_TS_   6/3/2024

Worklist: 6830

LAB CASE	ITEM	ITEM TYPE	DESCRIPTION
P2024-1316	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1368	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1404	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1430	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1452	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1502	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1513	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1525	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1527	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2024-1531	1	BCK	AM 27 Blood THC Quant by LC-QQQ
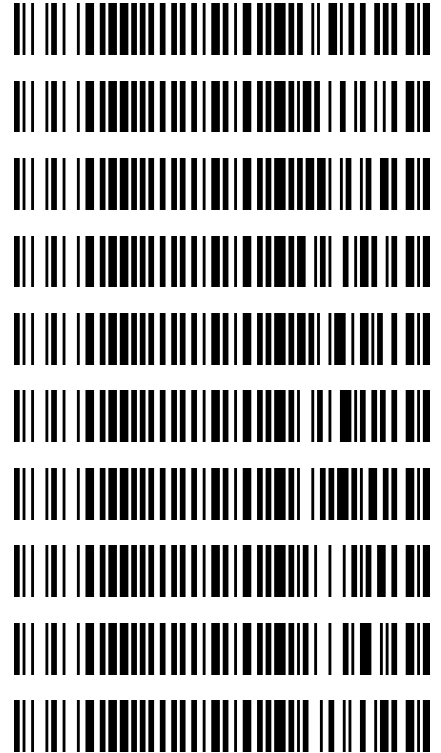
AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 06/03/2024   Analyst: Tamara Salazar
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 24C52816   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.
☒ 3. 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.
☒ 5. 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.
☒ 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)
☒ 9. Wait 5 minutes.
☒ 10. Add **2.25mL MTBE. (***Add in 3 increments of 750uL)*
☒ 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)*
☒ 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.
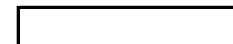
Post-Analytic
☒ 1. Create batch and process data.
☒ 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥0.98 for each analyte
☒ 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less).
         Ion ratios must 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:

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1		P2024-1430-1	P2024-1502-1	IS + QC_1
B	IS + Cal. 2			P2024-1404-1		IS + Cal. 7
C	IS + Cal. 3			P2024-1368-3		IS + Cal. 6
D	IS + Cal. 4			P2024-1316-1		IS + Cal. 5
E	IS + Cal. 5			P2024-1531-1		IS + Cal. 4
F	IS + Cal. 6			P2024-1527-1		IS + Cal. 3
G	IS + Cal. 7			P2024-1525-1	Neg Blood	IS + Cal. 2
H	IS + QC_1		P2024-1452-1	P2024-1513-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO
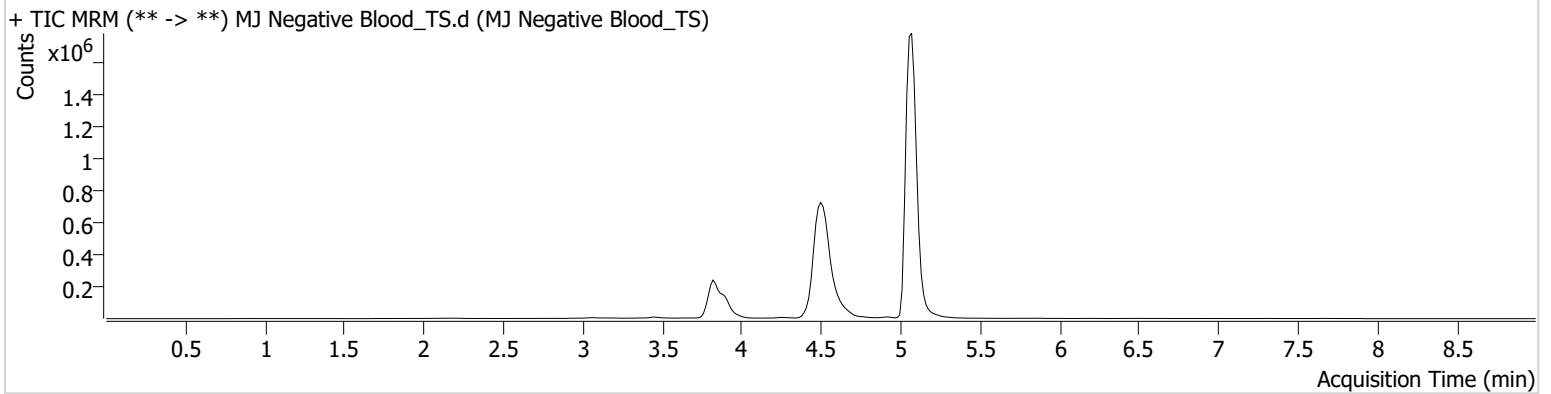
AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update   6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	MJ Negative Blood_TS.d
Type	Sample	**Sample**	MJ Negative Blood_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-G5	**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	6/4/2024 3:35:15 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ Negative Blood_TS.d (MJ Negative Blood_TS)

AM #27 Cannabinoids Quant. Results

Instrument	Falco (069901)	**Data File**	MJ QC Control_TS.d
Type	QC	**Sample**	MJ QC Control_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-A6	**Comment**	Only drugs and concentrations listed on the laboratory
Injection Volume	10		report itself are appropriate to be used for
Acq. Date-Time	6/4/2024 3:09:02 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 (** -> **) MJ QC Control_TS.d (MJ QC Control_TS)

