







Worklist: 3398

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>	
C2019-0775	1	152070	AM 27 Blood THC Quant by LC-QQQ	
C2019-0815	1	152071	AM 27 Blood THC Quant by LC-QQQ	
C2019-0819	1	152072	AM 27 Blood THC Quant by LC-QQQ	
C2019-0825	1	152073	AM 27 Blood THC Quant by LC-QQQ	
C2019-0827	1	152074	AM 27 Blood THC Quant by LC-QQQ	
C2019-0837	1	152075	AM 27 Blood THC Quant by LC-QQQ	
C2019-0840	1	152076	AM 27 Blood THC Quant by LC-QQQ	
C2019-0874	1	152077	AM 27 Blood THC Quant by LC-QQQ	
C2019-0884	1	152078	AM 27 Blood THC Quant by LC-QQQ	
C2019-0885	1	152079	AM 27 Blood THC Quant by LC-QQQ	
C2019-0886	1	152080	AM 27 Blood THC Quant by LC-QQQ	
C2019-0895	1	152081	AM 27 Blood THC Quant by LC-QQQ	
C2019-0919	1.2	152084	AM 27 Blood THC Quant by LC-QQQ	
C2019-0919	1.3	152083	AM 27 Blood THC Quant by LC-QQQ	
C2019-0928	2	152082	AM 27 Blood THC Quant by LC-QQQ	

BW

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

Extraction Date: 05/21/19
Plate lot#: 0539904

Analyst: Britany Wylie
Plate Expiration: 09/10/19

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 19A207P3

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.
- 3. Create worklist:

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. 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. *Shaker ID: 66759*
- 4. Pipette **500µL 0.1% formic acid in water** in wells of analytical plate.
- 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: 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.
Worklist path: D:\MassHunter\Data\2019\AM 27\052119 Batch Name: cann quant
- 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:

BW

Toxicology AM method 27 external prep information

working solution 1 ug/ml in meoh C-THC, THC-OH, THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 75 ul THC-OH in 9767.5 ul meOH

Ppd 3/13/19 Exp: 9/13/19 lot 91319 by AMN

Drug	lot	expiration
C-THC	FE01061702 cerillient	3/1/2022
THC-OH	318.1b18.1L1a	12/1/2021
THC	135.1b71.0L6	11/1/2021

AM 27 control 100 ul working solution lot (91319) in 9900 ul blood lot (19A207p3)

ppd 3/13/19 Exp 9/13/19 lot 31319 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-TH by AMN

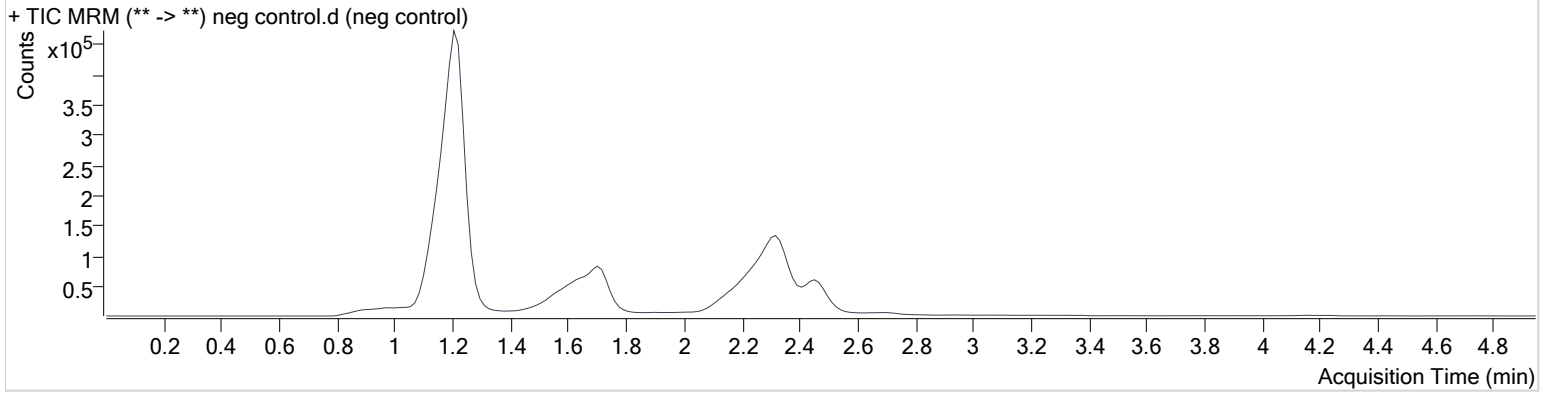
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	neg control.d
Type	Sample	Sample	neg control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2019 2:10:24 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.270	5451	∞			2015143	0.28 ng/ml Low

