9/9/2019

Worklist: 3665

REVIEWED
By Sarah Pickle at 11:40 am, Sep 10, 2019

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-1439	1	162861	AM 28 Blood Multi-Drug Quant Panel 2 by LC-Q(
C2019-1531	1	162862	AM 28 Blood Multi-Drug Quant Panel 2 by LC-Q(
C2019-1564	1	162863	AM 28 Blood Multi-Drug Quant Panel 2 by LC-Q(
C2019-1589	1	162860	AM 28 Blood Multi-Drug Quant Panel 2 by LC-Q(

REVIEWED
By Britany Wylie at 2:23 pm, Sep 11, 2019

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

Extraction Date 09-6-19 Analyst: Anne Nord
Plate lot#: 190730 (part IDP-112) Plate Expiration: 1/30/20

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: 445283-2 Hemostat
Column: Agilent 120 EC-C18 (2.1x 100-~~4~~2.7um) 9/18/19
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. Pipette **250µL blood (calibrated pipette) Pipette ID: 1926134** in wells of analytical (standards) plate.
☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
☒ 4. Pipette **250µL 00.5M 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** 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 R^2 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:
MDA, MDMA, Methocarbamol, Levamisole, and pseudoephedrine/ephedrine evaluated.
Limited curve range: methocarbamol 5-250

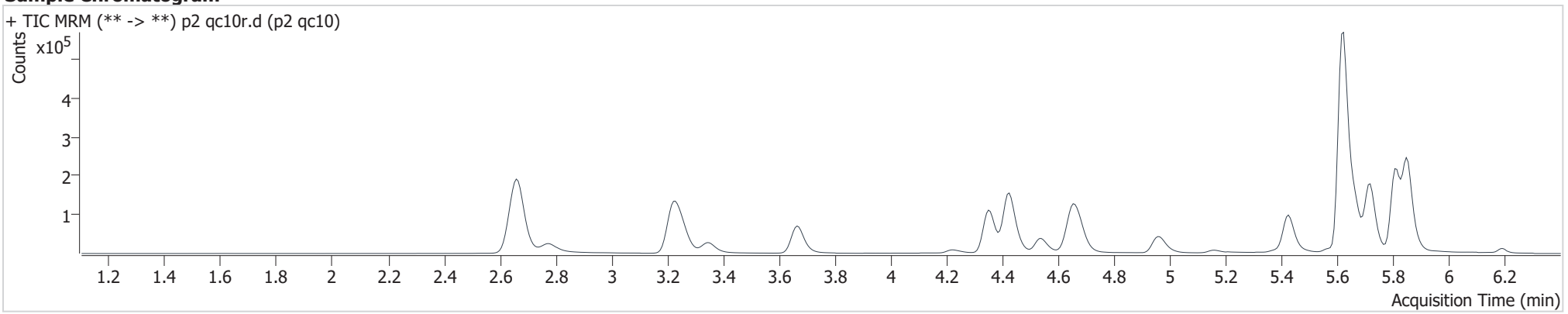
Part way through the run the pressure on the instrument maxed out. The guard column was replaced and the samples that had previously injected were re-injected. The re-injection data was used for evaluation.

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 qc10r.d
Type	QC	**Sample**	p2 qc10
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-A6	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 12:49:03 PM		
Sample Info.			

Sample Chromatogram



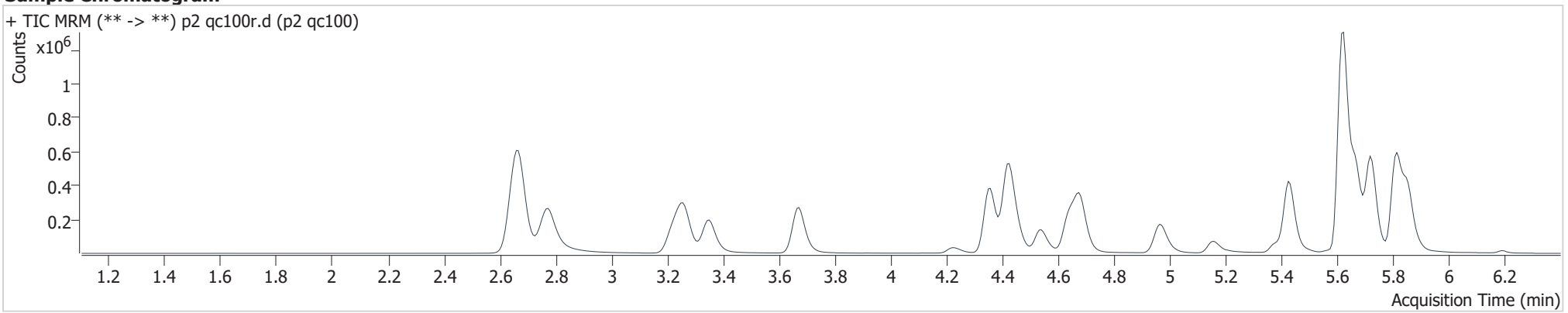
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.774	52447	2904.2	84.3	459.46	611917	10.202 ng/ml
MDA	3.266	50687	873.8	26.2	503.86	129859	10.531 ng/ml
MDMA	3.351	33205	337.52	77.7	1733.7	32277	9.789 ng/ml
Methocarbamol	4.453	14895	973.05	96.1	3063.9	129859	10.720 ng/ml
Pseudoephedrine	2.668	133550	3617.9	17.1	1028.7	611917	10.231 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 qc100r.d
Type	QC	**Sample**	p2 qc100
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-B6	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 12:59:52 PM		
Sample Info.			

Sample Chromatogram



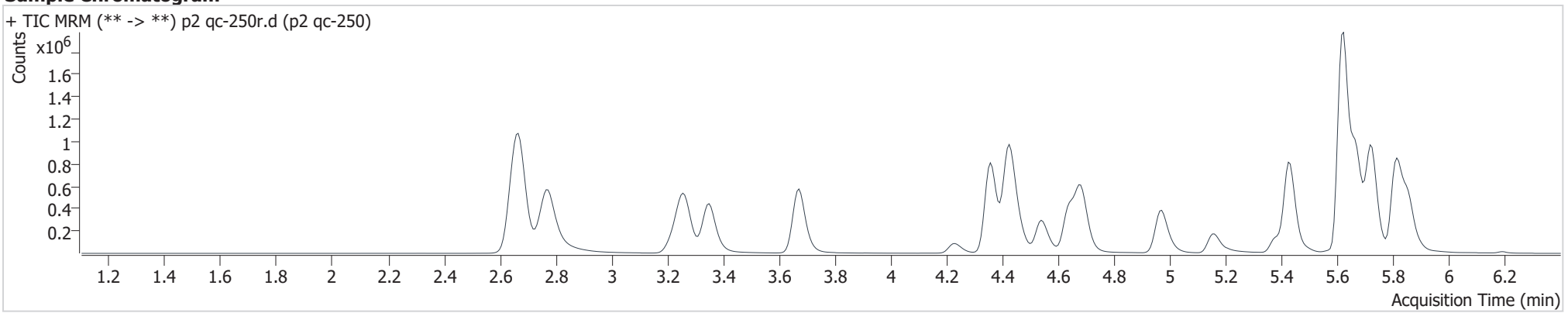
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.774	602478	36887	85.6	20348	719070	96.471 ng/ml
MDA	3.266	481230	15738	26.5	10384	128175	103.955 ng/ml
MDMA	3.351	353993	903.92	77.0	30471	34888	97.790 ng/ml
Methocarbamol	4.453	128600	10503	93.7	1122.4	128175	105.807 ng/ml
Pseudoephedrine	2.668	1520034	635.1	17.4	1107.3	719070	97.076 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 qc-250r.d
Type	QC	**Sample**	p2 qc-250
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-C6	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 1:10:39 PM		
Sample Info.			

