

REVIEWED

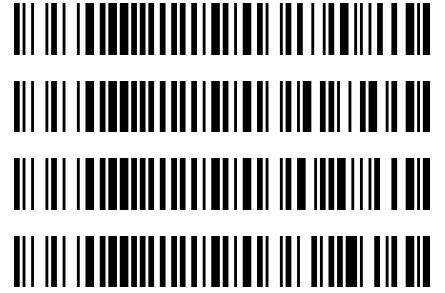
By Tamara Salazar at 9:58 am, Jun 08, 2021



6/4/2021

Worklist: 5019

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
P2021-1411	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
P2021-1445	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
P2021-1550	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
P2021-1691	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ



SC

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

Extraction Date: 06/03/21
Plate lot#: IDP-121-201207

Analyst: Sarah Collins
Plate Expiration: 06/07/2021

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: Lampire 20L20724

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

LCMS-QQQ ID: 069901

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: *Carbamazepine, Cocaethylene, and Phenazepam were the only compounds evaluated for this run.*

SC

	1	2	3	4	5	6	7	8	9	10	11	12
A	IS + Cal. 1	IS + QC_1		IS + Cal. 1	IS + QC_1				IS + Cal. 8		p2021-1550-1	IS + Cal. 8
B	IS + Cal. 2	IS + QC_2		IS + Cal. 2	IS + QC_2				IS + Cal. 7		p2021-1445-1	IS + Cal. 7
C	IS + Cal. 3	IS + QC_3		IS + Cal. 3	IS + QC_3				IS + Cal. 6		p2021-1411-1	IS + Cal. 6
D	IS + Cal. 4	IS + QC_4		IS + Cal. 4	IS + QC_4				IS + Cal. 5		negative blood	IS + Cal. 5
E	IS + Cal. 5			IS + Cal. 5				IS + QC_4	IS + Cal. 4		IS + QC_4	IS + Cal. 4
F	IS + Cal. 6			IS + Cal. 6				IS + QC_3	IS + Cal. 3		IS + QC_3	IS + Cal. 3
G	IS + Cal. 7			IS + Cal. 7				IS + QC_2	IS + Cal. 2		IS + QC_2	IS + Cal. 2
H	IS + Cal. 8			IS + Cal. 8				IS + QC_1	IS + Cal. 1	p2021-1691-1	IS + QC_1	IS + Cal. 1

All wells to contain 60 µl of Trapping Solution

SC

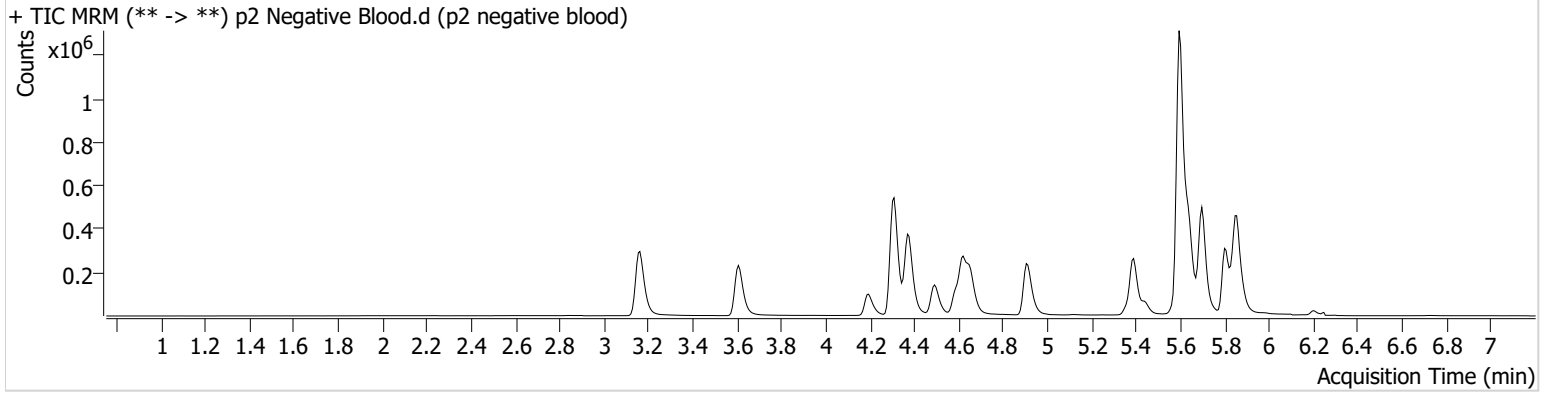


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Negative Blood.d
Type	Sample	Sample	p2 negative blood
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-D11	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 11:27:28 AM		
Sample Info.			

