SJ

Worklist: 4605

LAB CASE	ITEM	ITEM TYPE	DESCRIPTION	
M2020-3064	2	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
M2020-3924	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
M2020-3934	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
M2020-4093	2	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-2428	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-2860	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-2866	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-2926	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3015	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3016	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3019	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3037	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3055	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3073	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3076	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3077	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3098	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3099	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3102	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3103	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖
P2020-3105	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖‖

Worklist: 4605

LAB CASE	ITEM	ITEM TYPE	DESCRIPTION	
P2020-3108	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖‖ ‖‖ ‖‖‖‖‖‖‖‖‖‖‖‖‖ ‖ ‖‖‖‖ ‖‖
P2020-3109	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖ ‖‖ ‖‖‖‖‖‖‖‖‖‖‖‖ ‖ ‖‖ ‖‖ ‖‖‖‖ ‖‖
P2020-3114	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖ ‖‖ ‖‖‖‖‖‖‖‖‖‖‖‖ ‖ ‖‖‖‖‖‖ ‖ ‖‖
P2020-3115	1	BCK	AM 27 Blood THC Quant by LC-QQQ	‖‖ ‖‖ ‖‖‖‖‖‖‖‖‖‖‖‖ ‖ ‖‖ ‖‖‖ ‖‖‖ ‖‖
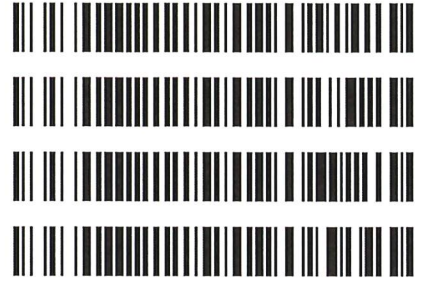
AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: <u>11/16/2020</u>   Analyst: <u>Sophia Jackson</u>
Plate lot#: IDP-108-2-200723   Plate Expiration: 01/23/2021
Mobile phase A: 0.1% Formic Acid in LCMS Water   **Mobile phase B**: 0.1% Formic acid in Acetonitrile
Blank Blood Lot: Lampire 20K20702   **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:** 3382167
☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 4. Pipette ==500µL 0.1% formic acid in water blood sample,== ==**500 µL saturated phosphate buffer in urine**== in wells of 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.
☒ 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. Case sample response for THC and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
☒ 5. Did all QCs pass for each analyte? (if not, describe in comments section)
☒ 6   Enter QCs into control charting.
☒ 7   Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports
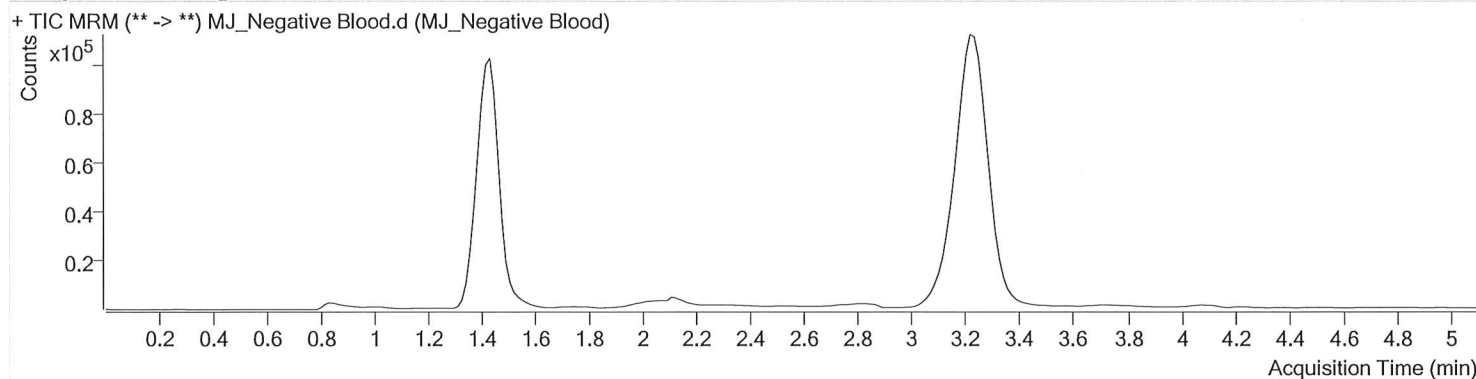
COMMENTS:
Curves limited: THC 3-100, THC-OH 3-100

