



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

Extraction Date: 6/16/2020
Plate lot#: 200303

Analyst: Anne Nord
Plate Expiration: 09-03-2020

REVIEWED
By: Emily White at 1:23 pm, Jul 17, 2020

Mobile phase A: 0.1% Formic Acid in LCMS Water MTBE
Mobile phase B: 0.1% Formic acid in Acetonitrile Hexane
LCMS Methanol

Blank Blood Lot: 20A52255 **Urine Blank:** 6920 **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.

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 250 ul 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, 500 ul saturated phosphate buffer in urine 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:

THC-OH not evaluated

Urine samples were re-extracted and run as an additional quality check, due to variation in response from screen to confirmation on Sample C2020-0994.



Toxicology AM method 27/26 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 2/13/20 Exp: 8/13/20 lot 21320 by AMN

Drug	lot	expiration
C-THC	FE07171501	9/1/2020
THC-OH	FE07721601	7/1/2021
THC	FE001041701	3/1/2022

AM 27/26 blood control 100 ul working solution lot (91319) in 9900 ul blood lot (20A52255)

ppd 02/13/20 Exp 08/13/20 lot b81320 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-THC by AMN

AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (6920)

ppd 4/17/20 Exp 9120 lot u101720 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC by BAW out of use 6/8/2020

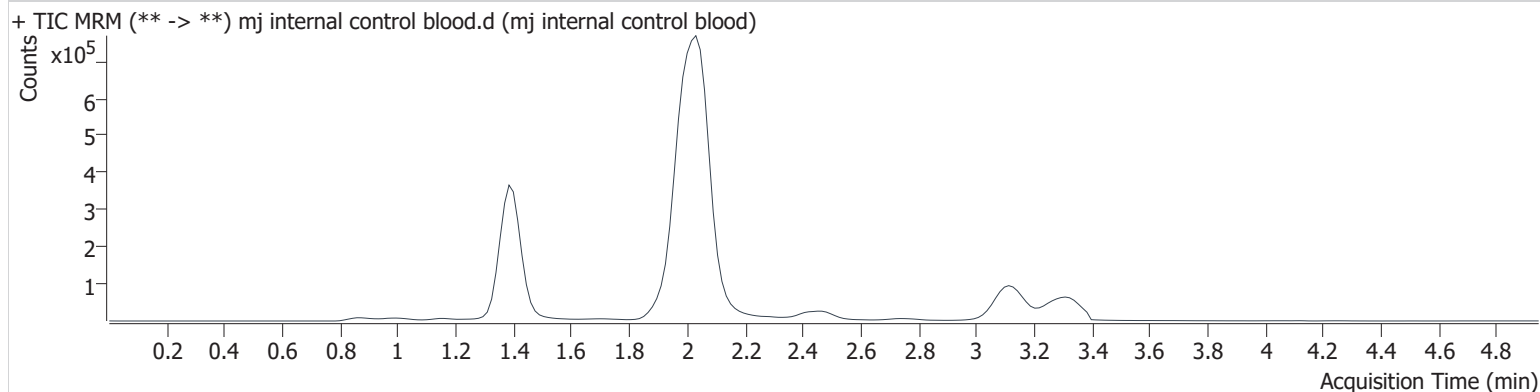
ppd 6/9/20 exp 8/13/20 lot 6920 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC by amn

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj internal control blood.d
Type	QC	Sample	mj internal control blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 11:40:00 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	89999	∞	9.7	∞	811556	5.399 ng/ml
THC-COOH	1.415	110060	297.9	39.0	425.5	645431	15.667 ng/ml
THC	3.153	18173	∞	28.1	387.8	481646	4.336 ng/ml

