



11/15/2019

Worklist: 3831

REVIEWED

By Britany Wylie at 3:38 pm, Nov 19, 2019

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2019-2126	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
M2018-2893	3	UCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ



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

Extraction Date 11/8/19 Analyst: Anne Nord
Plate lot#: 19730 (part IDP-112) Plate Expiration: 1/30/20

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: 19H52275 **Urine Blank lot:** 11719

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. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- 4. Pipette 250µL 0.5M ammonium hydroxide in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer 300µL of blood+base/urine+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)** *Manifold ID: 66792*
- 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. *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: Etizolam and Chlorpheniramine only compounds evaluated. Etizolam curve range 5-500



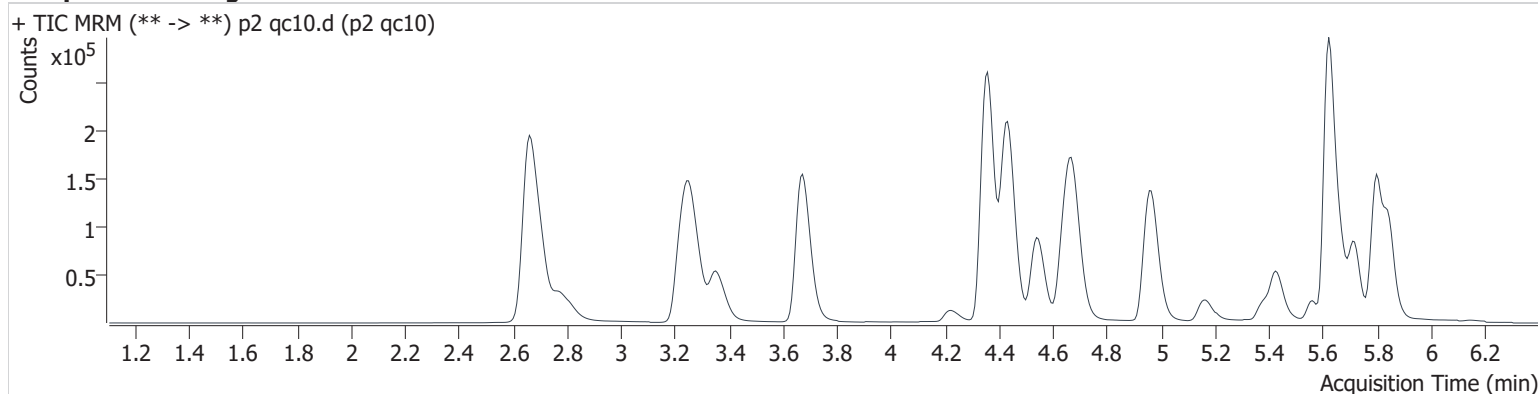
11/19/19

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 qc10.d
Type	QC	Sample	p2 qc10
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-A5	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 5:14:20 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	85919	2612.8	0.4	344.2	228967	9.953 ng/ml
Etizolam	5.824	16536	19050.5	24.1	2589.8	127832	9.879 ng/ml

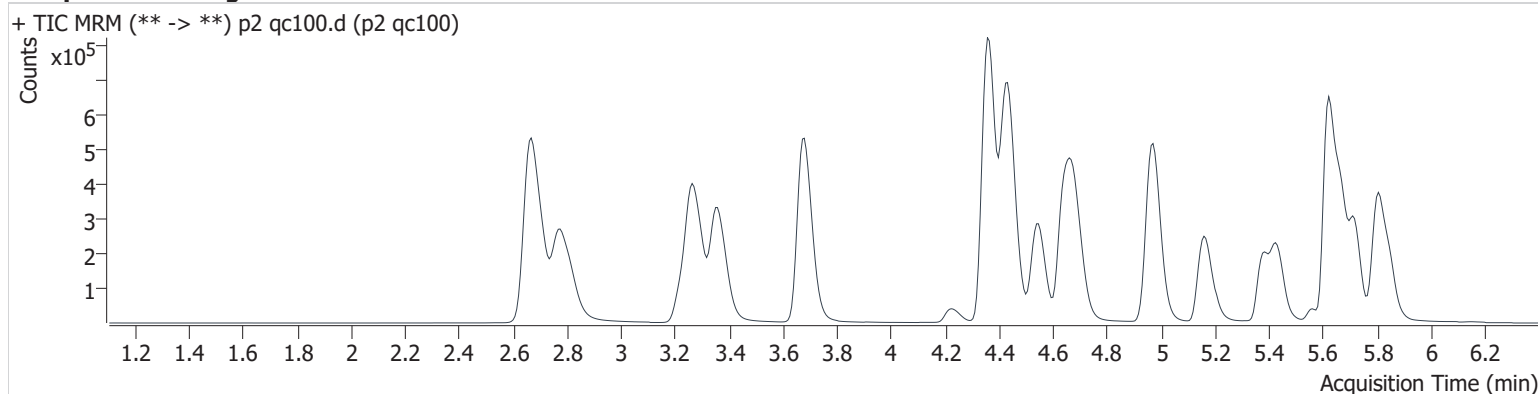


AM #28 Multi-Drug Quant. Results

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Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 qc100.d
Type	QC	Sample	p2 qc100
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-B5	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 5:25:09 PM		
Sample Info.			