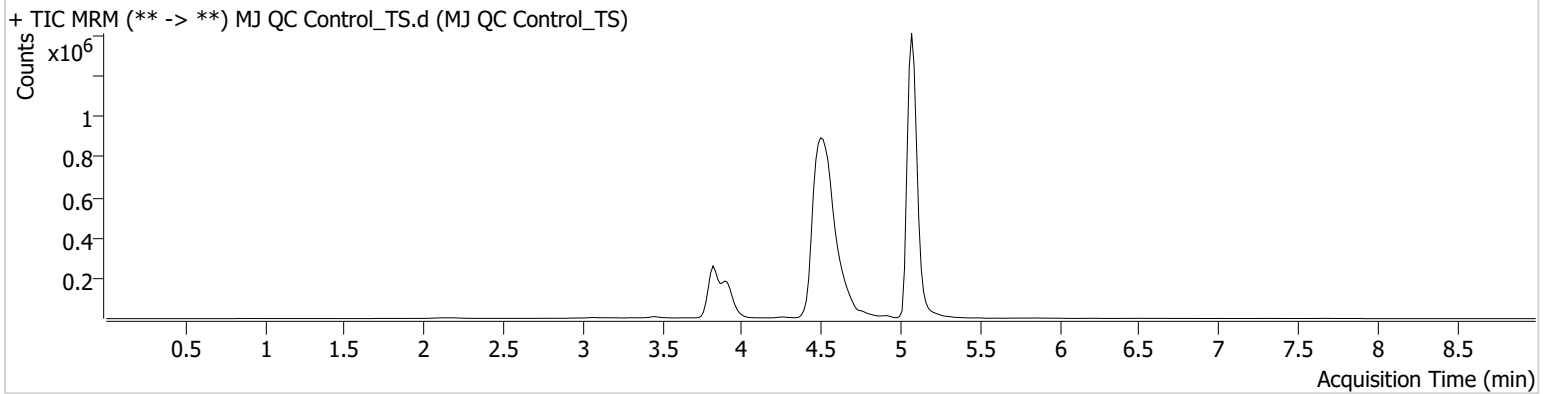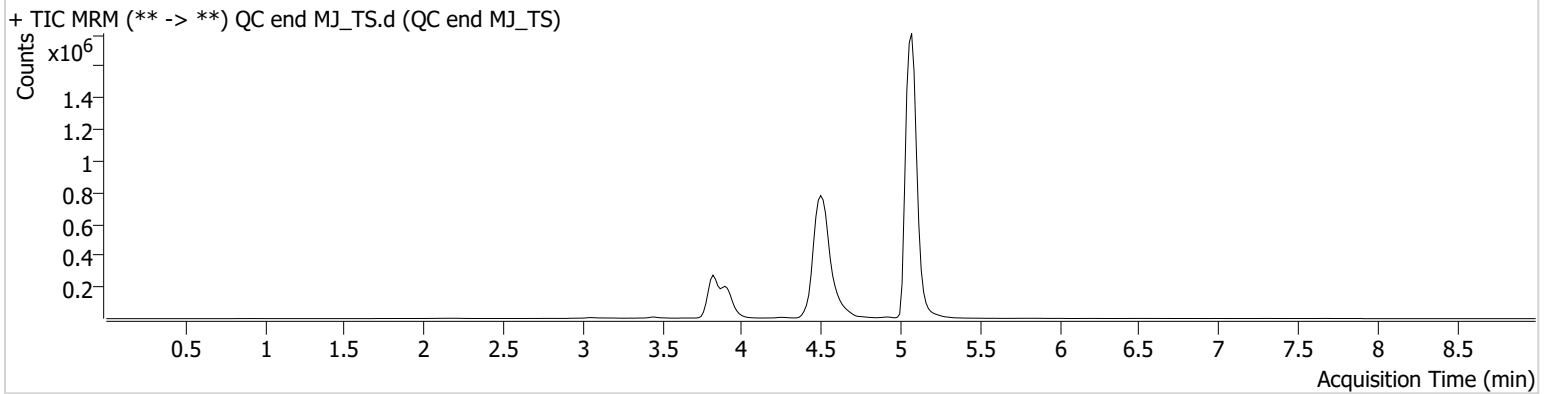| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	253311	∞	25.0	∞	5463455	5.0593 ng/ml
THC-COOH	3.924	51881	251.74	228.0	774.28	446801	15.0267 ng/ml
THC-OH	3.835	98744	∞	14.1	∞	1117537	4.7614 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	QC end MJ_TS.d
Type	QC	**Sample**	QC end MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-H5	**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	6/4/2024 8:23:59 AM		
Sample Info.			

