

REVIEWED

By Tamara Salazar at 3:19 pm, Aug 20, 2020

SJ
8/20/2020

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Worklist: 4442

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2020-2873	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
P2020-2077	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
P2020-2295	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
P2020-2304	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ



AM# 28: Multi-Drug Quantitation in Blood by LC-MS/MS

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Extraction Date: 08/18/20
Plate lot#: IDP-112-2-200514

Analyst: Sarah Pickle
Plate Expiration: 11/14/20

Mobile phase A: 5mM Amm Form + 0.01% FA
0.5M Ammonium Hydroxide

Mobile phase B: 0.01% Formic Acid in MeOH
Ethyl Acetate 20% Methanol in Water

Blank Blood Lot: Hemostat 445283-4
LCMS-QQQ ID: 069901

Column: Agilent 120 EC-C18 (2.1x 100-2.7um)

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., 900rpm for 15 minutes.
- 4. Pipette **250 µL 0.5 M ammonium hydroxide** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm 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. 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% LCMS MeOH in water** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Create batch and process data.
- 2. Make necessary changes to integration limits
- 3. Integration linear and R² values ≥0.98 for each analyte.
- 4. For unknown samples and controls: response ratio within 20% of average of controls and standards, RT within +/- 5% (tramadol RT +/-2%), S/N for primary transition >10 and secondary transitions >5.
- 5. Did all QCs pass for each analyte? Y / N _____ Add Control data to QC tracking spreadsheet.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS: *Hands of the analyst Sophie Jackson.*

Compounds evaluated: Chlordiazepoxide 5-500, Etizolam 5-500, Methocarbamol 5-250, Paroxetine.

Samples were inadvertently left on lab bench overnight unrefrigerated (8/12/20-8/13/20). Samples left overnight are from worklist 4426.

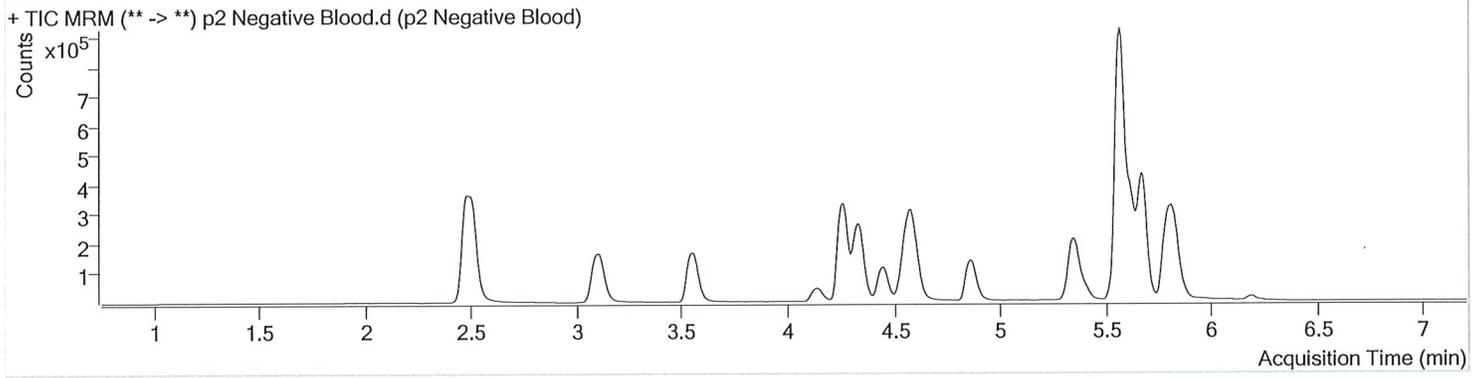


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Negative Blood.d
Type	Sample	Sample	p2 Negative Blood
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-A12	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 2:36:40 PM		
Sample Info.			

Sample Chromatogram



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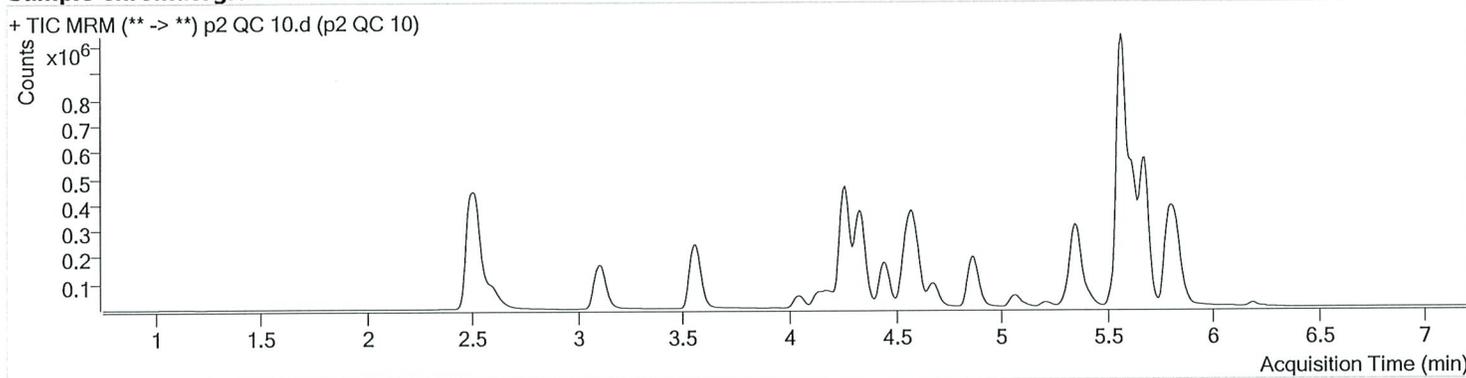


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 QC 10.d
Type	QC	Sample	p2 QC 10
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-A11	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 1:02:04 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	56104	558.89	79.2	147.59	473795	9.6660 ng/ml
Etizolam	5.797	58447	5339.73	26.9	∞	802663	9.4476 ng/ml
Methocarbamol	4.357	35003	912.77	77.2	685.52	1660879	9.4119 ng/ml
Paroxetine	5.552	10554	∞	49.2	3298.79	182243	10.9946 ng/ml