Sample Chromatogram



SC

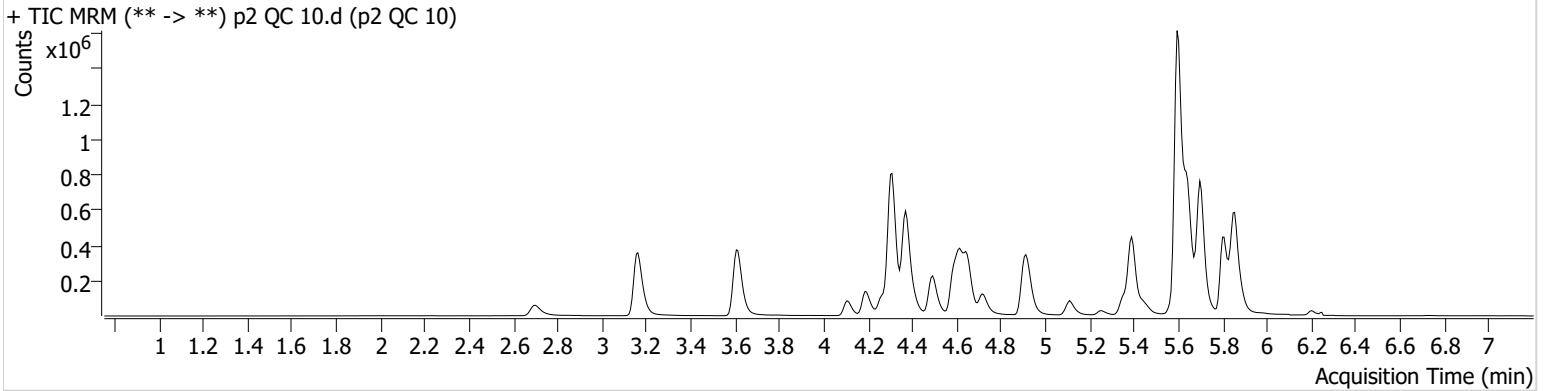


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 QC 10.d
Type	QC	Sample	p2 QC 10
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-H11	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 10:13:15 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	499079	3783.17	9.3	297.13	3083434	10.1069 ng/ml
Cocaethylene	4.714	179198	3123.50	65.3	6300.36	164744	10.5766 ng/ml
Phenazepam	5.886	19349	23662.31	67.9	19421.99	88575	9.7760 ng/ml

SC

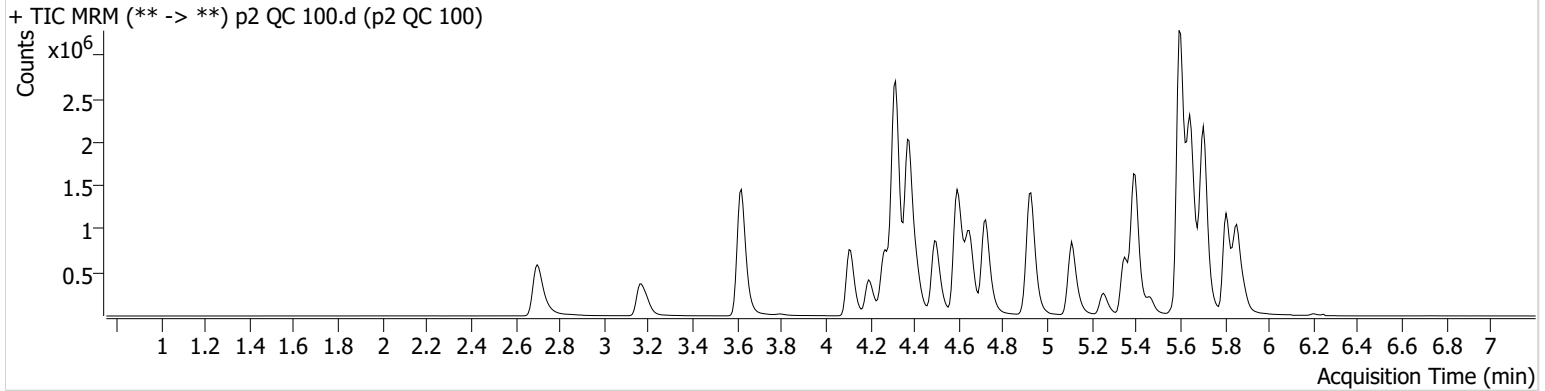


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 QC 100.d
Type	QC	Sample	p2 QC 100
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-G11	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 12:31:04 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	4473799	3190.70	9.5	4013.74	2674302	100.5334 ng/ml
Cocaethylene	4.721	1794658	3394635.01	65.3	2476.05	127854	136.1703 ng/ml
Phenazepam	5.886	168799	280322.71	68.8	70316.69	76548	102.3191 ng/ml

SC

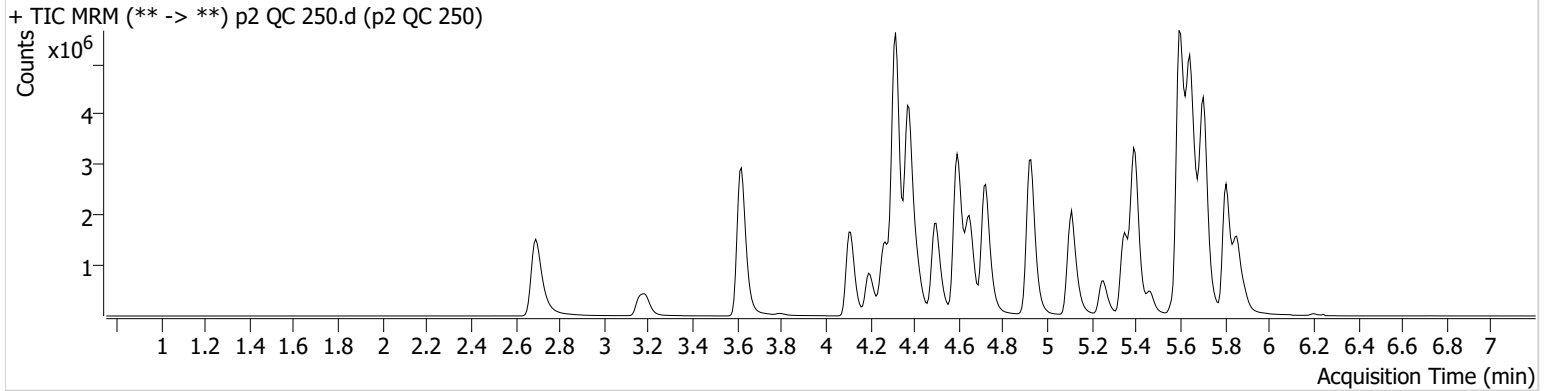


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 QC 250.d
Type	QC	Sample	p2 QC 250
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-F11	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 10:34:26 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	9752747	13648.32	9.9	2033.03	2174799	268.7890 ng/ml
Cocaethylene	4.721	4356114	215211.50	63.4	85971.79	167571	252.1590 ng/ml
Phenazepam	5.886	349726	416551.15	67.1	271503.90	64267	253.0862 ng/ml