Sample Chromatogram

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

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	357677	∞	24.5	∞	7930565	4.9298 ng/ml
THC-COOH	3.924	57523	1306.61	234.8	∞	495089	15.0355 ng/ml
THC-OH	3.820	107103	∞	13.8	161.74	1153773	4.9939 ng/ml

TS

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Last Cal. Update   6/4/2024 10:13 AM
Analyst Name   ISP\Datastor
Analyte   THC   **Internal Standard**   THC-D3
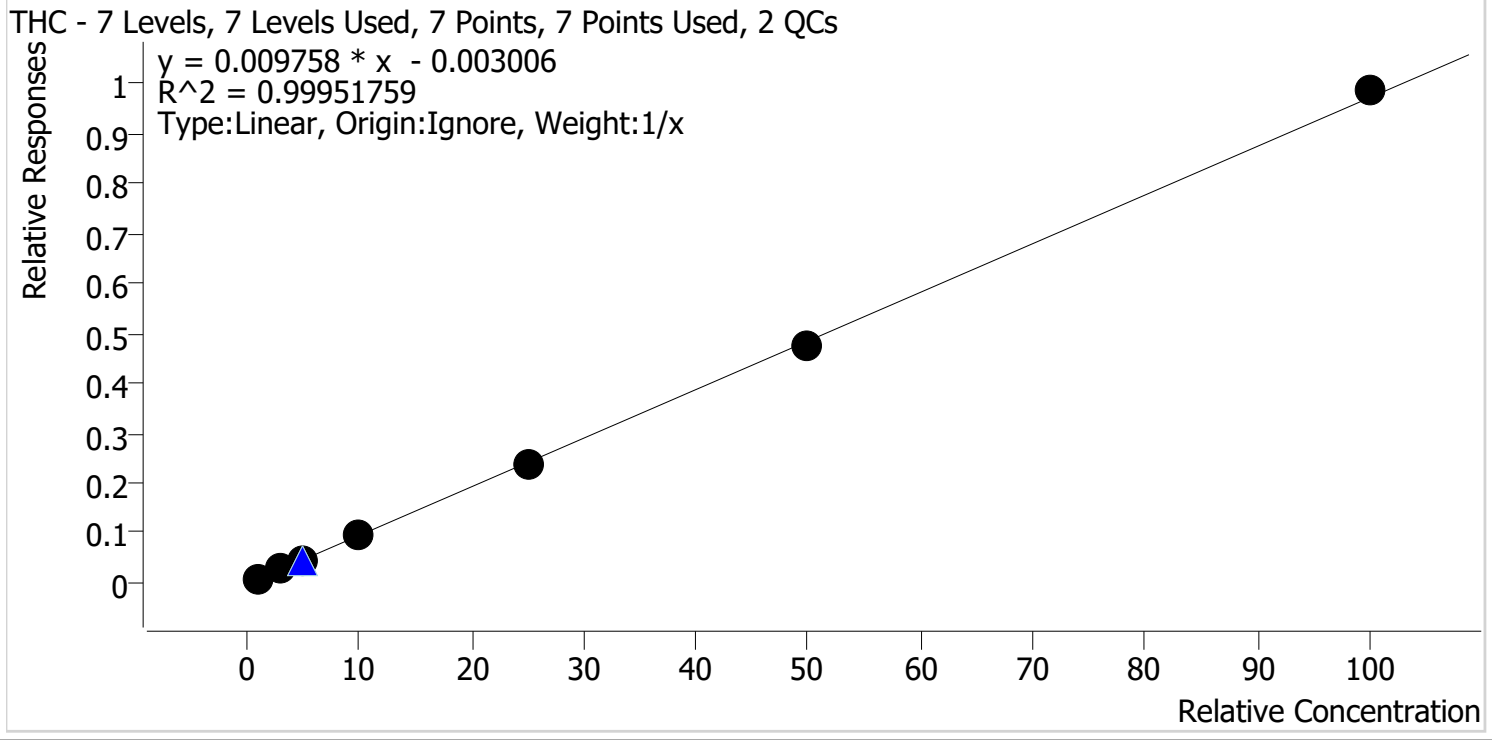
THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs

$y = 0.009758 * x - 0.003006$
$R^2 = 0.99951759$
Type:Linear, Origin:Ignore, Weight:1/x