AM #28 Multi-Drug Quant. Results

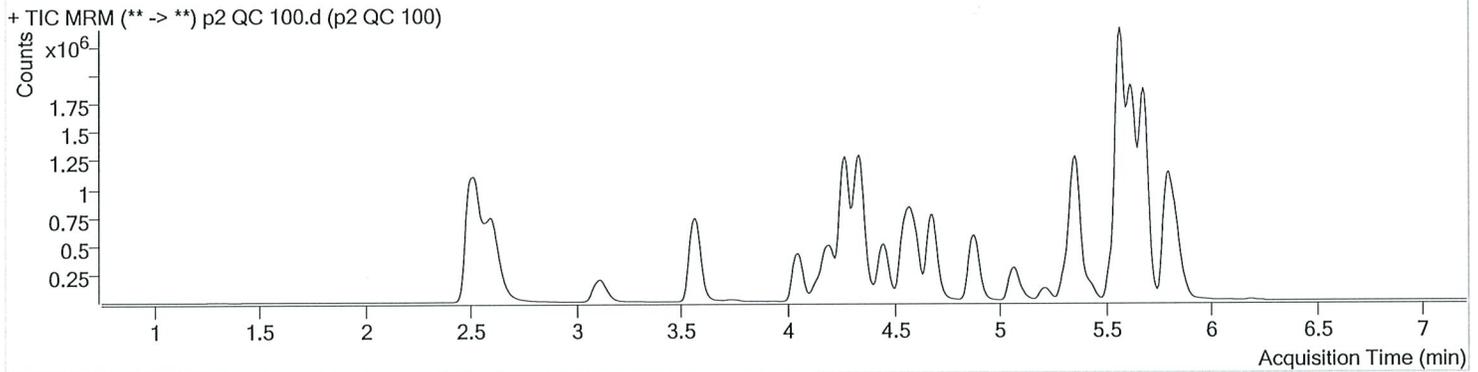


5/2

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
 Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 QC 100.d
Type	QC	Sample	p2 QC 100
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-B11	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 1:23:04 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	492433	6096.47	81.0	389.39	444209	105.6082 ng/ml
Etizolam	5.797	507452	4124.97	26.4	∞	711855	87.3720 ng/ml
Methocarbamol	4.357	321214	3698.39	77.0	8131.00	1532801	106.3172 ng/ml
Paroxetine	5.552	67524	∞	52.4	50093.01	128523	99.0127 ng/ml

AM #28 Multi-Drug Quant. Results

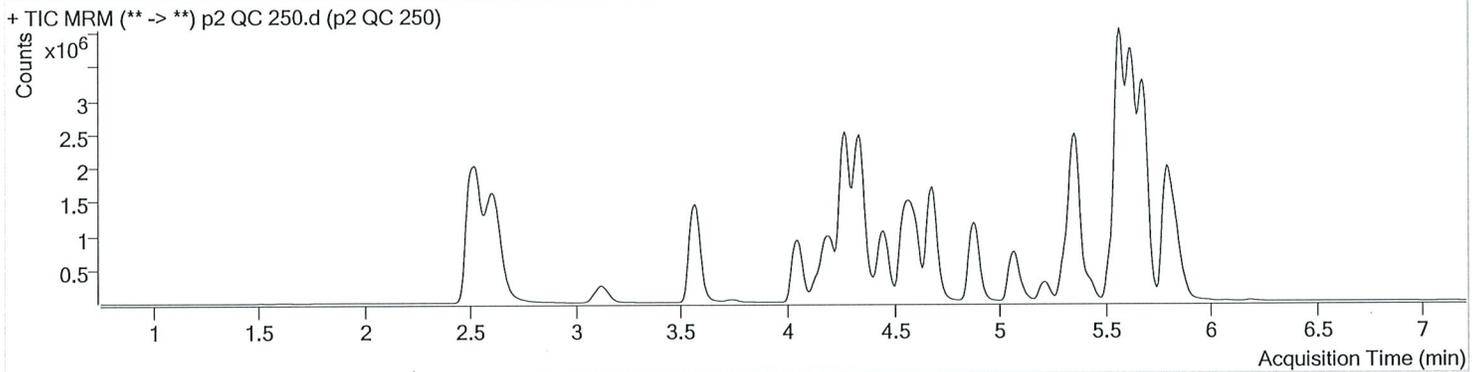


5/2

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 QC 250.d
Type	QC	Sample	p2 QC 250
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-C11	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 1:44:05 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	944487	2155.76	80.0	23859.90	349382	260.1340 ng/ml
Etizolam	5.797	993808	5378.86	26.9	∞	530675	228.5847 ng/ml
Methocarbamol	4.364	647801	12732.38	78.2	∞	1381853	239.5952 ng/ml
Paroxetine	5.552	173883	8831.83	50.1	340647.61	137926	237.4627 ng/ml

