

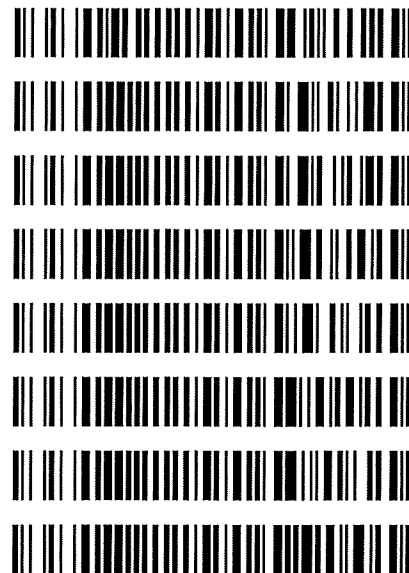
REVIEWED

By Anne Nord at 9:42 am, Sep 20, 2019

9/17/2019

Worklist: 3695

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
M2019-3634	6	164073	AM 27 Blood THC Quant by LC-QQQ
P2019-2650	1	164081	AM 27 Blood THC Quant by LC-QQQ
P2019-2652	1	164075	AM 27 Blood THC Quant by LC-QQQ
P2019-2655	1	164076	AM 27 Blood THC Quant by LC-QQQ
P2019-2685	1	164077	AM 27 Blood THC Quant by LC-QQQ
P2019-2710	1	164078	AM 27 Blood THC Quant by LC-QQQ
P2019-2736	1	164079	AM 27 Blood THC Quant by LC-QQQ
P2019-2746	1	164080	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 9/16/19

Analyst: Sarah Pickle

Plate lot#: Lot # 190716 Item # IDP-108

Plate Expiration: 1/16/20

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 445283-2

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: #3**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 067105*
- 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) Manifold ID: 067104
- 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.
SPE Dry ID: 067103
- 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.
Worklist path: D:\MassHunter\Data\2019\AM 27\091619 MDS SP TS Batch Name: THCQ SP correct
- 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r² 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: 10ng/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: *Curve Ranges: THC: 1-100, THC-COOH 10-250, THC-OH 3-100
Calibrator labels are backwards (Calibrator 7 is actually Calibrator 1 etc.)*

8

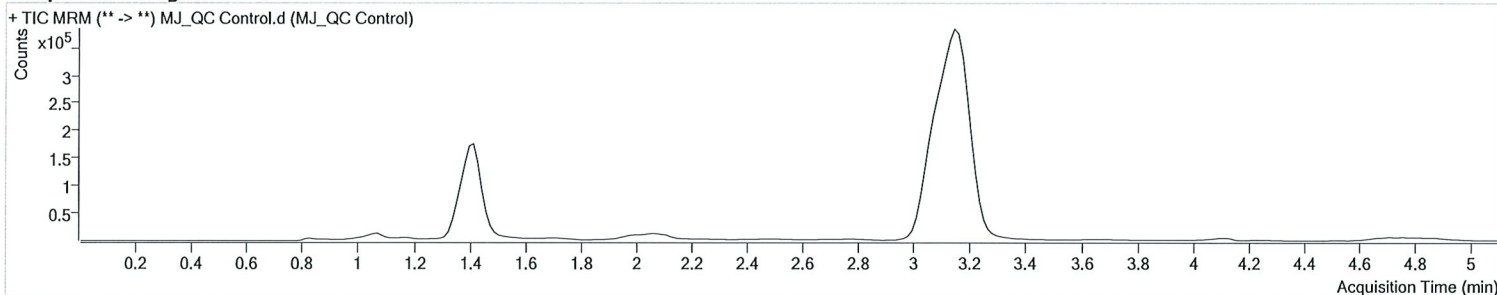
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Calibration Last Update 9/19/2019 10:08:51 AM

Instrument	Falco	Data File	MJ_QC Control.d
Type	Sample	Sample	MJ_QC Control
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-A6		
Injection Volume	10		
Acq. Date-Time	9/17/2019 12:23:16 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.164	111490	315.48	29.3	113.02	3215274	4.2832 ng/ml
THC-COOH	1.444	58044	∞	51.7	521.91	169052	15.5335 ng/ml
THC-OH	1.423	52696	∞	11.1	125.05	639588	4.6239 ng/ml