SC

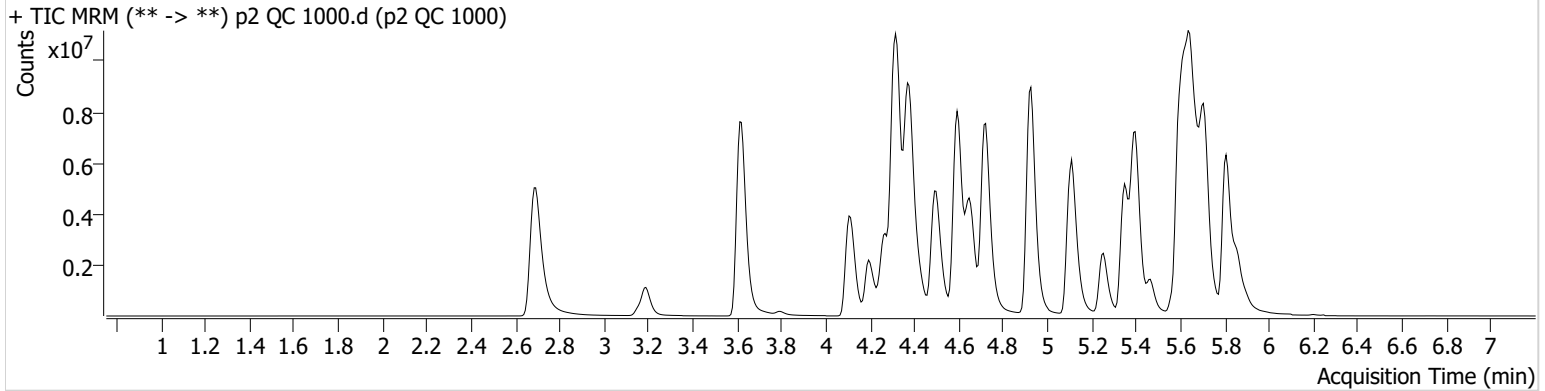


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 QC 1000.d
Type	QC	Sample	p2 QC 1000
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-E11	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 10:55:40 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	17696837	200892.17	11.9	16323.96	1099213	963.8885 ng/ml
Cocaethylene	4.721	13377203	499275.07	64.8	429756.73	129853	999.1995 ng/ml
Phenazepam	5.893	704305	630565.13	67.0	434197.02	35944	912.3524 ng/ml

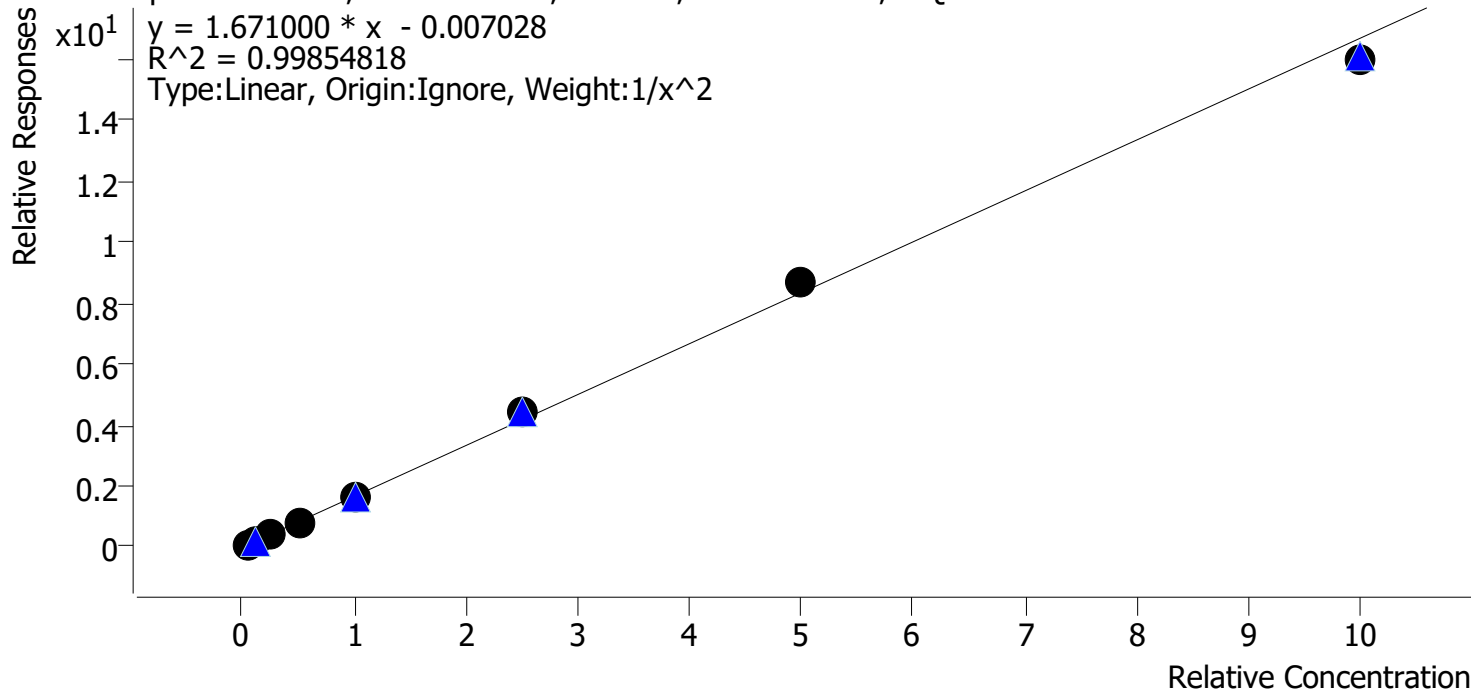
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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Last Cal. Update 6/7/2021 8:59 AM
Analyst Name ISP\datastor
Analyte Carbamazepine **Internal Standard** Carbamazepine-13C6

