5/10/2024

Worklist: 6809

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
M2024-1606	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2024-1784	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-0980	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1062	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ	
P2024-1104	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1217	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1367	3	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1369	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1393	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1405	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1406	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2024-1418	3	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: <u>05/10/2024</u>   Analyst: <u>Sarah Collins</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 23A52595   Blank Urine Lot: N/A
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*
☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 4. 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.
☒ 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 6. Transfer **700-800μL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
    Amount transferred: 800uL
☒ 7. 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)
☒ 8. Wait 5 minutes.
☒ 9. Add **2.25mL MTBE. (***Add in 3 increments of 750uL***)**
☒ 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***)**
☒ 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.
☒ 16. Reconstitute in **100μL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic
☒ 1. Create batch and process data.
☒ 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 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. Did all QCs pass for each analyte? (if not, describe in comments section)
☒ 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 ratio

Analytical Plate Map

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_2			p2024-1217-2	IS + QC_1 start
B	IS + Cal. 2			m2024-1606-1	p2024-1104-1	IS + Cal. 7
C	IS + Cal. 3			p2024-1418-3	p2024-1062-1	IS + Cal. 6
D	IS + Cal. 4			p2024-1406-1	p2024-0980-1	IS + Cal. 5
E	IS + Cal. 5			p2024-1405-2	m2024-1784-2	IS + Cal. 4
F	IS + Cal. 6			p2024-1393-1	m2024-1606-1*	IS + Cal. 3
G	IS + Cal. 7			p2024-1369-1	negative blood	IS + Cal. 2
H	IS + QC_1			p2024-1367-3	IS + QC_2 end	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Moved during analytical step 6 due to blood clot

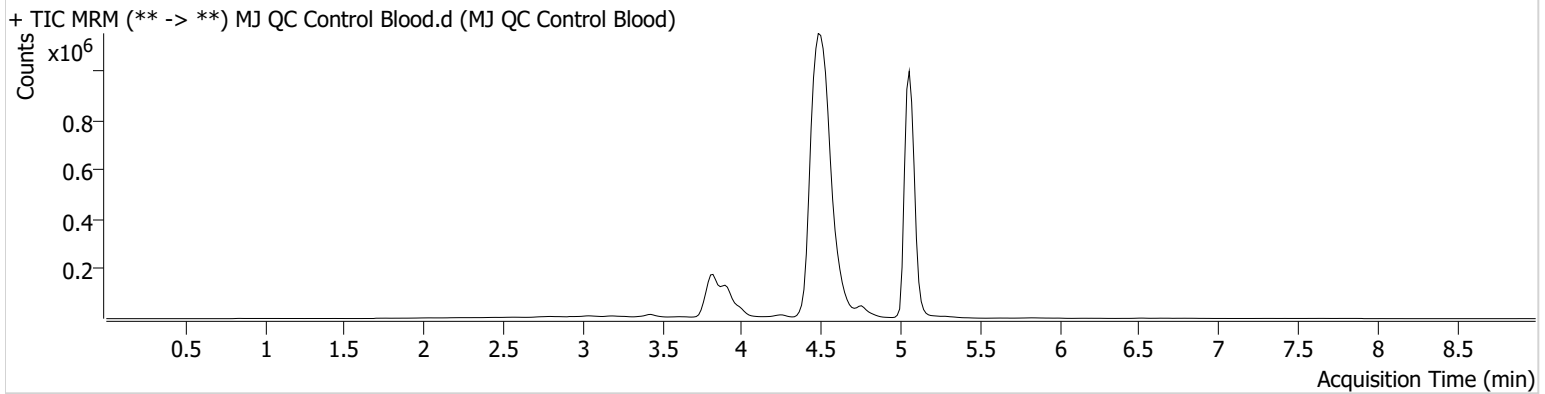Samples were moved on the SLE plate; column 4 to column 1, column 5 to column 2, and column 6 to column 3

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update   5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	MJ QC Control Blood.d
Type	QC	**Sample**	MJ QC Control Blood
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-A3	**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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 11:56:57 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ QC Control Blood.d (MJ QC Control Blood)

