

REVIEWED

By Sarah Pickle at 1:40 pm, Jun 05, 2019

15

5/29/2019

Worklist: 3415

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
M2019-1778	1	152792	AM 27 Blood THC Quant by LC-QQQ	
M2019-1833	2	152793	AM 27 Blood THC Quant by LC-QQQ	
M2019-2010	1	152794	AM 27 Blood THC Quant by LC-QQQ	
P2019-1321	1	152795	AM 27 Blood THC Quant by LC-QQQ	
P2019-1326	1	152796	AM 27 Blood THC Quant by LC-QQQ	
P2019-1364	1	152797	AM 27 Blood THC Quant by LC-QQQ	
P2019-1365	1	152798	AM 27 Blood THC Quant by LC-QQQ	
P2019-1371	1	152799	AM 27 Blood THC Quant by LC-QQQ	
P2019-1416	1	152800	AM 27 Blood THC Quant by LC-QQQ	

TS

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

Extraction Date: 06/03/19
Plate lot#: 0539904

Analyst: Tamara Salazar
Plate Expiration: 09/10/19

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: Hemostat 445283-2

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: ~~069910~~ 069901 TS

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: D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS
Batch Name: THCQ wklst 3415 TS
- 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:



Idaho State Police Forensic Services

TS

AM #27 Quantitative Analysis of THC and Metabolites in Blood by LCMS-QQQ

Methanol External Control Solution (Lot: WS041619)

10 ul of 1mg/mL THC, 100 ul of 100 ug/mL THC-OH, C-THC in 9790 ul MeOH

Approximate concentration 1ug/mL.

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>	<i>Expiration Date</i>
Methanol (LCMS)	Fisher	184782	
THC	Cerilliant	FE09101501	11/30/2020
C-THC	Cerilliant	FE07171501	09/30/2019
THC-OH	Cerilliant	FE01121503	01/31/2020
Prepared:	04/16/2019		
Prepared By:	Tamara Salazar		
Expires:	01/31/2020		

Blood External Control Solution (Lot: 041619)

100 ul of methanol external control solution was added to 9900 ul of blood.

Approximately 10ng/mL of each compound.

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>
Negative Blood	Hemostat	445283-1
Methanol External Control Solution	-	WS041619
Prepared:	04/16/19	
Prepared by:	Tamara Salazar	
Expires:	01/31/2020	

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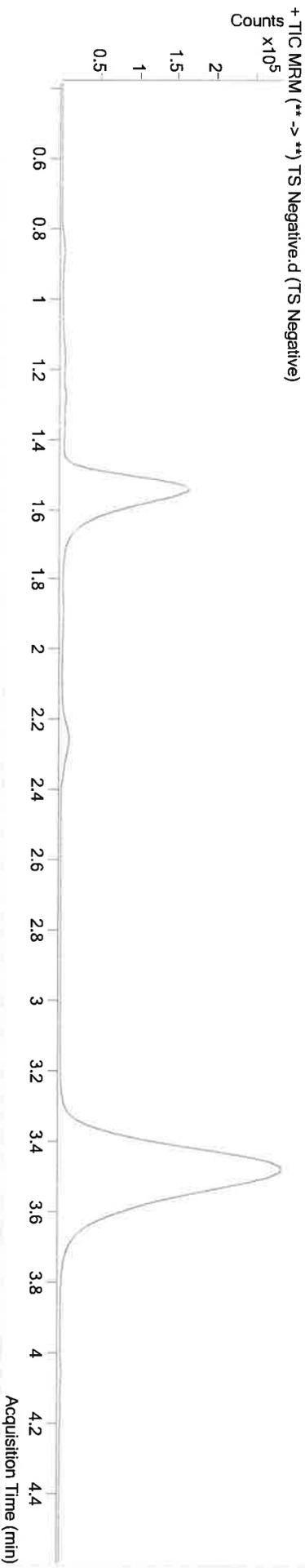
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415 TS.batch.bin
Calibration Last Update 6/4/2019 3:25:16 PM

Instrument Type	FALCO-LCMS (Property ID 069901)	Data File	TS Negative.d
Acq. Method	Sample	Sample	TS Negative
Sample Position	AM 27 THC quant.m	Comment	
Injection Volume	P4-H5		
Acq. Date-Time	10		
Sample Info.	6/3/2019 5:07:16 PM		