BW

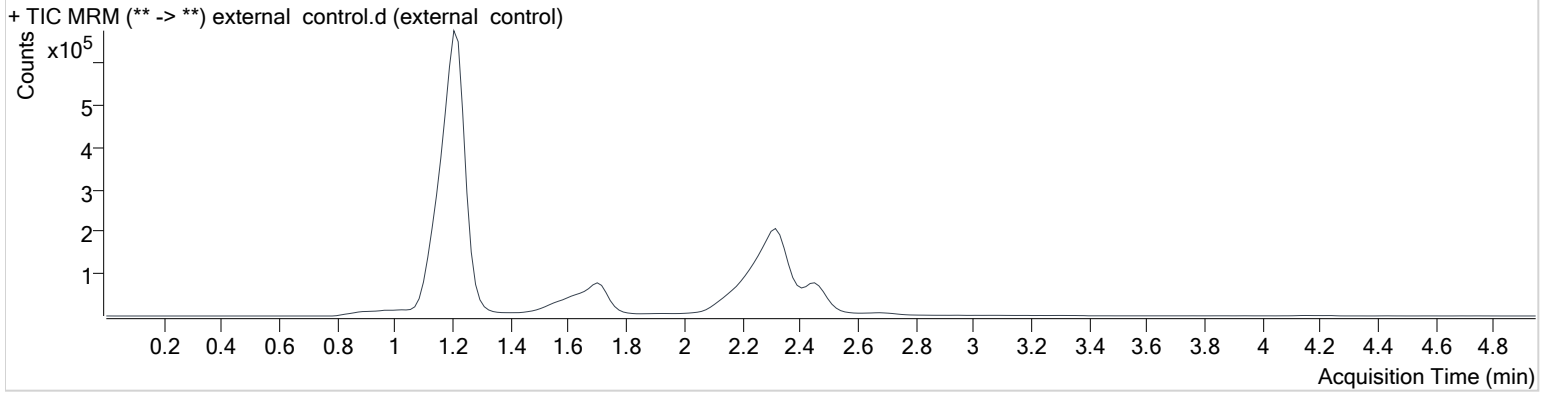
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	external control.d
Type	Sample	Sample	external control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-B2	Comment	THC, THCOH 7.5ng CTHC 15ng
Injection Volume	10		
Acq. Date-Time	5/21/2019 2:18:08 PM		

Sample Info.

Sample Chromatogram



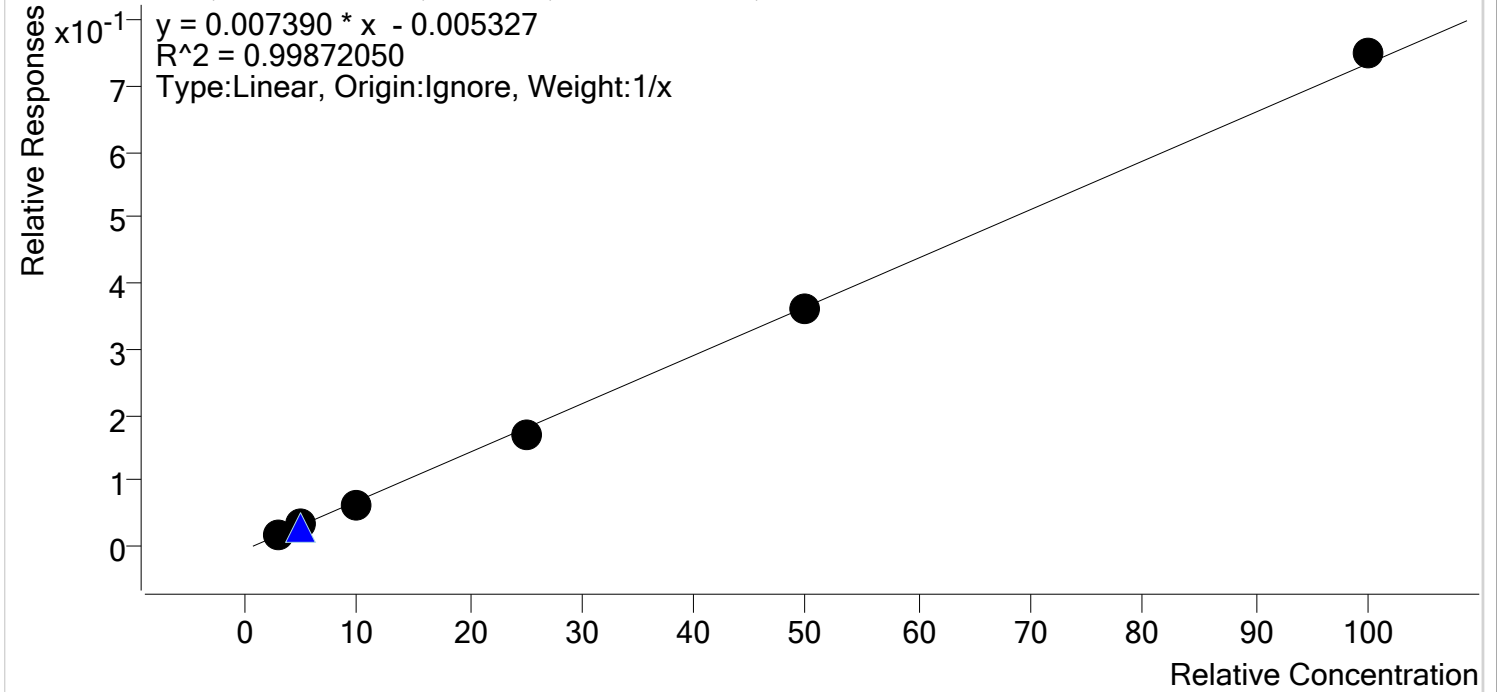
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	149294	∞	33.9	2222	938539	15.75 ng/ml
THC-OH	1.224	254771	∞	10.2	191	2263257	8.17 ng/ml
THC	2.341	68632	526	23.3	128	1095506	9.20 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Last Cal. Update 5/22/2019 7:54 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✓	3.0	3.3	109.1
cal 3	3	✓	5.0	5.0	100.9
cal 4	4	✓	10.0	9.4	94.4
cal 5	5	✓	25.0	23.6	94.3
cal 6	6	✓	50.0	49.6	99.3
cal 7	7	✓	100.0	102.0	102.0

