8/30/2019

LAB_CASE	ITEM	TASK_ID	DESCRIPTION	
P2019-2308	1	162121	AM 27 Blood THC Quant by LC-QQQ	
P2019-2330	1	162122	AM 27 Blood THC Quant by LC-QQQ	
P2019-2335	1	162123	AM 27 Blood THC Quant by LC-QQQ	
P2019-2339	1	162124	AM 27 Blood THC Quant by LC-QQQ	
P2019-2340	1	162125	AM 27 Blood THC Quant by LC-QQQ	
P2019-2348	1	162126	AM 27 Blood THC Quant by LC-QQQ	
P2019-2397	1	162127	AM 27 Blood THC Quant by LC-QQQ	
P2019-2398	1	162128	AM 27 Blood THC Quant by LC-QQQ	
P2019-2399	2	162129	AM 27 Blood THC Quant by LC-QQQ	
P2019-2409	1	162130	AM 27 Blood THC Quant by LC-QQQ	
P2019-2491	1	162131	AM 27 Blood THC Quant by LC-QQQ	

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

Extraction Date: 09/03/19   Analyst: <u>Tamara Salazar</u>

Plate lot#: 0539904   Plate Expiration: 09/10/19

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 445283-2   **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.

☒ 3. Create worklist:

Analytic:

☒ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.

☒ 2. Pipette **1000µL blood/urine (calibrated pipette) Pipette ID: 3** in wells of analytical (standards) plate.

☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.   *Shaker ID: 067105*

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

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

☒ 6. Transfer **800µL 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 85-100 PSI- Selector to the right)*** Manifold ID: 067104

☒ 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: 067103*

☒ 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.
       Worklist path: <u>D:\MassHunter\Data\2019\AM 27\090319 MDQ THCQ TS reinjects</u>
       Batch Name THCQ wklst 3644

☒ 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: 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
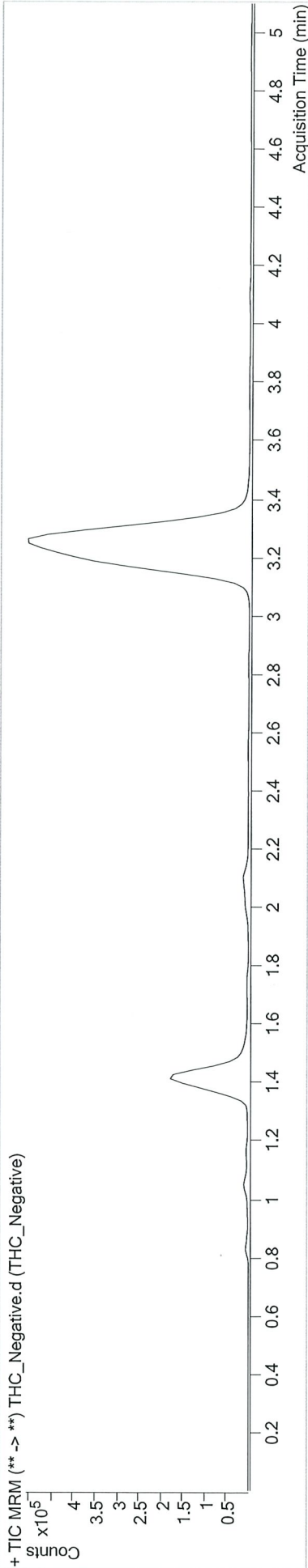
COMMENTS: *Curves limited: THC-COOH 5-100*

AM #27 Cannabinoids Quant. Results

TS

Batch results
Calibration Last Update   9/9/2019 8:47:30 AM

Instrument	Falco	
Type	Sample	
Acq. Method	AM 27 THC quant.m	
Sample Position	P3-H5	
Injection Volume	10	
Acq. Date-Time	9/5/2019 5:14:42 PM	
Sample Info.		

