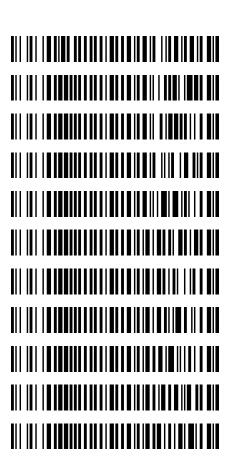
REVIEWED By Sarah Collins at 2:50 pm, Nov 12, 2024

Worklist: 6968

LAB CASE	ITEM	ITEM TYPE	DESCRIPTION
M2024-3974	2	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-2839	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3101	1	UCK	AM 27 Blood THC Quant by LC-QQQ
P2024-3136	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3164	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3239	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3245	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3257	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3261	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3263	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-3288	1	BCK	AM 27 Blood THC Quant by LC-QQQ



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11/12/2024

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

Extraction Date: 11/06/2024 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: POC021022 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 6. 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 r² values ≥ 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: THC-OH - 3-100--calibrator 1 dropped due to signal-to-noise

	1	2	3	4	5	6
А					P2024-3239-1	IS + QC_1
В				P2024-3101-1	P2024-3164-1	IS + Cal. 7
с				Urine Neg	P2024-3136-1	IS + Cal. 6
D				P2024-3288-1	P2024-2839-1	IS + Cal. 5
E				P2024-3263-1	M2024-3974-2	IS + Cal. 4
F				P2024-3261-1	Blood Neg	IS + Cal. 3
G				P2024-3257-1	IS + QC_2	IS + Cal. 2
Н				P2024-3245-1	IS + QC_1	IS + Cal. 1

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All wells to contain 100 μl of residual DMSO

SLE plate map

	1	2	3	4	5	6
А				P2024-3136-1	P2024-3239-1	IS + QC_1
В				P2024-3101-1	P2024-3164-1	IS + Cal. 7
с				Urine Neg	P2024-3136-1*	IS + Cal. 6
D				P2024-3288-1	P2024-2839-1	IS + Cal. 5
E				P2024-3263-1	M2024-3974-2	IS + Cal. 4
F				P2024-3261-1	Blood Neg	IS + Cal. 3
G				P2024-3257-1	IS + QC_2	IS + Cal. 2
Н				P2024-3245-1	IS + QC_1	IS + Cal. 1

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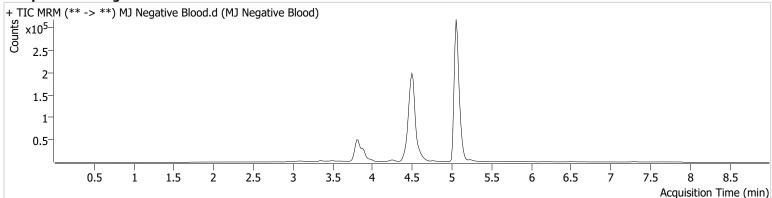
*Sample moved during step 6 of the extraction due to clotting.



 Batch results
 D:\MassHunter\Data\2024\AM 27 28\110624 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P3-F5 10 11/6/2024 3:55:09 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.

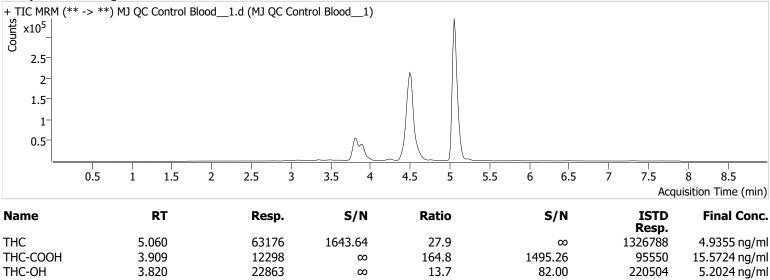




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 Calibration Last Update
 11/7/2024 6:32:08 AM