Sample Chromatogram



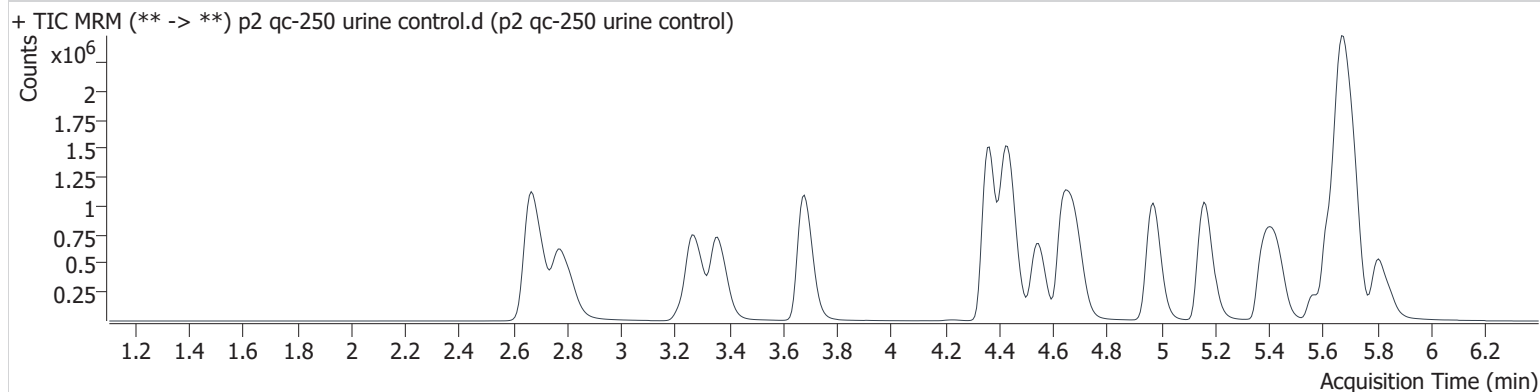
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	959601	7578.4	0.4	955.8	274907	103.519 ng/ml
Etizolam	5.824	143834	1600.8	23.1	5513.3	114033	90.855 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 qc-250 urine control.d
Type	Sample	Sample	p2 qc-250 urine control
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-C5	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 5:35:58 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	4062773	183720.9	0.4	608.2	555843	218.205 ng/ml
Etizolam	5.824	245893	1654.0	24.0	60126.5	51270	343.707 ng/ml

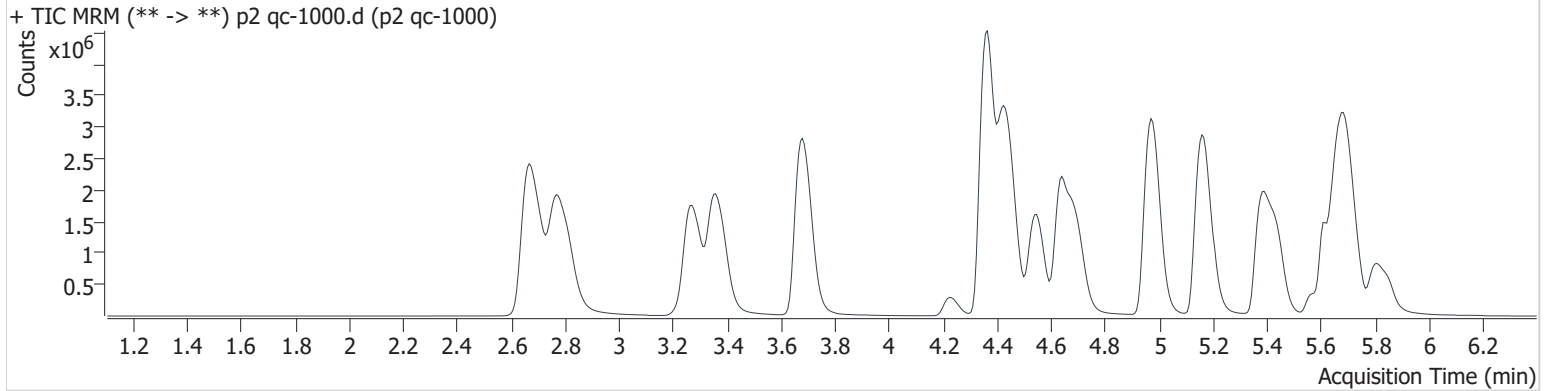
AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 qc-1000.d
Type	QC	Sample	p2 qc-1000
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-D5	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 5:46:47 PM		

Sample Info.