Sample Chromatogram



TS

AM #27 Cannabinoids Quant. Results



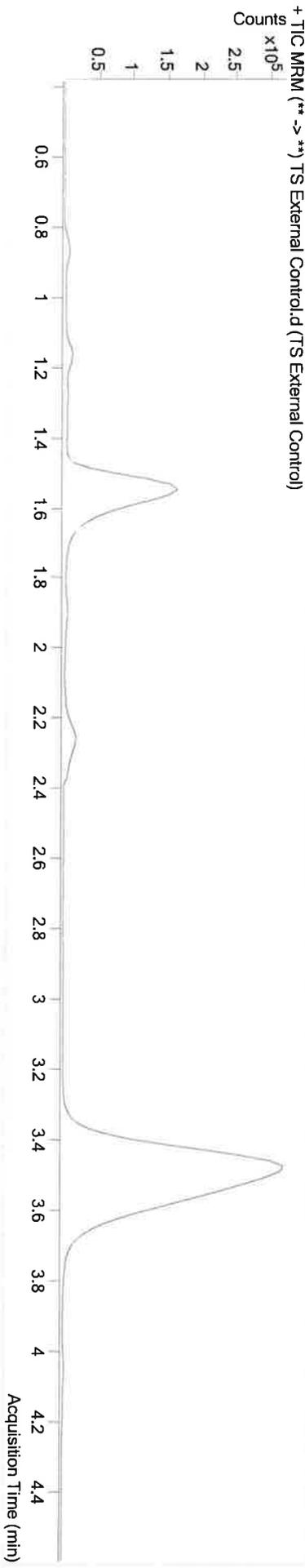
Batch results
Calibration Last Update D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415 TS.batch.bin
 6/4/2019 3:25:16 PM

Instrument FALCO-LCMS (Property ID 069901)
Type Sample
Acq. Method AM 27 THC quant.m
Sample Position P4-G5
Injection Volume 10
Acq. Date-Time 6/3/2019 5:22:28 PM
Sample Info.

Data File TS External Control.d
Sample TS External Control
Comment

Sample Chromatogram

+ TIC MRM (** -> **) TS External Control.d (TS External Control)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.491	185648	1621.21	28.3	410.95	3093509	7.3381 ng/ml
THC-COOH	1.580	41785	∞	42.6	27.04	200732	9.3605 ng/ml
THC-OH	1.558	88353	210.76	11.3	137.69	661469	8.4117 ng/ml

TS

AM #27 Cannabinoids Quant. Results

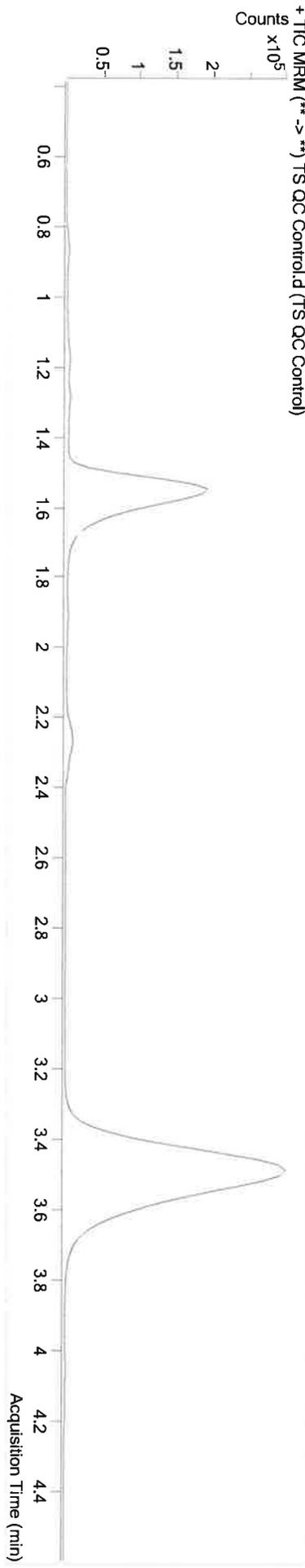


Batch results
Calibration Last Update: 6/4/2019 3:25:16 PM
D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415 TS.batch.bin

Instrument FALCO-LCMS (Property ID 069901)
Type Sample
Acq. Method AM 27 THC quant.m
Sample Position p4-A6
Injection Volume 10
Acq. Date-Time 6/3/2019 4:52:05 PM
Sample Info.