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	5.1	101.7
p2 Cal 2- 10ng	2	✓	10.0	9.8	97.6
p2 Cal 3 -25ng	3	✓	25.0	24.5	97.8
p2 Cal 4-50ng	4	✓	50.0	48.8	97.6
p2 Cal 5-100ng	5	✓	100.0	100.3	100.3
p2 Cal 6-250ng	6	✓	250.0	262.7	105.1
p2 Cal 7-500ng	7	✓	500.0	519.6	103.9
p2 Cal 8-1000ng	8	✓	1000.0	959.4	95.9

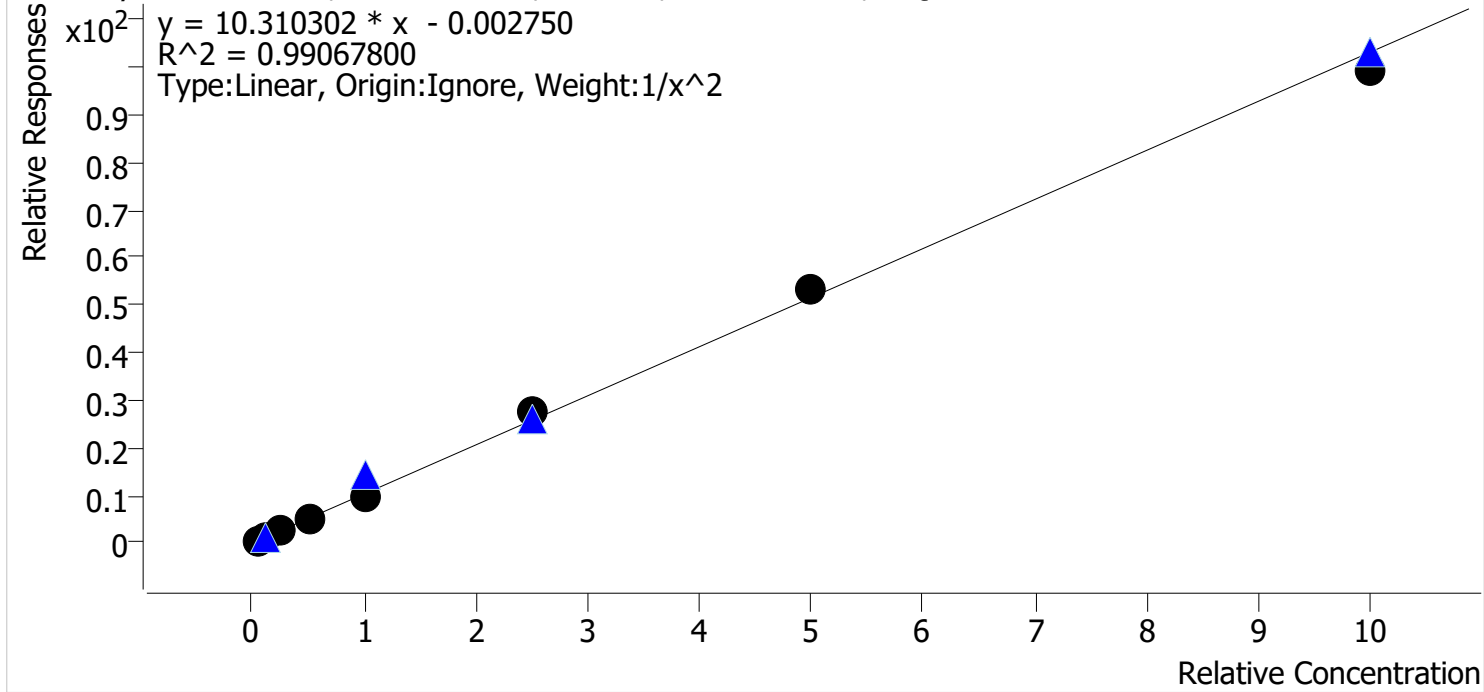
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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Last Cal. Update 6/7/2021 8:59 AM
Analyst Name ISP\datastor
Analyte Cocaethylene **Internal Standard** Doxepin-D3