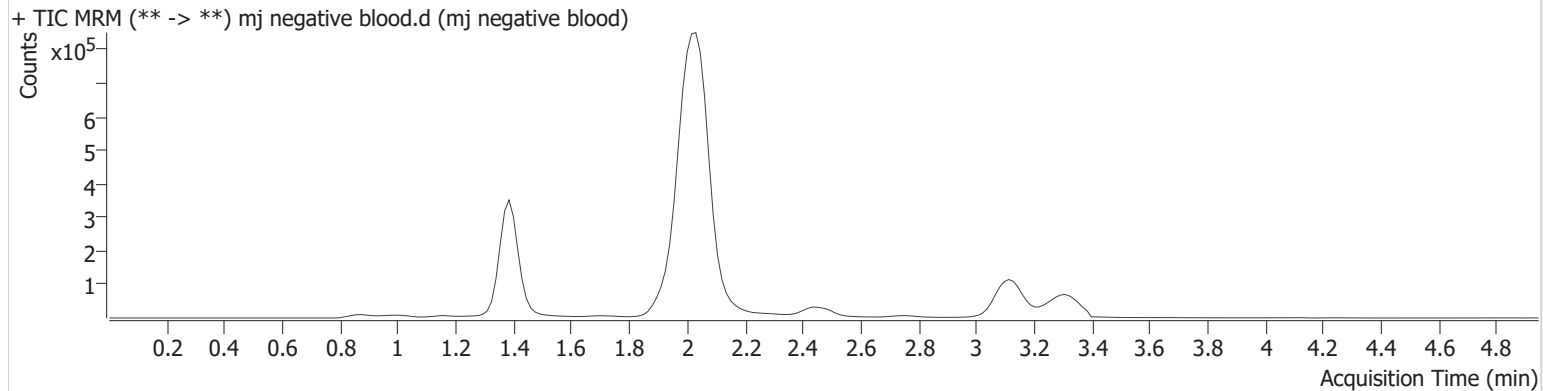


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj negative blood.d
Type	Sample	Sample	mj negative blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 11:47:43 AM		
Sample Info.			

Sample Chromatogram

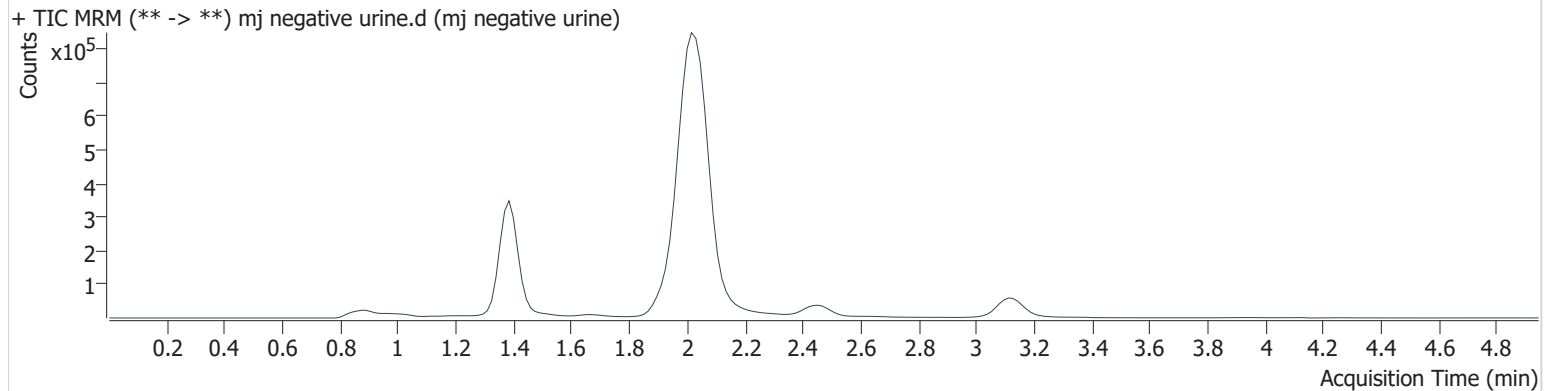


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-B2	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 11:55:26 AM		

Sample Chromatogram



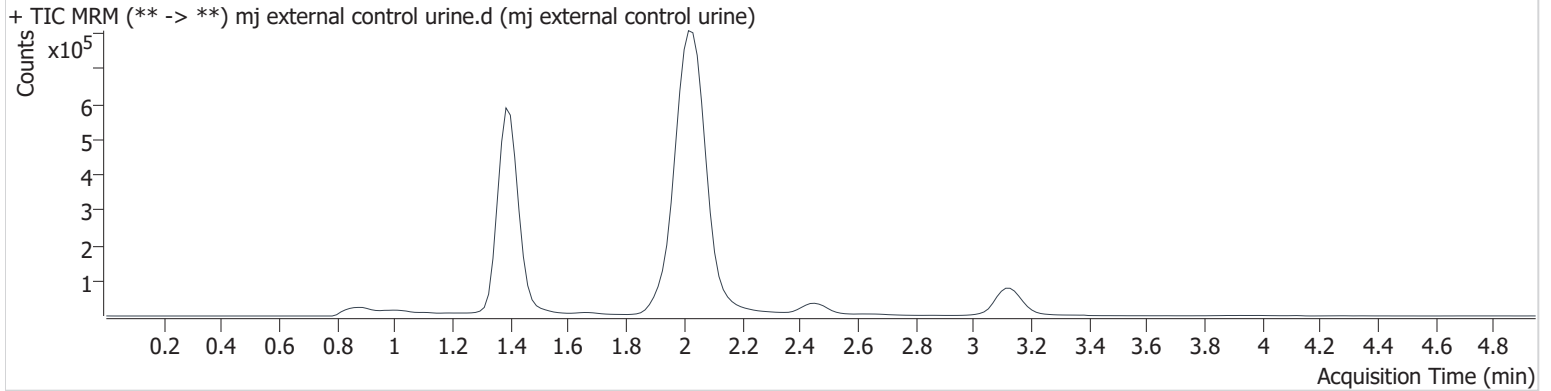
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj external control urine.d
Type	Sample	Sample	mj external control urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-C2	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 12:03:09 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	387667	∞	52.8 High	∞	1138415	16.603 ng/ml
THC-COOH	1.415	249215	1260.4	38.1	2819.6	540804	40.690 ng/ml
THC	3.138	66030	∞	24.7	∞	448848	15.831 ng/ml