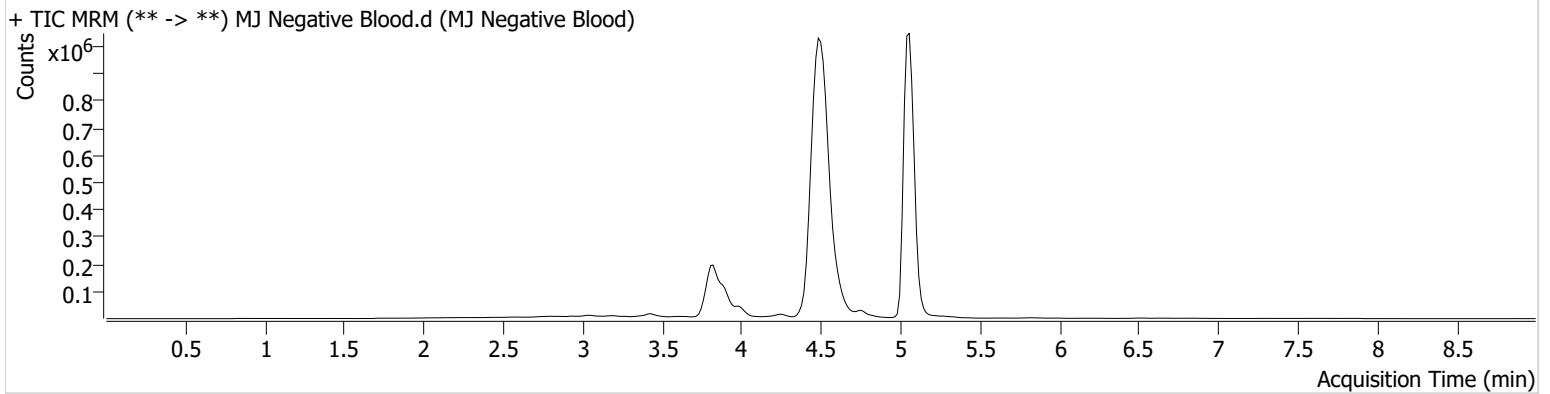| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	169195	∞	25.3	∞	4062806	4.9719 ng/ml
THC-COOH	3.909	32187	633.28	221.9	∞	341973	14.3918 ng/ml
THC-OH	3.820	66075	∞	13.4	109.28	873920	4.6611 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 5/14/2024 10:09:04 AM

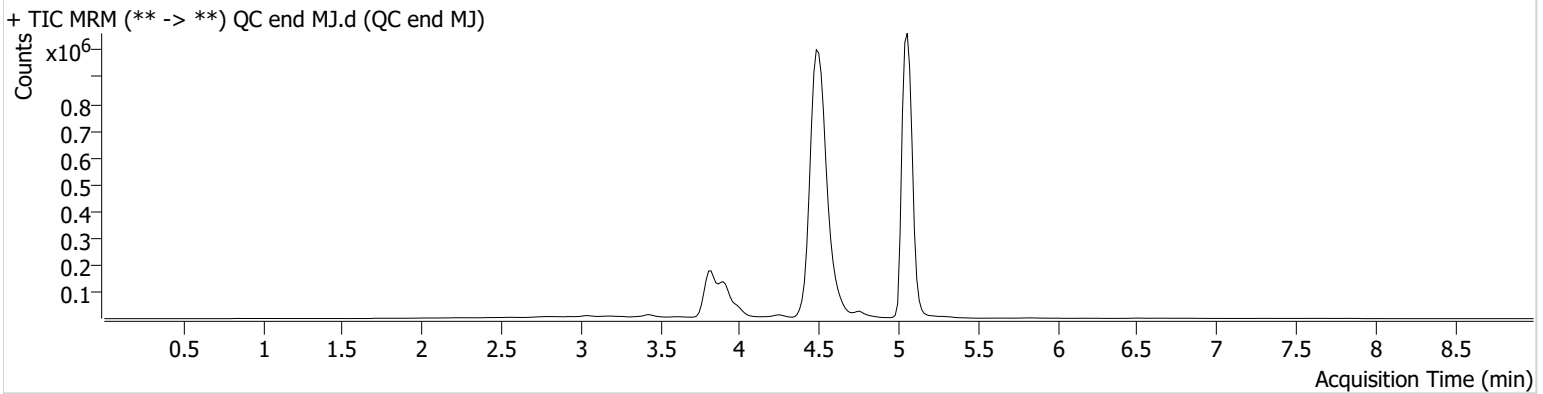|			
Instrument	Falco (069901)	**Data File**	MJ Negative Blood.d
Type	Sample	**Sample**	MJ Negative Blood
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-G2	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for 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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 12:23:10 PM		
Sample Info.			

Sample Chromatogram

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update   5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	QC end MJ.d
Type	QC	**Sample**	QC end MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-H2	**Comment**	Only drugs and concentrations listed on the laboratory
Injection Volume	10		report itself are appropriate to be used for
Acq. Date-Time	5/10/2024 6:03:38 PM		interpretation purposes. Any drugs or values included
Sample Info.			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

+ TIC MRM (** -> **) QC end MJ.d (QC end MJ)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	182793	∞	25.3	∞	4546323	4.8086 ng/ml
THC-COOH	3.909	32197	∞	232.4	∞	330406	14.8722 ng/ml
THC-OH	3.820	66824	∞	12.6	∞	805858	5.0751 ng/ml

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin

Last Cal. Update   5/14/2024 10:09 AM

Analyst Name   ISP\datastor

Analyte   THC   **Internal Standard**   THC-D3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs

$y = 0.008803 * x - 0.002125$

$R^2 = 0.99957758$

Type:Linear, Origin:Ignore, Weight:1/x
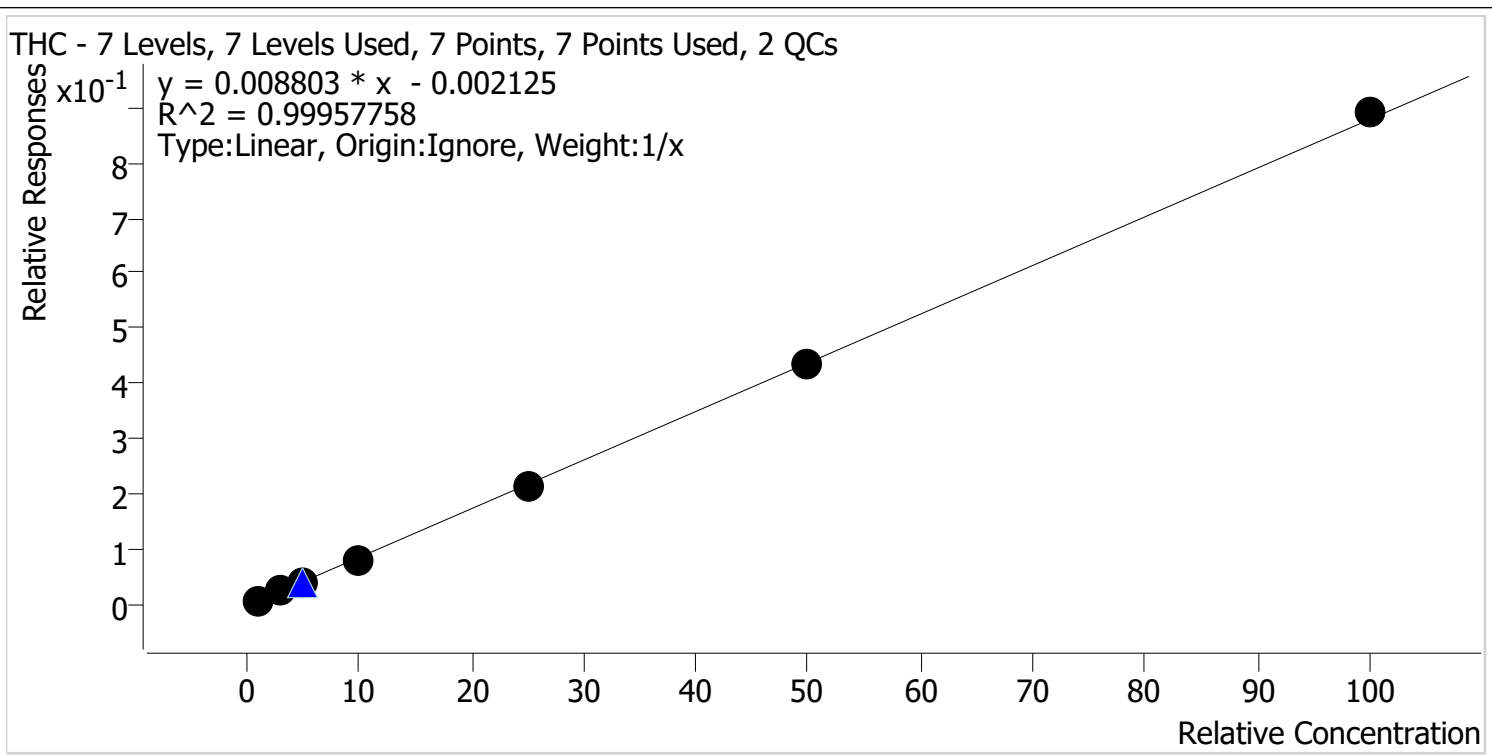
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	1.0	1.1	109.7
Cal 2 MJ	2	✔	3.0	2.9	98.1
Cal 3 MJ	3	✔	5.0	4.9	98.1
Cal 4 MJ	4	✔	10.0	9.6	96.2
Cal 5 MJ	5	✔	25.0	24.2	96.9
Cal 6 MJ	6	✔	50.0	49.8	99.7
Cal 7 MJ	7	✔	100.0	101.4	101.4

