Worklist: 4435

LAB CASE	ITEM	ITEM TYPE	DESCRIPTION	
M2020-2912	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2194	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2274	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2304	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2308	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2324	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2368	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-2369	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
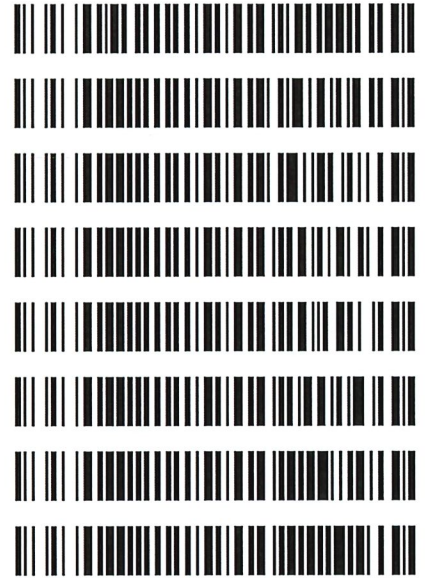
AM# 27: Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: <u>08/14//20</u>   Analyst: <u>Sarah Pickle</u>

Plate lot#: IDP-108-2-200303   Plate Expiration: 09/03/20

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: Hemostat 445283-4   **Column**: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 069901

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.   Pipette **1000 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: 3382167**

☒ 3.   Place on shaking incubator at ambient temp., 900rpm for 15 minutes.

☒ 4.   Pipette **500 µL 0.1% formic acid in LCMS water** in wells of analytical plate.

☒ 5.   Place on shaking incubator at ambient temp., 900rpm for 15 minutes.

☒ 6.   Transfer **800 µL of blood+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 85-95 PSI- Selector to the right)

☒ 8.   Wait 5 minutes.

☒ 9.   Add **2.25 mL MTBE. (*Add in 3 increments of 750 µL*)**

☒ 10.   Wait 5 minutes.

☒ 11.   Apply positive pressure for approx. 15 seconds. *(12-15 PSI- Selector to the left)*.

☒ 12.   Add **2.25 mL Hexane. (*Add in 3 increments of 750 µL*)**

☒ 13.   Wait 5 minutes.

☒ 14.   Apply positive pressure for approx. 15 seconds. *(12-15 PSI- Selector to the left)*.

☒ 15.   Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.

☒ 16.   Reconstitute in **100 µL 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 and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/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? Y / N

☒ 6   Enter QCs into control charting.

☒ 7   Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *Hands of the analyst Sophie Jackson.*

THC-OH was not evaluated.

Samples were inadvertently left on lab bench overnight unrefrigerated (8/12/20-8/13/20).
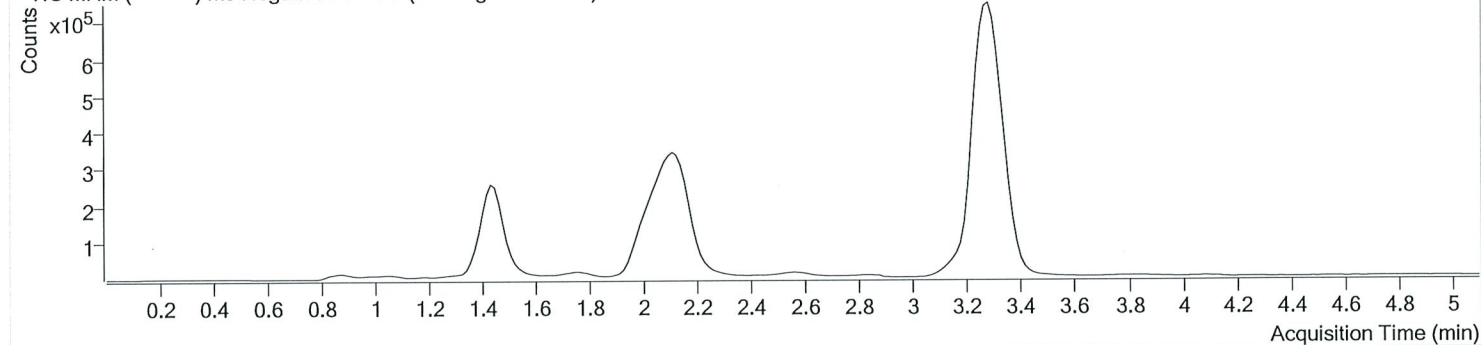
AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update 8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Negative Blood.d
Type	Sample	**Sample**	MJ Negative Blood
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-H5	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 12:14:06 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Negative Blood.d (MJ Negative Blood)