Sample Chromatogram



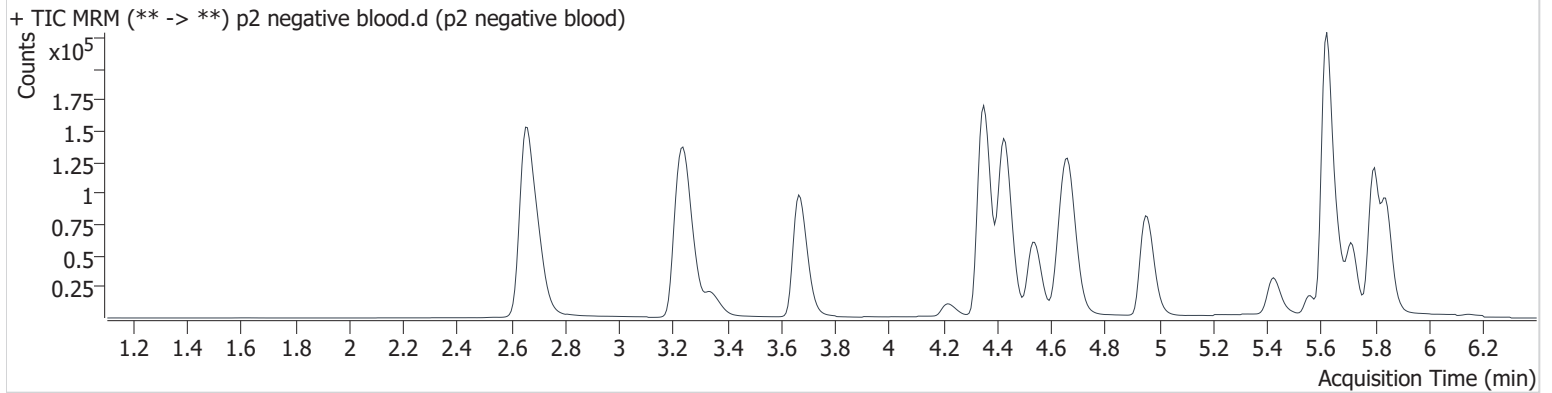
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	12151370	253451.3	0.4	1266.1	398273	915.015 ng/ml
Etizolam	5.831	581302	1572.6	23.2	9992.4	31984	1300.769 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 negative blood.d
Type	Sample	Sample	p2 negative blood
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-E5	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 6:19:00 PM		
Sample Info.			

Sample Chromatogram

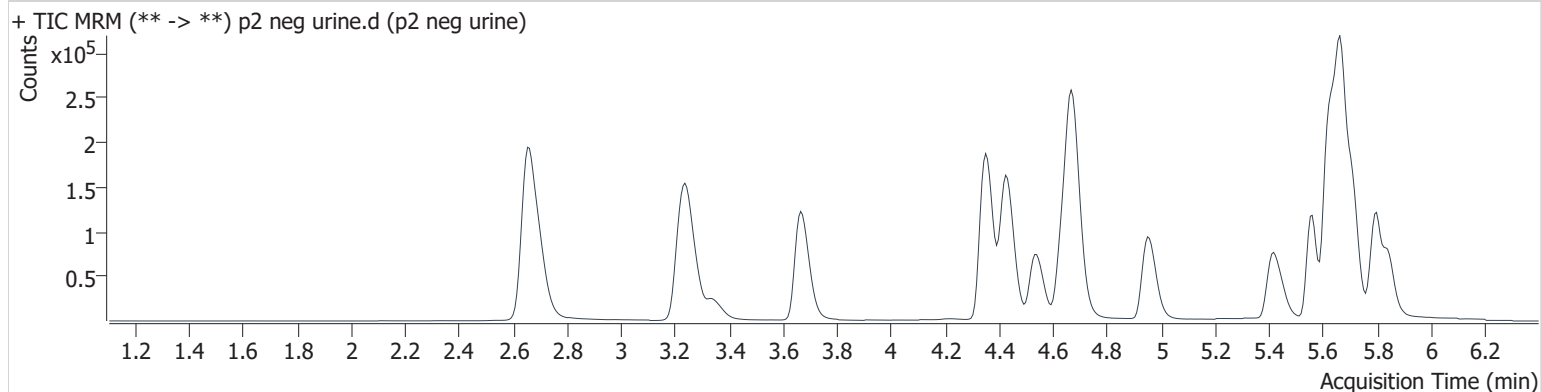


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 neg urine.d
Type	Sample	Sample	p2 neg urine
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-G5	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 6:51:20 PM		
Sample Info.			