Sample Chromatogram



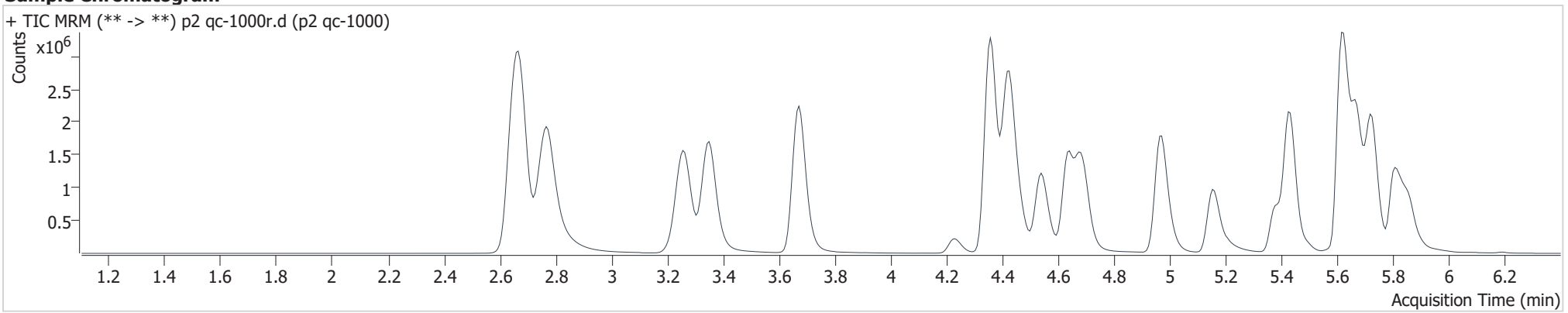
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.774	1323865	1336.2	85.5	1982.7	626428	242.767 ng/ml
MDA	3.266	1013118	20870	26.4	1859.5	112205	250.434 ng/ml
MDMA	3.351	830555	98615	77.0	2509.4	33669	237.949 ng/ml
Methocarbamol	4.453	246828	60107	94.0	15216	112205	233.836 ng/ml
Pseudoephedrine	2.668	3253033	2.3646E+05	17.4	893.05	626428	238.140 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 qc-1000r.d
Type	QC	**Sample**	p2 qc-1000
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-D6	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 1:21:26 PM		
Sample Info.			

Sample Chromatogram



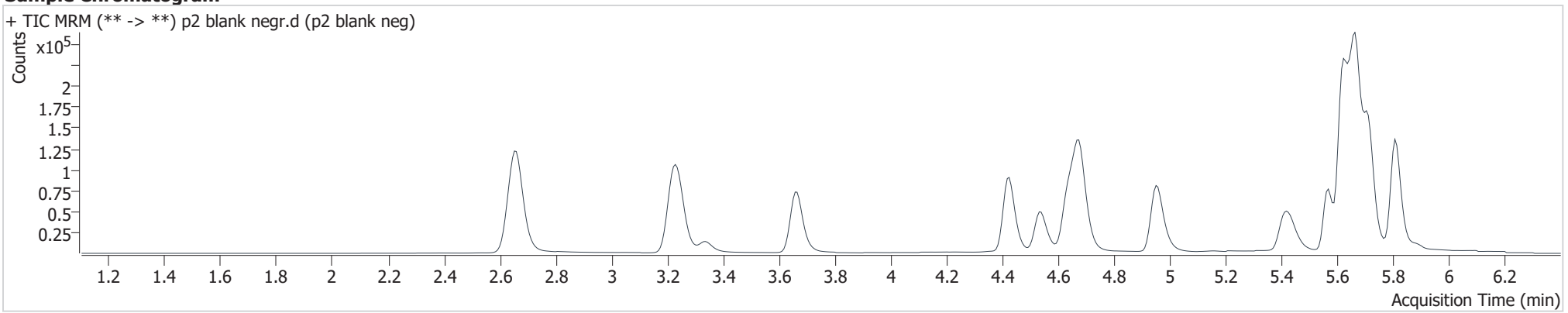
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
Levamisole	2.767	4794191	2800.9	85.6	81076	522877	1052.015	ng/ml	
MDA	3.266	3175724	9869.4	26.3	3822.8	91228	966.404	ng/ml	
MDMA	3.351	3509427	2499.9	77.4	1.379E+05	35393	956.874	ng/ml	9/9/19
Methocarbamol	4.459	531025	52409	92.5	52831	91228	~~621.315~~	~~ng/ml~~	outside curve range
Pseudoephedrine	2.668	11385037	4.1443E+05	16.8	563.28	522877	997.763	ng/ml	

AM #28 Multi-Drug Quant. Results

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update	9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 blank negr.d
Type	Sample	**Sample**	p2 blank neg
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	Vial 4	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 1:32:14 PM		
Sample Info.			

Sample Chromatogram

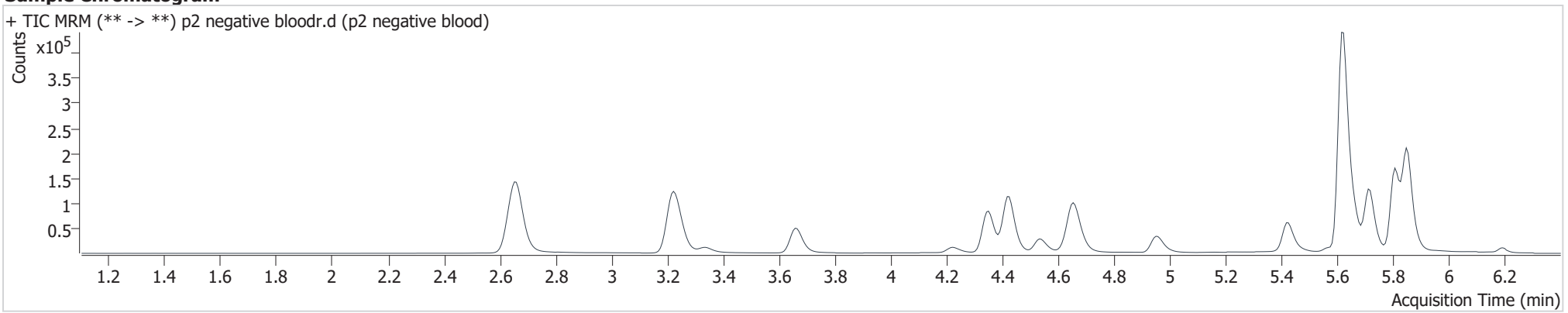


AM #28 Multi-Drug Quant. Results

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update	9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 negative bloodr.d
Type	Sample	**Sample**	p2 negative blood
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-E6	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 1:42:55 PM		
Sample Info.			