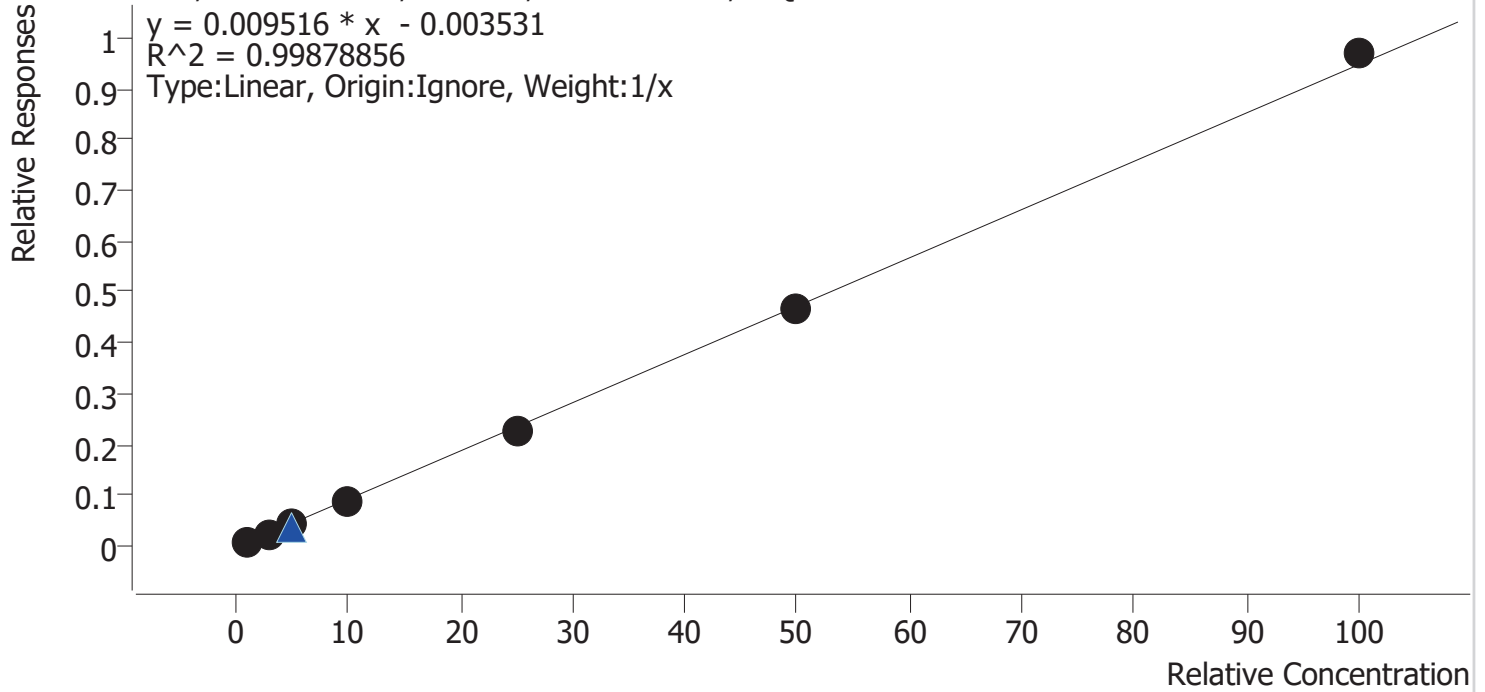
Compound Calibration Report



Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Last Cal. Update 6/16/2020 1:25 PM
Analyst Name ISP\datastor
Analyte THC

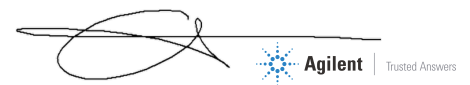
Internal Standard THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	1.2	120.1
mj cal2	2	✓	3.0	2.9	96.6
mj cal 3	3	✓	5.0	4.6	91.3
mj cal 4	4	✓	10.0	9.4	93.8
mj cal 5	5	✓	25.0	24.2	96.9
mj cal 6	6	✓	50.0	49.5	98.9
mj cal 7	7	✓	100.0	102.3	102.3