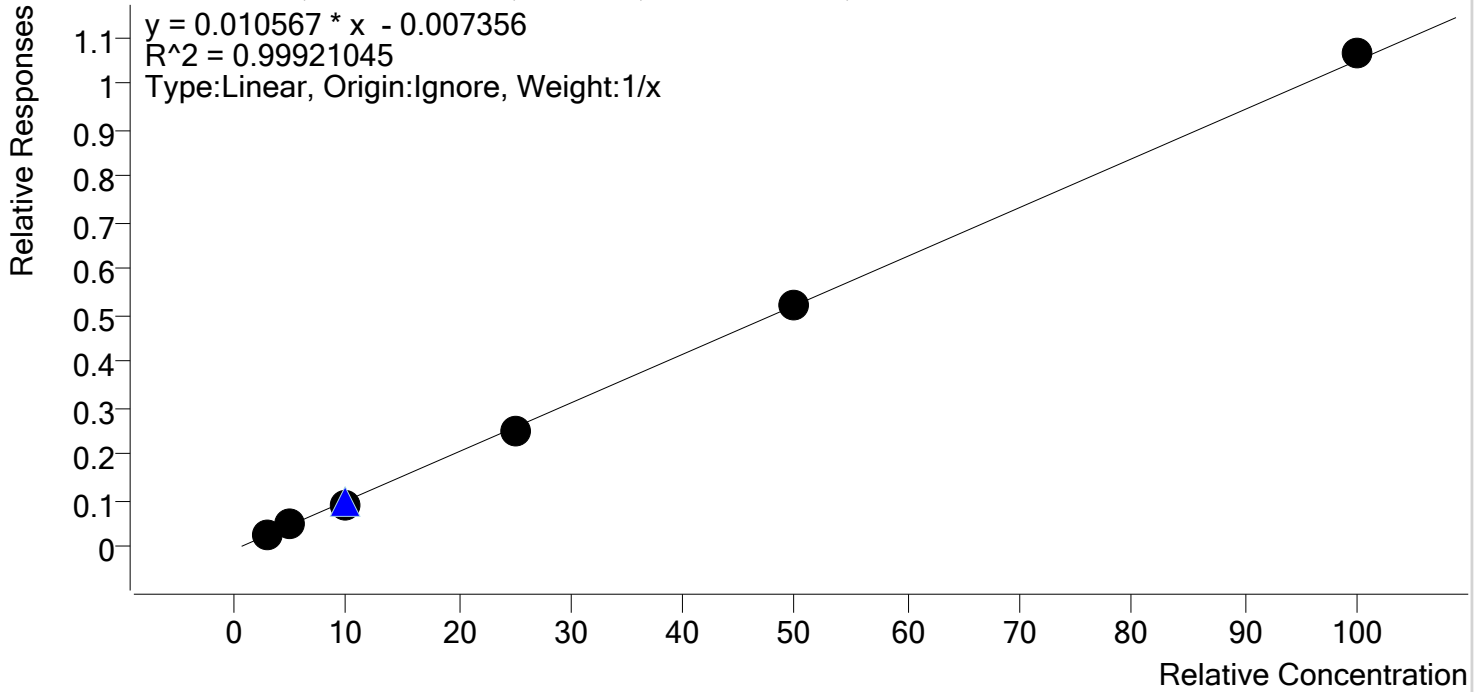
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Compound Calibration Report



Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Last Cal. Update 5/22/2019 7:54 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-d9

THC-COOH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✓	3.0	3.2	106.2
cal 3	3	✓	5.0	5.1	102.7
cal 4	4	✓	10.0	9.4	93.8
cal 5	5	✓	25.0	24.0	96.1
cal 6	6	✓	50.0	49.8	99.7
cal 7	7	✓	100.0	101.4	101.4

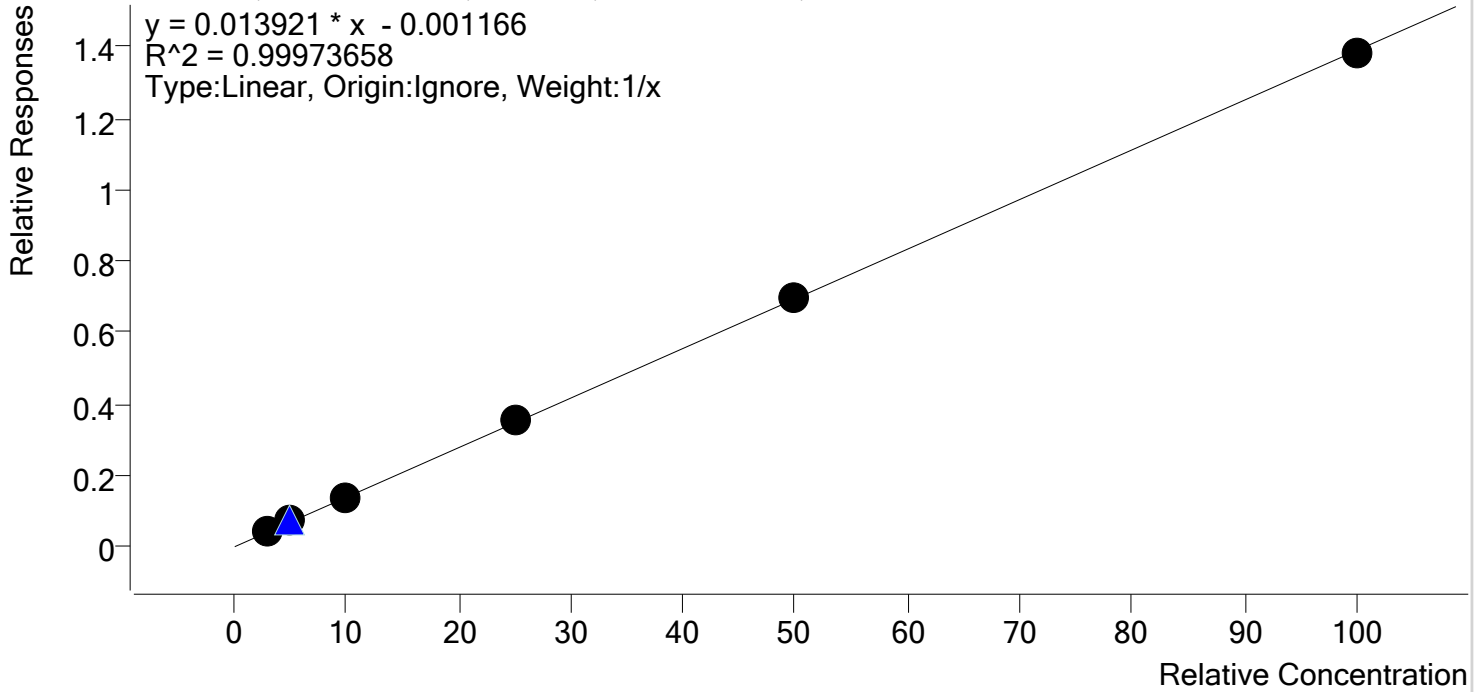
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Compound Calibration Report



Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Last Cal. Update 5/22/2019 7:54 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, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✓	3.0	2.8	94.9
cal 3	3	✓	5.0	5.2	104.6
cal 4	4	✓	10.0	9.9	98.5
cal 5	5	✓	25.0	25.5	102.2
cal 6	6	✓	50.0	50.2	100.4
cal 7	7	✓	100.0	99.3	99.3

