



2/2/2024

Worklist: 6669

REVIEWED

By Brittany Wylie at 2:41 pm, Feb 02, 2024

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
C2023-2857	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ





AM# 28 Panel 2: Multi-Drug Quantitation by LC-MS/MS

Extraction Date 02/01/24 Analyst: Anne Nord
Plate lot#: 230710 Plate re-test: 01/10/2024

Mobile phase A: 5mM Amm Form + 0.01% FA **Mobile phase B:** 0.01% Formic Acid in MeOH
0.5M Ammonium Hydroxide Ethyl Acetate 20% Methanol in Water

Blank Blood Lot: 23J52629 **Urine Blank 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 pipette 250 ul urine in blank well, add 40 ul BG Turbo, add 100 ul 500 mm sodium phosphate buffer, mix for at least 5 minutes at ambient temperature. Pipette 250µL blood (calibrated pipette) or 250µL hydrolyzed urine in wells of analytical (standards) plate. **Pipette ID: P31168J**
- 3. Pipette 250µL 0.5M ammonium hydroxide in wells of analytical plate.
- 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 5. Transfer 300µL of blood+base/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 900uL ethyl acetate.
- 9. Wait 5 minutes.
- 10. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 11. Add 900uL ethyl acetate.
- 12. Wait 5 minutes.
- 13. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 14. Remove plate containing eluate. Add 50 ul 1% HCl in MeOH,
- 15. Place on SPE Dry and evaporate to dryness at approx. 35°C.
SPE Dry ID: 66819
- 16. Reconstitute in 100µL 20% MeOH and heat seal plate with foil.

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: External control run for retest.
Compounds evaluated methocarbamol and phenytoin.

Pressure maxed out on instrument while running Cal 5, the column was disconnected and the lines preceding the column were flushed, the run was then restarted at Cal 5.

Mixing plate column 4 and 5, SLE and injection column 4 moved to 1 and column 5 moved to 2.

	1	2	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	negative blood							
F				IS + Cal. 6	C2023-2857-1							
G				IS + Cal. 7	external control blood							
H				IS + Cal. 8								

blank in front

plate position 2

c2023-____-__



**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	

AM #28 Multi-Drug Quant. Results

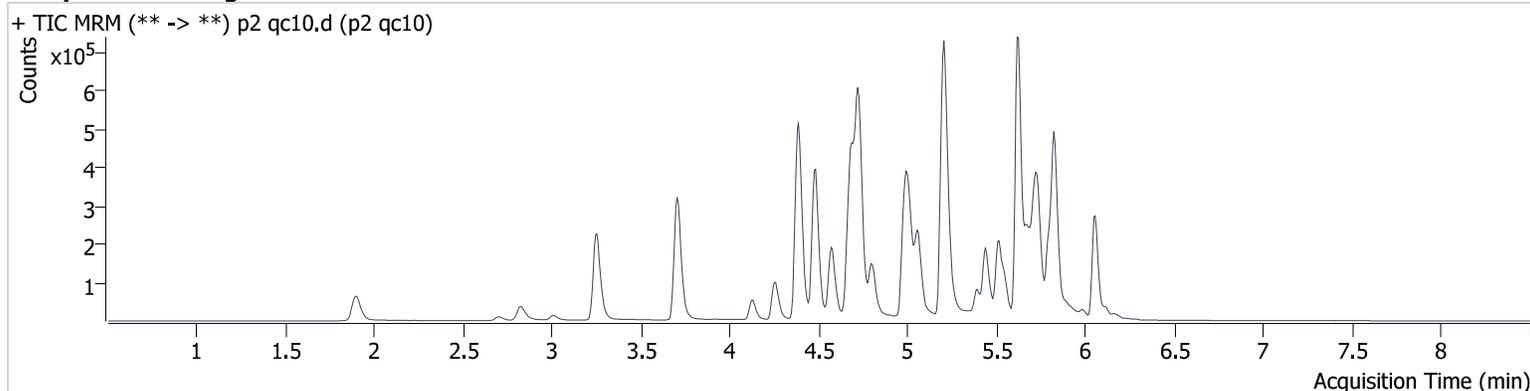
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-A2
Injection Volume 5
Acq. Date-Time 2/2/2024 9:09:01 AM
Sample Info.

Data File p2 qc10.d
Sample p2 qc10
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.
methocarbamol	4.482	8451	286.5	70.15	421.8	312073	10.121 ng/ml
Phenytoin	5.509	1541	1112.0	84.70	22.2	7721	10.423 ng/ml