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



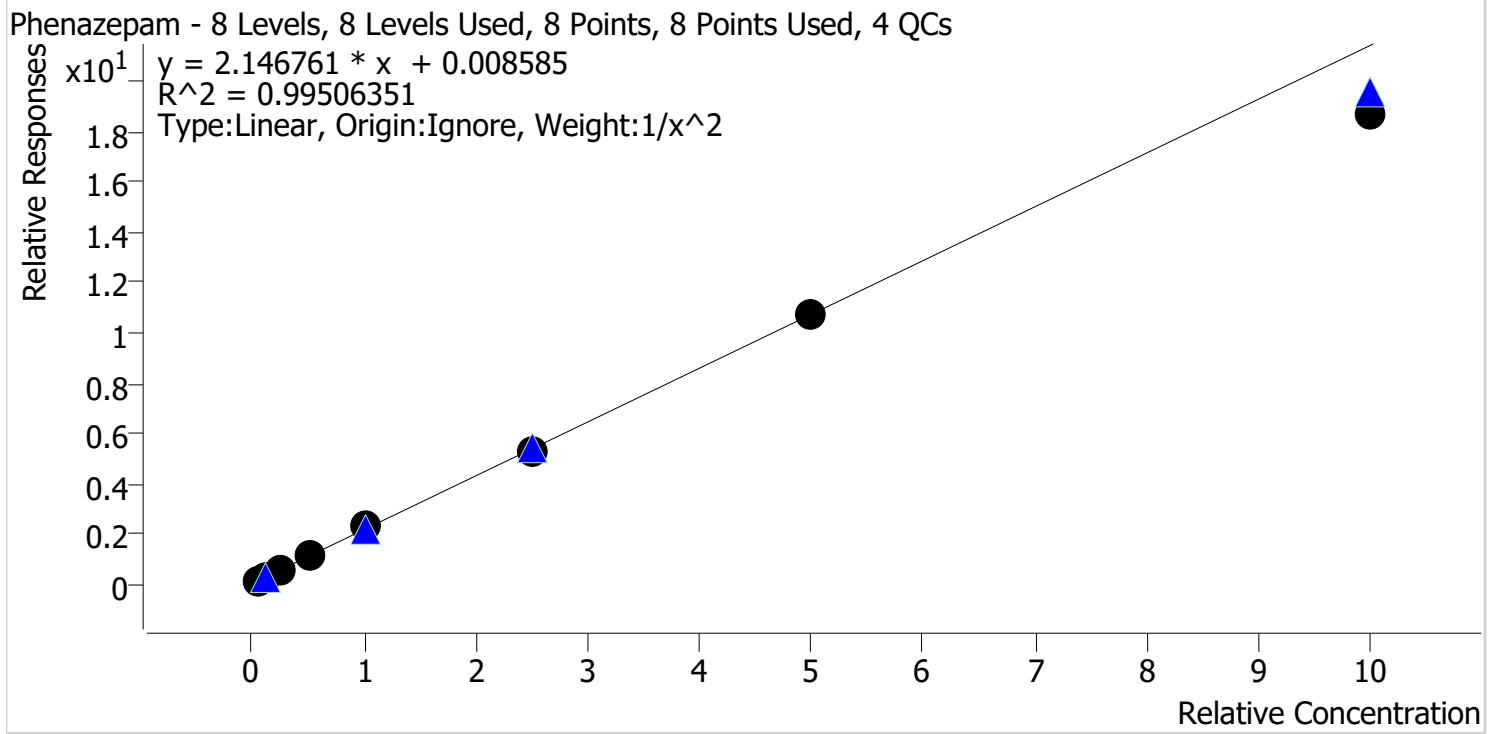
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	4.7	93.4
p2 Cal 2- 10ng	2	✓	10.0	11.6	116.3
p2 Cal 3 -25ng	3	✓	25.0	23.4	93.8
p2 Cal 4-50ng	4	✓	50.0	50.0	100.0
p2 Cal 5-100ng	5	✓	100.0	90.8	90.8
p2 Cal 6-250ng	6	✓	250.0	264.4	105.8
p2 Cal 7-500ng	7	✓	500.0	518.8	103.8
p2 Cal 8-1000ng	8	✓	1000.0	960.7	96.1

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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Last Cal. Update 6/7/2021 8:59 AM
Analyst Name ISP\datastor
Analyte Phenazepam Internal Standard Phenazepam-D4



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	4.9	97.5
p2 Cal 2- 10ng	2	✓	10.0	10.2	101.9
p2 Cal 3 -25ng	3	✓	25.0	26.0	103.9
p2 Cal 4-50ng	4	✓	50.0	53.4	106.7
p2 Cal 5-100ng	5	✓	100.0	104.5	104.5
p2 Cal 6-250ng	6	✓	250.0	247.6	99.0
p2 Cal 7-500ng	7	✓	500.0	497.6	99.5
p2 Cal 8-1000ng	8	✓	1000.0	869.7	87.0

SC

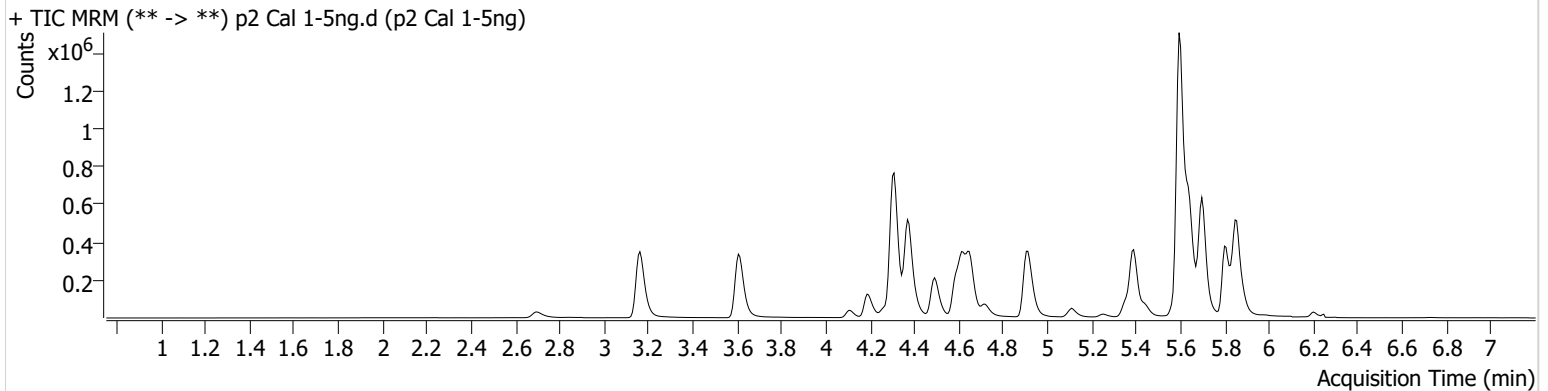


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 1-5ng.d
Type	Cal	Sample	p2 Cal 1-5ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-H12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 8:27:00 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	239634	1621.60	8.9	108.52	3073785	5.0861 ng/ml
Cocaethylene	4.721	91477	146729.63	67.3	112448.98	191090	4.6697 ng/ml
Phenazepam	5.886	9911	4894.32	68.9	8998.62	87544	4.8739 ng/ml