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update   5/14/2024 10:09 AM
Analyst Name   ISP\datastor
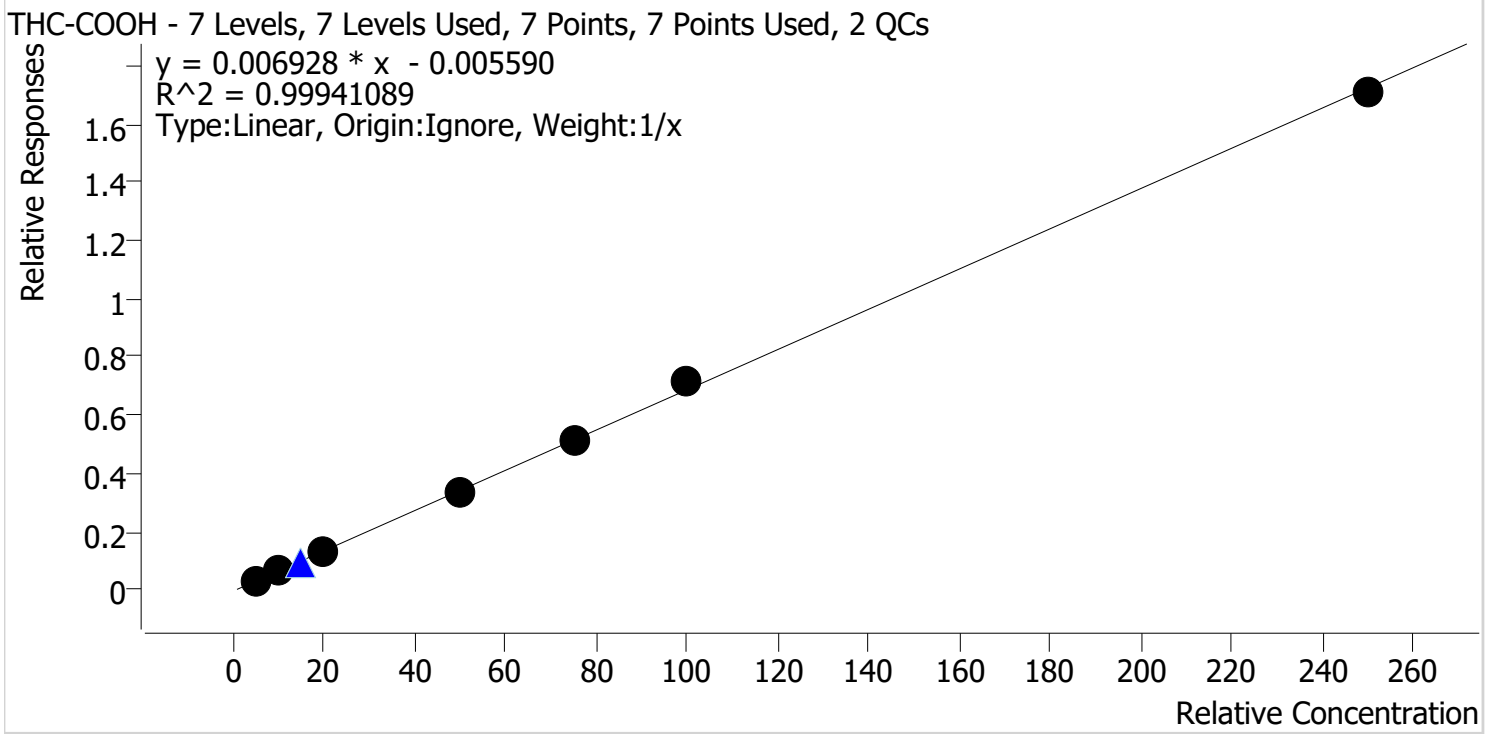**Analyte**   THC-COOH   **Internal Standard**   THC-COOH-D9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs

$y = 0.006928 * x - 0.005590$
$R^2 = 0.99941089$
Type:Linear, Origin:Ignore, Weight:1/x

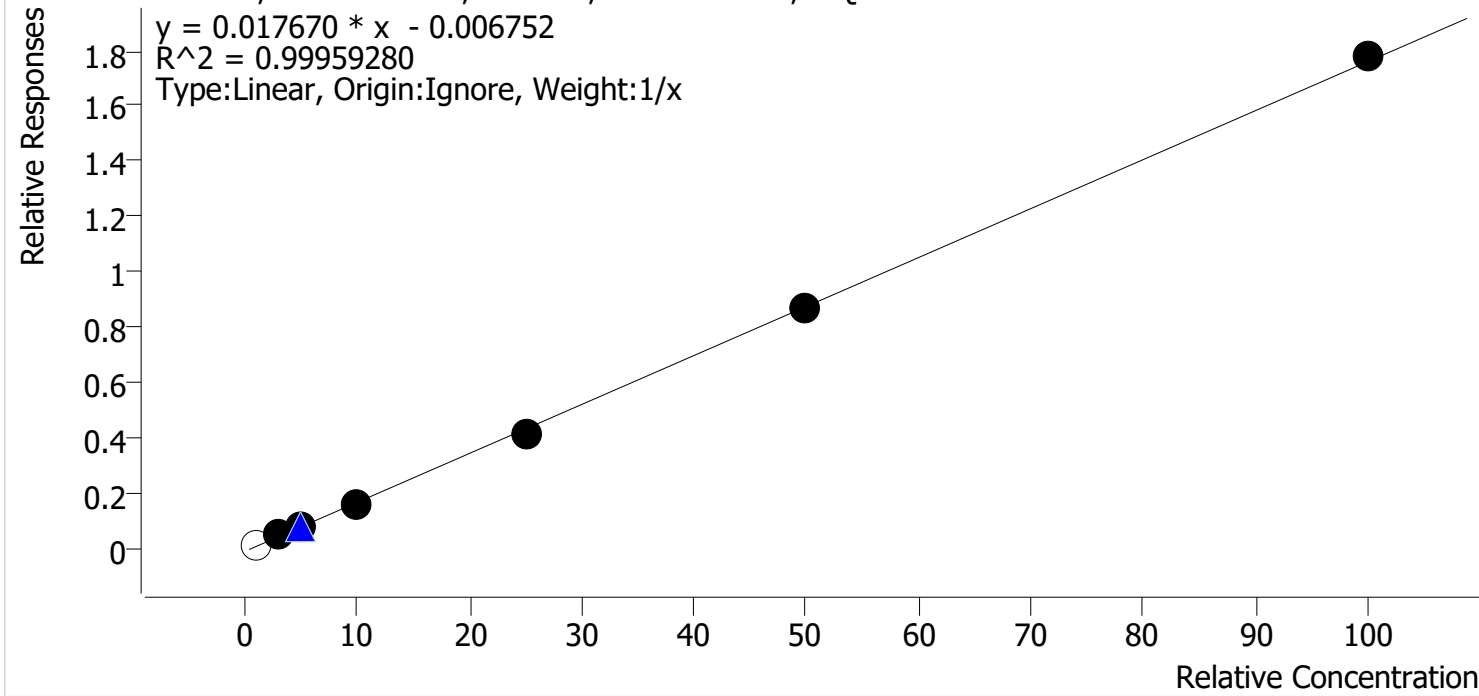| Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	5.0	5.1	102.5
Cal 2 MJ	2	✔	10.0	9.9	98.8
Cal 3 MJ	3	✔	20.0	19.5	97.6
Cal 4 MJ	4	✔	50.0	49.3	98.7
Cal 5 MJ	5	✔	75.0	74.4	99.2
Cal 6 MJ	6	✔	100.0	104.2	104.2
Cal 7 MJ	7	✔	250.0	247.6	99.0