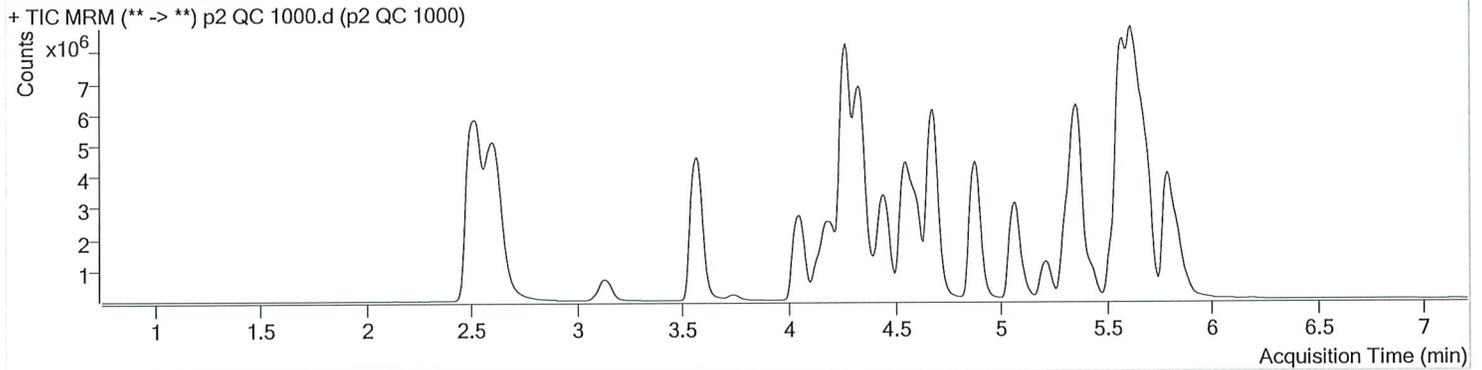


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
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Instrument	Falco	Data File	p2 QC 1000.d
Type	QC	Sample	p2 QC 1000
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-D11	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 2:05:06 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.840	1482013	24785.93	97.5 High	1906.39	174306	822.0457 ng/ml *
Etizolam	5.797	2070888	2322.29	26.2	3985.01	196816	1281.6177 ng/ml *
Methocarbamol	4.370	1336336	10941.55	80.1	31816.92	1148680	596.6953 ng/ml *
Paroxetine	5.552	545645	∞	51.1	∞	123043	835.0615 ng/ml

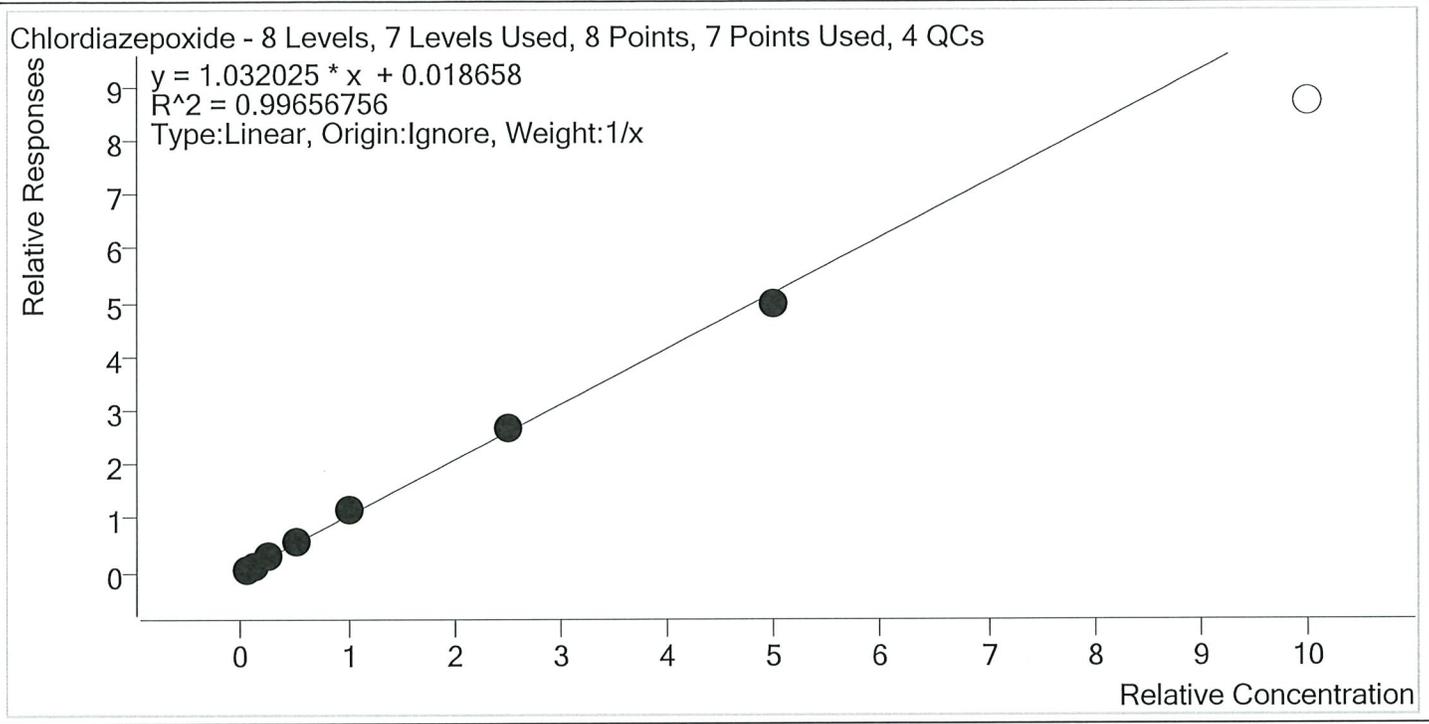
* Outside curve range

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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Last Cal. Update 8/20/2020 8:26 AM
Analyst Name ISP\datastor
Analyte Chlordiazepoxide **Internal Standard** Chlordiazepoxide-D5



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	3.8	75.8
p2 Cal 2- 10ng	2	✓	10.0	10.1	101.5
p2 Cal 3 -25ng	3	✓	25.0	26.4	105.4
p2 Cal 4-50ng	4	✓	50.0	54.6	109.2
p2 Cal 5-100ng	5	✓	100.0	109.9	109.9
p2 Cal 6-250ng	6	✓	250.0	256.1	102.5
p2 Cal 7-500ng	7	✓	500.0	479.1	95.8
p2 Cal 8-1000ng	8	×	1000.0	842.4	84.2

