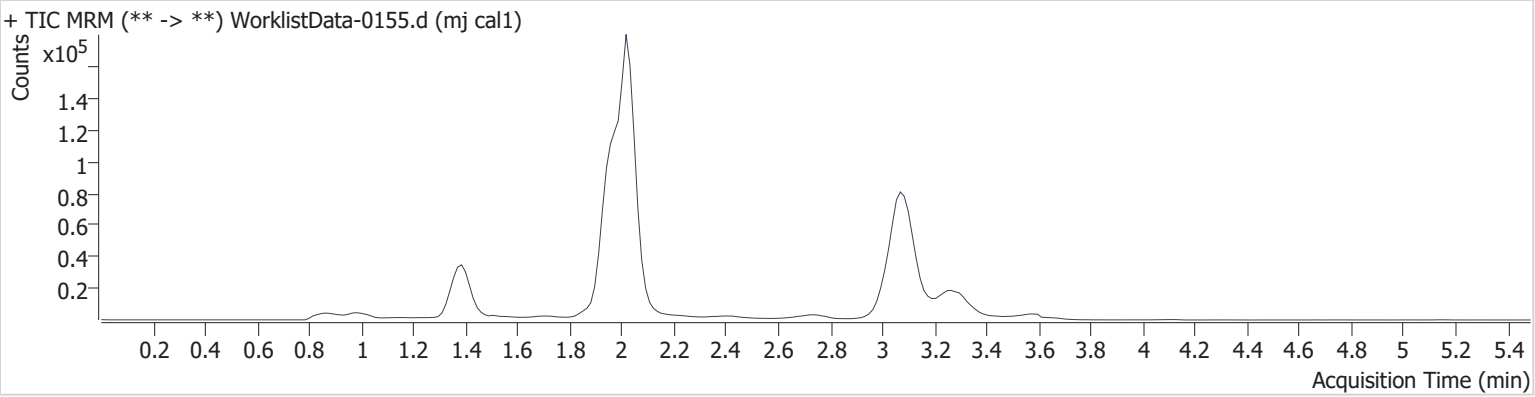
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✔	1.0	1.2	121.8
mj cal2	2	✔	3.0	2.7	91.1
mj cal 3	3	✔	5.0	4.7	93.7
mj cal 4	4	✔	10.0	9.7	96.7
mj cal 5	5	✔	25.0	24.1	96.5
mj cal 6	6	✔	50.0	48.7	97.3
mj cal 7	7	✔	100.0	102.9	102.9

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0155.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	2/16/2021 3:28:28 PM		
Sample Info.			

Sample Chromatogram



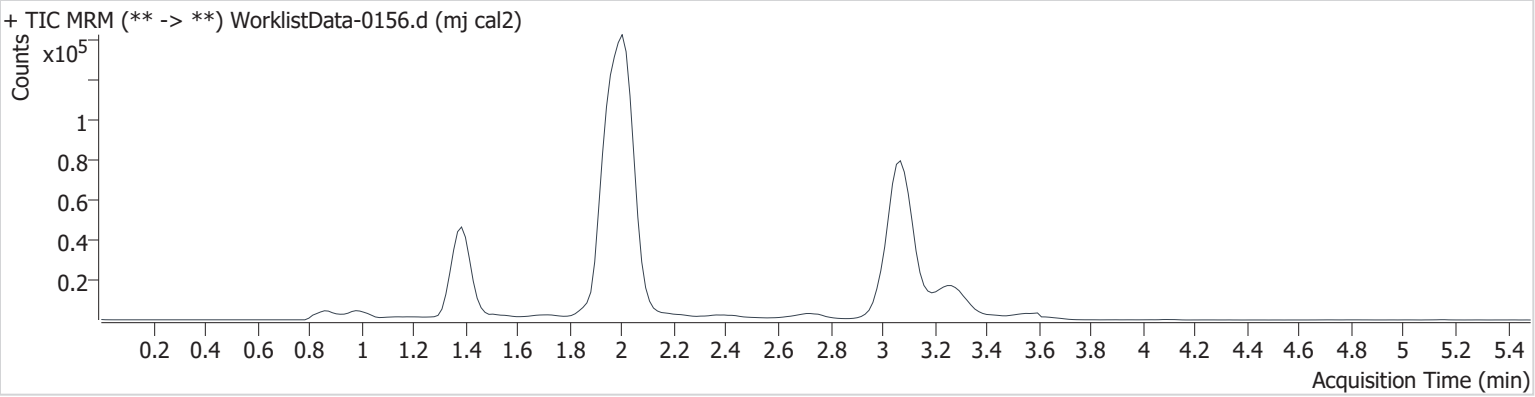
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.379	1348	∞	739.6	∞	77001	1.218 ng/ml	Low
THC-COOH	1.399	12363	∞	23.9	∞	65364	6.233 ng/ml	
THC	3.092	9034	345.7	36.7	∞	372407	1.159 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0156.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	2/16/2021 3:35:13 PM		
Sample Info.			