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update 8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ QC Control.d
Type	Sample	**Sample**	MJ QC Control
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-A6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:58:54 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ QC Control.d (MJ QC Control)
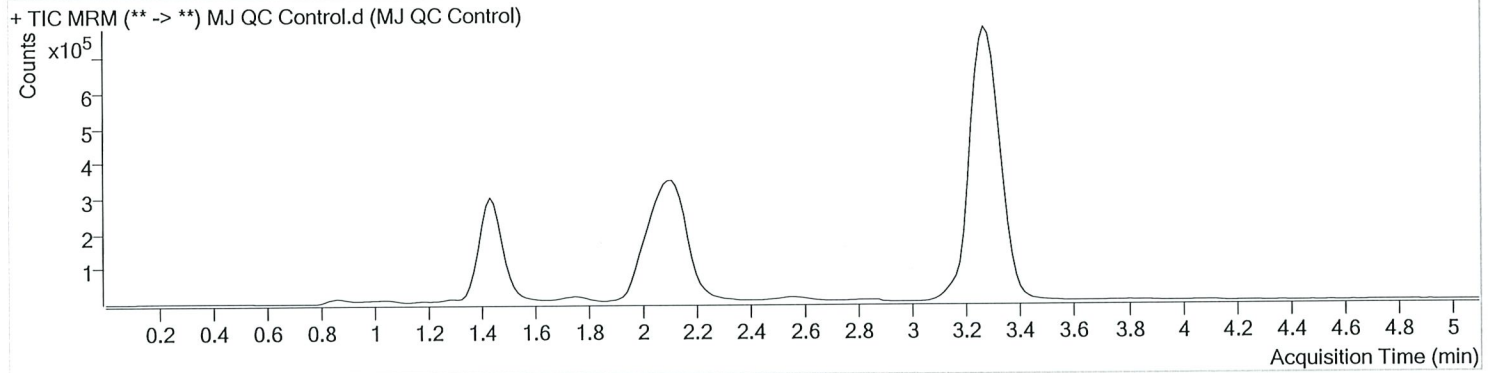
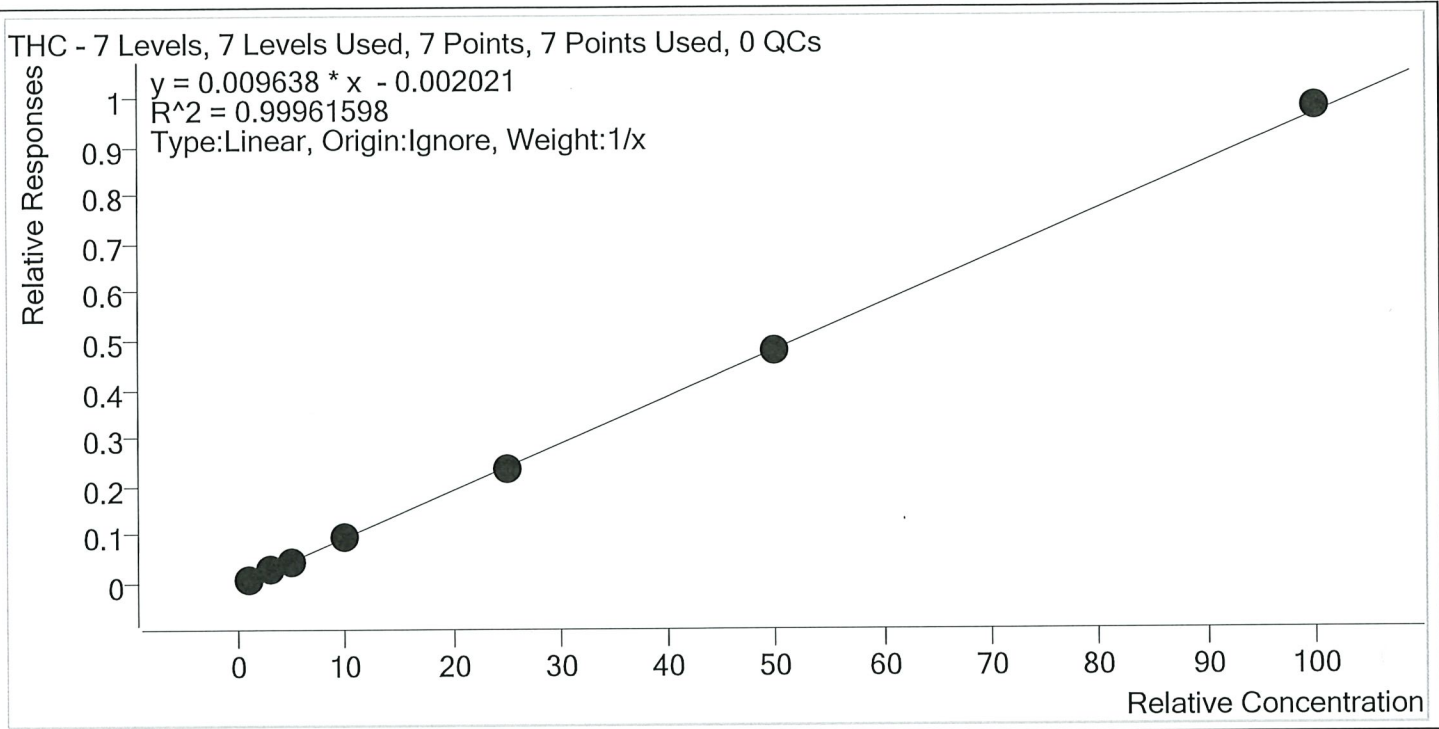| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
* THC-OH	1.453	157468	∞	8.9	∞	1072021	3.0876	ng/ml
THC-COOH	1.459	133856	∞	50.7	235.76	371522	15.4933	ng/ml
THC	3.285	232977	1200.17	26.8	∞	5924427	4.2898	ng/ml

