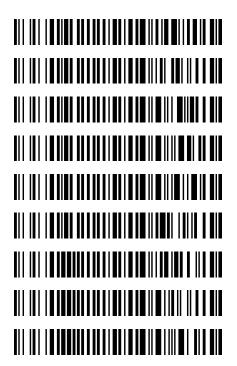
### REVIEWED

By Anne Nord at 11:29 am, Jan 29, 2024

## 1/25/202

### Worklist: 6662

LAB CASE	<u>ITEM</u>	ITEM TYPE	DESCRIPTION
M2023-4964	2	ВСК	AM 27 Blood THC Quant by LC-QQQ
M2023-5482	1	вск	AM 27 Blood THC Quant by LC-QQQ
M2024-0106	3	ВСК	AM 27 Blood THC Quant by LC-QQQ
M2024-0107	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
M2024-0108	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
M2024-0131	3	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2023-3880	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
P2024-0048	4	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-0050	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: 01/23/2023 Analyst: <u>Tamara Salazar</u>
Plate lot#: 231212 Plate Retest Date: 06/12/2024

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 23E52981 Blank Urine Lot:

Column: UCT Selectra DA 100 x 2.1mm 3um LCMS-QQQ ID: 069901

#### **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 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.
- Using a calibrated pipette, add 1000μl blood and urine (if applicable) (calibrated pipette) into the appropriate wells of analytical (standards) plate. Pipette ID: 42
- ☑ 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- Add 500μL of 0.1% formic acid in water to blood samples, and 500μL of saturated phosphate buffer to urine samples-in the wells of the analytical plate.
- ☑ 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- Σ 7. Transfer 700-800μL of blood+acid or urine+acid mixture to corresponding wells of SLE+ plate. Amount transferred: 750μL
- ✓ 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)
- $\boxtimes$  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.
- ☑ 17. Reconstitute in 100µL 100% MeOH and heat seal plate with foil. Place in autosampler and run worklist.

#### **Post-Analytic**

- $\boxtimes$  2. Make any necessary integration changes, Curve weighting of Linear 1/x with r<sup>2</sup> values  $\ge$ 0.98 for each analyte
- ✓ 4. Did all QCs pass for each analyte? (if not, describe in comments section)
- ☑ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: THC-OH 3-100 - calibrator 1 dropped due to S/N

Instrument stopped due to mobile phase running out on final QC. The mobile phase was remade on 01/24/24 and the run was restarted. The negative control was re-injected with the new mobile phase.

	1	2	3	4	5	6
А	IS + Cal. 1	IS + QC_1			M2024-0131-3	IS + QC_1
В	IS + Cal. 2				M2024-0108-1	IS + Cal. 7
С	IS + Cal. 3				M2024-0107-1	IS + Cal. 6
D	IS + Cal. 4				M2024-0106-3	IS + Cal. 5
E	IS + Cal. 5				M2023-5482-1	IS + Cal. 4
F	IS + Cal. 6			P2024-0050-1	M2023-4964-2	IS + Cal. 3
G	IS + Cal. 7			P2024-0048-4	Neg Blood	IS + Cal. 2
Н	IS + QC_1			P2023-3880-1	IS + QC_1	IS + Cal. 1

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

	1	2	3	4	5	6
А	IS + Cal. 1	IS + QC_1			M2024-0131-3	IS + QC_1
В	IS + Cal. 2				M2024-0108-1	IS + Cal. 7
С	IS + Cal. 3				M2024-0107-1	IS + Cal. 6
D	IS + Cal. 4				M2024-0106-3	IS + Cal. 5
E	IS + Cal. 5			P2024-0050-1	M2023-5482-1	IS + Cal. 4
F	IS + Cal. 6			P2024-0050-1*	M2023-4964-2	IS + Cal. 3
G	IS + Cal. 7			P2024-0048-4	Neg Blood	IS + Cal. 2
Н	IS + QC_1			P2023-3880-1	IS + QC_1	IS + Cal. 1

<sup>\*</sup>Sample moved during step 7 of the extraction due to clotting.

D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method **Sample Position Injection Volume**  Falco (069901) Sample

AM 27 Agilent Method.m

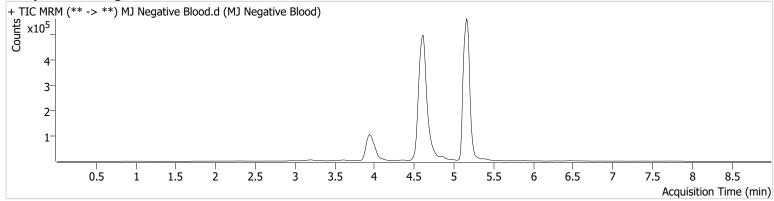
1/23/2024 2:40:57 PM

P1-G5 10

Acq. Date-Time Sample Info.