S

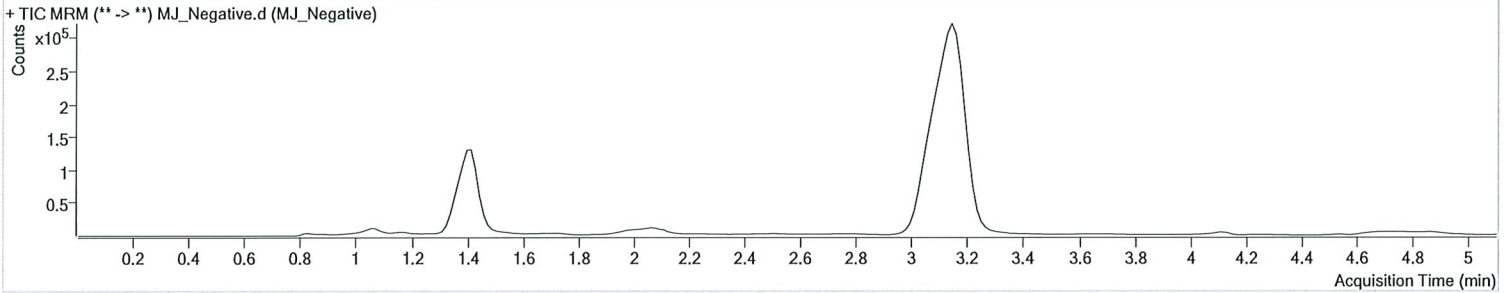
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Calibration Last Update 9/19/2019 10:08:51 AM

Instrument Type	Falco Sample	Data File Sample	MJ_Negative.d MJ_Negative
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-H5		
Injection Volume	10		
Acq. Date-Time	9/17/2019 12:38:29 AM		
Sample Info.			