AM #28 Multi-Drug Quant. Results

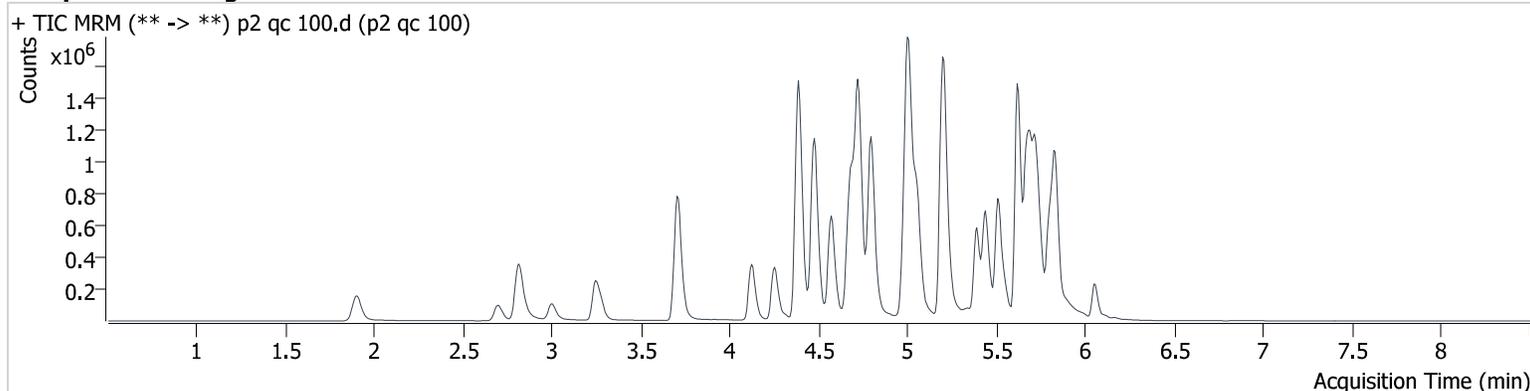
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-B2
Injection Volume 5
Acq. Date-Time 2/2/2024 10:06:36 AM
Sample Info.

Data File p2 qc 100.d
Sample p2 qc 100
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.475	67153	2017.4	69.10	∞	267192	90.653 ng/ml
Phenytoin	5.502	14258	3167.5	79.86	285.5	7173	98.392 ng/ml

AM #28 Multi-Drug Quant. Results

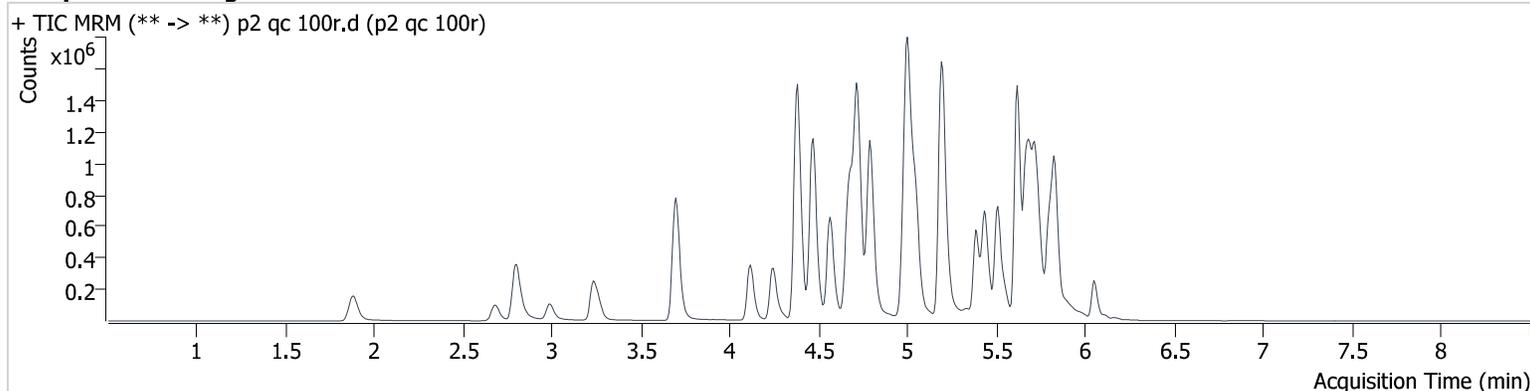
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-B2
Injection Volume 5
Acq. Date-Time 2/2/2024 11:10:47 AM
Sample Info.

Data File p2 qc 100r.d
Sample p2 qc 100r
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.462	65445	4236.3	70.66	2341.8	259595	90.931 ng/ml
Phenytoin	5.502	12851	4269.4	82.84	172.3	6823	93.270 ng/ml

AM #28 Multi-Drug Quant. Results

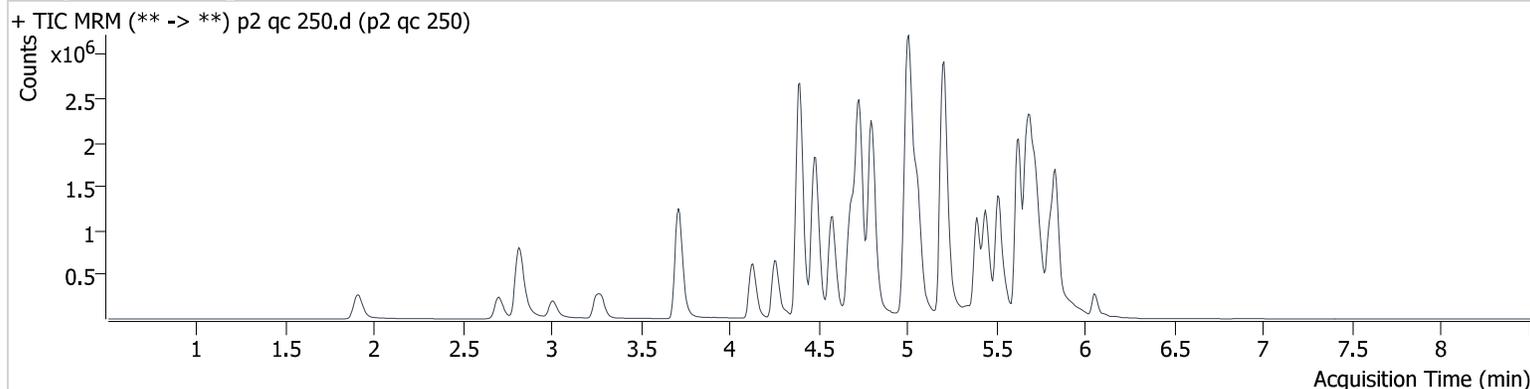
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-C2
Injection Volume 5
Acq. Date-Time 2/2/2024 9:18:13 AM
Sample Info.

