

REVIEWED

By Anne Nord at 10:51 am, May 17, 2021

5/13/2021

BWylee

Worklist: 4975

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2021-1030	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1071	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1106	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1121	1	BCK	AM 27 Blood THC Quant by LC-QQQ



AM# 27: Quantitation of THC and Metabolites in Blood and Urine

LC-MS/MS

Extraction Date: 05/13/21

Analyst: Britany Wylie

Plate lot#: 210412

Plate Expiration: 10-12-2021

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 20K20702

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQ 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.

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
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.
- 4. Pipette **500µL 0.1% formic acid in water blood sample** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **800µL of blood+acid or urine 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.
- 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 blood), 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? (if not is it describe in comments section)
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Blood only batch; curves limitations: THC & THC-OH 3-100

am 27
4/29/21 worklist 4975

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	1	2	3	4	5	6
A	IS + Cal. 1	neg blood				IS + QC_1
B	IS + Cal. 2	1030				IS + Cal. 7
C	IS + Cal. 3	1071				IS + Cal. 6
D	IS + Cal. 4	1121				IS + Cal. 5
E	IS + Cal. 5	1106				IS + Cal. 4
F	IS + Cal. 6					IS + Cal. 3
G	IS + Cal. 7					IS + Cal. 2
H	IS + QC_1					IS + Cal. 1

All wells to contain 100 µl of residual DMSO

Case #: C2021- -1

AM #27 Cannabinoids

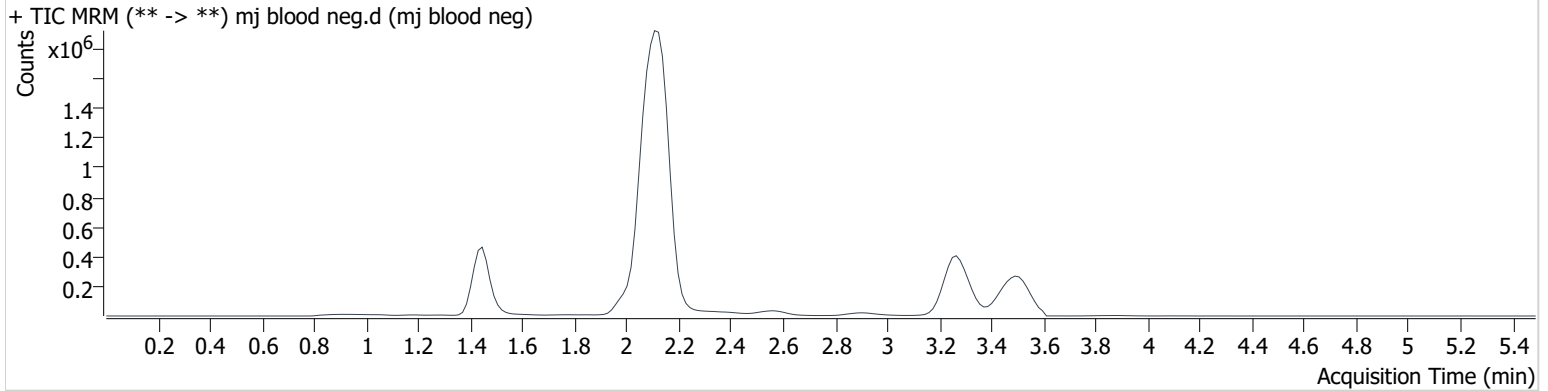
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj blood neg.d
Type	Sample	Sample	mj blood neg
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 6:30:32 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.508	38085	∞			1555463	1.049 ng/ml Low ³

negative control- no secondary transition peak found as well as calculated concentration less than 3