D:\MassHunter\Data\2019\AM 28(090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin

Data File   THC_Negative.d
Sample   THC_Negative
Comment

Sample Chromatogram

+ TIC MRM (** -> **) THC_Negative.d (THC_Negative)

THC_Negative

AM #27 Cannabinoids Quant. Results

Batch results
Calibration Last Update   D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin
9/9/2019 8:47:30 AM

Instrument	Falco
Type	Sample
Acq. Method	AM 27 THC quant.m
Sample Position	P3-A6
Injection Volume	10
Acq. Date-Time	9/5/2019 4:59:31 PM
Sample Info.	

Data File	THC_QC Control.d
Sample	THC_QC Control
Comment	

Sample Chromatogram

+ TIC MRM (** -> **) THC_QC Control.d (THC_QC Control)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC	3.300	180345	229.38	29.2	279.53	4845406	4.6634	ng/ml
THC-COOH	1.459	56138	∞	49.0	157.63	247867	10.4622	ng/ml
THC-OH	1.438	74644	∞	10.1	83.14	816583	5.0802	ng/ml

THC_QC Control

AM #27 Cannabinoids Quant. Calibration Curve Report
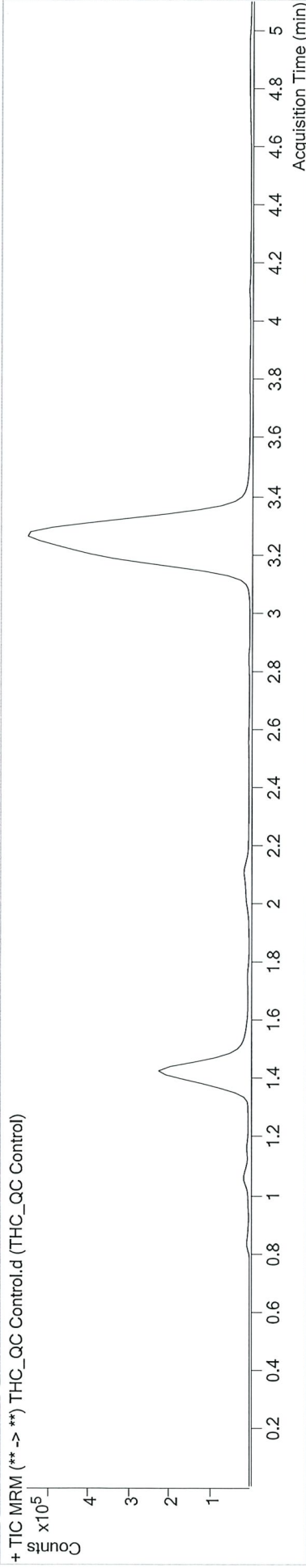
Batch results   D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin