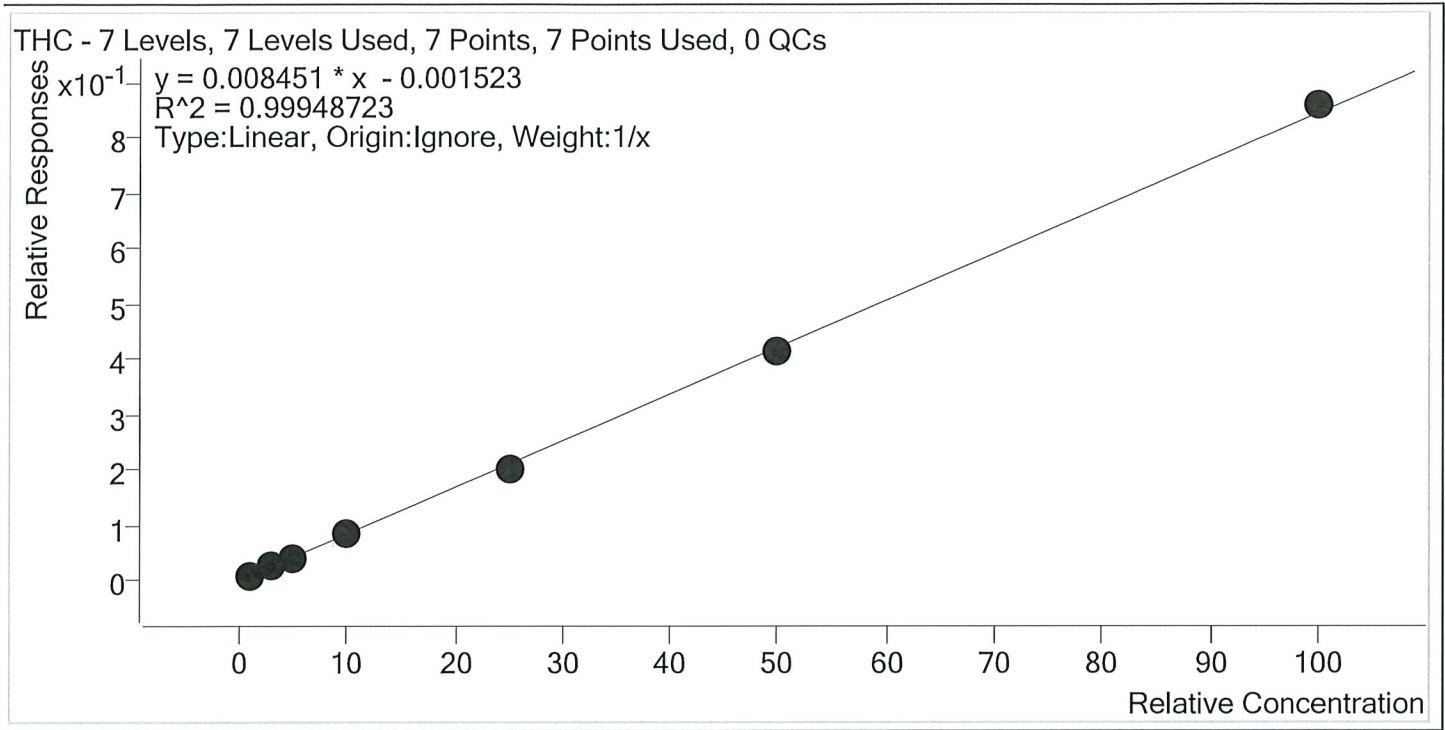
Sample Chromatogram





AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Last Cal. Update 9/19/2019 10:08 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