*Not evaluated

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Last Cal. Update	8/20/2020 8:23 AM
Analyst Name	ISP\datastor
Analyte	THC

Internal Standard   THC-D3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

$y = 0.009638 * x - 0.002021$
$R^2 = 0.99961598$
Type:Linear, Origin:Ignore, Weight:1/x

Relative Responses vs. Relative Concentration

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.1	109.7
MJ Cal 2	2	✓	3.0	2.9	96.7
MJ Cal 3	3	✓	5.0	4.9	98.0
MJ Cal 4	4	✓	10.0	9.8	97.6
MJ Cal 5	5	✓	25.0	24.4	97.6
MJ Cal 6	6	✓	50.0	49.5	98.9
MJ Cal 7	7	✓	100.0	101.5	101.5

Generated at 10:40 AM on 8/20/2020

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Last Cal. Update	8/20/2020 8:23 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, 0 QCs

$y = 0.022231 * x + 0.015863$
$R^2 = 0.99981707$
Type:Linear, Origin:Ignore, Weight:1/x
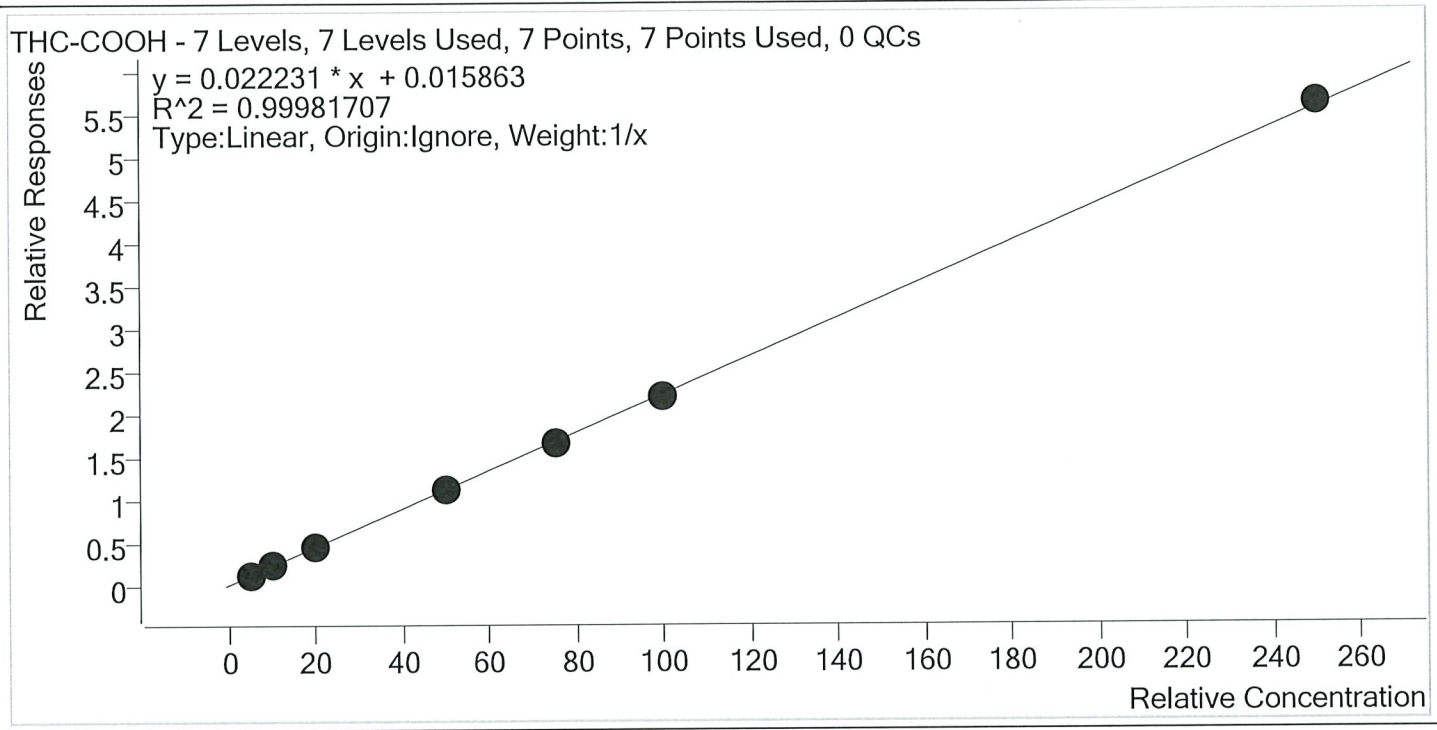
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	5.0	5.0	100.6
MJ Cal 2	2	✓	10.0	10.3	102.8
MJ Cal 3	3	✓	20.0	19.7	98.6
MJ Cal 4	4	✓	50.0	49.8	99.7
MJ Cal 5	5	✓	75.0	74.2	98.9
MJ Cal 6	6	✓	100.0	98.5	98.5
MJ Cal 7	7	✓	250.0	252.5	101.0