Sample Chromatogram



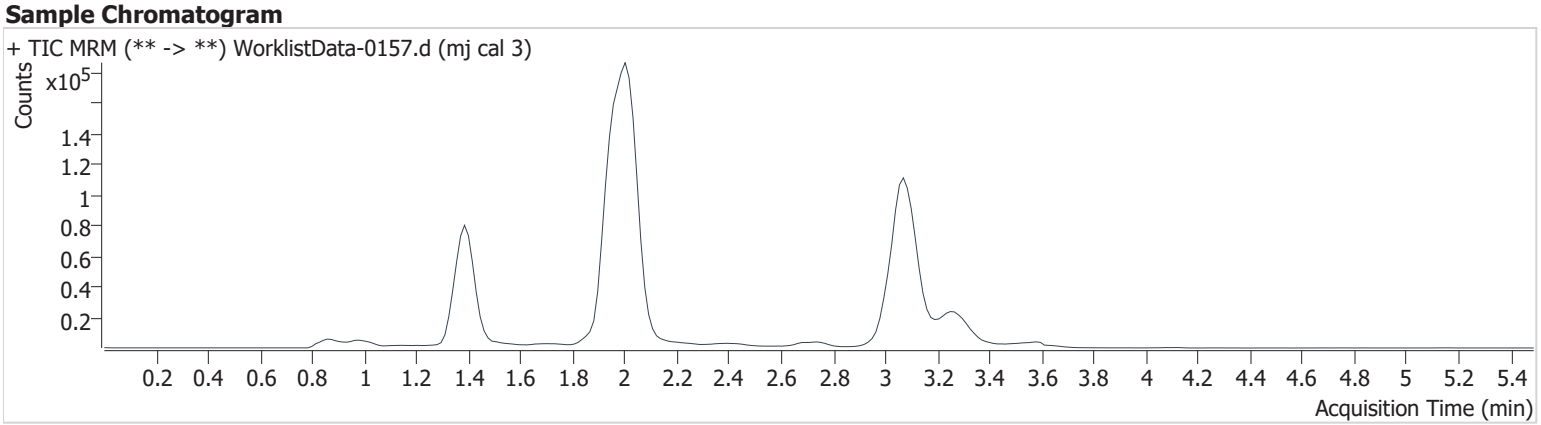
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.379	3733	∞	733.3	∞	81498	2.733 ng/ml	Low
THC-COOH	1.399	24621	∞	26.2	99.9	74316	9.445 ng/ml	
THC	3.092	29046	∞	32.8	∞	362200	3.063 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0157.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	2/16/2021 3:41:56 PM		
Sample Info.			

Sample Chromatogram



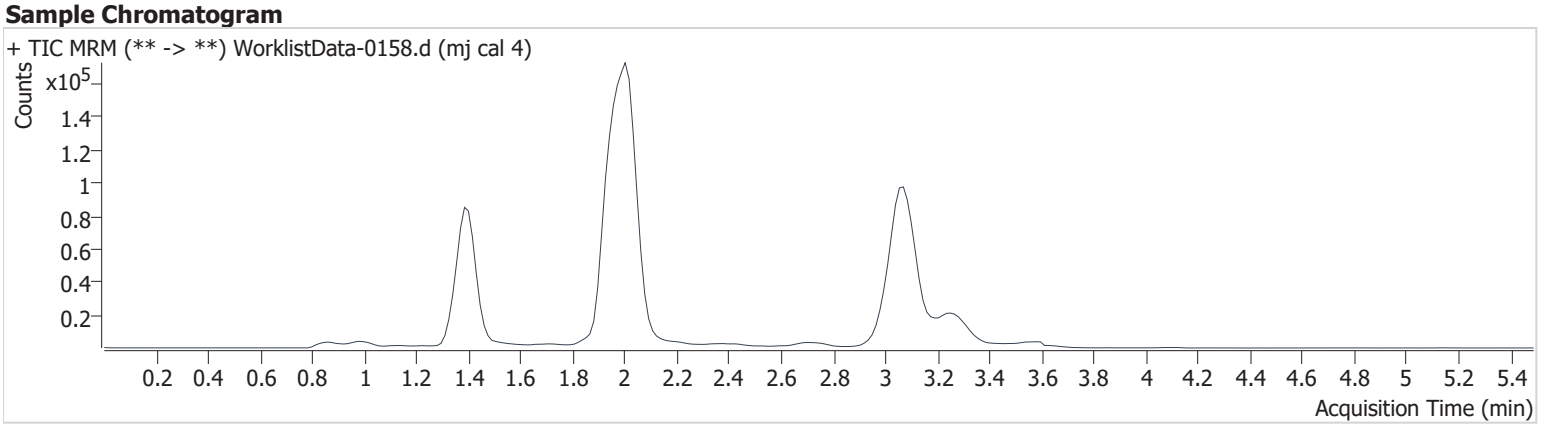
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	9194	∞	782.3	∞	111805	4.683 ng/ml
THC-COOH	1.399	71543	∞	24.5	∞	98455	18.375 ng/ml
THC	3.092	62104	∞	35.3	∞	484328	4.699 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0158.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	2/16/2021 3:48:40 PM		
Sample Info.			