Last Cal. Update   9/9/2019 8:47 AM

Analyst Name   ISP\datastor

Analyte   THC   **Internal Standard**   THC-D3
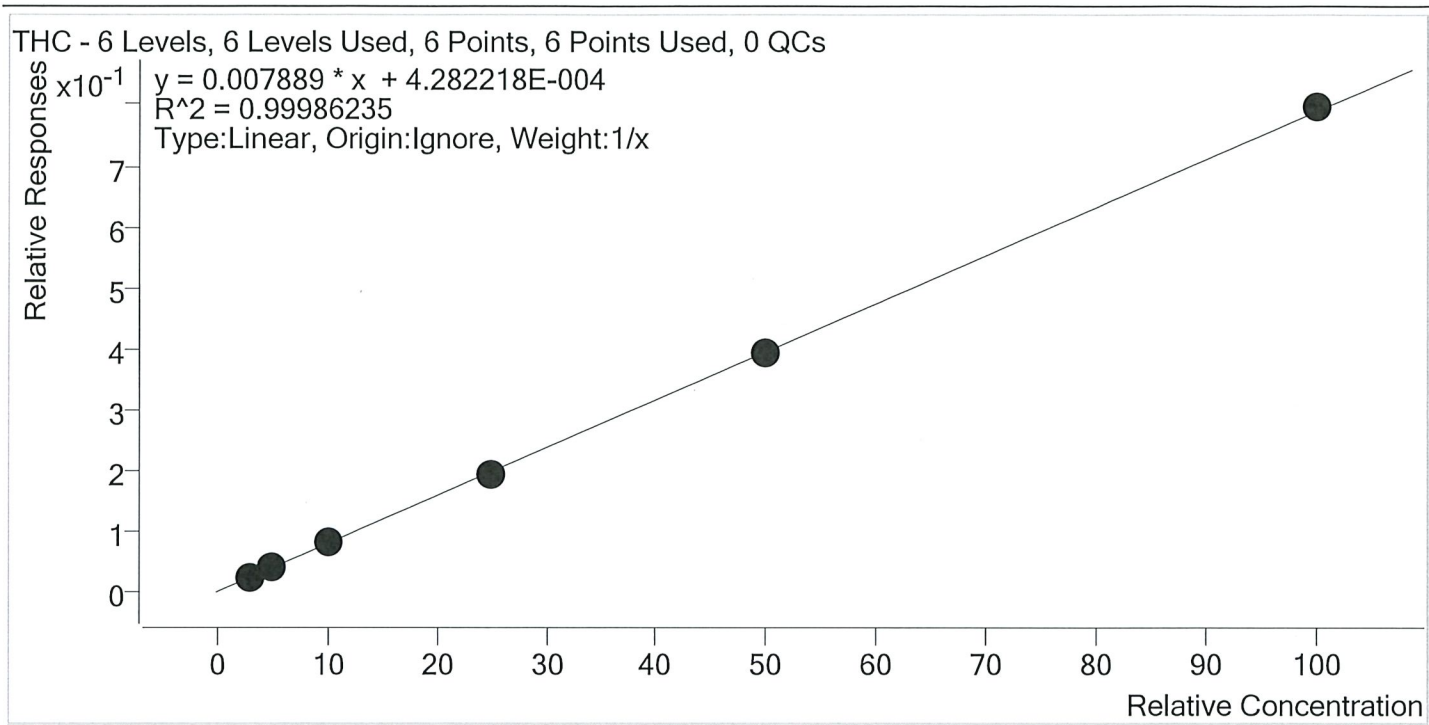
THC - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 0 QCs

$y = 0.007889 * x + 4.282218E\text{-}004$
$R^2 = 0.99986235$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
THC_Cal 1-3ng	1	✓	3.0	3.1	102.5
THC_Cal 2- 5ng	2	✓	5.0	4.9	98.7
THC_Cal 3 -10ng	3	✓	10.0	10.0	100.3
THC_Cal 4-25ng	4	✓	25.0	24.7	98.9
THC_Cal 5-50ng	5	✓	50.0	49.4	98.8
THC_Cal 6-100ng	6	✓	100.0	100.8	100.8

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin
Last Cal. Update	9/9/2019 8:47 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH

