

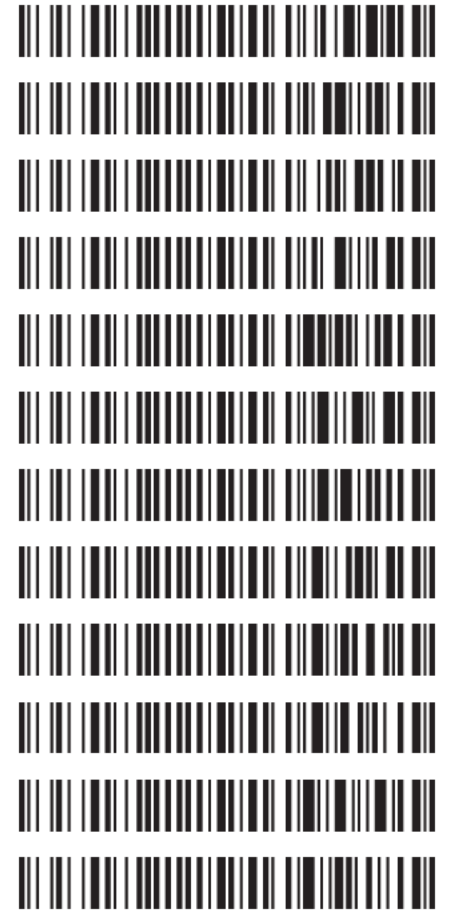
The highlighted items are the items included in this worklist. The am 27 tasks were assigned to both worklist 5862 and 5863 results and central data for am 27 tasks in worklist 5862.

**Worklist: 5863**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2022-0722	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
C2022-0808	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0888	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0906	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0942	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0967	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0967	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0974	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
C2022-0974	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0974	3	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
C2022-0984	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-1001	1	BCK	AM 27 Blood THC Quant by LC-QQQ

REVIEWED

By Britany Wylie at 11:43 am, May 12, 2022



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

Extraction Date 5/5/22

Analyst: Anne Nord

Plate lot#: 230316

Plate re-test: 09/16/22

**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:** 22B52020 **Urine Blank lot:**  
**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.
- 3. Create worklist:

## Analytic:

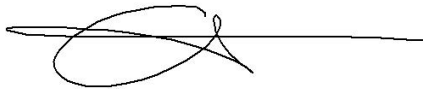
- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Urine hydrolysis pipette 250 ul urine in blank well, add 40 ul BG Turbo, add 100 ul 500 mm sodium phosphate buffer, mix for at least 5 minutes at ambient temperature. Pipette 250µL blood (calibrated pipette) Pipette ID: 1926134 or 250µL hydrolyzed urine in wells of analytical (standards) plate.
- 3. Pipette 250µL 00.5M 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, Place on SPE Dry and evaporate to dryness at approx. 35°C.  
*SPE Dry ID: 66819*
- 15. 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 R<sup>2</sup> 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: Blood only run

*Only Midazolam and Topiramate were evaluated.*



	1 mixing plate 5 SLE and injection	2 mixing plate 6 SLE and injection	3 mixing plate 7 SLE and injection
A	IS + Cal. 1	IS + QC_1	974-3
B	IS + Cal. 2	IS + QC_2	
C	IS + Cal. 3	qc3	
D	IS + Cal. 4	IS + QC_4	
E	IS + Cal. 5		
F	IS + Cal. 6	negative blood	
G	IS + Cal. 7	974-1	
H	IS + Cal. 8	722-1	

Run blanks before <sup>A</sup> no blanks needed in this batch 5/12/22

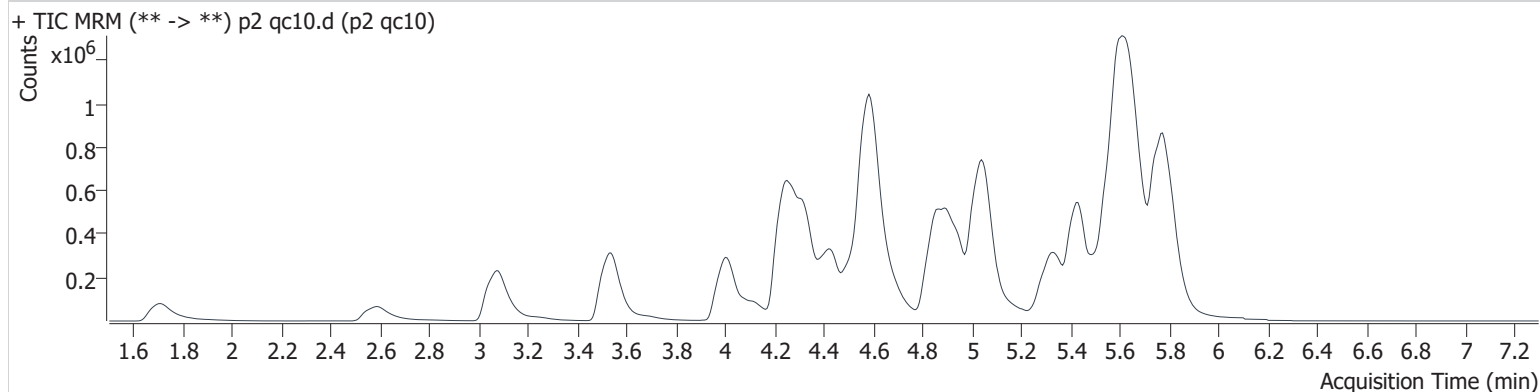
c2022-\_\_-\_\_

# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 qc10.d
<b>Type</b>	QC	<b>Sample</b>	p2 qc10
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-A6	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 4:16:16 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.774	60769	659.2	93.5	301.0	808821	10.673 ng/ml
Topiramate	4.930	6552	435.8	43.8	832.4	31241	10.915 ng/ml

