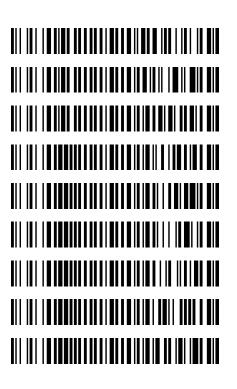
11/25/2024

75

#### Worklist: 6980

LAB CASE	<u>ITEM</u>	ITEM TYPE	DESCRIPTION
M2024-2586	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2024-4240	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2024-4396	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-3321	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-3365	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-3372	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-3395	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-3438	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-3458	1	BCK	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 11/25/2024 Plate lot#: 240919 Mobile phase A: 0.1% Formic Acid in LCMS Water Blank Blood Lot: Lampire 24C52816 Column: UCT Selectra DA 100 x 2.1mm 3um Analyst: <u>Tamara Salazar</u> Plate Retest Date: 03/19/2025 Mobile phase B: 0.1% Formic acid in Acetonitrile Blank Urine Lot: LCMS-QQQ ID: 069901

### **Pre-Analytic:**

- $\boxtimes$  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:

- $\boxtimes$  1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- ☑ 2. Using a calibrated pipette, pipette 1000µL blood or 1000µL urine in wells of analytical (standards) plate. Pipette ID: 42
- □ 3. Urine hydrolysis add **100 ul BG turbo, and 200 ul BG turbo buffer** to the urine samples in wells of the analytical plate.
- ☑ 4. Add **500µL of 0.1% formic acid in water** in the wells of the analytical plate.
- $\boxtimes$  5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- δ. Transfer 700-800μL of blood+acid or urine+acid mixture to corresponding wells of SLE+ plate. Amount transferred: 750 μL
- 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
- $\boxtimes$  8. Wait 5 minutes.
- 9. Add 2.25mL MTBE. (Add in 3 increments of 750uL)
- $\boxtimes$  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)
- $\boxtimes$  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: 067103
- ☑ 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 r<sup>2</sup> values  $\ge 0.98$  for each analyte
- 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
- $\boxtimes$  4. Did all QCs pass for each analyte? (if not, describe in comments section)
- $\boxtimes$  5. Enter QCs into control charting.
- ☑ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

### COMMENTS:

Calibrator 5 did not inject properly with the initial injection. The calibrator was reinjected.

	1	2	3	4	5	6
А	IS + Cal. 1	IS + QC_1			P2024-3365-1	IS + QC_1
в	IS + Cal. 2	IS + QC_2			P2024-3321-1	IS + Cal. 7
с	IS + Cal. 3				M2024-4396-3	IS + Cal. 6
D	IS + Cal. 4				M2024-4240-1	IS + Cal. 5
E	IS + Cal. 5			P2024-3458-1	M2024-2586-1	IS + Cal. 4
F	IS + Cal. 6			P2024-3438-1	Blood Neg	IS + Cal. 3
G	IS + Cal. 7			P2024-3395-1	IS + QC_2	IS + Cal. 2
н	IS + Cal. 7			P2024-3372-1	IS + QC_1	IS + Cal. 1

All wells to contain 100  $\mu l$  of residual DMSO

	1	2	3	4	5	6
А					P2024-3365-1	IS + QC_1
В					P2024-3321-1	IS + Cal. 7
с					M2024-4396-3	IS + Cal. 6
D				P2024-3458-1	M2024-4240-1	IS + Cal. 5
E				P2024-3458-1*	M2024-2586-1	IS + Cal. 4
F				P2024-3438-1	Blood Neg	IS + Cal. 3
G				P2024-3395-1	IS + QC_2	IS + Cal. 2
н				P2024-3372-1	IS + QC_1	IS + Cal. 1

\*Sample moved during step 6 of the extraction due to clotting.

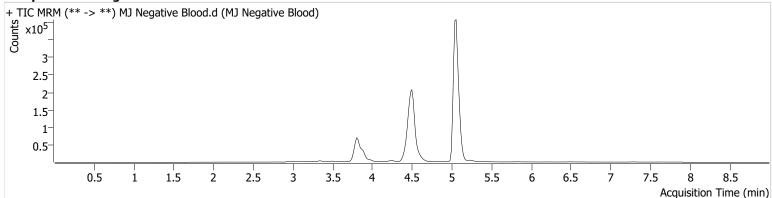


 Batch results
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 Calibration Last Update
 11/26/2024 3:28:26 PM

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

Falco (069901) Sample AM 27 Agilent Method.m P3-F5 10 11/25/2024 2:28:06 PM Data File Sample Operator Comment MJ Negative Blood.d MJ Negative Blood Tamara Salazar 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.