Data File p2 qc 250.d
Sample p2 qc 250
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.482	115009	6724.1	70.21	∞	178822	231.363 ng/ml
Phenytoin	5.509	33944	16746.2	75.90	1056.7	7234	231.445 ng/ml

AM #28 Multi-Drug Quant. Results

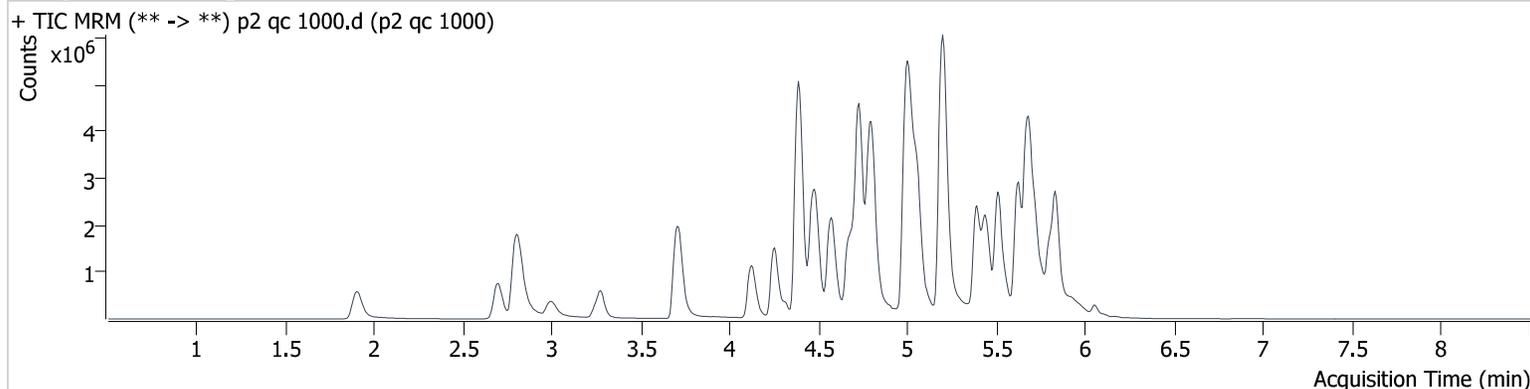
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type QC
Acq. Method mdqp2 2023.m
Sample Position P2-D2
Injection Volume 5
Acq. Date-Time 2/2/2024 9:27:25 AM
Sample Info.

Data File p2 qc 1000.d
Sample p2 qc 1000
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.489	177671	8854.3	73.22	∞	70906	900.249 ng/ml
Phenytoin	5.502	123082	732.8	77.54	829.5	5569	1087.877 ng/ml

AM #28 Multi-Drug Quant. Results

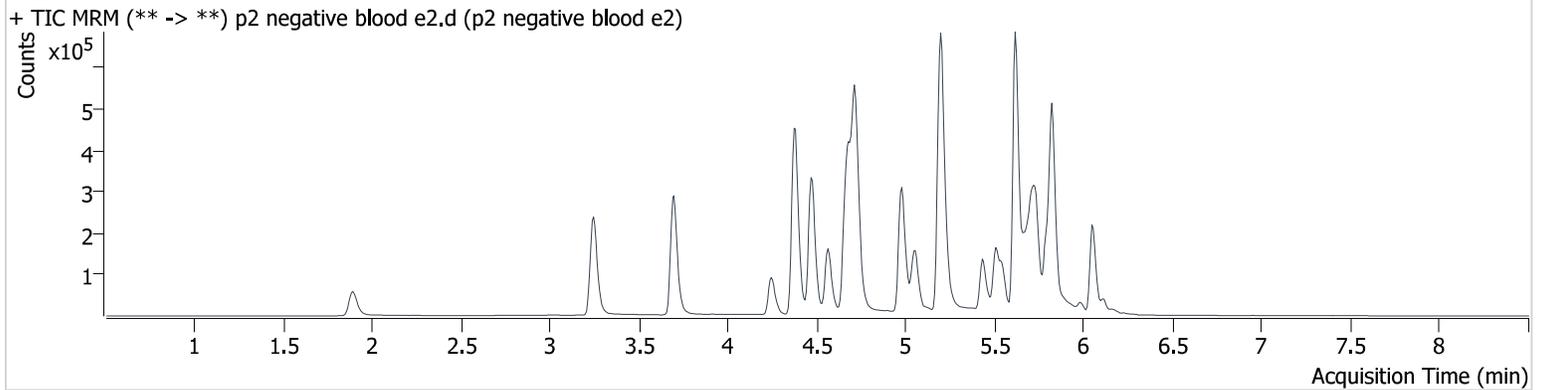
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Sample
Acq. Method mdqp2 2023.m
Sample Position P2-E2
Injection Volume 5
Acq. Date-Time 2/2/2024 9:48:00 AM
Sample Info.

