

REVIEWED

By Celena Shrum at 9:38 am, May 16, 2024



5/10/2024

Worklist: 6808

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
C2024-0831	2	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ



AM# 28 P2: Multi-Drug Quantitation by LC-MS/MS

Extraction Date: 05/08/24

Analyst: Anne Nord

Plate lot#: 230710

Plate Retest Date: 01/10/2024

Mobile phase A: 5mM Amm Form + 0.01% FA

Mobile phase B: 0.01% Formic Acid in MeOH

Blank Blood Lot: 24C52042

Blank Urine Lot: Blood only run

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

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: In blank well, add 250µL urine, 40µL BG Turbo, and 100µL Instant Buffer I. Place on plate shaker for 5 minutes.**
- 3. Using a calibrated pipette, pipette **250µL blood and urine** (if applicable) into wells of analytical (standards) plate. **Pipette ID: P31168J**
- 4. Pipette **250µL 0.5 M ammonium hydroxide** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **200-450µL of blood+base and urine+base (if applicable)** mixture to corresponding wells of SLE+ plate. Amount transferred: 300
- 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 **900uL ethyl acetate.**
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 12. Add **900uL ethyl acetate.**
- 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. If run contains urine or at the analyst's discretion, add 50µL 1% HCl in MeOH to wells and place plate cover on plate before drying (optional).
- 16. Reconstitute in **100µL 20% LC MeOH** 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? Yes, see comments Add Control data to QC tracking spreadsheet.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS: *Compounds evaluated*

Phenytoin

Doxepin

The external blood control was not checked on the worklist and was not run on 5/8/24, it was injected 5/9/24.

External control run to re-test plate.

External



5/16/24



**Idaho State Police
Forensic Services**

AM #28 P2 Confirmation Multidrug Control Prep Sheet

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>	<i>Expiration Date</i>
Phenytoin 1mg/ml	Cerilliant	FN04121905	06/30/24

Blood External Control Solution
2.5 ul of standard solution to 4997 ul of Blood.
Approximately 500 ng/ml of Phenytoin

<i>Negative blood source and lot number</i>	<i>Date prepared</i>	<i>Expiration</i>	<i>Lot number</i>	<i>Prepared by</i>	<i>Out of use</i>
Lampire 23J52629	02/01/24	06/30/24	B2124	Anne Nord	

	row 5 SLE and injection plate	row 9 SLE and injection plate										
	1 mixing plate	2 mixing plate	3	4	5	6	7	8	9	10	11	12
A	IS + Cal. 1	IS + QC_1										
B	IS + Cal. 2	IS + QC_2										
C	IS + Cal. 3	IS + QC 3										
D	IS + Cal. 4	IS + QC_4										
E	IS + Cal. 5	External control blood										
F	IS + Cal. 6	negative blood										
G	IS + Cal. 7	0831-2										
H	IS + Cal. 8											

blank in front

plate position 2

c2024-____-__

AM #28 Multi-Drug Quant. Results

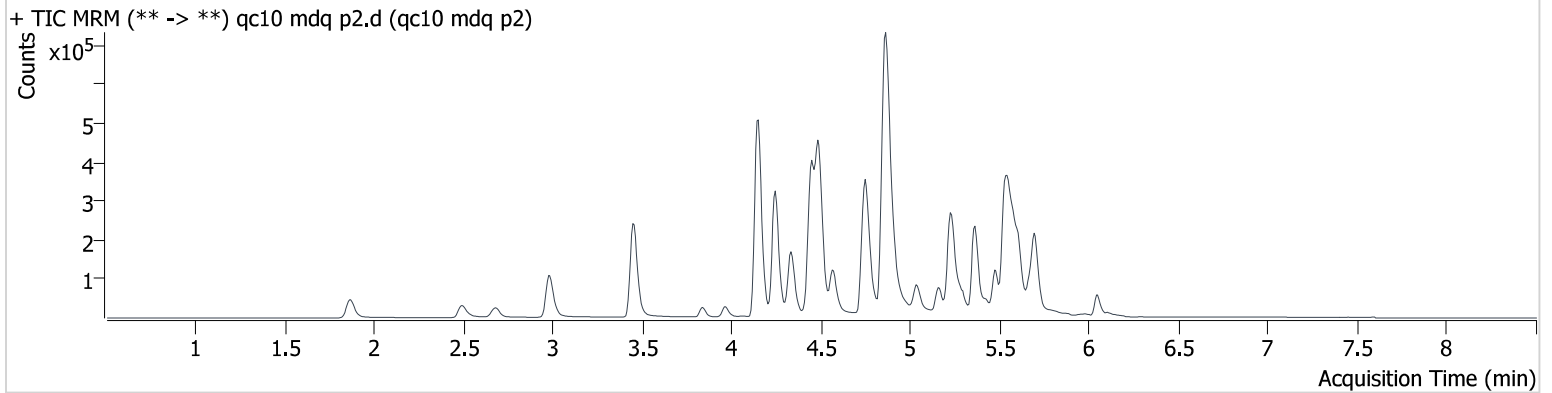
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-A9
Injection Volume 5
Acq. Date-Time 5/8/2024 10:10:04 PM
Sample Info.

Data File qc10 mdq p2.d
Sample qc10 mdq p2
Operator Anne Nord

Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.224	48043	2612.2	55.31	266.1	141729	10.649 ng/ml
Phenytoin	5.495	1099	118.9	78.28	120.9	5518	10.727 ng/ml