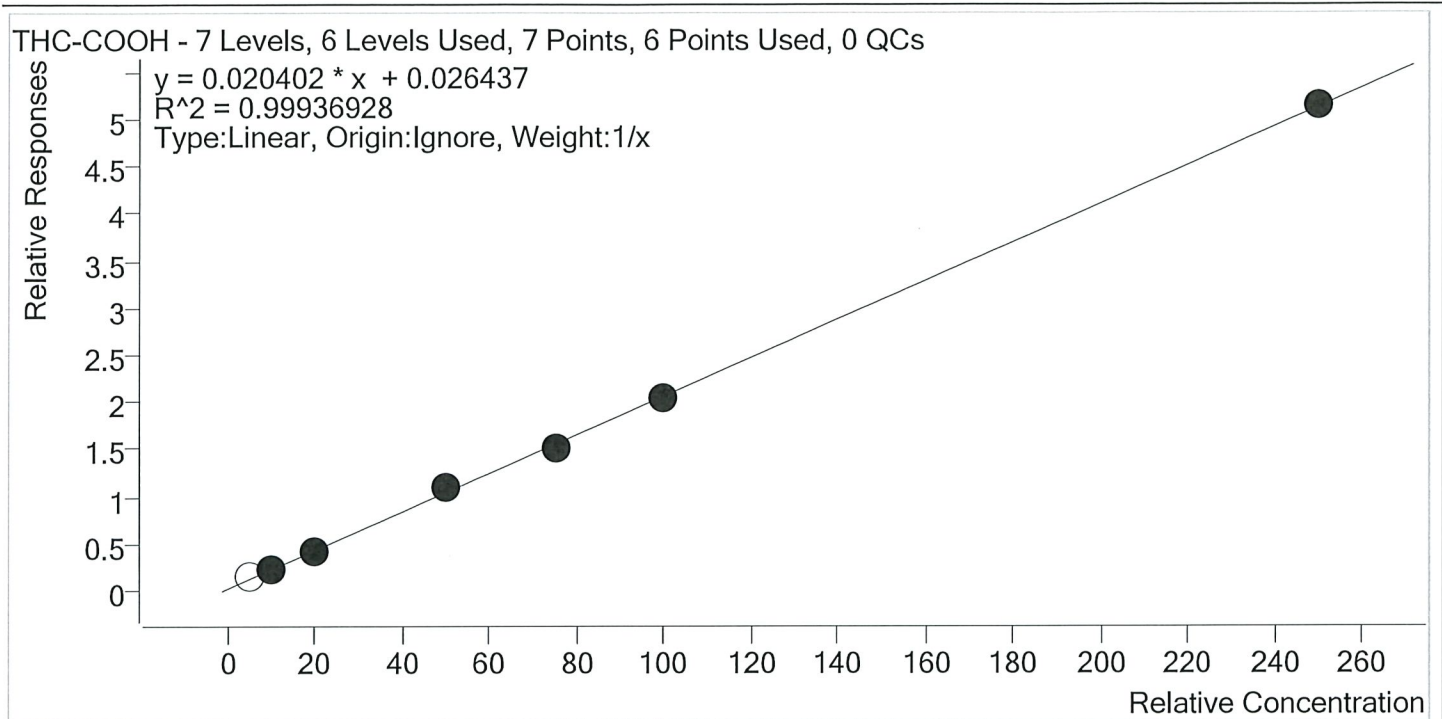


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 7 <u>1</u> §	1	✓	1.0	1.1	109.9
MJ Cal <u>6</u> <u>2</u> §	2	✓	3.0	3.0	98.7
MJ Cal <u>5</u> <u>3</u> §	3	✓	5.0	4.7	94.8
MJ Cal <u>4</u> <u>4</u> §	4	✓	10.0	9.9	99.3
MJ Cal <u>3</u> <u>5</u> §	5	✓	25.0	24.2	96.8
MJ Cal <u>2</u> <u>6</u> §	6	✓	50.0	49.4	98.8
MJ Cal <u>1</u> <u>7</u> §	7	✓	100.0	101.7	101.7



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Last Cal. Update 9/19/2019 10:08 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