Data File TS QC Control.d
Sample TS QC Control

Sample Chromatogram

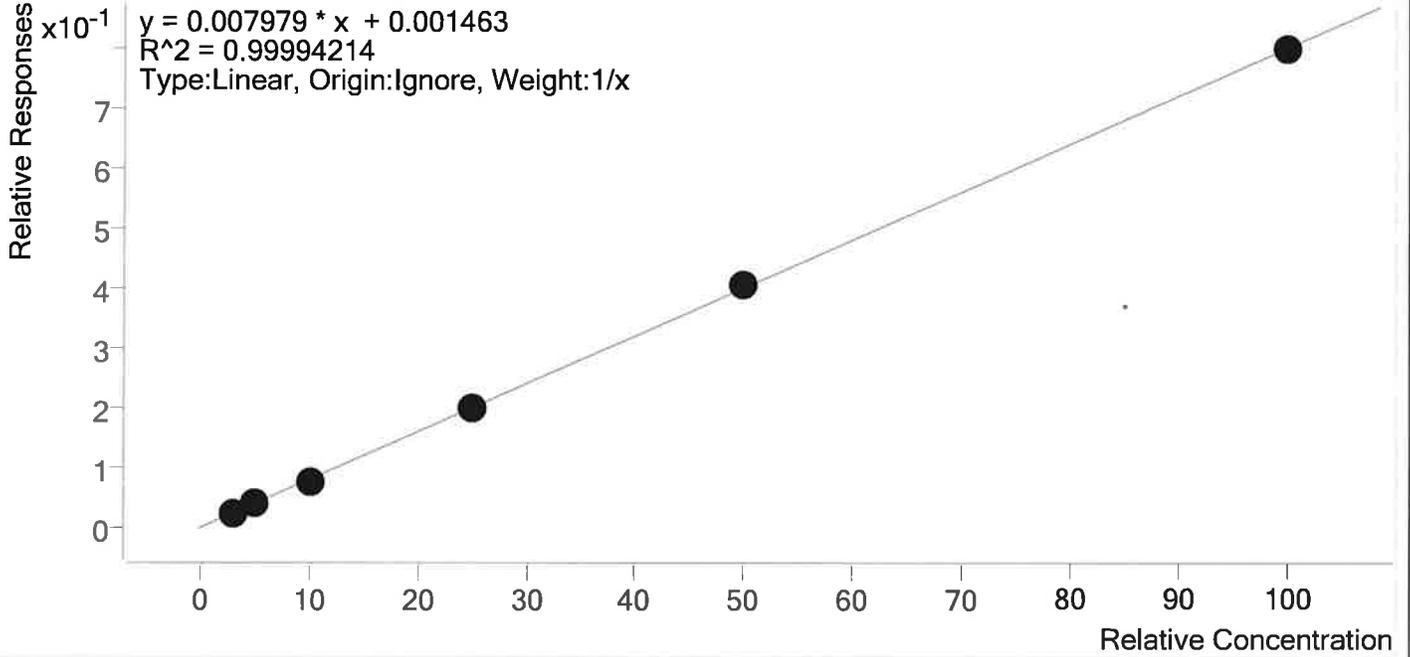


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.491	112642	889.34	28.2	174.72	2893254	4.6962 ng/ml
THC-COOH	1.595	48732	223.43	48.9	45.33	226351	9.7441 ng/ml
THC-OH	1.558	66349	248.02	11.9	92.81	804279	4.6716 ng/ml

Compound Calibration Report

Batch results D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415
 TS.batch.bin
Last Cal. Update 6/4/2019 3:25 PM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3