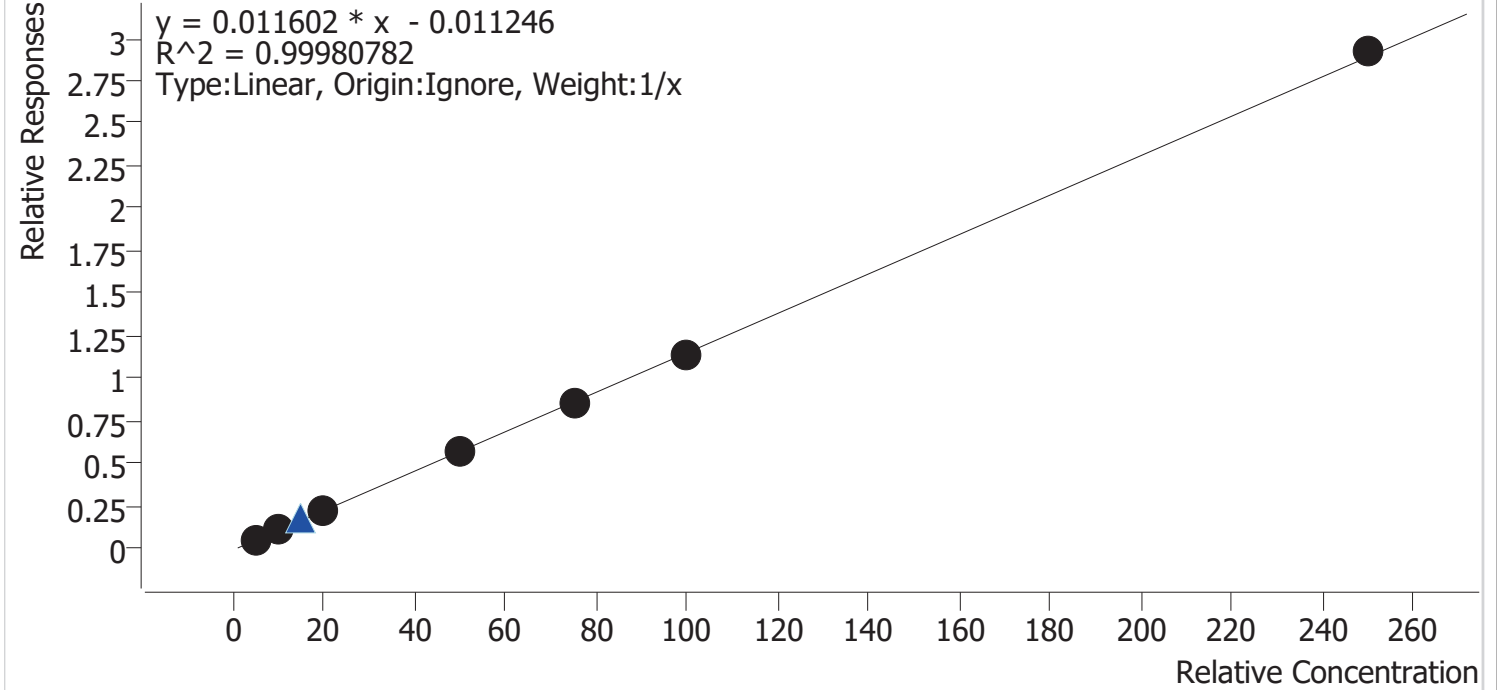
Compound Calibration Report



Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Last Cal. Update 6/16/2020 1:25 PM
Analyst Name ISP\datastor
Analyte THC-COOH

Internal Standard THC-COOH-d9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