BW

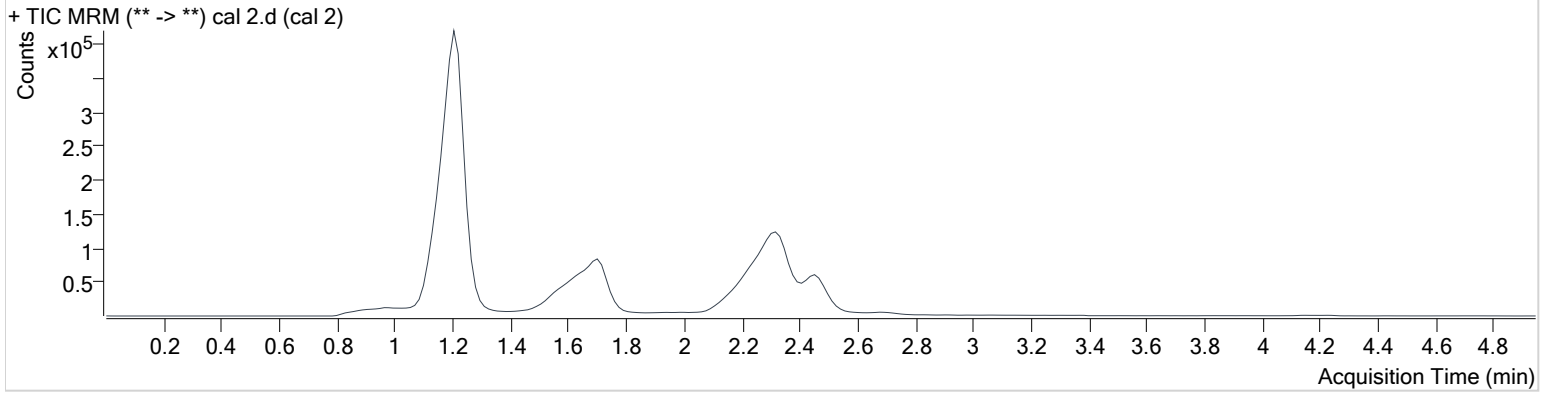
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	cal 2.d
Type	Cal	Sample	cal 2
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2019 1:01:02 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	17530	49	32.0	37	666000	3.19 ng/ml Low
THC-OH	1.209	59285	51	11.3	∞	1540367	2.85 ng/ml Low
THC	2.341	13601	58	20.1	20	721205	3.27 ng/ml

BW

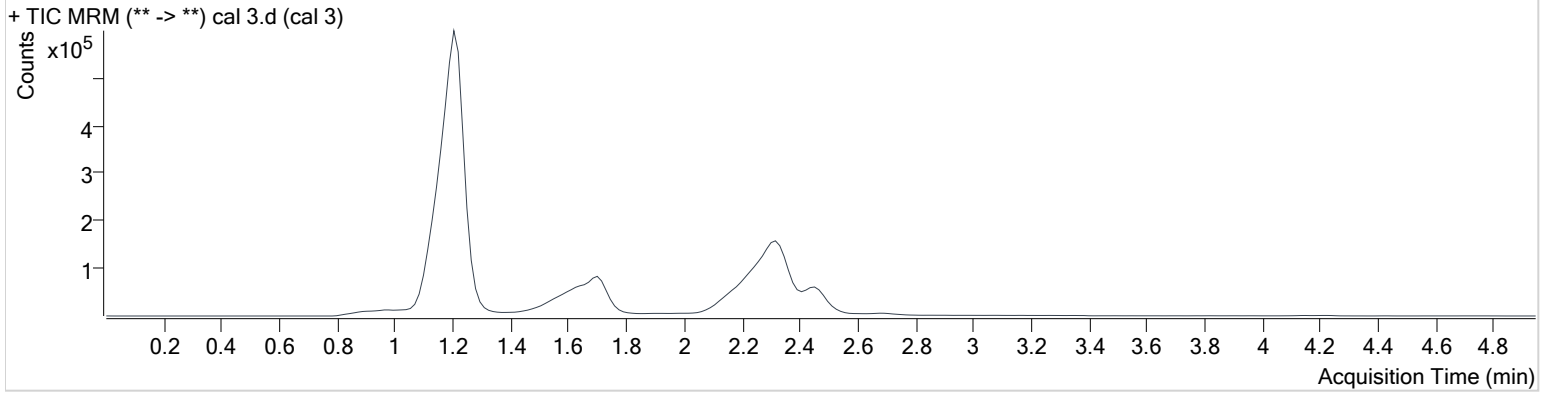
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-C1
Injection Volume 10
Acq. Date-Time 5/21/2019 1:08:47 PM
Sample Info.

Data File cal 3.d
Sample cal 3
Operator Britany Wylie
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	43954	301	31.6	74	937035	5.14 ng/ml Low
THC-OH	1.209	165142	505	9.8	73	2304092	5.23 ng/ml
THC	2.326	33108	321	23.9	82	1035674	5.05 ng/ml