SC

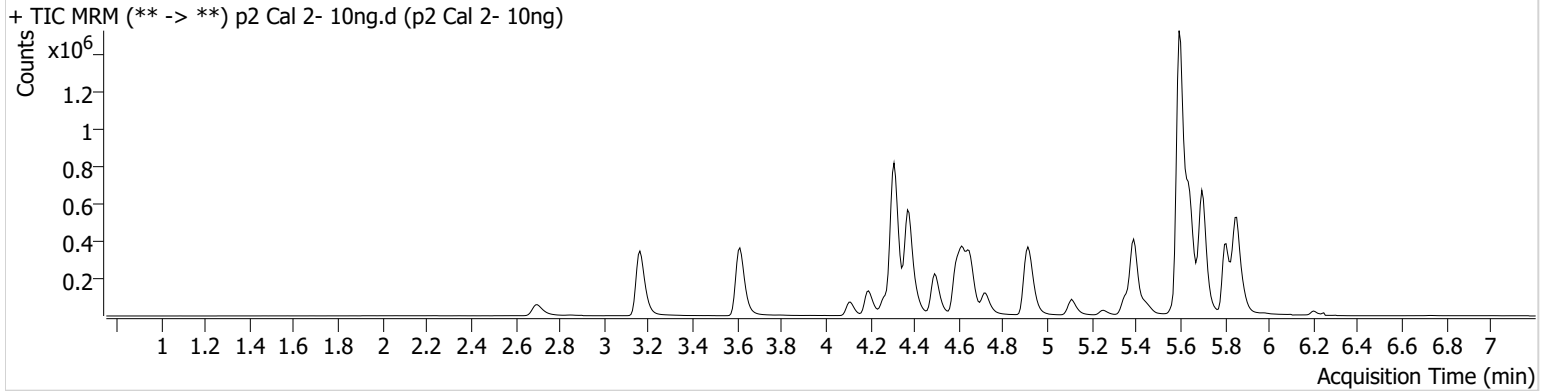


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 2- 10ng.d
Type	Cal	Sample	p2 Cal 2- 10ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-G12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 8:37:54 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	460415	3496.85	9.1	518.08	2949183	9.7633 ng/ml
Cocaethylene	4.721	176889	270560.94	64.6	197489.46	147811	11.6338 ng/ml
Phenazepam	5.886	18682	506.09	68.1	16847.73	82204	10.1863 ng/ml

SC

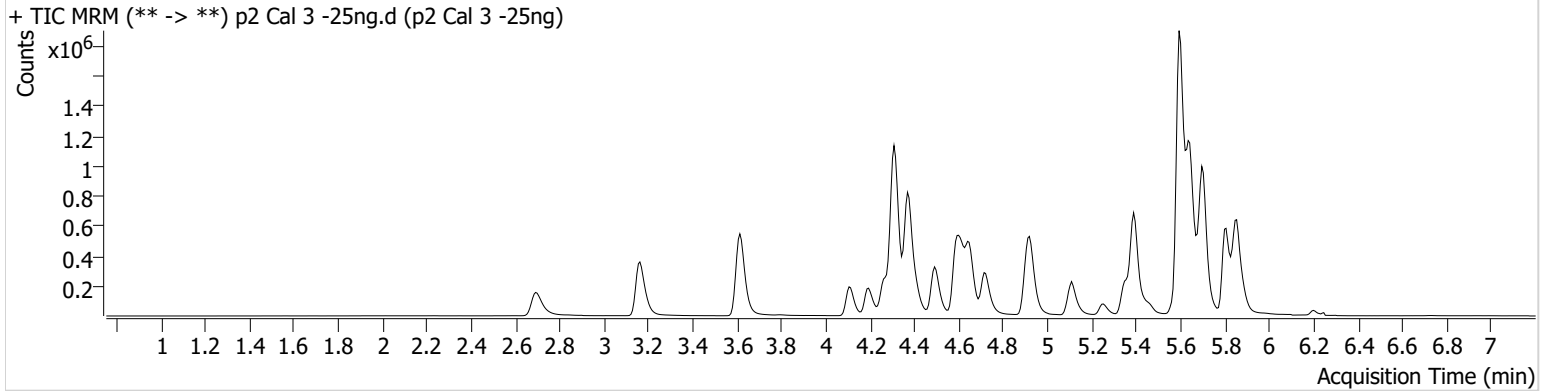


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 3 -25ng.d
Type	Cal	Sample	p2 Cal 3 -25ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-F12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 8:48:29 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	1178218	5071.63	9.3	390.93	2933090	24.4600 ng/ml
Cocaethylene	4.714	444350	762154.71	65.9	2783.47	183997	23.4498 ng/ml
Phenazepam	5.886	48021	84829.84	68.4	55266.36	84808	25.9759 ng/ml