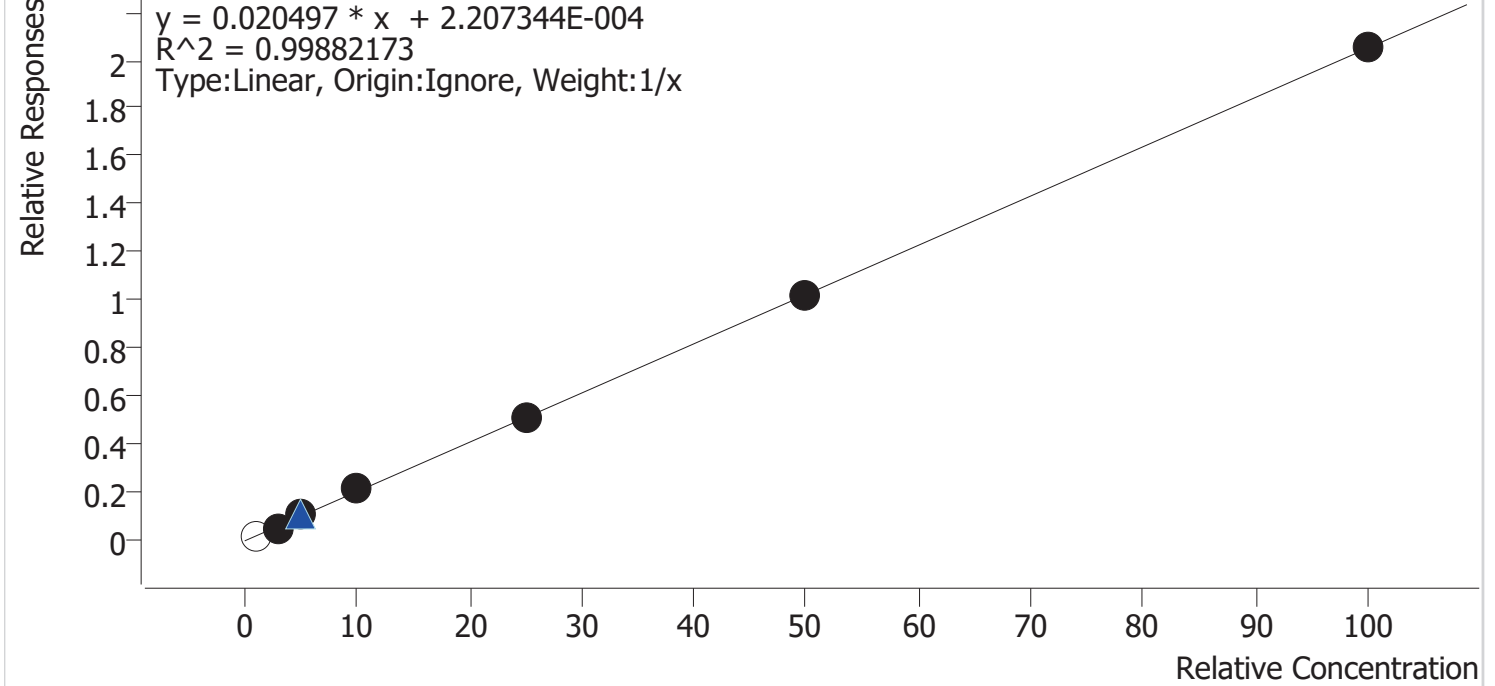
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.2	103.5
mj cal2	2	✓	10.0	9.9	98.8
mj cal 3	3	✓	20.0	20.0	100.0
mj cal 4	4	✓	50.0	49.8	99.6
mj cal 5	5	✓	75.0	73.9	98.5
mj cal 6	6	✓	100.0	98.6	98.6
mj cal 7	7	✓	250.0	252.7	101.1

Compound Calibration Report



Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Last Cal. Update 6/16/2020 1:25 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	×	1.0	1.0	104.0
mj cal2	2	✓	3.0	2.6	85.2
mj cal 3	3	✓	5.0	5.6	111.2
mj cal 4	4	✓	10.0	10.6	105.6
mj cal 5	5	✓	25.0	24.7	98.8
mj cal 6	6	✓	50.0	49.6	99.1
mj cal 7	7	✓	100.0	100.1	100.1

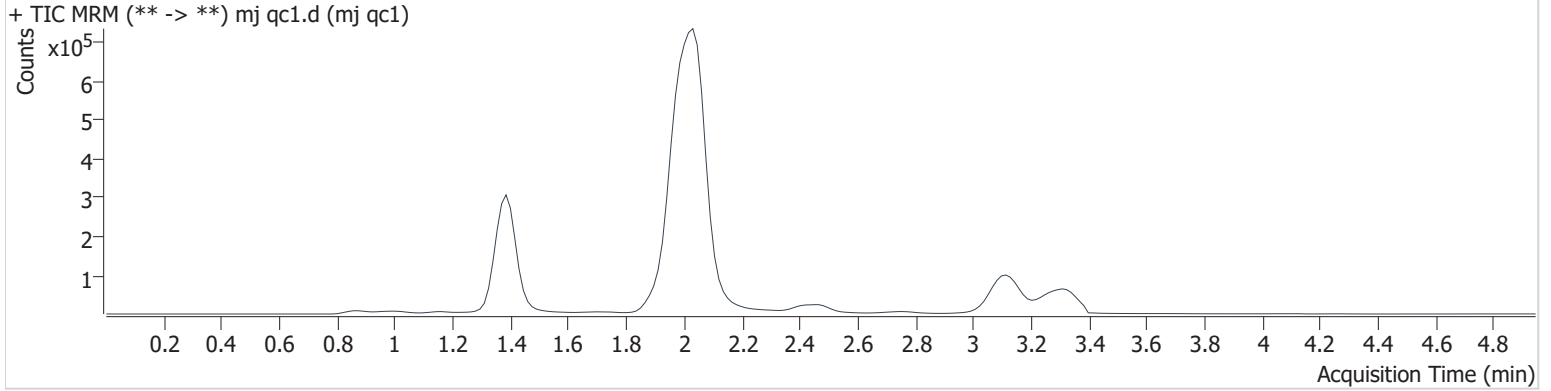
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj qc1.d
Type	Cal	Sample	mj qc1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 10:45:56 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	17443	9.6 Low	13.4 High	∞	809677	1.040 ng/ml	Low
THC-COOH	1.415	30460	23.0	42.5	148.7	623958	5.177 ng/ml	Low
THC	3.123	4223	∞	24.8	40.4	534477	1.201 ng/ml	Low

