

REVIEWED

By Anne Nord at 9:35 am, May 15, 2023

5/10/2023

Byylee

Worklist: 6364

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2023-0883	3	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-0894	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-0949	1	BLOOD	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-0949	2	BLOOD	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-0949	3	BLOOD	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-0951	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-0980	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-0989	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-1001	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-1003	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-1012	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-1023	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-1038	2	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	
C2023-1049	1	BCK	AM 25/AM 26 Blood MultiDrug/THC Screen by LC-QQQ	

AM# 25: Multi-Drug Screen in Blood and Urine by LC-MS/MS

BWylie

Extraction Date: 5/10/23 Analyst: Britany Wylie
Plate lot#: 220805 Plate retest date: 02/05/23

Mobile phase A: 10mM Ammonium Formate **Mobile phase B:** 0.1% Formic Acid in MeOH
0.5M Ammonium Hydroxide Ethyl Acetate LC 20% Methanol
Blank Blood Lot: 22B52016-1 **Blank Urine lot:** 12522 **Column:** Agilent Phenyl Hexyl (4.6x50mm, 2.7um)
LCMS-QQQ ID: 69679

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 pipette: 250 ul urine in blank well, add 40 ul BG Turbo, add 100 ul 500 mm sodium phosphate buffer mix for at least five minutes ambient temperature.
Pipette 250 µL blood (**calibrated pipette**) or 250 ul urine in wells of analytical (standards) plate. **Pipette ID: 390993**
- 3. Pipette **250 µL of 0.5 M ammonium hydroxide** in wells of analytical plate.
- 4. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
- 5. Transfer **300 µL of blood or urine+base** mixture to corresponding wells of SLE+ plate.
- 6. 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: 66792
- 7. Wait 5 minutes.
- 8. Add **900 µL ethyl acetate**.
- 9. Wait 5 minutes.
- 10. Apply positive pressure for approx. 10-15 seconds. *(12-15 PSI- Selector to the left)*.
- 11. Add **900 µL ethyl acetate**.
- 12. Wait 5 minutes.
- 13. Apply positive pressure for approx. 10-15 seconds. *(12-15 PSI- Selector to the left)*.
- 14. Remove plate containing eluate. add 50 ul 1% HCl in MeOH. Place on SPE Dry and evaporate to dryness at approx. 35°C.
SPE Dry ID: 66819
- 15. Reconstitute in **100 µL 20% LC MeOH** in LC Water and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Open quantitation software and create a new quantitation batch.
- 2. Make necessary changes to integration limits
- 3. Evaluate samples, S/N of primary transition >5 and S/N of secondary transition >3 or evaluation of peak symmetry and resolution. Within +/- 2% or 0.1 min RT of administrative control. Calculated concentration 5 or greater or 2-5 for discretionary range.
- 4. Did all QCs pass for each analyte? (If no is it described in comments?)
- 5. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *Blood Only Run - External controls run for plate re-test.*

BWylee

	1	2	3	4	5	6	7	8	9	10	11	12
A							0949-2					
B							0949-3					
C							0951-1					
D						neg blood	0980-1	1023-1				
E						ext blood	0989-1	1038-2				
F						0883-3	1001-1	1049-4				
G						0894-1	1003-1					cal1
H						0949-1	1012-1					

C2023-____-__

plate position 2

Byyle

Toxicology AM method 25/28 external control prep

working solution 10000 ng/ml in meoh diphendydramine, methamphetamine, alprazolam, , morphine

Stock solution 1mg/ml 50 ul each in 4800 ul MeOH (VWR 21050767)

ppd 7/7/22: Exp: 7/7/23 lot 7722 by AMN

Drug	lot	expiration
Methamphetamine	FE03132001	7/1/2025
alprazolam	FE06102008	6/1/2025
Diphendydramine	FN02212011	3/1/2025
Morphine	FE03232010	4/1/2025

AM 25/28 control 500 ul working solution (7722) in 4500 ul negative urine (1000ng/mL Expected concentration)

ppd 7/7/22, exp 7/7/23 lot u7722 negative urine 21522 by AMN

AM 25/28 Blood Control: 50ul working solution (7722) in 4950 ul neg blood (100ng/mL Expected concentration)

ppp 7/7/22, exp 7/7/23 lot b7722 neg blood 22B52016-3 by AMN

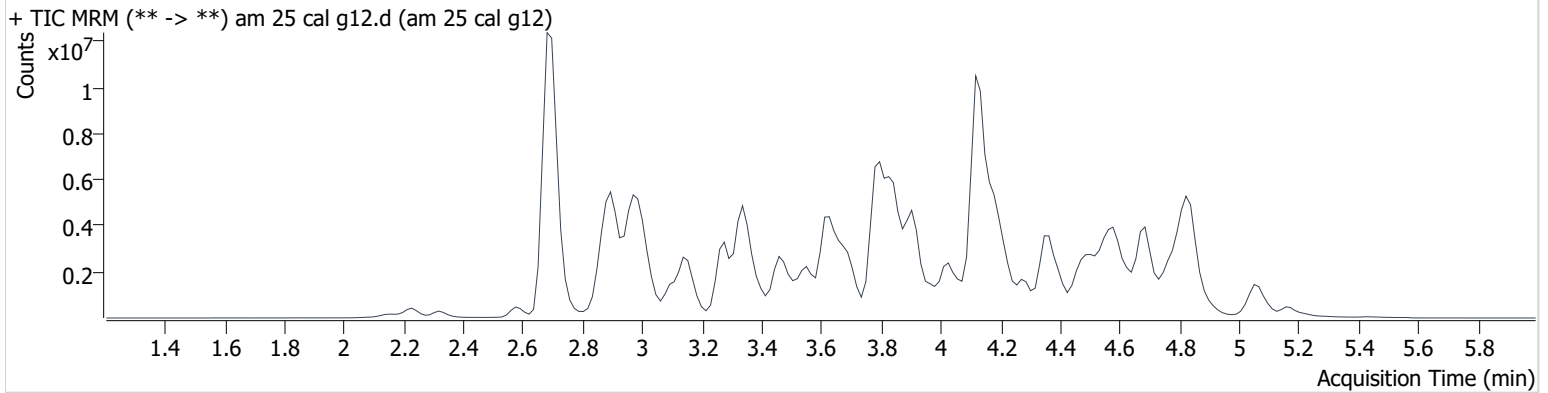
AM #25 Multi-Drug Screen Results

B. Wylie

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 25 51023.batch.bin
Calibration Last Update 5/10/2023 7:25:38 PM

Instrument	69679	Data File	am 25 cal g12.d
Type	Cal	Sample	am 25 cal g12
Acq. Method	mds713.m	Operator	Britany Wylie
Sample Position	P2-G12	Comment	
Injection Volume	2.5		
Acq. Date-Time	5/10/2023 3:45:14 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
10-OH-Carbamazepine	3.850	1441697	2260.4	14.3	4878894	10.000
6-MAM	2.831	18575	6895.7	3987.6	517545	10.000
7-aminoclonazepam	3.586	292067	1103.9	298450.1	1508098	10.000
7-aminoflunitrazepam	3.816	478447	297.1	89.8	1508098	10.000
9-Hydroxyrisperidone	3.916	2237377	7205.6	772.2	1508098	10.000
Acetyl Fentanyl	3.782	158560	65.5	28139.8	5837053	10.000
Acetyl Norfentanyl	2.871	151440	1761.4	209.1	10350389	10.000
a-hydroxyalprazolam	4.688	223434	302.1	2830.4	4878894	10.000
alpha-hydroxymidazolam	4.671	835372	336201.5	6058.4	7940825	10.000
alpha-PHP	3.820	1215326	500.8	2056.7	3412283	10.000
alpha-PVP	3.530	1704897	1657.4	566.7	3412283	10.000
Alprazolam	4.767	1103421	1146.2	935.3	7940825	10.000
Amitriptyline	4.586	930726	382.4	361.9	3036408	10.000
Amphetamine	2.906	1761422	221.0	4712.4	3412283	10.000
Benzoyllecgonine	3.431	111490	32.1	145.5	151477	10.000
Brompheniramine	4.183	43453	62453.5	188.1	3919080	10.000
Buprenorphine	4.130	33303	12196.3	63467.4	753909	10.000
Bupropion	3.790	1442331	499.2	435.4	5976190	10.000
Carbamazepine	4.344	2754045	1046.6	1110.9	2346935	10.000
Carisoprodol	4.297	597442	172266.7	100.5	2130944	10.000
Chlordiazepoxide	4.845	309241	378.9	96.7	7940825	10.000
Chlorpheniramine	4.063	2647105	∞	35.6	3919080	10.000
Chlorpromazine	4.810	1047801	126640.6	2727.6	4228313	10.000
Citalopram	4.228	1408858	1000.4	353193.1	25830211	10.000
Clomipramine	4.826	1201483	856.4	∞	2519915	10.000
Clonazepam	4.628	615860	368.7	247053.8	61623	10.000
Clonazolam	4.501	499719	853.7	558.7	4878894	10.000
clozapine	4.365	1891750	621067.2	490907.1	6288227	10.000
Cocaehtylene	3.844	1662455	3178.5	3437.2	9033631	10.000
Cocaine	3.615	2160955	1722452.5	730.3	9033631	10.000
Codeine	2.698	157313	754.0	590.6	2346935	10.000
Cyclobenzaprine	4.510	1506763	844.3	156.1	3036408	10.000
Desipramine	4.557	1915630	388835.1	1882.7	3036408	10.000
Dextromethorphan	4.172	888760	779.0	152702.7	3919080	10.000