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



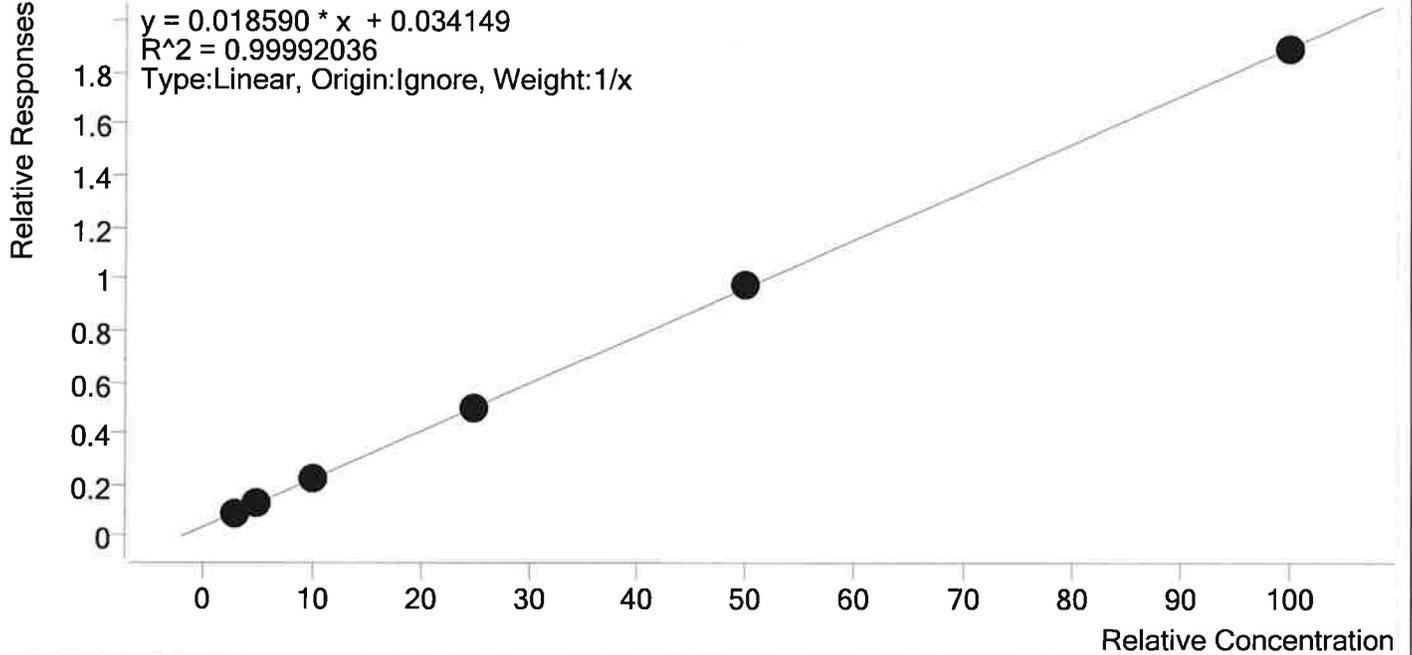
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
TS Cal 1-3ng	1	✓	3.0	3.0	101.1
TS Cal 2- 5ng	2	✓	5.0	5.0	100.1
TS Cal 3 -10ng	3	✓	10.0	9.8	98.1
TS Cal 4-25ng	4	✓	25.0	25.0	100.2
TS Cal 5-50ng	5	✓	50.0	50.4	100.8
TS Cal 6-100ng r	6	✓	100.0	99.7	99.7

Compound Calibration Report

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Batch results D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415
 TS.batch.bin
Last Cal. Update 6/4/2019 3:25 PM
Analyst Name ISP\Datator
Analyte THC-COOH **Internal Standard** THC-COOH-D9