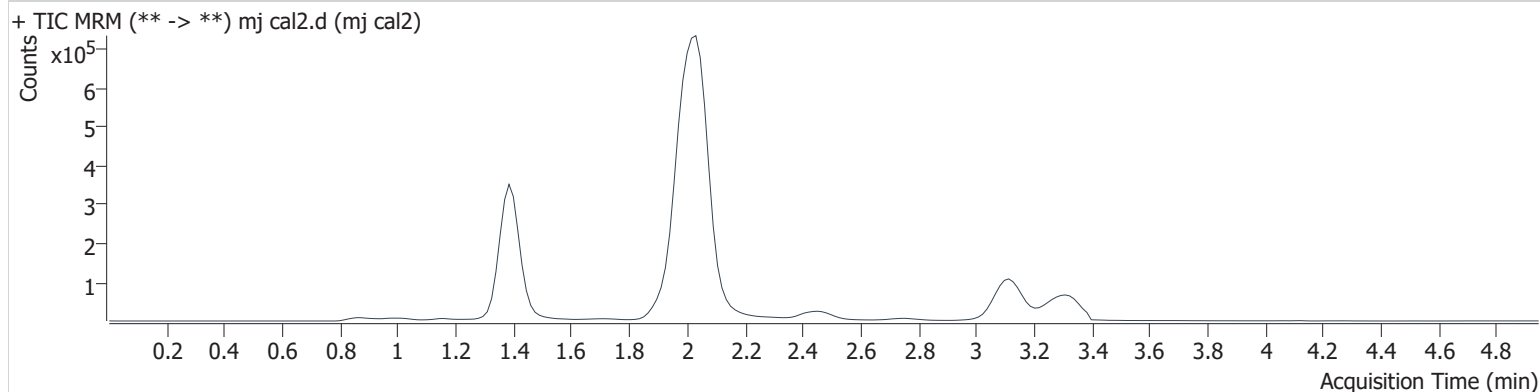
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 10:53:41 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.390	44491	∞	11.6	∞	845205	2.557 ng/ml	Low
THC-COOH	1.415	65511	139.2	40.6	258.1	634023	9.875 ng/ml	Low
THC	3.138	12831	∞	26.8	∞	533375	2.899 ng/ml	Low

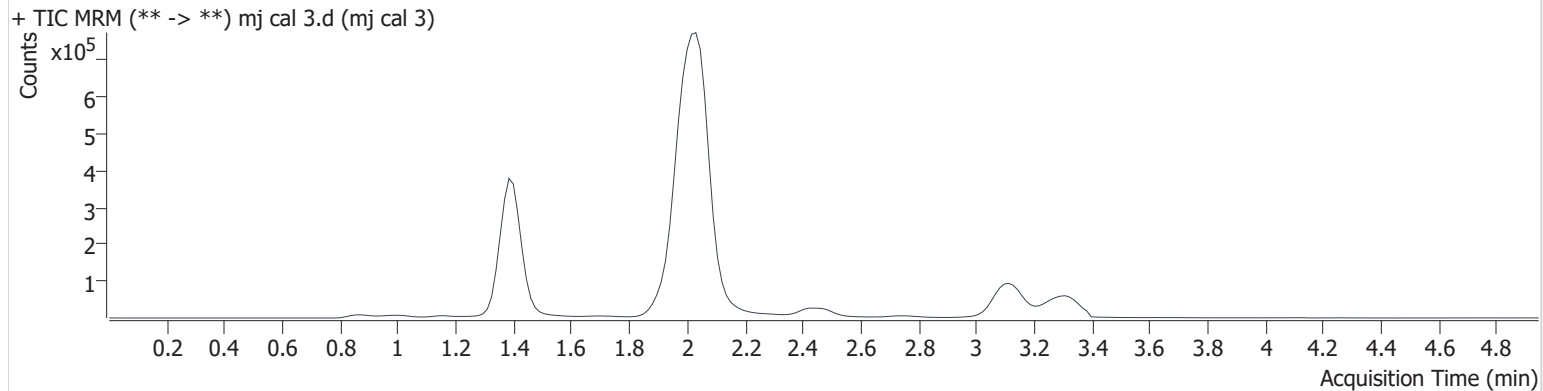
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-C1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 11:01:24 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	93133	∞	9.0	∞	815511	5.561 ng/ml
THC-COOH	1.415	138786	1403.4	39.5	75391.0	628743	19.995 ng/ml
THC	3.138	19237	∞	27.0	24539.95759108.5	481779	4.567 ng/ml