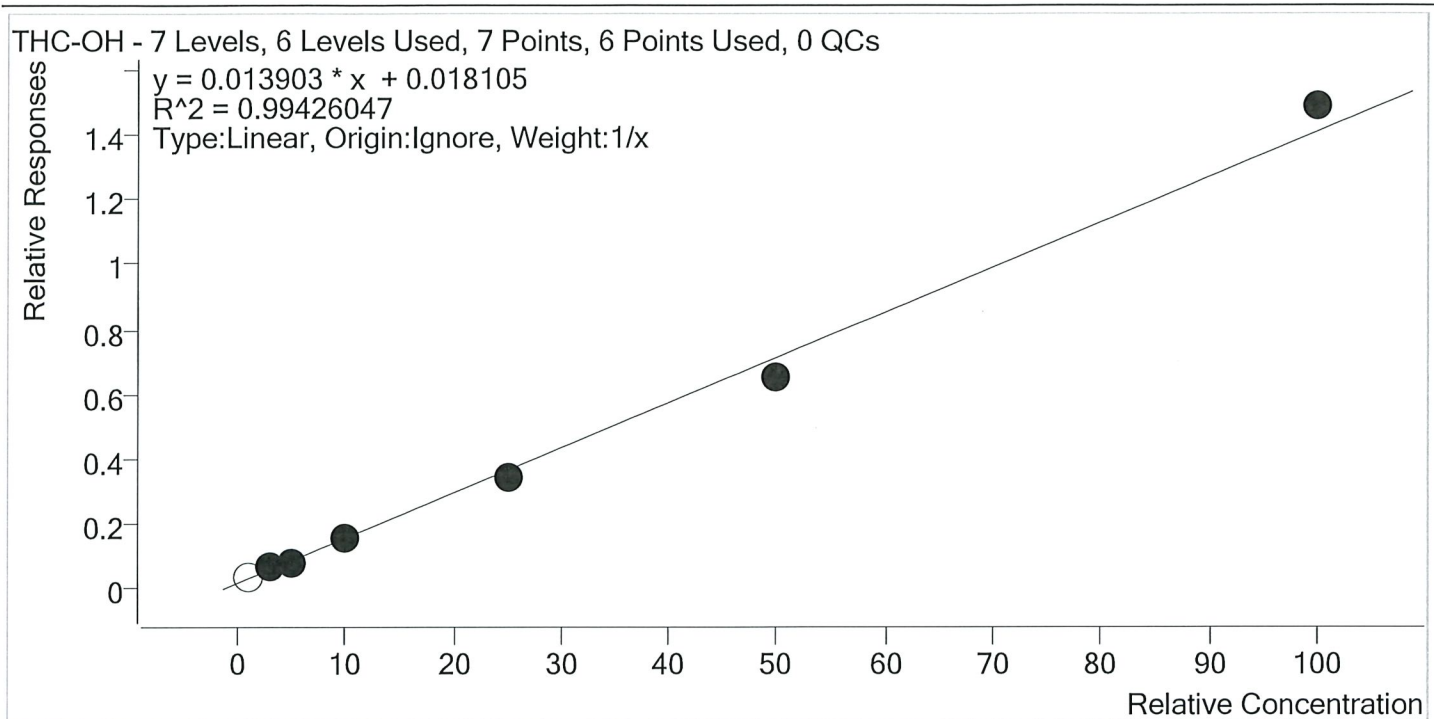


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1 7 1 §	1	×	5.0	6.7	134.3
MJ Cal 2 2 §	2	✓	10.0	10.1	100.9
MJ Cal 3 3 §	3	✓	20.0	19.6	97.8
MJ Cal 4 4 §	4	✓	50.0	52.3	104.6
MJ Cal 5 5 §	5	✓	75.0	72.5	96.6
MJ Cal 6 6 §	6	✓	100.0	99.8	99.8
MJ Cal 7 7 §	7	✓	250.0	250.8	100.3



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Last Cal. Update 9/19/2019 10:08 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 71 <u>1</u> P	1	x	1.0	1.4	144.5
MJ Cal 62 <u>2</u> P	2	✓	3.0	3.4	113.4
MJ Cal 53 <u>3</u> P	3	✓	5.0	4.8	96.8
MJ Cal 44 <u>4</u> P	4	✓	10.0	9.8	98.1
MJ Cal 35 <u>5</u> P	5	✓	25.0	23.7	94.8
MJ Cal 26 <u>6</u> P	6	✓	50.0	45.7	91.3
MJ Cal 17 <u>7</u> P	7	✓	100.0	105.6	105.6

PS

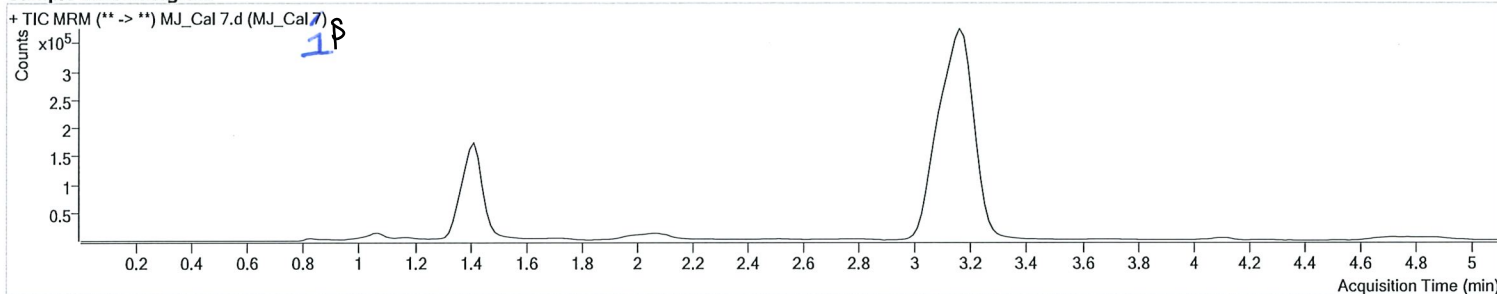
AM #27 Cannabinoids Quant. Results



Batch results Calibration Last Update D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin 9/19/2019 10:08:51 AM

Instrument Type	Falco Cal	Data File Sample	MJ_Cal 7.d MJ_Cal 7 1 PS
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-B6		
Injection Volume	10		
Acq. Date-Time	9/17/2019 12:15:40 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.179	25298	131.01	32.9	28.66	3257362	1.0992 ng/ml
THC-COOH	1.444	29429	∞	40.1 Low	126.20	180013	6.7172 ng/ml
THC-OH	1.438	26092	∞	6.4 Low	9.72 Low	683240	1.4446 ng/ml