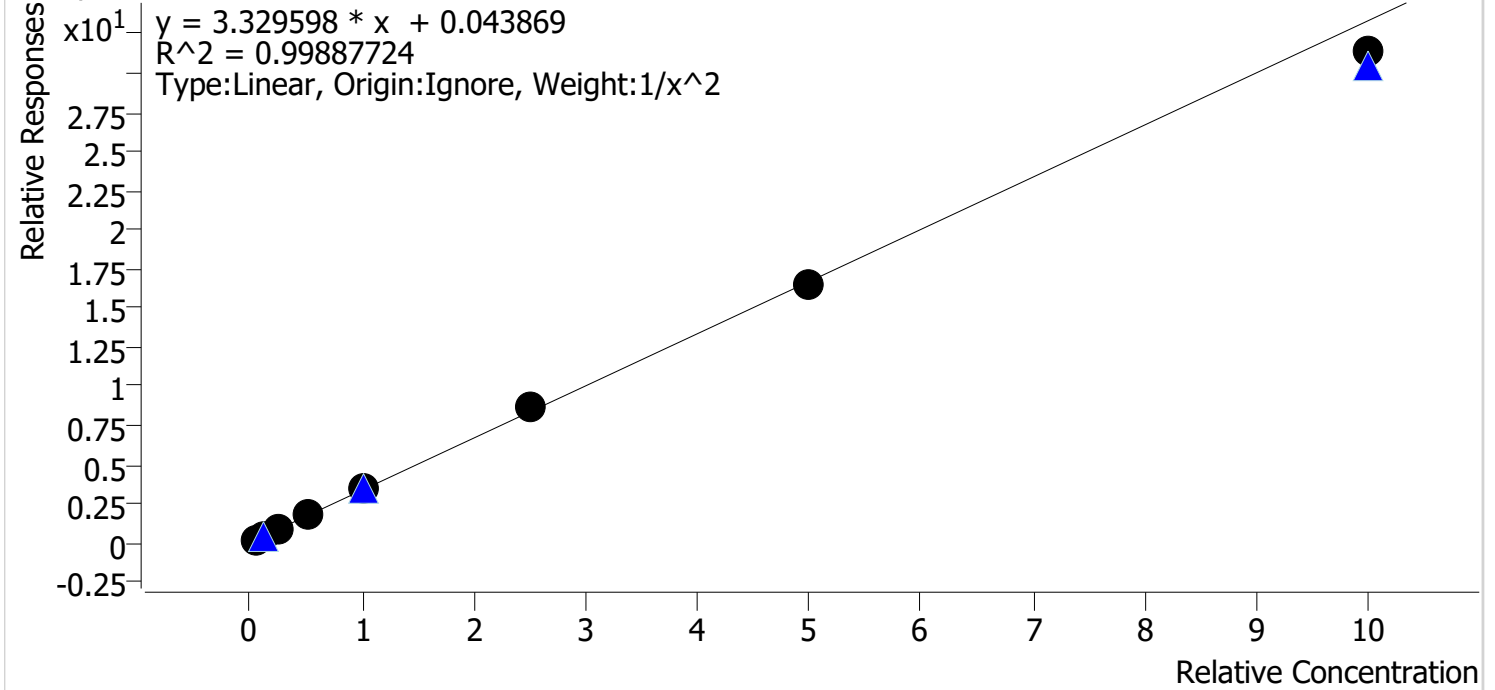
Sample Chromatogram



Compound Calibration Report

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Last Cal. Update 11/18/2019 4:20 PM
Analyst Name ISP\datastor
Analyte Chlorpheniramine **Internal Standard** acetyl-fentanyl-d5

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



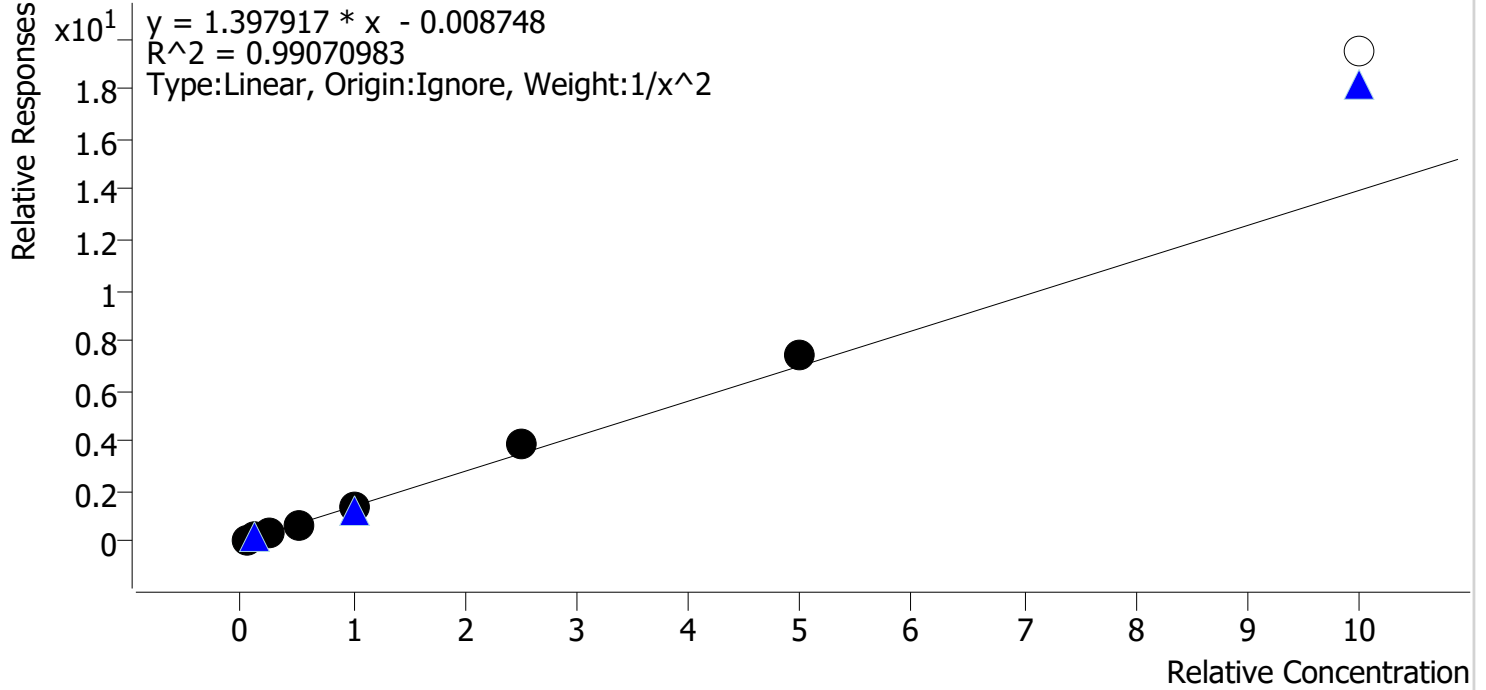
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1	1	✓	5.0	4.9	99.0
p2 cal 2	2	✓	10.0	10.1	100.5
p2 cal 3	3	✓	25.0	25.7	102.8
p2 cal 4	4	✓	50.0	50.7	101.5
p2 cal 5	5	✓	100.0	100.2	100.2
p2 cal 6	6	✓	250.0	258.2	103.3
p2 cal 7	7	✓	500.0	493.1	98.6
p2 cal 8	8	✓	1000.0	940.7	94.1

