REVIEWED By Tamara Salazar at 8:37 am, Mar 07, 2024

LAB CASE	ITEM	ITEM TYPE	DESCRIPTION
M2024-0391	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2024-0478	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2024-0580	4	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3296	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0195	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0255	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0276	2	BLOOD	AM 27 Blood THC Quant by LC-QQQ
P2024-0280	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0307	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0393	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0412	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0419	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0446	1	BCK	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 02/29/2024 Plate lot#: 231212 **Mobile phase A**: 0.1% Formic Acid in LCMS Water **Blank Blood Lot**: Lampire 23E52981 **LCMS-QQQ ID**: 069901 Analyst: Celena Shrum Plate Retest Date: 06/12/2024 **Mobile phase B:** 0.1% Formic acid in Acetonitrile **Column**: Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

Pre-Analytic:

- \boxtimes 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- \boxtimes 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 (if applicable): add 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.
- ☑ 3. Using a calibrated pipette, add 1000µl blood or 1000µl hydrolyzed urine into the appropriate wells of the analytical (standards) plate. Pipette ID: #42
- \boxtimes 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- S. Add 500µL of 0.1% formic acid in water to blood samples or 500µl of saturated phosphate buffer to urine samples to the appropriate wells of the analytical plate.
- \boxtimes 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- ☑ 7. Transfer **800µL of blood+acid mixture or urine+acid** to corresponding wells of SLE+ plate.
- ☑ 8. 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
- \boxtimes 9. Wait 5 minutes.
- ☑ 10. Add 2.25mL MTBE. (Add in 3 increments of 750uL)
- \boxtimes 11. Wait 5 minutes.
- ☑ 12. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- ⊠ 13. Add 2.25mL Hexane. (Add in 3 increments of 750uL)
- \boxtimes 14. Wait 5 minutes.
- ☑ 15. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- ☑ 16. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. SPE Dry ID: 067103
- Σ 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- \boxtimes 1. Create batch and process data.
- \boxtimes 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 lng/mL and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/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. THC concentrations of 1-3ng/mL will be reported qualitatively.
- \boxtimes 5. Did all QCs pass for each analyte? (if not, describe in comments section)
- \boxtimes 6. Enter QCs into control charting.
- ☑ 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Curve limits: THC-OH 3-100 -calibrator 1 dropped due to poor chromatography/accuracy

	1	2	3	4	5	6
а					P2024-0280-2	QC 1
b				M2024-0391-1	P2024-0307-1*	cal 100 ng
с				M2024-0478-1	P2024-0393-1	cal 50 ng
d				M2024-0580-4	P2024-0412-1*	cal 25 ng
e				P2023-3296-1	P2024-0419-3	cal 10ng
f			P2024-0307-1*	P2024-0195-1	P2024-0446-1	cal 5 ng
g			P2024-0255-1*	P2024-0255-1*	NEG Blood	cal 3 ng
h			P2024-0412-1*	P2024-0276-2	QC 2	cal 1ng

*Moved during SLE portion of the extraction due to blood clots



Batch resultsD:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P1-G5 10 2/29/2024 4:54:37 PM Data File Sample Operator Comment MJ Negative Blood.d MJ Negative Blood Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P1-A6 10 2/29/2024 4:28:24 PM Data File Sample Operator Comment MJ QC Control Blood.d MJ QC Control Blood Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.



D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 3/5/2024 9:52:07 AM

Instrument	Falco (069
Туре	QC
Acq. Method	AM 27 Ag
Sample Position	P1-H5
Injection Volume	10
Acq. Date-Time	2/29/2024
Sample Info.	

9901) ilent Method.m 4 11:01:50 PM

Data File Sample Operator Comment QC end MJ.d QC end MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.

