**REVIEWED** By Tamara Salazar at 10:10 am, Jun 20, 2024

LAB CASE	<u>ITEM</u>	ITEM TYPE	DESCRIPTION
M2024-1947	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2024-1982	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
M2024-2017	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2024-2070	2	ВСК	AM 27 Blood THC Quant by LC-QQQ
M2024-2117	2	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1171	3	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1487	2	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1516	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1517	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1519	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1554	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1601	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ
P2024-1737	2	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1748	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1774	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1776	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1779	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-1818	1	BCK	AM 27 Blood THC Quant by LC-QQQ



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6/20/2024

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

Extraction Date: 06/14/2024 Plate lot#: 240513 **Mobile phase A**: 0.1% Formic Acid in LCMS Water **Blank Blood Lot**: Lampire 24C52816 **LCMS-QQQ ID**: 069901 Analyst: Celena Shrum Plate Retest Date: 11/13/2024 **Mobile phase B:** 0.1% Formic acid in Acetonitrile **Column**: UCT Selectra DA 100 x 2.1mm 3um

## **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).
- I6. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. SPE Dry ID: 067103
- □ 17. 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 r<sup>2</sup> values  $\ge 0.98$  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 1ng/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.
- 27. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Run stopped due to high pressure and was restarted shortly after (~2 hours), after the issue was remediated. M2024-1947-1 and M2024-1982-1 were reinjected due to low ISTD responses. M2024-2017-1 was reinjected as the blank run before it did

not properly inject. New mobile phase had to be made prior to reinjecting this sample, so per the method, the negative control was also reinjected and the sample and negative control were bracketed by QC's.

	1	2	3	4	5	6
A	IS + Cal. 1	QC2	P2024-1487-2	P2024-1774-1		
В	IS + Cal. 2	NEG Blood	P2024-1516-1	P2024-1776-1		
С	IS + Cal. 3	M2024-1947-1	P2024-1517-1	P2024-1779-1		
D	IS + Cal. 4	M2024-1982-1	P2024-1519-1	P2024-1818-1		
E	IS + Cal. 5	M2024-2017-1	P2024-1554-1	M2024-2070-2*		
F	IS + Cal. 6	M2024-2070-2*	P2024-1601-1			
G	IS + Cal. 7	M2024-2117-2	P2024-1737-2			
н	QC1	P2024-1171-3	P2024-1748-1			

\*Moved during the SLE portion of the extraction due to clotting



 Batch results
 D:\MassHunter\Data\2024\AM 27 28\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P1-B2 10 6/15/2024 9:05:04 AM Data File Sample Operator Comment MJ Negative Blood\_CS.d MJ Negative Blood\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P1-H1 10 6/15/2024 8:38:49 AM

Data File Sample Operator Comment MJ QC Control Blood\_CS.d MJ QC Control Blood\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P1-H1 10 6/15/2024 7:10:24 PM Data File Sample Operator Comment QC end MJ\_CS.d QC end MJ\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument	Falco (069901)
Туре	QC
Acq. Method	AM 27 Agilent Method.m
Sample Position	P1-A2
Injection Volume	10
Acq. Date-Time	6/17/2024 2:02:43 PM
Sample Info.	

Data File Sample Operator Comment MJ QC Control Blood\_CS\_r.d MJ QC Control Blood\_CS\_r 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.

### Starting bracket for reinjected sample





 Batch results
 D:\MassHunter\Data\2024\AM 27 28\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument
Туре
Acq. Method
Sample Position
Injection Volume
Acq. Date-Time
Sample Info.

Falco (069901) Sample AM 27 Agilent Method.m P1-B2 10 6/17/2024 2:29:15 PM

Data File Sample Operator Comment MJ Negative Blood\_CS\_r.d MJ Negative Blood\_CS\_r 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.

### Negative control for reinjected sample





 Batch results
 D:\MassHunter\Data\2024\AM 27 28\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument	F
Туре	Ç
Acq. Method	A
Sample Position	Р
Injection Volume	1
Acq. Date-Time	6
Sample Info.	

Falco (069901) QC AM 27 Agilent Method.m P1-A2 L0 5/17/2024 3:21:41 PM Data File Sample Operator Comment

QC end MJ\_CS\_r 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.