Data File p2 negative blood e2.d
Sample p2 negative blood e2
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

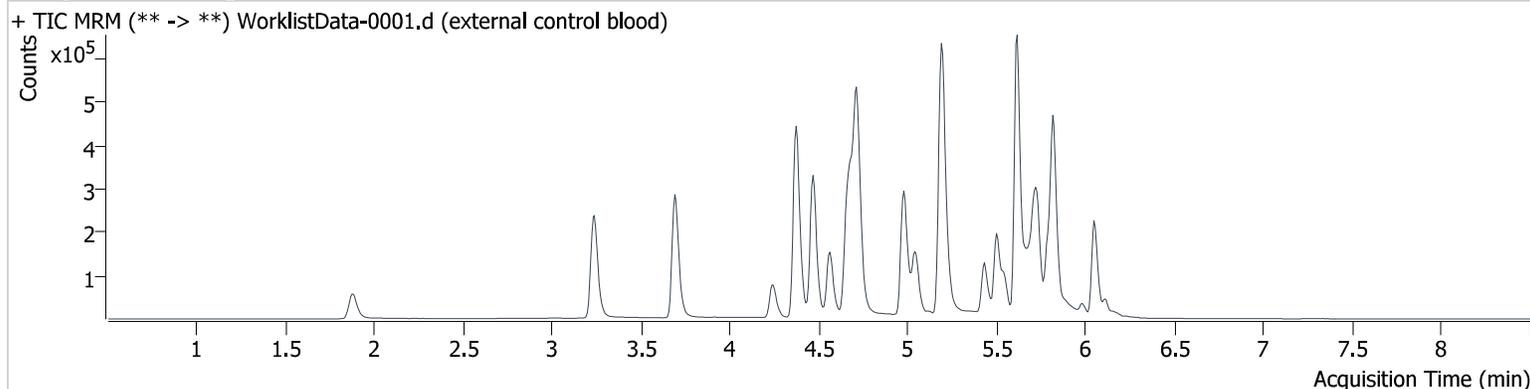
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Sample
Acq. Method mdqp2 2023.m
Sample Position P2-G2
Injection Volume 5
Acq. Date-Time 2/2/2024 11:01:25 AM
Sample Info.

Data File WorklistData-0001.d
Sample external control blood
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