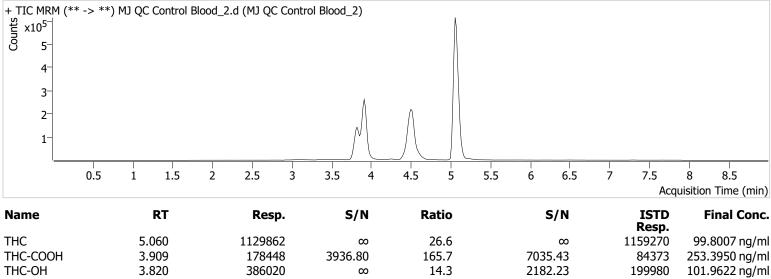
Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P3-A6 10 11/6/2024 3:28:56 PM Data File Sample Operator Comment MJ QC Control Blood__1.d MJ QC Control Blood__1 Tamara Salazar Only drugs and concentrations listed on the 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\110624 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P3-G5 10 11/6/2024 10:01:58 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.

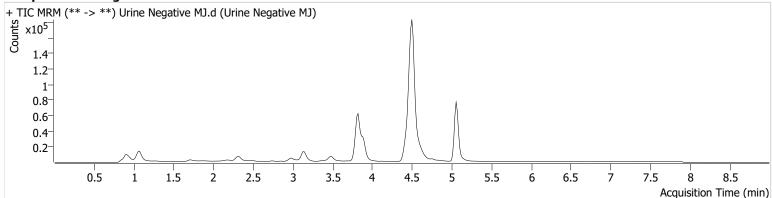




 Batch results
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 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P3-C4 10 11/6/2024 9:09:35 PM Data File Sample Operator Comment Urine Negative MJ.d Urine Negative 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.

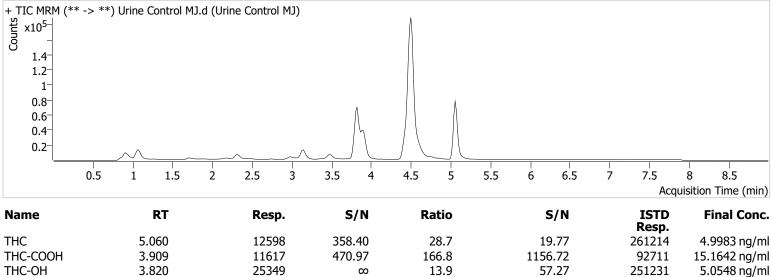




 Batch results
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 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P3-H5 10 11/6/2024 8:43:22 PM Data File Sample Operator Comment Urine Control MJ.d Urine Control 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.







AM #27 Cannabinoids Quant. Calibration Curve Report

Last	h resu Cal. U yst Na	pdate		24 6:32 AN		∖AM 27 28∖	110624	AM 27 28 T	S\QuantResu	lts\AM 27.bat	ch.bin
Anal	yte		THC					Intern	al Standard	THC-D	3
Relative Responses H	- 7 Lo 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 Type:Lir	evels Used 9772 * x 9999519 hear, Origin	- 6.1339 6	11E-00)4	, 2 QCs	60	70 8	30 90 Bolotiuo	100
										Kelduve	Concentration
		Sample		Leve	el	Enable	d	Expected	Final Co	ncentration	Accuracy

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	1.0	1.0	104.5
Cal 2 MJ	2	V	3.0	3.0	98.9
Cal 3 MJ	3	V	5.0	4.9	97.7
Cal 4 MJ	4	~	10.0	9.9	98.6
Cal 5 MJ	5	~	25.0	25.0	99.9
Cal 6 MJ	6	~	50.0	50.2	100.4
Cal 7 MJ	7	v	100.0	100.1	100.1