Sample Chromatogram



Compound Calibration Report

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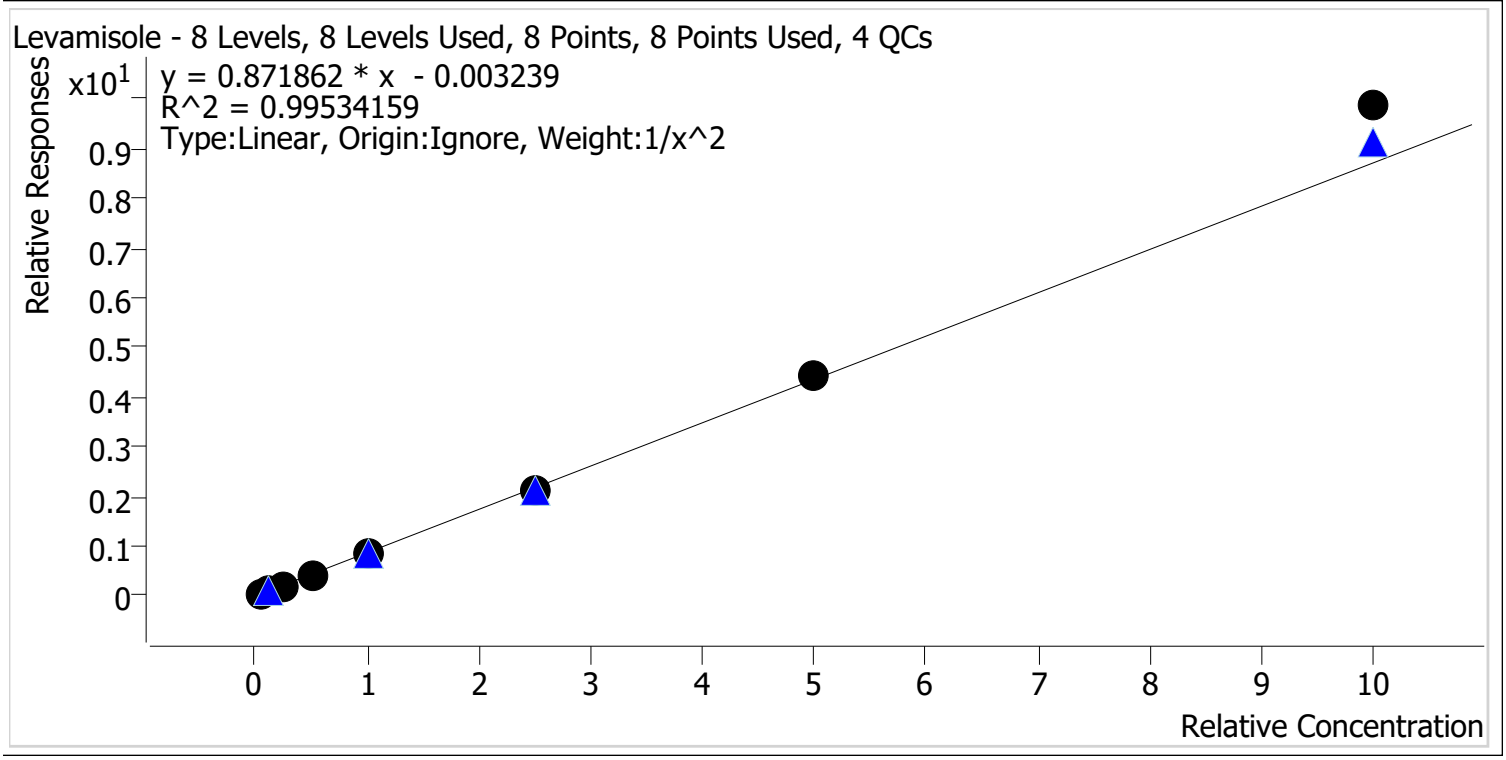
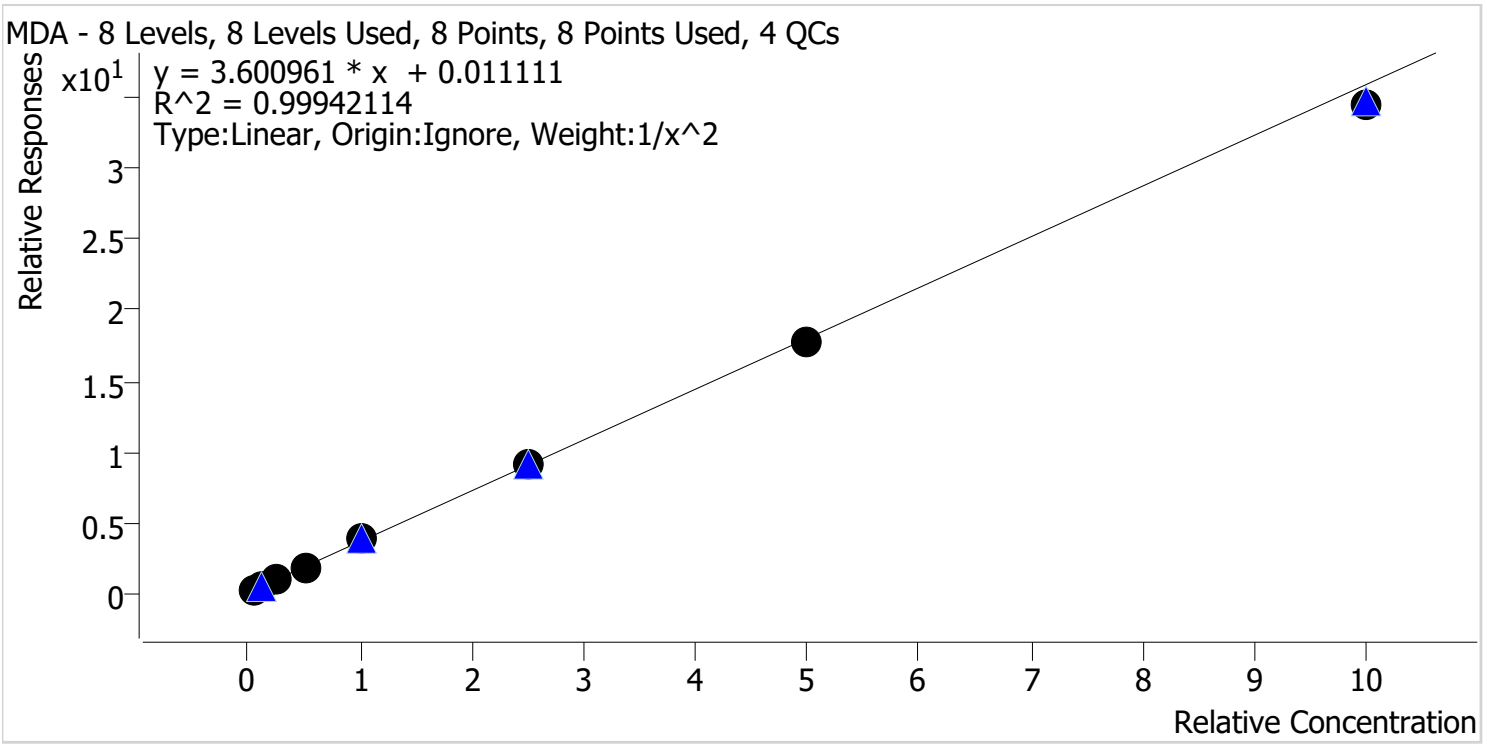
Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Last Cal. Update	9/9/2019 9:01 AM
Analyst Name	ISP\datastor
Analyte	Levamisole
Internal Standard	Pseudoephedrine-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1	1	✔	5.0	5.1	102.3
p2 cal 2	2	✔	10.0	9.8	98.1
p2 cal 3	3	✔	25.0	24.2	96.6
p2 cal 4	4	✔	50.0	47.2	94.4
p2 cal 5	5	✔	100.0	96.9	96.9
p2 cal 6	6	✔	250.0	242.9	97.2
p2 cal 7	7	✔	500.0	506.6	101.3
p2 cal 8	8	✔	1000.0	1132.4	113.2