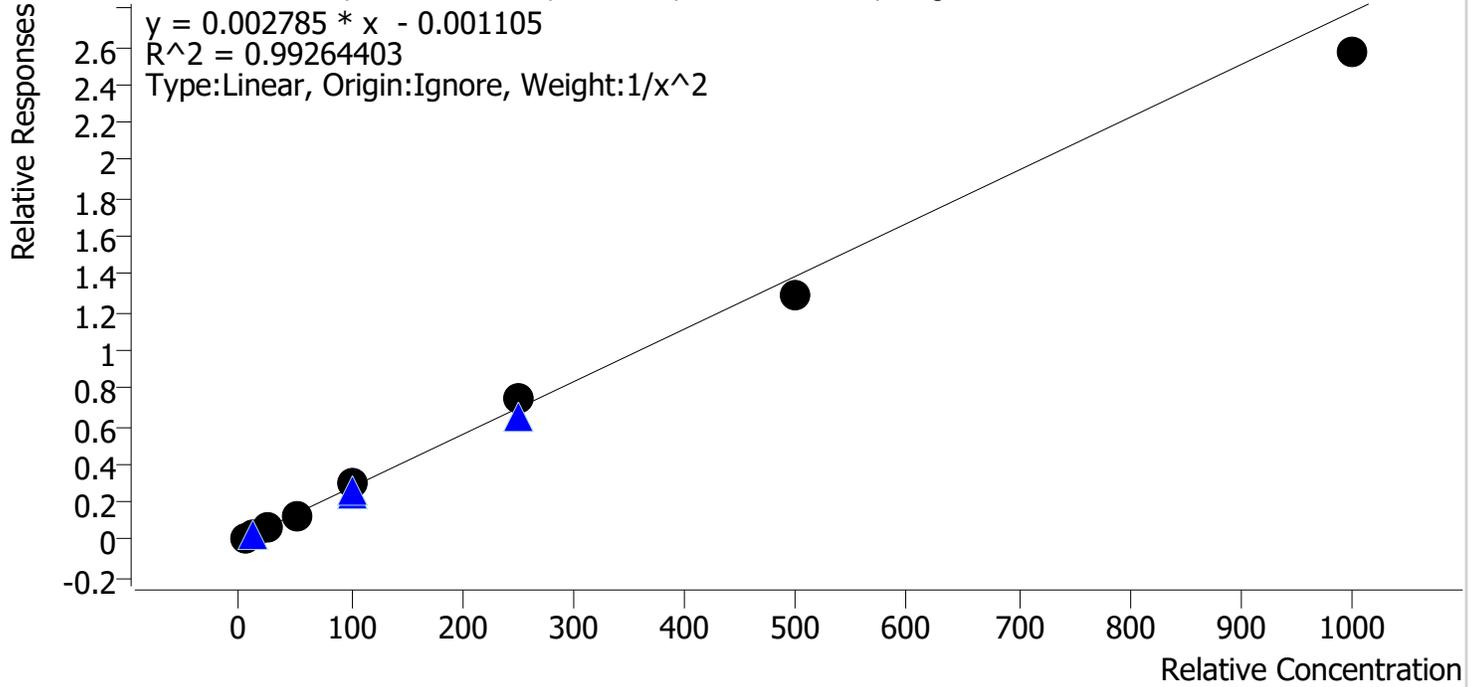


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Phenytoin	5.502	95196	69085.5	80.64	912.2	7896	593.717 ng/ml

Compound Calibration Report

Batch results D:\MassHunter\Data\2024\lam 27-28\020124\QuantResults\lam 28 p2 for review.batch.bin
Last Cal. Update 2/2/2024 12:19 PM
Analyst Name ISP\datastor
Analyte methocarbamol **Internal Standard** Midazolam-D4

methocarbamol - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 5 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✓	5.0	4.8	95.3
p2 cal 2 mdq	2	✓	10.0	11.0	109.9
p2 cal 3 mdq	3	✓	25.0	24.6	98.4
p2 cal 4 mdq	4	✓	50.0	47.8	95.5
p2 cal 5 mdq	5	✓	100.0	109.5	109.5
p2 cal 6 mdq	6	✓	250.0	266.2	106.5
p2 cal 7 mdq	7	✓	500.0	462.6	92.5
p2 cal 8 mdq	8	✓	1000.0	923.1	92.3

AM #28 Multi-Drug Quant. Results

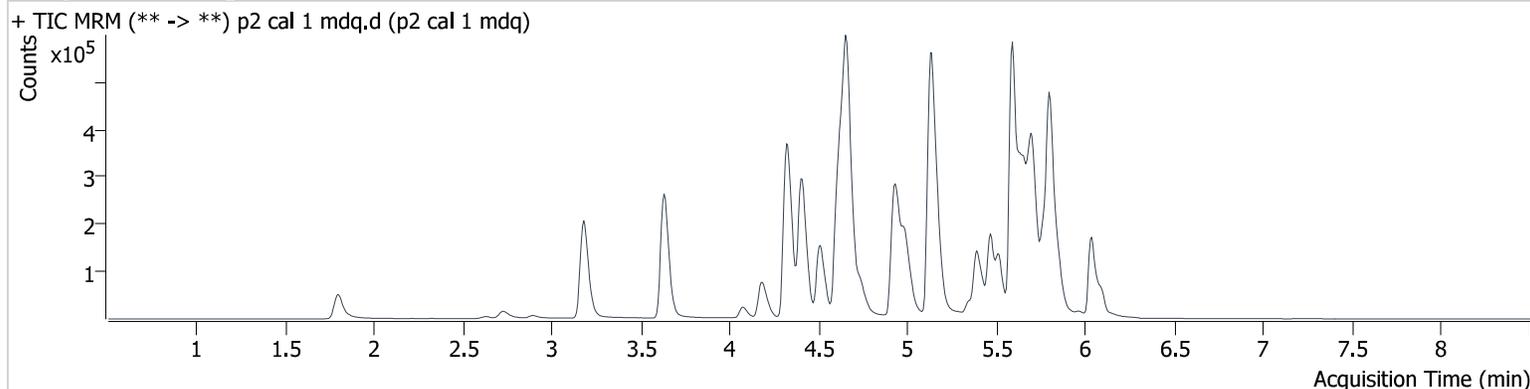
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-A1
Injection Volume 5
Acq. Date-Time 2/1/2024 6:21:00 PM
Sample Info.

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

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.408	4227	153.9	70.01	54.3	347520	4.765 ng/ml
Phenytoin	5.460	742	470.1	88.59	24.2	7980	5.175 ng/ml