**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.



D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method **Sample Position**  Falco (069901) Sample

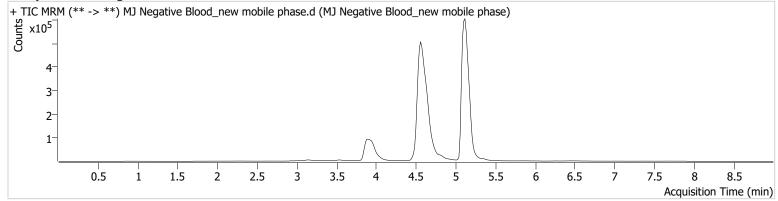
AM 27 Agilent Method.m

P1-G5 **Injection Volume** 1/24/2024 10:14:31 AM

Acq. Date-Time Sample Info.

**Data File Sample** Operator Comment MJ Negative Blood\_new mobile phase.d MJ Negative Blood\_new mobile phase 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.



D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method Falco (069901) QC

**Sample Position** P1-A6

**Injection Volume** 10

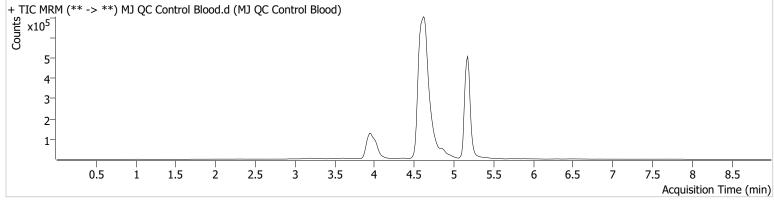
Acq. Date-Time Sample Info.

AM 27 Agilent Method.m

1/23/2024 2:14:44 PM

**Data File Sample** Operator Comment MJ QC Control Blood.d MJ QC Control 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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	106837	$\infty$	28.6	œ	2287086	5.0158 ng/ml
THC-COOH	4.015	32678	266.57	140.9	œ	238367	15.3180 ng/ml
THC-OH	3.941	57166	$\infty$	14.6	$\infty$	560977	5.2378 ng/ml

D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method Falco (069901)

AM 27 Agilent Method.m

**Sample Position** P1-A6 **Injection Volume** 10

Acq. Date-Time Sample Info.

QC

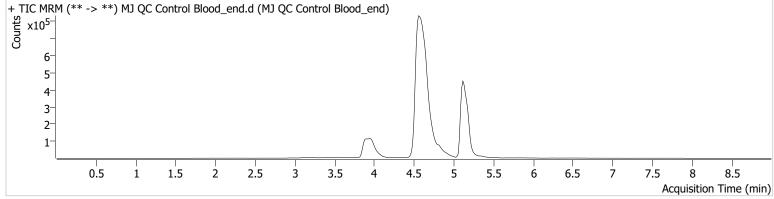
1/24/2024 9:48:07 AM

**Data File Sample** Operator Comment

MJ QC Control Blood\_end.d MJ QC Control Blood end 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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	112958	∞	26.4	∞	2545366	4.7817 ng/ml
THC-COOH	3.969	26813	∞	180.3	∞	245711	12.4498 ng/ml
THC-OH	3.896	47417	∞	15.3	∞	618939	4.0914 ng/ml

D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method Falco (069901)

QC

AM 27 Agilent Method.m

**Sample Position Injection Volume** Acq. Date-Time

P1-A6

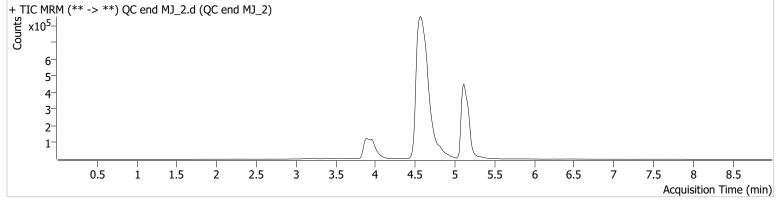
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Sample Info.

