

**APPROVED**  
By Anne Nord at 11:18 am, Mar 03, 2021

SJ

3/2/2021

**Worklist: 4811**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
P2020-3400	1	BCK	AM 25 Blood Multi-Drug Screen by LC-QQQ



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

Extraction Date: 3/2/21  
Plate lot#: IDP-107-2-200511

Analyst: Sophia Jackson  
Plate Expiration: 11/11/2020 – ok with external control

**Mobile phase A:** 10mM Amm Form  
0.5M Ammonium Hydroxide  
**Blank Blood Lot:** Lampire 20L20724  
**LCMS-QQQ ID:** 069901

**Mobile phase B:** 0.1% Formic Acid in MeOH  
Ethyl Acetate LC Methanol  
**Column:** Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

### 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. Pipette **250 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: #16**
- 3. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
- 4. Pipette **250 µL of 0.5 M ammonium hydroxide** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
- 6. Transfer **300 µL of blood+base** 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 **900 µL ethyl acetate**.
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 10-15 seconds. *(12-15 PSI- Selector to the left)*.
- 12. Add **900 µL ethyl acetate**.
- 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.
- 16. Reconstitute in **100 µL 20% LC MeOH** 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? Y / N \_\_\_\_\_
- 5. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

### COMMENTS:

*Due to extraction occurring after the expiration of the analytical plate, an external control was included with this run*

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	1	2	3	4	5	6	7	8	9	10	11	12
A						Negative control						
B						External control						
C						SJ 3/3/21 P2020-3400-1**						
D						P2020-3400-1						
E				SJ 3/3/21 Negative-control*								
F				SJ 3/3/21 External-control*								
G												IS + Cal. 1
H				SJ 3/3/21 P2020-3400-1*								

All wells to contain 60 µl of residual DMSO

\*Negative control, external control, and P2020-3400-1 moved during analytical step 6 in order to better fit on the SLE plate  
 \*\*P2020-3400-1 moved during analytical step 6 due to blood clot



# Idaho State Police Forensic Services

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**AM #25 Blood Multi-Drug Screen by LCMS-QQQ  
And  
AM #28 Blood Multi-Drug Confirmatory Analysis by  
LCMS-QQQ---Panel 1**

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**Methanol External Control Solution (Lot: 121020)**

*100 µL of 1mg/mL stock was added to each drug to 9600 µL of LC MeOH.*

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>	<i>Expiration Date</i>
Methanol (LCMS)	Fisher	197468	
Alprazolam	Cerilliant	FE07061604	07/31/2021
Clonazepam	Cerilliant	FE07131603	10/31/2021
Hydrocodone	Cerilliant	FE04241902	09/30/2024
Morphine	Cerilliant	FE06231704	07/31/2022
Prepared:	12/10/2020		
Prepared By:	Tamara Salazar		
Expires:	07/31/2021		

**Blood External Control Solution (Lot: WS121020)**

*100 µL of methanol external control solution was added to 9900 µL of blood.  
Approximately 100 ng/mL of each compound.*

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>
Negative Blood	Lampire	20L20725
Methanol External Control Solution		121020
Prepared:	12/10/2020	
Prepared by:	Tamara Salazar	
Expires:	07/31/2021	