AM #28 Multi-Drug Quant. Results

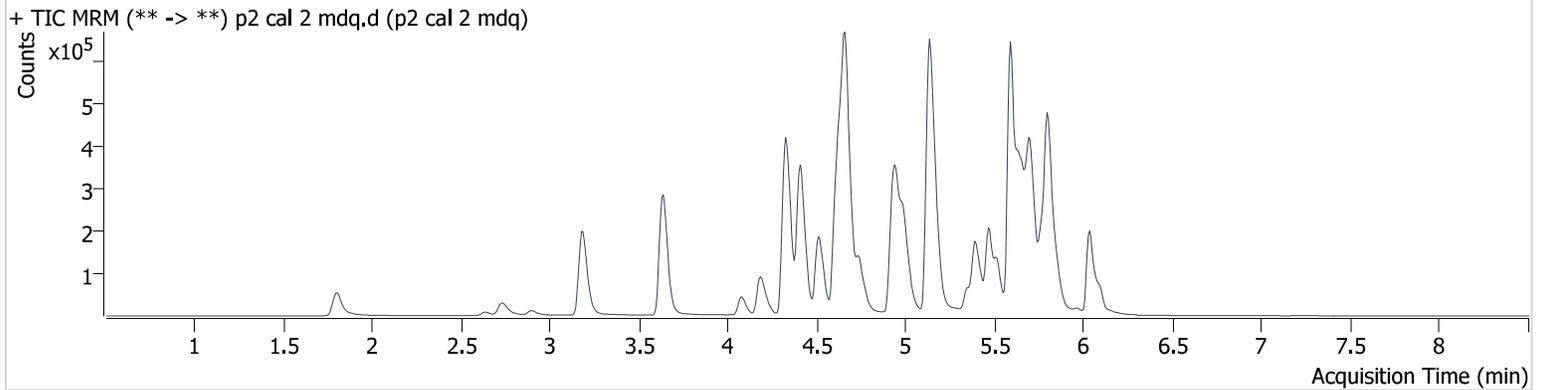
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-B1
Injection Volume 5
Acq. Date-Time 2/1/2024 6:30:14 PM
Sample Info.

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

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.408	9761	409.1	68.84	352.0	330778	10.994 ng/ml
Phenytoin	5.467	1447	2920.8	73.63	12.4	7809	9.718 ng/ml

AM #28 Multi-Drug Quant. Results

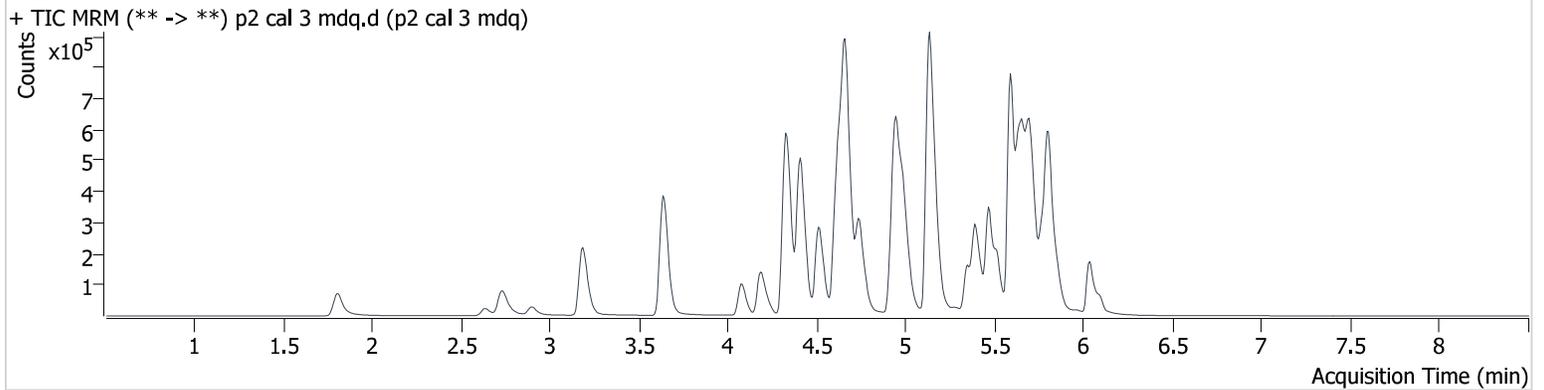
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-C1
Injection Volume 5
Acq. Date-Time 2/1/2024 6:39:28 PM
Sample Info.

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

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.408	22863	521.3	70.08	1067.8	339163	24.605 ng/ml
Phenytoin	5.467	3655	1731.1	87.82	31.8	8067	22.895 ng/ml

AM #28 Multi-Drug Quant. Results

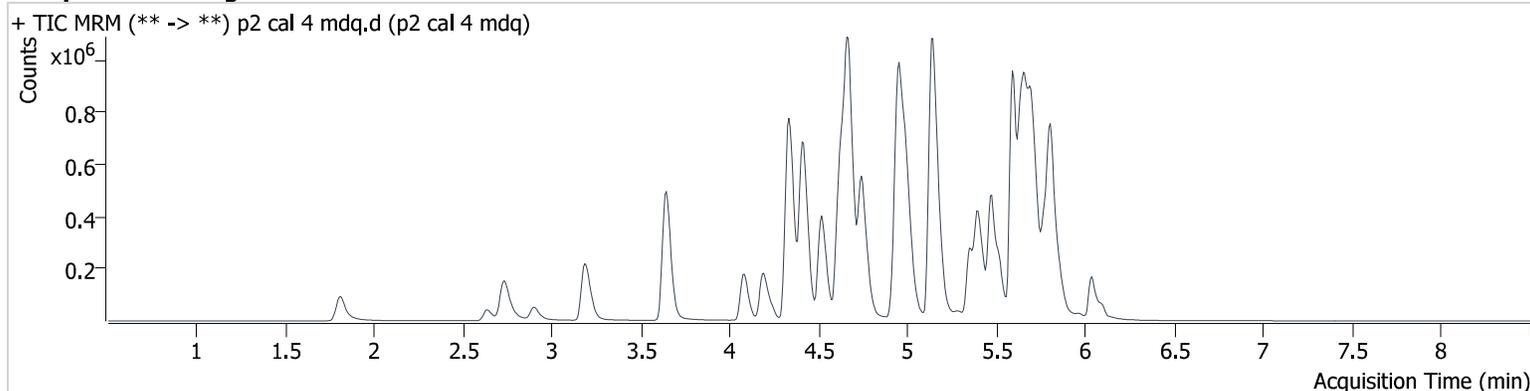
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-D1
Injection Volume 5
Acq. Date-Time 2/1/2024 6:48:41 PM
Sample Info.