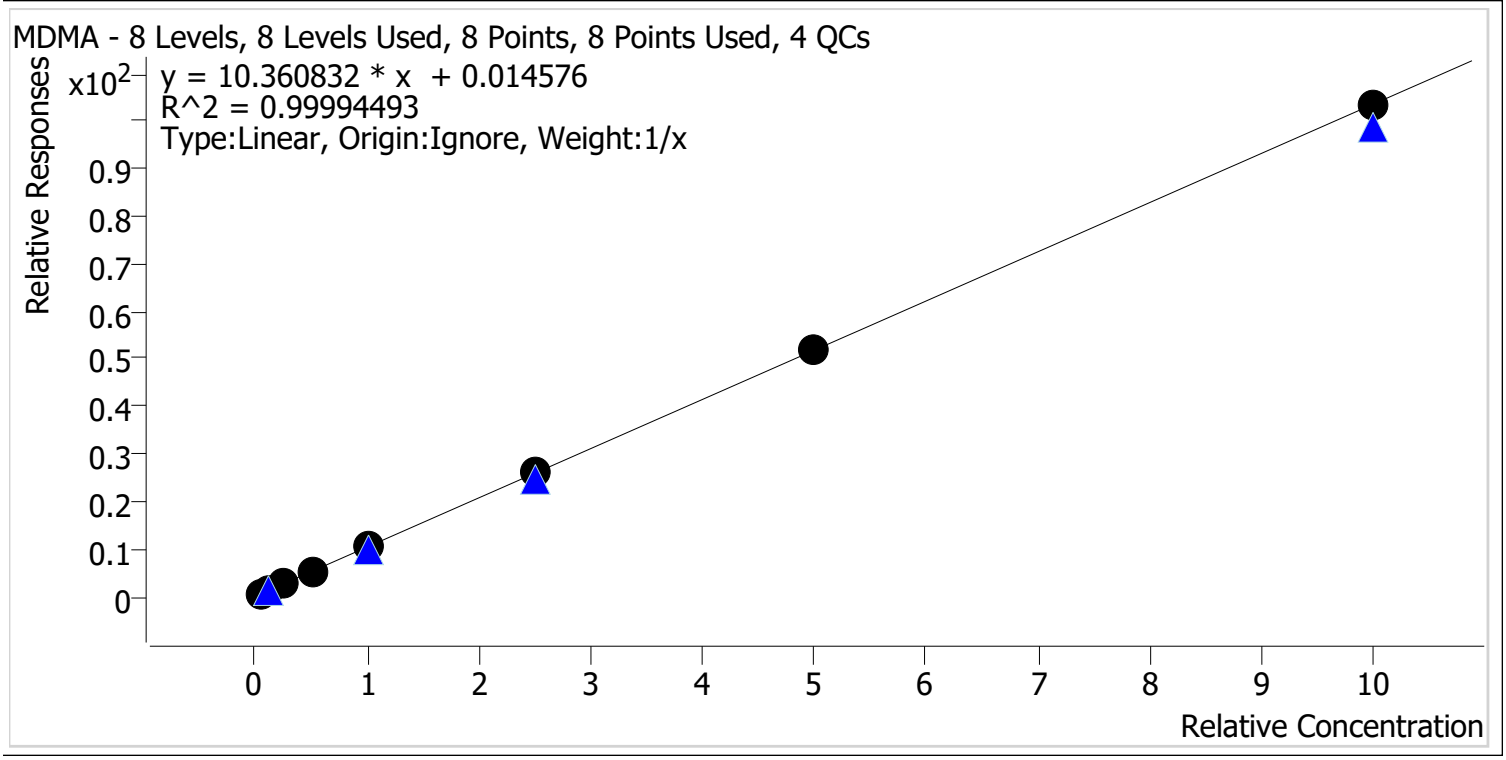
Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Last Cal. Update	9/9/2019 9:01 AM
Analyst Name	ISP\datastor
Analyte	MDA
Internal Standard	MDA-D5



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1	1	✔	5.0	5.0	99.0
p2 cal 2	2	✔	10.0	10.1	101.4
p2 cal 3	3	✔	25.0	25.1	100.3
p2 cal 4	4	✔	50.0	50.3	100.6
p2 cal 5	5	✔	100.0	103.5	103.5
p2 cal 6	6	✔	250.0	249.8	99.9
p2 cal 7	7	✔	500.0	494.7	98.9
p2 cal 8	8	✔	1000.0	963.1	96.3