BW

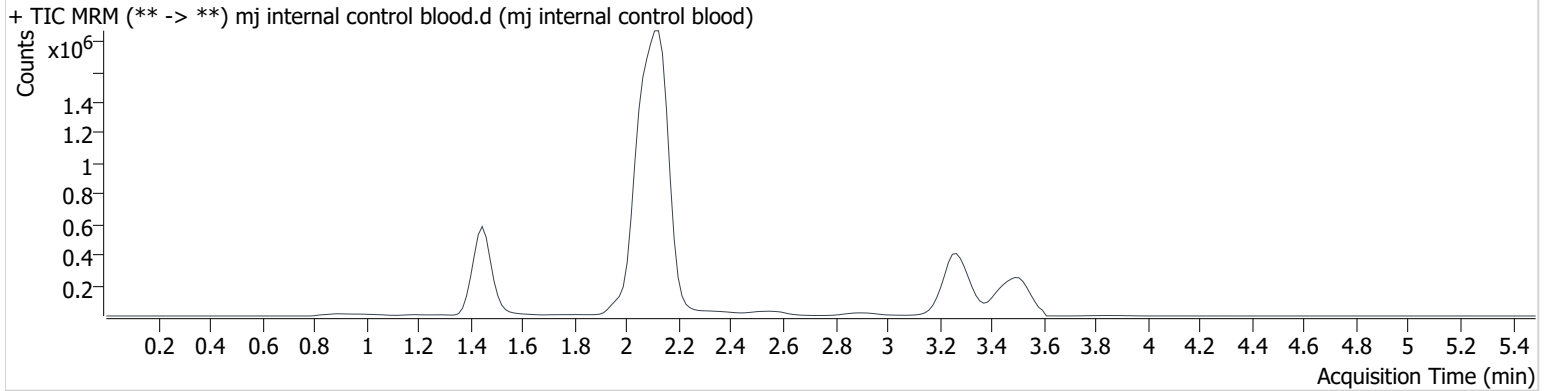
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj internal control blood.d
Type	QC	Sample	mj internal control blood
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 6:23:50 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	280268	∞	9.4	∞	1702945	5.648 ng/ml
THC-COOH	1.476	135227	195.3	35.4	90379.3	620768	15.051 ng/ml
THC	3.302	131834	∞	26.7	∞	1202512	4.557 ng/ml

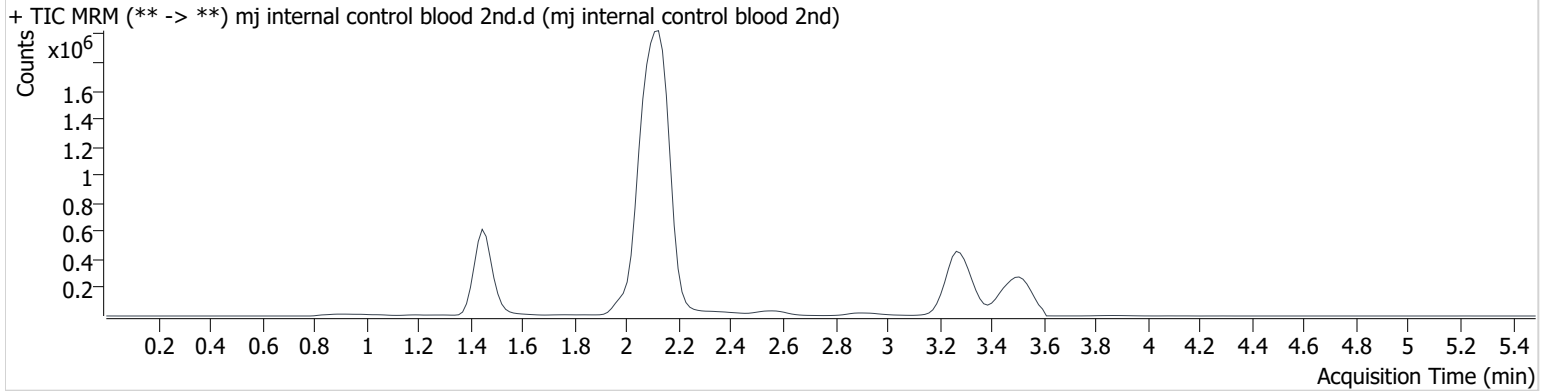
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj internal control blood 2nd.d
Type	QC	Sample	mj internal control blood 2nd
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 7:30:35 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	239300	∞	10.9	∞	1696549	4.876 ng/ml
THC-COOH	1.476	140334	480.5	35.5	1132.4	641395	15.112 ng/ml
THC	3.302	149815	∞	25.6	∞	1344998	4.617 ng/ml