BW

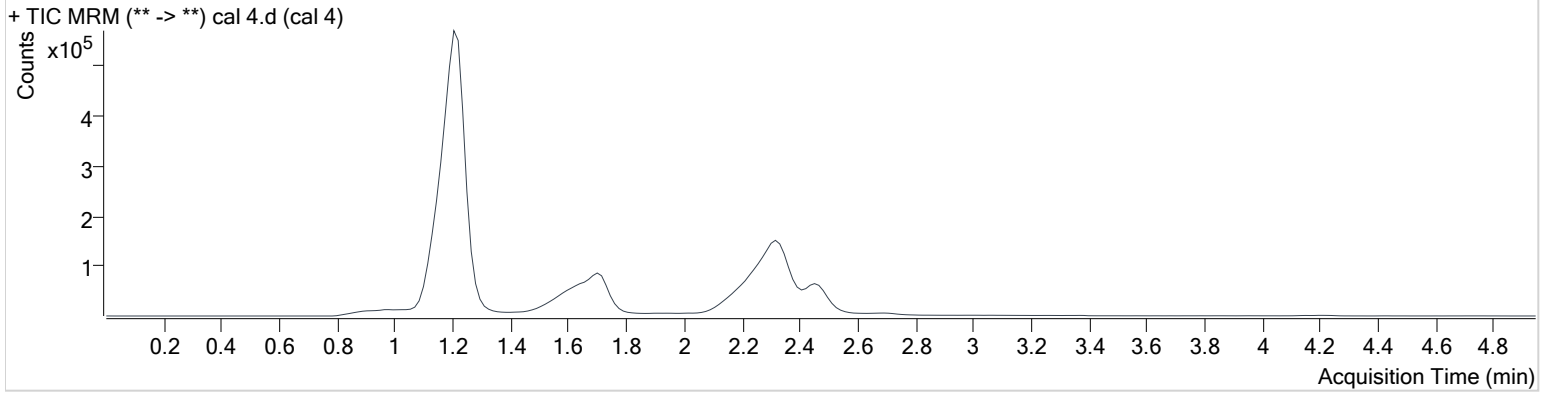
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	cal 4.d
Type	Cal	Sample	cal 4
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2019 1:16:30 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	75542	∞	37.2	∞	822926	9.38 ng/ml Low
THC-OH	1.224	272883	∞	10.3	2332433 8088122 2	2006451	9.85 ng/ml
THC	2.341	56373	388	23.5	28	875071	9.44 ng/ml

BW

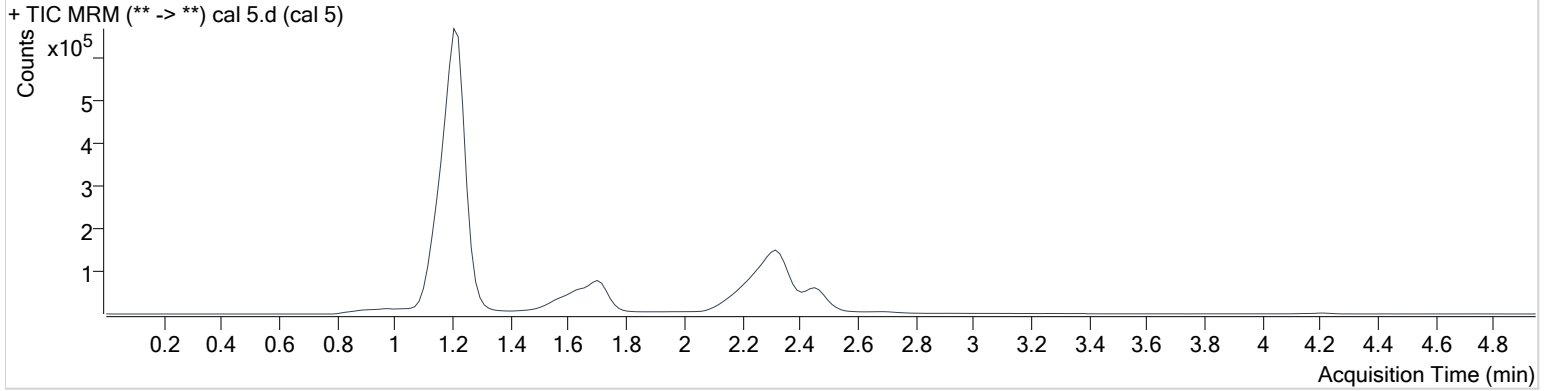
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	cal 5.d
Type	Cal	Sample	cal 5
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2019 1:24:13 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	187009	976	35.9	2408	758472	24.03 ng/ml
THC-OH	1.209	635854	467	10.9	21226	1794280	25.54 ng/ml
THC	2.341	136688	1264	24.4	128	809149	23.58 ng/ml