am 25 cal g12

AM #25 Multi-Drug Screen Results

BWylee

Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
Dextrophan	3.404	1113338	366.6	∞	3412283	10.000
Diazepam	5.044	623817	485.3	904.4	7940825	10.000
Dihydrocodeine	2.667	477788	5313.6	656.4	2346935	10.000
Dimethyltryptamine	2.979	933925	536.8	1226.6	3412283	10.000
Diphenhydramine	4.127	3901081	2297.6	325.3	25830211	10.000
Doxepin	4.294	946434	320.0	160.9	6288227	10.000
Doxylamine	3.679	3531558	2786.1	277.4	3412283	10.000
Duloxetine	4.508	34779	17094.9	69134.0	2519915	10.000
EDDP	4.171	647791	2224.0	1518630.4	1064948	10.000
Estazolam	4.677	2126408	844.7	1443.4	7940825	10.000
Etizolam	4.747	103671	389.9	333629.9	7940825	10.000
Fentanyl	4.027	136928	173.8	22207.7	6631372	10.000
Flualprazolam	4.595	368821	349.4	100852.7	7940825	10.000
Flunitrazepam	4.735	1331701	2063.1	622640.7	4878894	10.000
Fluorofentanyl	4.056	122553	40438.5	548.8	6631372	10.000
Fluoxetine	4.491	1612030	5179.5	1791842.9	2519915	10.000
Flurazepam	4.209	1252229	1004629.9	377602.8	753909	10.000
Hydrocodone	2.927	490053	981.9	383.9	2346935	10.000
Hydromorphone	2.322	496231	509.0	725.3	82550	10.000
hydroxyzine	4.623	1713536	26991.5	1196823.7	6288227	10.000
Imipramine	4.555	2626659	1038.4	599.8	3036408	10.000
Ketamine	3.375	1198634	505.3	214.8	4225508	10.000
Lamotrigine	3.558	120004	174.5	2505.6	3412283	10.000
Levamisole	2.871	875683	3907.8	292.1	9033631	10.000
Levetiracetam	2.585	536819	630.4	355.3	1508098	10.000
Lorazepam	4.596	186530	765.8	75.1	4878894	10.000
Maprotiline	4.586	642545	633.3	285.8	3036408	10.000
MDA	3.025	1502766	1160.6	356.5	9585204	10.000
MDEA	3.270	1809066	581.8	701.7	9585204	10.000
MDMA	3.102	2091133	1151.4	805.5	9585204	10.000
Meperidine	3.635	1048846	287.8	461.9	82550	10.000
Meprobamate	3.715	436696	310.2	73.8	2130944	10.000
Methadone	4.521	2088168	506.5	607.0	5837053	10.000
Methamphetamine	3.012	4577292	∞	∞	9585204	10.000
Methocarbamol	3.651	261315	985.7	450.6	2130944	10.000
Methylphenidate	3.560	3524788	1623.1	490.8	6886700	10.000
Metoprolol	3.479	360407	1408.5	811.1	3412283	10.000
Midazolam	4.549	209213	75471.6	1469.4	1508098	10.000
Mirtazapine	3.695	1184282	433.5	13114.1	753909	10.000
Mitragynine	4.208	142075	62842.6	5438.4	6631372	10.000
Morphine	2.141	127512	693.0	∞	82550	10.000
Norbuprenorphine	3.886	30409	10901.2	12953.7	753909	10.000
Nordiazepam	4.894	614653	636.3	1339.0	7940825	10.000
Norfentanyl	3.360	2605405	880.4	453.8	10350389	10.000
Norhydrocodone	2.943	41265	49.7	356.8	2346935	10.000
norketamine	3.361	251386	38.8	2986.4	4225508	10.000
Normeperidine	3.667	1296589	2673.3	333.5	82550	10.000
Noroxycodone	2.896	557026	735.8	249.2	2346935	10.000
Nortriptyline	4.603	1083837	552609.0	393.9	2519915	10.000
O-desmethyl-tramadol	2.900	3098550	1480.2	206.2	2973878	10.000
O-Desmethylvenlafaxine	3.281	792862	612.8	684.4	2973878	10.000
Olanzapine	3.383	322154	67673.2	1982.8	2519915	10.000
Oxazepam	4.708	1188261	210.6	187.7	4878894	10.000
Oxycodone	2.894	1098803	118.8	1254.5	4225508	10.000
Oxymorphone	2.227	677145	600.4	845.0	82550	10.000
Paroxetine	4.503	278340	193.8	32061.8	2519915	10.000
Phenazepam	4.823	901734	412795.1	1117.1	7940825	10.000
Phencyclidine	3.975	1883696	1805.9	222.2	2973878	10.000
Phentermine	3.180	733036	∞	∞	6886700	10.000
Phenytoin	4.236	131072	237.4	51.6	61623	10.000

AM #25 Multi-Drug Screen Results

BWylee

Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
primidone	3.515	133376	341.8	50.6	61623	10.000
Promethazine	4.463	3141635	79150.3	271.1	3036408	10.000
Pseudoephedrine	2.692	36413034	2996.1	21787.2	9585204	10.000
Quetiapine	4.393	2039004	2028155.4	422218.5	3919080	10.000
Risperidone	4.085	2047602	738368.2	299.6	3919080	10.000
Sertraline	4.783	642739	340357.3	∞	2519915	10.000
Sufentanil	4.316	102995	27178.1	170.8	6631372	10.000
Tapentadol	3.484	2124611	987.9	3695.0	4225508	10.000
Temazepam	4.859	1991726	436.6	99.3	7940825	10.000
Topiramate	3.904	19666	6974.7	4024.3	110183	10.000
Tramadol	3.465	2927259	961.1	21.2	517545	10.000
Trazodone	4.194	1735092	1421.7	19270.9	5837053	10.000
Venlafaxine	3.878	2715946	80619.9	217.1	2973878	10.000
Zaleplon	4.477	929187	570849.4	886.2	4878894	10.000
Zolpidem	3.814	2715133	1270.9	1255.9	11250874	10.000
Zopiclone	3.887	156570	466.8	44578.8	699598	10.000

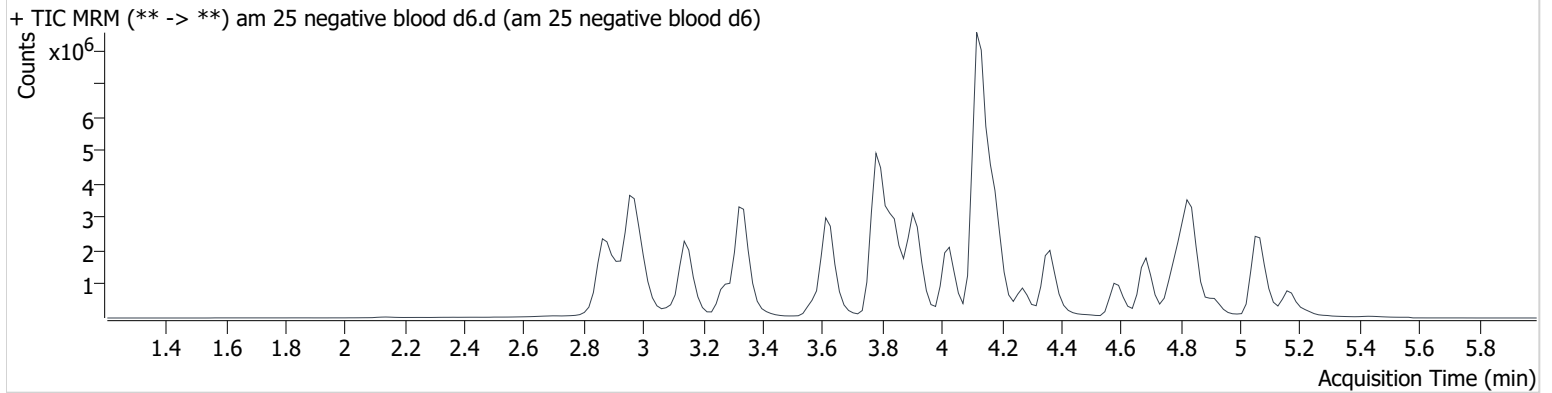
AM #25 Multi-Drug Screen Results

BWylie

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 25 51023.batch.bin
Calibration Last Update 5/10/2023 7:25:38 PM

Instrument	69679	Data File	am 25 negative blood d6.d
Type	Sample	Sample	am 25 negative blood d6
Acq. Method	mds713.m	Operator	Britany Wylie
Sample Position	P2-D6	Comment	
Injection Volume	2.5		
Acq. Date-Time	5/10/2023 3:52:08 PM		
Sample Info.			