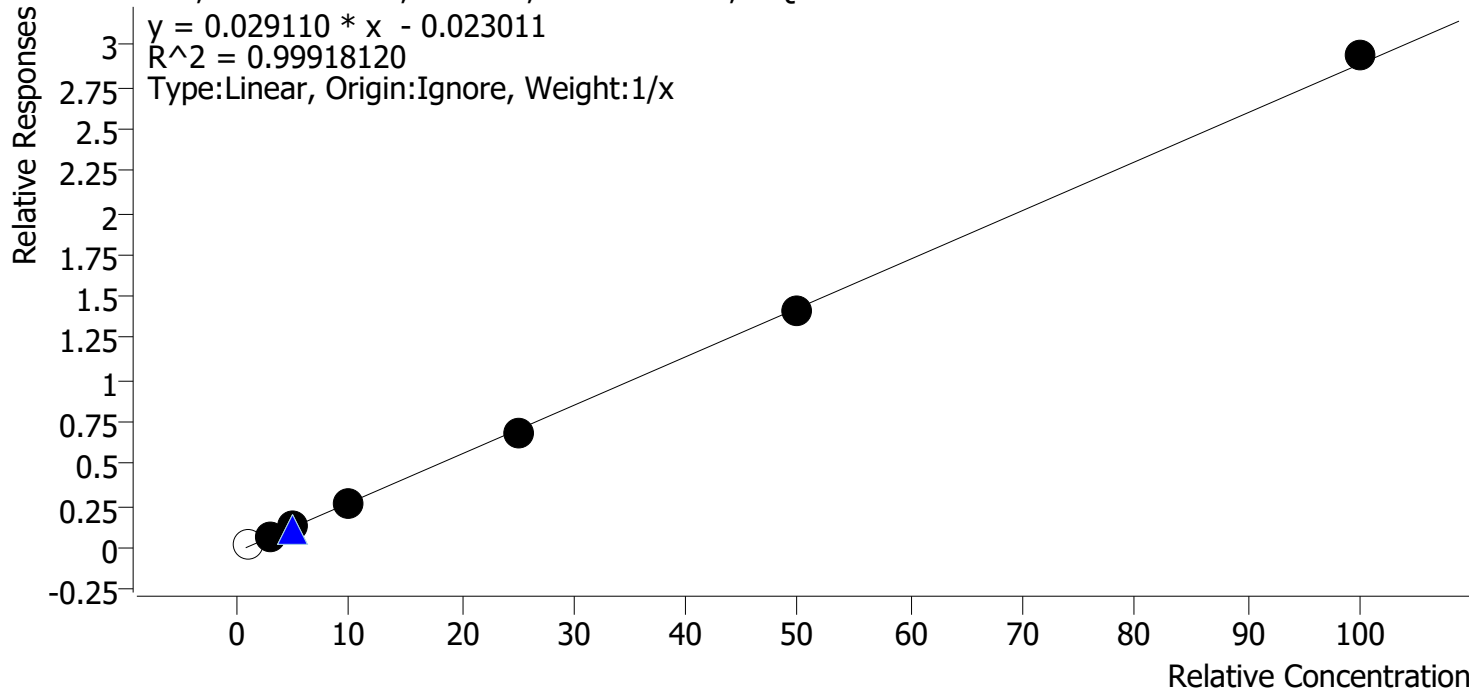
Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Last Cal. Update 5/14/2021 8:35 AM
Analyst Name ISP\datastor
Analyte THC

Internal Standard **THC-d3**

THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	x	1.0	1.6	162.8
mj cal2	2	✓	3.0	3.2	108.3
mj cal 3	3	✓	5.0	5.0	99.7
mj cal 4	4	✓	10.0	9.5	95.1
mj cal 5	5	✓	25.0	24.0	96.2
mj cal 6	6	✓	50.0	49.5	99.0
mj cal 7	7	✓	100.0	101.7	101.7