SC

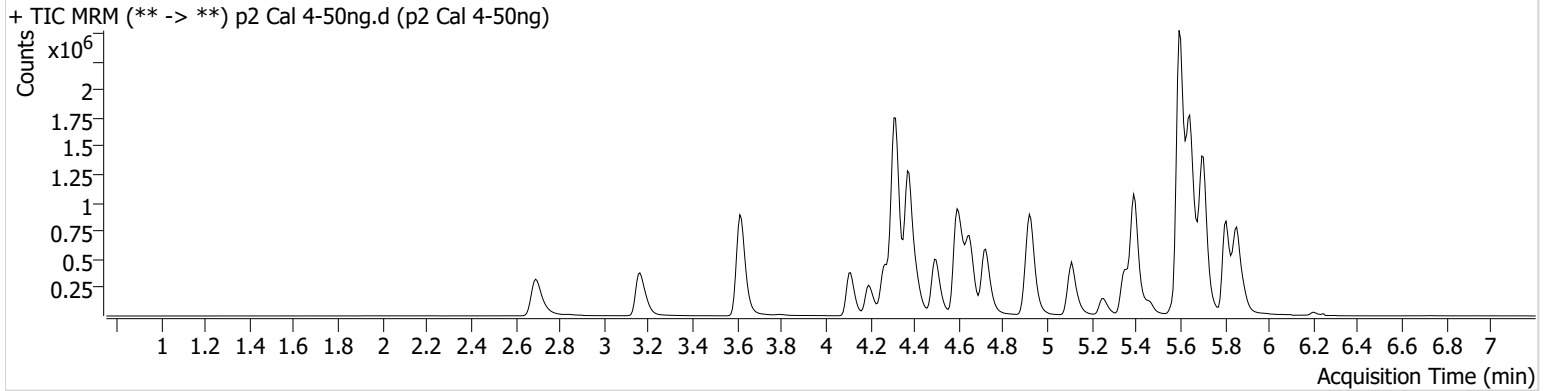


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 4-50ng.d
Type	Cal	Sample	p2 Cal 4-50ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-E12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 8:59:05 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	2397411	87859.29	9.6	2537.14	2965630	48.7987 ng/ml
Cocaethylene	4.721	948860	874.27	65.2	1128640.47	184111	50.0131 ng/ml
Phenazepam	5.886	96679	120077.20	69.4	96142.47	83761	53.3660 ng/ml

SC

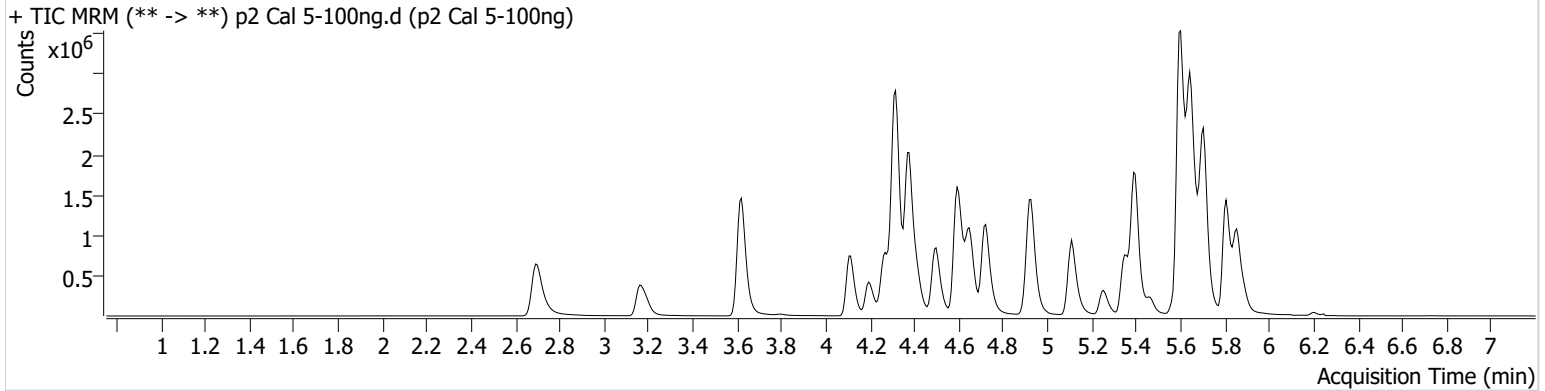


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 5-100ng.d
Type	Cal	Sample	p2 Cal 5-100ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-D12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 9:09:41 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	4676499	13094.22	9.7	1891.58	2802657	100.2767 ng/ml
Cocaehtylene	4.720	1843560	263233.52	64.6	52702.17	196927	90.8254 ng/ml
Phenazepam	5.886	184111	191634.20	65.7	211315.11	81747	104.5119 ng/ml

SC

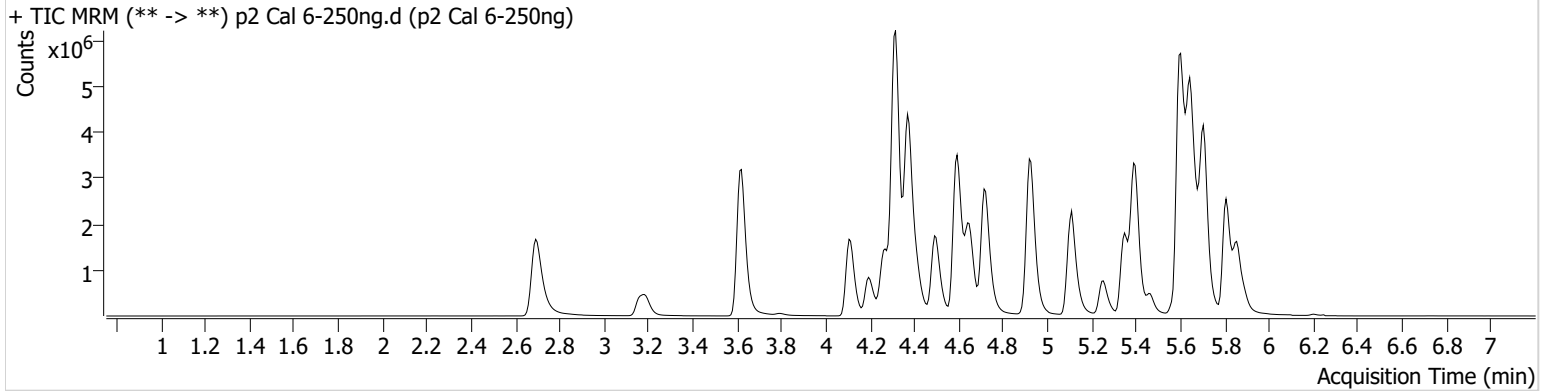


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 6-250ng.d
Type	Cal	Sample	p2 Cal 6-250ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-C12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 9:20:18 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	9887874	152831.29	10.3	4024.45	2256322	262.6766 ng/ml
Cocaethylene	4.714	4645373	31981.89	63.2	291775.71	170398	264.4413 ng/ml
Phenazepam	5.886	362023	406989.48	67.5	268807.49	68008	247.5652 ng/ml