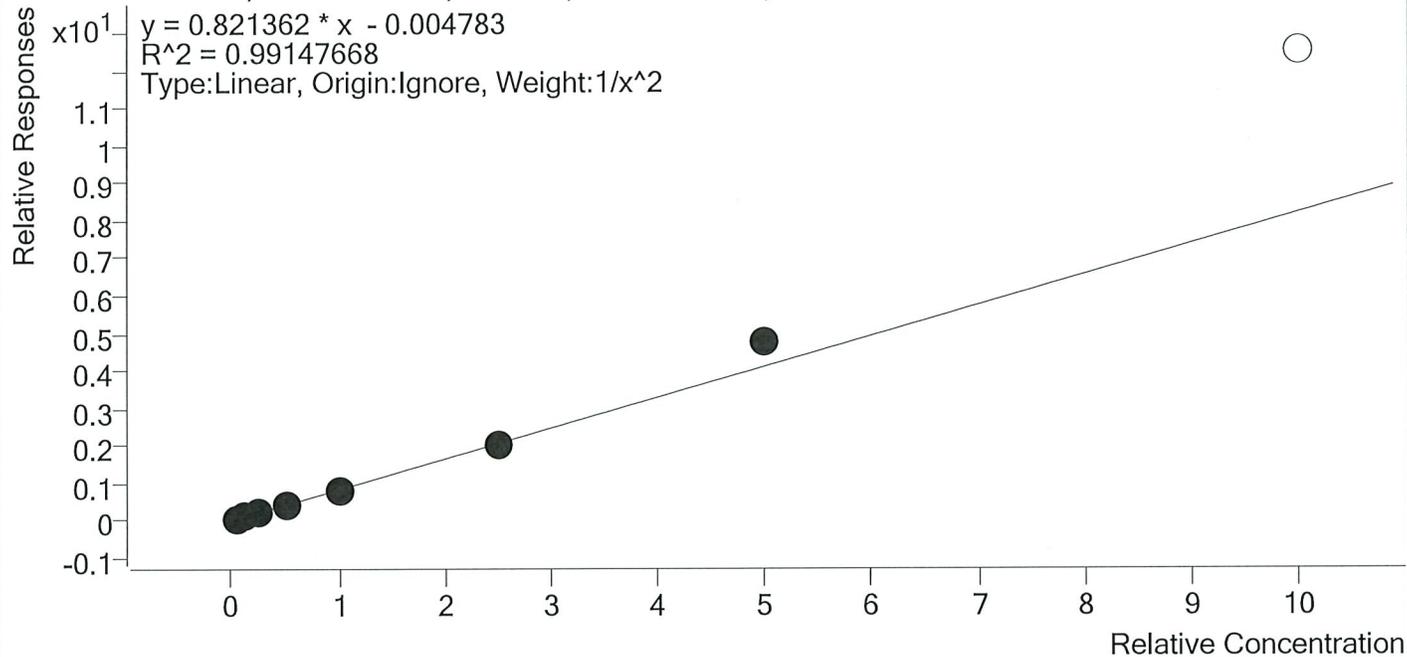
29



AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
 Last Cal. Update 8/20/2020 8:26 AM
 Analyst Name ISP\datastor
 Analyte Etizolam Internal Standard Estazolam-D5

Etizolam - 8 Levels, 7 Levels Used, 8 Points, 7 Points Used, 4 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	5.0	100.3
p2 Cal 2- 10ng	2	✓	10.0	10.4	103.9
p2 Cal 3 -25ng	3	✓	25.0	23.1	92.5
p2 Cal 4-50ng	4	✓	50.0	46.9	93.8
p2 Cal 5-100ng	5	✓	100.0	95.3	95.3
p2 Cal 6-250ng	6	✓	250.0	247.0	98.8
p2 Cal 7-500ng	7	✓	500.0	576.9	115.4
p2 Cal 8-1000ng	8	×	1000.0	1526.7	152.7

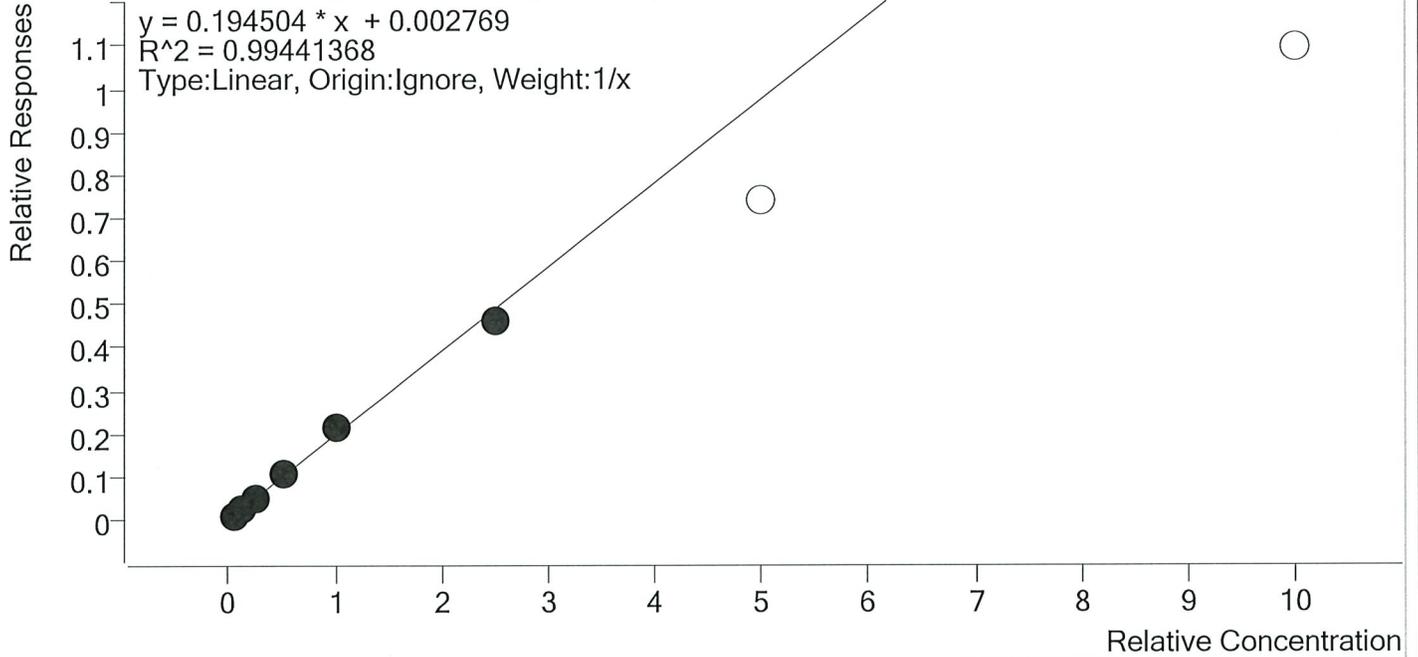
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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
 Last Cal. Update 8/20/2020 8:26 AM
 Analyst Name ISP\datastor
 Analyte Methocarbamol Internal Standard Pseudoephedrine-D3