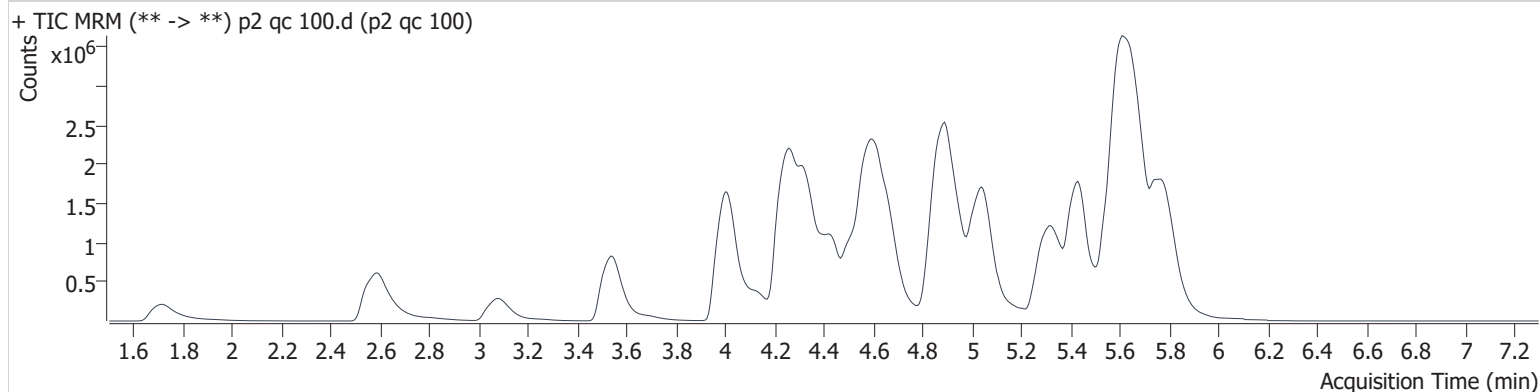
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 qc 100.d
<b>Type</b>	QC	<b>Sample</b>	p2 qc 100
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-B6	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 4:27:08 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.774	492512	842.4	93.9	1106.6	713397	94.921 ng/ml
Topiramate	4.937	36564	14592.6	41.8	1159.9	21710	88.595 ng/ml

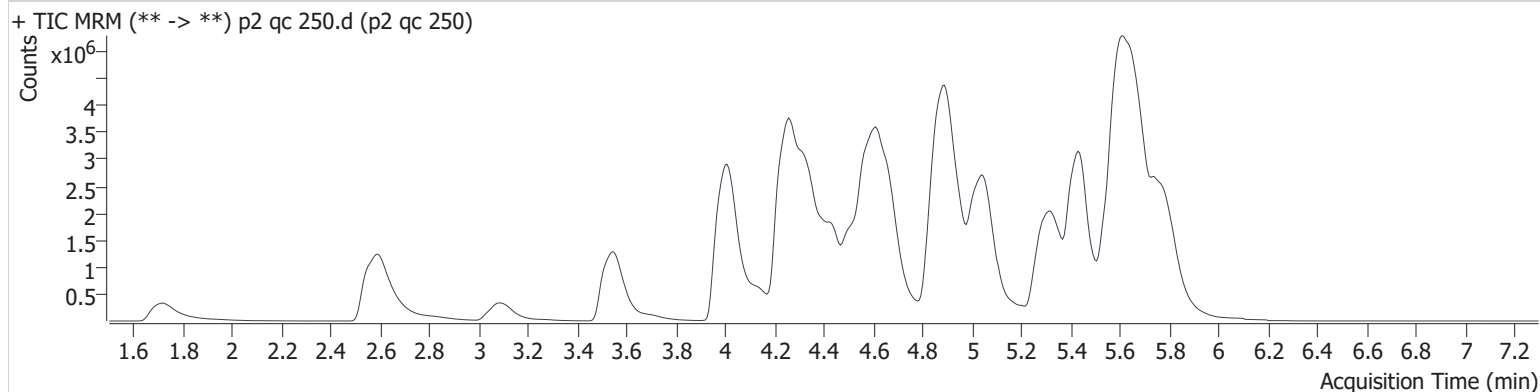
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 qc 250.d
<b>Type</b>	QC	<b>Sample</b>	p2 qc 250
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-C6	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 5:42:57 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.781	891913	1747.4	95.4	1605.2	506349	241.589 ng/ml
Topiramate	4.944	49409	34272.9	40.9	4209.1	11410	228.005 ng/ml

