BW

AM# 30: THC and Metabolites Screen in Blood by LC-QTOF

Extraction Date: 11/4/2020 Analyst: Britany Wylie
Plate lot#: 200723 Plate Expiration: 1-23-2021

Mobile phase A: 0.1% Formic acid in Water **Mobile phase B:** 0.1% Formic acid in MeOH
0.1% Formic Acid in Water MTBE Hexane
1N KOH Saturated Phosphate Buffer
Blank Blood Lot: 20G20792 **Urine Blank:** 10120
Column: Phenomenex Phenyl Hexyl (4.6x50mm: 2.6 um) **LCMS-QTOF ID**: 70044

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 **1000 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: K52558g**
Pipette 1000 ul urine to analytical (standards) plate.
☒ 3. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
☒ 4. Pipette **500 µL 0.1% formic acid in blood** wells **500 ul saturated phosphate buffer in urine** wells of analytical plate.
☒ 5. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
☒ 6. Transfer **800 µL 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 **2.25 mL MTBE** (add in 3 increments of 750 µL).
☒ 10. Wait 5 minutes.
☒ 11. Apply positive pressure for approx. 10-15 seconds. ***(12-15 PSI- Selector to the left).***
☒ 12. Add **2.25 mL hexane** (add in 3 increments of 750 µL).
☒ 13. Wait 5 minutes.
☒ 14. Apply positive pressure for approx. 10-15 seconds. ***(12-15 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 **100 µL 100% LCMS MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

☒ 1. Create batch and process data.
☒ 2. Calculated sample concentration of 3 ng/mL or greater for THC and THC-OH, a calculated sample concentration of 10 ng/mL or greater for Carboxy-THC.
☒ 3. Retention time within +/- 2% or +/-0.100 min whichever is greater of the average retention time of the calibrators.
☒ 4. Did all QCs pass for each analyte? Yes
☒ 5. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

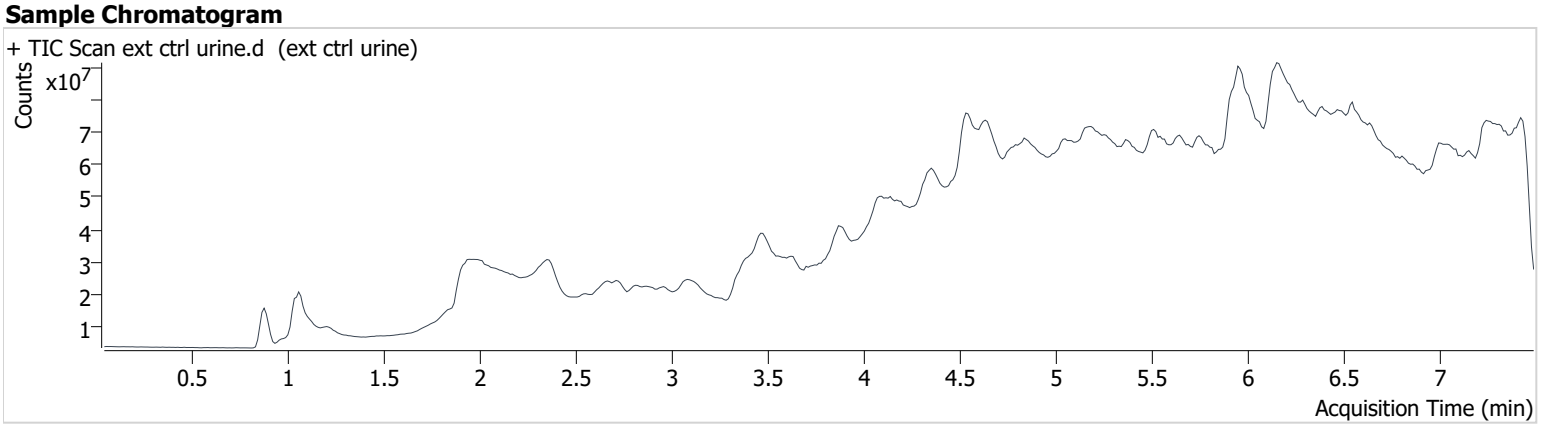
COMMENTS: *samples were ran on the QTOF due to variability in low end calibrators only c-THC was evaluated, the samples were ran on the QTOF and then reconstituted and run on the LCQQQ.*
Hydroxy-THC and THC not evaluated in this run. Low response and variability in the lower end calibrators.

AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\11420\QuantResults\thcs qtof.batch.bin
Calibration Last Update 11/6/2020 11:31:15 AM

Instrument	70044	**Data File**	ext ctrl urine.d
Type	Sample	**Sample**	ext ctrl urine
Acq. Method	THC Screen 1122.m	**Operator**	Britany Wylie
Sample Position	P2-B2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/4/2020 4:36:39 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.613	787962	-0.18	83.5	1799934	41.5956 ng/ml

Toxicology AM method 27/26 external prep information

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working solution 15 ug/ml in meoh C-THC, THC-OH, 7.5 ug/ml THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH
Ppd 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

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

		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	

AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine **out of use**

ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

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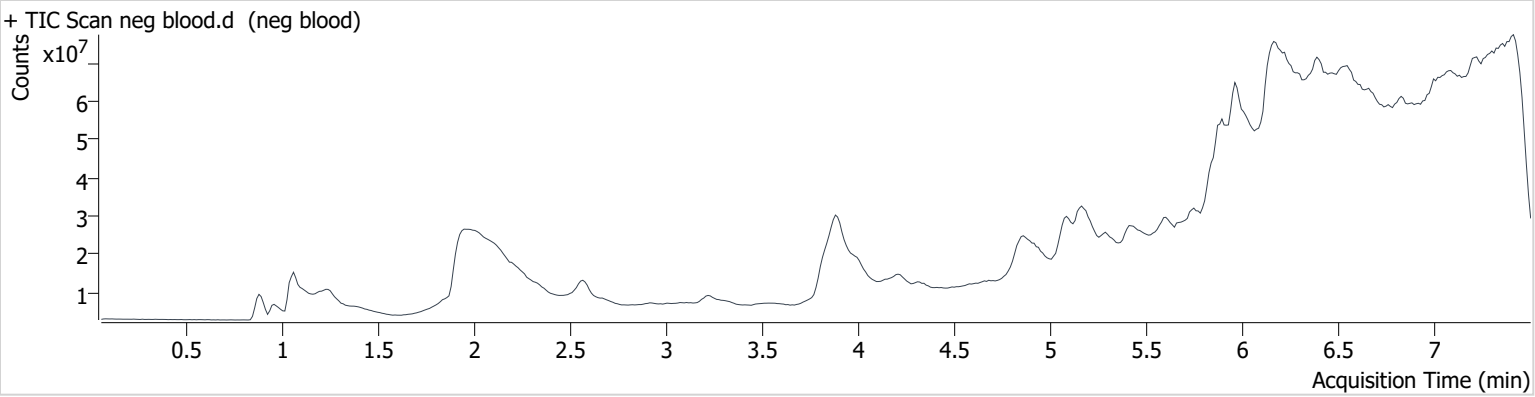
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\11420\QuantResults\thcs qtof.batch.bin
Calibration Last Update 11/6/2020 11:31:15 AM

Instrument	70044	**Data File**	neg blood.d
Type	Sample	**Sample**	neg blood
Acq. Method	THC Screen 1122.m	**Operator**	Britany Wylie
Sample Position	P2-C4	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/4/2020 7:19:41 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.	
THC-COOH	6.611	103448	-5.58	0.0	1905010	5.1820 ng/ml	*neg due to mass score <70