Methocarbamol - 8 Levels, 6 Levels Used, 8 Points, 6 Points Used, 4 QCs



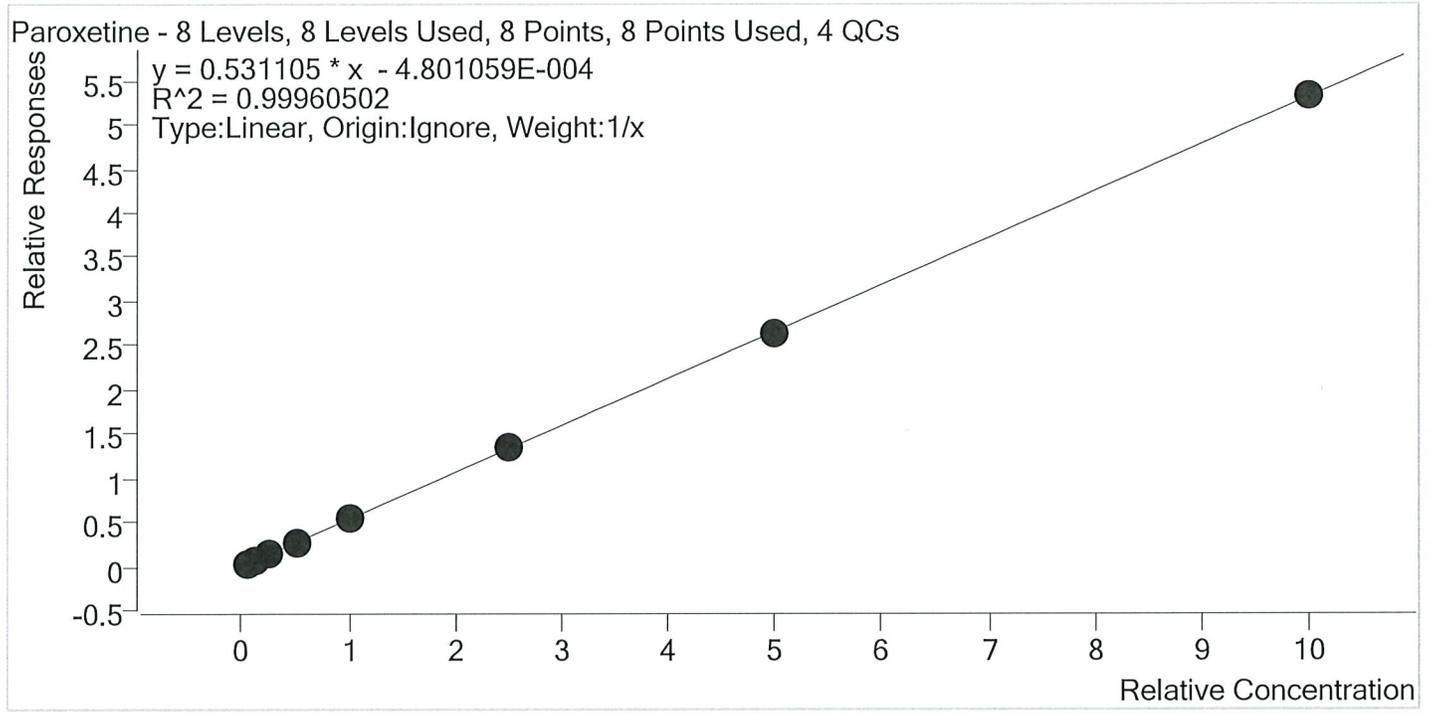
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	4.0	79.6
p2 Cal 2- 10ng	2	✓	10.0	10.6	106.3
p2 Cal 3 -25ng	3	✓	25.0	25.8	103.2
p2 Cal 4-50ng	4	✓	50.0	53.8	107.5
p2 Cal 5-100ng	5	✓	100.0	108.5	108.5
p2 Cal 6-250ng	6	✓	250.0	237.3	94.9
p2 Cal 7-500ng	7	×	500.0	378.5	75.7
p2 Cal 8-1000ng	8	×	1000.0	560.8	56.1

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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
 Last Cal. Update 8/20/2020 8:26 AM
 Analyst Name ISP\datastor
 Analyte Paroxetine Internal Standard Doxepin-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	5.2	104.2
p2 Cal 2- 10ng	2	✓	10.0	9.1	90.6
p2 Cal 3 -25ng	3	✓	25.0	26.3	105.3
p2 Cal 4-50ng	4	✓	50.0	47.5	95.0
p2 Cal 5-100ng	5	✓	100.0	105.8	105.8
p2 Cal 6-250ng	6	✓	250.0	250.0	100.0
p2 Cal 7-500ng	7	✓	500.0	494.4	98.9
p2 Cal 8-1000ng	8	✓	1000.0	1001.7	100.2