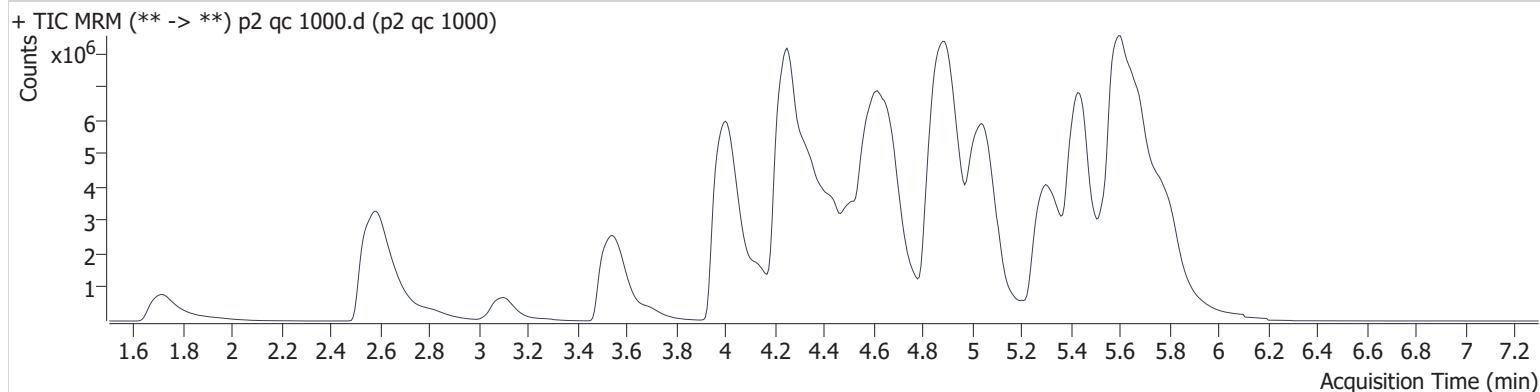
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 qc 1000.d
<b>Type</b>	QC	<b>Sample</b>	p2 qc 1000
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-D6	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 4:37:58 PM		

**Sample Info.**

## Sample Chromatogram



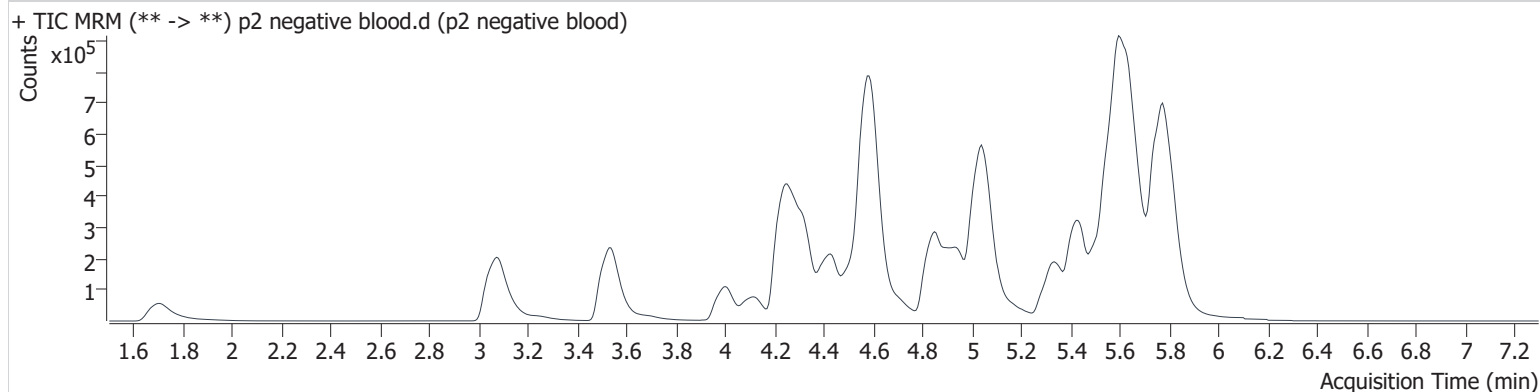
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.788	1719581	475.5	94.2	671.2	233957	1006.852 ng/ml
Topiramate	4.950	56216	25388.0	40.4	8428.2	2843	1041.727 ng/ml

# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

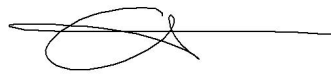
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<b>Type</b>	Sample	<b>Sample</b>	p2 negative blood
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-F6	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 4:59:33 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