cal 1 dropped did not meet accuracy requirements, poor peak shape

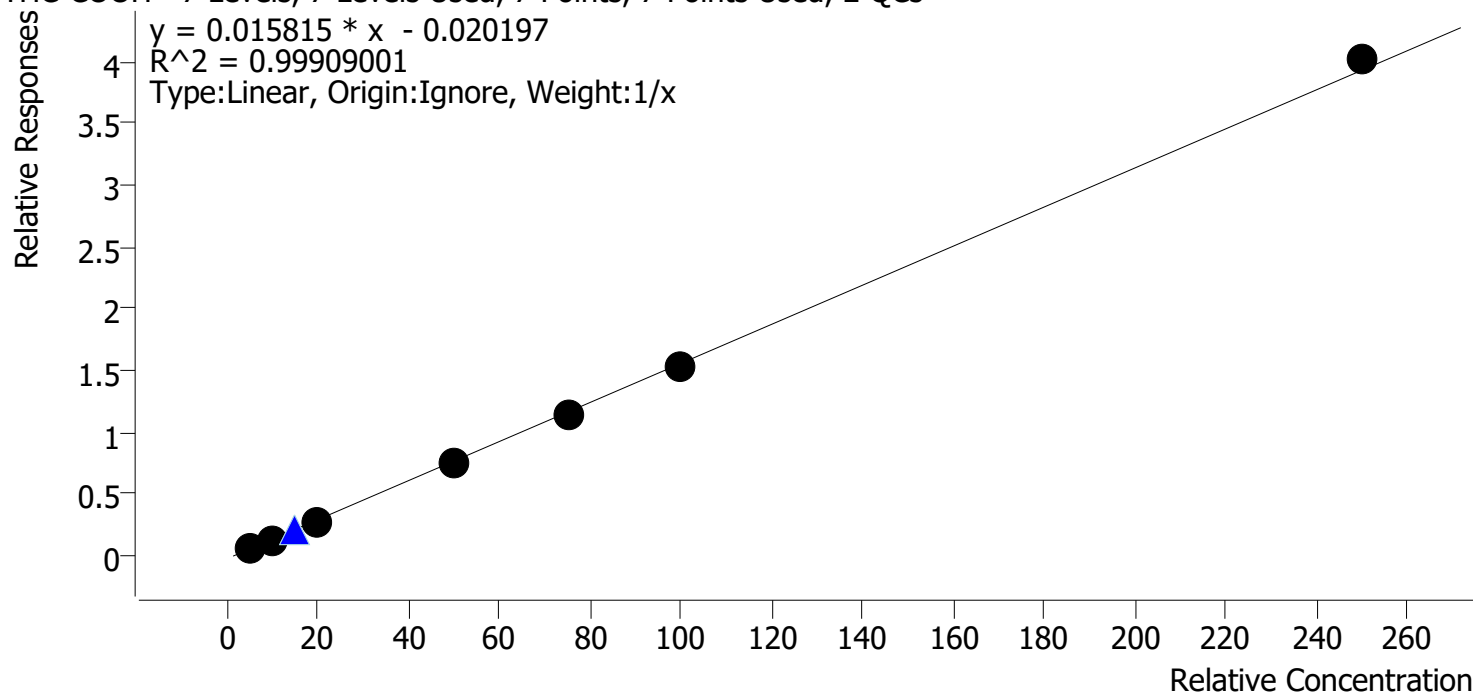
Compound Calibration Report

Byylee

Batch results	D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Last Cal. Update	5/14/2021 8:35 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, 2 QCs



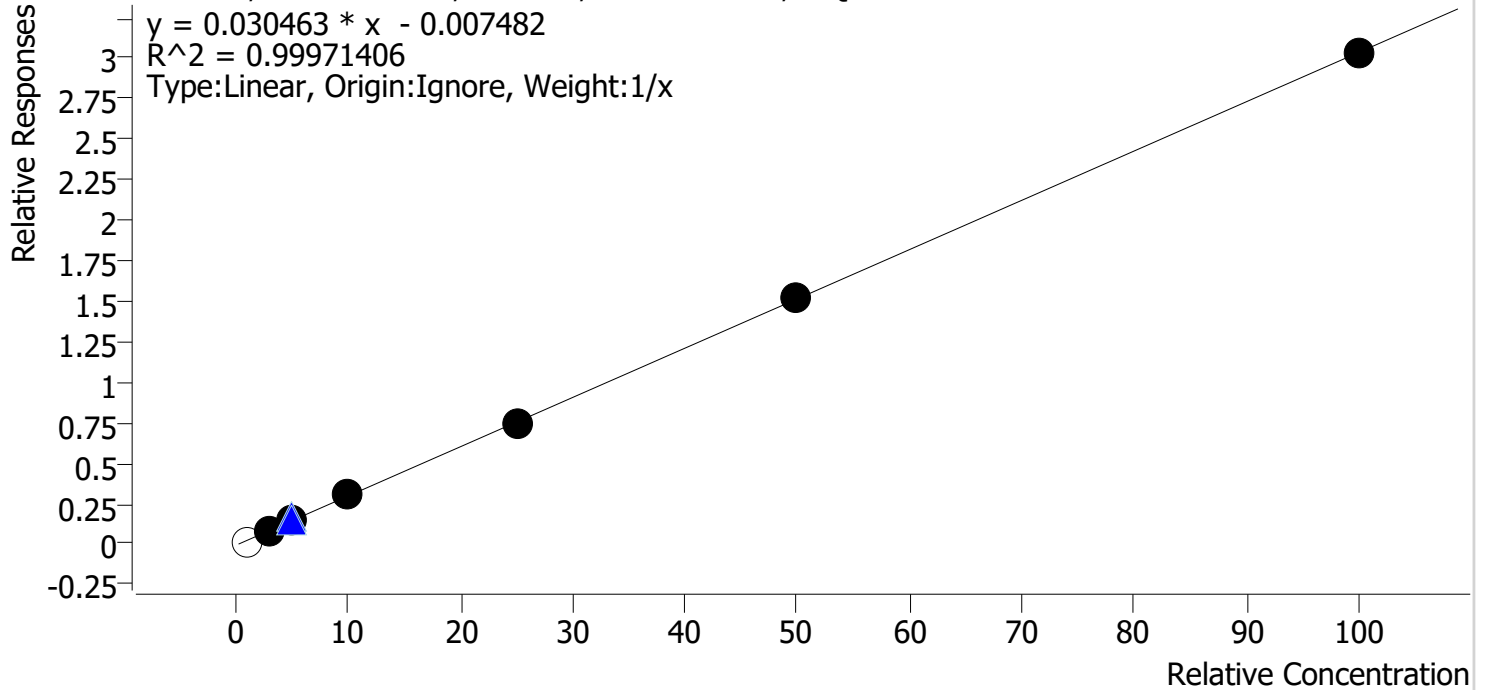
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.6	111.4
mj cal2	2	✓	10.0	9.8	98.0
mj cal 3	3	✓	20.0	19.1	95.6
mj cal 4	4	✓	50.0	48.1	96.2
mj cal 5	5	✓	75.0	74.1	98.8
mj cal 6	6	✓	100.0	97.9	97.9
mj cal 7	7	✓	250.0	255.4	102.2