Compound Calibration Report

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Last Cal. Update 11/18/2019 4:20 PM
Analyst Name ISP\datastor
Analyte Etizolam

Internal Standard Estazolam-D5

Etizolam - 8 Levels, 7 Levels Used, 8 Points, 7 Points Used, 3 QCs



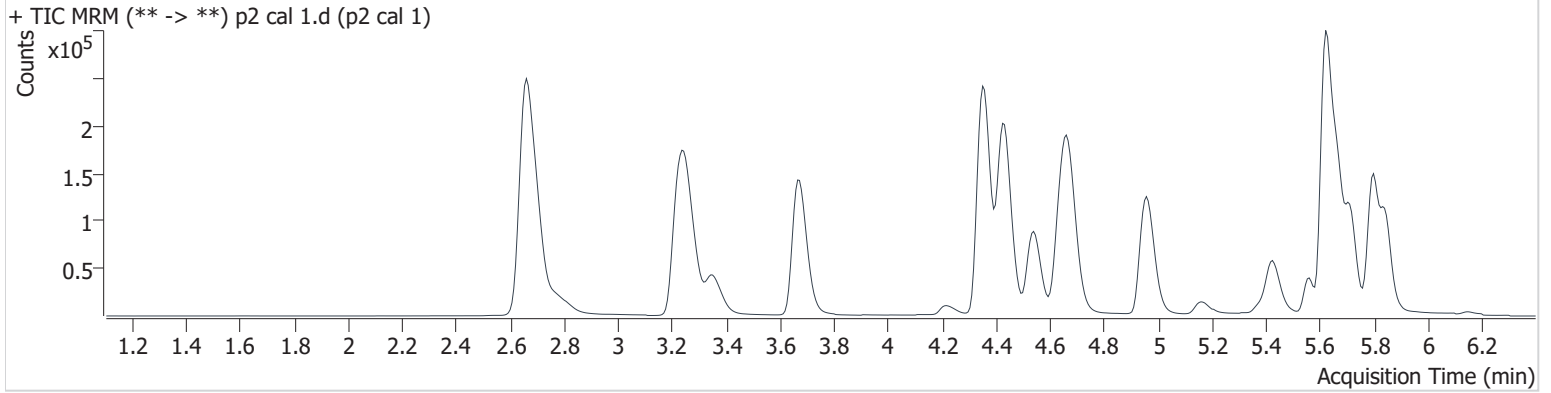
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1	1	✓	5.0	5.2	103.9
p2 cal 2	2	✓	10.0	9.8	97.9
p2 cal 3	3	✓	25.0	22.0	88.1
p2 cal 4	4	✓	50.0	46.1	92.3
p2 cal 5	5	✓	100.0	99.2	99.2
p2 cal 6	6	✓	250.0	278.3	111.3
p2 cal 7	7	✓	500.0	536.1	107.2
p2 cal 8	8	x	1000.0	1395.2	139.5

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 1.d
Type	Cal	Sample	p2 cal 1
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-A4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 3:37:07 PM		
Sample Info.			