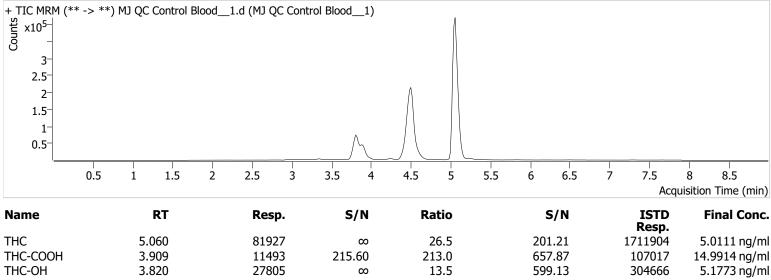




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 Calibration Last Update
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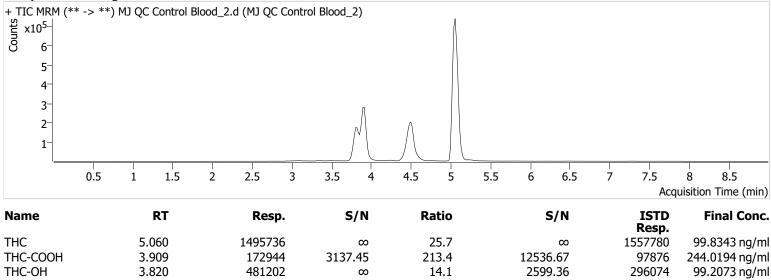
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Batch resultsD:\MassHunter\Data\2024\AM 27 28\112524 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update11/26/2024 3:28:26 PM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P3-G5 10 11/25/2024 7:03:34 PM Data File Sample Operator Comment MJ QC Control Blood\_2.d MJ QC Control Blood\_2 Tamara Salazar 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.







### AM #27 Cannabinoids Quant. Calibration Curve Report

Batch Last C Analys	al. U	pdate		024 3:28 Pl		AM 27 28∖′	112524 /	AM 27 28 TS	S\QuantRes	sults\A	M 27.batcl	h.bin
Analy	te		THC					Interna	al Standaro	1	THC-D3	
Relative Responses	- 7 Le 1- 0.9- 0.8- 0.7- 0.6- 0.5- 0.4- 0.3- 0.2- 0.1- 0-	y = 0.00 $R^{2} = 0$	)9621 * x ).9999535	d, 7 Points - 3.5596 in:Ignore, 20	82E-00	4	2 QCs	60	70		90 Pelative C	100 Concentration
		Sample		Leve		Enable		Expected	<b>Final O</b>		tration	Accuracy

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	1.0	1.0	103.0
Cal 2 MJ	2	~	3.0	3.0	99.8
Cal 3 MJ	3	~	5.0	4.9	98.2
Cal 4 MJ	4	~	10.0	9.8	98.1
Cal 5 MJ_r	5	~	25.0	25.2	100.6
Cal 6 MJ	6	~	50.0	50.1	100.3
Cal 7 MJ	7	~	100.0	100.0	100.0





### AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	#27 Canna					AM 27.batch.bin	RENSI
Last Cal. Update	11/26/2024 3:2		AIVI Z7 Z0\11Z0	24 AIVI 27 20 13	QuantResults	AIVI 27.Datch.Din	
Analyst Name	ISP\Datastor	-0110					
Analyte	THC-COOH			Interna	I Standard	THC-COOH-D9	1
$1 = \frac{6}{18} + \frac{1}{18} + \frac{1}{18} = \frac{1}{18} + \frac{1}{$	vels, 7 Levels Use 007246 * x - 0.0 0.99985053 inear, Origin:Ign	01236			180 200	) 220 240 Relative Concen	260 tratio
0			En al la d	<b>F</b>	<b></b>	Relative Concen	itratio

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	5.0	5.2	104.9
Cal 2 MJ	2	~	10.0	9.7	97.1
Cal 3 MJ	3	~	20.0	20.0	100.1
Cal 4 MJ	4	~	50.0	48.8	97.7
Cal 5 MJ_r	5	~	75.0	74.6	99.4
Cal 6 MJ	6	~	100.0	100.3	100.3
Cal 7 MJ	7	~	250.0	251.4	100.5