Compound Calibration Report

Byylee

Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Last Cal. Update 5/14/2021 8:35 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, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	x	1.0	0.7	73.2
mj cal2	2	✓	3.0	2.9	97.3
mj cal 3	3	✓	5.0	4.9	98.1
mj cal 4	4	✓	10.0	10.6	105.7
mj cal 5	5	✓	25.0	24.7	98.9
mj cal 6	6	✓	50.0	50.2	100.3
mj cal 7	7	✓	100.0	99.7	99.7

cal 1 dropped did not meet accuracy requirements, poor peak shape

AM #27 Cannabinoids

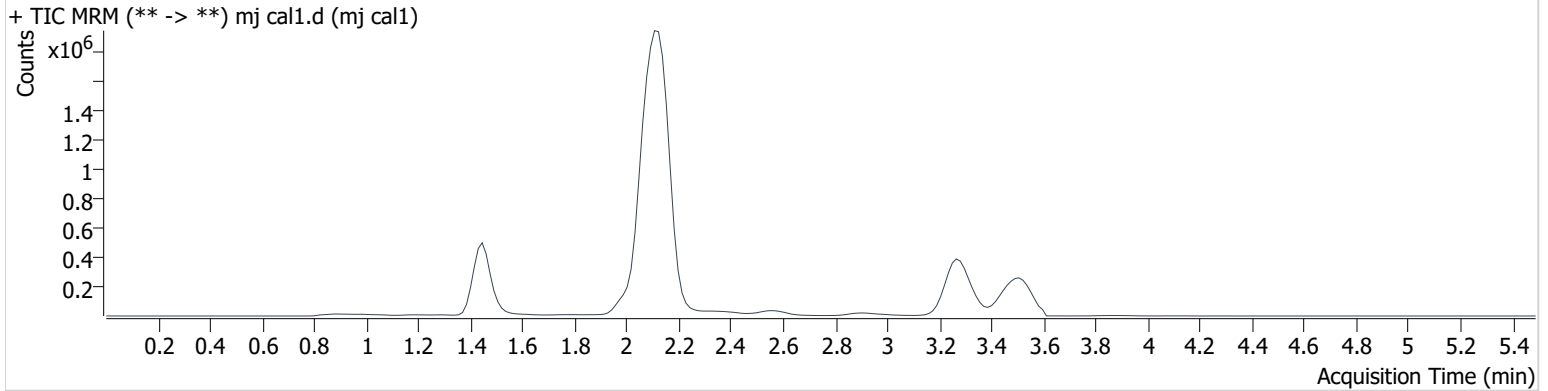
BWylie

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Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj cal1.d
Type	Cal	Sample	mj cal1
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 5:36:54 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	23868	∞	29.4 High	∞	1610161	0.732 ng/ml Low
THC-COOH	1.476	39087	24.5	33.5	71.0	575829	5.569 ng/ml
THC	3.317	25529	∞	35.7 High	∞	1047627	1.628 ng/ml