Sample Chromatogram



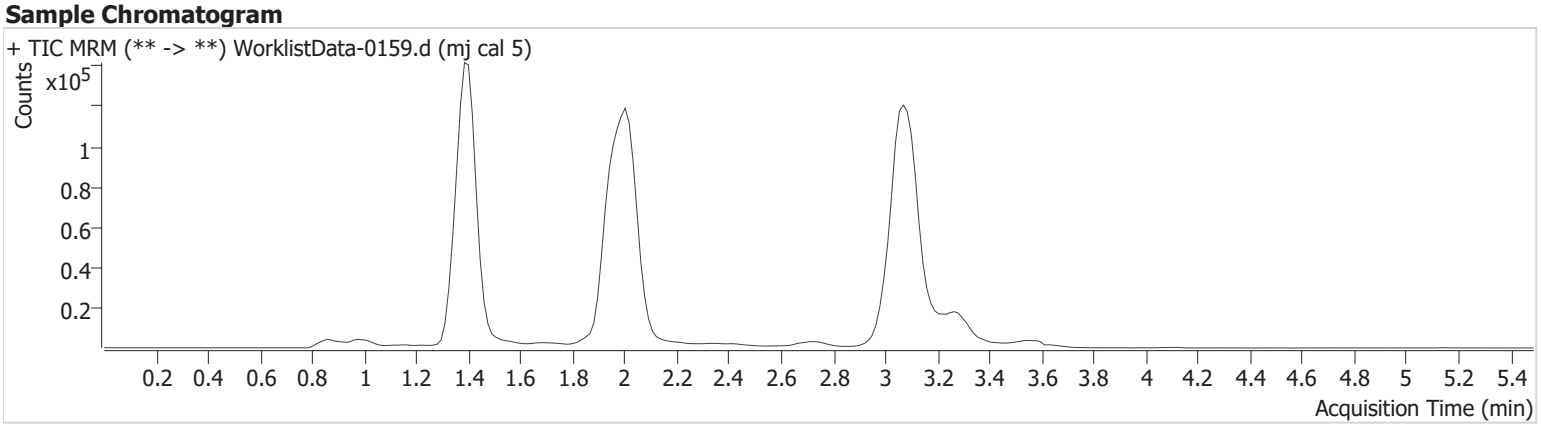
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	10813	∞	616.1	∞	61647	9.672 ng/ml
THC-COOH	1.399	107628	∞	23.6	∞	55685	45.621 ng/ml
THC	3.092	97852	∞	33.1	∞	378861	9.126 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0159.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	2/16/2021 3:55:24 PM		
Sample Info.			