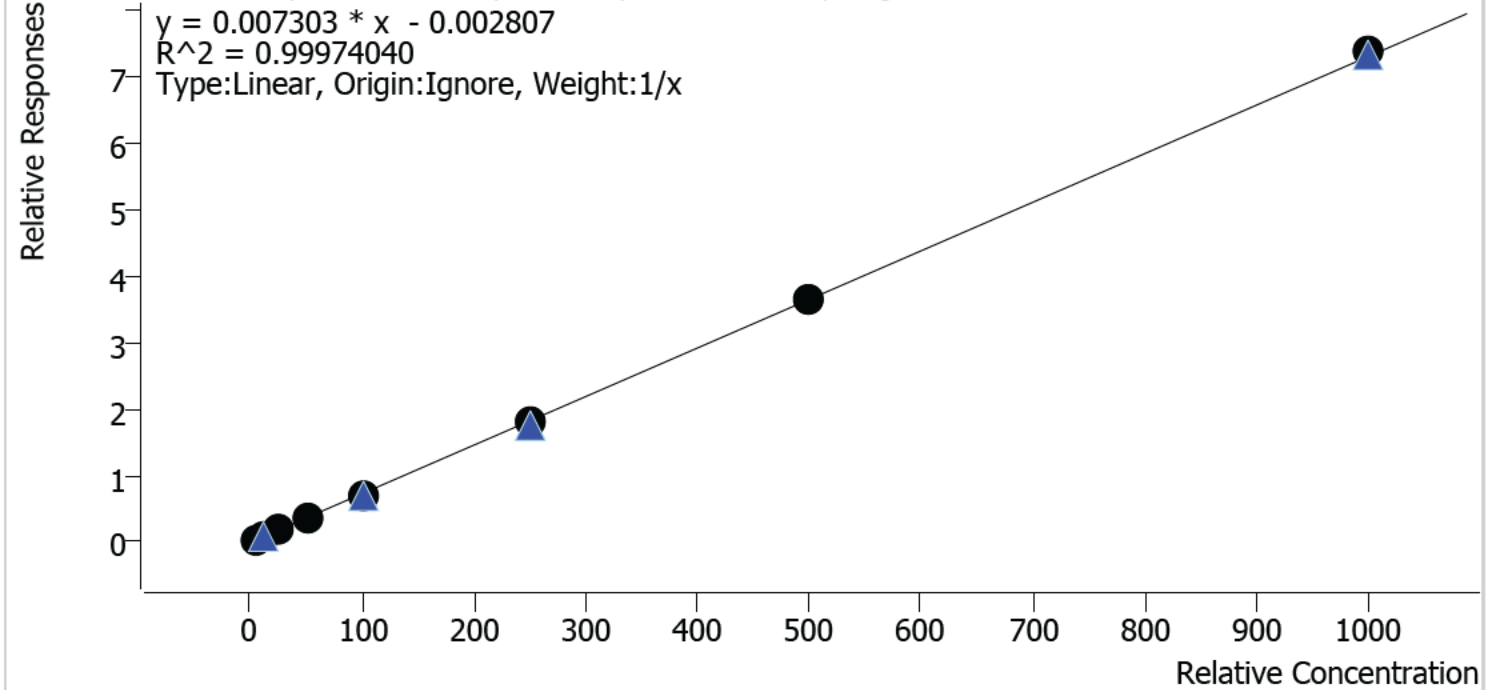


# Compound Calibration Report



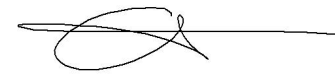
**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Last Cal. Update** 5/10/2022 10:32 AM  
**Analyst Name** ISP\datastor  
**Analyte** Midazolam **Internal Standard** **Midazolam-D4**