MJ_Cal 7 1 PS

P

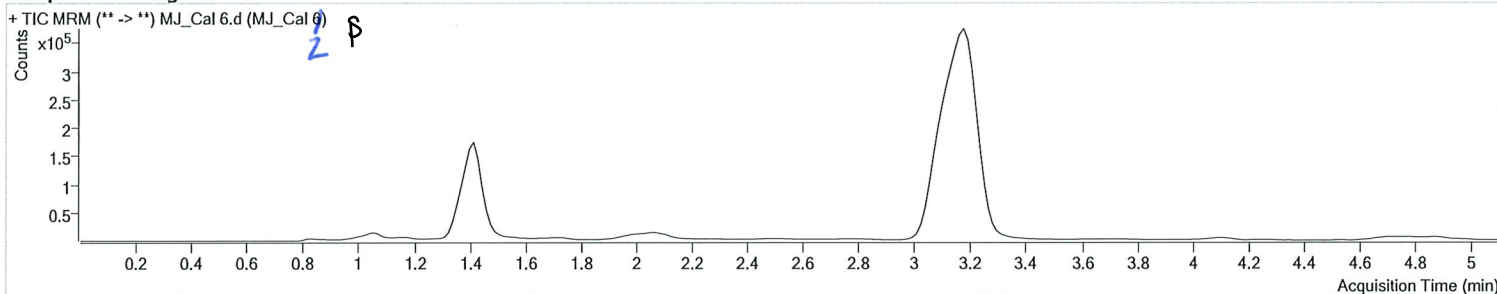
AM #27 Cannabinoids Quant. Results



Batch results Calibration Last Update D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin 9/19/2019 10:08:51 AM

Instrument Type	Falco Cal	Data File Sample	MJ_Cal 6.d MJ_Cal 6 2 P
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-C6		
Injection Volume	10		
Acq. Date-Time	9/17/2019 12:08:04 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.194	76626	137.61	29.9	27.67	3259959	2.9615 ng/ml
THC-COOH	1.444	40217	175.65	54.1	∞	173187	10.0864 ng/ml
THC-OH	1.423	41822	∞	10.0	27.68	639560	3.4013 ng/ml

MJ_Cal 6 2 P

AM #27 Cannabinoids Quant. Results



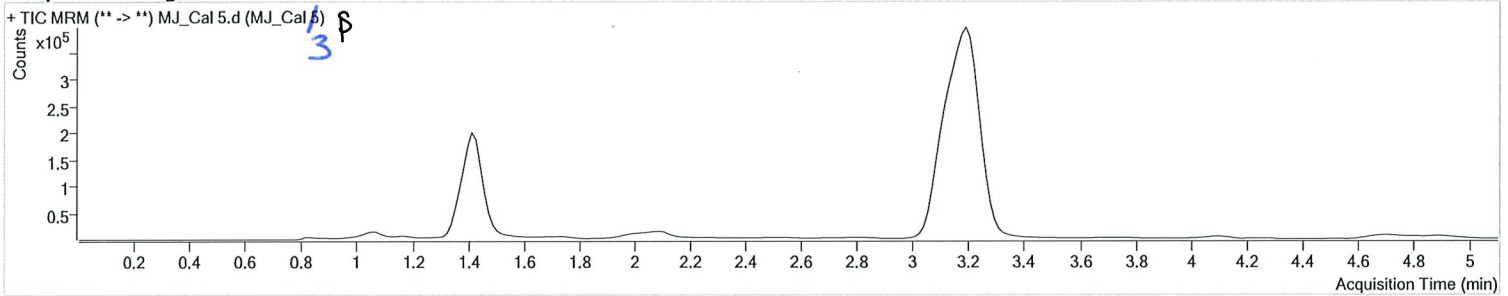
Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Calibration Last Update 9/19/2019 10:08:51 AM

Instrument Type Falco
Acq. Method AM 27 THC quant.m
Sample Position P3-D6
Injection Volume 10
Acq. Date-Time 9/17/2019 12:00:28 AM
Sample Info.

Data File Sample MJ_Cal 5.d
 MJ_Cal 5 3 β

Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.209	129376	495.07	29.5	91.54	3356941	4.7405 ng/ml
THC-COOH	1.444	78896	600.07	57.7	433.88	185443	19.5575 ng/ml
THC-OH	1.423	59483	∞	10.6	83.63	696515	4.8405 ng/ml