AM #28 Multi-Drug Quant. Results

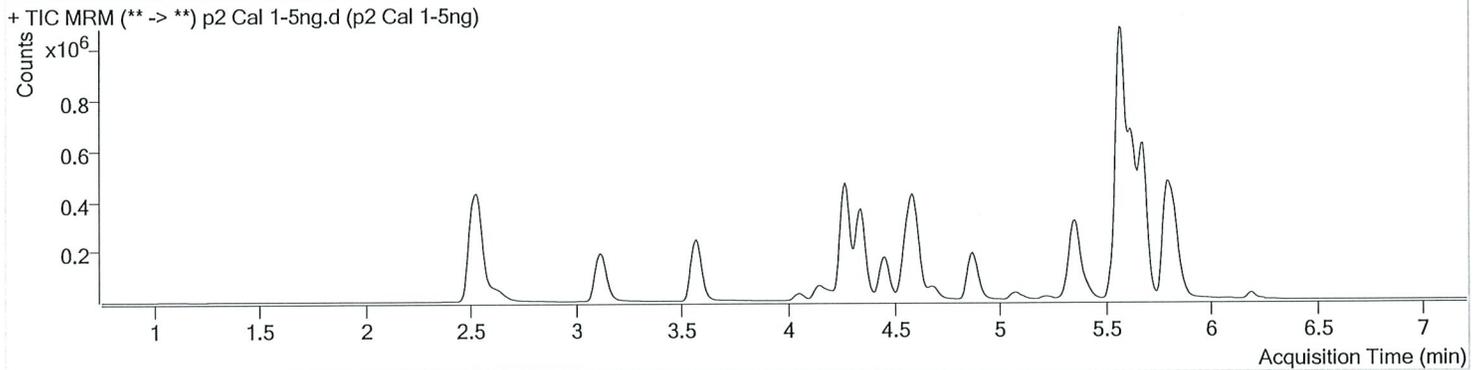


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Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
 Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Cal 1-5ng.d
Type	Cal	Sample	p2 Cal 1-5ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-A10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 11:16:40 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	32365	337.88	77.4	122.33	560257	3.7896 ng/ml
Etizolam	5.797	33065	∞	25.7	11369.89	908405	5.0138 ng/ml
Methocarbamol	4.364	18351	∞	77.2	∞	1746717	3.9781 ng/ml
Paroxetine	5.552	8485	1109.20	55.6	∞	312071	5.2096 ng/ml

AM #28 Multi-Drug Quant. Results

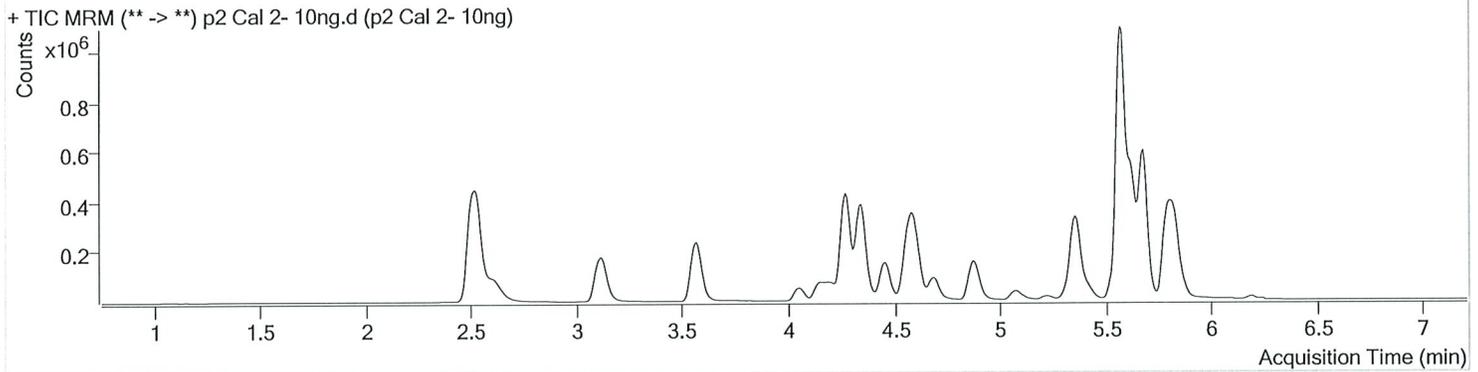


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Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Cal 2- 10ng.d
Type	Cal	Sample	p2 Cal 2- 10ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-B10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 11:27:20 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	64191	2487.05	75.2	232.99	520252	10.1476 ng/ml
Etizolam	5.797	70081	45708.12	24.8	20247.56	870187	10.3875 ng/ml
Methocarbamol	4.364	37770	∞	78.7	∞	1611628	10.6258 ng/ml
Paroxetine	5.552	7483	∞	59.6	15677.06	157044	9.0621 ng/ml

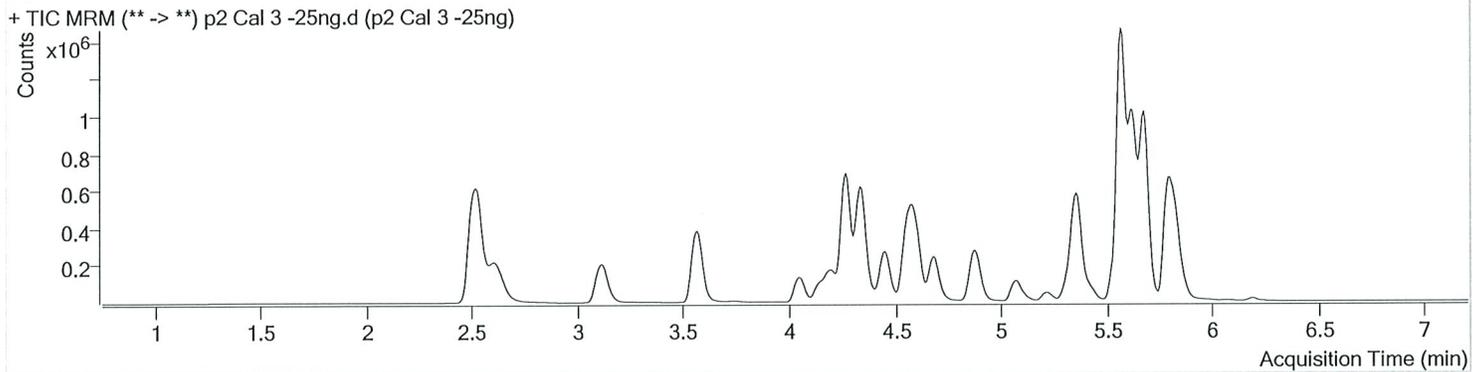
AM #28 Multi-Drug Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Cal 3 -25ng.d
Type	Cal	Sample	p2 Cal 3 -25ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-C10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 11:37:50 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	163259	4150.81	78.7	505.31	561779	26.3514 ng/ml
Etizolam	5.797	170462	∞	26.0	∞	920272	23.1338 ng/ml
Methocarbamol	4.364	93433	1764.15	80.5	712.24	1764414	25.8017 ng/ml
Paroxetine	5.552	30934	3194.13	49.9	∞	221999	26.3266 ng/ml