### AM #27 Cannabinoids Quant Calibration Curve Penert

	ŀ	\M #∠	27 Ca	nnabi	noid	s Quar	<u>nt. C</u>	alıbratı	on Cu	rve	Repor	
Batch re Last Cal Analyst	I. Upda	ite		024 3:28 PM		\AM 27 28\ <sup>.</sup>	112524	AM 27 28 T	S\QuantRe	sults\Al	M 27.batch	n.bin
Analyte			THC-OF	1				Intern	al Standar	d	THC-OH-	·D3
1. 1. 1. 0. 0. 0. 0.	y = 6− R′	= 0.0163 ^2 = 0.9	814 * x 999155	+ 0.0068	00	7 Points U nt:1/x 40	sed, 2	QCs 60	70	80 R	90 Lelative C	100 oncentratior
	Sa	mple		Leve	I	Enable	d	Expected	Final C	Concer	tration	Accuracy

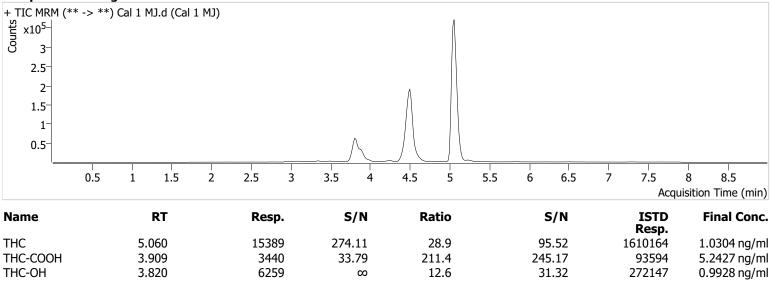
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	1.0	1.0	99.3
Cal 2 MJ	2	~	3.0	3.0	98.6
Cal 3 MJ	3	~	5.0	5.1	102.0
Cal 4 MJ	4	~	10.0	9.9	99.3
Cal 5 MJ_r	5	~	25.0	25.1	100.3
Cal 6 MJ	6	~	50.0	50.6	101.2
Cal 7 MJ	7	~	100.0	99.4	99.4



 Batch results
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 Calibration Last Update
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Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-H6 10 11/25/2024 12:16:54 PM Data File Sample Operator Comment Cal 1 MJ.d Cal 1 MJ Tamara Salazar 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.

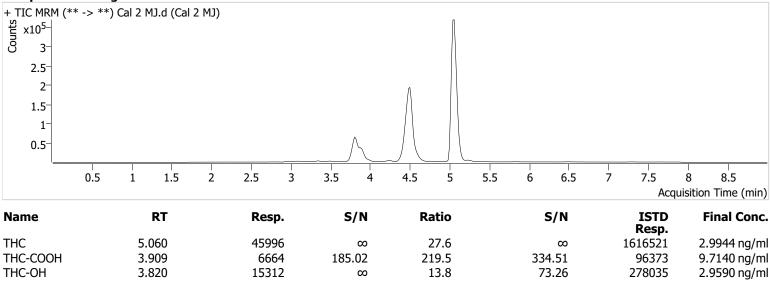




 Batch results
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Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-G6 10 11/25/2024 12:30:09 PM Data File Sample Operator Comment Cal 2 MJ.d Cal 2 MJ Tamara Salazar Only 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.



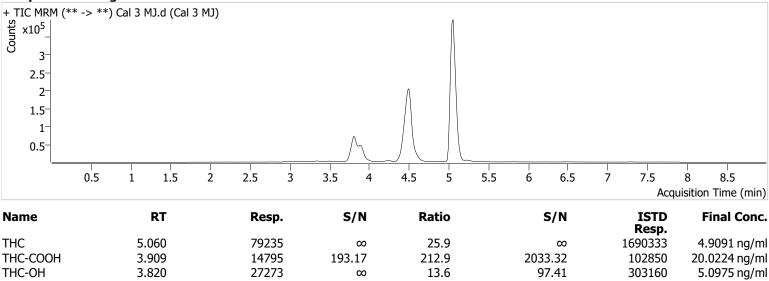


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Instrument
Туре
Acq. Method
Sample Position
Injection Volume
Acq. Date-Time
Sample Info.

Falco (069901) Cal AM 27 Agilent Method.m P3-F6 10 11/25/2024 12:43:16 PM Data File Sample Operator Comment Cal 3 MJ.d Cal 3 MJ Tamara Salazar 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.