AM #28 Multi-Drug Quant. Results

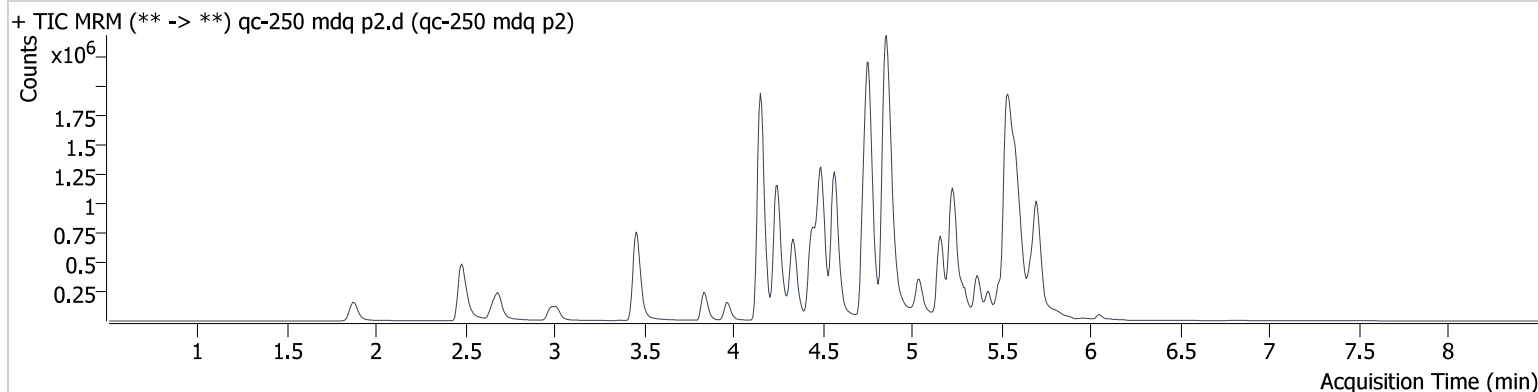
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-C9
Injection Volume 5
Acq. Date-Time 5/8/2024 10:19:18 PM
Sample Info.

Data File qc-250 mdq p2.d
Sample qc-250 mdq p2
Operator Anne Nord

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.224	596777	33705.5	54.95	1831.0	75177	251.988 ng/ml
Phenytoin	5.495	20115	2281.9	82.86	1177.2	4073	256.357 ng/ml

AM #28 Multi-Drug Quant. Results

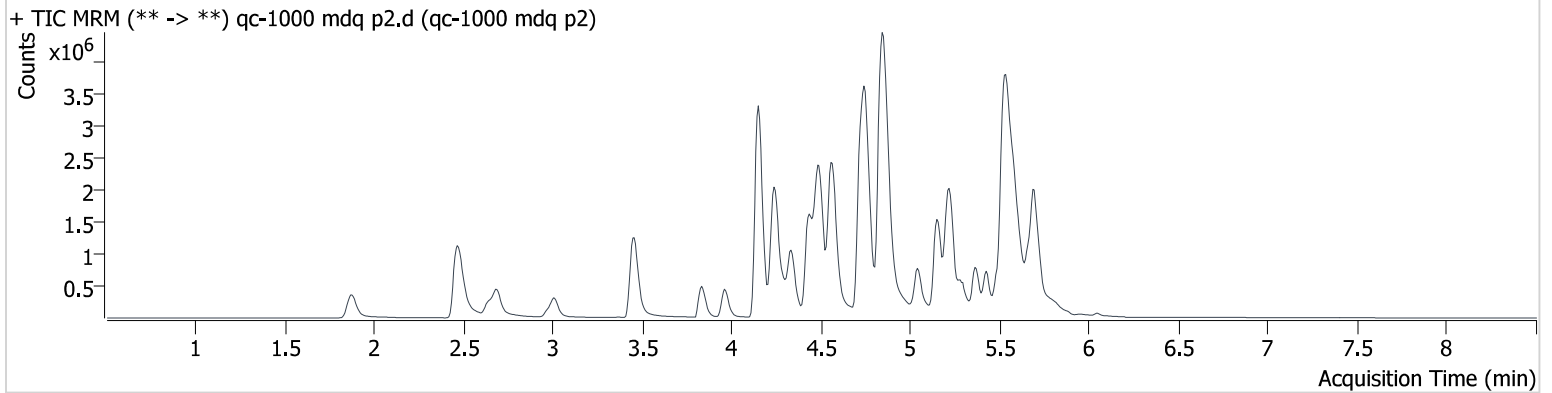
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-D9
Injection Volume 5
Acq. Date-Time 5/8/2024 10:28:32 PM
Sample Info.

Data File qc-1000 mdq p2.d
Sample qc-1000 mdq p2
Operator Anne Nord

Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	1201839	46510.5	54.87	20030.8	40649	938.849 ng/ml
Phenytoin	5.495	68778	2330.0	79.04	5167.6	4087	872.552 ng/ml

AM #28 Multi-Drug Quant. Results

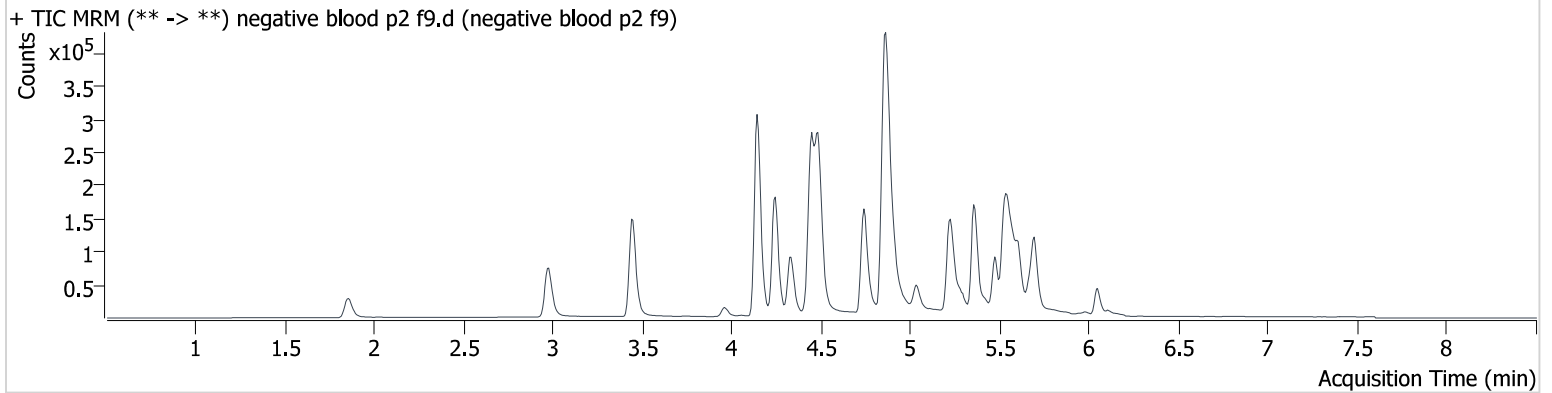
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Sample
Acq. Method mdqp2 2023.m
Sample Position P2-F9
Injection Volume 5
Acq. Date-Time 5/8/2024 10:46:59 PM
Sample Info.