THC-COOH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 0 QCs



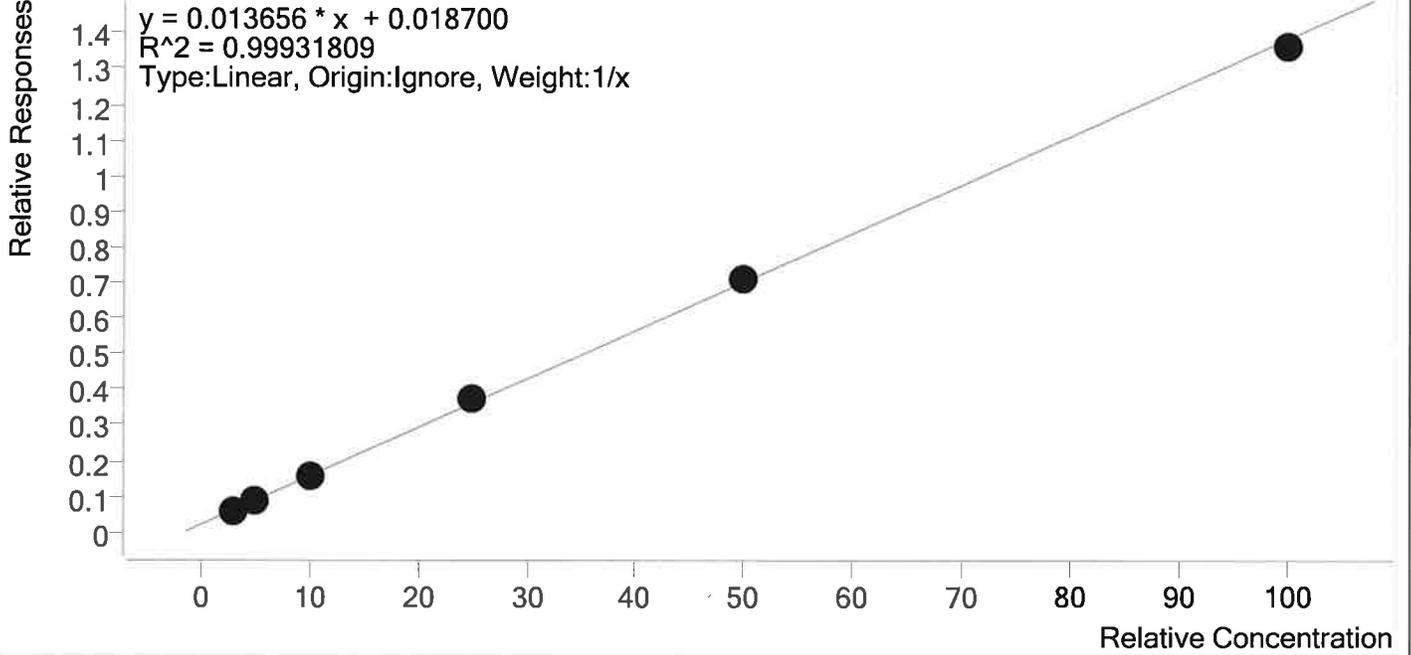
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
TS Cal 1-3ng	1	✓	3.0	3.1	101.7
TS Cal 2- 5ng	2	✓	5.0	4.9	97.6
TS Cal 3 -10ng	3	✓	10.0	10.1	100.8
TS Cal 4-25ng	4	✓	25.0	24.8	99.3
TS Cal 5-50ng	5	✓	50.0	50.5	101.0
TS Cal 6-100ng r	6	✓	100.0	99.7	99.7

TS

Compound Calibration Report

Batch results D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415
 TS.batch.bin
Last Cal. Update 6/4/2019 3:25 PM
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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
TS Cal 1-3ng	1	✓	3.0	2.9	97.3
TS Cal 2- 5ng	2	✓	5.0	4.9	98.4
TS Cal 3 -10ng	3	✓	10.0	10.0	99.8
TS Cal 4-25ng	4	✓	25.0	26.2	104.9
TS Cal 5-50ng	5	✓	50.0	50.6	101.1
TS Cal 6-100ng r	6	✓	100.0	98.4	98.4

TS

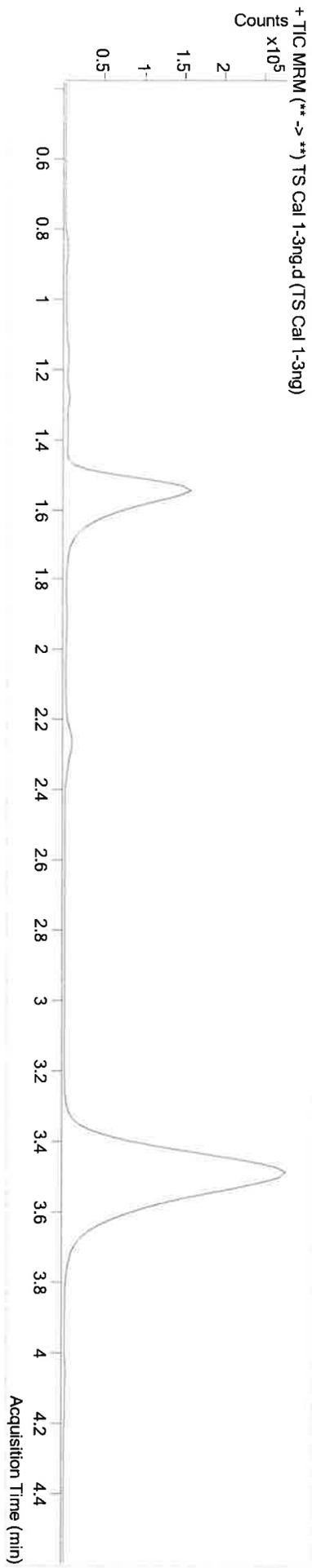
AM #27 Cannabinoids Quant. Results



Batch results Calibration Last Update D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415 TS.batch.bin
6/4/2019 3:25:16 PM