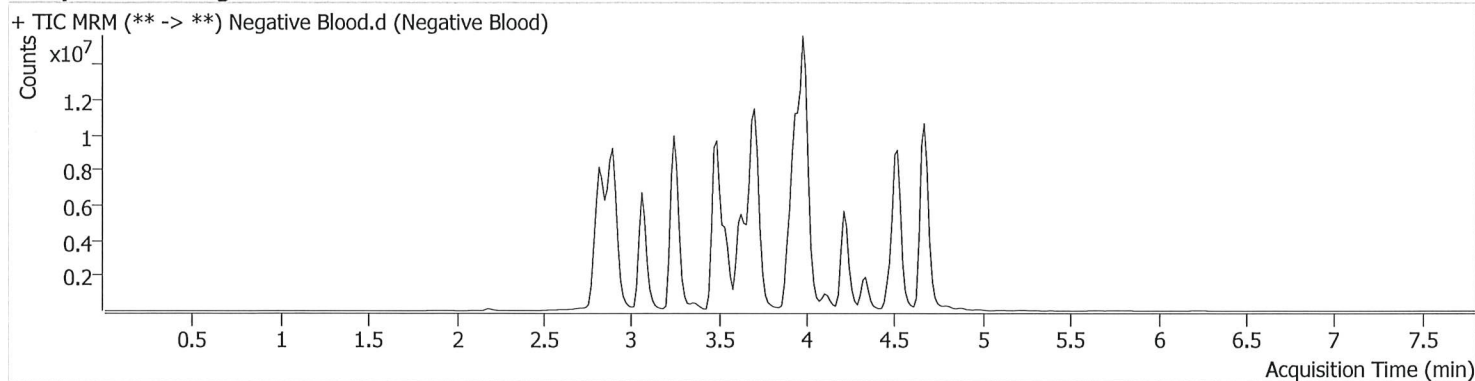


# AM #25 Multi-Drug Screen Results

**Batch results** D:\MassHunter\Data\2021\AM 25-26\03022021 AM 25 SJ\QuantResults\AM 25.batch.bin  
**Calibration Last Update** 3/3/2021 11:33:22 AM

<b>Instrument</b>	Instrument 1	<b>Data File</b>	Negative Blood.d
<b>Type</b>	Sample	<b>Sample</b>	Negative Blood
<b>Acq. Method</b>	AM 25 MDS.m	<b>Operator</b>	Sophia Jackson
<b>Sample Position</b>	P2-A6	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	3/2/2021 11:31:29 AM		
<b>Sample Info.</b>			

## Sample Chromatogram





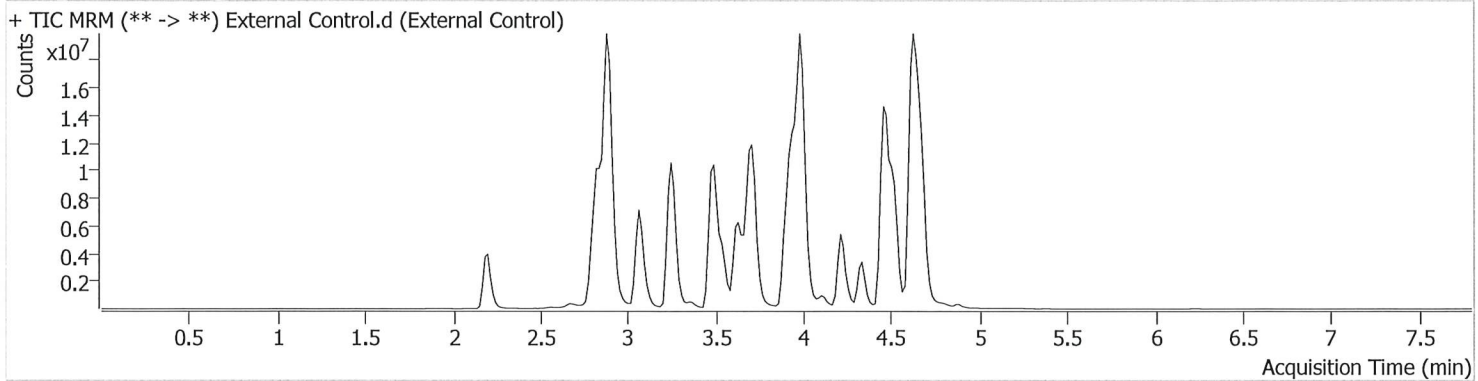
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**Batch results** D:\MassHunter\Data\2021\AM 25-26\03022021 AM 25 SJ\QuantResults\AM 25.batch.bin  
**Calibration Last Update** 3/3/2021 11:33:22 AM