Internal Standard   THC-COOH-D9
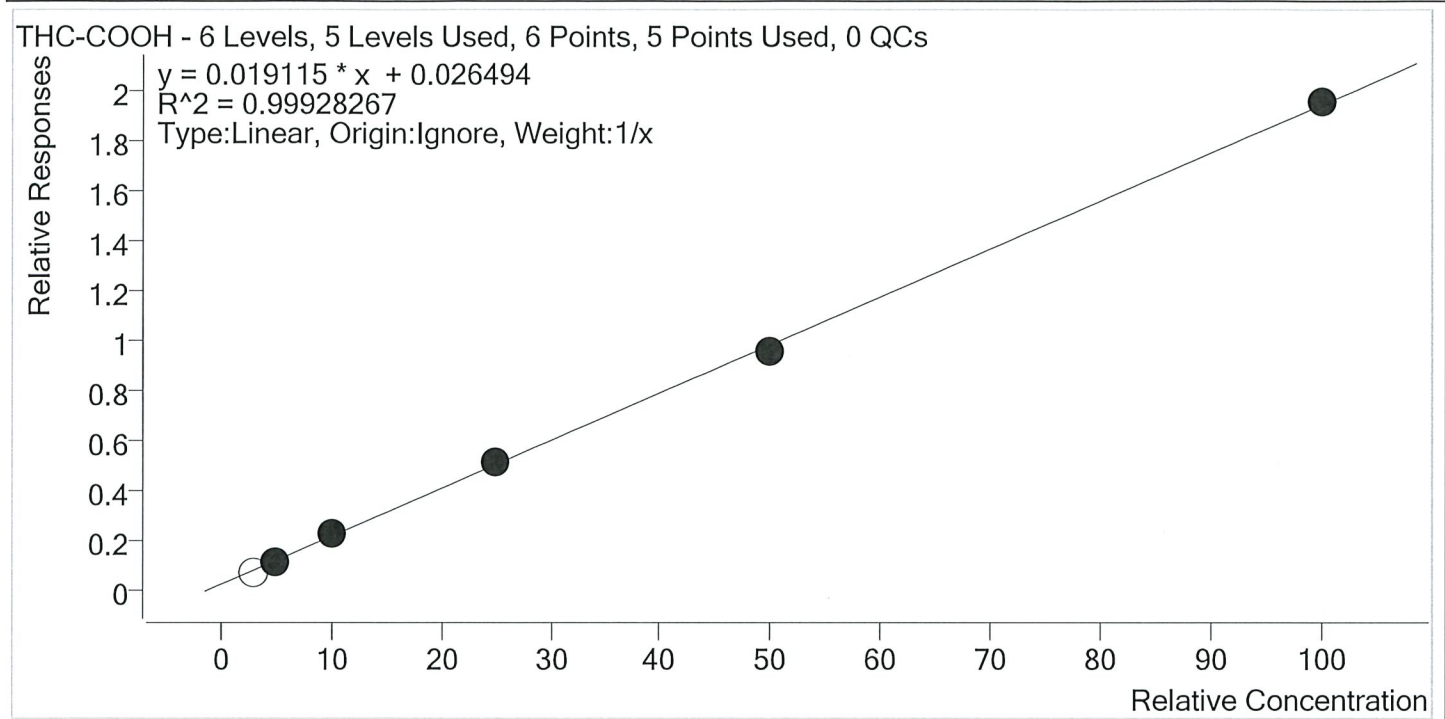
THC-COOH - 6 Levels, 5 Levels Used, 6 Points, 5 Points Used, 0 QCs

$y = 0.019115 * x + 0.026494$
$R^2 = 0.99928267$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
THC_Cal 1-3ng	1	✗	3.0	2.6	87.7
THC_Cal 2- 5ng	2	✓	5.0	4.7	94.5
THC_Cal 3 -10ng	3	✓	10.0	10.6	106.2
THC_Cal 4-25ng	4	✓	25.0	25.3	101.1
THC_Cal 5-50ng	5	✓	50.0	48.8	97.6
THC_Cal 6-100ng	6	✓	100.0	100.6	100.6

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin
Last Cal. Update	9/9/2019 8:47 AM
Analyst Name	ISP\datastor
Analyte	THC-OH