Sample Chromatogram



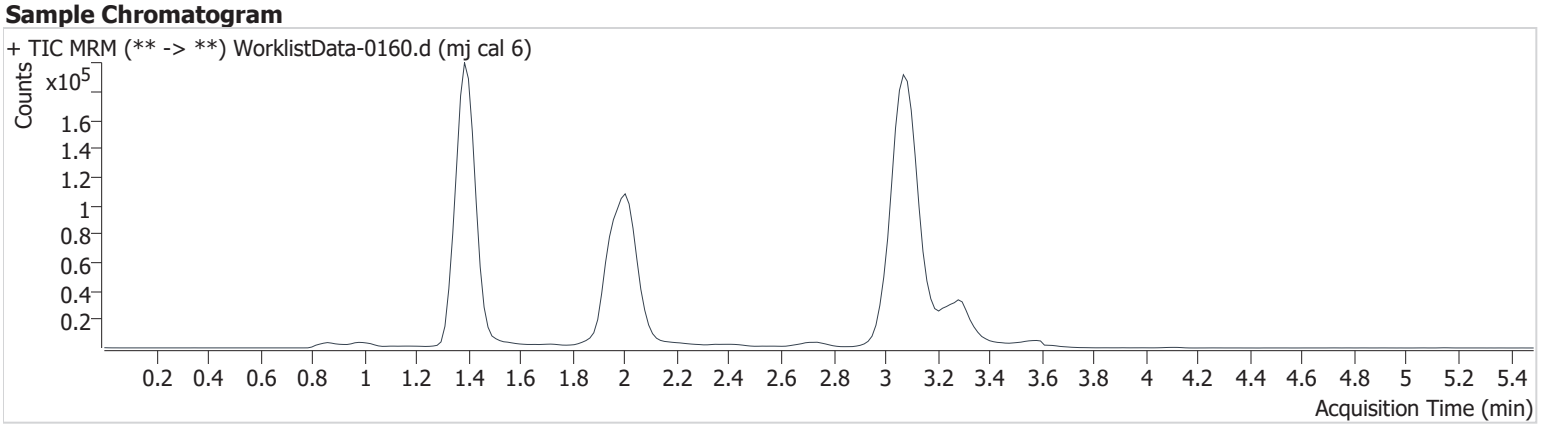
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	30188	∞	612.0	∞	67816	24.114 ng/ml
THC-COOH	1.399	182718	∞	23.2	9680.4	60578	70.095 ng/ml
THC	3.092	265243	∞	34.8	∞	384777	23.802 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0160.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	2/16/2021 4:02:08 PM		
Sample Info.			

Sample Chromatogram



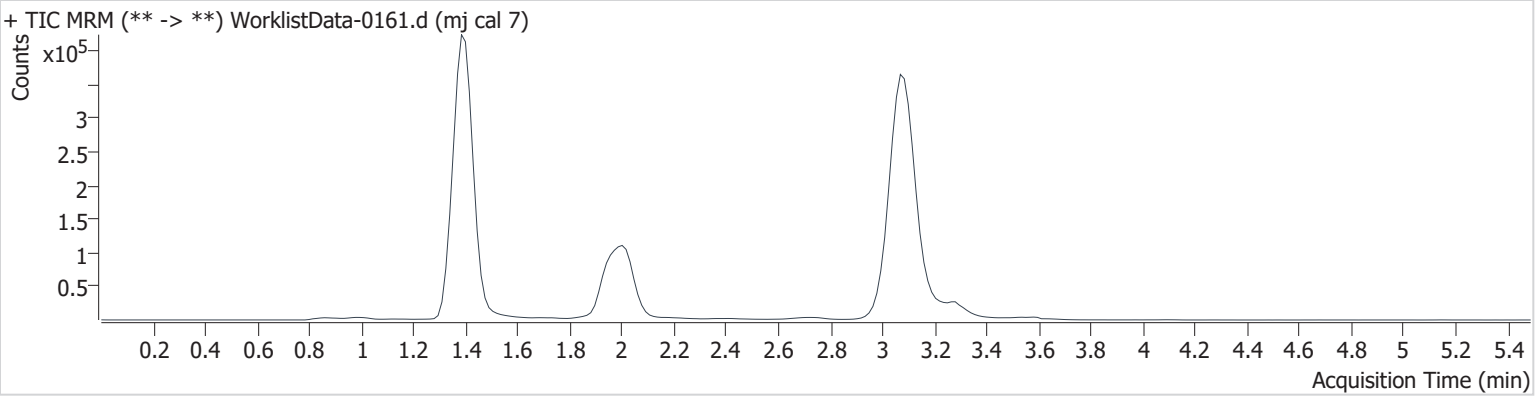
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	56558	∞	623.4	∞	62578	48.671 ng/ml
THC-COOH	1.399	229845	∞	22.1	∞	52757	100.375 ng/ml
THC	3.092	603614	∞	31.1	∞	418724	49.413 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\021621\QuantResults\cann quant.batch.bin
Calibration Last Update 2/17/2021 7:46:55 AM

Instrument	69679	**Data File**	WorklistData-0161.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	2/16/2021 4:08:52 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.379	117829	∞	601.1	∞	61470	102.909 ng/ml
THC-COOH	1.399	565983	∞	22.5	14131.0	49575	259.856 ng/ml
THC	3.092	1443698	∞	31.7	∞	479980	102.738 ng/ml