AM #27 Cannabinoids Quant. Calibration Curve Report

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Batc	h resu	lts		D:\Ma	assHunt	er\Data	\2024\A	M 27 28	8\11062	4 AM 27	′ 28 TS∖	QuantR	esults\/	AM 27.ba	atch.bin	
Last	Cal. U	pdate		11/7/2	2024 6:	32 AM										
Anal	yst Na	me		ISP\D	atastor											
Anal	yte			THC-	соон					I	nternal	Standa	Ird	THC-0	COOH-[09
	-COO							, 7 Poin	ts Use	d, 2 QC	Ìs					
Relative Responses	2.2	y = 0	0.008	8352 *	x - 0.	001353	3									
loc	2-	y = 0.008352 * x - 0.001353 R^2 = 0.99990029 Type:Linear, Origin:Ignore, Weight:1/x													Y	
est		турс	. בוו וע		iginiig	nore, v	veigne	. 1/ ^								
8 2 2	1.8-															
Itič	1.6-															
cela	1.4-															
Ľ.	1.2-															
	1-															
	0.8-						_									
	0.6-															
	0.4-															
	0.2-															
	0-															
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			0	20	40	60	80	100	120	140	160	180	200	220	240	260
														Relative	e Conce	entration

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	5.0	5.1	101.5
Cal 2 MJ	2	~	10.0	10.0	99.9
Cal 3 MJ	3	~	20.0	19.9	99.7
Cal 4 MJ	4	~	50.0	48.9	97.9
Cal 5 MJ	5	~	75.0	75.9	101.2
Cal 6 MJ	6	~	100.0	99.5	99.5
Cal 7 MJ	7	~	250.0	250.7	100.3





AM #27 Cannabinoids Quant. Calibration Curve Report

Batch resu Last Cal. U Analyst Na	pdate		24 6:32 AN		∖AM 27 28\`	110624	AM 27 28 T	S\QuantRes	sults\A	M 27.batc	h.bin
Analyte		THC-OF	ł				Intern	al Standaro	k	THC-OF	I-D3
THC-OH - 2 1.8 1.8 1.6 1.4 1.4 1.2 1.2 1- 0.8 0.6 0.4 0.2 0.2	y = 0.01 R^2 = 0 Type:Lin	6 Levels (8878 * x .9999035 ear, Origi	+ 0.0054 5	174		sed, 2	QCs				
	0	10	20	30	40	50	60	70	80 F	90 Relative (100 Concentration
	Sample		Leve		Enable	d	Expected	Final C	once	ntration	Accuracy

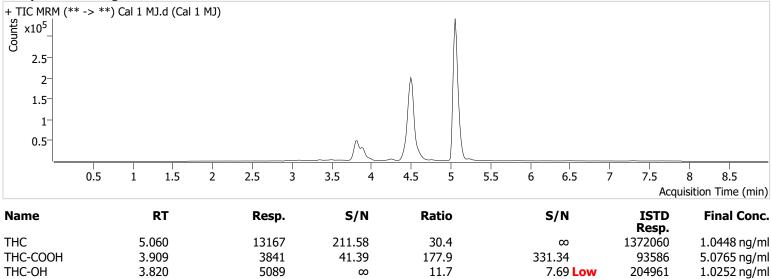
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	×	1.0	1.0	102.5
Cal 2 MJ	2	~	3.0	2.9	98.0
Cal 3 MJ	3	~	5.0	5.0	100.4
Cal 4 MJ	4	~	10.0	10.0	100.3
Cal 5 MJ	5	~	25.0	25.3	101.0
Cal 6 MJ	6	~	50.0	50.5	100.9
Cal 7 MJ	7	~	100.0	99.3	99.3



 Batch results
 D:\MassHunter\Data\2024\AM 27 28\110624 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-H6 10 11/6/2024 1:43:52 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.

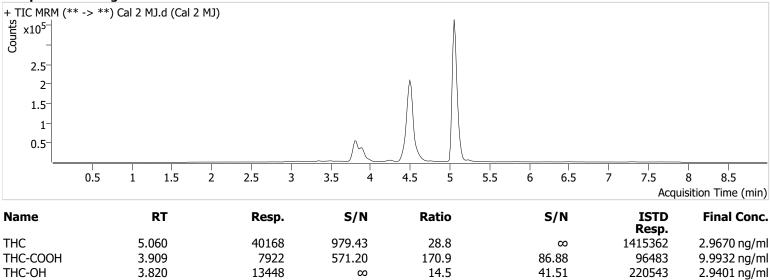




 Batch results
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 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-G6 10 11/6/2024 1:57:08 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.