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

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.414	40503	779.0	70.72	488.4	307060	47.767 ng/ml
Phenytoin	5.467	7233	3312.9	82.26	95.3	7633	47.219 ng/ml

AM #28 Multi-Drug Quant. Results

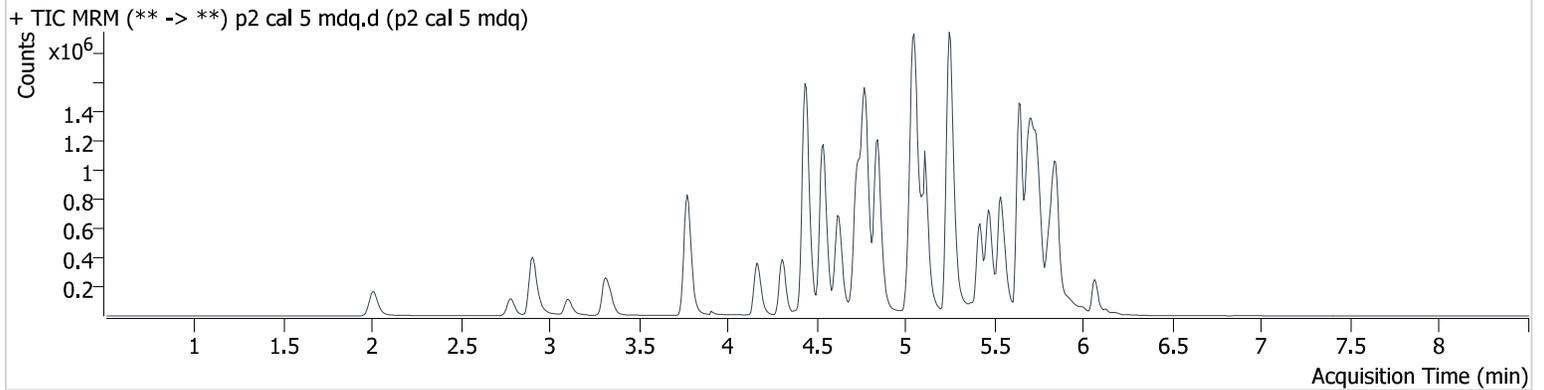
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-E1
Injection Volume 5
Acq. Date-Time 2/2/2024 8:22:49 AM
Sample Info.

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

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.537	66306	4570.9	81.52	703.5	218219	109.515 ng/ml
Phenytoin	5.536	14973	6343.5	80.20	181.0	7436	99.671 ng/ml

AM #28 Multi-Drug Quant. Results

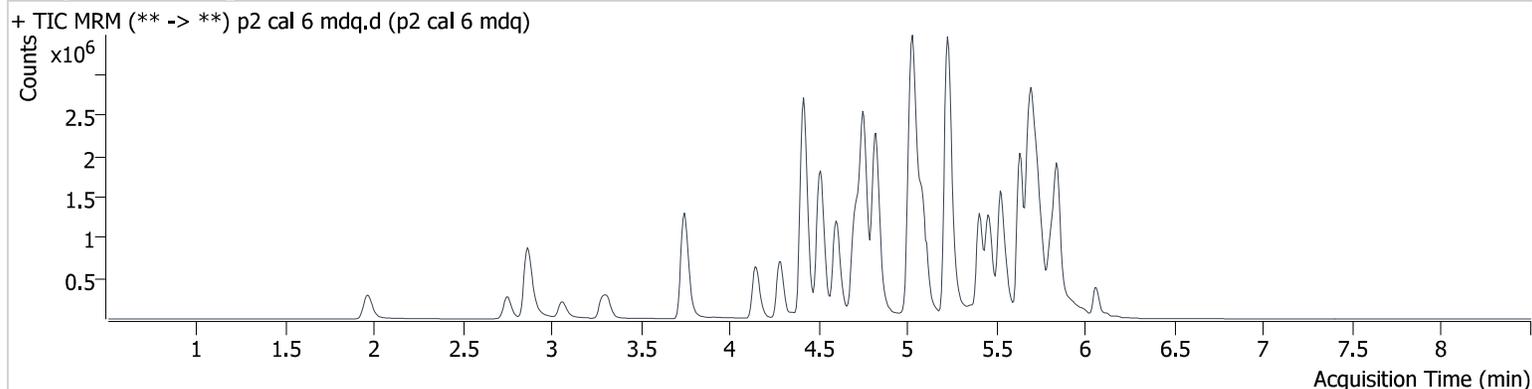
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-F1
Injection Volume 5
Acq. Date-Time 2/2/2024 8:32:11 AM
Sample Info.