Sample Chromatogram



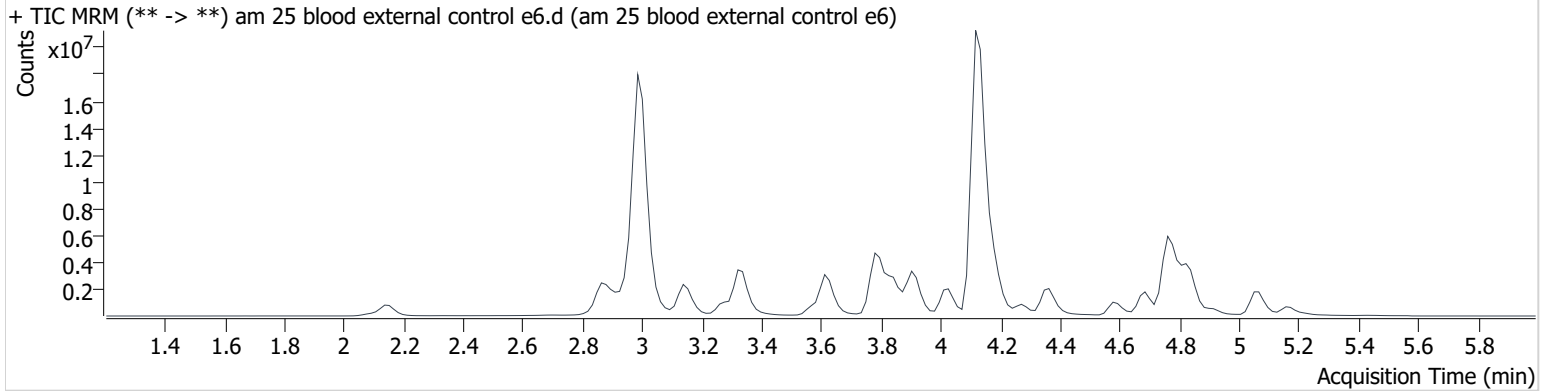
AM #25 Multi-Drug Screen Results

B. Wylie

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 25 51023.batch.bin
Calibration Last Update 5/10/2023 7:25:38 PM

Instrument	69679	Data File	am 25 blood external control e6.d
Type	Sample	Sample	am 25 blood external control e6
Acq. Method	mds713.m	Operator	Britany Wylie
Sample Position	P2-E6	Comment	
Injection Volume	2.5		
Acq. Date-Time	5/10/2023 3:58:50 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
Alprazolam	4.767	8463340	996.7	2041.0	8891115	68.503
Diphenhydramine	4.127	33653369	116821.4	6241.0	25761423	86.497
Hydromorphone	2.141 Low	530060	1797.4	1322.7	69405	12.705
Methamphetamine	2.996	26855212	∞	∞	9585081	58.671
Morphine	2.141	1009160	7955.2	11586.4	69405	94.131

Byyle

Toxicology AM method 25/28 external control prep

working solution 10000 ng/ml in meoh diphendydramine, methamphetamine, alprazolam, , morphine

Stock solution 1mg/ml 50 ul each in 4800 ul MeOH (VWR 21050767)

ppd 7/7/22: Exp: 7/7/23 lot 7722 by AMN

Drug	lot	expiration
Methamphetamine	FE03132001	7/1/2025
alprazolam	FE06102008	6/1/2025
Diphendydramine	FN02212011	3/1/2025
Morphine	FE03232010	4/1/2025

AM 25/28 control 500 ul working solution (7722) in 4500 ul negative urine (1000ng/mL Expected concentration)

ppd 7/7/22, exp 7/7/23 lot u7722 negative urine 21522 by AMN

AM 25/28 Blood Control: 50ul working solution (7722) in 4950 ul neg blood (100ng/mL Expected concentration)

ppp 7/7/22, exp 7/7/23 lot b7722 neg blood 22B52016-3 by AMN

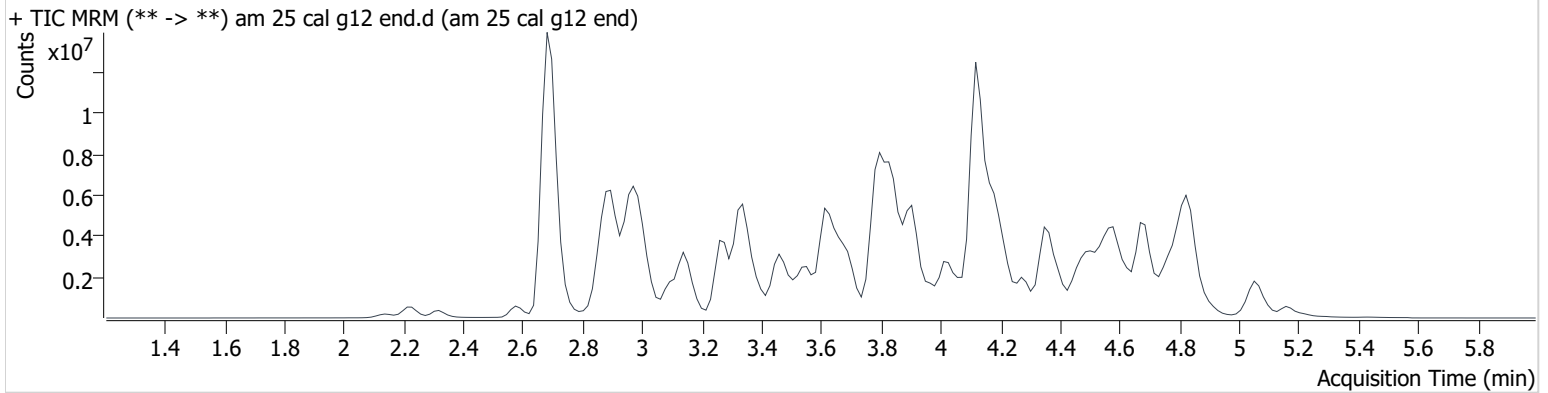
AM #25 Multi-Drug Screen Results

B. Wylie

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 25 51023.batch.bin
Calibration Last Update 5/10/2023 7:25:38 PM

Instrument	69679	Data File	am 25 cal g12 end.d
Type	Sample	Sample	am 25 cal g12 end
Acq. Method	mds713.m	Operator	Britany Wylie
Sample Position	P2-G12	Comment	
Injection Volume	2.5		
Acq. Date-Time	5/10/2023 5:39:28 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
10-OH-Carbamazepine	3.850	1729894	4234.4	309.3	5780283	10.128
6-MAM	2.831	22965	24674.7	31739.2	607286	10.537
7-aminoclonazepam	3.586	344287	196.6	509634.3	1780860	9.982
7-aminoflunitrazepam	3.816	587940	145982.8	1050.5	1780860	10.406
9-Hydroxyrisperidone	3.901	2643329	∞	31732.8	1780860	10.005
Acetyl Fentanyl	3.782	175254	292.3	23294.9	7067189	9.129
Acetyl Norfentanyl	2.855	180254	706.2	460.0	12356916	9.970
a-hydroxyalprazolam	4.688	280130	419.6	66265.6	5780283	10.582
alpha-hydroxymidazolam	4.671	948237	1083.6	552.3	9165018	9.835
alpha-PHP	3.820	1465829	16138.1	2042.1	4076076	10.097
alpha-PVP	3.530	1975893	1983.7	452.0	4076076	9.702
Alprazolam	4.767	1305940	483.6	588.2	9165018	10.254
Amitriptyline	4.586	1067564	341.6	360.1	3454393	10.082
Amphetamine	2.906	2023246	3450.6	8497.3	4076076	9.616
Benzoyllecgonine	3.431	127492	34.0	28.6	159666	10.849
Brompheniramine	4.183	50848	177.1	60.4	4552456	10.074
Buprenorphine	4.115	40201	18129.9	44016.7	844640	10.775
Bupropion	3.790	1631528	711.4	315.2	7044142	9.597
Carbamazepine	4.344	3183803	3051.1	566.7	2789595	9.726
Carisoprodol	4.297	683103	925.9	106.8	2469521	9.866
Chlordiazepoxide	4.845	387321	565.7	43.1	9165018	10.852
Chlorpheniramine	4.063	3191673	3922.9	73.5	4552456	10.380
Chlorpromazine	4.795	1234813	383464.8	3003.9	4872480	10.227
Citalopram	4.228	1608224	688.4	226278.7	30173232	9.772
Clomipramine	4.811	1406219	744.4	961.0	2808520	10.501
Clonazepam	4.628	743043	2061.7	68210.7	77022	9.653
Clonazolam	4.501	604576	514.9	∞	5780283	10.212
clozapine	4.365	2234117	8492.4	715011.0	7801386	9.519
Cocaehtylene	3.844	1912132	1343.8	445126.0	10685316	9.724
Cocaine	3.615	2518897	879.0	1003.3	10685316	9.855
Codeine	2.698	179585	4785.2	1598.4	2789595	9.604
Cyclobenzaprine	4.495	1834676	591.7	84.9	3454393	10.703
Desipramine	4.557	2125014	739328.5	994.3	3454393	9.751
Dextromethorphan	4.172	1075196	1999.4	746.3	4552456	10.415