Internal Standard   THC-OH-D3
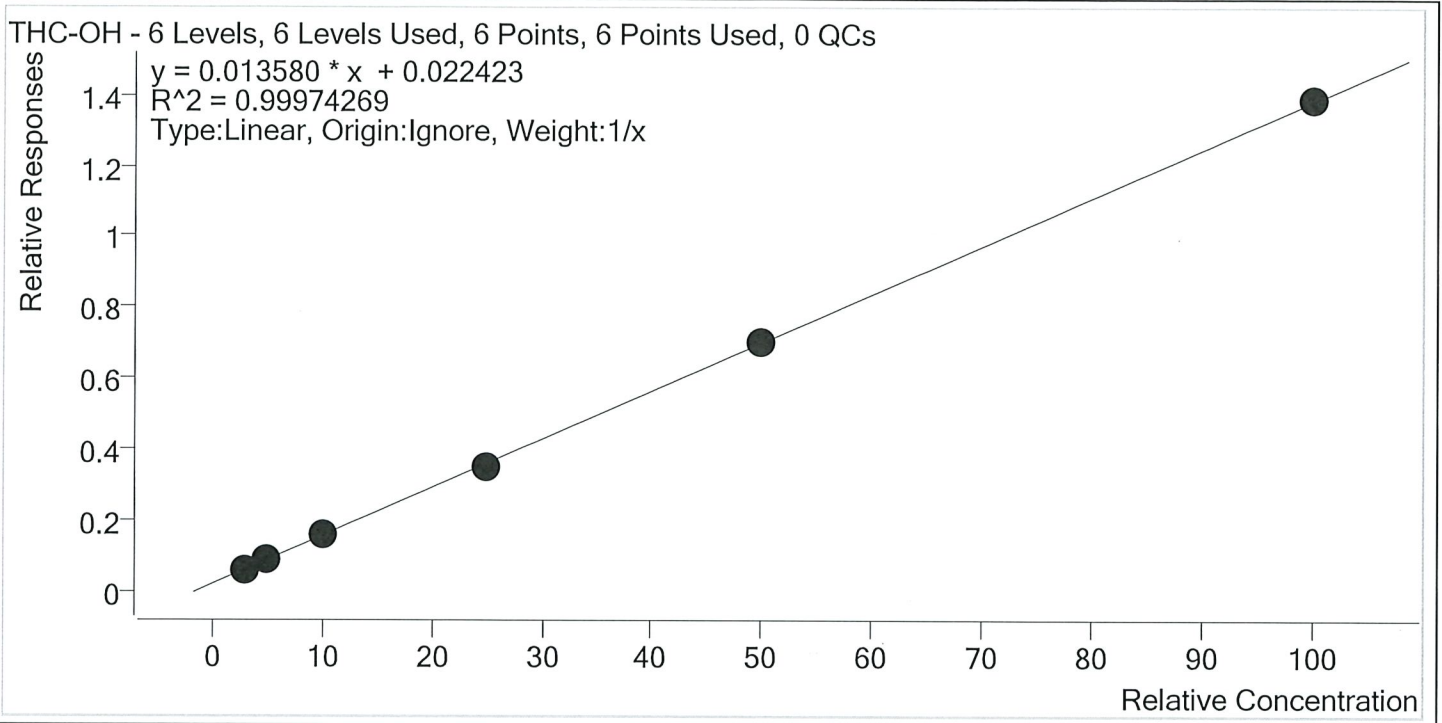
THC-OH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 0 QCs

$y = 0.013580 * x + 0.022423$
$R^2 = 0.99974269$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
THC_Cal 1-3ng	1	✓	3.0	3.0	101.4
THC_Cal 2- 5ng	2	✓	5.0	5.0	100.2
THC_Cal 3 -10ng	3	✓	10.0	10.1	100.7
THC_Cal 4-25ng	4	✓	25.0	24.1	96.5
THC_Cal 5-50ng	5	✓	50.0	50.5	101.0
THC_Cal 6-100ng	6	✓	100.0	100.3	100.3

Generated at 8:48 AM on 9/9/2019

AM #27 Cannabinoids Quant. Results

Batch results
Calibration Last Update   D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin
9/9/2019 8:47:30 AM

Instrument	Falco
Type	Cal
Acq. Method	AM 27 THC quant.m
Sample Position	P3-G6
Injection Volume	10
Acq. Date-Time	9/5/2019 4:14:04 PM
Sample Info.	

Data File   THC_Cal 1-3ng.d
Sample   THC_Cal 1-3ng

Comment

Sample Chromatogram

+ TIC MRM (** -> **) THC_Cal 1-3ng.d (THC_Cal 1-3ng)

Name	RT	Resp.	S/N	Ratio	ISTD Resp.	S/N	Final Conc.	
THC	3.285	109415	∞	29.4	4430784	71.33	3.0758	ng/ml
THC-COOH	1.459	19415	∞	47.9	252817	164.64	2.6315	ng/ml
THC-OH	1.438	54568	∞	10.5	856432	∞	3.0408	ng/ml

Page 1 of 1

Generated at 8:48 AM on 9/9/2019

THC_Cal 1-3ng

TS

AM #27 Cannabinoids Quant. Results

Batch results
Calibration Last Update   9/9/2019 8:47:30 AM

Instrument	Falco
Type	Cal
Acq. Method	AM 27 THC quant.m
Sample Position	P3-F6
Injection Volume	10
Acq. Date-Time	9/5/2019 4:21:38 PM
Sample Info.	