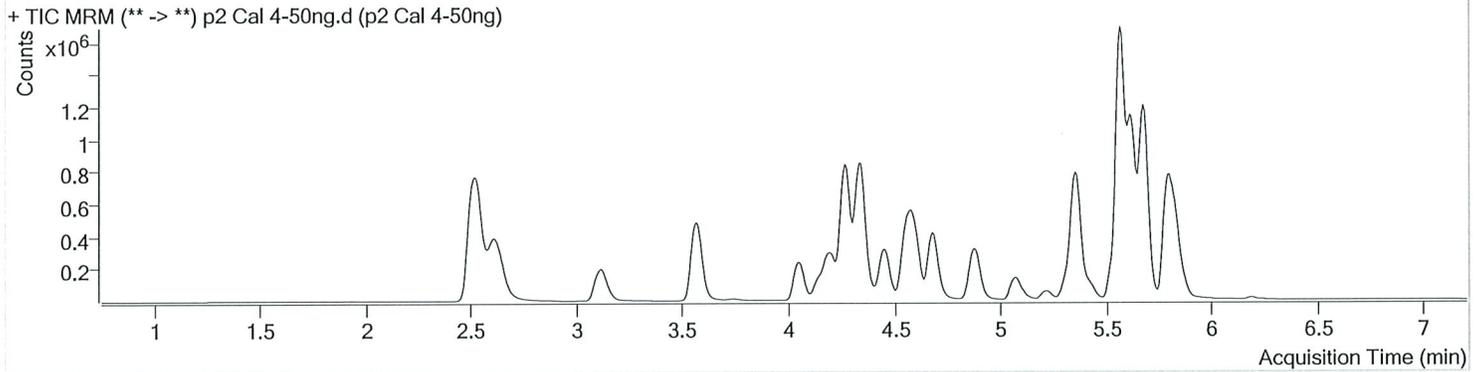


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Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Cal 4-50ng.d
Type	Cal	Sample	p2 Cal 4-50ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-D10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 11:48:19 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	289373	∞	78.3	866.01	497231	54.5830 ng/ml
Etizolam	5.797	306544	∞	26.0	51021.20	805436	46.9192 ng/ml
Methocarbamol	4.364	173711	10860.80	78.4	∞	1618526	53.7561 ng/ml
Paroxetine	5.552	30920	649.95	52.4	18944.70	122795	47.5008 ng/ml

P 55

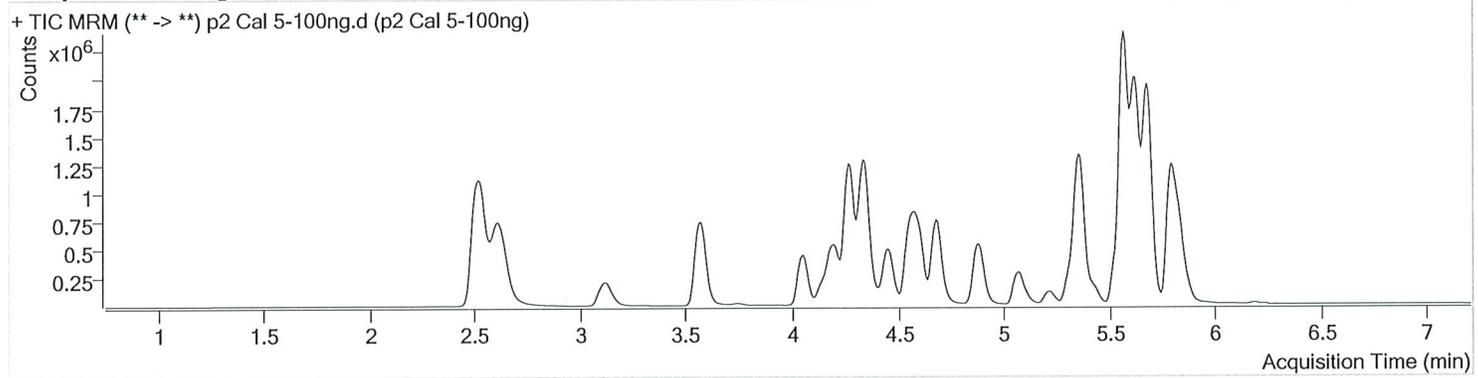


AM #28 Multi-Drug Quant. Results

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Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Cal 5-100ng.d
Type	Cal	Sample	p2 Cal 5-100ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-E10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 11:58:49 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	526344	2911.35	78.2	2348.46	456616	109.8857 ng/ml
Etizolam	5.797	563422	2681.12	25.7	6215.59	724197	95.3025 ng/ml
Methocarbamol	4.364	324212	4217.97	78.0	3269.84	1515806	108.5419 ng/ml
Paroxetine	5.552	74769	122224.00	50.7	41605.23	133124	105.8421 ng/ml

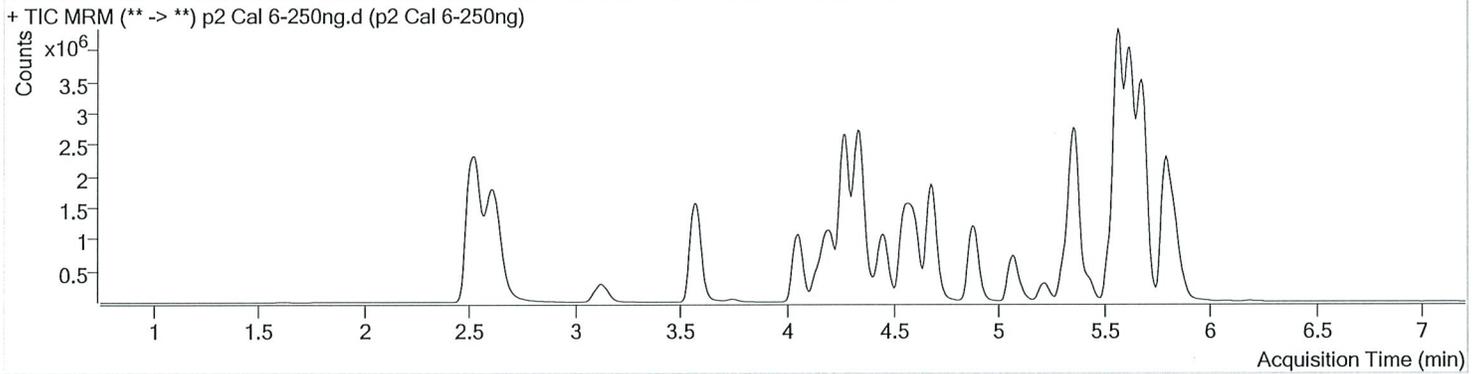


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Cal 6-250ng.d
Type	Cal	Sample	p2 Cal 6-250ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-F10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 12:09:19 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	1053086	5806.59	82.8	2221.96	395598	256.1324 ng/ml
Etizolam	5.797	1181884	36561.46	25.9	2931.76	583938	247.0010 ng/ml
Methocarbamol	4.364	704577	53503.41	79.8	∞	1517437	237.2964 ng/ml
Paroxetine	5.552	175688	1565.53	51.8	∞	132377	249.9792 ng/ml