P

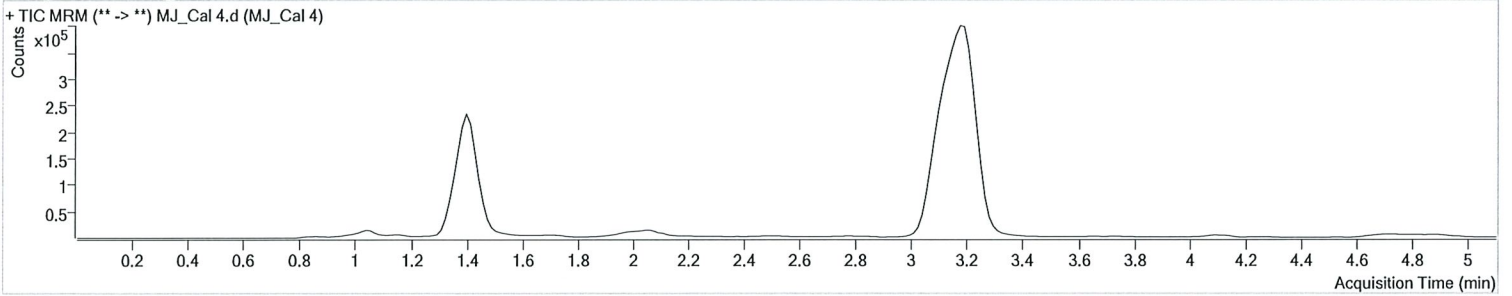
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Calibration Last Update 9/19/2019 10:08:51 AM

Instrument Falco **Data File** MJ_Cal 4.d
Type Cal **Sample** MJ_Cal 4
Acq. Method AM 27 THC quant.m
Sample Position P3-E6 **Comment**
Injection Volume 10
Acq. Date-Time 9/16/2019 11:52:52 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.194	270027	633.60	28.4	187.81	3277745	9.9283 ng/ml
THC-COOH	1.429	189207	∞	59.6	2313.22	173049	52.2961 ng/ml
THC-OH	1.408	103105	∞	12.2	224.48	667537	9.8076 ng/ml

P

AM #27 Cannabinoids Quant. Results

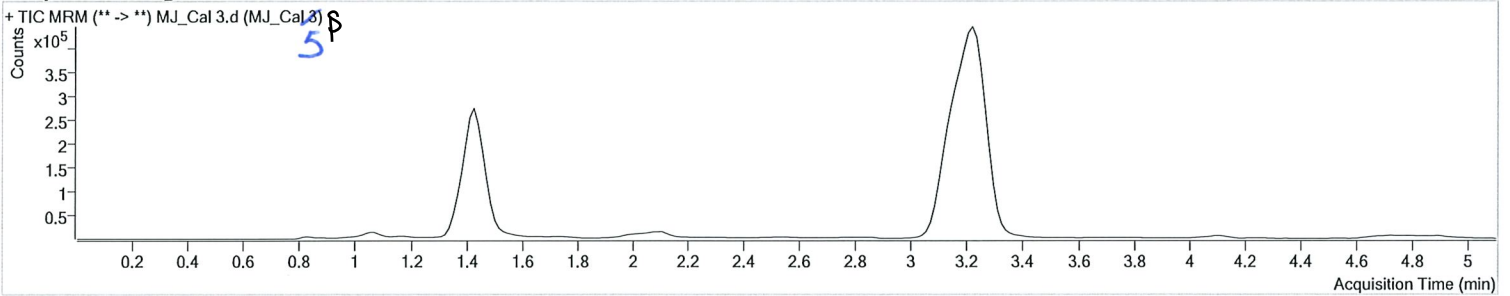


Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Calibration Last Update 9/19/2019 10:08:51 AM

Instrument Type Falco
Acq. Method AM 27 THC quant.m
Sample Position P3-F6
Injection Volume 10
Acq. Date-Time 9/16/2019 11:45:15 PM
Sample Info.

Data File MJ_Cal 3.d
Sample MJ_Cal 3 5 P
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.239	630621	1043.29	28.0	288.90	3106843	24.1981 ng/ml
THC-COOH	1.459	257702	854.74	62.7	2326.53	171219	72.4769 ng/ml
THC-OH	1.438	226331	∞	12.8	799.33	650826	23.7116 ng/ml