Data File negative blood p2 f9.d
Sample negative blood p2 f9
Operator Anne Nord

Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



AM #28 Multi-Drug Quant. Results

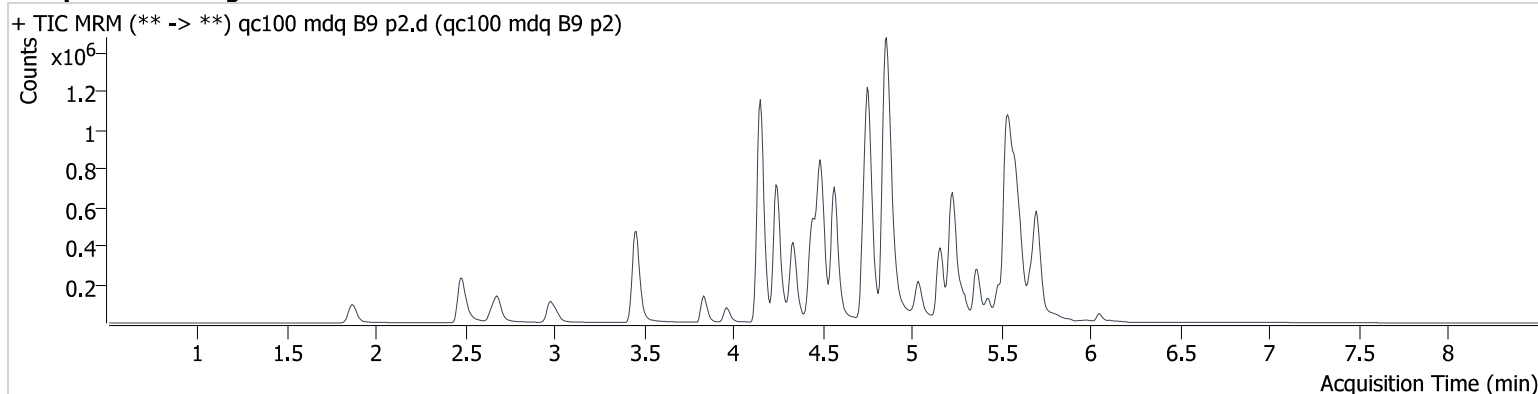
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Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Sample
Acq. Method mdqp2 2023.m
Sample Position P2-B9
Injection Volume 5
Acq. Date-Time 5/8/2024 11:05:26 PM
Sample Info.

Data File qc100 mdq B9 p2.d
Sample qc100 mdq B9 p2
Operator Anne Nord

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	316571	7115.1	56.70	2931.0	102295	98.165 ng/ml
Phenytoin	5.488	8892	1087.0	81.56	857.4	5011	92.376 ng/ml

AM #28 Multi-Drug Quant. Results

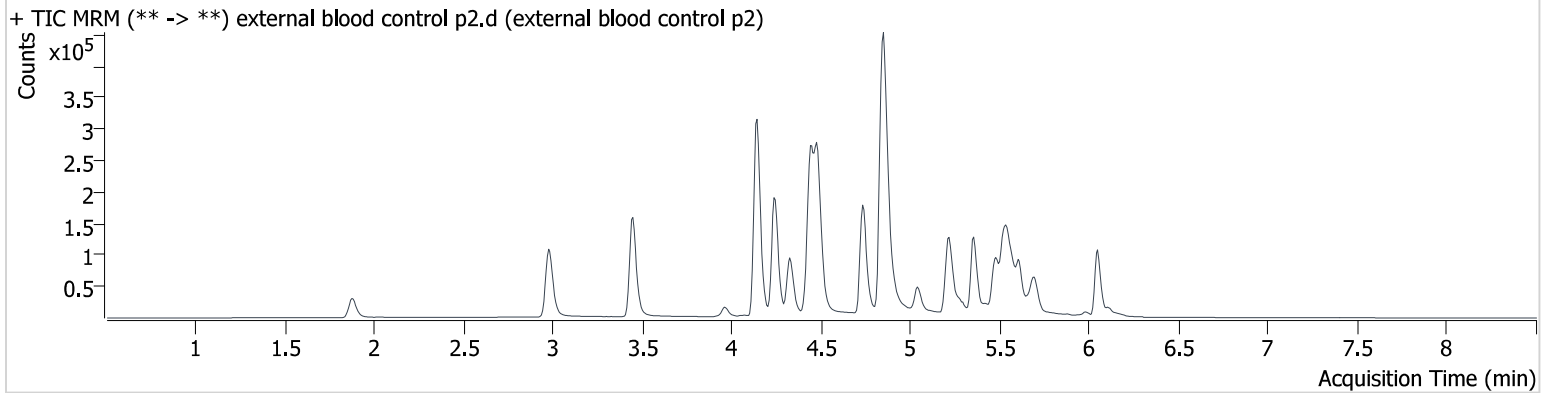
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Sample
Acq. Method mdqp2 2023.m
Sample Position P2-E9
Injection Volume 5
Acq. Date-Time 5/9/2024 1:29:32 PM
Sample Info.