Data File D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin

Data File   THC_Cal 2- 5ng.d
Sample   THC_Cal 2- 5ng

Comment

Sample Chromatogram

+ TIC MRM (** -> **) THC_Cal 2- 5ng.d (THC_Cal 2- 5ng)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC	3.285	184443	2132.61	29.2	391.60	4687871	4.9328	ng/ml
THC-COOH	1.459	31236	∞	53.2	240.45	267420	4.7246	ng/ml
THC-OH	1.438	83035	150.29	10.5	230.25	918008	5.0095	ng/ml

THC_Cal 2- 5ng

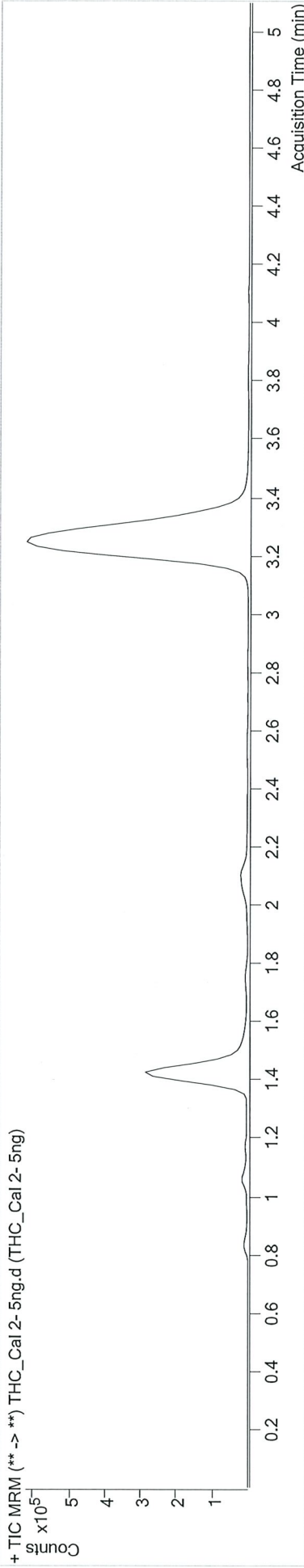Generated at 8:48 AM on 9/9/2019

Page 1 of 1

AM #27 Cannabinoids Quant. Results

Batch results
Calibration Last Update   D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin
9/9/2019 8:47:30 AM

Instrument	Falco	**Data File**	THC_Cal 3 -10ng.d
Type	Cal	**Sample**	THC_Cal 3 -10ng
Acq. Method	AM 27 THC quant.m	**Comment**	
Sample Position	P3-E6		
Injection Volume	10		
Acq. Date-Time	9/5/2019 4:29:13 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) THC_Cal 3 -10ng.d (THC_Cal 3 -10ng)
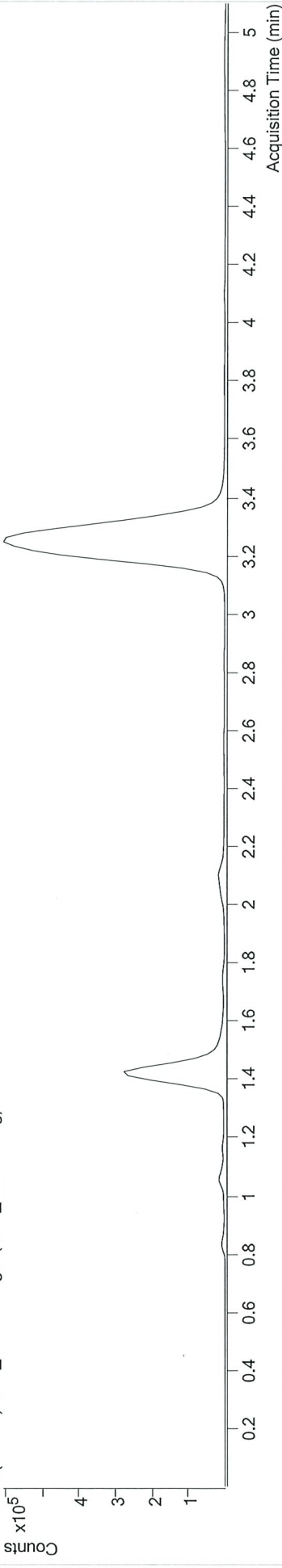
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC	3.270	364683	1547.85	28.2	∞	4585610	10.0261	ng/ml
THC-COOH	1.459	58739	129.23	49.4	493.90	255936	10.6204	ng/ml
THC-OH	1.438	137121	∞	11.6	197.23	861604	10.0681	ng/ml