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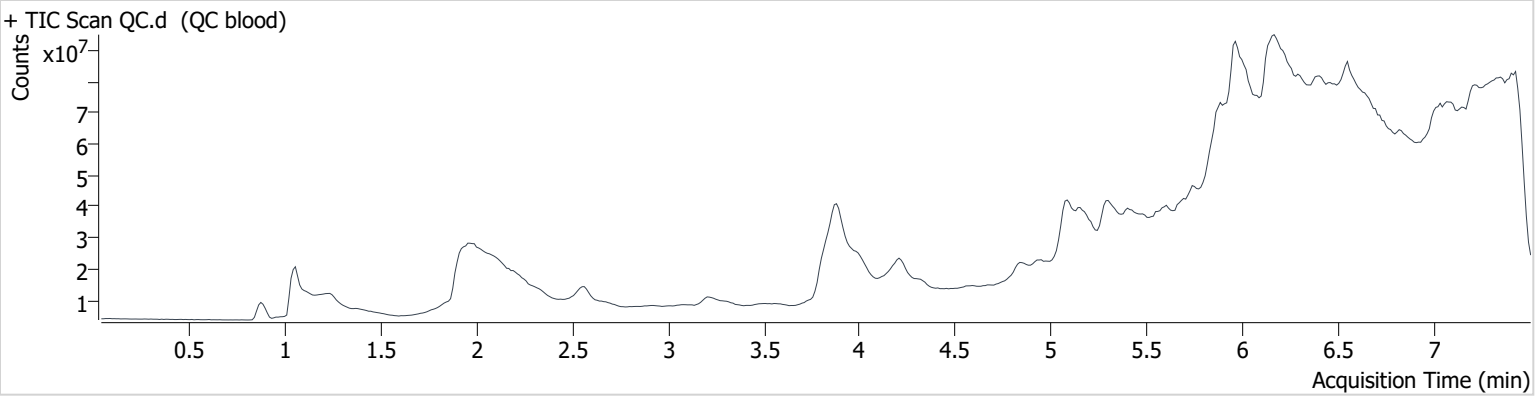
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\11420\QuantResults\thcs qtof.batch.bin
Calibration Last Update 11/6/2020 11:31:15 AM

Instrument	70044	**Data File**	QC.d
Type	QC	**Sample**	QC blood
Acq. Method	THC Screen 1122.m	**Operator**	Britany Wylie
Sample Position	P2-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/4/2020 4:17:28 PM		
Sample Info.			

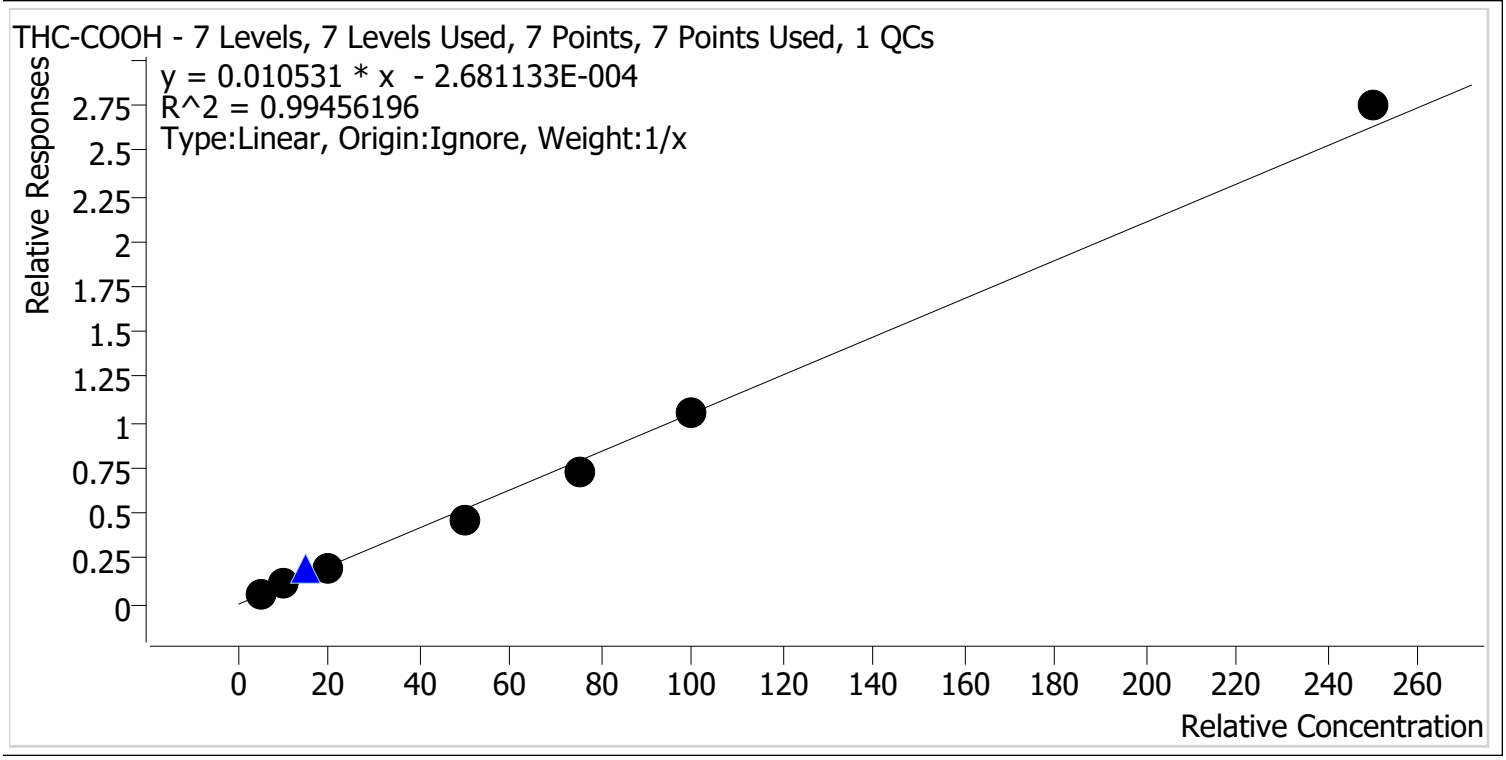
Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.610	459630	-0.25	82.9	2389893	18.2880 ng/ml