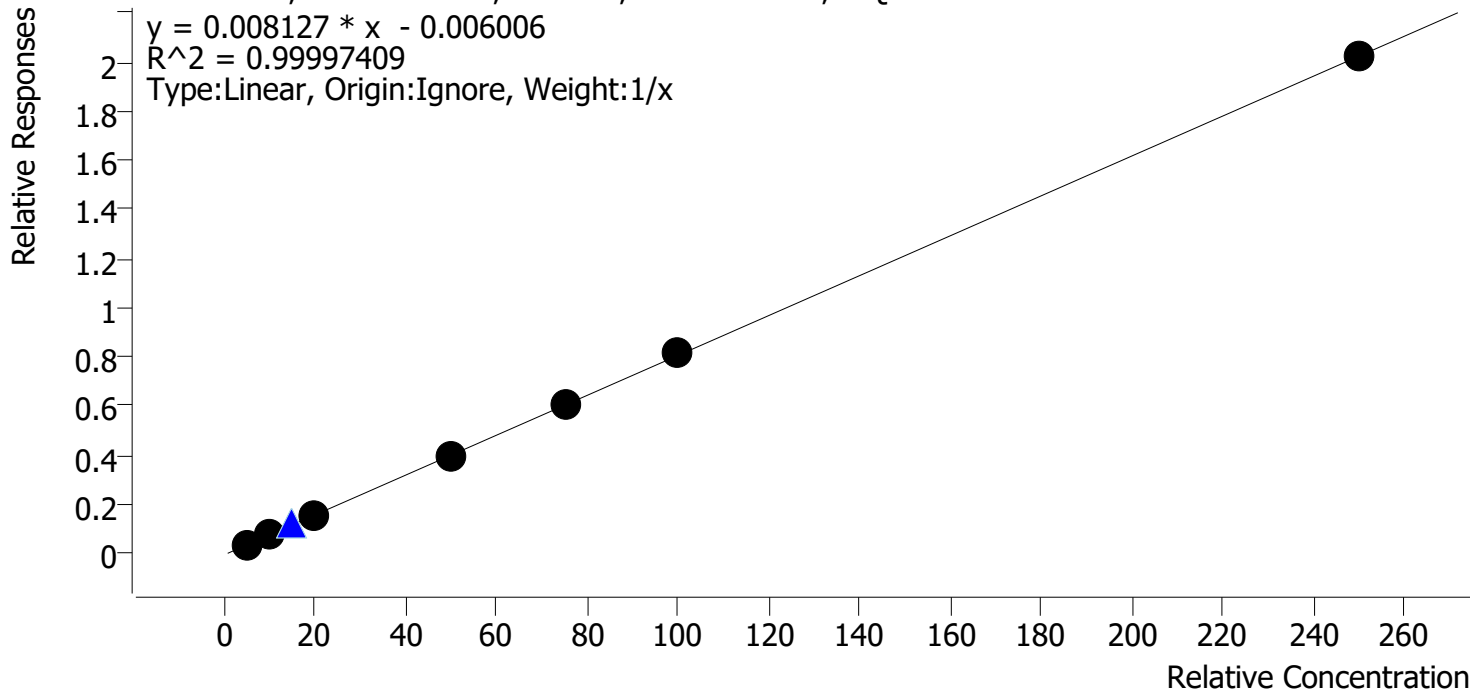| Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ_TS	1	✔	1.0	1.1	111.6
Cal 2 MJ_TS	2	✔	3.0	3.0	98.5
Cal 3 MJ_TS	3	✔	5.0	4.6	92.8
Cal 4 MJ_TS	4	✔	10.0	9.8	97.6
Cal 5 MJ_TS	5	✔	25.0	24.9	99.8
Cal 6 MJ_TS	6	✔	50.0	49.2	98.3
Cal 7 MJ_TS	7	✔	100.0	101.4	101.4

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Last Cal. Update   6/4/2024 10:13 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

$y = 0.008127 * x - 0.006006$
$R^2 = 0.99997409$
Type:Linear, Origin:Ignore, Weight:1/x

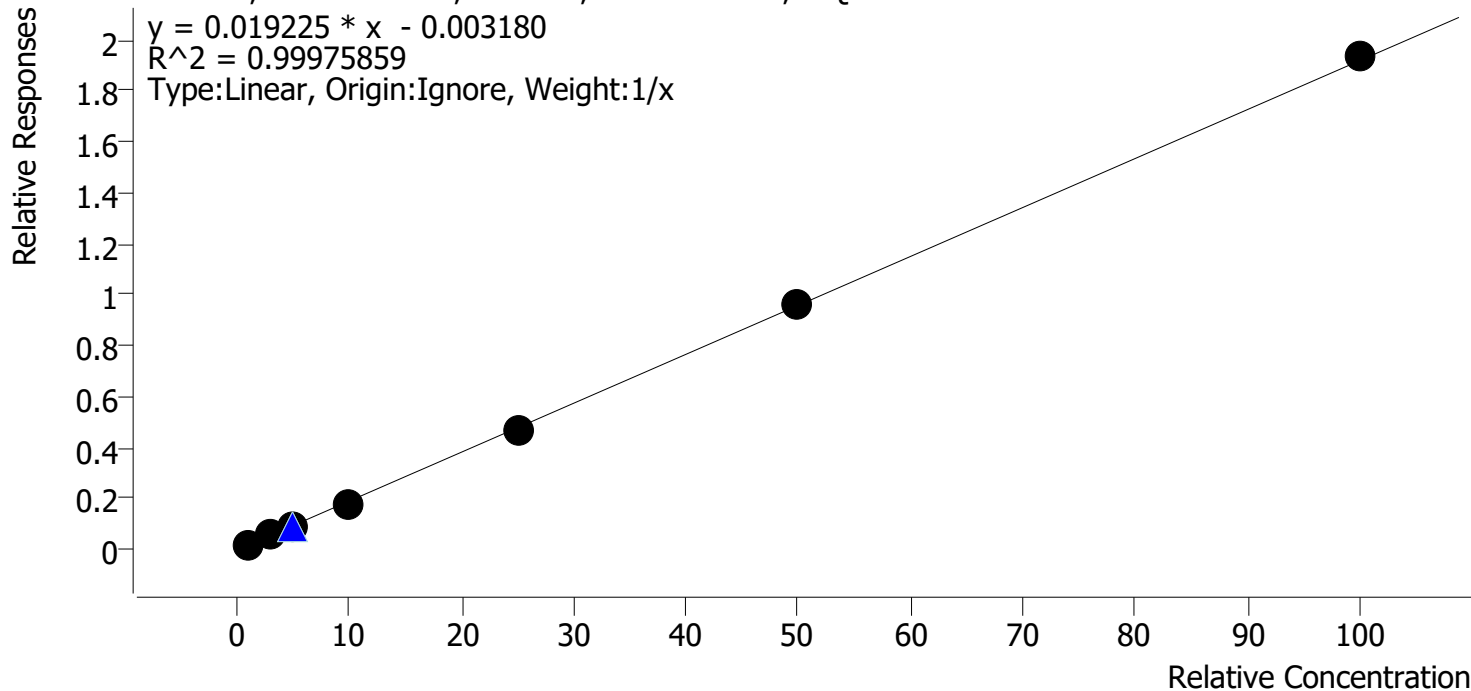| Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ_TS	1	✔	5.0	5.1	101.3
Cal 2 MJ_TS	2	✔	10.0	9.9	98.7
Cal 3 MJ_TS	3	✔	20.0	20.0	99.8
Cal 4 MJ_TS	4	✔	50.0	50.1	100.1
Cal 5 MJ_TS	5	✔	75.0	74.6	99.4
Cal 6 MJ_TS	6	✔	100.0	100.7	100.7
Cal 7 MJ_TS	7	✔	250.0	249.7	99.9