Instrument	FALCO-LCMS (Property ID 069901)	Data File	TS Cal 1-3ng.d
Type	Cal	Sample	TS Cal 1-3ng
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P4-G6		
Injection Volume	10		
Acq. Date-Time	6/3/2019 3:57:47 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.506	67066	246.34	29.6	64.31	2613331	3.0331 ng/ml
THC-COOH	1.580	17934	∞	42.6	97.35	197328	3.0517 ng/ml
THC-OH	1.558	40348	138.01	10.9	34.52	688954	2.9191 ng/ml

TS

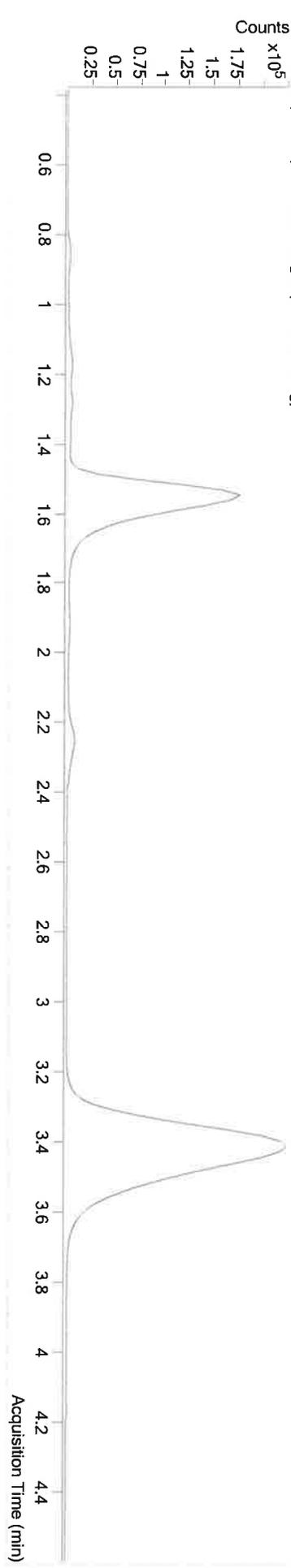
AM #27 Cannabinoids Quant. Results



Batch results
 Calibration Last Update: 6/4/2019 3:25:16 PM
 D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ_wk1st_3415 TS.batch.bin

Instrument Type: FALCO-LCMS (Property ID 069901)
Acq. Method: AM 27 THC quant.m
Sample Position: P4-F6
Injection Volume: 10
Acq. Date-Time: 6/3/2019 4:05:22 PM
Sample Info:

Sample Chromatogram
 + TIC MRM (** -> **) TS Cal 2- 5ng.d (TS Cal 2- 5ng)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.431	89294	580.08	28.9	83.40	2156473	5.0064 ng/ml
THC-COOH	1.580	26257	80.52	49.1	403.95	210273	4.8800 ng/ml
THC-OH	1.558	65886	181.86	11.5	81.31	766904	4.9218 ng/ml

TS

AM #27 Cannabinoids Quant. Results

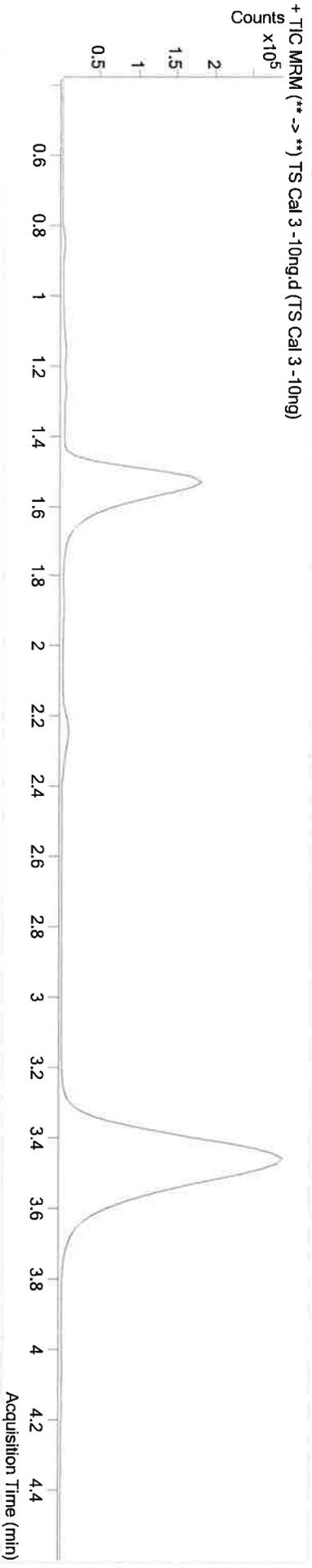


Batch results
D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415 TS.batch.bin
Calibration Last Update 6/4/2019 3:25:16 PM