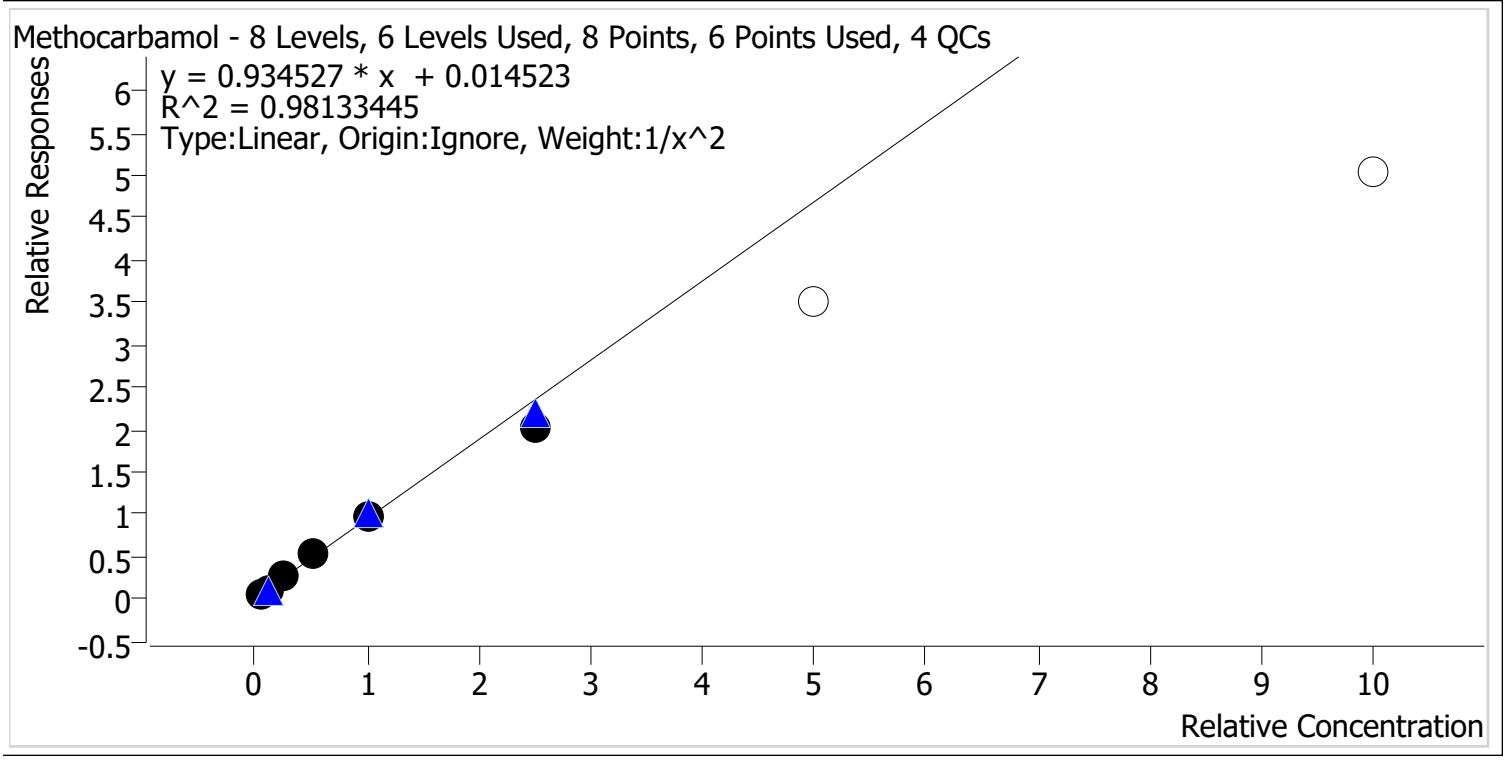
Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Last Cal. Update	9/9/2019 9:01 AM
Analyst Name	ISP\datastor
Analyte	MDMA
Internal Standard	MDMA-D6



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1	1	✔	5.0	5.1	101.1
p2 cal 2	2	✔	10.0	10.0	99.9
p2 cal 3	3	✔	25.0	24.7	98.7
p2 cal 4	4	✔	50.0	49.2	98.3
p2 cal 5	5	✔	100.0	102.2	102.2
p2 cal 6	6	✔	250.0	248.8	99.5
p2 cal 7	7	✔	500.0	503.0	100.6
p2 cal 8	8	✔	1000.0	997.2	99.7

Compound Calibration Report

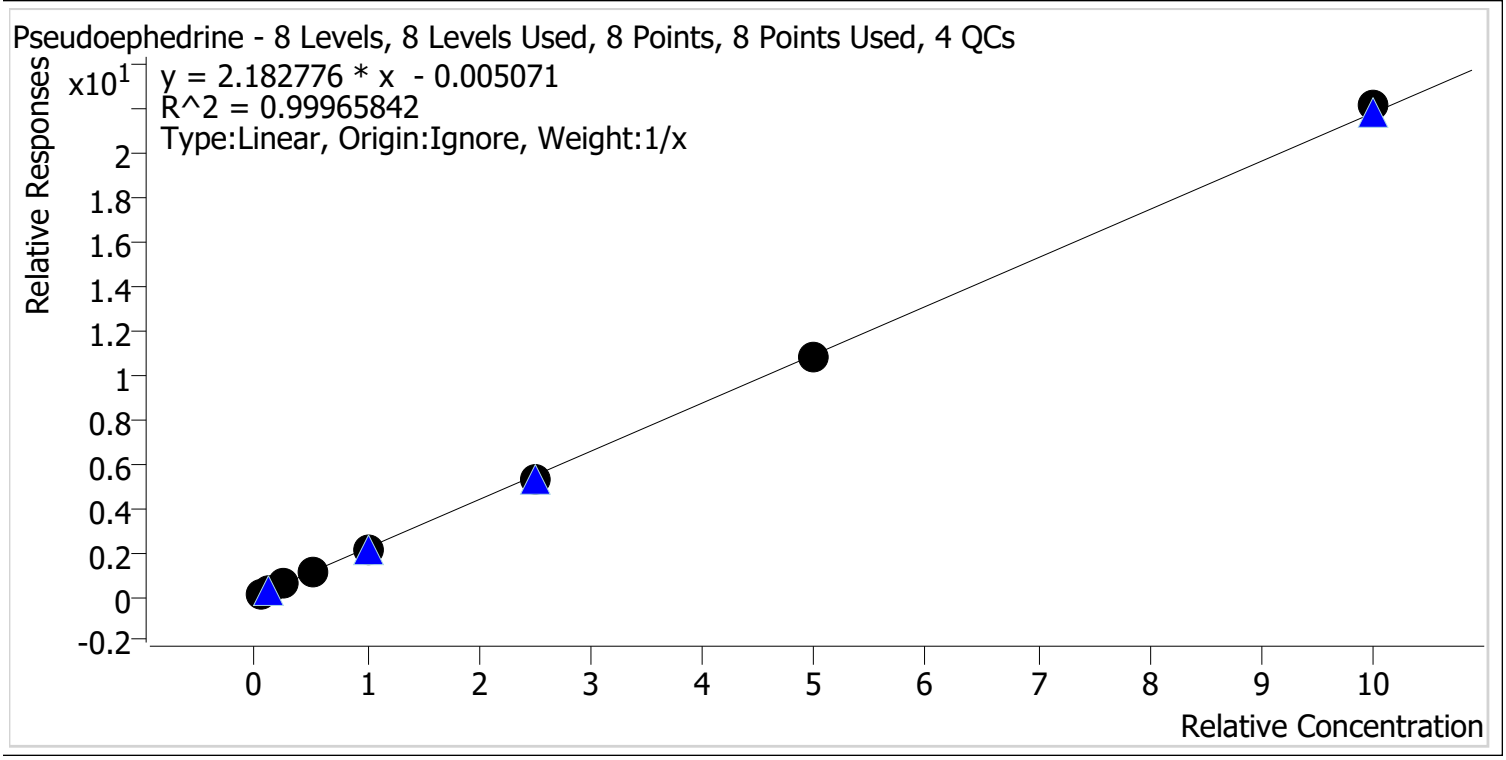
Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Last Cal. Update	9/9/2019 9:01 AM
Analyst Name	ISP\datastor
Analyte	Methocarbamol
Internal Standard	MDA-D5