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin

Last Cal. Update   6/4/2024 10:13 AM

Analyst Name   ISP\Datastor

Analyte   THC-OH   **Internal Standard**   THC-OH-D3

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

$y = 0.019225 * x - 0.003180$

$R^2 = 0.99975859$

Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ_TS	1	✔	1.0	1.1	109.9
Cal 2 MJ_TS	2	✔	3.0	2.9	97.5
Cal 3 MJ_TS	3	✔	5.0	4.8	96.6
Cal 4 MJ_TS	4	✔	10.0	9.6	96.4
Cal 5 MJ_TS	5	✔	25.0	24.7	98.9
Cal 6 MJ_TS	6	✔	50.0	50.0	100.0
Cal 7 MJ_TS	7	✔	100.0	100.8	100.8

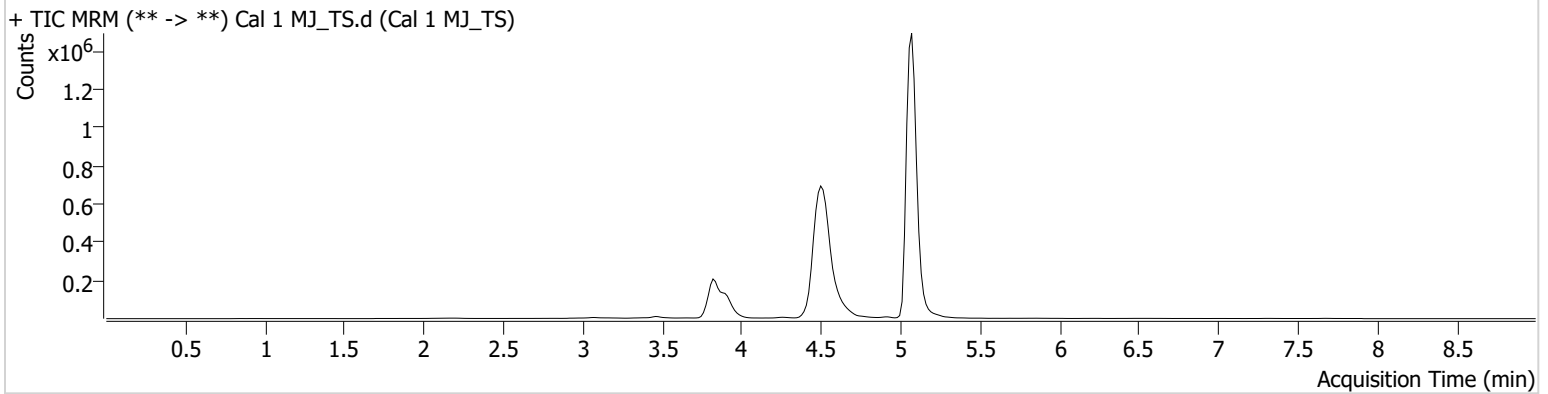   Generated at 10:30 AM on 6/4/2024

AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	Cal 1 MJ_TS.d
Type	Cal	**Sample**	Cal 1 MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-H6	**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	6/4/2024 1:24:00 AM		
Sample Info.			