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update   5/14/2024 10:09 AM
Analyst Name   ISP\datastor
Analyte   THC-OH   **Internal Standard**   THC-OH-D3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 2 QCs

$y = 0.017670 * x - 0.006752$
$R^2 = 0.99959280$
Type:Linear, Origin:Ignore, Weight:1/x

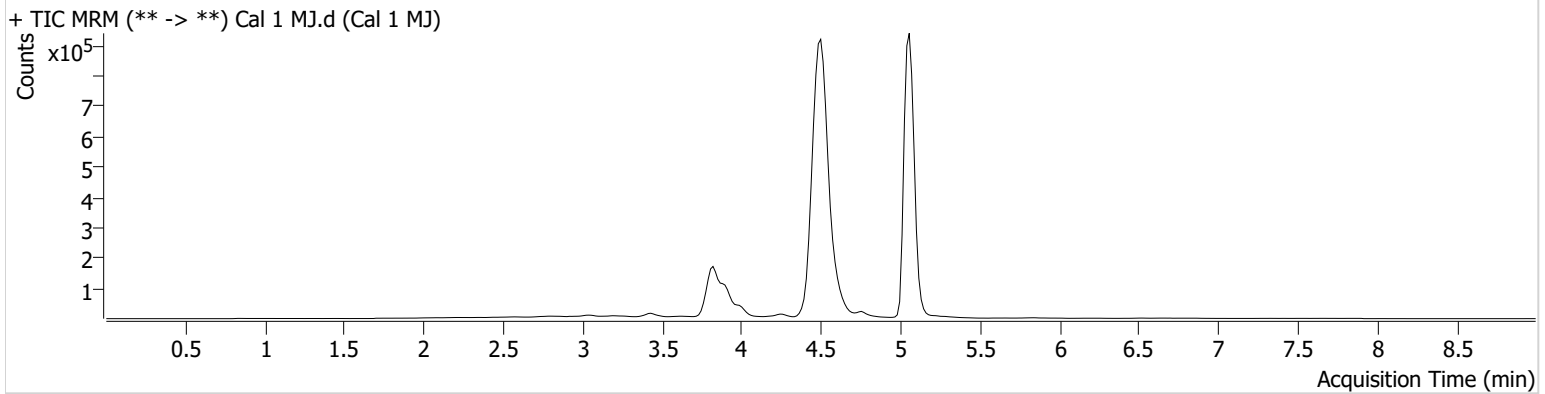| Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✗	1.0	1.4	136.6
Cal 2 MJ	2	✔	3.0	3.2	106.8
Cal 3 MJ	3	✔	5.0	4.8	97.0
Cal 4 MJ	4	✔	10.0	9.9	98.8
Cal 5 MJ	5	✔	25.0	24.2	96.7
Cal 6 MJ	6	✔	50.0	49.9	99.8
Cal 7 MJ	7	✔	100.0	101.0	101.0

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	Cal 1 MJ.d
Type	Cal	**Sample**	Cal 1 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-H3	**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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 10:11:59 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) Cal 1 MJ.d (Cal 1 MJ)