Data File external blood control p2.d
Sample external blood control p2
Operator Anne Nord

Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Phenytoin	5.495	48873	4389.0	81.36	4813.7	4115	615.949 ng/ml

AM #28 Multi-Drug Quant. Results

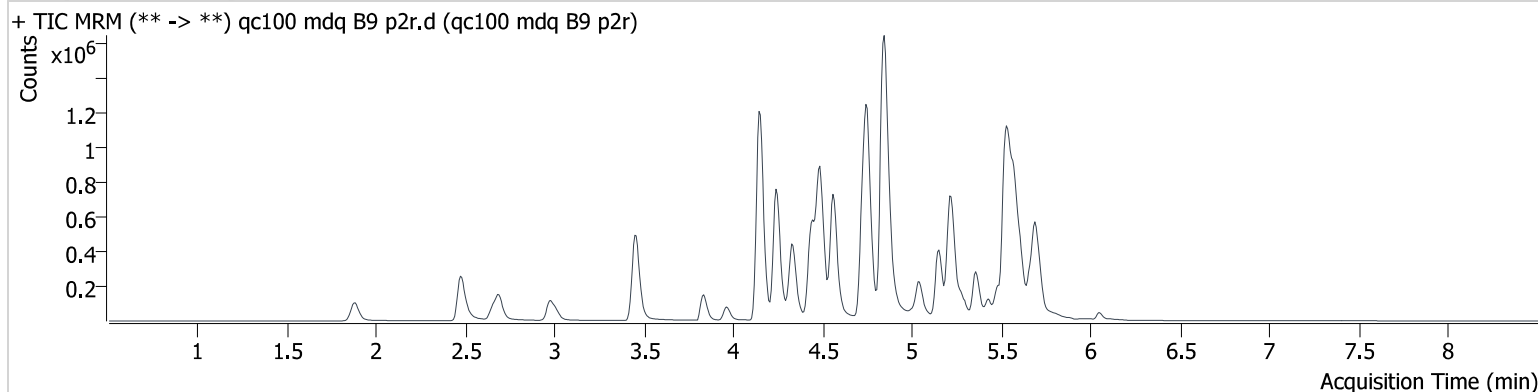
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Sample
Acq. Method mdqp2 2023.m
Sample Position P2-B9
Injection Volume 5
Acq. Date-Time 5/9/2024 1:38:54 PM
Sample Info.

Data File qc100 mdq B9 p2r.d
Sample qc100 mdq B9 p2r
Operator Anne Nord

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Sample Chromatogram

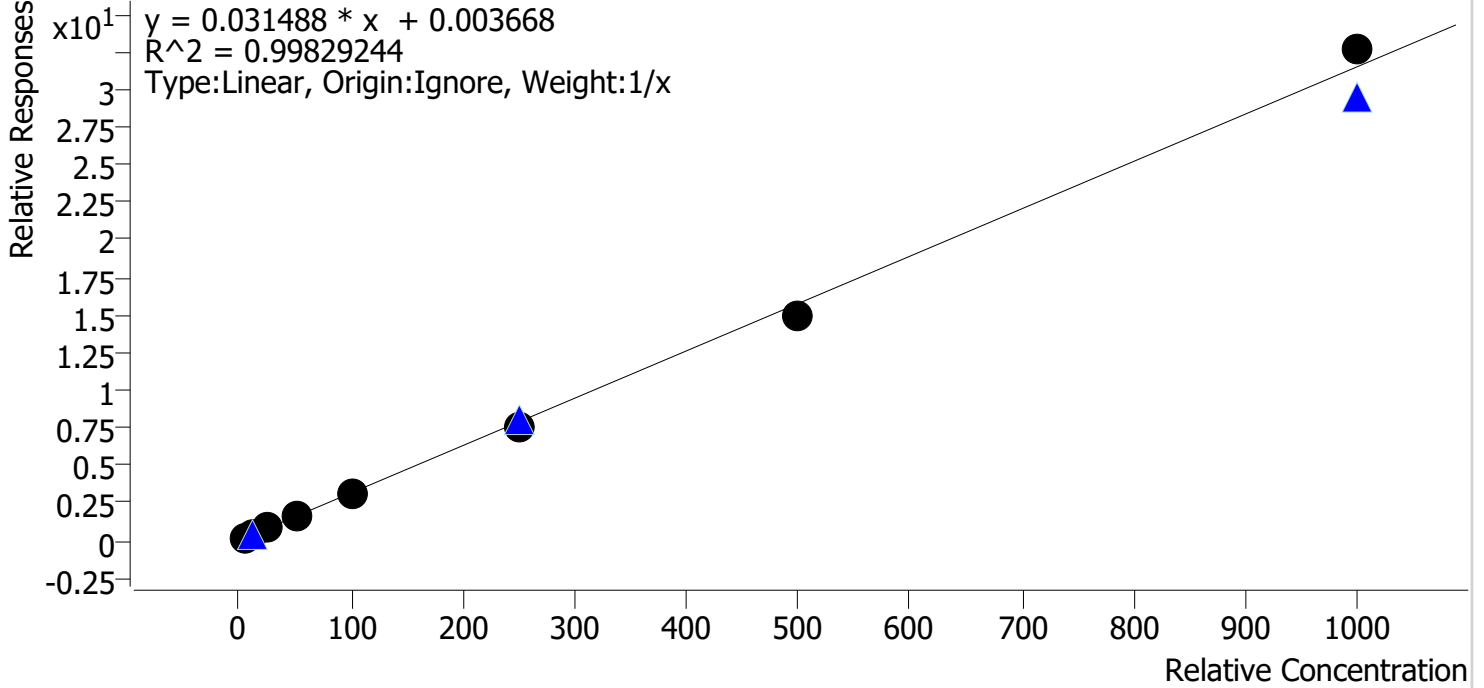


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	335870	9667.7	55.95	3608.9	107354	99.243 ng/ml
Phenytoin	5.495	9202	575.7	85.38	778.4	4981	96.151 ng/ml

Compound Calibration Report

Batch results D:\MassHunter\Data\2024\lam 27-28\050824r\QuantResults\lam 28 p2.batch.bin
Last Cal. Update 5/9/2024 4:03 PM
Analyst Name ISP\datastor
Analyte Doxepin **Internal Standard** Doxepin-D3