<b>Instrument</b>	Instrument 1	<b>Data File</b>	External Control.d
<b>Type</b>	Sample	<b>Sample</b>	External Control
<b>Acq. Method</b>	AM 25 MDS.m	<b>Operator</b>	Sophia Jackson
<b>Sample Position</b>	P2-B6	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	3/2/2021 11:14:39 AM		

**Sample Info.**

**Sample Chromatogram**



Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
Alprazolam	4.626	34609725	1445.26	6357.88	33338713	69.2121
Clonazepam	4.455	27356319	2360.66	2487.00	33338713	78.2971
Hydrocodone	2.882	21608357	12165.64	2657.94	15784344	78.5458
Morphine	2.201	3811051	∞	1775.72	290212	99.9080

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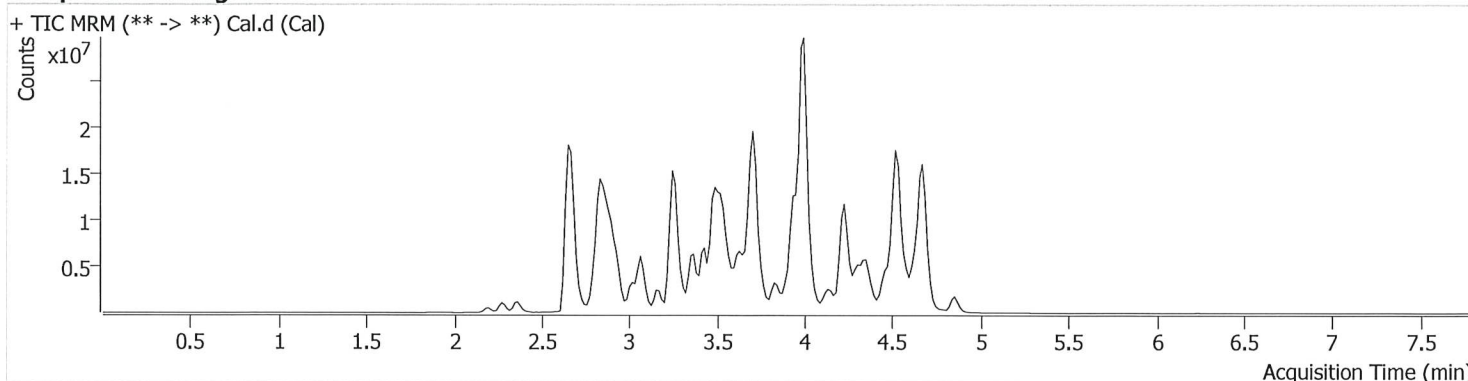


# AM #25 Multi-Drug Screen Results

**Batch results** D:\MassHunter\Data\2021\AM 25-26\03022021 AM 25 SJ\QuantResults\AM 25.batch.bin  
**Calibration Last Update** 3/3/2021 11:33:22 AM

<b>Instrument</b>	Instrument 1	<b>Data File</b>	Cal.d
<b>Type</b>	Cal	<b>Sample</b>	Cal
<b>Acq. Method</b>	AM 25 MDS.m	<b>Operator</b>	Sophia Jackson
<b>Sample Position</b>	P2-G12	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	3/2/2021 10:57:38 AM		