Sample Chromatogram

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

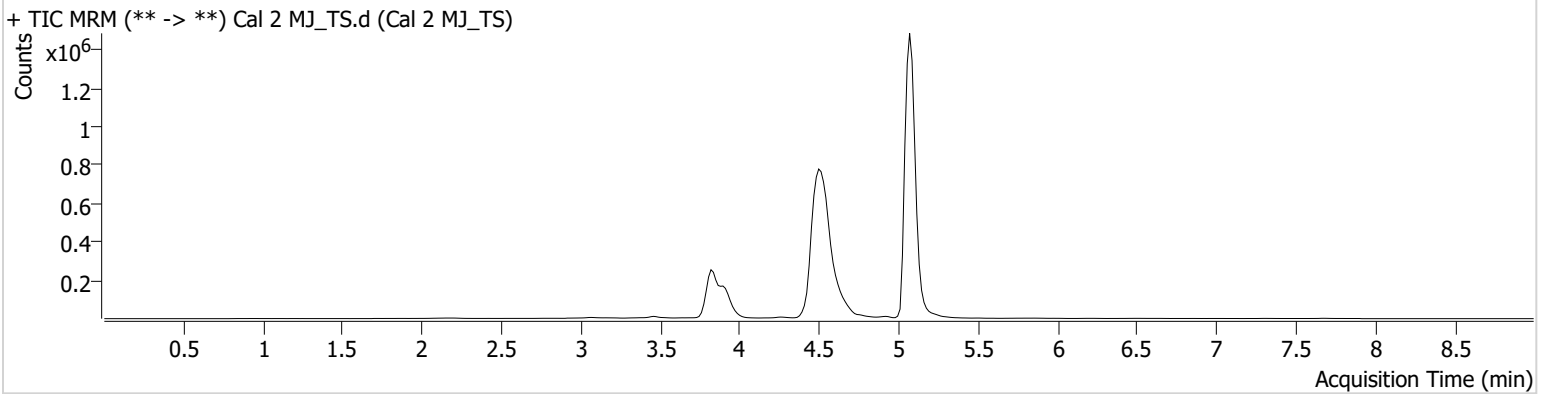| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	49228	550.15	27.4	542.68	6245865	1.1157 ng/ml
THC-COOH	3.924	13874	195.75	239.6	167.15	394853	5.0627 ng/ml
THC-OH	3.835	16902	92.91	13.6	28.87	941990	1.0987 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	Cal 2 MJ_TS.d
Type	Cal	**Sample**	Cal 2 MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-G6	**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	6/4/2024 1:37:17 AM		
Sample Info.			

Sample Chromatogram

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

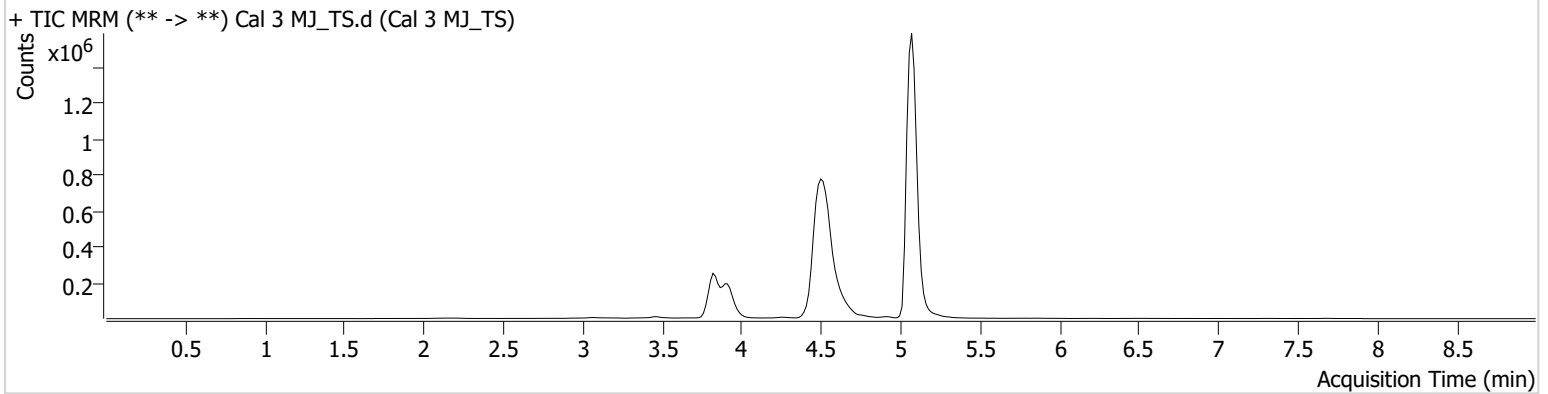| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	158953	∞	25.3	∞	6157192	2.9535 ng/ml
THC-COOH	3.924	33004	908.37	233.7	∞	444599	9.8731 ng/ml
THC-OH	3.835	59382	∞	13.3	259.01	1119238	2.9251 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update   6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	Cal 3 MJ_TS.d
Type	Cal	**Sample**	Cal 3 MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-F6	**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	6/4/2024 1:50:23 AM		
Sample Info.			