Sample Chromatogram



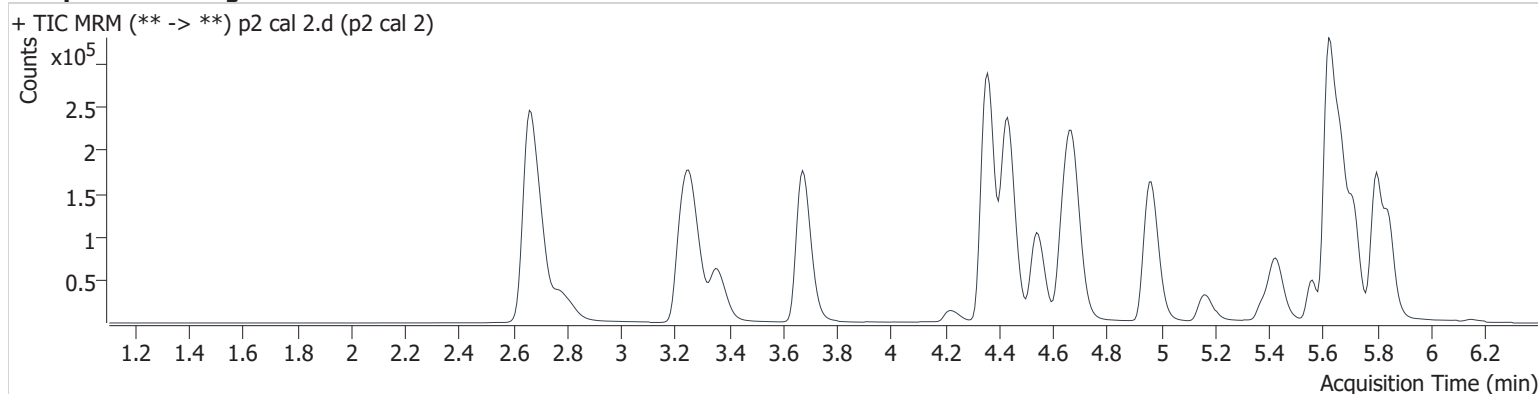
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	50241	689.0	0.5	113.9	240770	4.949 ng/ml
Etizolam	5.817	8551	7890.3	24.2	1554.8	133832	5.197 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 2.d
Type	Cal	Sample	p2 cal 2
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-B4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 3:47:56 PM		
Sample Info.			

Sample Chromatogram



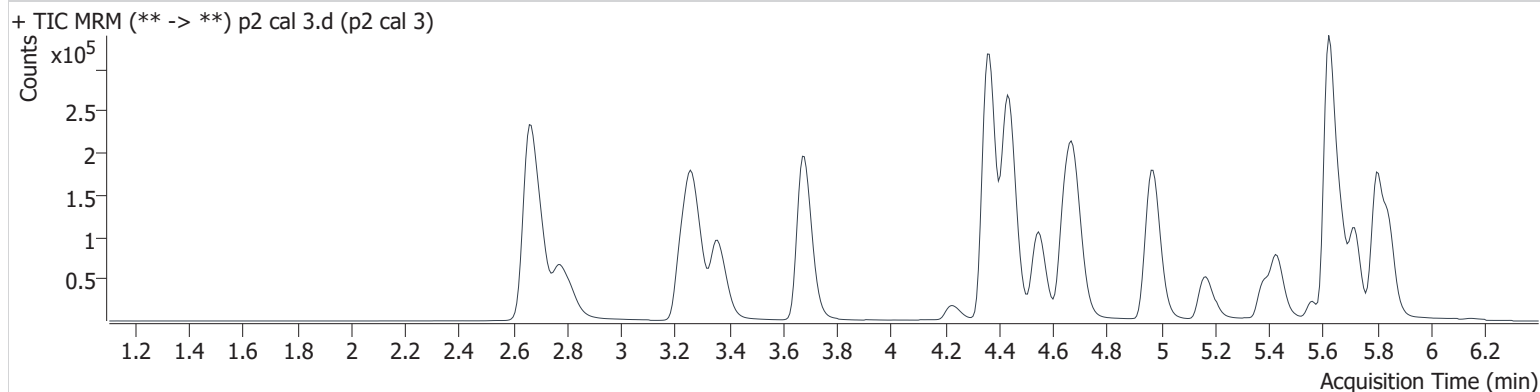
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	118716	10443.5	0.4	53.2	313568	10.053 ng/ml
Etizolam	5.817	16930	18111.1	23.1	4225.3	132123	9.792 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 3.d
Type	Cal	Sample	p2 cal 3
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-C4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 3:58:46 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.168	199382	2258.1	0.4	321.4	221605	25.704 ng/ml
Etizolam	5.824	34693	22086.2	23.9	8015.5	115975	22.025 ng/ml

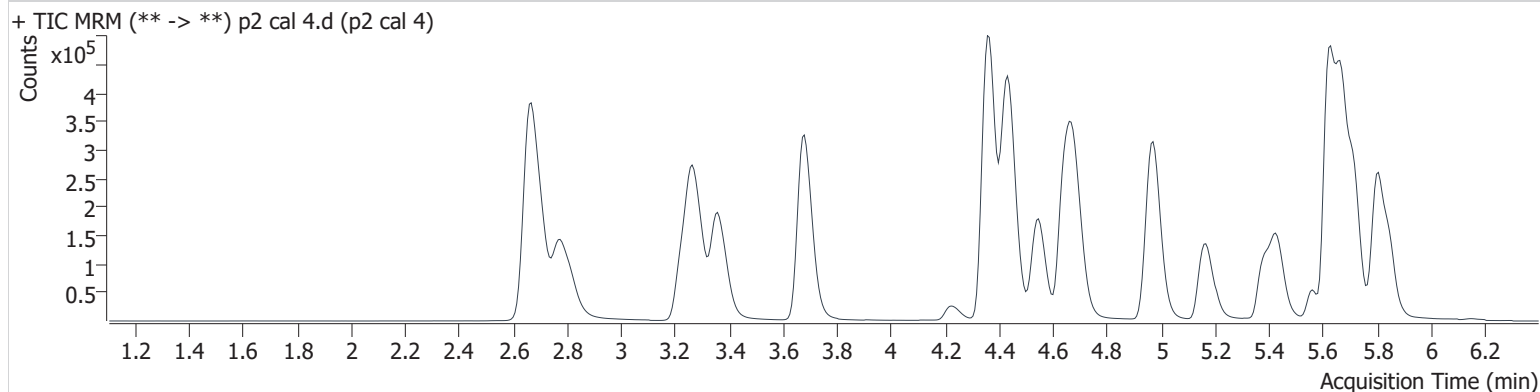


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 4.d
Type	Cal	Sample	p2 cal 4
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-D4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 4:09:35 PM		
Sample Info.			