AM #27 Cannabinoids

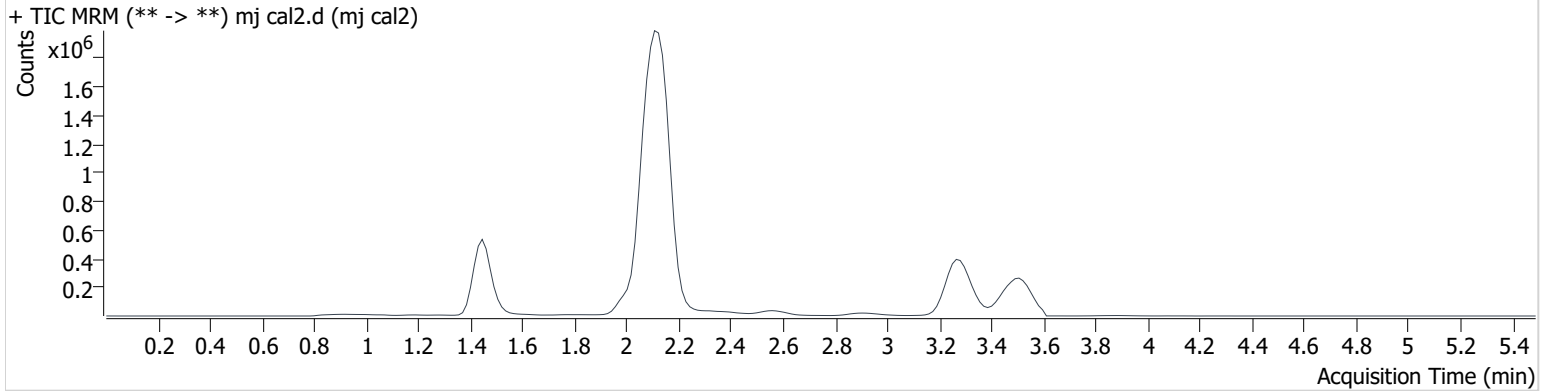
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Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 5:43:38 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	131014	∞	12.3	∞	1608692	2.919 ng/ml Low
THC-COOH	1.476	78505	59.0	35.8	86.2	582441	9.800 ng/ml
THC	3.302	74907	∞	26.8	∞	1046267	3.250 ng/ml

AM #27 Cannabinoids

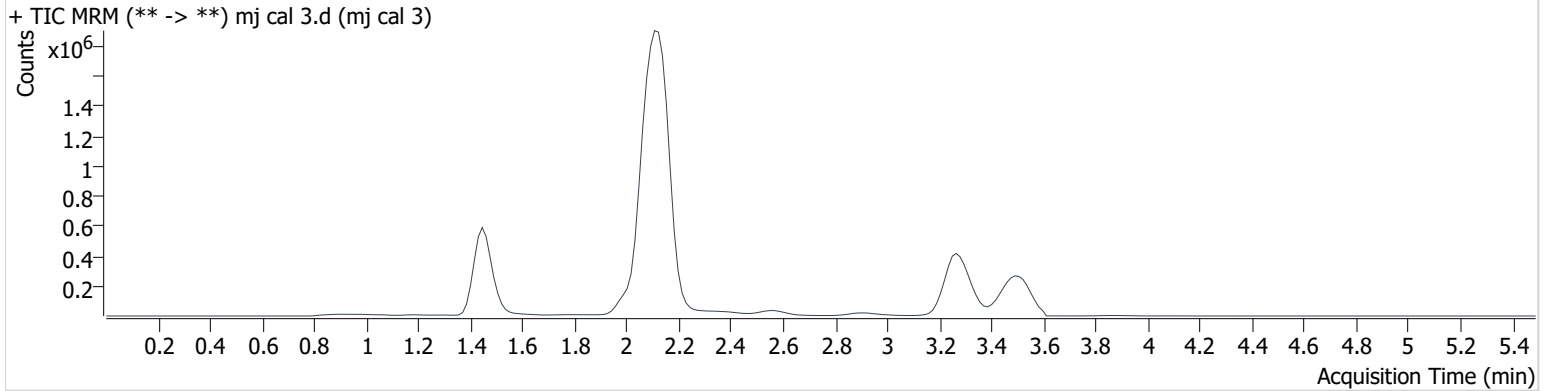
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Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-C1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 5:50:20 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	222408	∞	11.2	∞	1566913	4.905 ng/ml
THC-COOH	1.476	164509	119.6	36.8	141.6	583270	19.111 ng/ml
THC	3.317	130476	∞	24.6	∞	1068937	4.984 ng/ml