am 25 cal g12 end

AM #25 Multi-Drug Screen Results

B. Wylee

Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
Dextrophan	3.404	1329998	946694.9	186643.3	4076076	10.001
Diazepam	5.029	722663	1302.2	4798.7	9165018	10.037
Dihydrocodeine	2.652	569404	859.0	682.5	2789595	10.026
Dimethyltryptamine	2.979	1131015	541.5	539.7	4076076	10.138
Diphenhydramine	4.127	4550356	959.5	1784.4	30173232	9.985
Doxepin	4.294	1081276	∞	55.7	7801386	9.209
Doxylamine	3.664	4163688	150.8	47837.9	4076076	9.870
Duloxetine	4.508	40686	573.7	1957.2	2808520	10.496
EDDP	4.171	772793	1415.3	205.9	1217439	10.435
Estazolam	4.677	2553095	749.6	645.2	9165018	10.403
Etizolam	4.747	120865	96376.1	600358.1	9165018	10.101
Fentanyl	4.027	169461	230.4	36172.6	7771676	10.560
Flualprazolam	4.595	439596	277.6	257.6	9165018	10.327
Flunitrazepam	4.735	1540206	2549.9	737.2	5780283	9.762
Fluorofentanyl	4.056	146822	2079.5	140.9	7771676	10.222
Fluoxetine	4.491	1872094	42082.2	246.2	2808520	10.420
Flurazepam	4.209	1466190	1940.1	183906.5	844640	10.451
Hydrocodone	2.927	585786	1086.6	537.1	2789595	10.057
Hydromorphone	2.322	592872	1344.1	1026.4	98368	10.026
hydroxyzine	4.623	1999284	2080.6	373307.0	7801386	9.405
Imipramine	4.555	2911569	371.2	1292.5	3454393	9.743
Ketamine	3.375	1417489	1211.3	212.9	4996900	10.000
Lamotrigine	3.558	132762	185.5	117262.2	4076076	9.262
Levamisole	2.871	1009152	3672.3	582.1	10685316	9.743
Levetiracetam	2.585	620360	401.1	1020.1	1780860	9.786
Lorazepam	4.596	231788	9344.5	158.1	5780283	10.489
Maprotiline	4.586	732513	796.6	214.1	3454393	10.021
MDA	3.025	1794075	1572.6	456.3	11604940	9.861
MDEA	3.255	2106046	562.6	269.6	11604940	9.615
MDMA	3.102	2417774	8044.9	1289.0	11604940	9.550
Meperidine	3.620	1281411	3426.5	6852.3	98368	10.253
Meprobamate	3.715	505192	416.5	211.7	2469521	9.982
Methadone	4.521	2420277	1231.7	638.7	7067189	9.573
Methamphetamine	2.996	5325805	∞	∞	11604940	9.610
Methocarbamol	3.651	309730	499.6	938.3	2469521	10.228
Methylphenidate	3.560	4209178	806.9	1962.6	8082359	10.175
Metoprolol	3.464	425030	316.2	54276.0	4076076	9.873
Midazolam	4.564	245037	60418.2	52287.2	1780860	9.918
Mirtazapine	3.695	1399858	484889.1	1212.5	844640	10.551
Mitragynine	4.208	163308	62870.2	200509.8	7771676	9.808
Morphine	2.141	158594	∞	5980.5	98368	10.438
Norbuprenorphine	3.886	34977	9925.0	14178.8	844640	10.267
Nordiazepam	4.894	748509	5592.0	320.4	9165018	10.551
Norfentanyl	3.345	3011042	1231.6	262.2	12356916	9.680
Norhydrocodone	2.928	55219	12592.5	461.1	2789595	11.258
norketamine	3.361	290988	524.4	6461.0	4996900	9.788
Normeperidine	3.667	1572223	1766.8	683.2	98368	10.176
Noroxycodone	2.896	658721	376.1	303.2	2789595	9.949
Nortriptyline	4.588	1283193	336538.0	800.9	2808520	10.623
O-desmethyl-tramadol	2.900	3697811	1030.8	458.0	3422542	10.370
O-Desmethylvenlafaxine	3.281	963542	187.7	984.9	3422542	10.560
Olanzapine	3.414	359566	83722.2	252.8	2808520	10.014
Oxazepam	4.693	1348760	371.4	290.3	5780283	9.581
Oxycodone	2.878	1326715	278.7	51399.2	4996900	10.210
Oxymorphone	2.227	806558	38.5	6400.0	98368	9.996
Paroxetine	4.503	325582	85.4	51211.1	2808520	10.495
Phenazepam	4.807	1037842	292233.7	1023.7	9165018	9.972
Phencyclidine	3.975	2148111	677.6	179.7	3422542	9.909
Phentermine	3.164	813000	∞	∞	8082359	9.450
Phenytoin	4.236	154920	199.5	34.4	77022	9.456

AM #25 Multi-Drug Screen Results

BWylee

Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
primidone	3.499	166642	112509.1	101.4	77022	9.996
Promethazine	4.463	3530496	3840.3	343.6	3454393	9.878
Pseudoephedrine	2.692	40391145	1652.9	2601.0	11604940	9.162
Quetiapine	4.393	2332649	951715.6	11190.3	4552456	9.848
Risperidone	4.085	2344920	306462.3	260.5	4552456	9.859
Sertraline	4.782	724014	1707.3	∞	2808520	10.107
Sufentanil	4.316	116140	52274.9	116.0	7771676	9.622
Tapentadol	3.484	2458773	3289.3	396.0	4996900	9.786
Temazepam	4.844	2274515	607.9	197.7	9165018	9.894
Topiramate	3.904	22739	9319.4	8398.5	127304	10.008
Tramadol	3.449	3350024	1302.5	27.9	607286	9.753
Trazodone	4.194	2015107	1037131.7	450504.4	7067189	9.592
Venlafaxine	3.878	3083449	999.7	358.6	3422542	9.865
Zaleplon	4.477	1144761	554862.7	8403.4	5780283	10.399
Zolpidem	3.814	3178955	1644100.3	310.8	13128899	10.033
Zopiclone	3.886	187453	39799.6	49960.3	798961	10.483

AM# 26: THC and Metabolites Screen in Blood by LC-MS/MS

Wylie

Extraction Date: 5/10/23 Analyst: Britany Wylie

Plate lot#: 230113 Plate retest date: 7/13/23

Mobile phase A: 10mM Ammonium Formate
0.1% Formic Acid in Water

Mobile phase B: 0.1% Formic acid in MeOH
MTBE Hexane

Blank Blood Lot: 22B52016-1 **Urine Blank:** 12522

Column: Agilent Phenyl Hexyl (4.6x50mm: 2.7 um)

LCMS-QQQ ID: 69679

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 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette **1000 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: K52558G**
Pipette 1000 ul urine to analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
- 4. Pipette **500 µL 0.1% formic acid in blood** wells **500 ul saturated phosphate buffer in urine** wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
- 6. Transfer **800 µL 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**) Manifold ID: 66792
- 8. Wait 5 minutes.
- 9. Add **2.25 mL MTBE** (add in 3 increments of 750 µL).
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 10-15 seconds. (**12-15 PSI- Selector to the left**).
- 12. Add **2.25 mL hexane** (add in 3 increments of 750 µL).
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 10-15 seconds. (**12-15 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: 66819
- 16. Reconstitute in **100 µL 100% LCMS MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Create batch and process data.
- 2. Calculated sample concentration of 3 ng/mL or greater for THC and THC-OH, a calculated sample concentration of 10 ng/mL or greater for Carboxy-THC.
- 3. Retention time within +/- 2% or +/-0.100 min whichever is greater of the average retention time of the calibrators.
- 4. Did all QCs pass for each analyte? Yes
- 5. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *calibrators and a few samples were reinjected (poor chromatography)- noticed that the autosampler was not cooling. Stopped run and turned cooler back on, reinjected Cals, QC's and samples. Upon review of data C2023-0949-2&3 were not injected/properly. Started instrument and reinjected with QC, 949-2 & 3 were reconstituted prior to injection on 5-11-23*

Byyle

	1	2	3	4	5	6
a	cal 1	Internal control urine	0980-1			
b	cal 2	negative blood	0989-1			
c	cal 3	0883-3	1001-1			
d	cal 4	0894-1	1003-1			
e	Cal 5	0949-1	1012-1			
f	cal 6	0949-2	1023-1			
g	cal 7	0949-3	1038-2			
h	Internal control (blood)	0951-1	1049-1			

Plate position 3

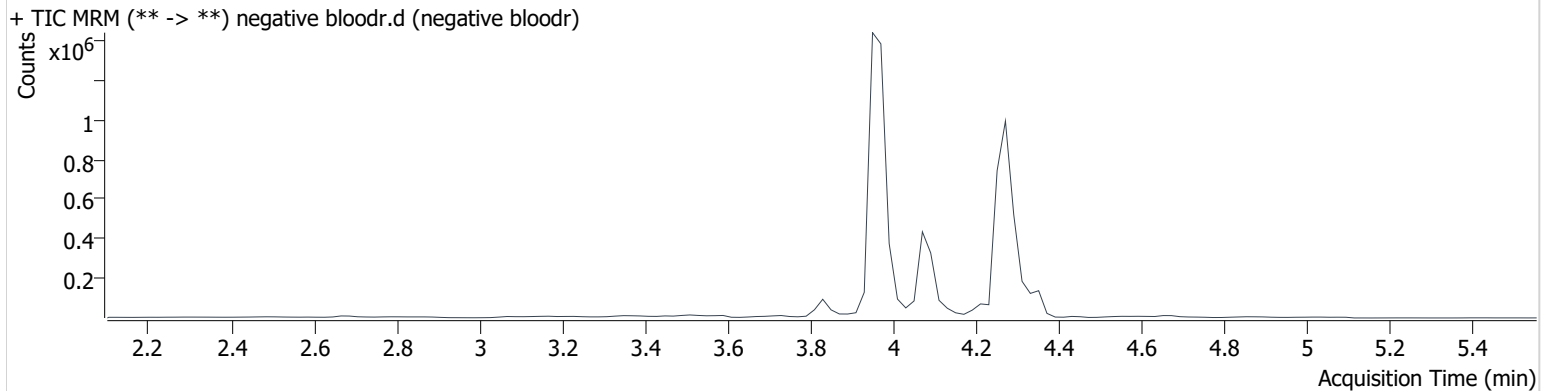
c2023-____-

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	negative bloodr.d
Type	Sample	Sample	negative bloodr
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-B2	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 9:16:47 PM		
Sample Info.			