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update   11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Negative Blood.d
Type	Sample	**Sample**	MJ_Negative Blood
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 2:49:01 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Negative Blood.d (MJ_Negative Blood)

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update   11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_QC Control.d
Type	Sample	**Sample**	MJ_QC Control
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 2:33:40 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_QC Control.d (MJ_QC Control)
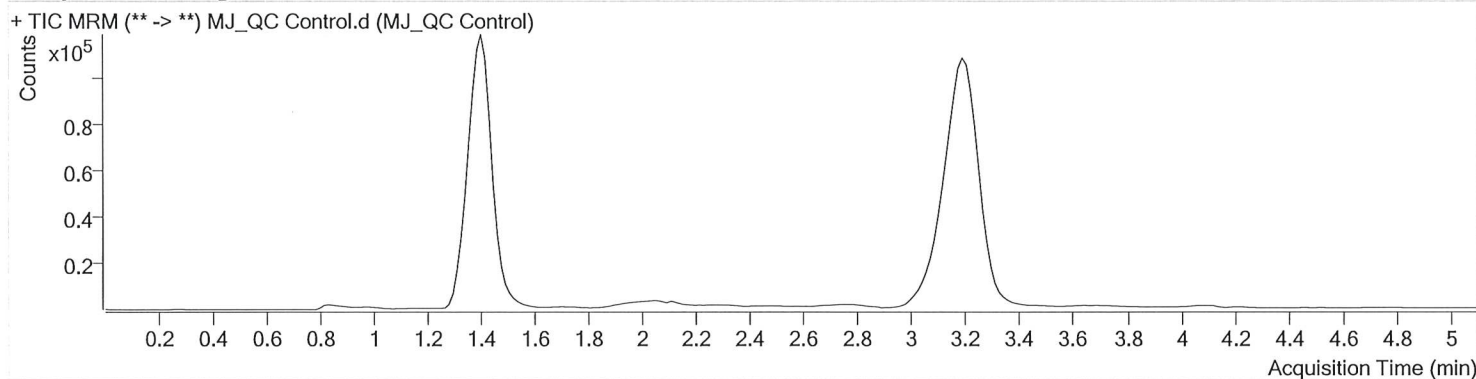
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.408	27201	∞	16.0	37.57	487428	4.7242	ng/ml
THC-COOH	1.429	51865	∞	75.1	798.27	112576	15.7374	ng/ml
THC	3.209	34126	157.22	29.8	∞	938265	4.3619	ng/ml

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin

Last Cal. Update   11/18/2020 12:47 PM

Analyst Name   ISP\datastor

Analyte   THC   **Internal Standard**   THC-D3
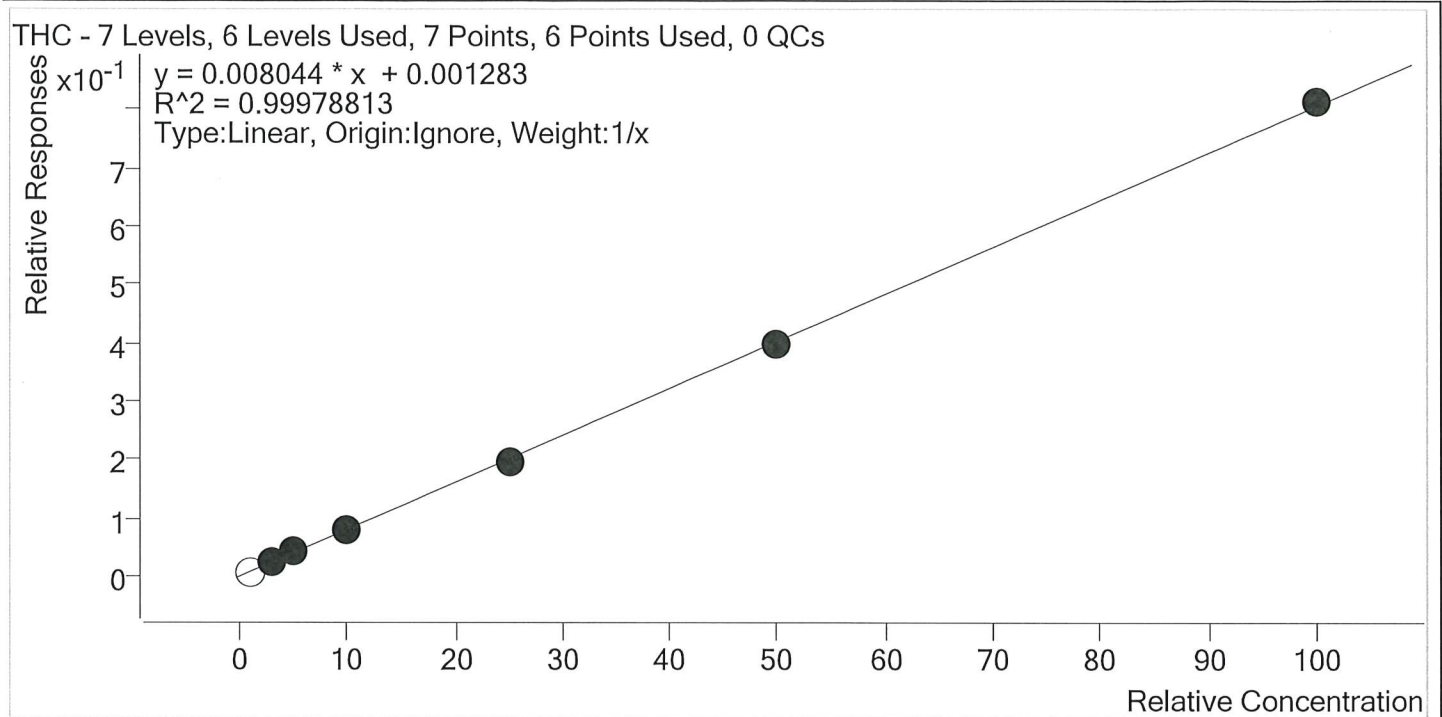
THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs

$y = 0.008044 * x + 0.001283$

$R^2 = 0.99978813$

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

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	✗	1.0	0.9	91.8
MJ_Cal 2	2	✓	3.0	3.0	100.7
MJ_Cal 3	3	✓	5.0	5.2	103.2
MJ_Cal 4	4	✓	10.0	9.8	98.0
MJ_Cal 5	5	✓	25.0	24.4	97.8
MJ_Cal 6	6	✓	50.0	49.7	99.4
MJ_Cal 7	7	✓	100.0	100.8	100.8

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin

Last Cal. Update   11/18/2020 12:47 PM

Analyst Name   ISP\datastor

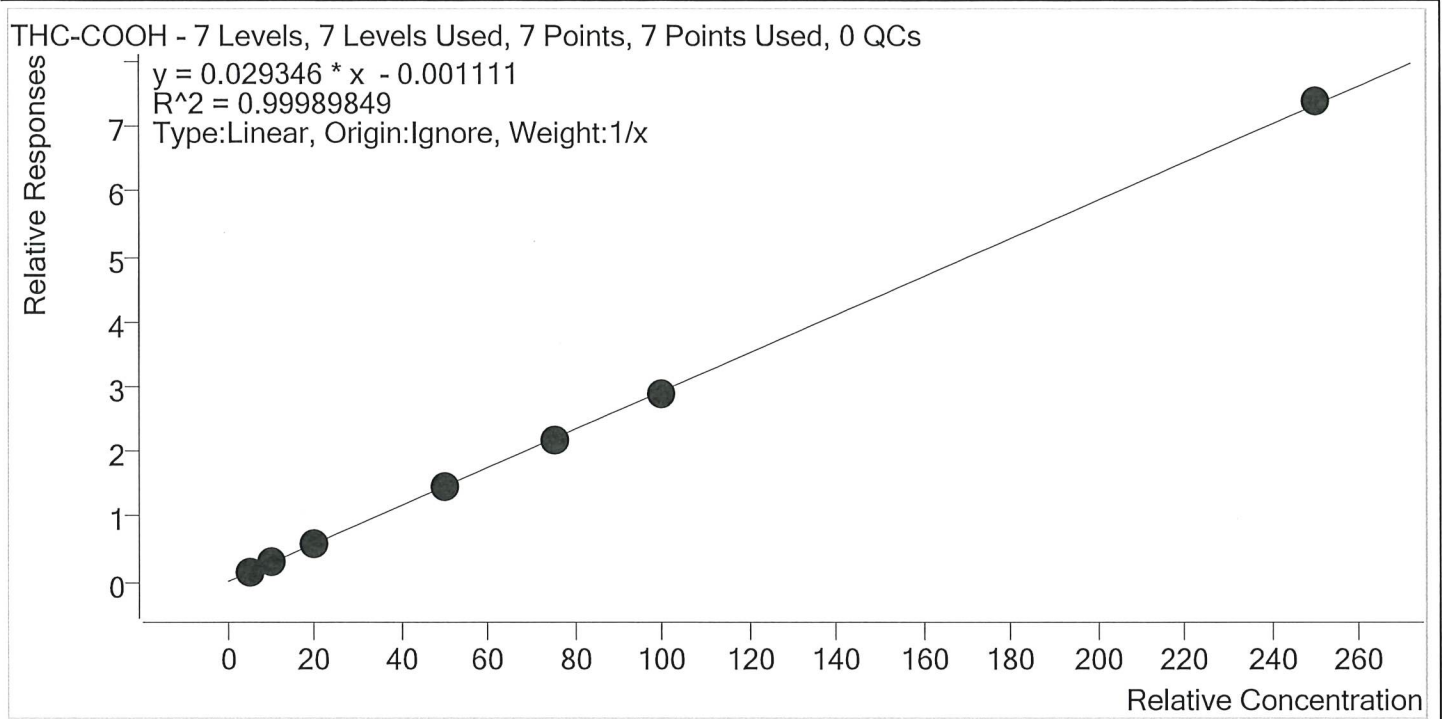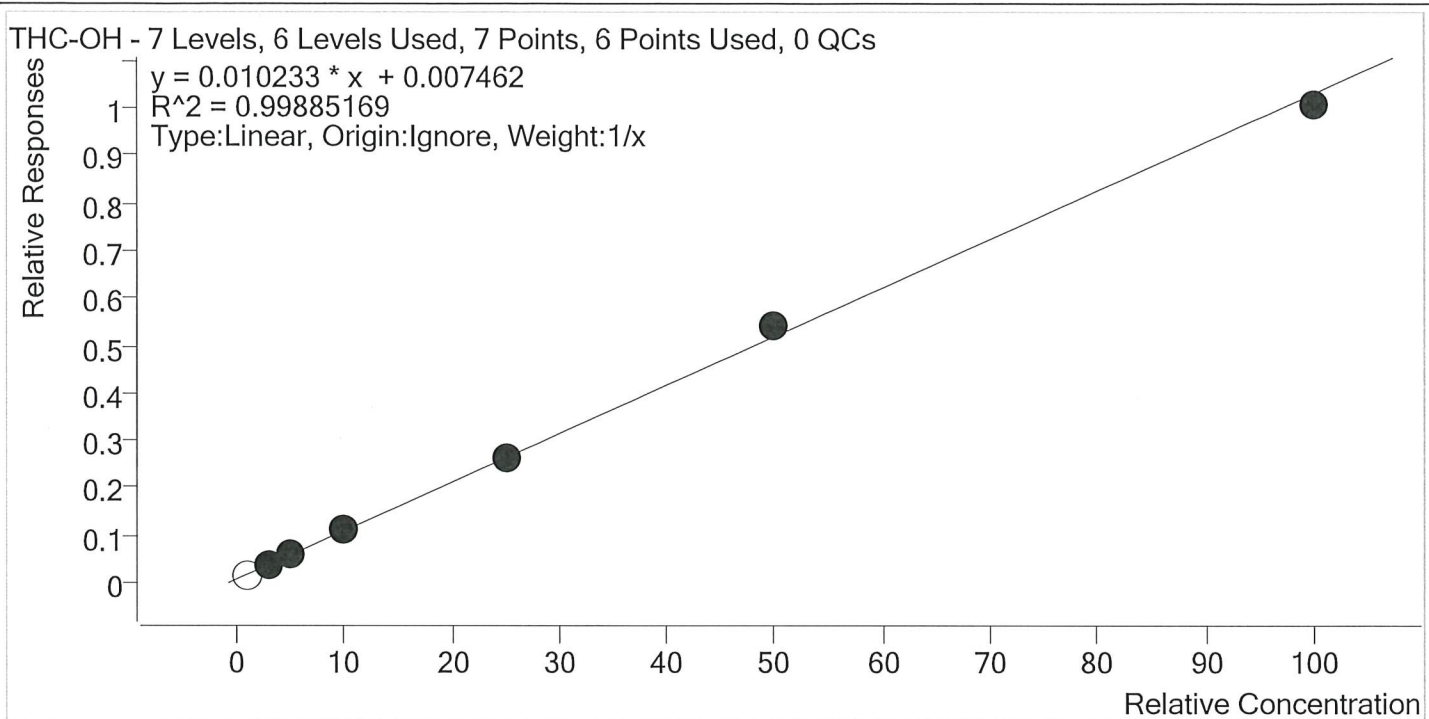**Analyte**   THC-COOH   **Internal Standard**   THC-COOH-D9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