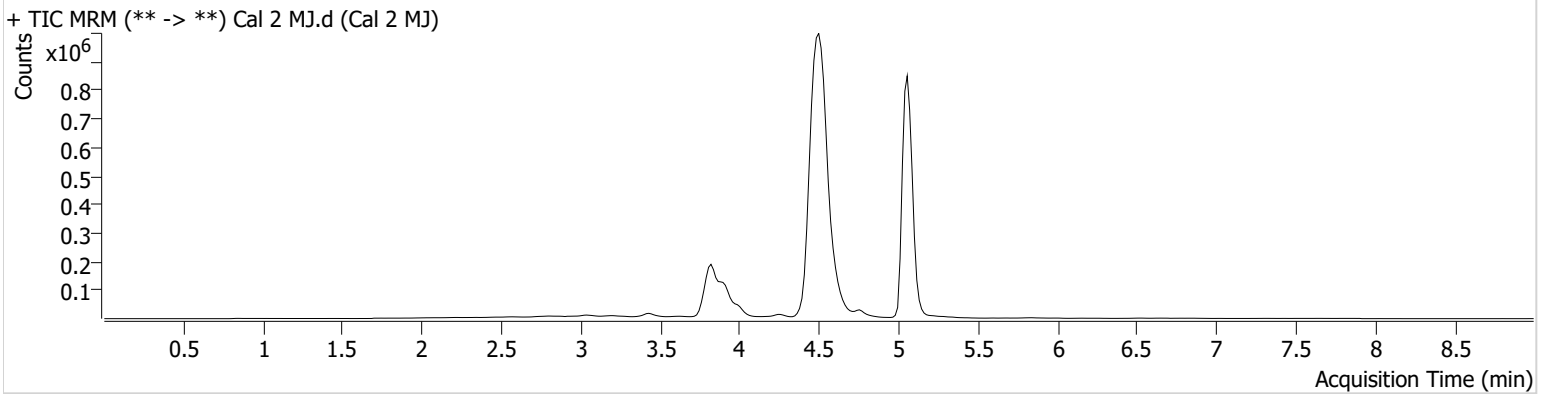| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	31061	378.51	27.4	∞	4124237	1.0969 ng/ml
THC-COOH	3.909	10127	573.77	225.5	150.55	338584	5.1241 ng/ml
THC-OH	3.820	14264	∞	5.5 **Low**	6.35 **Low**	820327	1.3662 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	Cal 2 MJ.d
Type	Cal	**Sample**	Cal 2 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-G3	**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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 10:25:14 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) Cal 2 MJ.d (Cal 2 MJ)

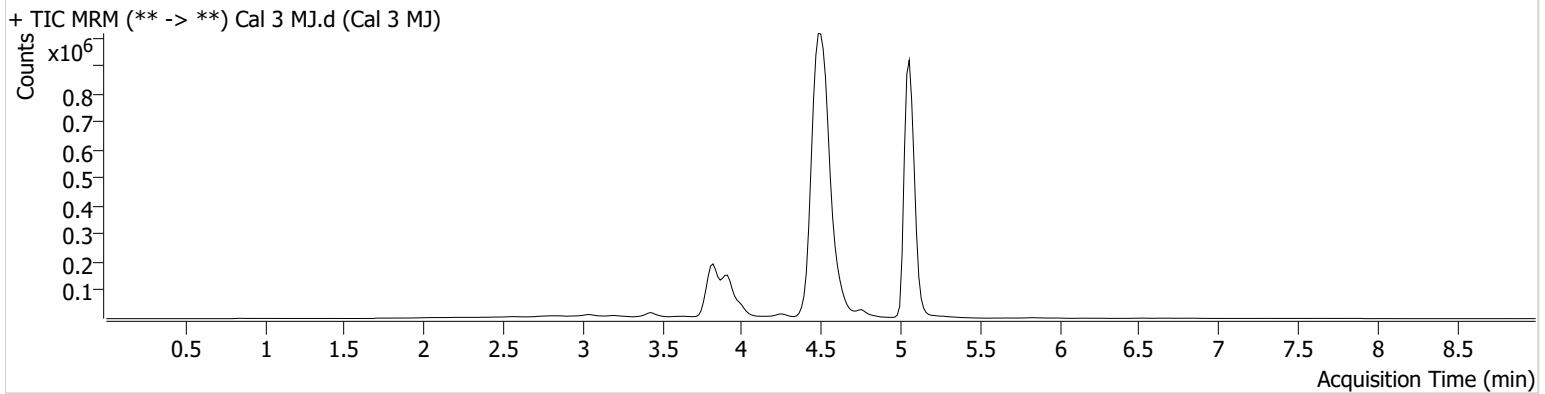| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	86035	582.34	25.2	∞	3617138	2.9432 ng/ml
THC-COOH	3.909	22039	91.12	217.1	2872.32	350437	9.8842 ng/ml
THC-OH	3.820	43935	∞	13.2	∞	881576	3.2026 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update   5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	Cal 3 MJ.d
Type	Cal	**Sample**	Cal 3 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-F3	**Comment**	Only drugs and concentrations listed on the laboratory
Injection Volume	10		report itself are appropriate to be used for
Acq. Date-Time	5/10/2024 10:38:20 AM		interpretation purposes. Any drugs or values included
Sample Info.			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

+ TIC MRM (** -> **) Cal 3 MJ.d (Cal 3 MJ)