MJ_Cal 3 5 P

5

AM #27 Cannabinoids Quant. Results

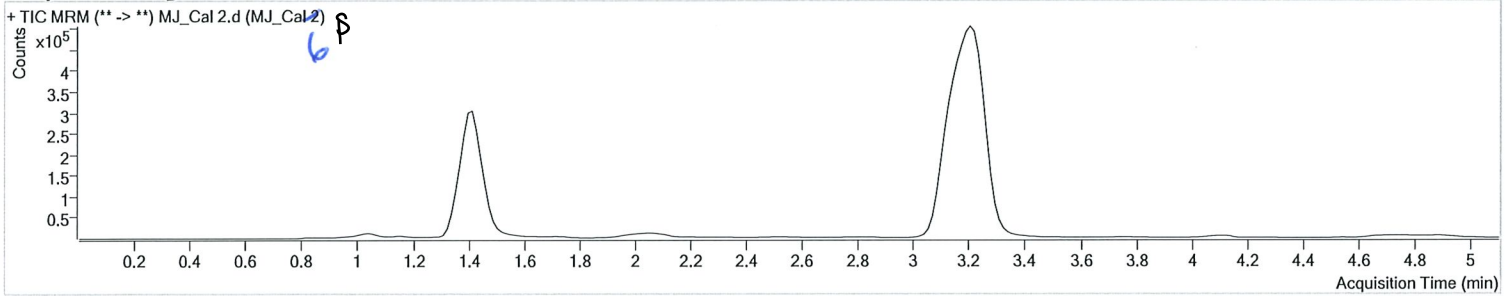


Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Calibration Last Update 9/19/2019 10:08:51 AM

Instrument Type Falco
Acq. Method AM 27 THC quant.m
Sample Position P3-G6
Injection Volume 10
Acq. Date-Time 9/16/2019 11:37:31 PM
Sample Info.

Data File MJ_Cal 2.d
Sample MJ_Cal 2 6 §
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.224	1244070	6108.37	27.4	∞	2990373	49.4075 ng/ml
THC-COOH	1.429	315015	563.23	62.7	1822.30	152712	99.8128 ng/ml
THC-OH	1.408	379475	∞	13.9	∞	581293	45.6536 ng/ml

MJ_Cal 2 6 §

P

AM #27 Cannabinoids Quant. Results



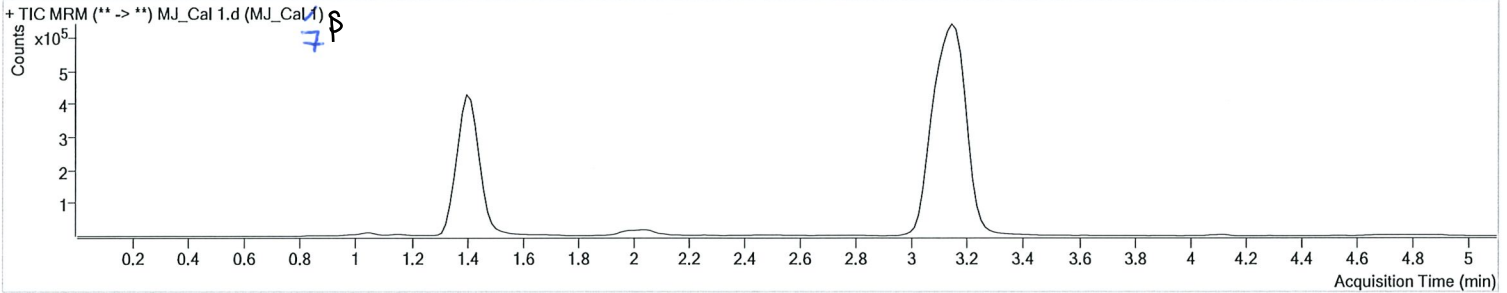
Batch results D:\MassHunter\Data\2019\AM 25\091619 MDS SP TS\QuantResults\THCQ SP correct.batch.bin
Calibration Last Update 9/19/2019 10:08:51 AM

Instrument Type Falco
Acq. Method AM 27 THC quant.m
Sample Position P3-H6
Injection Volume 10
Acq. Date-Time 9/17/2019 9:27:53 AM
Sample Info.

Data File Sample MJ_Cal 1.d §
MJ_Cal 7 §

Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.164	2198595	10363.65	26.6	∞	2563485	101.6649 ng/ml
THC-COOH	1.414	635391	4335.07	61.3	4957.24	123554	250.7704 ng/ml
THC-OH	1.393	603736	∞	14.1	∞	406276	105.5854 ng/ml

MJ_Cal 7 §