Sample Chromatogram





AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results Last Cal. Update Analyst Name	D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin 3/5/2024 9:52 AM ISP\datastor							
Analyte	THC	Internal Standard	THC-D3					
THC - 7 Levels,	vels Used, 7 Points, 7 Points Used, 2 QCs 378 * x - 0.002572 99959057 ar, Origin:Ignore, Weight:1/x	60 70 80	90 100 Relative Concentration					

Level	Enabled	Expected Concentration	Final Concentration	Accuracy
1	~	1.0	1.1	111.5
2	~	3.0	3.0	98.7
3	V	5.0	4.7	94.2
4	V	10.0	9.6	96.2
5	V	25.0	24.4	97.7
6	V	50.0	50.5	101.0
7	~	100.0	100.7	100.7
	Level 1 2 3 4 5 6 7	Level Enabled 1 י 2 י 3 י 4 · 5 · 6 · 7 ·	Level Enabled Expected Concentration 1 ✓ 1.0 2 ✓ 3.0 3 ✓ 5.0 4 ✓ 10.0 5 ✓ 25.0 6 ✓ 50.0 7 ✓ 100.0	LevelEnabledExpected ConcentrationFinal Concentration1'1.01.12'3.03.03'5.04.74'10.09.65'25.024.46'50.050.57'100.0100.7



AM #27 Cannabinoids Quant. Calibration Curve Report

		~~		_				
Analyte	ТНС-СООН	Internal Standard	THC-COOH-D9					
Analyst Name	ISP\datastor							
Last Cal. Update	3/5/2024 9:52 AM							
Batch results D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin								



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	5.0	5.4	108.5
Cal 2 MJ	2	~	10.0	9.7	96.8
Cal 3 MJ	3	~	20.0	19.0	95.0
Cal 4 MJ	4	~	50.0	48.8	97.6
Cal 5 MJ	5	~	75.0	75.7	101.0
Cal 6 MJ	6	~	100.0	101.0	101.0
Cal 7 MJ	7	r	250.0	250.4	100.2



AM #27 Cannabinoids Quant Calibration Curve Report

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Batc	h resu	lts	ts D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin										
Last	Cal. U	pdate	3/5/2024 9:52 AM										
Anal	yst Na	me	ISP\data	stor									
Anal	yte		THC-OH					Intern	al Standard	ł	THC-OH	-D3	
Relative Responses	C-OH - 1.6 1.2 1.2 0.8 0.6 0.4 0.2 0 ⁻	7 Levels, y = 0.01 R^2 = 0 Type:Lin	6 Levels U 6584 * x .99992256 ear, Origir	Jsed, 7 F - 0.0032 h:Ignore,	Points, 6 03 , Weight	Points U :1/x	sed, 2 Q	Cs					
		0	10	20	30	40	50	60	70	80	90	100	

Relative Concentration									
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy				
Cal 1 MJ	1	×	1.0	1.3	132.9				
Cal 2 MJ	2	~	3.0	3.0	100.3				
Cal 3 MJ	3	~	5.0	5.1	101.7				
Cal 4 MJ	4	~	10.0	9.9	98.9				
Cal 5 MJ	5	~	25.0	24.6	98.4				
Cal 6 MJ	6	~	50.0	50.3	100.6				
Cal 7 MJ	7	~	100.0	100.1	100.1				



 Batch results
 D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-H6 10 2/29/2024 2:43:23 PM Data File Sample Operator Comment Cal 1 MJ.d Cal 1 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





 Batch results
 D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-G6 10 2/29/2024 2:56:39 PM Data File Sample Operator Comment Cal 2 MJ.d Cal 2 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





 Batch results
 D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-F6 10 2/29/2024 3:09:46 PM Data File Sample Operator Comment Cal 3 MJ.d Cal 3 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





 Batch results
 D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-E6 10 2/29/2024 3:22:53 PM Data File Sample Operator Comment Cal 4 MJ.d Cal 4 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





 Batch results
 D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-D6 10 2/29/2024 3:35:59 PM Data File Sample Operator Comment Cal 5 MJ.d Cal 5 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





 Batch results
 D:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-C6 10 2/29/2024 3:49:05 PM Data File Sample Operator Comment Cal 6 MJ.d Cal 6 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2024\AM 27 28\022924 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update3/5/2024 9:52:07 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-B6 10 2/29/2024 4:02:11 PM Data File Sample Operator Comment Cal 7 MJ.d Cal 7 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.