BW

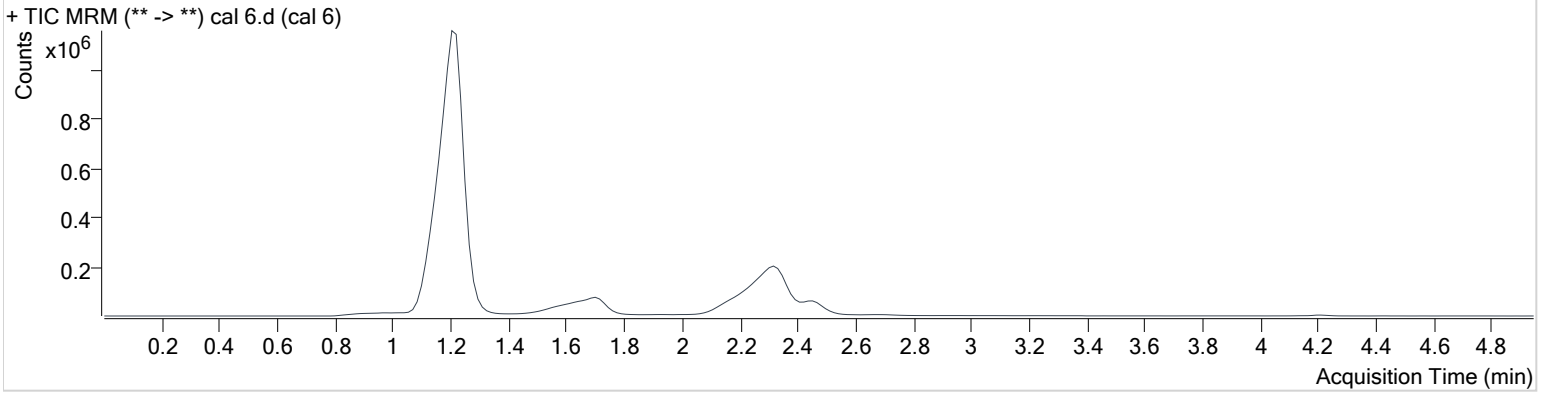
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	cal 6.d
Type	Cal	Sample	cal 6
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-F1	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2019 1:31:57 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	517250	∞	38.1	∞	996284	49.83 ng/ml
THC-OH	1.209	1769914	∞	11.6	119	2537393	50.19 ng/ml
THC	2.341	385857	1200	24.2	374	1067494	49.63 ng/ml

BW

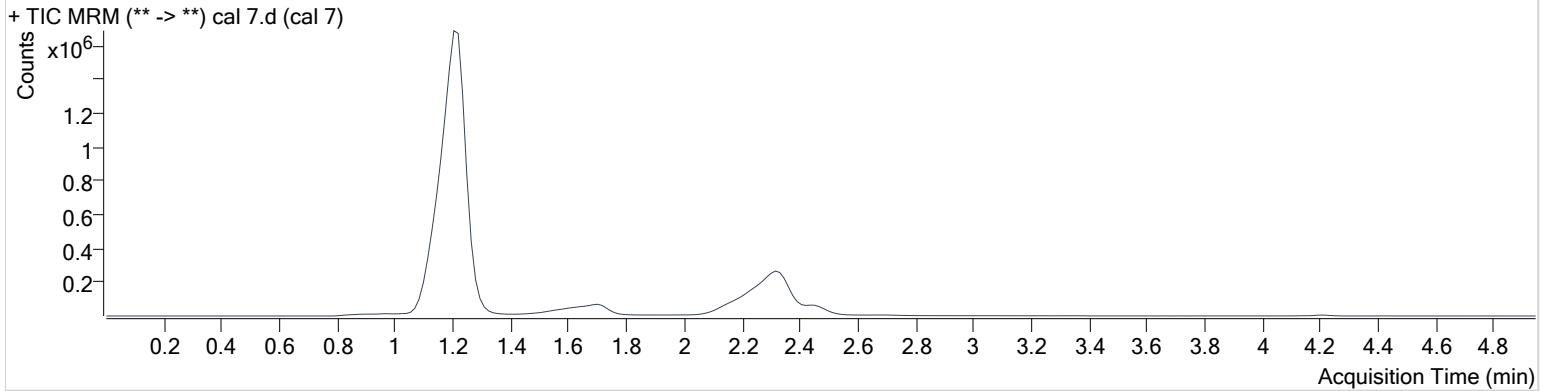
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	cal 7.d
Type	Cal	Sample	cal 7
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2019 1:47:19 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	1024175	3675	38.2	1105	962175	101.43 ng/ml
THC-OH	1.209	3484597	1361	11.8	∞	2521914	99.34 ng/ml
THC	2.341	838583	4102	24.7	1653	1119998	102.03 ng/ml