**Data File Sample** Operator Comment

QC end MJ\_2.d QC end MJ 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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	113998	$\infty$	23.1	$\infty$	2570545	4.7786 ng/ml
THC-COOH	3.969	29351	336.21	173.7	$\infty$	259282	12.8679 ng/ml
THC-OH	3.896	51667	∞	13.8	40.43	676089	4.0828 ng/ml



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

Batch results D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin

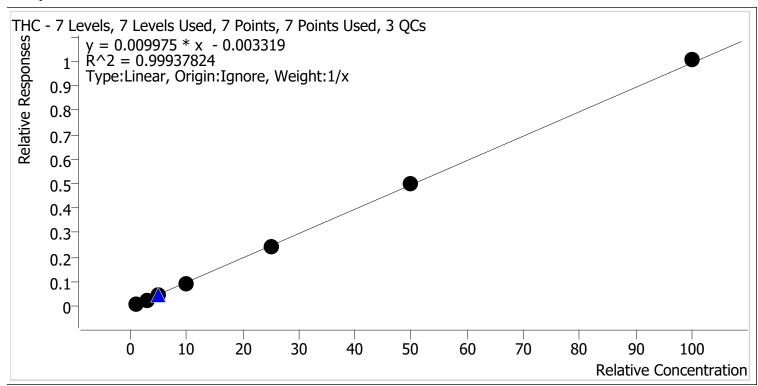
Last Cal. Update

1/25/2024 9:41 AM

Analyst Name

ISP\datastor

Analyte THC Internal Standard THC-D3



Sample	Level	Enabled	Expected Final Concentration		Accuracy
Cal 1 MJ	1	~	1.0	1.1	114.8
Cal 2 MJ	2	~	3.0	2.9	97.2
Cal 3 MJ	3	~	5.0	4.7	94.5
Cal 4 MJ	4	~	10.0	9.4	94.3
Cal 5 MJ	5	~	25.0	24.4	97.6
Cal 6 MJ	6	~	50.0	50.1	100.2
Cal 7 MJ	7	~	100.0	101.3	101.3



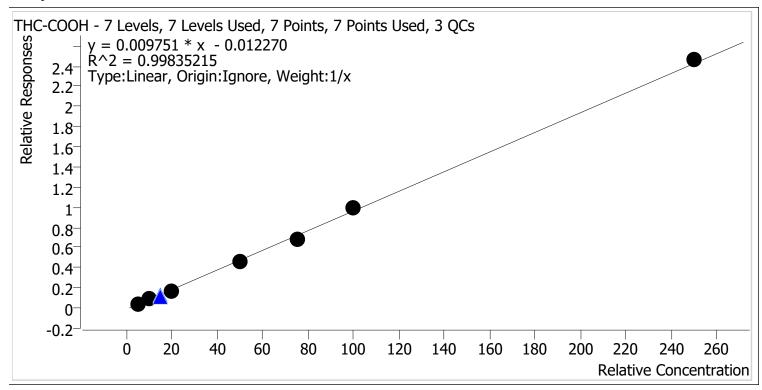
### AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin

**Last Cal. Update** 1/25/2024 9:41 AM

Analyst Name ISP\datastor
Analyte THC-COOH

Internal Standard THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	5.0	5.4	109.0
Cal 2 MJ	2	~	10.0	10.4	103.7
Cal 3 MJ	3	~	20.0	18.2	90.8
Cal 4 MJ	4	~	50.0	49.3	98.6
Cal 5 MJ	5	<b>&gt;</b>	75.0	70.4	93.8
Cal 6 MJ	6	~	100.0	102.5	102.5
Cal 7 MJ	7	~	250.0	253.8	101.5



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

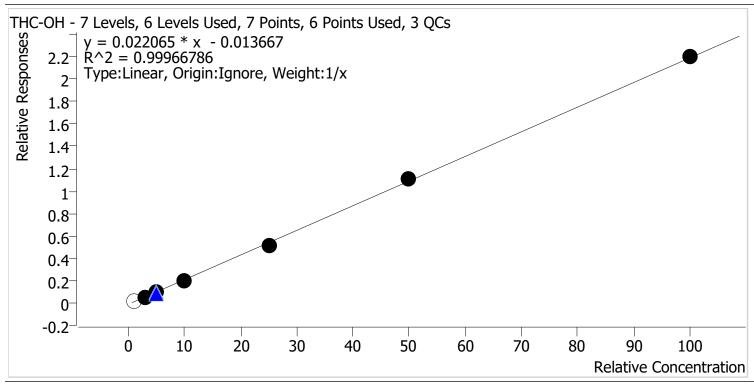
Batch results D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin

Last Cal. Update

1/25/2024 9:41 AM

Analyst Name ISP\datastor

Analyte THC-OH Internal Standard THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	Х	1.0	1.4	138.5
Cal 2 MJ	2	V	3.0	3.1	104.0
Cal 3 MJ	3	~	5.0	5.0	100.4
Cal 4 MJ	4	V	10.0	9.7	97.5
Cal 5 MJ	5	V	25.0	24.2	96.6
Cal 6 MJ	6	~	50.0	50.6	101.1
Cal 7 MJ	7	~	100.0	100.4	100.4

D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method Falco (069901)

Cal

AM 27 Agilent Method.m

**Sample Position Injection Volume** Acq. Date-Time

P1-H6

10

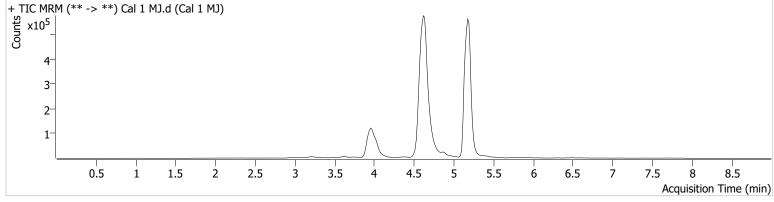
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Sample Info.

**Data File** Cal 1 MJ.d Sample Cal 1 MJ Operator Tamara Salazar Comment

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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.195	24647	$\infty$	30.4	$\infty$	3029103	1.1484 ng/ml
THC-COOH	4.030	10079	75.67	157.7	œ	246733	5.4477 ng/ml
THC-OH	3.941	99 <del>4</del> 0	$\infty$	15.3	8.49 <b>Low</b>	588806	1.3845 ng/ml

D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method Falco (069901)

Cal

AM 27 Agilent Method.m

**Sample Position Injection Volume** Acq. Date-Time

P1-G6

10 1/23/2024 12:42:59 PM

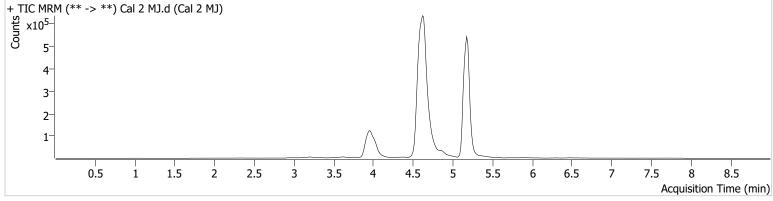
Sample Info.

**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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	70937	∞	26.6	œ	2752019	2.9168 ng/ml
THC-COOH	4.030	21838	289. <del>4</del> 6	146.0	148.01	245759	10.3714 ng/ml
THC-OH	3.956	32159	∞	1 <del>4</del> .7	25.90	582745	3.1204 ng/ml

Batch results D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin 1/25/2024 9:41:43 AM

Instrument Type Acq. Method Falco (069901)

Cal

AM 27 Agilent Method.m

Sample Position Injection Volume Acq. Date-Time P1-F6 10

1/23/2024 12:56:06 PM

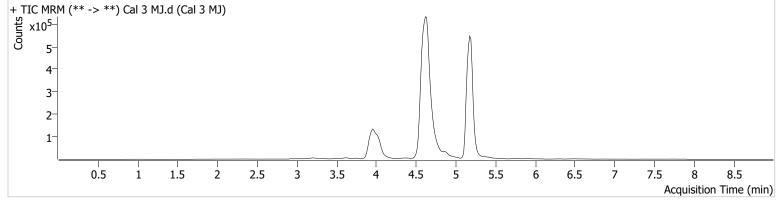
Sample Info.

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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.195	120278	355.97	27.5	44.44	2743998	4.7271 ng/ml
THC-COOH	4.030	<del>4</del> 1513	793.90	157.4	∞	251822	18.1650 ng/ml
THC-OH	3.956	57647	54.98	15.4	256.77	593950	5.0181 ng/ml

D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method Falco (069901) Cal

AM 27 Agilent Method.m

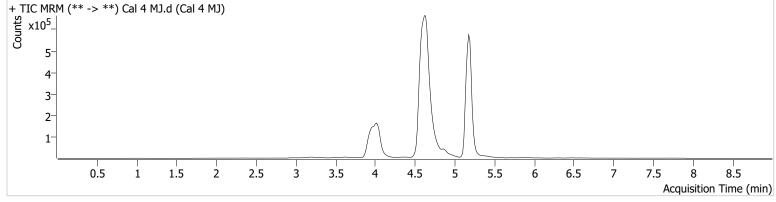
**Sample Position Injection Volume** Acq. Date-Time Sample Info.