$y = 0.029346 * x - 0.001111$
$R^2 = 0.99989849$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	✓	5.0	5.1	102.5
MJ_Cal 2	2	✓	10.0	10.0	99.8
MJ_Cal 3	3	✓	20.0	20.0	99.9
MJ_Cal 4	4	✓	50.0	49.1	98.2
MJ_Cal 5	5	✓	75.0	74.5	99.3
MJ_Cal 6	6	✓	100.0	99.4	99.4
MJ_Cal 7	7	✓	250.0	251.9	100.7

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin

Last Cal. Update   11/18/2020 12:47 PM

Analyst Name   ISP\datastor

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

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs

$y = 0.010233 * x + 0.007462$
$R^2 = 0.99885169$
Type:Linear, Origin:Ignore, Weight:1/x

| Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	✗	1.0	0.9	88.4
MJ_Cal 2	2	✓	3.0	2.9	97.7
MJ_Cal 3	3	✓	5.0	5.0	99.2
MJ_Cal 4	4	✓	10.0	10.0	100.2
MJ_Cal 5	5	✓	25.0	25.2	100.7
MJ_Cal 6	6	✓	50.0	52.3	104.6
MJ_Cal 7	7	✓	100.0	97.6	97.6

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update   11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Cal 1.d
Type	Cal	**Sample**	MJ_Cal 1
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 1:28:52 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Cal 1.d (MJ_Cal 1)
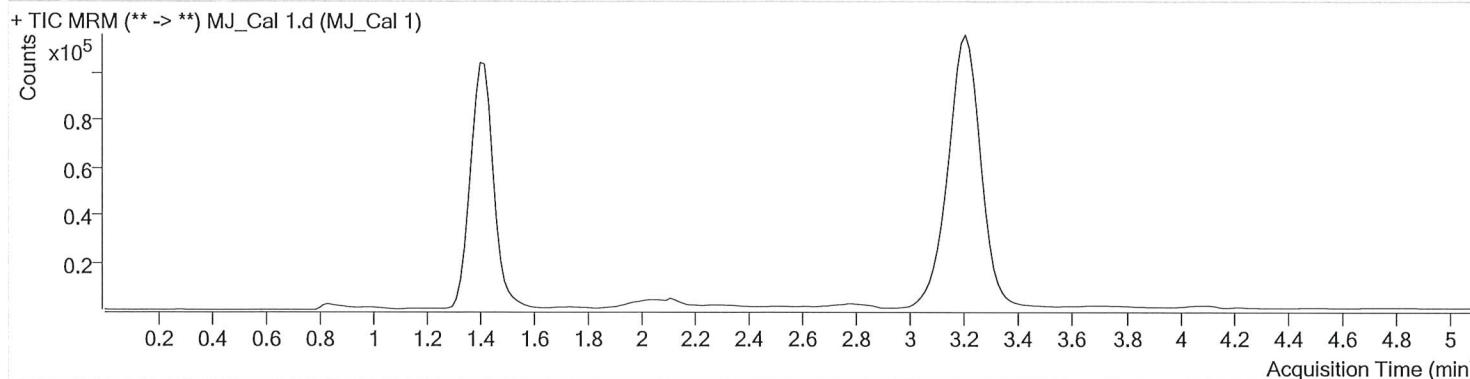
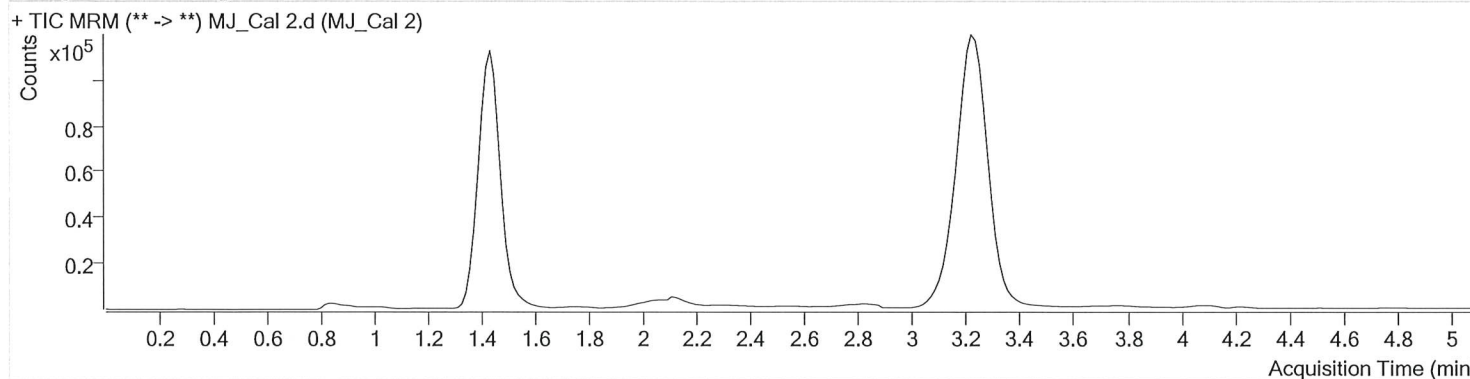| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.423	7719	7.29 **Low**	15.7	8.14 **Low**	467451	0.8845	ng/ml **Low**
THC-COOH	1.444	16472	∞	70.9	175.12	110303	5.1267	ng/ml
THC	3.209	8383	55.25	36.3 **High**	6.60 **Low**	967666	0.9175	ng/ml **Low**