AM #27 Cannabinoids

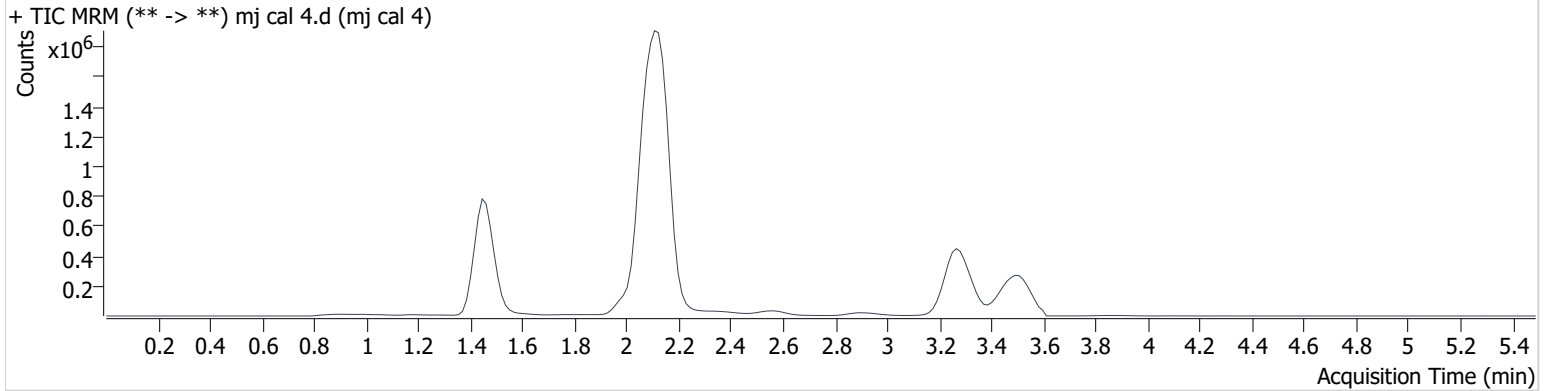
BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj cal 4.d
Type	Cal	Sample	mj cal 4
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 5:57:02 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	517899	∞	10.6	∞	1647308	10.566 ng/ml
THC-COOH	1.476	445600	1033474.4	36.4	608.7	602021	48.079 ng/ml
THC	3.302	287219	∞	23.9	∞	1131370	9.512 ng/ml

AM #27 Cannabinoids

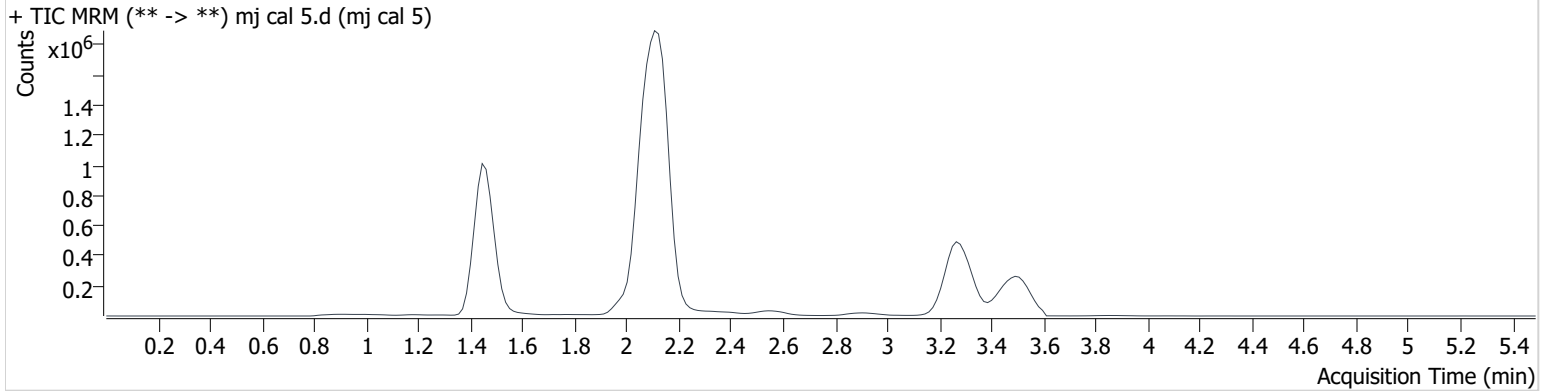
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Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 6:03:44 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	1153434	∞	11.5	∞	1547196	24.718 ng/ml
THC-COOH	1.476	663558	507.2	37.0	1266.9	576045	74.115 ng/ml
THC	3.302	719884	∞	23.3	∞	1063334	24.048 ng/ml