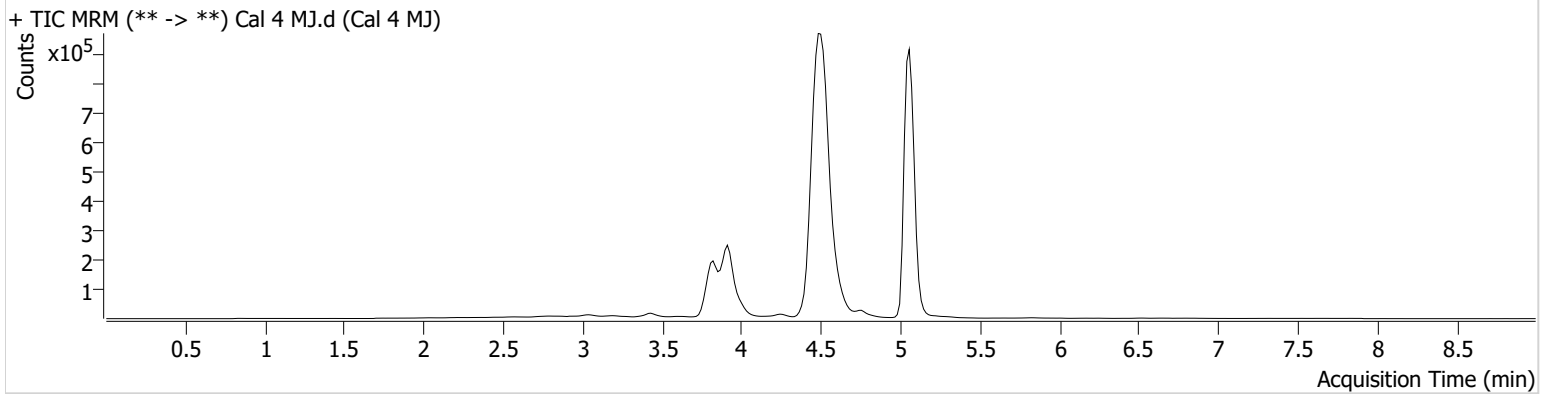| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	157664	∞	23.4	107.28	3841469	4.9035 ng/ml
THC-COOH	3.909	45322	195.49	231.4	∞	349462	19.5258 ng/ml
THC-OH	3.820	69878	∞	13.3	∞	885311	4.8491 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update   5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	Cal 4 MJ.d
Type	Cal	**Sample**	Cal 4 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-E3	**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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 10:51:26 AM		
Sample Info.			

Sample Chromatogram

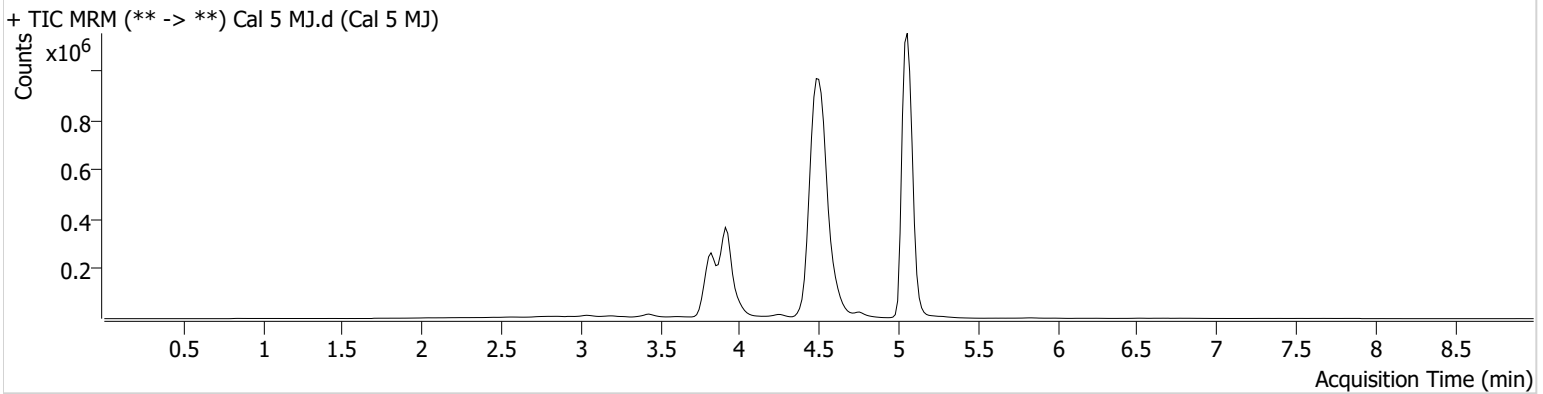| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	303614	3699.07	24.7	∞	3679066	9.6156 ng/ml
THC-COOH	3.909	115479	∞	214.5	865.40	343507	49.3295 ng/ml
THC-OH	3.820	138758	∞	13.2	∞	826831	9.8797 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update   5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	Cal 5 MJ.d
Type	Cal	**Sample**	Cal 5 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-D3	**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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 11:04:34 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) Cal 5 MJ.d (Cal 5 MJ)