Sample Chromatogram



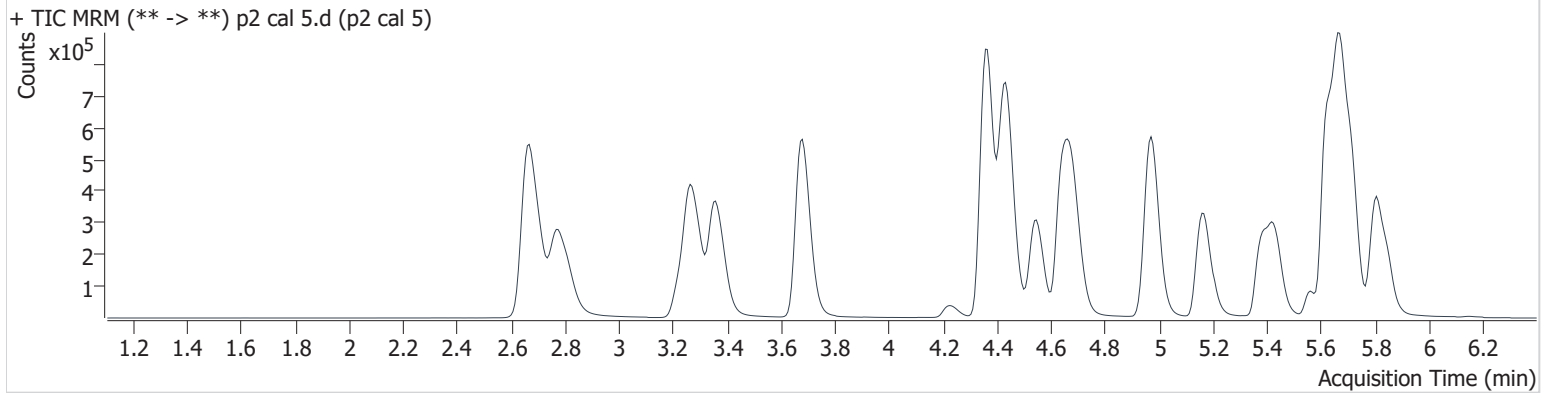
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.168	526873	3143.0	0.4	1101.3	303920	50.749 ng/ml
Etizolam	5.817	71509	27316.1	23.7	24391.8	112371	46.148 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 5.d
Type	Cal	Sample	p2 cal 5
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-E4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 4:20:23 PM		
Sample Info.			

Sample Chromatogram



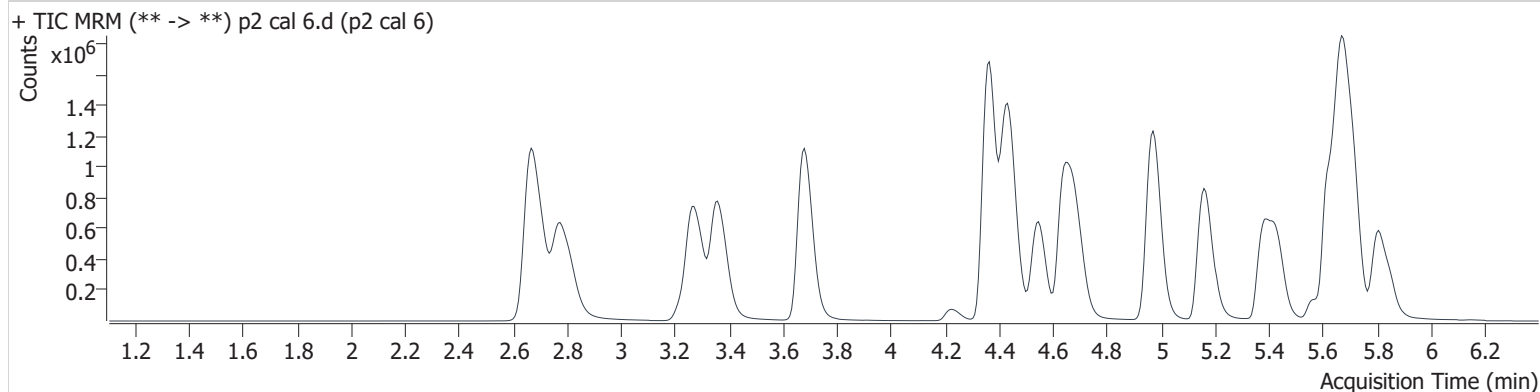
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	1284967	241690.4	0.4	1340.0	380173	100.195 ng/ml
Etizolam	5.824	135970	11422.2	23.8	6054.6	98685	99.188 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 6.d
Type	Cal	Sample	p2 cal 6
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-F4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 4:31:12 PM		
Sample Info.			