P1-E6 10

1/23/2024 1:09:11 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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.195	233441	$\infty$	26.8	∞	2571131	9.4349 ng/ml
THC-COOH	4.030	109896	1456.16	148.6	∞	234525	49.3152 ng/ml
THC-OH	3.956	113517	$\infty$	14.8	$\infty$	563629	9.7471 ng/ml

D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin **Batch results** Calibration Last Update 1/25/2024 9:41:43 AM

Instrument **Type** Acq. Method Falco (069901) Cal

**Sample Position Injection Volume** Acq. Date-Time

Sample Info.

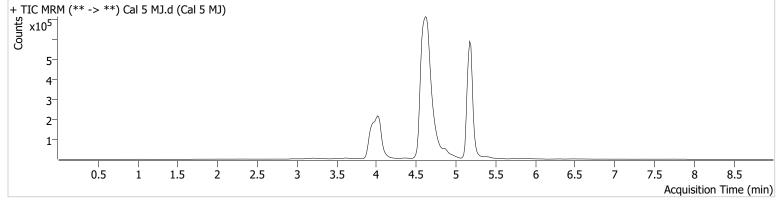
AM 27 Agilent Method.m P1-D6

10

1/23/2024 1:22:18 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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.195	521739	$\infty$	26.3	$\infty$	2174329	24.3885 ng/ml
THC-COOH	4.030	1575 <del>44</del>	1306.20	153.1	$\infty$	233765	70.3755 ng/ml
THC-OH	3.956	291141	∞	14.4	∞	560467	24.1617 ng/ml

Batch results D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin 1/25/2024 9:41:43 AM

Instrument Type Acq. Method Falco (069901)

Cal

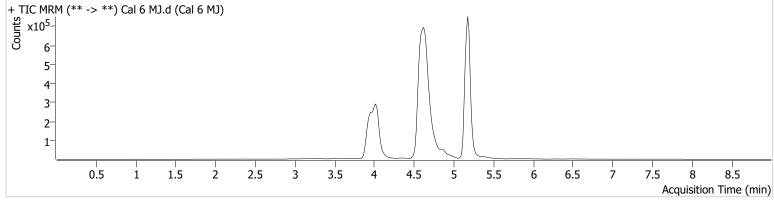
AM 27 Agilent Method.m

Sample Position Injection Volume Acq. Date-Time P1-C6

Sample Info.

10 1/23/2024 1:35:23 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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	1086344	$\infty$	27.0	$\infty$	2188296	50.1011 ng/ml
THC-COOH	4.030	223932	2683.38	147.0	3572.17	226790	102.5225 ng/ml
THC-OH	3.9 <del>4</del> 1	599334	$\infty$	14.5	∞	543964	50.5532 ng/ml

Batch results D:\MassHunter\Data\2024\AM 27 28\012324 AM 27 28 TS\QuantResults\AM 27.batch.bin 1/25/2024 9:41:43 AM

Instrument Type Acq. Method Falco (069901)

Cal AM 27 Agilent Method.m

Sample Position Injection Volume Acq. Date-Time AM 27 Agilent Method.m P1-B6

10 1/23

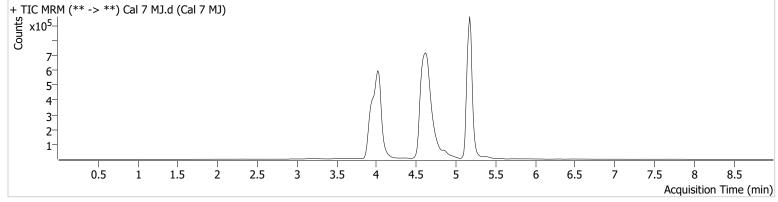
1/23/2024 1:48:30 PM

Sample Info.

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.



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.180	1966105	$\infty$	27.4	∞	1952496	101.2832 ng/ml
THC-COOH	4.030	540189	5990.19	145.4	∞	219367	253.8028 ng/ml
THC-OH	3.941	1249338	∞	14.7	∞	567456	100.3994 ng/ml