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update   11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Cal 2.d
Type	Cal	**Sample**	MJ_Cal 2
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 1:36:36 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Cal 2.d (MJ_Cal 2)

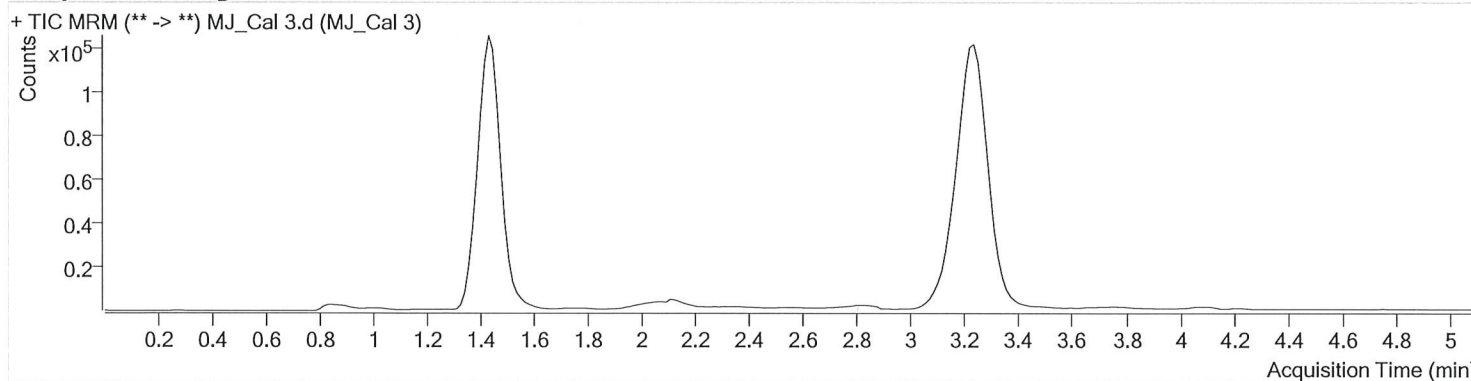| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.438	16799	49.48	15.3	23.42	448420	2.9318	ng/ml **Low**
THC-COOH	1.459	31779	∞	73.3	∞	108904	9.9815	ng/ml
THC	3.239	24310	153.77	31.6	59.10	950642	3.0195	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update 11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Cal 3.d
Type	Cal	**Sample**	MJ_Cal 3
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 1:44:11 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Cal 3.d (MJ_Cal 3)