AM #27 Cannabinoids

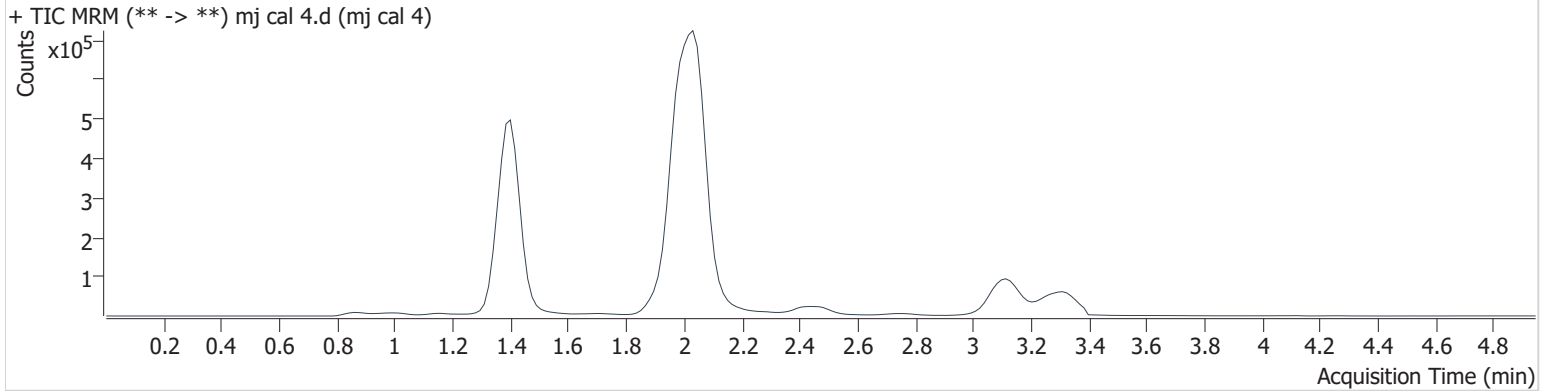


Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj cal 4.d
Type	Cal	Sample	mj cal 4
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 11:09:07 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	177235	∞	10.0	∞	818053	10.559 ng/ml
THC-COOH	1.415	348705	752.3	40.9	736.4	615821	49.776 ng/ml
THC	3.138	39480	∞	25.5	∞	460545	9.380 ng/ml

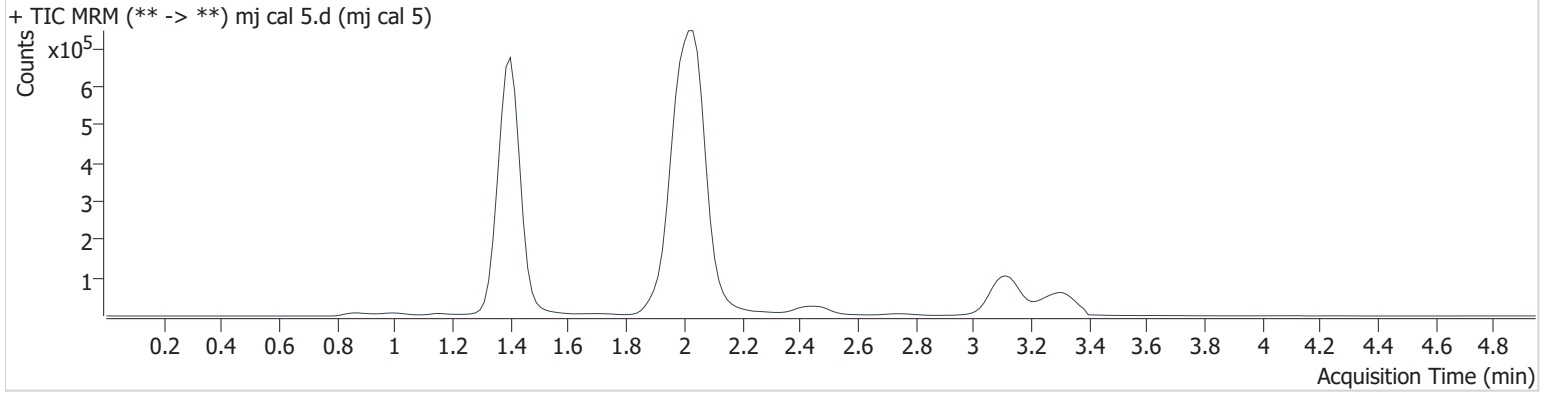
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 11:16:50 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	419580	∞	10.8	∞	828705	24.690 ng/ml
THC-COOH	1.415	532947	446.8	41.6	2295.5	630296	73.851 ng/ml
THC	3.138	107812	∞	23.5	∞	474989	24.224 ng/ml