AM #27 Cannabinoids Quant. Calibration Curve Report

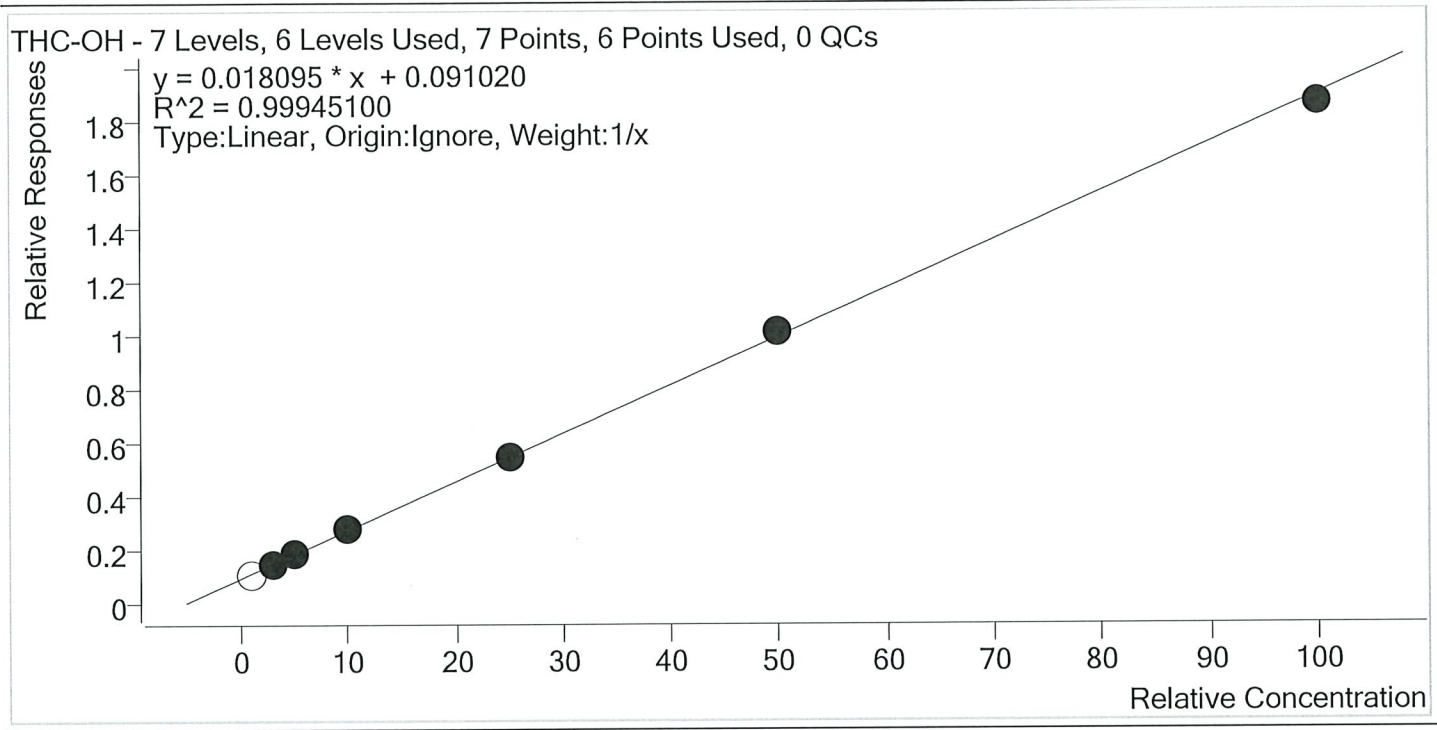|	
Batch results	D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Last Cal. Update	8/20/2020 8:23 AM
Analyst Name	ISP\datastor
Analyte	THC-OH