Doxepin - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 3 QCs

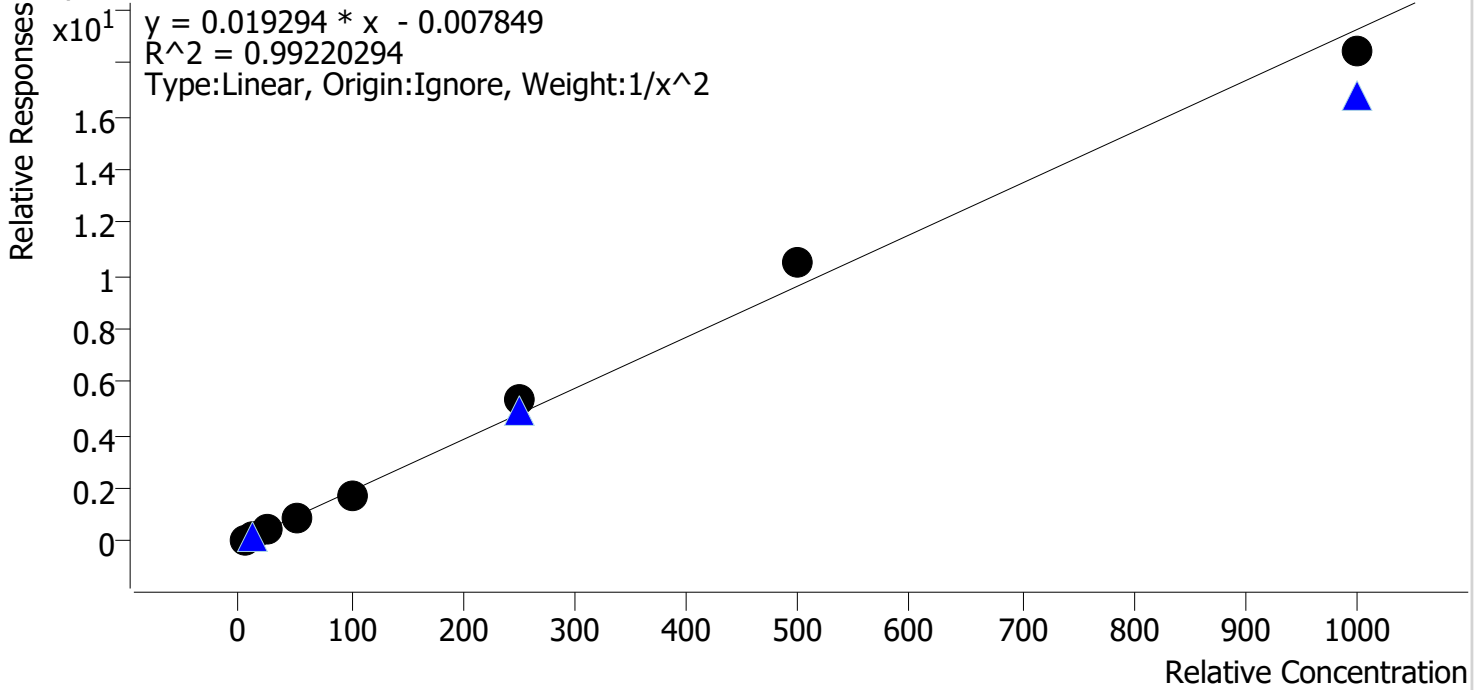


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 1 mdq p2	1	✓	5.0	5.3	106.2
cal 2 mdq p2	2	✓	10.0	10.3	103.3
cal 3 mdq p2	3	✓	25.0	24.6	98.5
cal 4 mdq p2	4	✓	50.0	48.9	97.7
cal 5 mdq p2	5	✓	100.0	99.5	99.5
cal 6 mdq p2	6	✓	250.0	240.7	96.3
cal 7 mdq p2	7	✓	500.0	474.5	94.9
cal 8 mdq p2	8	✓	1000.0	1036.2	103.6

Compound Calibration Report

Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Last Cal. Update 5/9/2024 4:03 PM
Analyst Name ISP\datastor
Analyte Phenytoin **Internal Standard** Phenytoin-D10

Phenytoin - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 3 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 1 mdq p2	1	✓	5.0	4.9	98.5
cal 2 mdq p2	2	✓	10.0	10.8	107.8
cal 3 mdq p2	3	✓	25.0	22.8	91.1
cal 4 mdq p2	4	✓	50.0	47.2	94.3
cal 5 mdq p2	5	✓	100.0	93.7	93.7
cal 6 mdq p2	6	✓	250.0	275.6	110.3
cal 7 mdq p2	7	✓	500.0	543.2	108.6
cal 8 mdq p2	8	✓	1000.0	956.8	95.7

AM #28 Multi-Drug Quant. Results

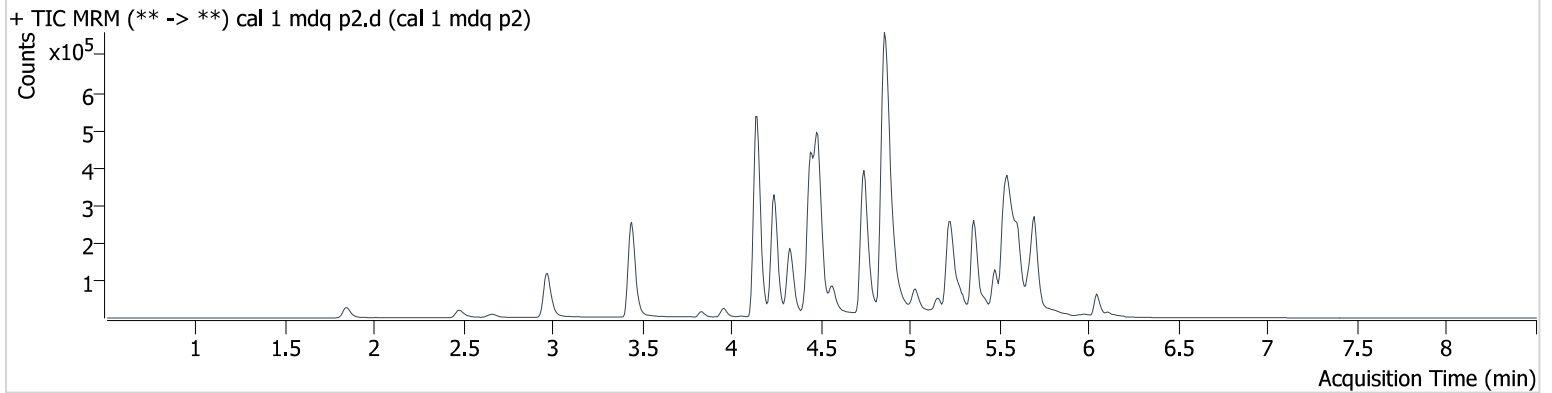
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-A5
Injection Volume 5
Acq. Date-Time 5/8/2024 8:38:00 PM
Sample Info.