Sample Chromatogram

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

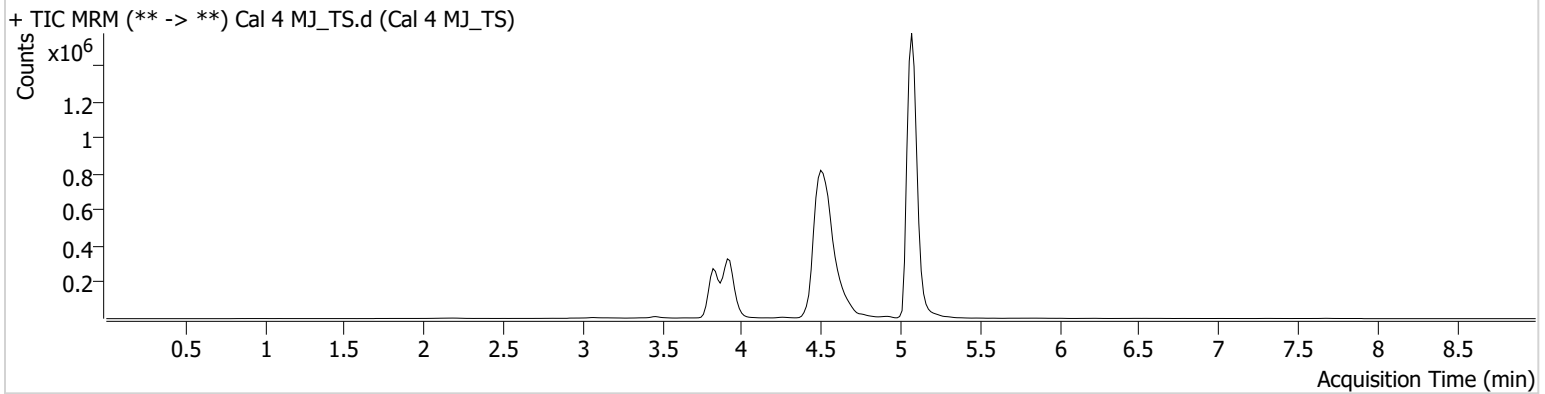| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	271188	∞	25.5	∞	6414119	4.6407 ng/ml
THC-COOH	3.924	66240	240.65	213.5	∞	423858	19.9686 ng/ml
THC-OH	3.835	94116	∞	13.6	457.90	1049959	4.8280 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update   6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	Cal 4 MJ_TS.d
Type	Cal	**Sample**	Cal 4 MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-E6	**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	6/4/2024 2:03:29 AM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) Cal 4 MJ_TS.d (Cal 4 MJ_TS)

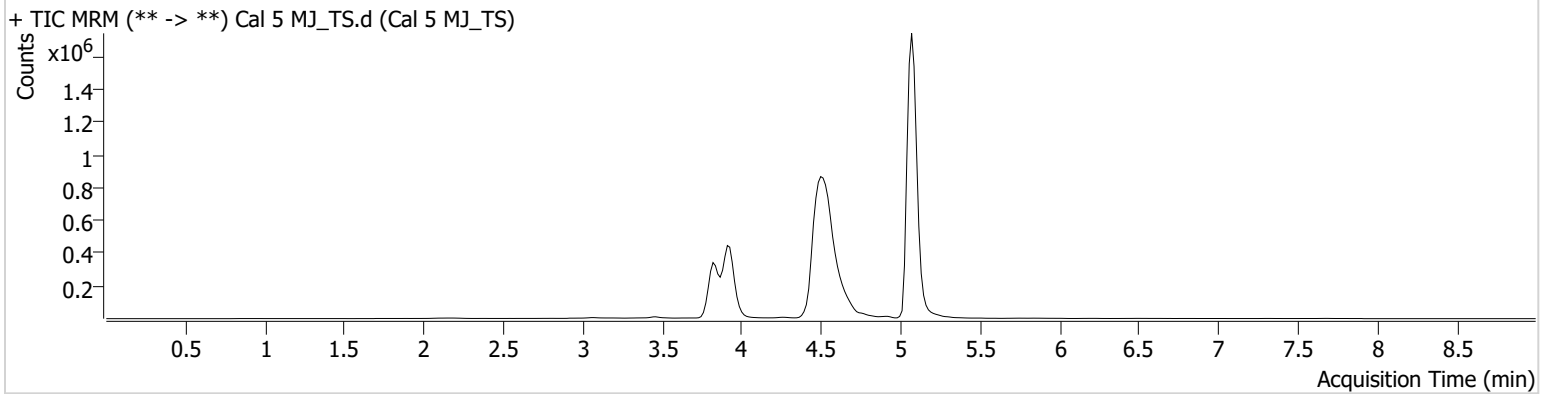| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	541798	∞	24.2	∞	5871708	9.7637 ng/ml
THC-COOH	3.924	169854	3420.07	176.5	5038.00	423695	50.0667 ng/ml
THC-OH	3.835	191007	∞	13.4	295.39	1048562	9.6407 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update   6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	Cal 5 MJ_TS.d
Type	Cal	**Sample**	Cal 5 MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-D6	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/4/2024 2:16:36 AM		
Sample Info.			

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.