Sample Chromatogram

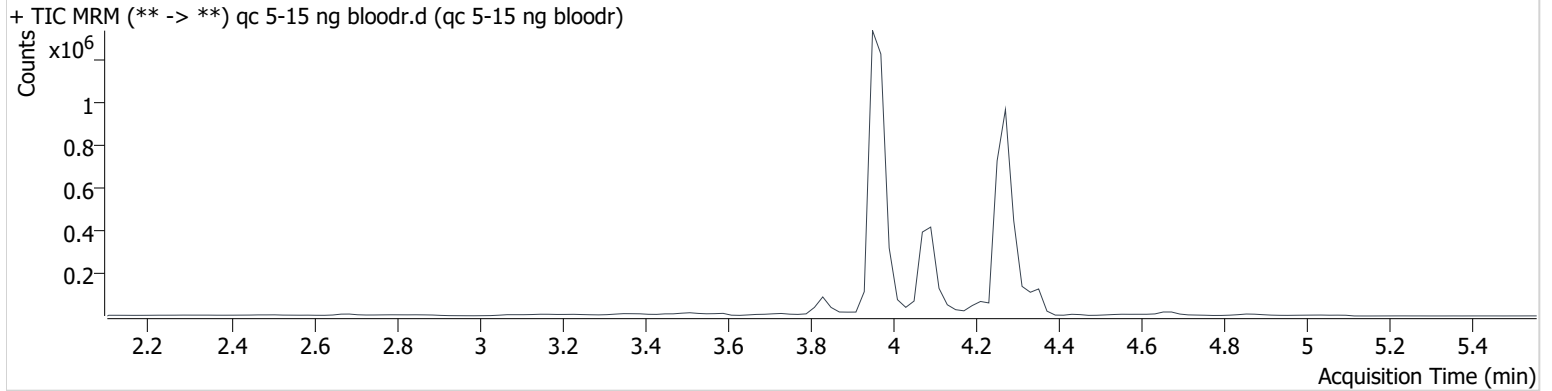


AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	qc 5-15 ng bloodr.d
Type	QC	Sample	qc 5-15 ng bloodr
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 9:10:19 PM		
Sample Info.			

Sample Chromatogram



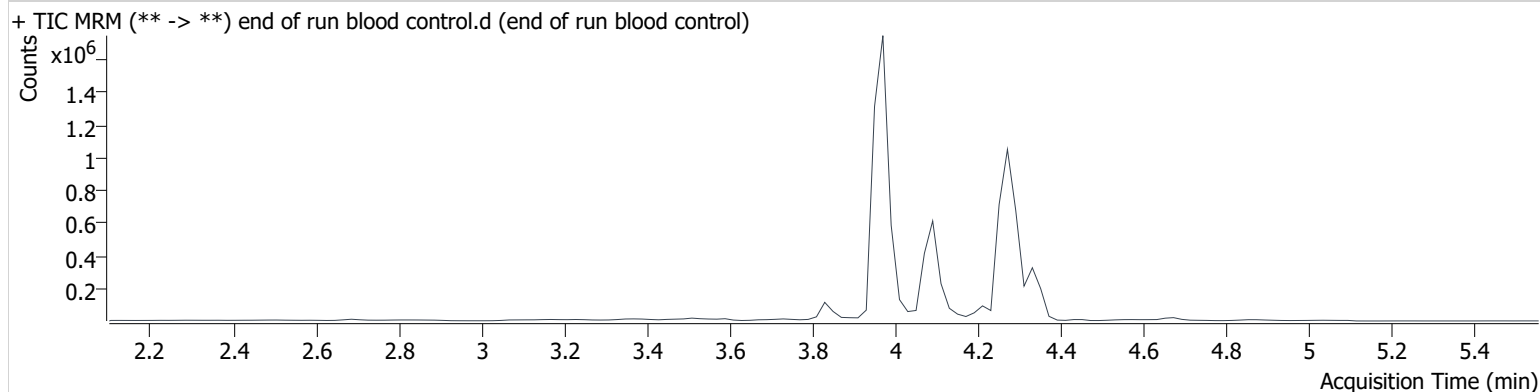
Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.345	14319	200737	9.641 ng/ml
THC-COOH	4.093	132250	663440	15.849 ng/ml
THC-OH	3.979	31830	3392317	4.432 ng/ml

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	end of run blood control.d
Type	Sample	Sample	end of run blood control
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 10:40:56 PM		
Sample Info.			

Sample Chromatogram



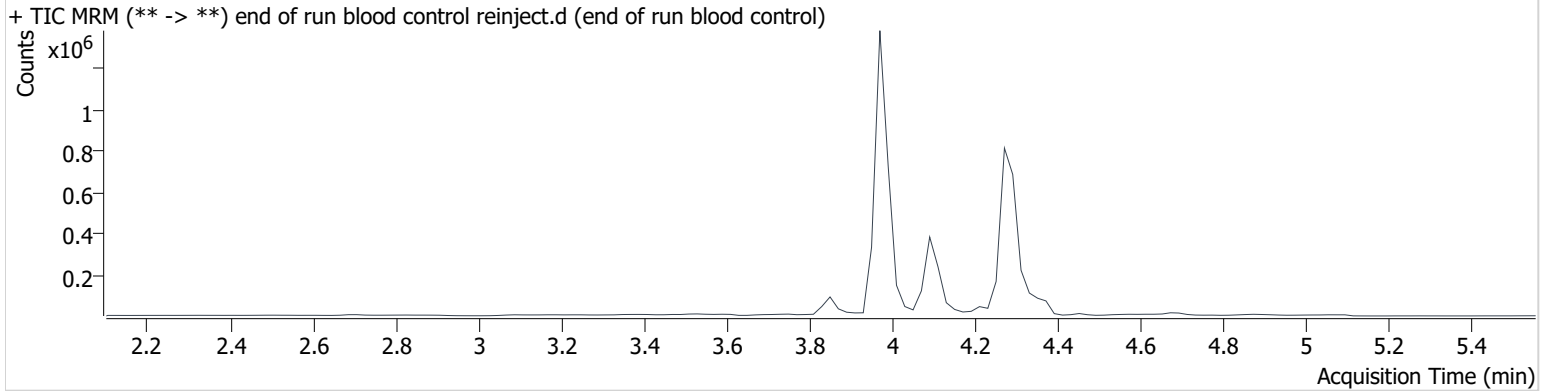
Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.345	24873	492110	6.881 ng/ml
THC-COOH	4.093	174655	878430	15.808 ng/ml
THC-OH	3.979	41801	4261533	4.629 ng/ml

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	end of run blood control reinject.d
Type	Sample	Sample	end of run blood control
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	5		
Acq. Date-Time	5/11/2023 9:11:36 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.385	6634	187905	4.858 ng/ml
THC-COOH	4.113	103220	540531	15.182 ng/ml
THC-OH	3.979	28243	2894687	4.605 ng/ml