THC_Cal 3 -10ng

Page 1 of 1

Generated at 8:48 AM on 9/9/2019

AM #27 Cannabinoids Quant. Results

Batch results
Calibration Last Update   D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin
9/9/2019 8:47:30 AM

Instrument	Falco
Type	Cal
Acq. Method	AM 27 THC quant.m
Sample Position	P3-D6
Injection Volume	10
Acq. Date-Time	9/5/2019 4:36:48 PM
Sample Info.	

Data File	THC_Cal 4-25ng.d
Sample	THC_Cal 4-25ng
Comment	

Sample Chromatogram

+ TIC MRM (** -> **) THC_Cal 4-25ng.d (THC_Cal 4-25ng)

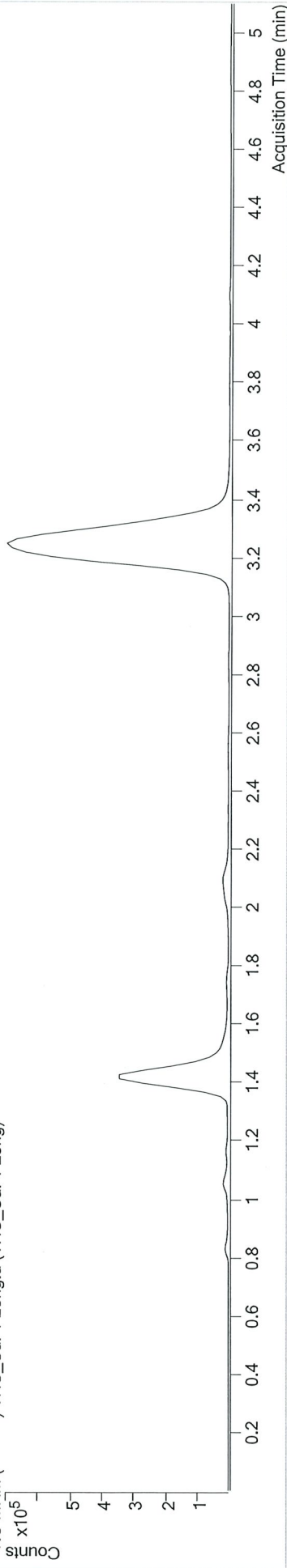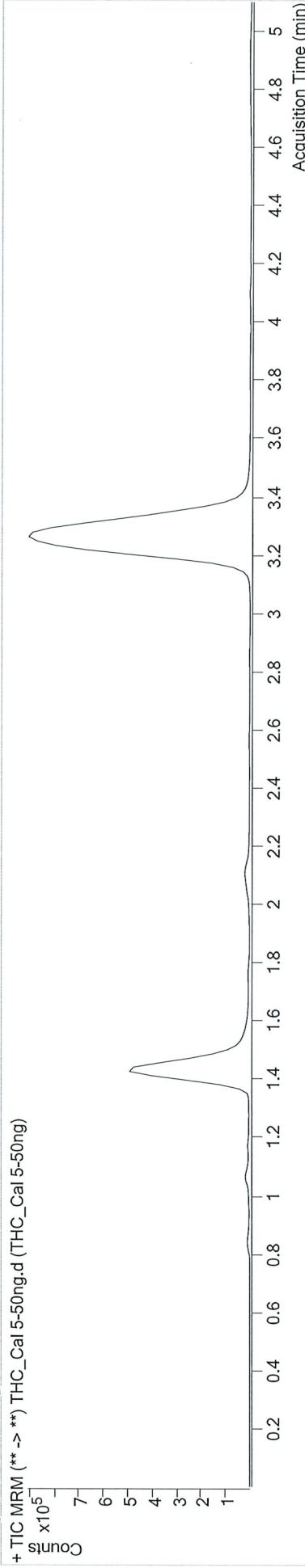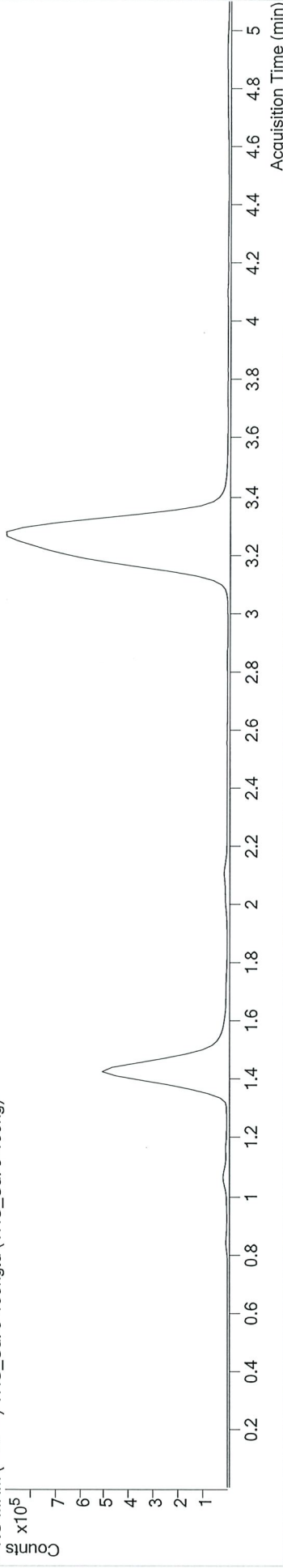| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC	3.270	915401	∞	28.0	996.58	4682489	24.7252	ng/ml
THC-COOH	1.459	133098	∞	55.3	896.06	261139	25.2775	ng/ml
THC-OH	1.423	313806	∞	12.8	∞	896201	24.1335	ng/ml