Sample Chromatogram

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

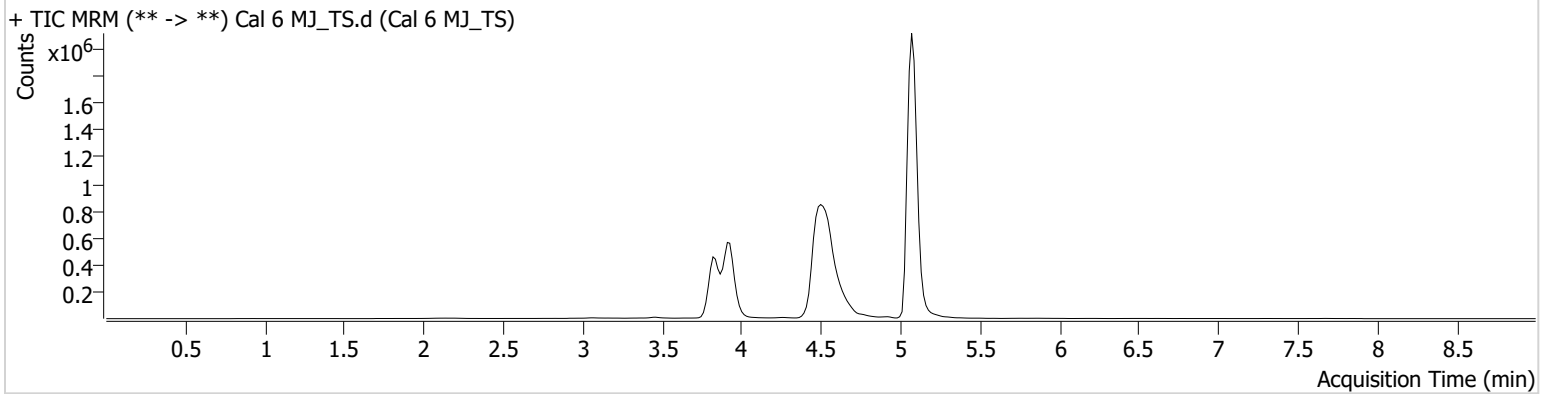| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	1328687	6800.82	24.7	∞	5526694	24.9444 ng/ml
THC-COOH	3.924	255934	8216.49	188.4	∞	426478	74.5802 ng/ml
THC-OH	3.835	498690	∞	13.8	∞	1056674	24.7139 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update   6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	Cal 6 MJ_TS.d
Type	Cal	**Sample**	Cal 6 MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-C6	**Comment**	Only drugs and concentrations listed on the laboratory
Injection Volume	10		report itself are appropriate to be used for
Acq. Date-Time	6/4/2024 2:29:43 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 6 MJ_TS.d (Cal 6 MJ_TS)

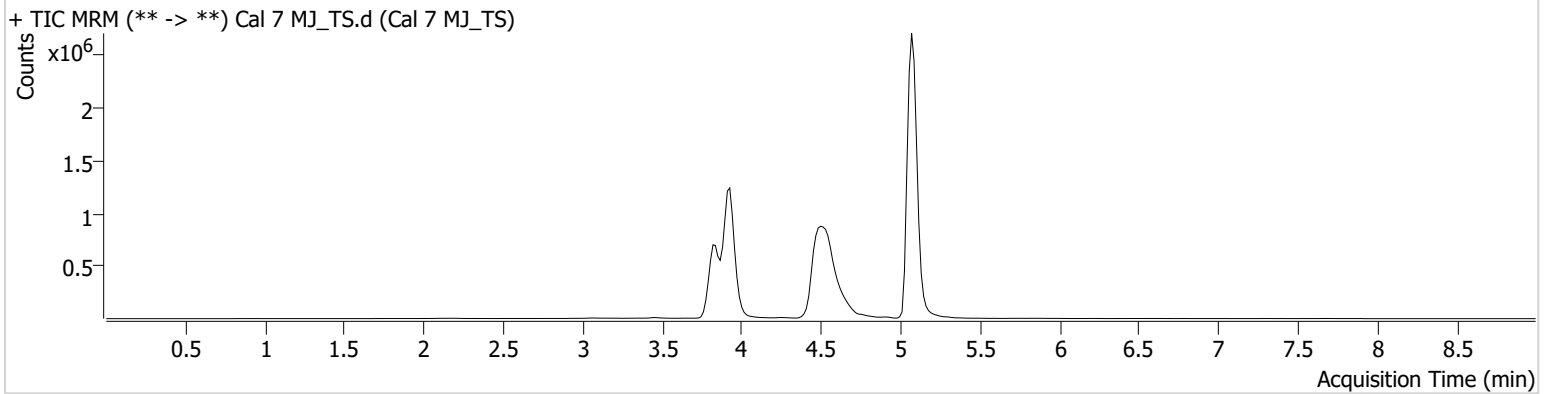| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	2619337	∞	26.0	∞	5494116	49.1635 ng/ml
THC-COOH	3.924	341091	∞	189.6	∞	419868	100.6991 ng/ml
THC-OH	3.835	1009373	5254.99	13.9	2797.39	1053396	50.0075 ng/ml

AM #27 Cannabinoids Quant. Results

Batch results   D:\MassHunter\Data\2024\AM 25 26\060324 AM 25 26 CS\QuantResults\AM 27.batch.bin
Calibration Last Update   6/4/2024 10:13:56 AM

Instrument	Falco (069901)	**Data File**	Cal 7 MJ_TS.d
Type	Cal	**Sample**	Cal 7 MJ_TS
Acq. Method	AM 27 Agilent Method.m	**Operator**	Tamara Salazar
Sample Position	P5-B6	**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	6/4/2024 2:42:49 AM		
Sample Info.			

Sample Chromatogram

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

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.075	4936682	20852.91	25.6	∞	5003330	101.4183 ng/ml
THC-COOH	3.924	829111	4616.39	181.2	11374.78	409698	249.7497 ng/ml
THC-OH	3.835	2087040	∞	13.9	∞	1078895	100.7861 ng/ml