reinjected QC with samples that failed to properly inject on 5/10/23

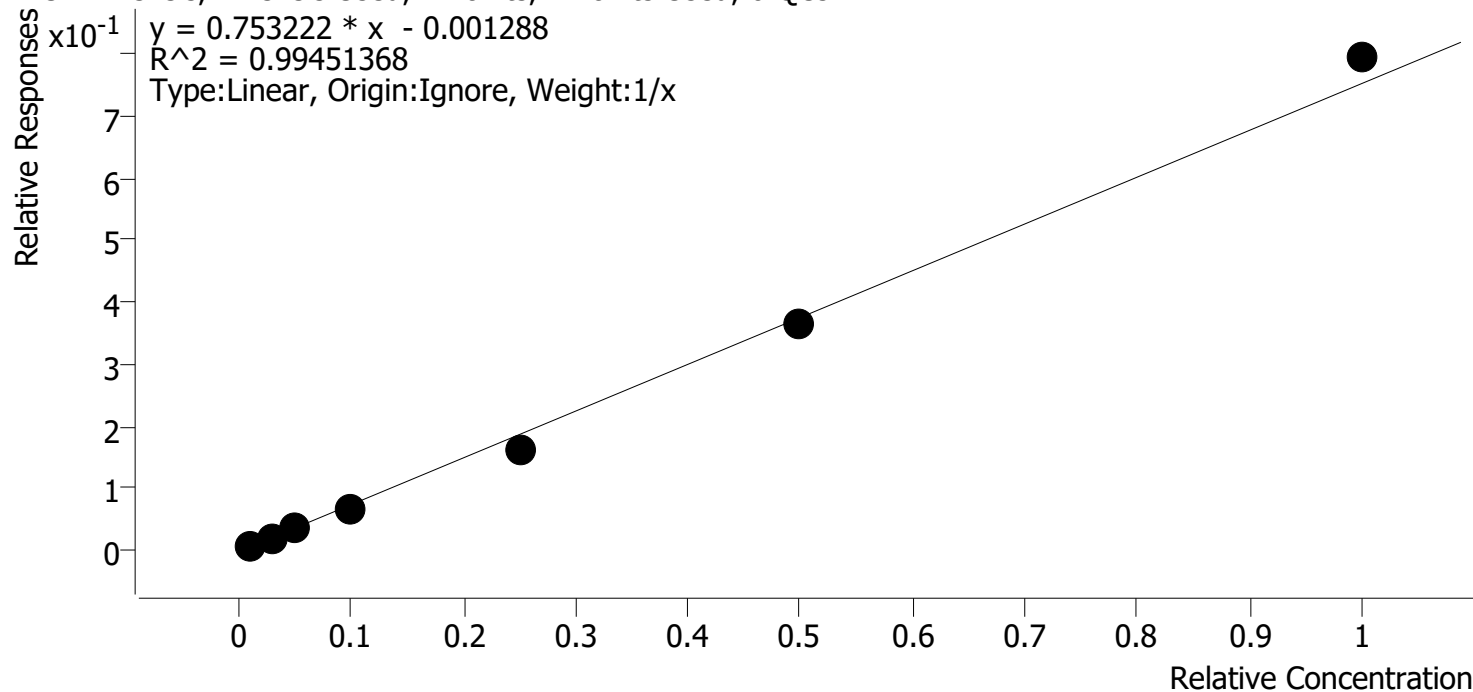
BW
5-12-23

Compound Calibration Report

Boyle

Batch results D:\MassHunter\Data\2023\lam 25-26\051023\QuantResults\lam 26 51023.batch.bin
Last Cal. Update 5/11/2023 9:29 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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

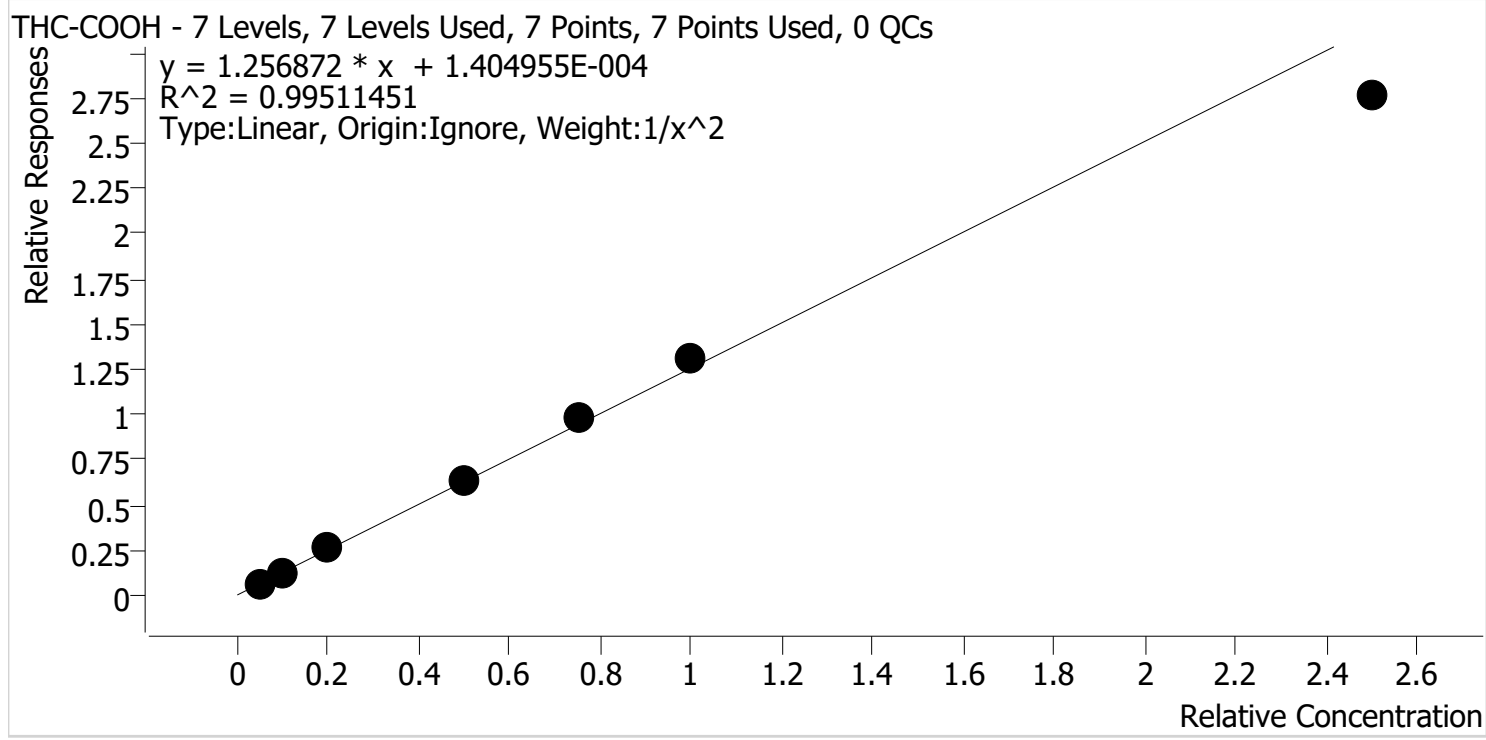


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 1r	1	✓	1.0	1.2	122.7
cal 2r	2	✓	3.0	3.1	102.3
cal 3r	3	✓	5.0	4.8	95.5
cal 4r	4	✓	10.0	8.9	89.3
cal 5r	5	✓	25.0	21.9	87.5
cal-6r	6	✓	50.0	48.6	97.2
cal-7r	7	✓	100.0	105.5	105.5

Compound Calibration Report

B. Wylee

Batch results D:\MassHunter\Data\2023\lam 25-26\051023\QuantResults\lam 26 51023.batch.bin
Last Cal. Update 5/11/2023 9:29 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-d9



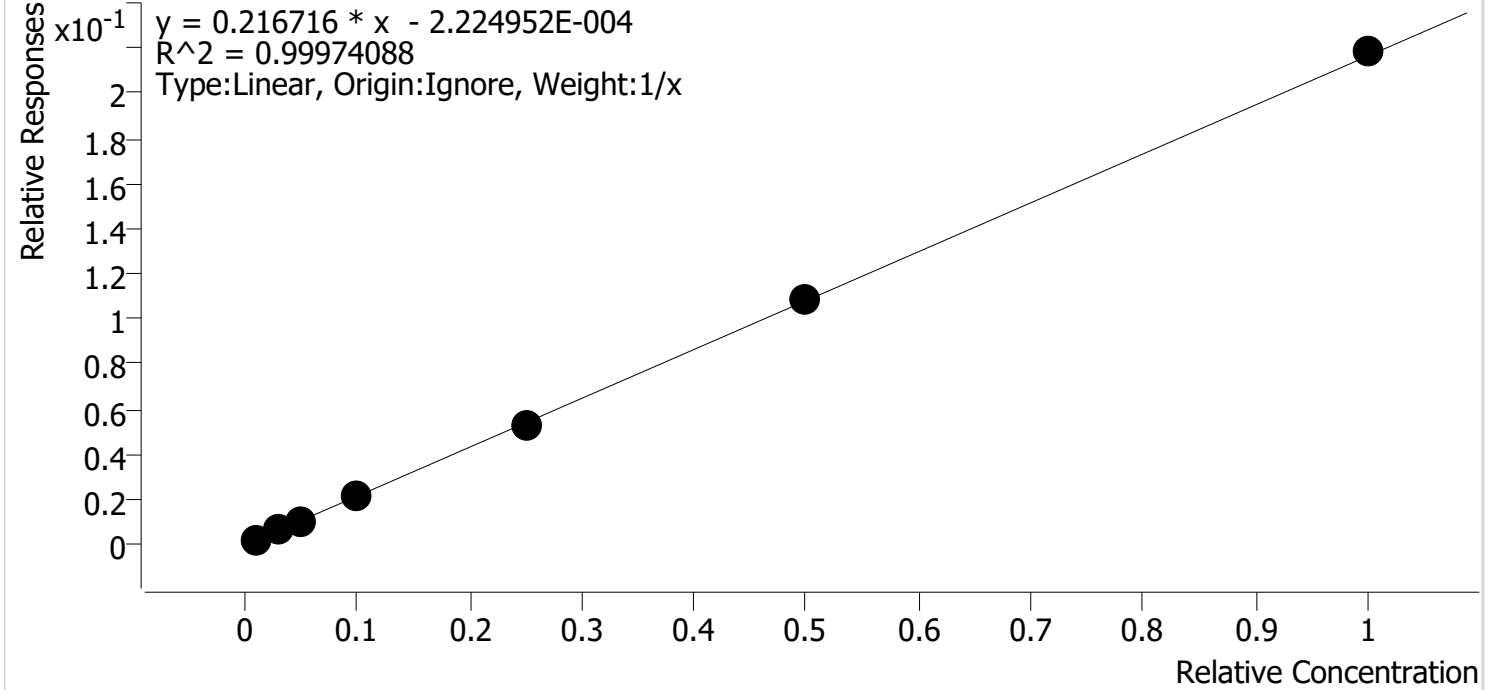
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 1r	1	✓	5.0	5.0	99.4
cal 2r	2	✓	10.0	9.9	99.2
cal 3r	3	✓	20.0	20.5	102.3
cal 4r	4	✓	50.0	50.8	101.5
cal 5r	5	✓	75.0	78.7	104.9
cal-6r	6	✓	100.0	104.7	104.7
cal-7r	7	✓	250.0	220.1	88.0