QC end MJ\_CS\_r.d

### End bracket for reinjected sample





## AM #27 Cannabinoids Quant. Calibration Curve Report

Batch res Last Cal. Analyst N	ults Update ame	D:\M 6/20/ ISP\I	assHunter\D /2024 7:53 A Datastor	ata∖2024 M	\AM 27 28\	061424	AM 27 28 T	S CS\Quan	tResult	s∖AM 27 C	S.batch.bin
Analyte		THC					Intern	al Standar	d	THC-D3	
1 - C - 7 1 9.0 8.0 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.1	Levels, 7 y = 0.0 R^2 = Type:L	Levels U 010049 * 0.99994 inear, O	sed, 7 Poin x - 0.0018 466 rigin:Ignore	ts, 7 Po 826 e, Weigh	ints Used, nt:1/x 40	4 QCs	60	70	80 R	90 elative C	- 100 oncentration
	Sampla				Enchlo	A	Exposted	Einal (	`onoon	tration	A

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ_CS	1	~	1.0	1.0	104.6
Cal 2 MJ_CS	2	~	3.0	3.0	98.5
Cal 3 MJ_CS	3	~	5.0	4.9	98.3
Cal 4 MJ_CS	4	~	10.0	9.9	99.2
Cal 5 MJ_CS	5	~	25.0	24.8	99.0
Cal 6 MJ_CS	6	~	50.0	50.0	99.9
Cal 7 MJ_CS	7	~	100.0	100.4	100.4



## AM #27 Cannabinoids Quant. Calibration Curve Report

		_		1
Analyte	THC-COOH	Internal Standard	THC-COOH-D9	
Analyst Name	ISP\Datastor			
Last Cal. Update	6/20/2024 7:53 AM			
Batch results	D:\MassHunter\Data\2024\AM 27 28\061424 AM 27	7 28 TS CS\QuantRes	ults\AM 27 CS.batch.bin	



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ_CS	1	~	5.0	5.2	104.6
Cal 2 MJ_CS	2	~	10.0	9.9	99.0
Cal 3 MJ_CS	3	~	20.0	19.5	97.4
Cal 4 MJ_CS	4	~	50.0	49.4	98.8
Cal 5 MJ_CS	5	~	75.0	75.2	100.2
Cal 6 MJ_CS	6	~	100.0	99.6	99.6
Cal 7 MJ_CS	7	~	250.0	251.3	100.5



## AM #27 Cannabinoids Quant. Calibration Curve Report

<i>i</i> 、			
Batch results Last Cal. Update Analyst Name	D:\MassHunter\Data\2024\AM 27 28 6/20/2024 7:53 AM ISP\Datastor	3\061424 AM 27 28 TS CS\QuantRes	ults\AM 27 CS.batch.bin
Analyte	THC-OH	Internal Standard	THC-OH-D3
THC-OH - 7 Levels, y = 0.01 $R^2 = 0$ Type:Lin $R^2 = 0$ Type:Lin $R^2 = 0$ $R^2 = 0$ R	7 Levels Used, 7 Points, 7 Points 6766 * x - 6.321495E-004 .99958858 ear, Origin:Ignore, Weight:1/x	Used, 4 QCs	

0 10 20 30 40 50 60 70 80 90 100 Relative Concentration

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ_CS	1	~	1.0	1.0	102.1
Cal 2 MJ_CS	2	~	3.0	2.9	98.0
Cal 3 MJ_CS	3	~	5.0	5.0	99.7
Cal 4 MJ_CS	4	~	10.0	9.8	97.8
Cal 5 MJ_CS	5	~	25.0	25.2	100.9
Cal 6 MJ_CS	6	~	50.0	51.4	102.8
Cal 7 MJ_CS	7	~	100.0	98.6	98.6



 Batch results
 D:\MassHunter\Data\2024\AM 27 28\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-A1 10 6/15/2024 6:53:38 AM

Data File Sample Operator Comment Cal 1 MJ\_CS.d Cal 1 MJ\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-B1 10 6/15/2024 7:07:01 AM Data File Sample Operator Comment Cal 2 MJ\_CS.d Cal 2 MJ\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-C1 10 6/15/2024 7:20:08 AM Data File Sample Operator Comment Cal 3 MJ\_CS.d Cal 3 MJ\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument
Туре
Acq. Method
Sample Position
<b>Injection Volume</b>
Acq. Date-Time
Sample Info.

Falco (069901) Cal AM 27 Agilent Method.m P1-D1 10 6/15/2024 7:33:14 AM Data File Sample Operator Comment Cal 4 MJ\_CS.d Cal 4 MJ\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-E1 10 6/15/2024 7:46:21 AM Data File Sample Operator Comment Cal 5 MJ\_CS.d Cal 5 MJ\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.binCalibration Last Update6/20/2024 7:53:11 AM

Instrument
Туре
Acq. Method
Sample Position
Injection Volume
Acq. Date-Time
Sample Info.

Falco (069901) Cal AM 27 Agilent Method.m P1-F1 10 6/15/2024 7:59:27 AM Data File Sample Operator Comment Cal 6 MJ\_CS.d Cal 6 MJ\_CS 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\061424 AM 27 28 TS CS\QuantResults\AM 27 CS.batch.bin

 Calibration Last Update
 6/20/2024 7:53:11 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-G1 10 6/15/2024 8:12:33 AM Data File Sample Operator Comment Cal 7 MJ\_CS.d Cal 7 MJ\_CS 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.