Sample Chromatogram



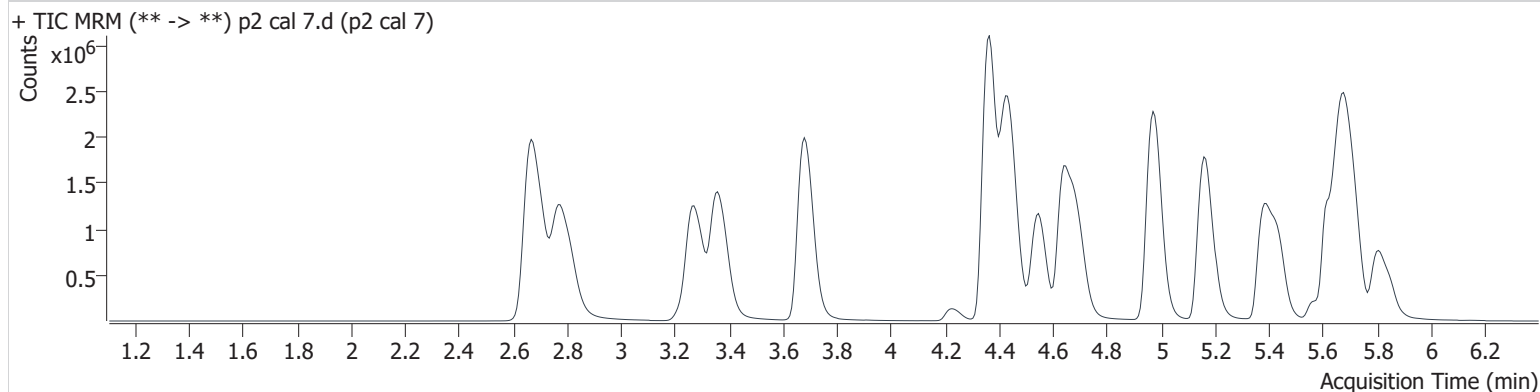
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	3412579	245253.6	0.4	979.7	394947	258.191 ng/ml
Etizolam	5.824	258237	11497.0	23.6	4106.1	66519	278.336 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 7.d
Type	Cal	Sample	p2 cal 7
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-G4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 4:42:01 PM		
Sample Info.			

Sample Chromatogram



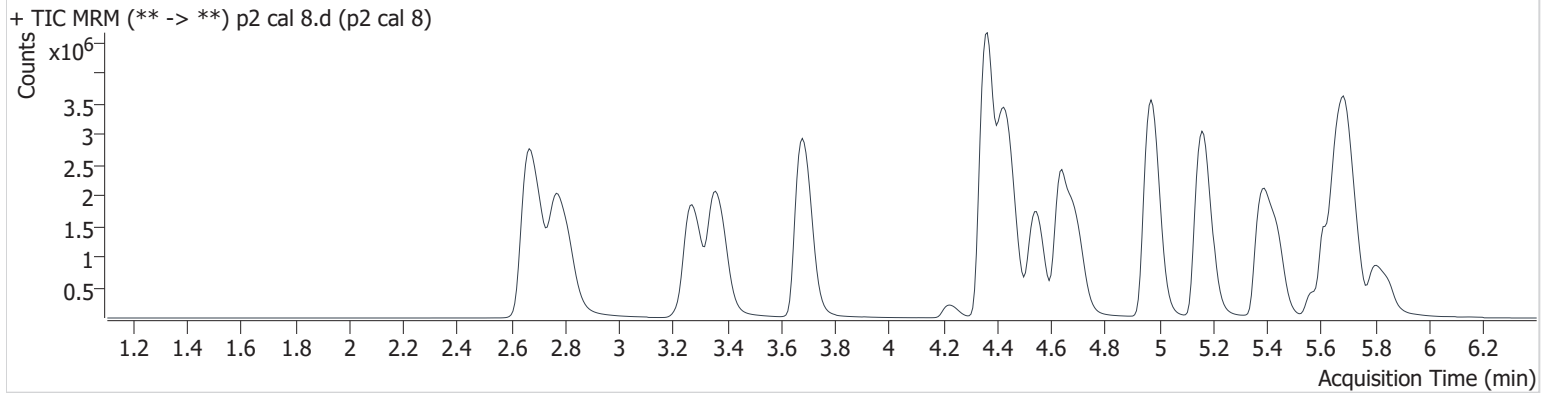
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	7203556	320892.8	0.4	1016.1	437589	493.095 ng/ml
Etizolam	5.824	413594	4662.1	23.0	3186.4	55249	536.134 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 110819\QuantResults\p2.batch.bin
Calibration Last Update 11/18/2019 4:20:46 PM

Instrument	69679	Data File	p2 cal 8.d
Type	Cal	Sample	p2 cal 8
Acq. Method	am 28 p2.m	Operator	Anne Nord
Sample Position	P2-H4	Comment	
Injection Volume	2.5		
Acq. Date-Time	11/9/2019 4:52:50 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Chlorpheniramine	5.161	13001954	44532.5	0.4	1335.4	414511	940.748 ng/ml
Etizolam	5.831	568986	1240.4	23.6	32099.1	29185	1395.250 ng/ml