Compound Calibration Report

B. Wylee

Batch results D:\MassHunter\Data\2023\lam 25-26\051023\QuantResults\lam 26 51023.batch.bin
Last Cal. Update 5/11/2023 9:29 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, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 1r	1	✓	1.0	1.1	110.3
cal 2r	2	✓	3.0	2.9	96.0
cal 3r	3	✓	5.0	4.8	96.8
cal 4r	4	✓	10.0	9.8	98.1
cal 5r	5	✓	25.0	24.5	97.9
cal-6r	6	✓	50.0	50.0	100.0
cal-7r	7	✓	100.0	100.9	100.9

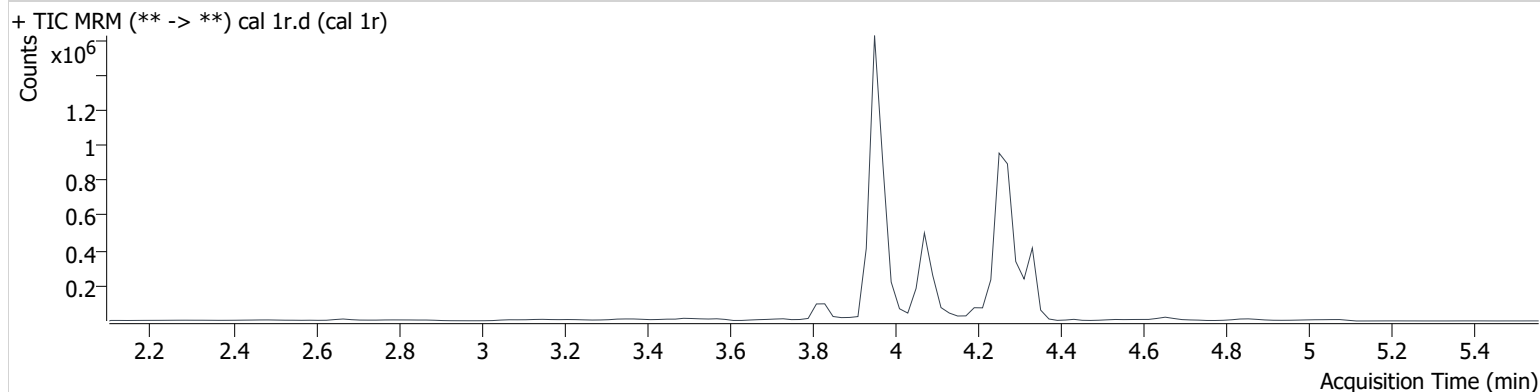
AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	cal 1r.d
Type	Cal	Sample	cal 1r
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-A1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 8:18:18 PM		

Sample Info.

Sample Chromatogram



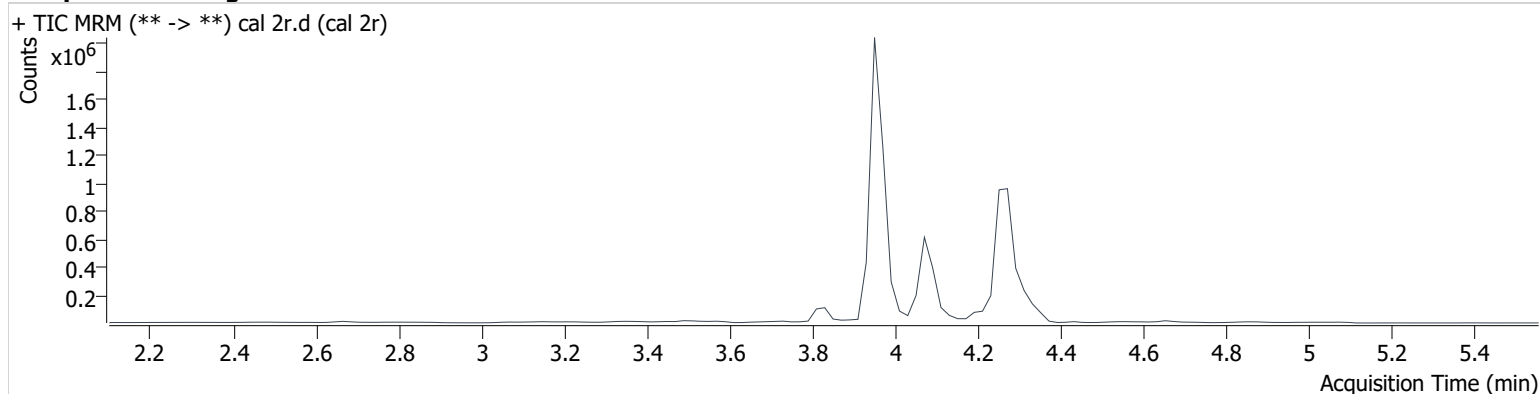
Name	RT	Resp.	ISTD Resp.	Final Conc.	
THC	4.345	5077	638128	1.227 ng/ml	Low
THC-COOH	4.093	53650	857310	4.968 ng/ml	Low
THC-OH	3.959	8253	3807971	1.103 ng/ml	Low

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	cal 2r.d
Type	Cal	Sample	cal 2r
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-B1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 8:24:57 PM		
Sample Info.			

Sample Chromatogram



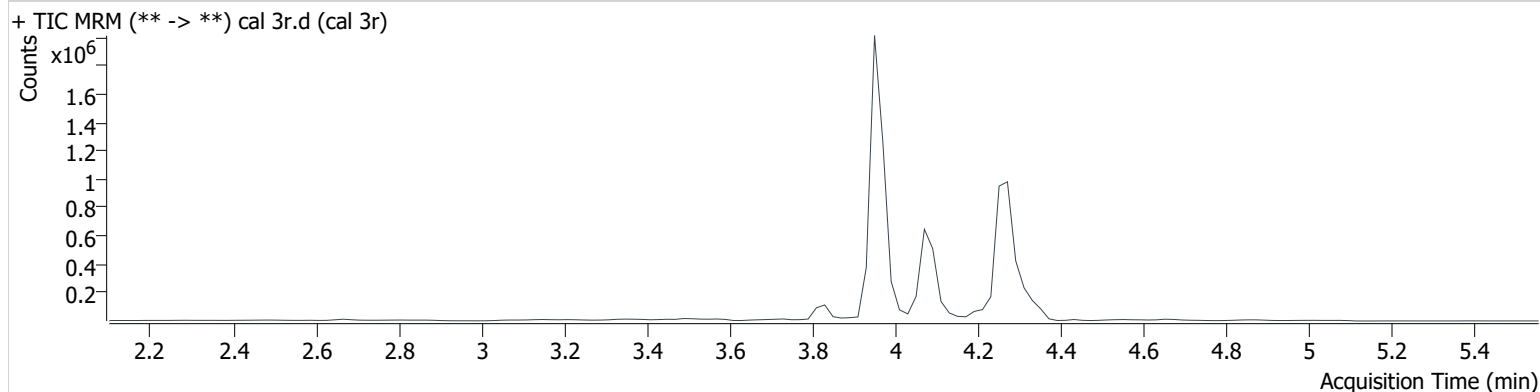
Name	RT	Resp.	ISTD Resp.	Final Conc.	
THC	4.325	6147	281697	3.068 ng/ml	
THC-COOH	4.093	123620	990553	9.918 ng/ml	Low
THC-OH	3.959	28254	4693136	2.881 ng/ml	Low

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	cal 3r.d
Type	Cal	Sample	cal 3r
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-C1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 8:31:26 PM		

Sample Chromatogram



Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.325	9676	279041	4.775 ng/ml
THC-COOH	4.093	233805	908580	20.463 ng/ml
THC-OH	3.959	45229	4406578	4.839 ng/ml