Data File cal 1 mdq p2.d
Sample cal 1 mdq p2
Operator Anne Nord

Comment
Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	28724	223.4	54.48	560.1	168172	5.308 ng/ml
Phenytoin	5.488	652	74.8	65.54	43.6	7480	4.924 ng/ml

AM #28 Multi-Drug Quant. Results

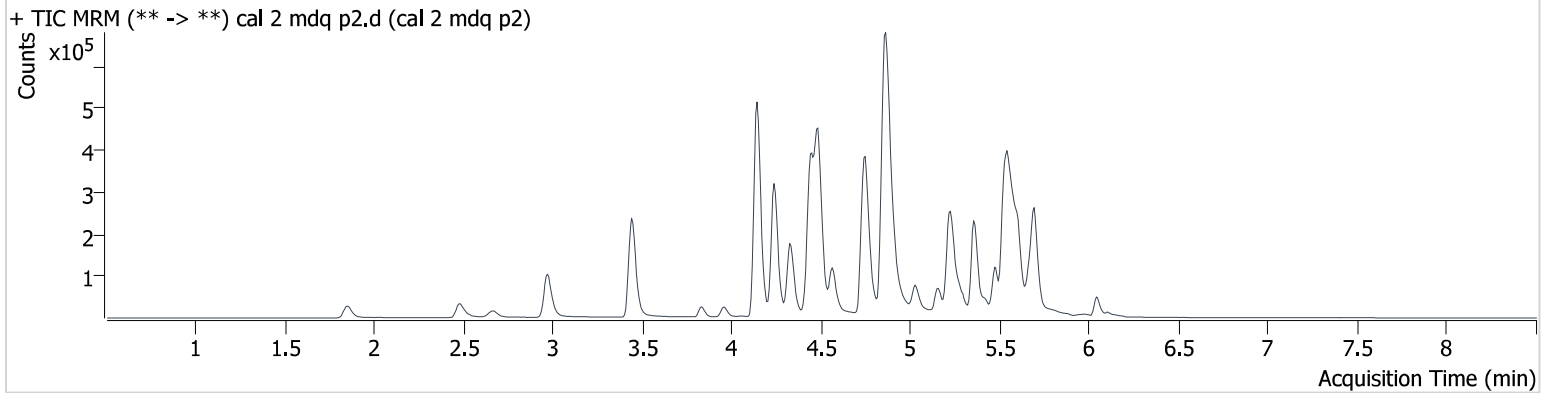
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-B5
Injection Volume 5
Acq. Date-Time 5/8/2024 8:47:11 PM
Sample Info.

Data File cal 2 mdq p2.d
Sample cal 2 mdq p2
Operator Anne Nord

Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	47140	1777.6	56.84	1192.7	143322	10.329 ng/ml
Phenytoin	5.488	1118	327.0	73.97	103.6	5585	10.785 ng/ml

AM #28 Multi-Drug Quant. Results

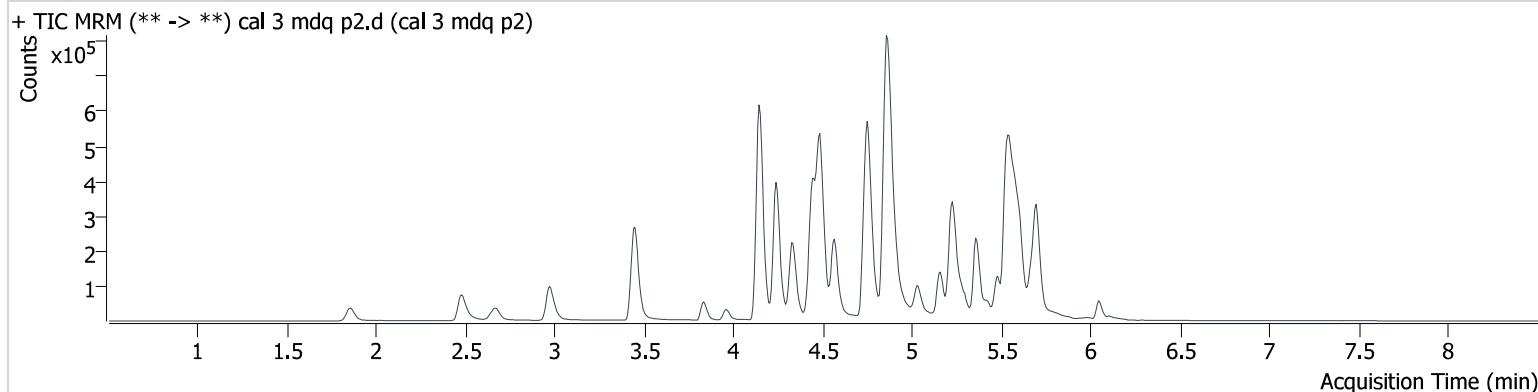
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-C5
Injection Volume 5
Acq. Date-Time 5/8/2024 8:56:25 PM
Sample Info.