Instrument FALCO-LCMS (Property ID 069901)
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P4-E6
Injection Volume 10
Acq. Date-Time 6/3/2019 4:13:00 PM
Sample Info.

Data File
Sample
Comment
TS Cal 3 -10ng.d
TS Cal 3 -10ng

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.476	215611	∞	27.4	∞	2704264	9.8094 ng/ml
THC-COOH	1.565	46665	135.77	49.2	135.26	210703	10.0764 ng/ml
THC-OH	1.543	116861	∞	12.3	139.40	753863	9.9822 ng/ml

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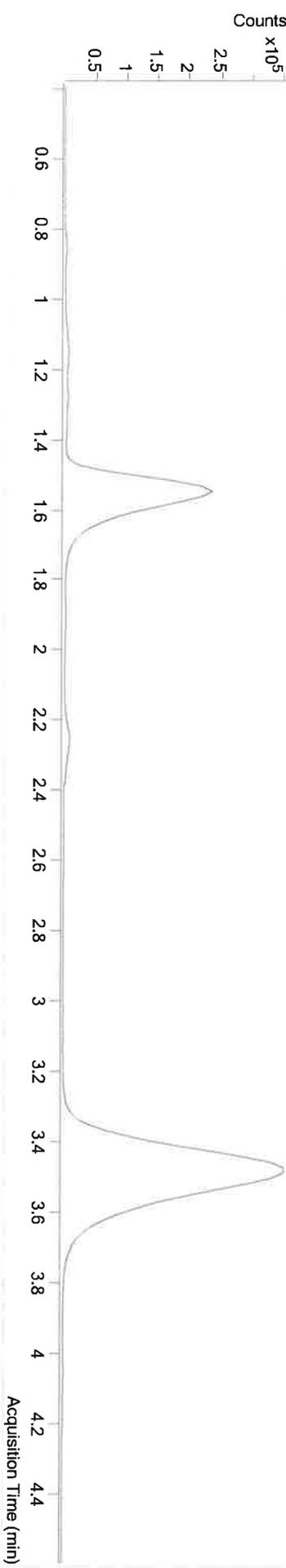
AM #27 Cannabinoids Quant. Results



Batch results
 Calibration Last Update: 6/4/2019 3:25:16 PM
 D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415 TS.batch.bin

Instrument: FALCO-LCMS (Property ID 069901)
Type: Cal
Acq. Method: AM 27 THC quant.m
Sample Position: P4-D6
Injection Volume: 10
Acq. Date-Time: 6/3/2019 4:20:36 PM
Sample Info.

Sample Chromatogram
 + TIC MRM (**->**) TS Cal 4-25ng.d (TS Cal 4-25ng)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.491	576909	∞	27.3	738.87	2866606	25.0401 ng/ml
THC-COOH	1.580	107617	∞	56.8	117.15	217221	24.8129 ng/ml
THC-OH	1.558	295418	2994.77	12.7	382.39	783720	26.2333 ng/ml