AM #27 Cannabinoids

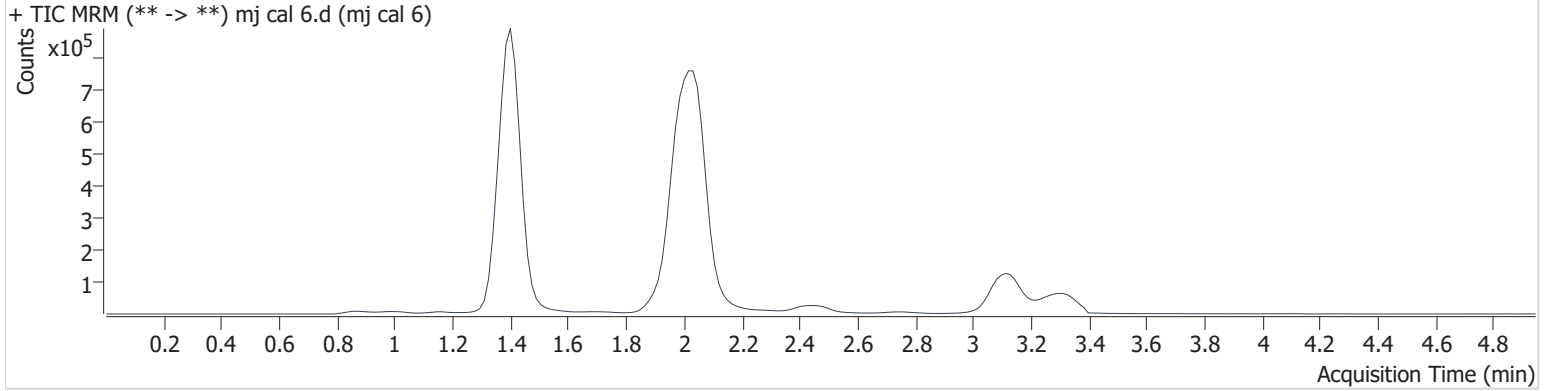


Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-F1
Injection Volume 10
Acq. Date-Time 6/16/2020 11:24:34 AM
Sample Info.

Data File mj cal 6.d
Sample mj cal 6
Operator Anne Nord
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	879965	∞	11.3	∞	866100	49.557 ng/ml
THC-COOH	1.415	712297	157.0	42.7	1571.4	628674	98.629 ng/ml
THC	3.138	225130	∞	23.7	∞	481818	49.473 ng/ml

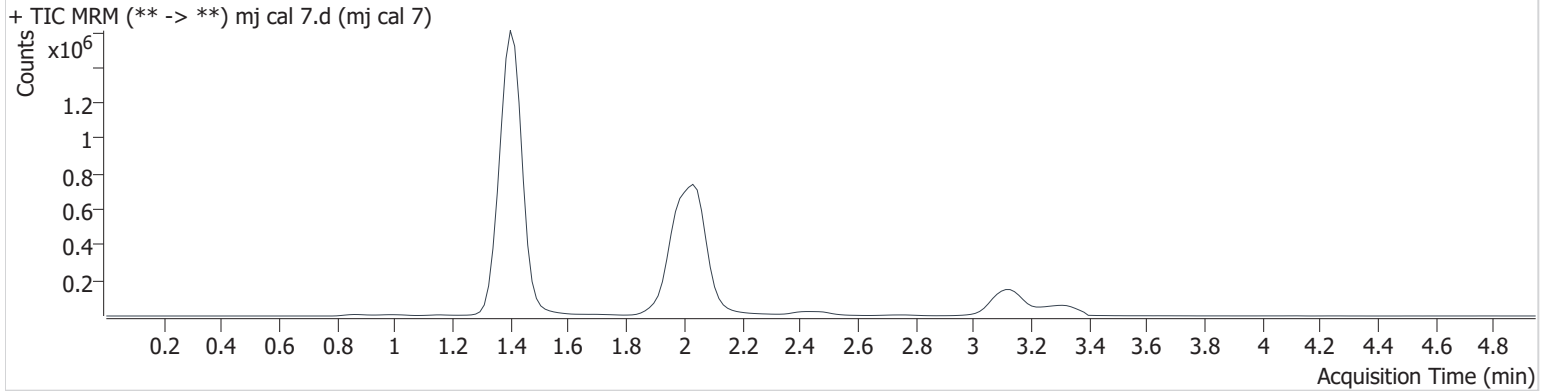
AM #27 Cannabinoids



Batch results D:\MassHunter\Data\2020\am28-27 061620\QuantResults\mj.batch.bin
Calibration Last Update 6/16/2020 1:25:47 PM

Instrument	69679	Data File	mj cal 7.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	6/16/2020 11:32:17 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	1712146	∞	11.7	∞	834575	100.076 ng/ml
THC-COOH	1.415	1710554	2984874.3	41.9	2917.6	585713	252.696 ng/ml
THC	3.138	437759	∞	25.0	∞	451522	102.256 ng/ml