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1	1	✔	5.0	5.2	104.5
p2 cal 2	2	✔	10.0	8.5	85.0
p2 cal 3	3	✔	25.0	27.8	111.2
p2 cal 4	4	✔	50.0	54.0	108.1
p2 cal 5	5	✔	100.0	104.4	104.4
p2 cal 6	6	✔	250.0	217.0	86.8
p2 cal 7	7	✗	500.0	375.0	75.0
p2 cal 8	8	✗	1000.0	537.6	53.8

Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Last Cal. Update	9/9/2019 9:01 AM
Analyst Name	ISP\datastor
Analyte	Pseudoephedrine
Internal Standard	Pseudoephedrine-D3



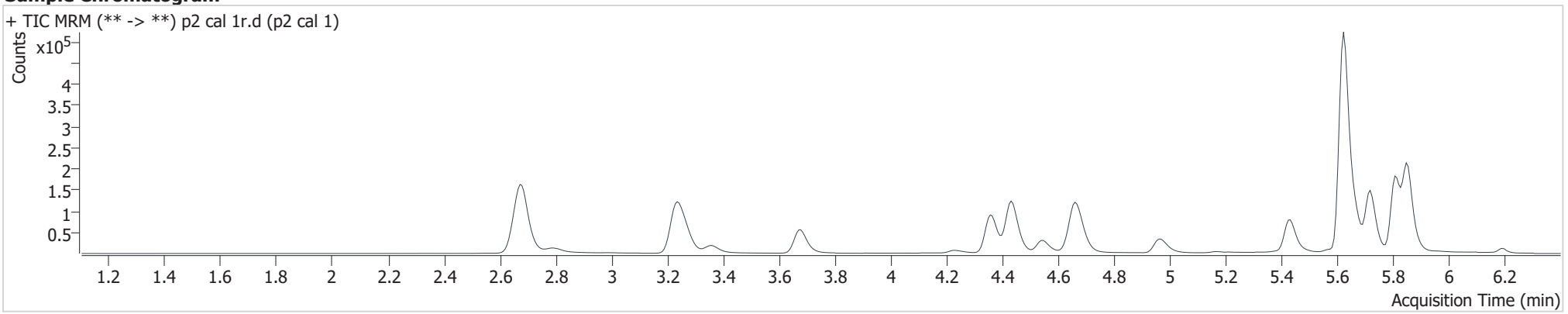
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1	1	✔	5.0	5.4	107.2
p2 cal 2	2	✔	10.0	10.3	102.6
p2 cal 3	3	✔	25.0	24.4	97.5
p2 cal 4	4	✔	50.0	47.9	95.7
p2 cal 5	5	✔	100.0	99.3	99.3
p2 cal 6	6	✔	250.0	243.5	97.4
p2 cal 7	7	✔	500.0	494.3	98.9
p2 cal 8	8	✔	1000.0	1015.0	101.5

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 cal 1r.d
Type	Cal	**Sample**	p2 cal 1
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-A5	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 11:01:18 AM		
Sample Info.			

Sample Chromatogram



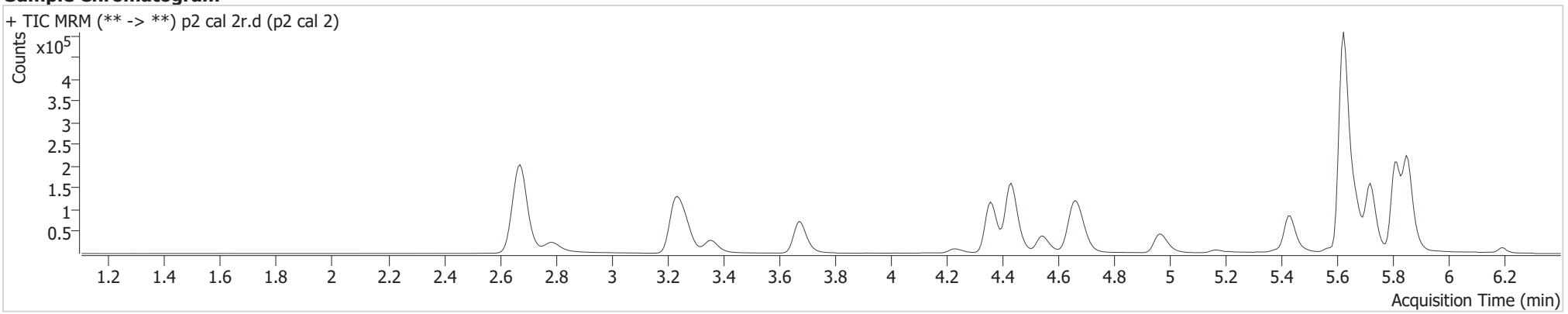
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.794	23617	3130.2	85.0	873.44	570924	5.116 ng/ml
MDA	3.279	24234	439.22	26.8	259.11	127951	4.951 ng/ml
MDMA	3.365	16288	3072.6	80.6	346.67	30262	5.054 ng/ml
Methocarbamol	4.459	8106	331.76	95.0	149.82	127951	5.225 ng/ml
Pseudoephedrine	2.688	63884	1178.5	16.9	246.37	570924	5.359 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 cal 2r.d
Type	Cal	**Sample**	p2 cal 2
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-B5	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 11:12:07 AM		
Sample Info.			