Internal Standard THC-OH-D3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs

$y = 0.018095 * x + 0.091020$
$R^2 = 0.99945100$
Type:Linear, Origin:Ignore, Weight:1/x

Relative Responses vs Relative Concentration

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✗	1.0	0.8	81.5
MJ Cal 2	2	✓	3.0	2.9	95.1
MJ Cal 3	3	✓	5.0	5.1	101.1
MJ Cal 4	4	✓	10.0	10.1	101.2
MJ Cal 5	5	✓	25.0	25.5	101.9
MJ Cal 6	6	✓	50.0	51.2	102.4
MJ Cal 7	7	✓	100.0	98.3	98.3

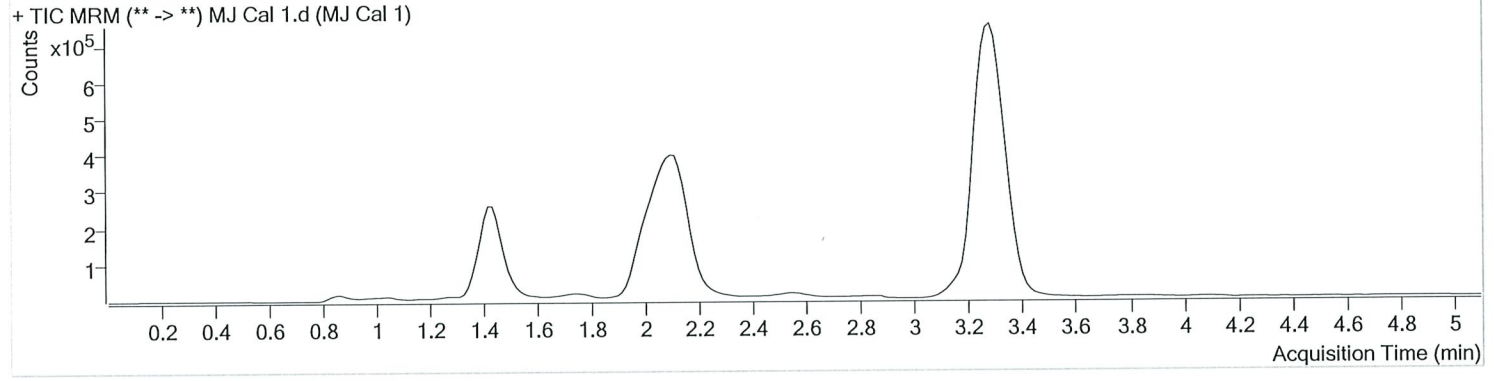Generated at 10:40 AM on 8/20/2020

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update   8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Cal 1.d
Type	Cal	**Sample**	MJ Cal 1
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-H6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:05:40 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Cal 1.d (MJ Cal 1)