Midazolam - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 4 QCs

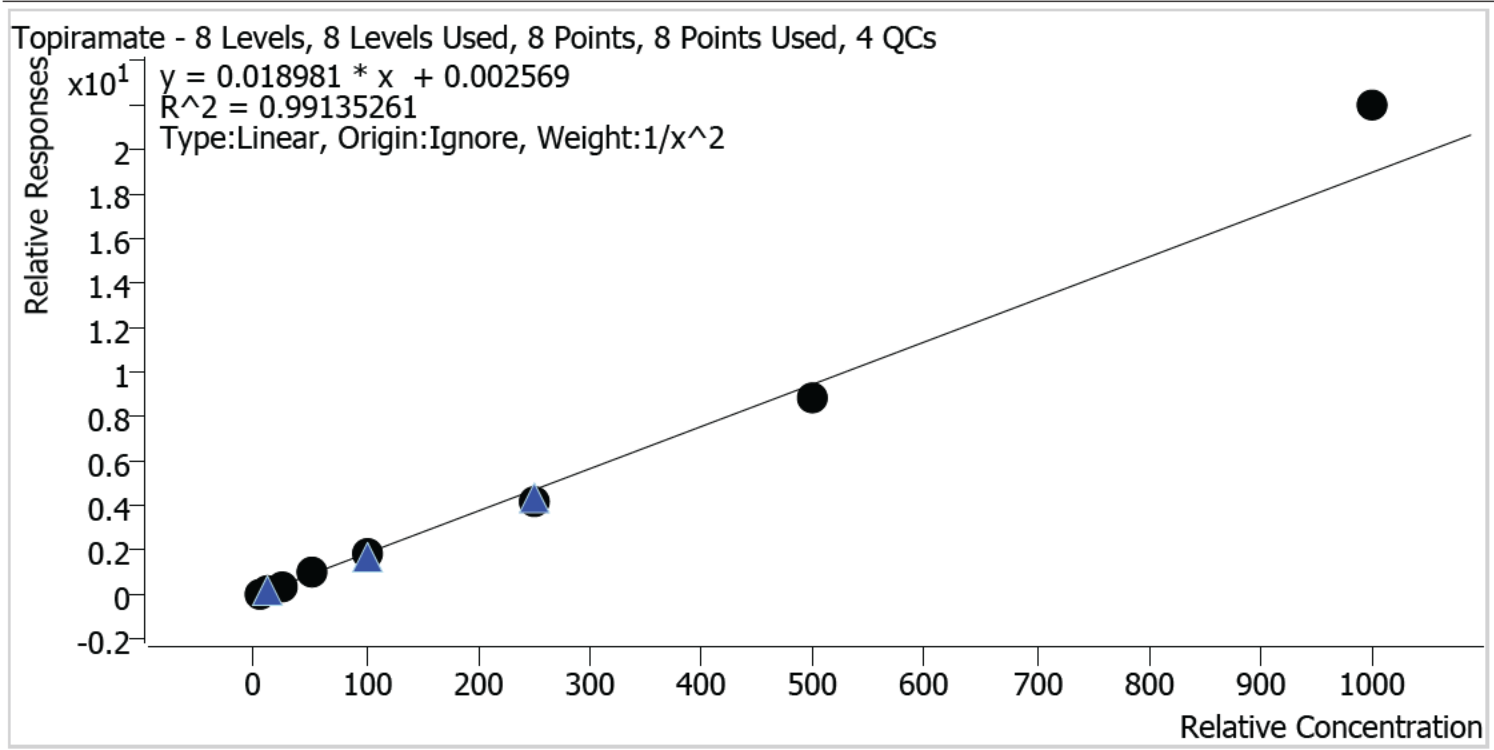


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✓	5.0	5.2	103.7
p2 cal 2 mdq	2	✓	10.0	10.5	105.3
p2 cal 3 mdq	3	✓	25.0	24.7	98.8
p2 cal 4 mdq	4	✓	50.0	48.7	97.4
p2 cal 5 mdq	5	✓	100.0	95.9	95.9
p2 cal 6 mdq	6	✓	250.0	244.6	97.8
p2 cal 7 mdq	7	✓	500.0	500.1	100.0
p2 cal 8 mdq	8	✓	1000.0	1010.3	101.0

# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Last Cal. Update** 5/10/2022 10:32 AM  
**Analyst Name** ISP\datastor  
**Analyte** Topiramate **Internal Standard** Topiramate-d12



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✓	5.0	5.0	99.1
p2 cal 2 mdq	2	✓	10.0	10.3	102.7
p2 cal 3 mdq	3	✓	25.0	24.2	96.9
p2 cal 4 mdq	4	✓	50.0	51.7	103.3
p2 cal 5 mdq	5	✓	100.0	100.7	100.7
p2 cal 6 mdq	6	✓	250.0	220.9	88.4
p2 cal 7 mdq	7	✓	500.0	465.8	93.2
p2 cal 8 mdq	8	✓	1000.0	1157.8	115.8

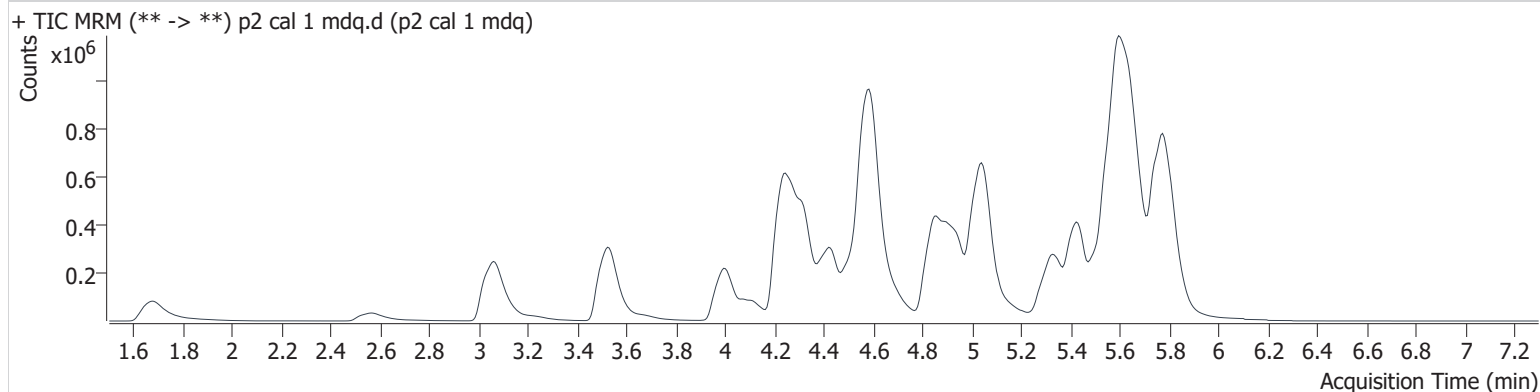
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 1 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 1 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-A5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 2:49:38 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.767	30373	283.2	93.0	6605.6	866633	5.184 ng/ml
Topiramate	4.937	3622	243.6	37.6	414.1	37468	4.957 ng/ml