Sample Chromatogram



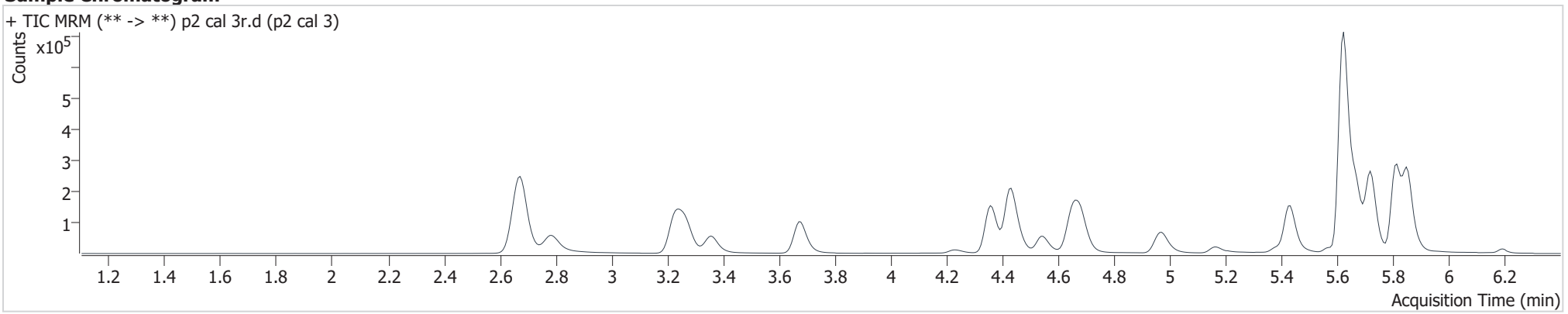
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.787	52989	8133.1	84.7	723.09	643935	9.810 ng/ml
MDA	3.279	53633	899.11	26.9	2330.2	142489	10.144 ng/ml
MDMA	3.365	35982	8078.1	78.6	760.85	34288	9.988 ng/ml
Methocarbamol	4.459	13387	2462.2	93.9	826.22	142489	8.499 ng/ml
Pseudoephedrine	2.682	140879	601.94	17.3	3643.4	643935	10.255 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 cal 3r.d
Type	Cal	**Sample**	p2 cal 3
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-C5	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 11:22:54 AM		
Sample Info.			

Sample Chromatogram



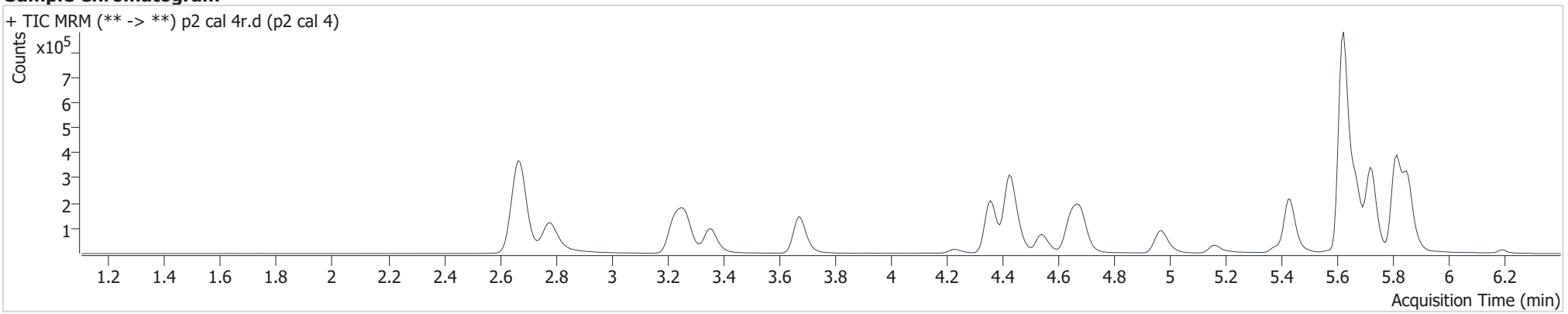
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.787	128288	5229.8	85.5	1350.8	618683	24.155 ng/ml
MDA	3.273	124436	2630.7	26.9	1239.1	136190	25.065 ng/ml
MDMA	3.358	86164	565.16	78.7	4059	33516	24.672 ng/ml
Methocarbamol	4.459	37363	2157.9	94.1	2458.9	136190	27.803 ng/ml
Pseudoephedrine	2.682	325898	613.49	17.4	15667	618683	24.365 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 cal 4r.d
Type	Cal	**Sample**	p2 cal 4
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-D5	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 11:33:42 AM		
Sample Info.			

Sample Chromatogram



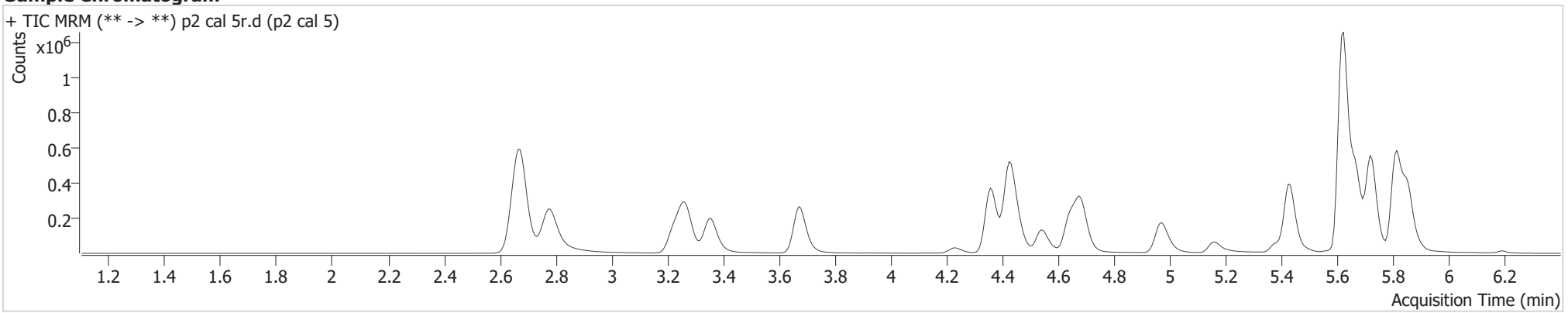
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.780	273820	42907	85.7	36658	670759	47.194 ng/ml
MDA	3.273	232541	1883.8	26.9	317.06	127658	50.278 ng/ml
MDMA	3.358	166117	799.37	79.0	6606.3	32512	49.175 ng/ml
Methocarbamol	4.453	66331	9036.8	92.5	5386.6	127658	54.046 ng/ml
Pseudoephedrine	2.675	697350	19155	17.5	14432	670759	47.862 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 cal 5r.d
Type	Cal	**Sample**	p2 cal 5
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-E5	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 11:44:29 AM		
Sample Info.			