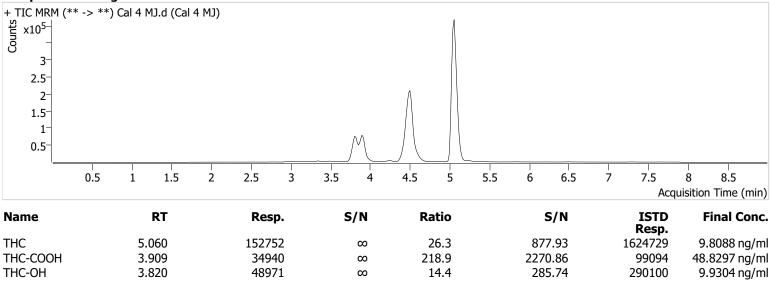


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 Calibration Last Update
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Instrument
Туре
Acq. Method
Sample Position
Injection Volume
Acq. Date-Time
Sample Info.

Falco (069901) Cal AM 27 Agilent Method.m P3-E6 10 11/25/2024 12:56:22 PM Data File Sample Operator Comment Cal 4 MJ.d Cal 4 MJ Tamara Salazar 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.



Cal 5 MJ.d



Batch resultsD:\MassHunter\Data\2024\AM 27 28\112524 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update11/26/2024 3:28:26 PM

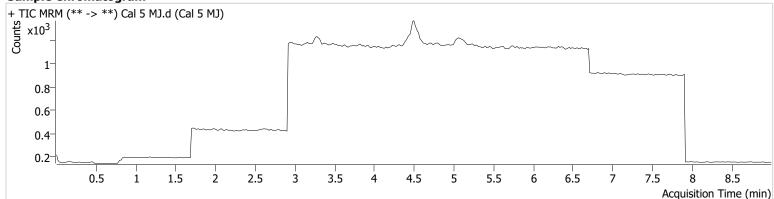
Instrument	Falco (069901)
Туре	Sample
Acq. Method	AM 27 Agilent M
Sample Position	P3-D6
Injection Volume	10
Acq. Date-Time	11/25/2024 1:09
Sample Info.	Calibrator did not i

co (069901) nple 27 Agilent Method.m D6 25/2024 1:09:28 PM

Data File Sample Operator Comment

Calibrator did not inject properly. Please refer to re-injection data.

Cal 5 MJ Tamara Salazar 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.

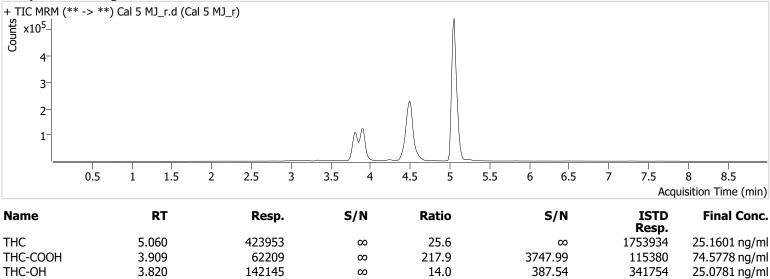




Batch resultsD:\MassHunter\Data\2024\AM 27 28\112524 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update11/26/2024 3:28:26 PM

Instrument	Falco (069901)
Туре	Cal
Acq. Method	AM 27 Agilent Method.m
Sample Position	P3-D6
Injection Volume	10
Acq. Date-Time	11/25/2024 2:41:30 PM
Sample Info.	Re-inject data.

Data File Sample Operator Comment Cal 5 MJ\_r.d Cal 5 MJ\_r Tamara Salazar 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.



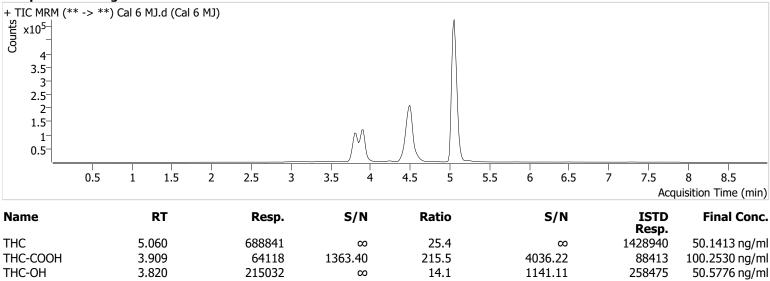


 Batch results
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 Calibration Last Update
 11/26/2024 3:28:26 PM

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

Falco (069901) Cal AM 27 Agilent Method.m P3-C6 10 11/25/2024 1:22:34 PM Data File Sample Operator Comment Cal 6 MJ.d Cal 6 MJ Tamara Salazar 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\112524 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 11/26/2024 3:28:26 PM

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

Falco (069901) Cal AM 27 Agilent Method.m P3-B6 10 11/25/2024 1:35:40 PM Data File Sample Operator Comment Cal 7 MJ.d Cal 7 MJ Tamara Salazar 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.