## Sample Chromatogram



Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
6-MAM	2.801	72212	51092.00	57877.29	2213564	10.0000
7-aminoclonazepam	3.554	2432892	∞	1403.30	9833433	10.0000
7-aminoflunitrazepam	3.768	3391773	1124.05	415.22	9833433	10.0000
Acetyl Fentanyl	3.672	206278	107.94	64236.73	29476546	10.0000
Acetyl Norfentanyl	2.840	378613	907.82	573.08	29476546	10.0000
a-hydroxyalprazolam	4.515	544261	292313.29	352681.02	9833433	10.0000
alpha-hydroxymidazolam	4.514	3762747	477.48	2345.42	9833433	10.0000
Alpha-PHP	3.696	2267208	40612.82	8128.09	29476546	10.0000
alpha-PVP	3.437	4429779	995.89	597.90	6549507	10.0000
Alprazolam	4.626	4846563	1170.28	755.05	32312226	10.0000
Amitriptyline	4.324	802514	136.18	206.29	3199401	10.0000
Amphetamine	2.844	2500236	1048.48	503.64	6549507	10.0000
Benzoylcegonine	3.385	1541390	686.70	63613.71	701890	10.0000
Brompheniramine	3.949	62015	72.53	783.02	28617887	10.0000
Buprenorphine	3.991	349478	110585.27	48134.71	1362914	10.0000
Bupropion	3.635	3282169	445.11	453.71	11415266	10.0000
Carbamazepine	4.234	15784219	∞	2673.74	1484043	10.0000
Carisoprodol	4.233	2698147	1948566.24	306.91	14594922	10.0000
Chlordiazepoxide	4.627	1881994	1316.73	1755.45	32312226	10.0000
Chlorpheniramine	3.846	4026871	278.85	16.52	28617887	10.0000
Citalopram	3.994	1752595	2160.41	881.36	28617887	10.0000
Clomipramine	4.517	1633309	8449.71	385.41	28617887	10.0000
Clonazepam	4.455	3386337	1592.17	471.05	32312226	10.0000
Clonazolam	4.375	3041232	4568005.89	1192.26	32312226	10.0000
Cocaehtylene	3.688	4272218	2705021.42	2696.08	29386279	10.0000
Cocaine	3.475	6304854	957.90	1373.39	29386279	10.0000
Codeine	2.699	489839	5272.39	3923.49	12091500	10.0000
Cyclobenzaprine	4.262	1274923	483.37	50.84	3199401	10.0000
Desipramine	4.294	1997888	909.19	462.73	3199401	10.0000
Dextromethorphan	3.985	1042367	5630.93	588.77	5456884	10.0000
Dextroprhan	3.295	2777327	982.30	1050565.30	5456884	10.0000
Diazepam	4.858	2542775	1588.37	1435.37	32312226	10.0000
Dihydrocodeine	2.667	1174136	451.33	368.48	12091500	10.0000
Diphenhydramine	3.940	5103899	2750.12	1079.26	28617887	10.0000

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# AM #25 Multi-Drug Screen Results

Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
Doxepin	4.061	894331	224.54	41.32	11515513	10.0000
Doxylamine	3.524	9315543	808.91	528.45	5456884	10.0000
EDDP	3.999	5249986	671.12	766.47	2716780	10.0000
Estazolam	4.535	10770722	1244.49	170213.87	32312226	10.0000
Etizolam	4.636	592650	908483.35	2164445.82	32312226	10.0000
Fentanyl	3.901	131404	32.95	1204.83	7941144	10.0000
Flualprazolam	4.484	2012758	3073.65	3668.58	32312226	10.0000
Flunitrazepam	4.579	6064141	1034.38	1054.43	32312226	10.0000
Fluoxetine	4.257	1275807	1109.62	51.87	3322658	10.0000
Flurazepam	4.022	2237463	2262.43	242.22	32312226	10.0000
Hydrocodone	2.882	2107427	1926.51	2487.05	12091500	10.0000
Hydromorphone	2.367	1918044	749.17	4978.84	275611	10.0000
Imipramine	4.291	2451623	2192.83	892.85	3199401	10.0000
Ketamine	3.266	4365627	3467.01	154.28	18182371	10.0000
Lamotrigine	3.434	472806	161.42	2844.52	28617887	10.0000
Levamisole	2.841	2828780	2783.34	388.27	29386279	10.0000
Levetiracetam	2.659	1540281	386.97	1018.85	28617887	10.0000
Lorazepam	4.439	1104880	1925.03	∞	32312226	10.0000
Maprotiline	4.324	591869	43.55	323.62	3199401	10.0000
MDA	2.948	3097545	1163.90	42.97	15480690	10.0000
MDEA	3.161	4710052	3108.73	1910.18	15480690	10.0000
MDMA	3.024	5810370	880.38	400.61	15480690	10.0000
Meperidine	3.511	2296509	444.54	598.05	5456884	10.0000
Meprobamate	3.668	1542348	863.24	552.27	14594922	10.0000
Methadone	4.303	3321077	521.98	225.17	2716780	10.0000
Methamphetamine	2.935	3503319	161.12	4427.60	15480690	10.0000
Methocarbamol	3.573	1219864	562.64	371.37	2716780	10.0000
Methylphenidate	3.436	10001723	1738.60	879.71	15118572	10.0000
Metoprolol	3.371	676780	335.00	407089.12	5456884	10.0000
Midazolam	4.423	726032	311938.05	440429.96	32312226	10.0000
Mirtazapine	3.586	1792811	1260.67	1947.58	5456884	10.0000
Mitragynine	4.037	241272	179535.13	613123.23	5456884	10.0000
Morphine	2.201	362264	∞	1429.53	275611	10.0000
Norbuprenorphine	3.746	46926	39426.66	7193.82	1362914	10.0000
Nordiazepam	4.707	2743487	1055.97	1060.86	32312226	10.0000
Norfentanyl	3.267	7277649	1277.48	283.06	29476546	10.0000
Norhydrocodone	2.868	48735	86.34	28008.02	275611	10.0000
Norketamine	3.252	919989	409.74	1607.35	18182371	10.0000
Normeperidine	3.528	2043169	461.13	344.59	28617887	10.0000
Noroxycodone	2.835	1474780	336.42	241.24	18182371	10.0000
Nortriptyline	4.340	801457	203433.89	196.82	3199401	10.0000
O-desmethyl-tramadol	2.869	8776701	1706.29	661.15	28617887	10.0000
Olanzapine	3.229	935476	710.97	228.96	1484043	10.0000
Oxazepam	4.521	4818141	491.30	273.33	25707071	10.0000
Oxycodone	2.833	3946809	842.63	1414.08	18182371	10.0000
Oxymorphone	2.271	2088535	1199.34	612.16	275611	10.0000
Paroxetine	4.254	198945	1317.15	10080.35	3322658	10.0000
Phenazepam	4.651	4251812	7892.31	1882894.80	32312226	10.0000
Phencyclidine	3.834	3720051	1081.70	403.83	5456884	10.0000
Phentermine	3.087	1252446	87.05	37.31	15118572	10.0000
Phenytoin	4.141	2611605	3792.39	924.73	1484043	10.0000
Promethazine	4.214	2826249	1665.39	298.14	28617887	10.0000
Pseudoephedrine	2.660	44220853	948.57	46468.67	15480690	10.0000
Quetiapine	4.160	1943608	1035.71	2033.60	35742329	10.0000
Sertraline	4.473	634631	633381.68	1596.28	3322658	10.0000
Sufentanil	4.160	107497	97096.78	44.84	29476546	10.0000
Tapentadol	3.375	5126188	1251.96	417.65	18182371	10.0000
Temazepam	4.673	8179734	659.95	938.41	32312226	10.0000
Tramadol	3.356	9566230	1971.83	60.05	28617887	10.0000
Trazodone	3.992	2312980	1022.55	1683.03	11515513	10.0000

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# AM #25 Multi-Drug Screen Results

Name	RT	Resp.	S/N	S/N	ISTD Resp.	Calc. Conc.
Venlafaxine	3.722	6062440	5930.85	356.02	3322658	10.0000
Zaleplon	4.351	5551891	1775.04	429.53	35742329	10.0000
Zolpidem	3.705	9942440	727207.67	1003.51	35742329	10.0000
Zopiclone	3.655	1185706	1099370.96	256710.50	6197080	10.0000