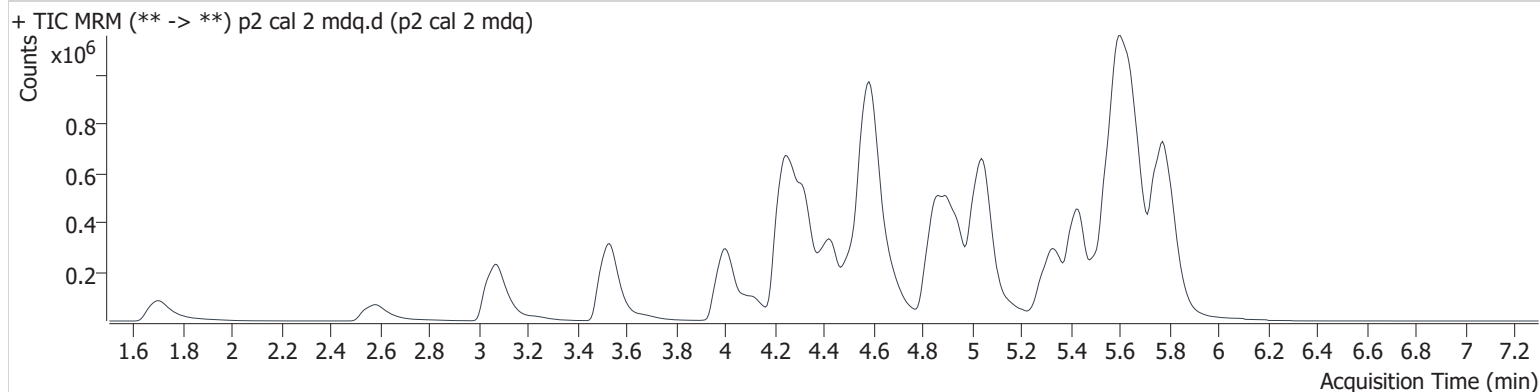
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 2 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 2 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-B5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 3:00:28 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.774	57293	768.4	95.0	968.6	773325	10.529 ng/ml
Topiramate	4.944	6142	3558.9	37.5	1219.2	31115	10.265 ng/ml

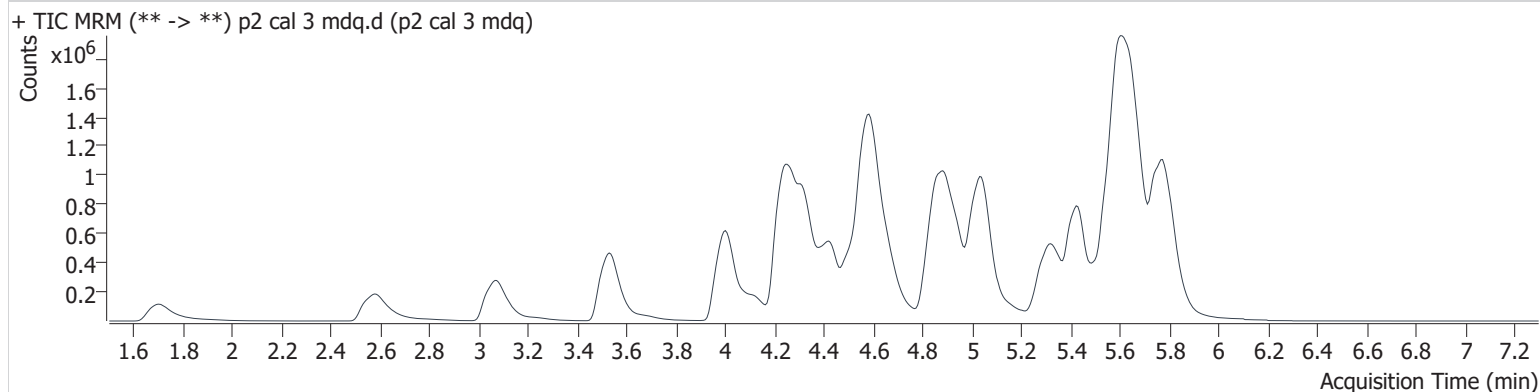
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 3 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 3 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-C5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 3:11:18 PM		

**Sample Info.**

## Sample Chromatogram



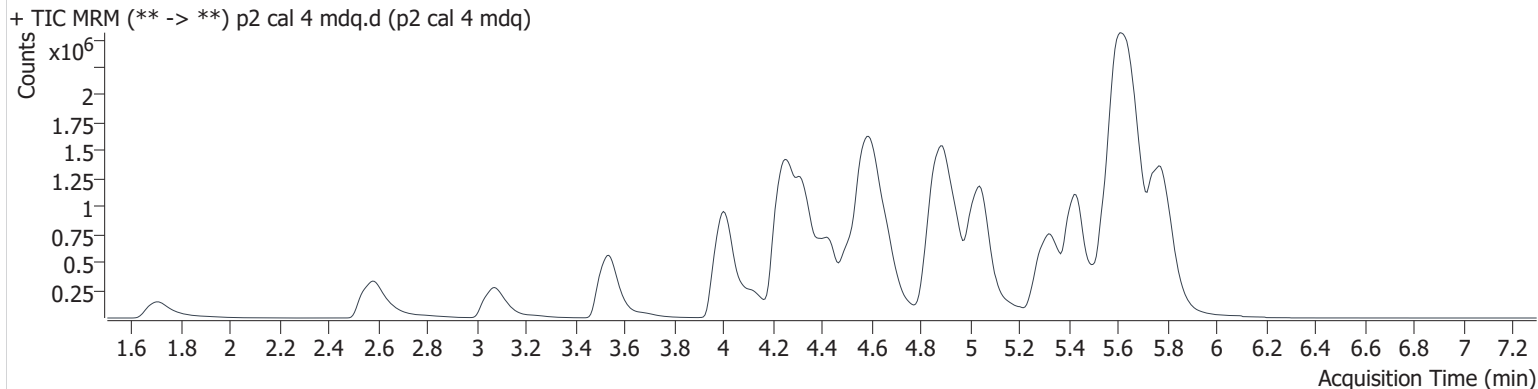
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.774	155835	4538.7	95.6	1719.0	877150	24.712 ng/ml
Topiramate	4.930	14364	5077.7	39.2	2292.4	31053	24.235 ng/ml

# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 4 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 4 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-D5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 3:22:07 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



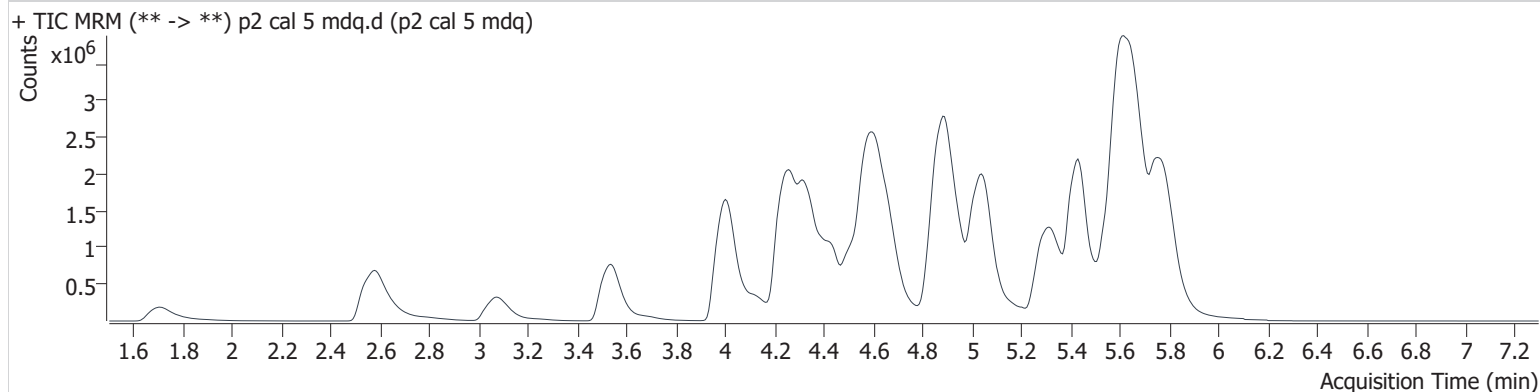
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.774	273360	853.0	94.8	1648.5	775073	48.680 ng/ml
Topiramate	4.937	26134	10897.0	39.1	1924.4	26581	51.663 ng/ml

# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 5 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 5 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-E5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 3:32:57 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



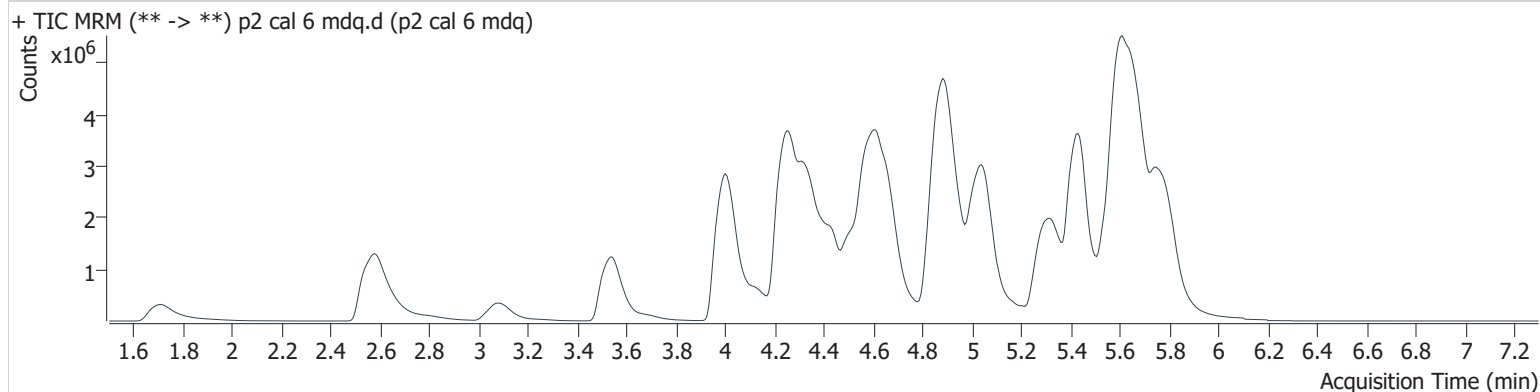
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.774	475876	1012.7	94.0	222.0	681957	95.939 ng/ml
Topiramate	4.944	36213	3207.9	41.5	7086.0	18929	100.656 ng/ml

# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 6 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 6 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-F5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 3:43:47 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.781	867996	786.5	94.3	842.9	486676	244.610 ng/ml
Topiramate	4.950	48237	12642.4	38.9	7669.7	11498	220.890 ng/ml

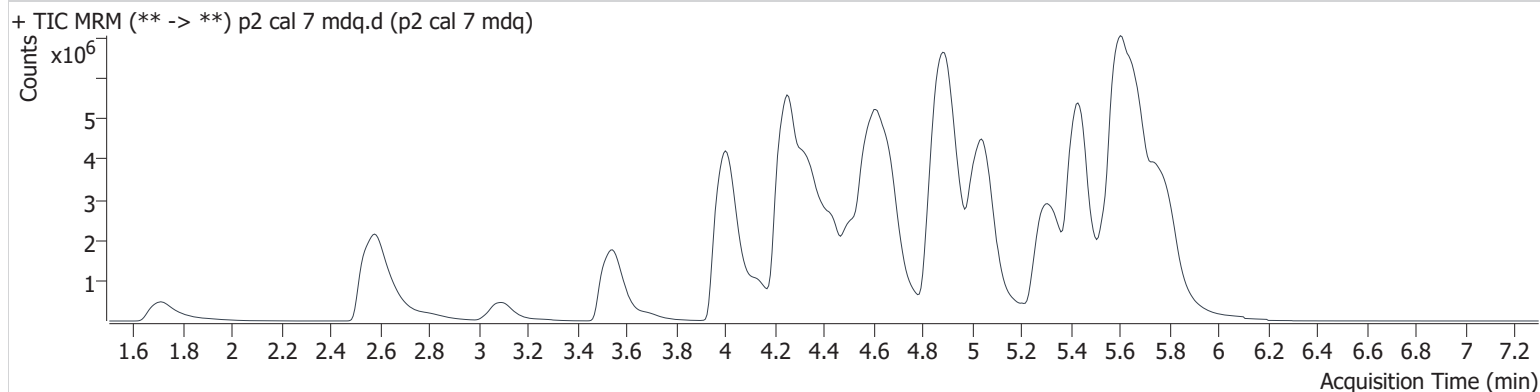


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 7 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 7 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-G5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 3:54:36 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.781	1281026	704.3	93.5	3305.5	351055	500.070 ng/ml
Topiramate	4.944	53002	4629.2	40.2	5256.6	5994	465.761 ng/ml

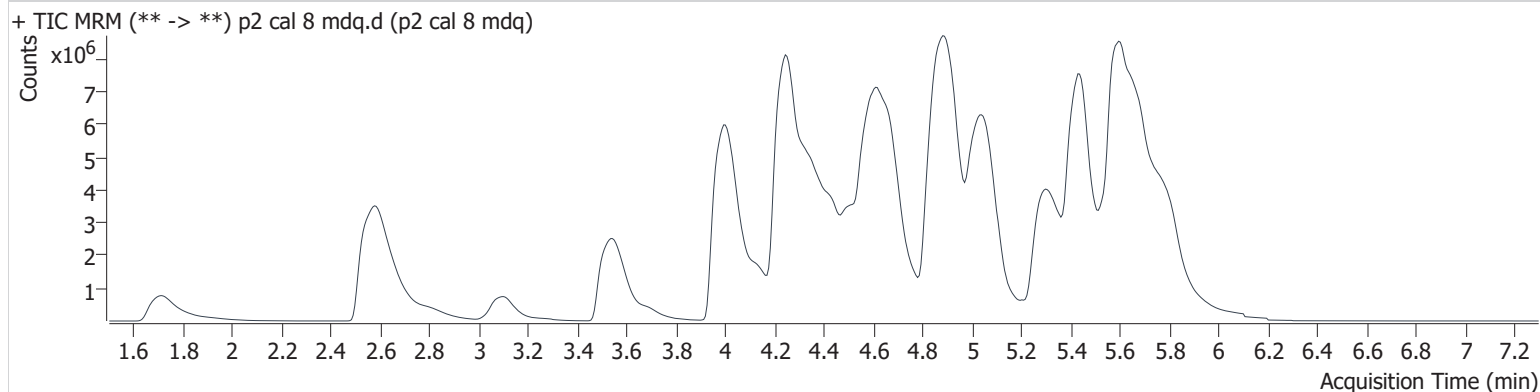
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2022\am 27-28\050622\QuantResults\mdq p2.batch.bin  
**Calibration Last Update** 5/10/2022 10:32:58 AM

<b>Instrument</b>	69679	<b>Data File</b>	p2 cal 8 mdq.d
<b>Type</b>	Cal	<b>Sample</b>	p2 cal 8 mdq
<b>Acq. Method</b>	mdqp2 1-21-21.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P2-H5	<b>Comment</b>	
<b>Injection Volume</b>	5		
<b>Acq. Date-Time</b>	5/6/2022 4:05:26 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Midazolam	5.794	1749830	∞	93.8	563.1	237266	1010.276 ng/ml
Topiramate	4.950	52860	23228.5	40.1	9188.6	2405	1157.754 ng/ml