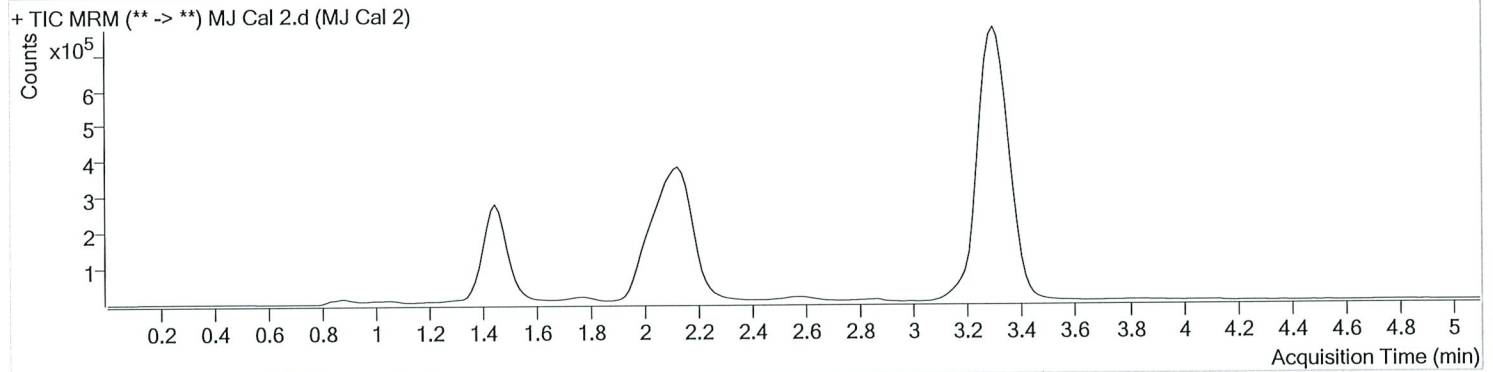| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.483	113631	∞	4.5 **Low**	11.95	1074285	0.8153	ng/ml **Low**
THC-COOH	1.459	48135	∞	47.4	∞	377093	5.0284	ng/ml
THC	3.285	53744	46.51	29.5	106.73	6283457	1.0971	ng/ml **Low**

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update   8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Cal 2.d
Type	Cal	**Sample**	MJ Cal 2
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-G6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:13:25 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Cal 2.d (MJ Cal 2)

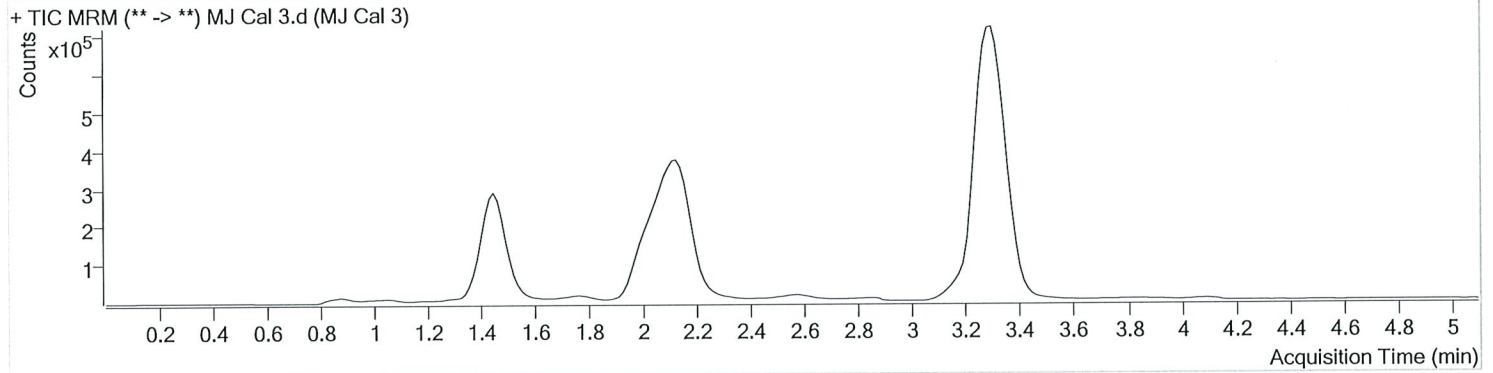| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.498	153002	∞	6.8 **Low**	∞	1072668	2.8526	ng/ml **Low**
THC-COOH	1.474	92763	∞	52.2	∞	379473	10.2825	ng/ml
THC	3.315	158729	423.79	25.0	50.30	6115999	2.9024	ng/ml **Low**

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update   8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Cal 3.d
Type	Cal	**Sample**	MJ Cal 3
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-F6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:20:59 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Cal 3.d (MJ Cal 3)