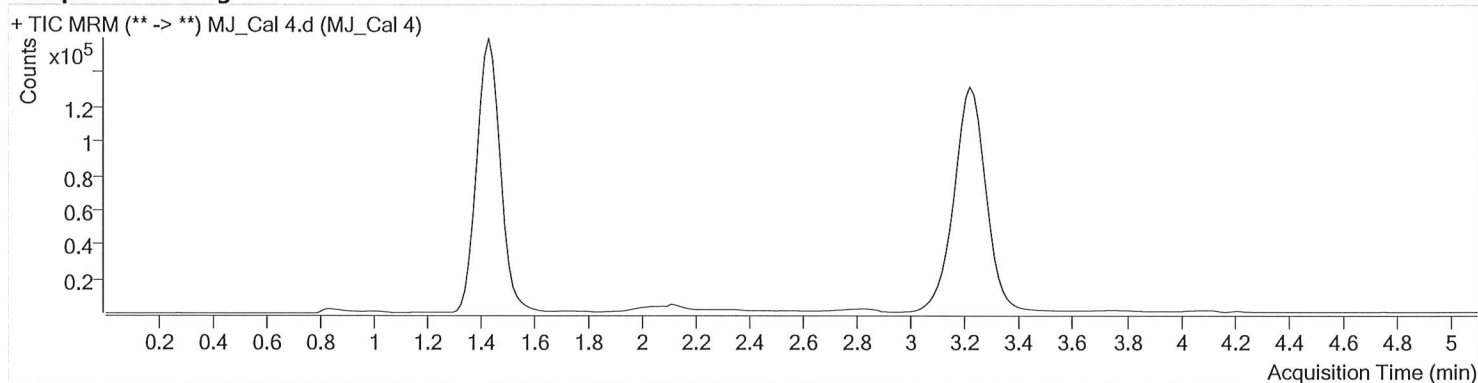| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.438	27294	∞	16.6	52.46	469013	4.9578	ng/ml
THC-COOH	1.459	66221	∞	73.9	781.11	113146	19.9821	ng/ml
THC	3.239	41118	213.13	29.1	89.69	960652	5.1614	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update   11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Cal 4.d
Type	Cal	**Sample**	MJ_Cal 4
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 1:51:47 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Cal 4.d (MJ_Cal 4)

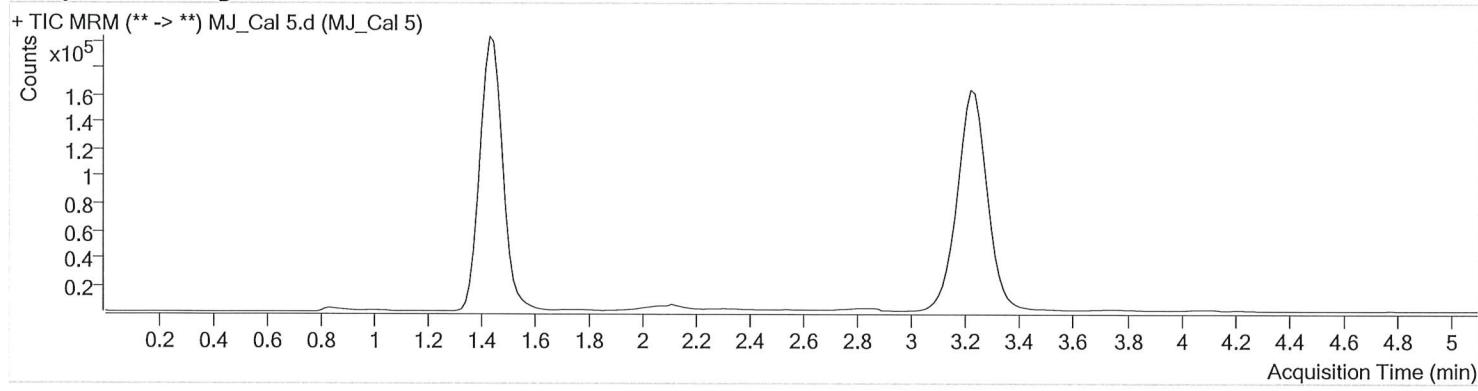| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.423	51305	411.71	16.2	180.73	466245	10.0243	ng/ml
THC-COOH	1.459	158874	2941.38	76.3	2219.02	110303	49.1201	ng/ml
THC	3.224	78494	511.95	29.8	181.64	979323	9.8043	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update 11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Cal 5.d
Type	Cal	**Sample**	MJ_Cal 5
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 1:59:23 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Cal 5.d (MJ_Cal 5)