AM #28 Multi-Drug Quant. Results

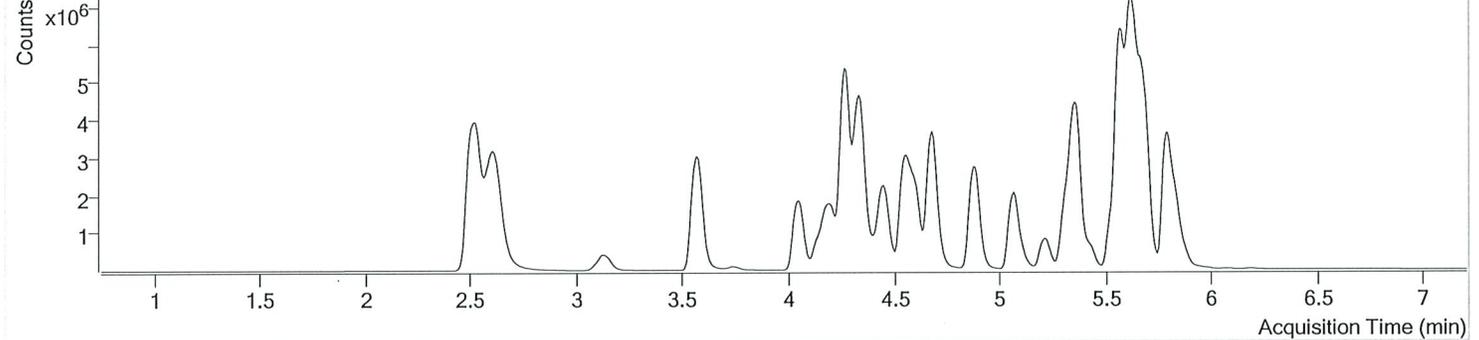


Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
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Instrument	Falco	Data File	p2 Cal 7-500ng.d
Type	Cal	Sample	p2 Cal 7-500ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-G10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 12:19:52 PM		

Sample Chromatogram

+ TIC MRM (** -> **) p2 Cal 7-500ng.d (p2 Cal 7-500ng)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.834	1394207	4123.90	86.3	6932.12	280909	479.1104 ng/ml
Etizolam	5.797	1593343	12098.39	27.0	4082.76	336623	576.8594 ng/ml
Methocarbamol	4.364	1035616	36384.14	80.5	4361.21	1401614	378.4517 ng/ml
Paroxetine	5.552	496977	2923.35	50.3	∞	189311	494.3778 ng/ml

DS

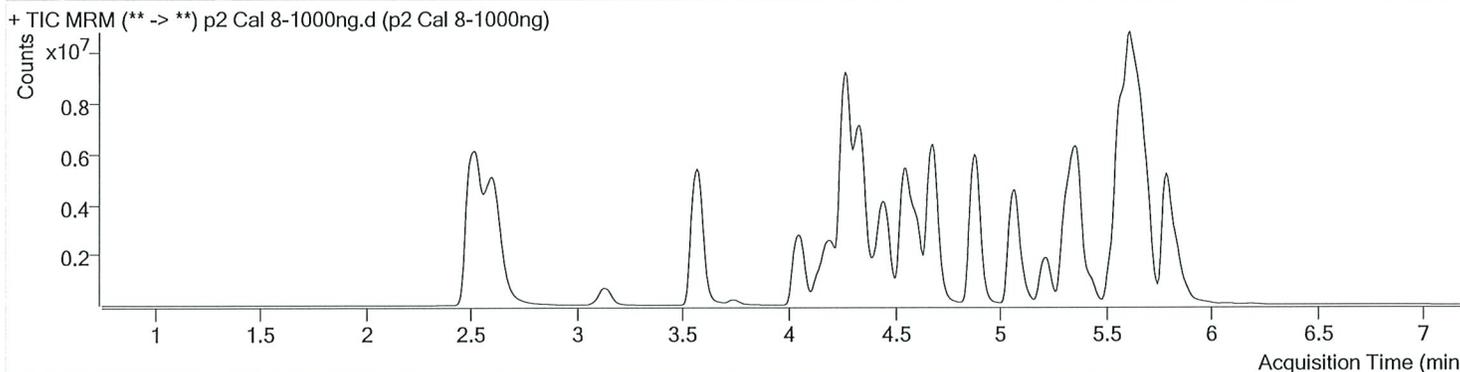


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\081820 AM 28 P1 P2 SJ SP\QuantResults\AM 28 P2 casework.batch.bin
Calibration Last Update 8/20/2020 8:26:44 AM

Instrument	Falco	Data File	p2 Cal 8-1000ng.d
Type	Cal	Sample	p2 Cal 8-1000ng
Acq. Method	MDQ P2 061720.m	Operator	Sarah Pickle
Sample Position	P2-H10	Comment	
Injection Volume	2		
Acq. Date-Time	8/18/2020 12:30:21 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlordiazepoxide	5.841	1426642	2629.81	95.7 High	15459.54	163742	842.4283 ng/ml
Etizolam	5.797	1810182	3573.65	27.2	∞	144415	1526.6544 ng/ml
Methocarbamol	4.370	1252748	132526.05	80.5	∞	1145577	560.8015 ng/ml
Paroxetine	5.552	1053111	∞	50.4	∞	197968	1001.7018 ng/ml