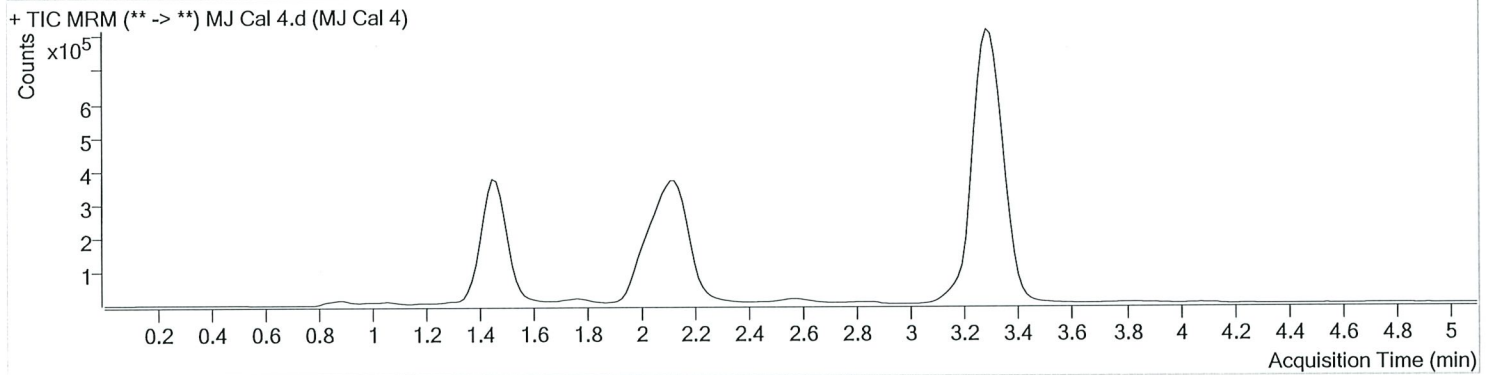| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	189497	∞	8.2	∞	1038319	5.0558	ng/ml
THC-COOH	1.474	165885	∞	56.6	∞	365221	19.7177	ng/ml
THC	3.300	254045	646.15	26.0	248.74	5621889	4.8982	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update   8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Cal 4.d
Type	Cal	**Sample**	MJ Cal 4
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-E6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:28:33 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Cal 4.d (MJ Cal 4)

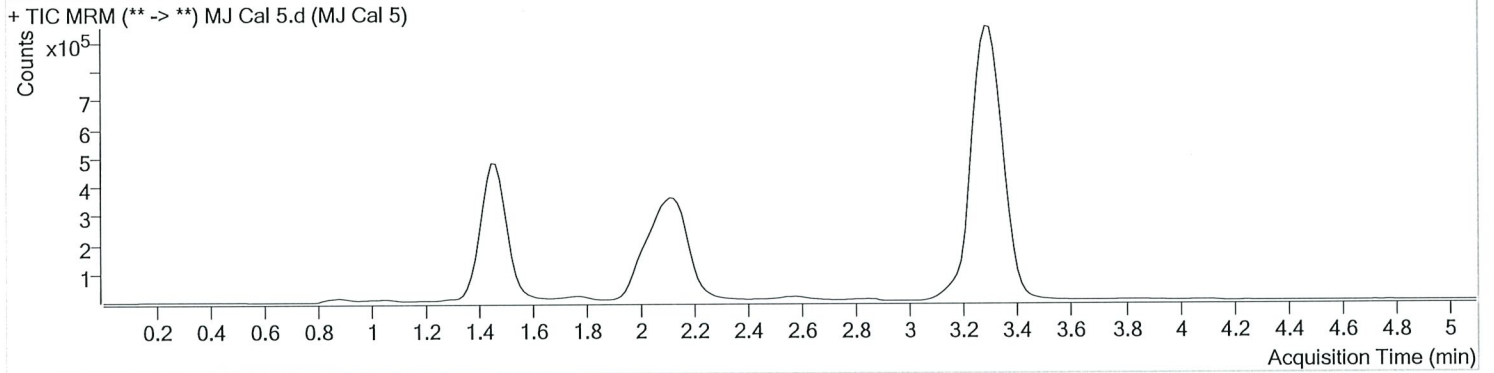| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	291819	∞	9.6	242.51	1064808	10.1155	ng/ml
THC-COOH	1.474	414965	∞	55.7	∞	369325	49.8280	ng/ml
THC	3.300	542990	3120.07	25.6	88.94	5899046	9.7600	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update   8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Cal 5.d
Type	Cal	**Sample**	MJ Cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-D6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:36:07 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Cal 5.d (MJ Cal 5)