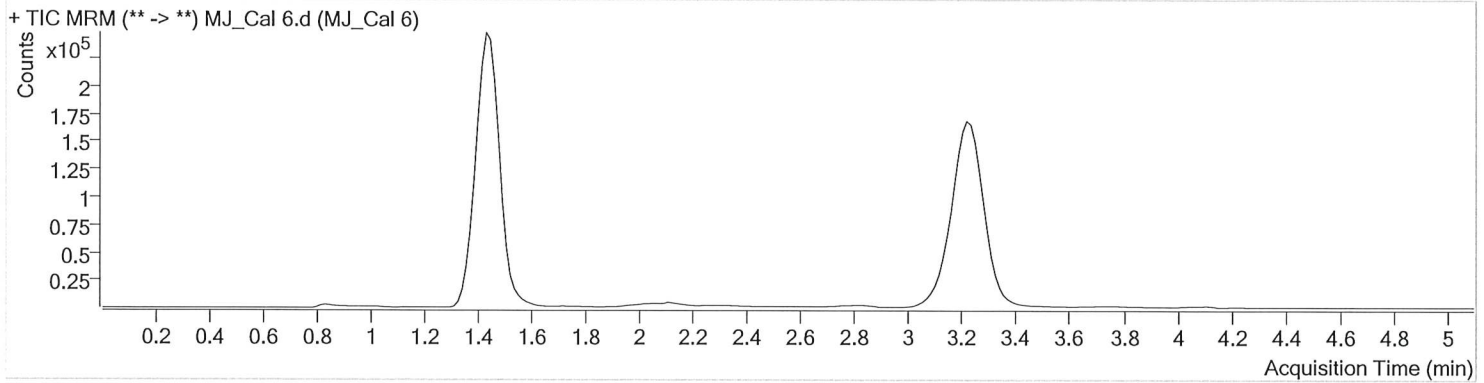| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.423	121151	∞	15.7	555.68	457155	25.1688	ng/ml
THC-COOH	1.459	233138	2067.47	76.7	2789.48	106714	74.4852	ng/ml
THC	3.239	199643	688.33	28.5	544.35	1008601	24.4467	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update 11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Cal 6.d
Type	Cal	**Sample**	MJ_Cal 6
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 2:07:00 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Cal 6.d (MJ_Cal 6)

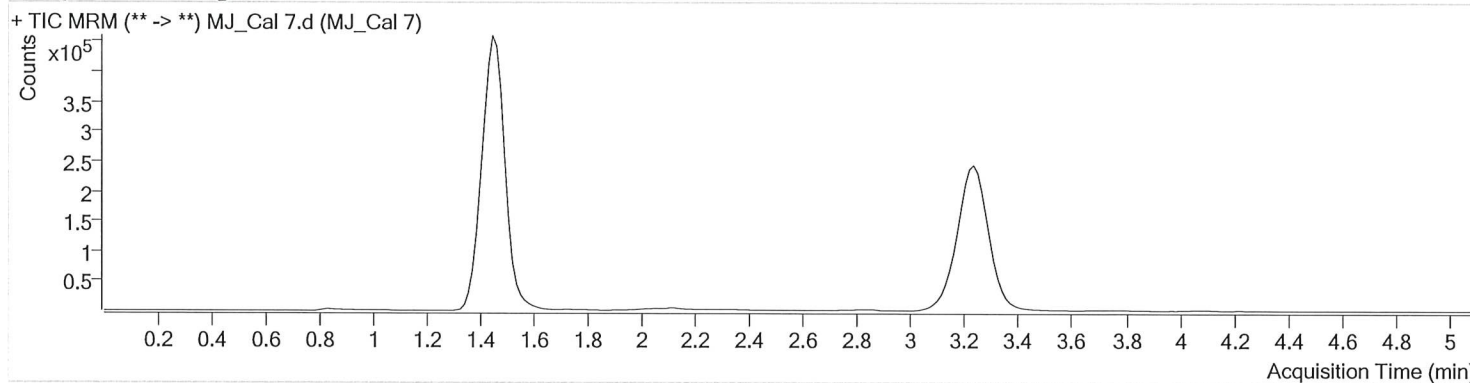| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.423	251518	∞	15.9	1428.73	463675	52.2810	ng/ml
THC-COOH	1.459	309879	∞	77.3	4375.41	106229	99.4423	ng/ml
THC	3.239	374146	1433.71	28.2	7730.06	932378	49.7242	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2020\AM 27-28\111620 AM 27 28 SJ\QuantResults\AM 27.batch.bin
Calibration Last Update   11/18/2020 12:47:51 PM

Instrument	Instrument 1	**Data File**	MJ_Cal 7.d
Type	Cal	**Sample**	MJ_Cal 7
Acq. Method	AM 27 THCQ.m	**Operator**	Sophia Jackson
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/16/2020 2:14:34 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_Cal 7.d (MJ_Cal 7)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.438	472181	∞	17.9	6015.17	469105	97.6363	ng/ml
THC-COOH	1.459	752604	13710.29	77.8	11532.80	101842	251.8621	ng/ml
THC	3.239	796583	2536.00	27.6	806.74	980405	100.8439	ng/ml