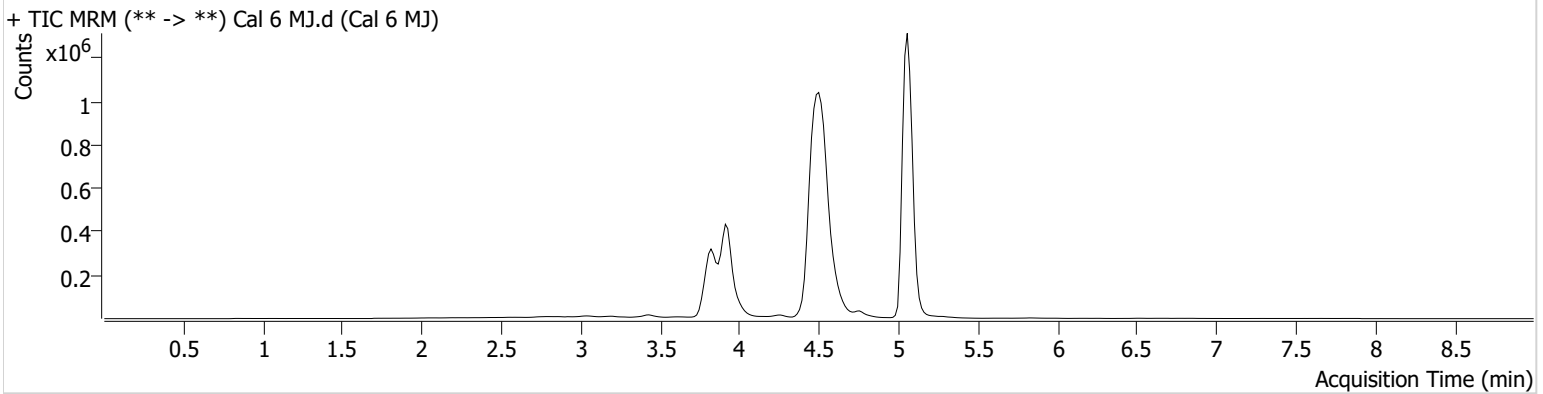| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	875820	∞	25.1	∞	4146986	24.2315 ng/ml
THC-COOH	3.909	187027	∞	211.6	4027.99	366886	74.3850 ng/ml
THC-OH	3.820	380839	∞	13.2	∞	906285	24.1641 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update   5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	Cal 6 MJ.d
Type	Cal	**Sample**	Cal 6 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-C3	**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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 11:17:39 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) Cal 6 MJ.d (Cal 6 MJ)

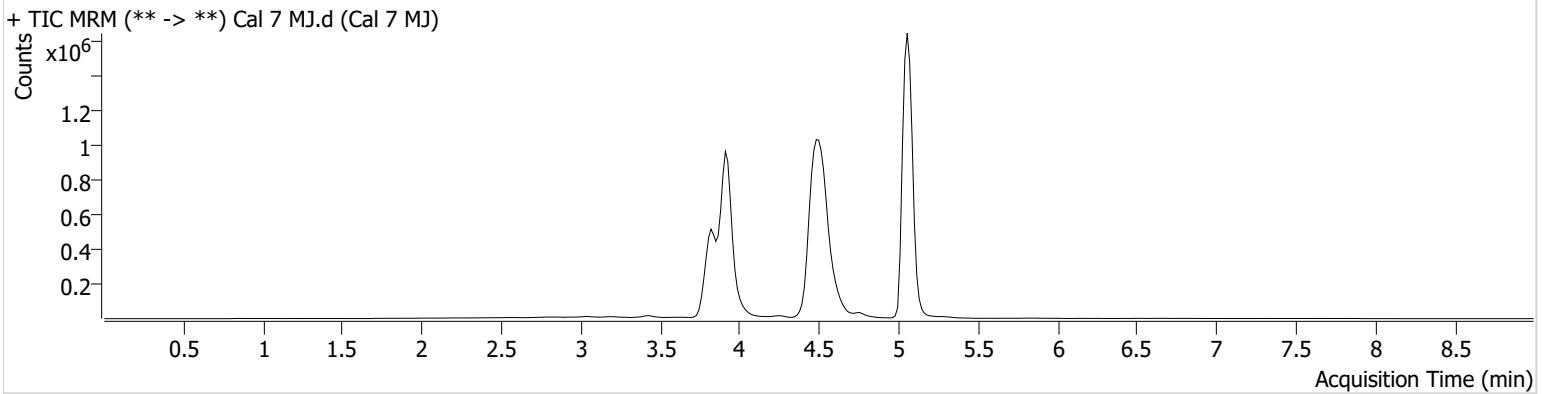| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	1616779	∞	25.0	956.12	3702443	49.8448 ng/ml
THC-COOH	3.909	232092	4337.18	213.0	∞	324064	104.1792 ng/ml
THC-OH	3.820	725231	∞	13.1	∞	828761	49.9063 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 27 28\051024 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 5/14/2024 10:09:04 AM

Instrument	Falco (069901)	**Data File**	Cal 7 MJ.d
Type	Cal	**Sample**	Cal 7 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Sarah Collins
Sample Position	P1-B3	**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.
Injection Volume	10		
Acq. Date-Time	5/10/2024 11:30:45 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) Cal 7 MJ.d (Cal 7 MJ)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.060	3036440	∞	25.1	∞	3410861	101.3645 ng/ml
THC-COOH	3.909	576579	∞	215.6	14557.78	337248	247.5722 ng/ml
THC-OH	3.820	1566792	∞	13.2	∞	881282	100.9982 ng/ml