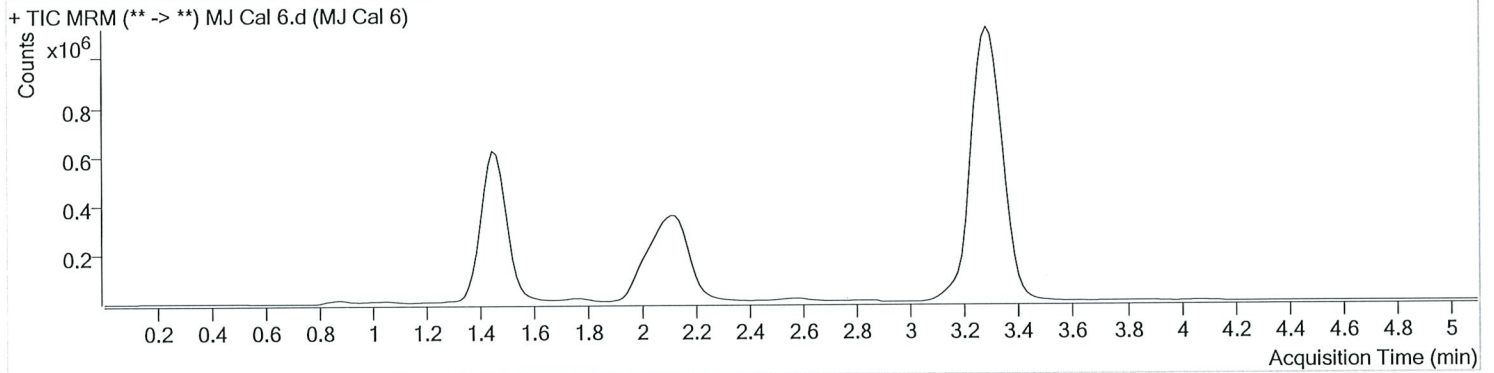| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	599444	∞	10.8	323.43	1085662	25.4839	ng/ml
THC-COOH	1.474	621144	∞	55.7	∞	373099	74.1747	ng/ml
THC	3.300	1410102	2762.79	25.9	∞	6050075	24.3918	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update   8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Cal 6.d
Type	Cal	**Sample**	MJ Cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-C6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:43:42 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Cal 6.d (MJ Cal 6)

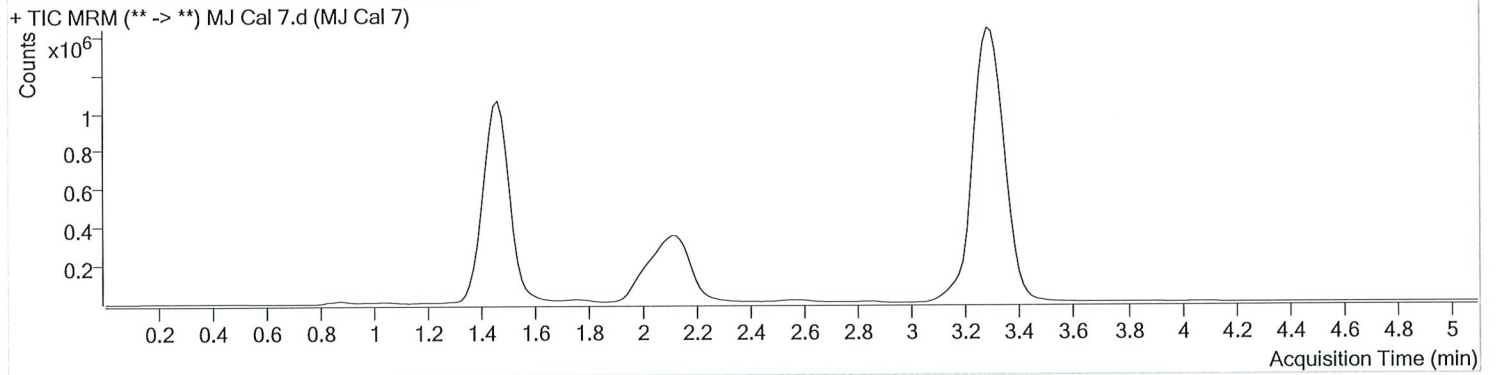| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.438	1087639	∞	12.0	∞	1068643	51.2166	ng/ml
THC-COOH	1.474	811683	∞	59.9	∞	368164	98.4589	ng/ml
THC	3.285	2746465	22684.21	25.7	67.28	5786333	49.4563	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\081420 AM 25 reinjects AM 27 SJ SP\QuantResults\AM 27 SJ SP.batch.bin
Calibration Last Update 8/20/2020 8:23:52 AM

Instrument	Falco	**Data File**	MJ Cal 7.d
Type	Cal	**Sample**	MJ Cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Sarah Pickle
Sample Position	P3-B6	**Comment**	
Injection Volume	10		
Acq. Date-Time	8/14/2020 11:51:17 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	1.438	1912258	∞	12.1	∞	1022979	98.2757	ng/ml
THC-COOH	1.474	1940284	∞	60.3	∞	344673	252.5098	ng/ml
THC	3.300	5235347	19891.33	25.2	2430.91	5362999	101.4942	ng/ml