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020\am 30\11420\QuantResults\thcs qtof.batch.bin
Last Cal. Update	11/6/2020 11:31 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
CAL 1	1	✔	5.0	5.5	110.2
CAL 2	2	✔	10.0	11.5	114.6
CAL 3	3	✔	20.0	17.8	89.0
CAL 4	4	✔	50.0	44.6	89.2
CAL 5	5	✔	75.0	68.7	91.6
CAL 6	6	✔	100.0	101.0	101.0
CAL 7	7	✔	250.0	260.9	104.4

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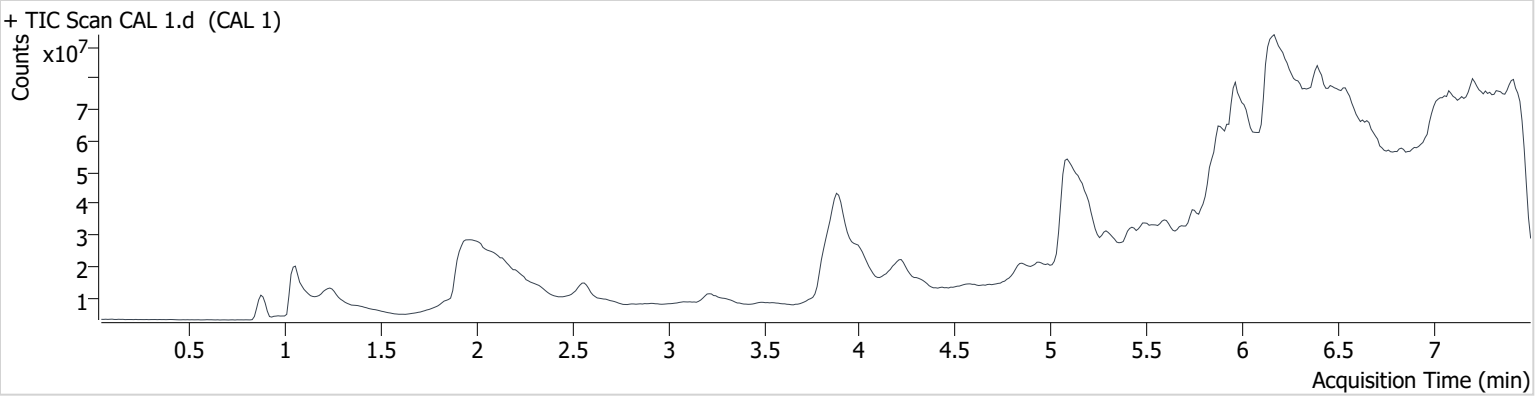
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\11420\QuantResults\thcs qtof.batch.bin
Calibration Last Update 11/6/2020 11:31:15 AM

Instrument	70044	**Data File**	CAL 1.d
Type	Cal	**Sample**	CAL 1
Acq. Method	THC Screen 1122.m	**Operator**	Britany Wylie
Sample Position	P2-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/4/2020 3:10:13 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	Mass Accuracy	Mass Abundance Score	ISTD Resp.	Final Conc.
THC-COOH	6.610	117922	-4.83	53.3	2040790	5.5124 ng/ml