AM #27 Cannabinoids

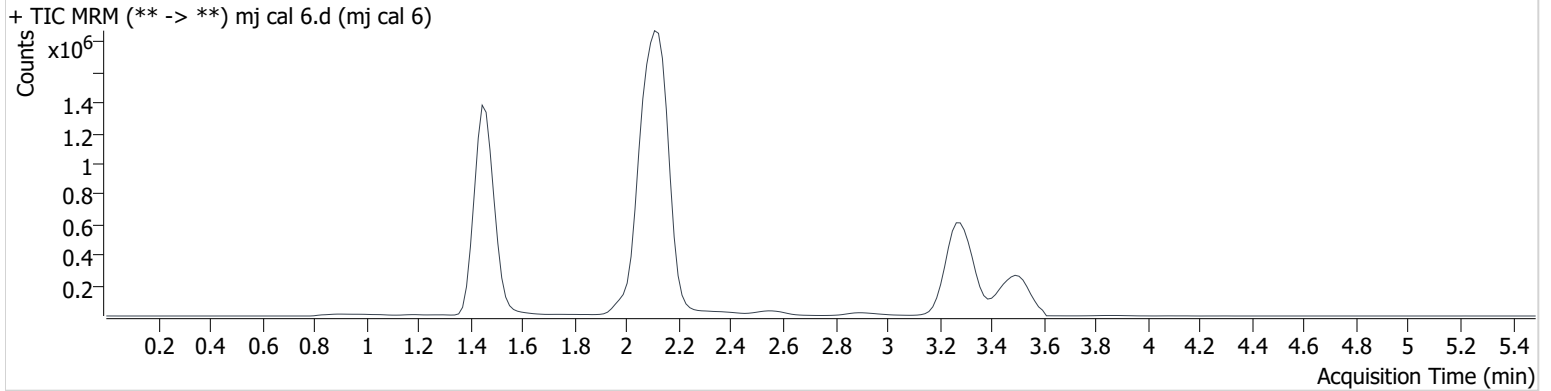
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Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj cal 6.d
Type	Cal	Sample	mj cal 6
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-F1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 6:10:26 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	2364426	∞	11.5	∞	1554703	50.170 ng/ml
THC-COOH	1.476	875975	1175.4	36.4	464.7	573096	97.926 ng/ml
THC	3.302	1524080	∞	23.4	∞	1075294	49.481 ng/ml

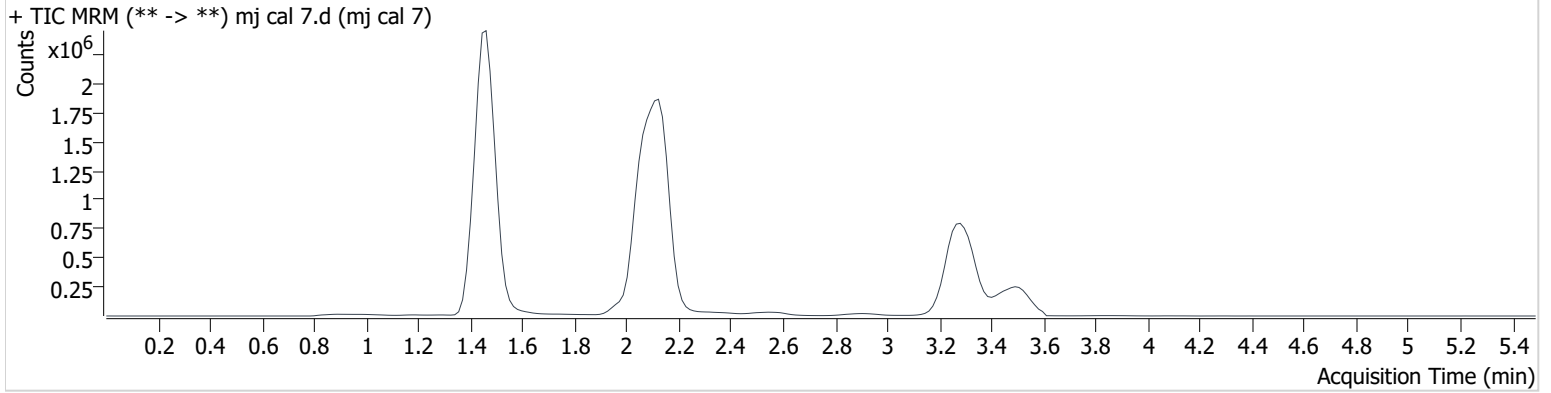
AM #27 Cannabinoids

BWylie

Batch results D:\MassHunter\Data\2021\am 27-28\051321\QuantResults\cannq.batch.bin
Calibration Last Update 5/14/2021 8:35:48 AM

Instrument	69679	Data File	mj cal 7.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	5/13/2021 6:17:08 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.451	4665249	∞	12.1	∞	1539530	99.722 ng/ml
THC-COOH	1.476	2158813	3320.6	36.8	997.8	537160	255.400 ng/ml
THC	3.302	3047351	∞	23.9	∞	1037145	101.726 ng/ml