Data File cal 3 mdq p2.d
Sample cal 3 mdq p2
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	103782	1935.2	57.57	315.7	133158	24.635 ng/ml
Phenytoin	5.488	2190	1371.5	92.18	137.4	5077	22.766 ng/ml

AM #28 Multi-Drug Quant. Results

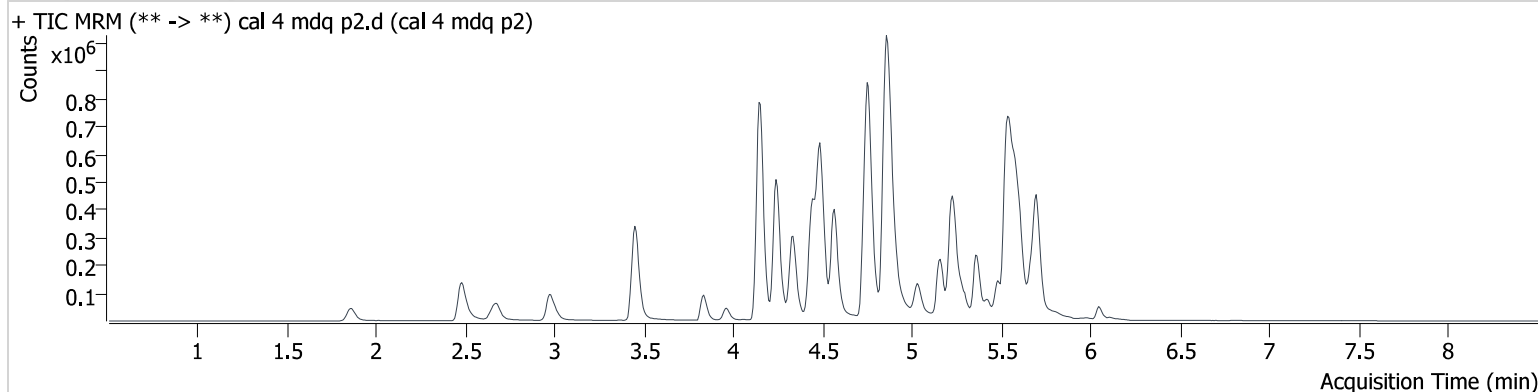
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-D5
Injection Volume 5
Acq. Date-Time 5/8/2024 9:05:37 PM
Sample Info.

Data File cal 4 mdq p2.d
Sample cal 4 mdq p2
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	181320	7625.6	57.82	740.0	117568	48.863 ng/ml
Phenytoin	5.488	4232	793.3	87.94	369.6	4692	47.155 ng/ml

AM #28 Multi-Drug Quant. Results

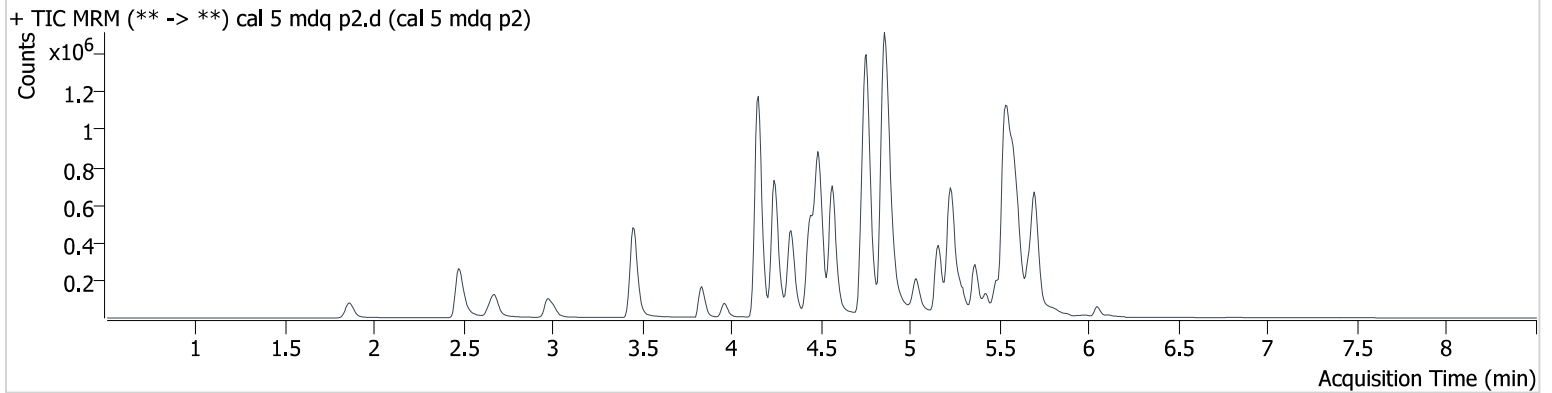
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-E5
Injection Volume 5
Acq. Date-Time 5/8/2024 9:14:49 PM
Sample Info.

Data File cal 5 mdq p2.d
Sample cal 5 mdq p2
Operator Anne Nord

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	327951	∞	55.50	1020.1	104550	99.502 ng/ml
Phenytoin	5.488	8725	2922.0	78.13	812.5	4846	93.715 ng/ml

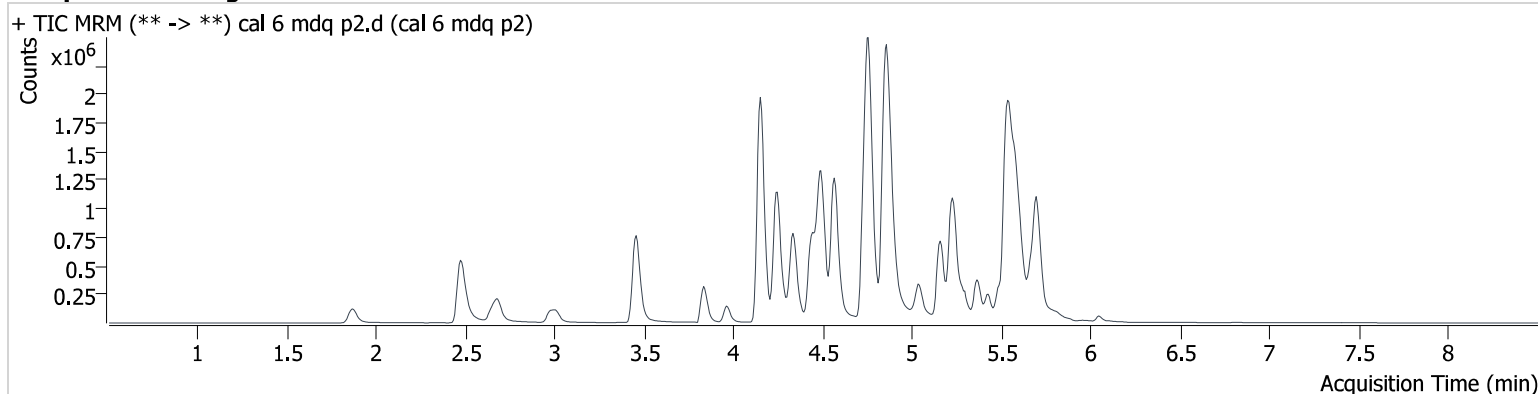
AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-F5
Injection Volume 5
Acq. Date-Time 5/8/2024 9:24:01 PM
Sample Info.