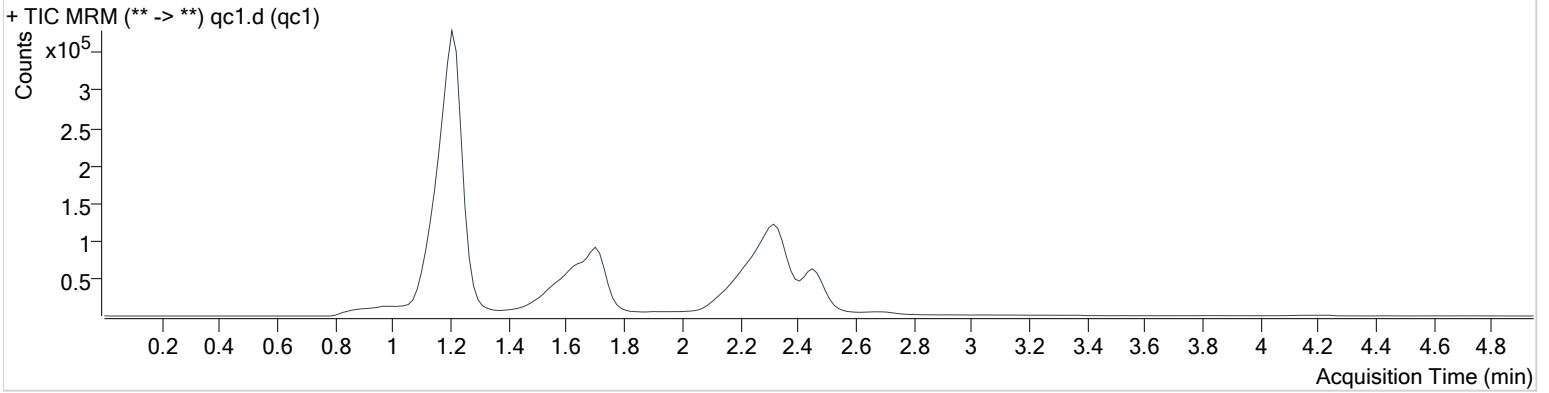
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument 69679 **Data File** qc1.d
Type QC **Sample** qc1
Acq. Method AM 27 THC quant.m **Operator** Britany Wylie
Sample Position P3-A1 **Comment**
Injection Volume 10
Acq. Date-Time 5/21/2019 12:53:16 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	6549	24	45.7 High	24	658221	1.64 ng/ml Low
THC-OH	1.224	20319	∞	13.0 High	11	1450789	1.09 ng/ml Low
THC	2.341	4889	25	12.6 Low	11	714663	1.65 ng/ml Low

BW

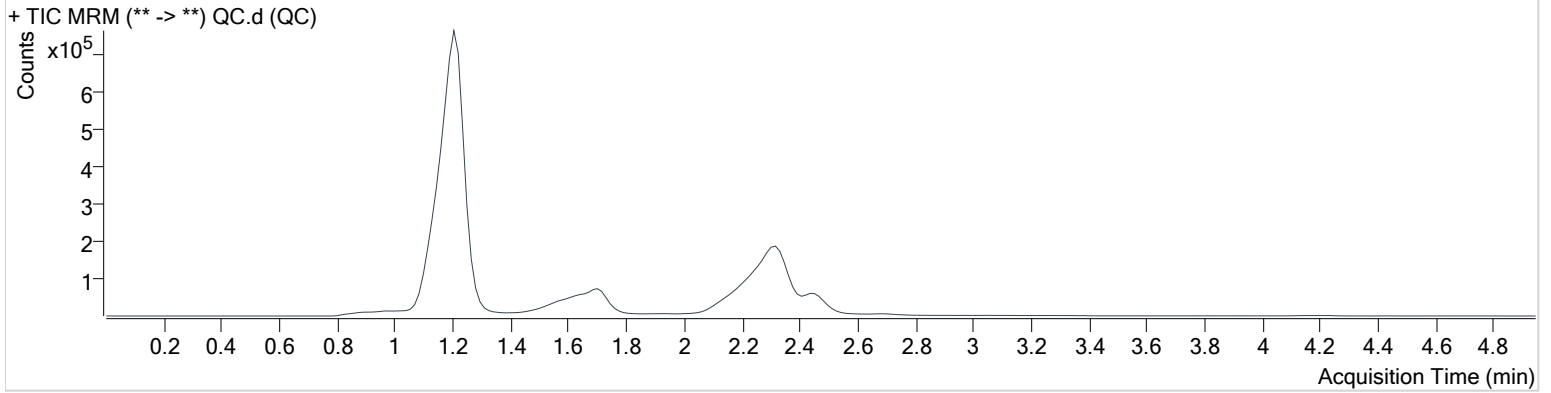
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\052119\QuantResults\cann quant.batch.bin
Calibration Last Update 5/22/2019 7:54:30 AM

Instrument	69679	Data File	QC.d
Type	QC	Sample	QC
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	THC,THCOH 5ng CTHC 10
Injection Volume	10		
Acq. Date-Time	5/21/2019 2:02:40 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	106253	310	35.5	247	1135831	9.55 ng/ml Low
THC-OH	1.209	205905	∞	10.3	∞	2944113	5.11 ng/ml
THC	2.341	40068	260	23.3	21	1304219	4.88 ng/ml

BW