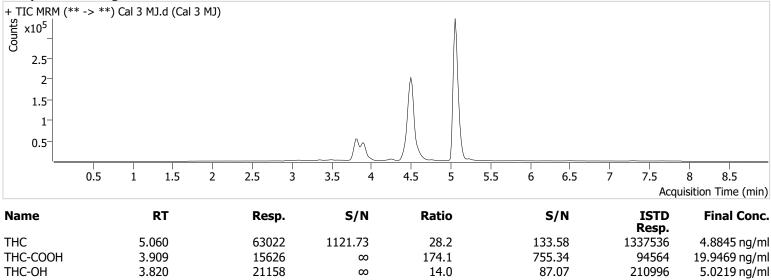




 Batch results
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 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-F6 10 11/6/2024 2:10:14 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.



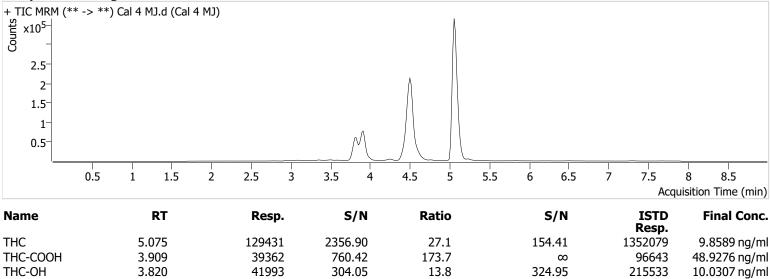


 Batch results
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 Calibration Last Update
 11/7/2024 6:32:08 AM

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

Falco (069901) Cal AM 27 Agilent Method.m P3-E6 10 11/6/2024 2:23:23 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.

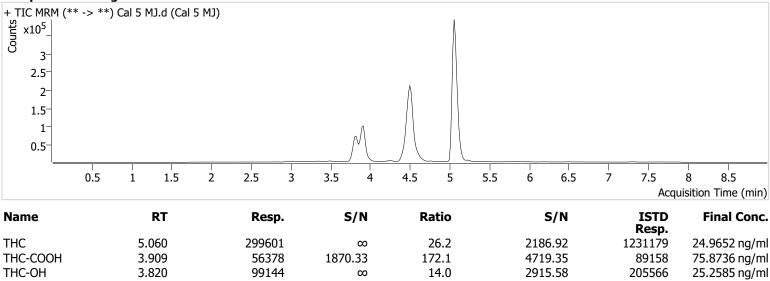




 Batch results
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 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-D6 10 11/6/2024 2:36:29 PM Data File Sample Operator Comment Cal 5 MJ.d 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.

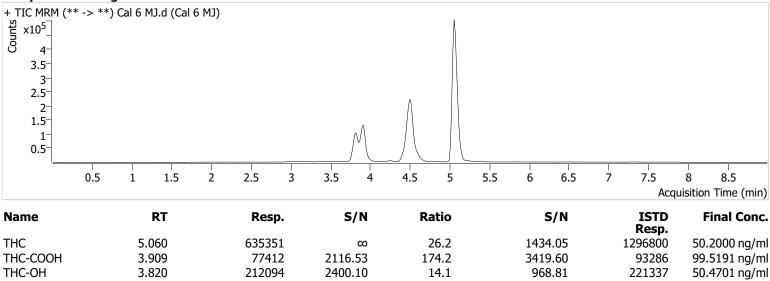




 Batch results
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 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-C6 10 11/6/2024 2:49:37 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\110624 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 11/7/2024 6:32:08 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P3-B6 10 11/6/2024 3:02:42 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.