Data File cal 6 mdq p2.d
Sample cal 6 mdq p2
Operator Anne Nord
Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.224	601687	23103.2	55.17	6614.6	79358	240.671 ng/ml
Phenytoin	5.488	20775	5550.9	82.63	2266.8	3912	275.645 ng/ml

AM #28 Multi-Drug Quant. Results

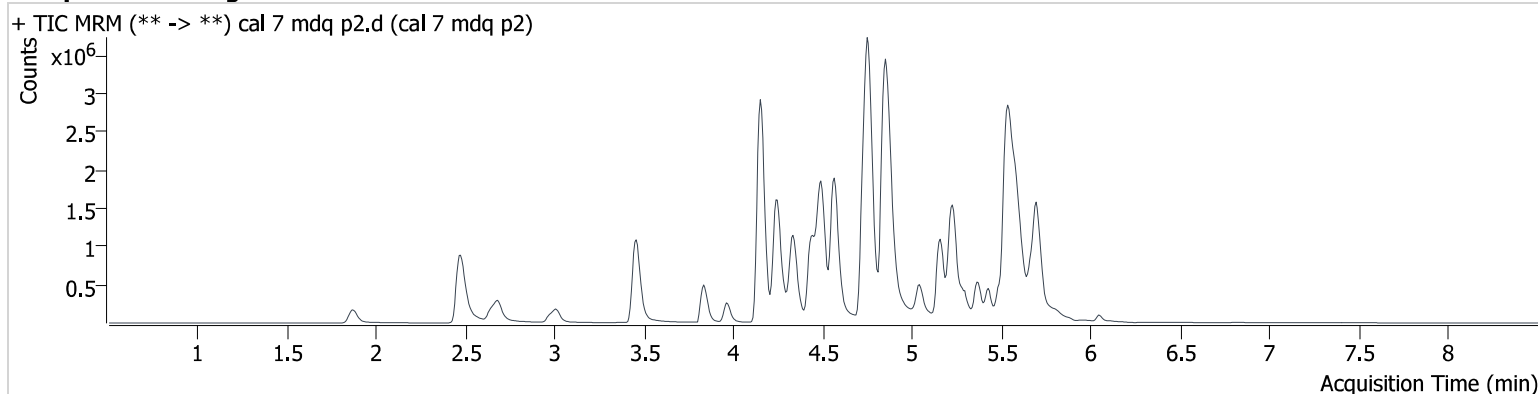
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-G5
Injection Volume 5
Acq. Date-Time 5/8/2024 9:33:12 PM
Sample Info.

Data File cal 7 mdq p2.d
Sample cal 7 mdq p2
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	897373	16423.9	55.62	10249.5	60050	474.466 ng/ml
Phenytoin	5.495	42392	4839.1	74.55	1080.6	4048	543.244 ng/ml

AM #28 Multi-Drug Quant. Results

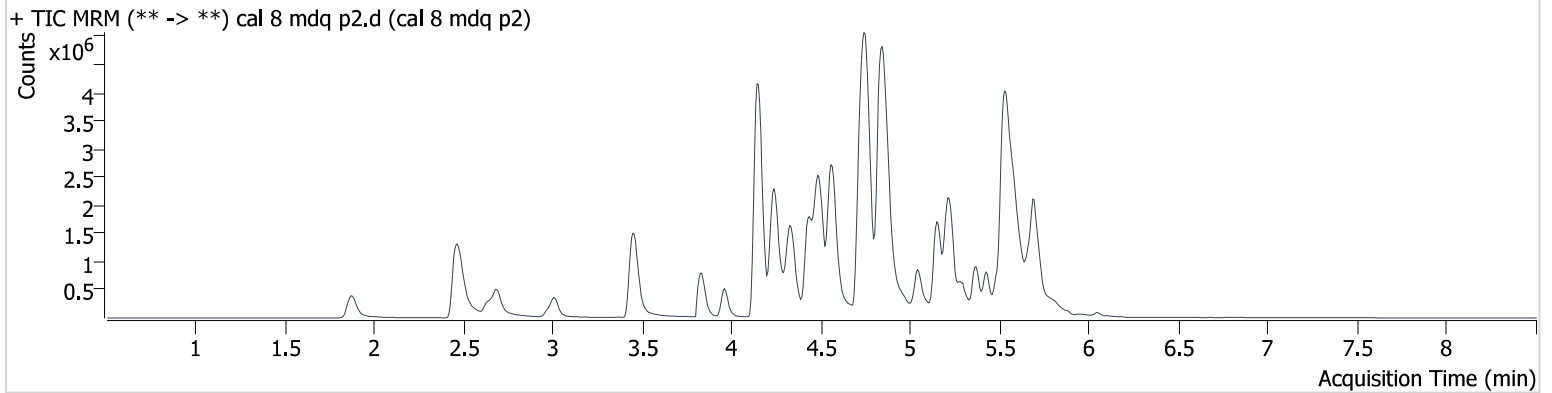
Batch results D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 28 p2.batch.bin
Calibration Last Update 5/9/2024 4:03:59 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-H5
Injection Volume 5
Acq. Date-Time 5/8/2024 9:51:38 PM
Sample Info.

Data File cal 8 mdq p2.d
Sample cal 8 mdq p2
Operator Anne Nord

Comment Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values 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



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Doxepin	5.217	1392448	395.5	50.45	476.4	42671	1036.227 ng/ml
Phenytoin	5.495	82508	969.3	80.97	3351.2	4471	956.804 ng/ml