THC_Cal 4-25ng

Generated at 8:48 AM on 9/9/2019

AM #27 Cannabinoids Quant. Results

TS

Batch results D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin
Calibration Last Update 9/9/2019 8:47:30 AM

Instrument	Falco	**Data File**	THC_Cal 5-50ng.d
Type	Cal	**Sample**	THC_Cal 5-50ng
Acq. Method	AM 27 THC quant.m	**Comment**	
Sample Position	P3-C6		
Injection Volume	10		
Acq. Date-Time	9/5/2019 4:44:22 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) THC_Cal 5-50ng.d (THC_Cal 5-50ng)

Name	RT	Resp.	S/N	Ratio	ISTD Resp.	S/N	Final Conc.	
THC	3.285	1977139	4189.20	27.5	5065845	2586.84	49.4159	ng/ml
THC-COOH	1.474	272680	∞	58.8	284147	∞	48.8169	ng/ml
THC-OH	1.438	699141	∞	13.0	987438	264.66	50.4876	ng/ml

Generated at 8:48 AM on 9/9/2019

THC_Cal 5-50ng

AM #27 Cannabinoids Quant. Results

Batch results
Calibration Last Update 9/9/2019 8:47:30 AM

D:\MassHunter\Data\2019\AM 28\090319 MDQ THCQ TS reinjects\QuantResults\THCQ wklst 3644.batch.bin

Instrument	Falco
Type	Cal
Acq. Method	AM 27 THC quant.m
Sample Position	P3-B6
Injection Volume	10
Acq. Date-Time	9/5/2019 4:51:57 PM
Sample Info.	

Data File	THC_Cal 6-100ng.d
Sample	THC_Cal 6-100ng
Comment	

Sample Chromatogram

+ TIC MRM (** -> **) THC_Cal 6-100ng.d (THC_Cal 6-100ng)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC	3.285	3427144	12704.41	27.9	5096.83	4306176	100.8242	ng/ml
THC-COOH	1.474	433712	323.95	59.8	3299.46	222560	100.5606	ng/ml
THC-OH	1.438	1067928	1006.39	13.3	3545.65	771658	100.2604	ng/ml