Data File p2 cal 6 mdq.d
Sample p2 cal 6 mdq
Operator Anne Nord
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.510	106960	1356.1	75.92	∞	144533	266.158 ng/ml
Phenytoin	5.523	34733	4203.1	79.04	784.0	6789	252.318 ng/ml

AM #28 Multi-Drug Quant. Results

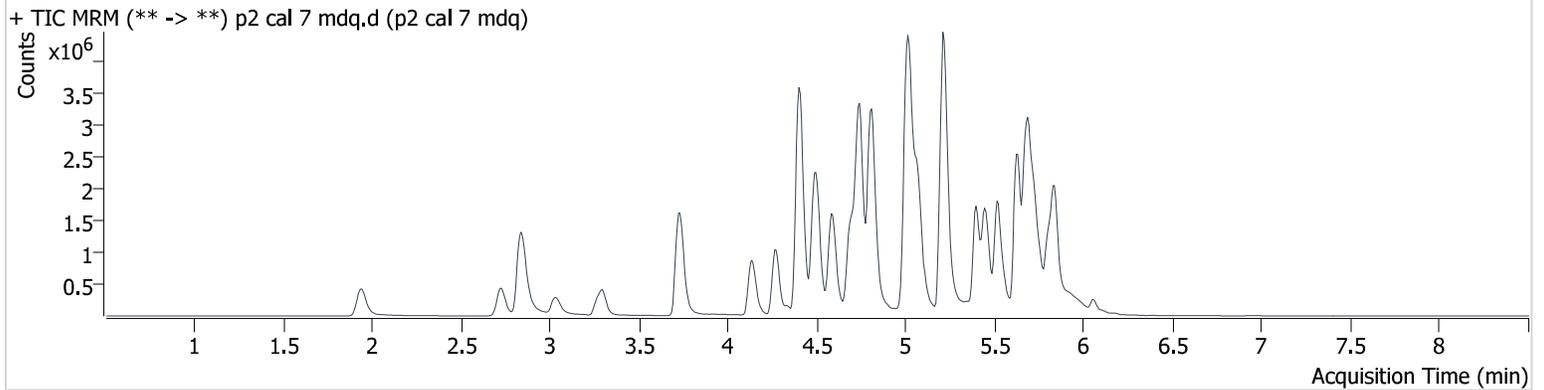
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-G1
Injection Volume 5
Acq. Date-Time 2/2/2024 8:41:24 AM
Sample Info.

Data File p2 cal 7 mdq.d
Sample p2 cal 7 mdq
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.
methocarbamol	4.503	134742	∞	76.36	471.8	104696	462.578 ng/ml
Phenytoin	5.516	73860	47943.2	77.37	791.8	6317	575.830 ng/ml

AM #28 Multi-Drug Quant. Results

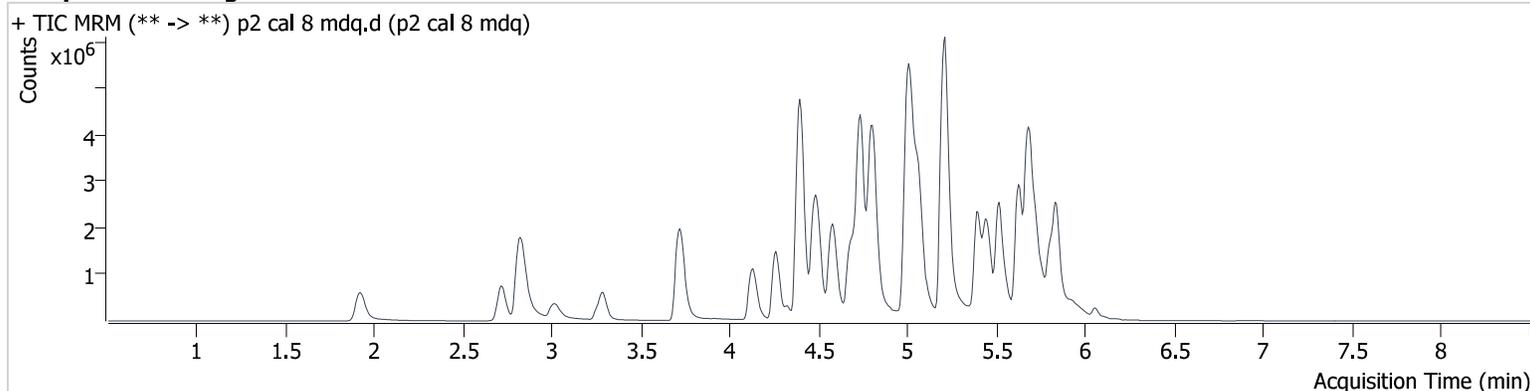
Batch results D:\MassHunter\Data\2024\am 27-28\020124\QuantResults\am 28 p2 for review.batch.bin
Calibration Last Update 2/2/2024 12:19:31 PM

Instrument 69679
Type Cal
Acq. Method mdqp2 2023.m
Sample Position P2-H1
Injection Volume 5
Acq. Date-Time 2/2/2024 8:50:36 AM
Sample Info.

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

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
methocarbamol	4.496	167728	8200.3	72.50	∞	65279	923.120 ng/ml
Phenytoin	5.509	135434	42774.7	80.33	1101.2	6836	975.291 ng/ml