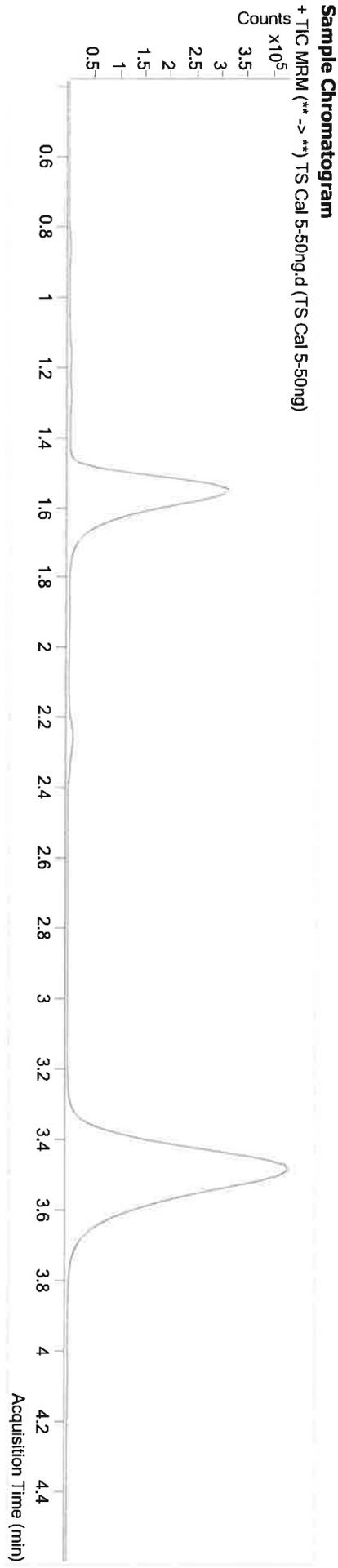
TS

AM #27 Cannabinoids Quant. Results



Batch results
 Calibration Last Update: D:\MassHunter\Data\2019\AM 27\060319 THCQ SP TS\QuantResults\THCQ wk1st 3415 TS.batch.bin
 6/4/2019 3:25:16 PM

Instrument	FALCO-LCMS (Property ID 069901)	Data File	TS Cal 5-50ng.d
Type	Cal	Sample	TS Cal 5-50ng
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P4-C6		
Injection Volume	10		
Acq. Date-Time	6/3/2019 4:28:13 PM		
Sample Info.			



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.491	1156571	∞	27.2	∞	2865431	50.4047 ng/ml
THC-COOH	1.580	214384	566.00	60.4	∞	220424	50.4809 ng/ml
THC-OH	1.558	560375	1293.45	13.7	2524.80	790183	50.5616 ng/ml

TS

AM #27 Cannabinoids Quant. Results

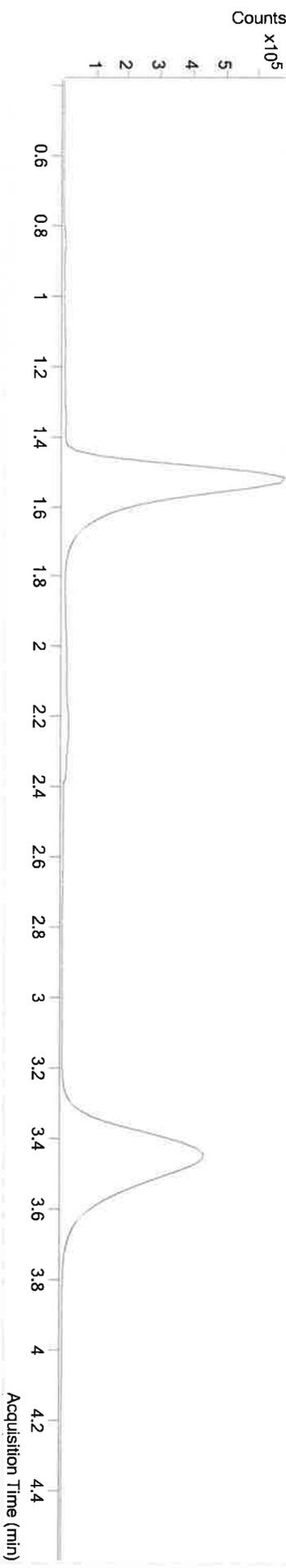


Batch results
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Calibration Last Update 6/4/2019 3:25:16 PM

Instrument FALCO-LCMS (Property ID 069901)
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P4-B6
Injection Volume 10
Acq. Date-Time 6/3/2019 4:44:28 PM
Sample Info.

Data File TS Cal 6-100ng_r.d
Sample TS Cal 6-100ng_r

Sample Chromatogram
+ TIC MRM (** -> **) TS Cal 6-100ng_r.d (TS Cal 6-100ng_r)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.461	1881694	∞	27.0	∞	2360986	99.7064 ng/ml
THC-COOH	1.549	471223	313.08	59.6	188.31	249647	99.6980 ng/ml
THC-OH	1.528	1758215	∞	14.1	6088.36	1290711	98.3820 ng/ml

Calibrator did not inject properly with initial injection. The calibrator was reinjected. TS