SC

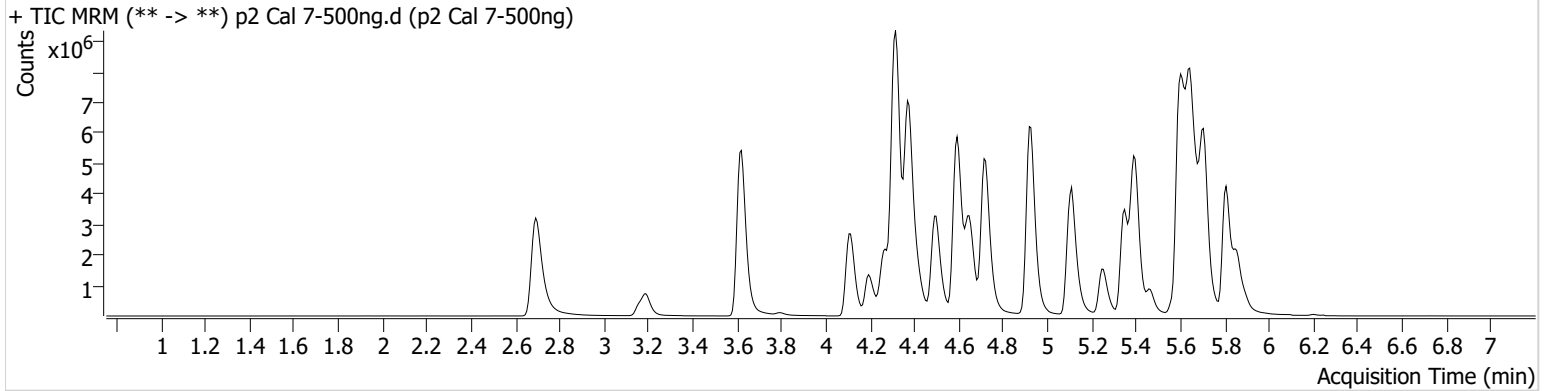


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 7-500ng.d
Type	Cal	Sample	p2 Cal 7-500ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-B12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 9:30:54 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	14710197	147329.04	10.8	5865.78	1695575	519.6092 ng/ml
Cocaethylene	4.714	8812118	360558.91	64.3	133319.05	164738	518.8454 ng/ml
Phenazepam	5.886	551944	483536.00	65.6	240623.26	51632	497.5534 ng/ml

SC

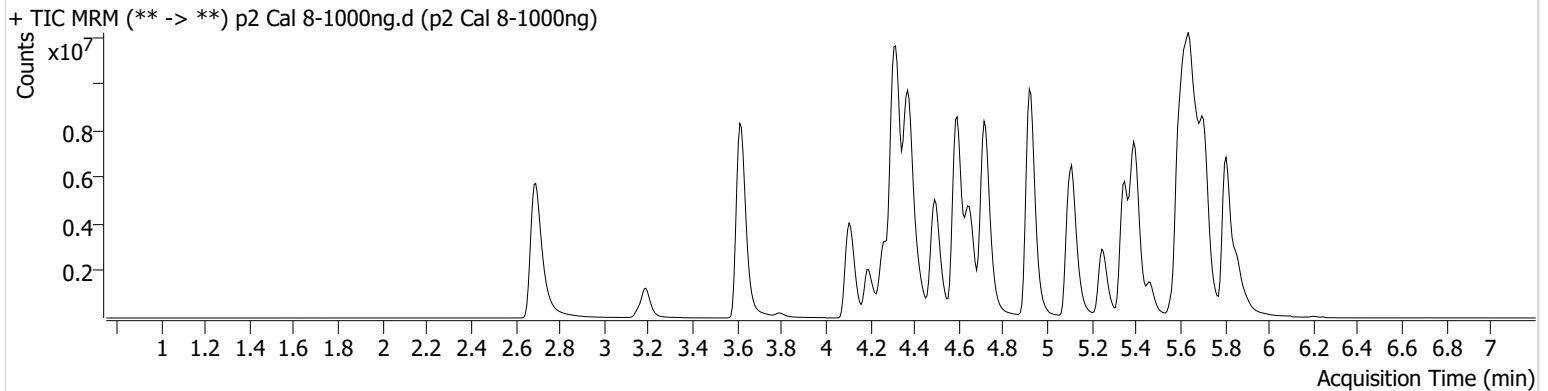


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\060321 AM 27 28 SC\QuantResults\AM 28 P2.batch.bin
Calibration Last Update 6/7/2021 8:59:45 AM

Instrument	Falco (069901)	Data File	p2 Cal 8-1000ng.d
Type	Cal	Sample	p2 Cal 8-1000ng
Acq. Method	AM 28 MDQ P2.m	Operator	Sarah Collins
Sample Position	P6-A12	Comment	
Injection Volume	2		
Acq. Date-Time	6/4/2021 9:41:29 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Carbamazepine	5.598	17963808	9861.95	11.8	10652.45	1121030	959.3902 ng/ml
Cocaine	4.714	15144342	136219.46	64.8	267495.46	152895	960.7235 ng/ml
Phenazepam	5.893	715777	566907.73	67.9	643063.98	38318	869.7436 ng/ml