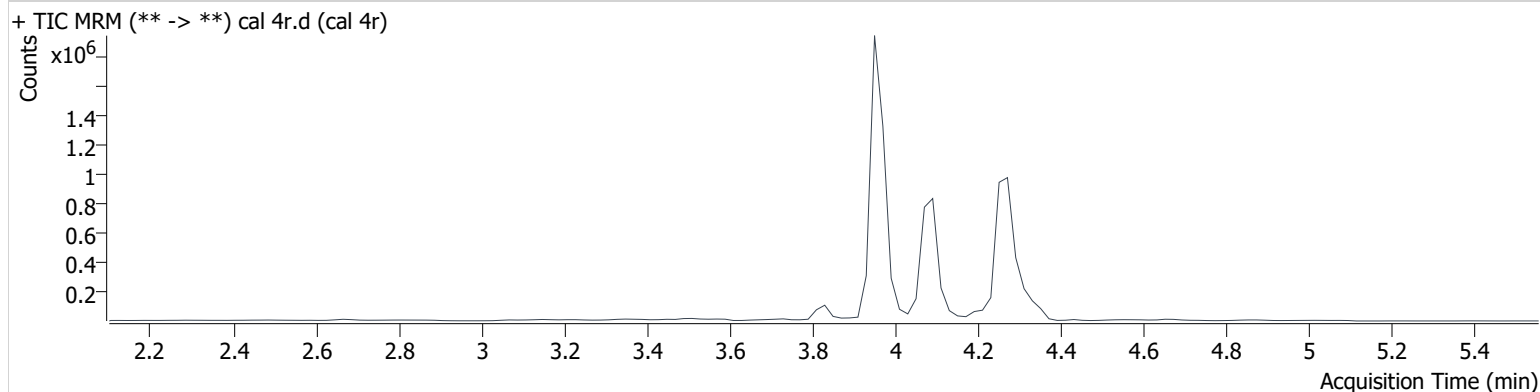
AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	cal 4r.d
Type	Cal	Sample	cal 4r
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-D1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 8:37:54 PM		

Sample Info.

Sample Chromatogram



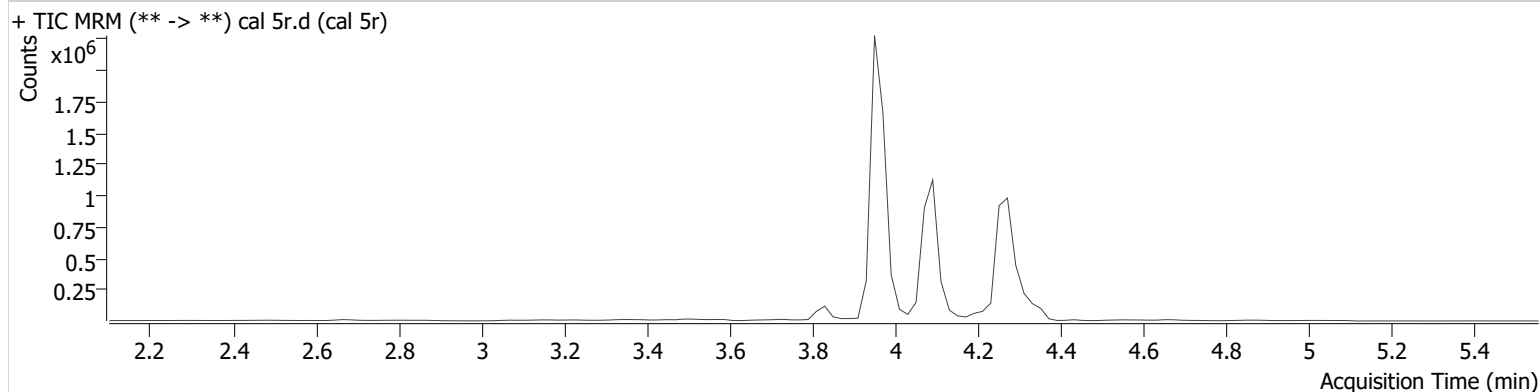
Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.325	16654	252570	8.925 ng/ml
THC-COOH	4.093	517582	811058	50.762 ng/ml
THC-OH	3.959	84520	4016047	9.814 ng/ml

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	cal 5r.d
Type	Cal	Sample	cal 5r
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-E1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 8:44:22 PM		
Sample Info.			

Sample Chromatogram



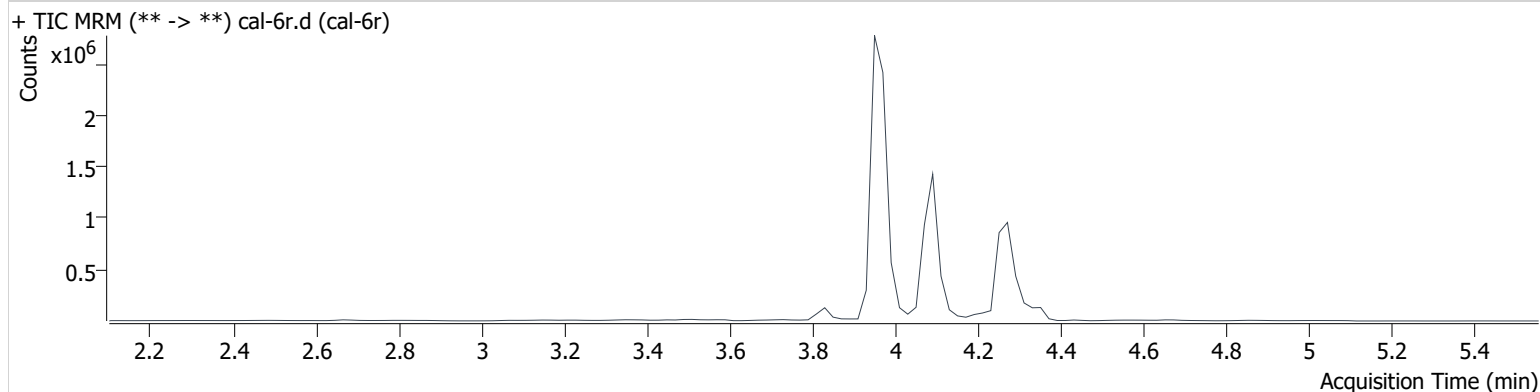
Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.365	39551	241942	21.874 ng/ml
THC-COOH	4.093	764305	772604	78.697 ng/ml
THC-OH	3.959	203019	3844007	24.473 ng/ml

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	cal-6r.d
Type	Cal	Sample	cal-6r
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-F1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 8:50:50 PM		
Sample Info.			

Sample Chromatogram



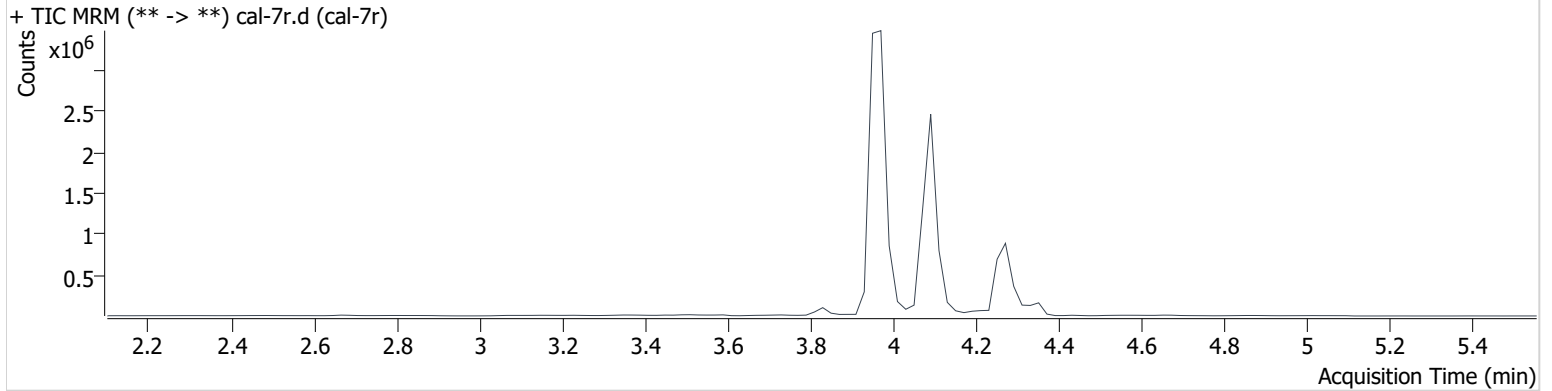
Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.365	69726	191069	48.620 ng/ml
THC-COOH	4.093	987204	750386	104.661 ng/ml
THC-OH	3.959	407518	3767804	50.010 ng/ml

AM #26 Cannabinoids Screen Results *BWylie*

Batch results D:\MassHunter\Data\2023\am 25-26\051023\QuantResults\am 26 51023.batch.bin
Calibration Last Update 5/11/2023 9:29:19 AM

Instrument	69679	Data File	cal-7r.d
Type	Cal	Sample	cal-7r
Acq. Method	am 26 cann scr 5-5-20.m	Operator	Britany Wylie
Sample Position	P3-G1	Comment	
Injection Volume	5		
Acq. Date-Time	5/10/2023 8:57:19 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	ISTD Resp.	Final Conc.
THC	4.365	124383	156764	105.511 ng/ml
THC-COOH	4.093	1869317	675736	220.086 ng/ml
THC-OH	3.959	731028	3347173	100.881 ng/ml