Sample Chromatogram



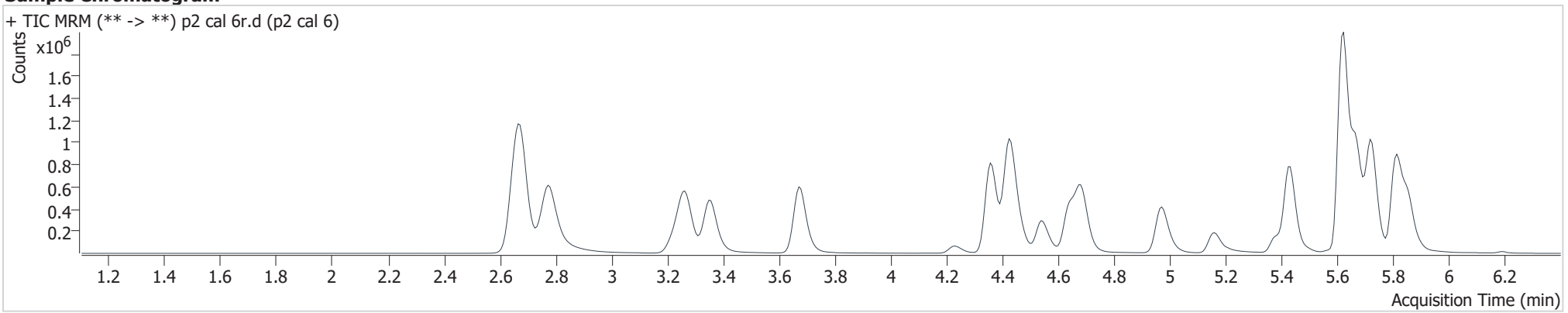
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.780	577004	79749	85.7	26096	685880	96.862 ng/ml
MDA	3.273	475335	10766	26.6	10354	127127	103.527 ng/ml
MDMA	3.358	359583	43900	76.5	22864	33920	102.176 ng/ml
Methocarbamol	4.453	125903	21619	93.2	2794.4	127127	104.422 ng/ml
Pseudoephedrine	2.675	1483623	721.89	17.4	578.02	685880	99.331 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 cal 6r.d
Type	Cal	**Sample**	p2 cal 6
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-F5	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 11:55:16 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.774	1416350	1.3929E+05	85.8	61569	669805	242.907 ng/ml
MDA	3.266	1056271	23451	26.7	27452	117274	249.816 ng/ml
MDMA	3.351	901800	71659	78.5	60320	34968	248.773 ng/ml
Methocarbamol	4.459	239488	20346	93.2	609.15	117274	216.966 ng/ml
Pseudoephedrine	2.675	3556642	898.12	17.3	1.8386E+05	669805	243.499 ng/ml

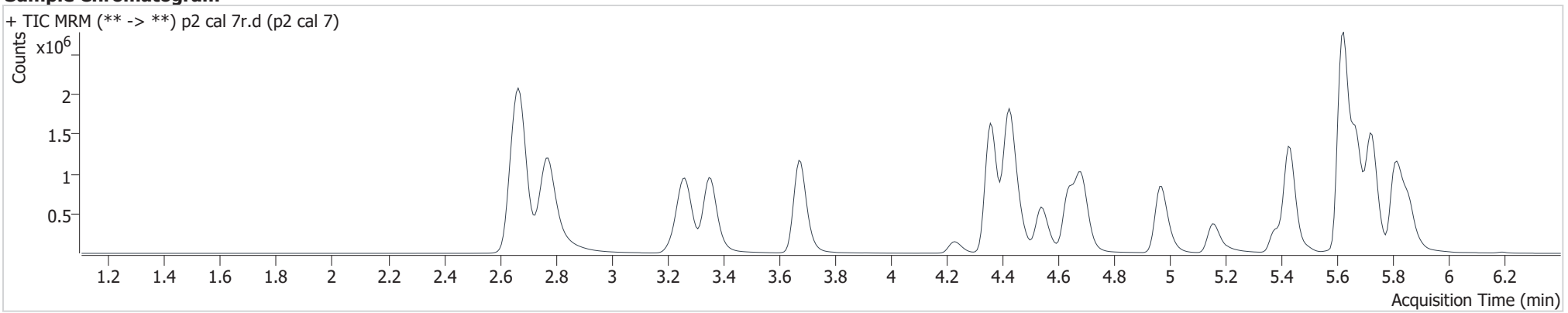
AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument 69679
Type Cal
Acq. Method am 28 p2.m
Sample Position P2-G5
Injection Volume 2.5
Acq. Date-Time 9/7/2019 12:06:04 PM
Sample Info.

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

Sample Chromatogram



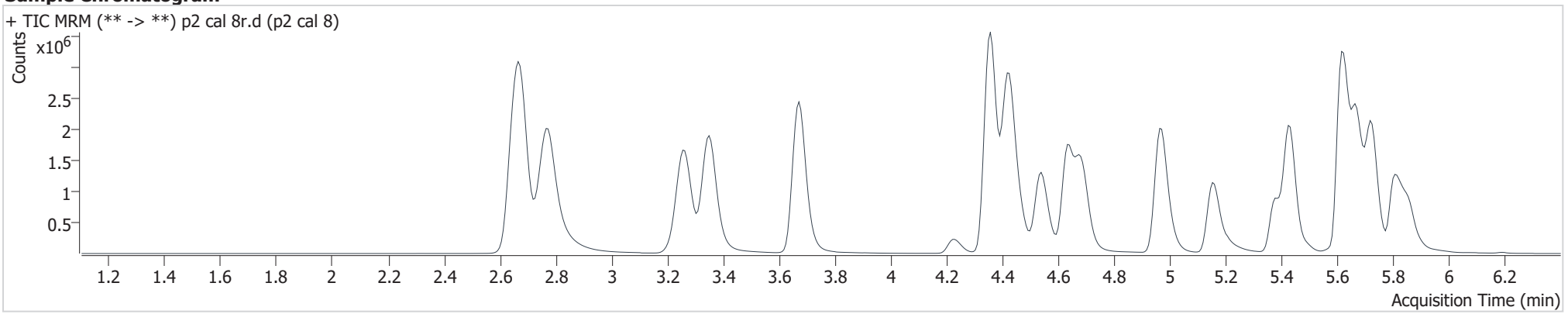
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.774	2857246	2.7007E+05	85.9	1405.4	647424	506.559 ng/ml
MDA	3.266	1896987	24669	26.6	741.63	106413	494.742 ng/ml
MDMA	3.351	1861984	1794.6	78.4	9323	35720	502.974 ng/ml
Methocarbamol	4.459	374481	2349.4	93.6	47467	106413	375.013 ng/ml
Pseudoephedrine	2.668	6981892	3004.2	17.0	855.4	647424	494.288 ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2019\am28-27 090619\QuantResults\mdq p2.batch.bin
Calibration Last Update 9/9/2019 9:01:23 AM

Instrument	69679	**Data File**	p2 cal 8r.d
Type	Cal	**Sample**	p2 cal 8
Acq. Method	am 28 p2.m	**Operator**	Anne Nord
Sample Position	P2-H5	**Comment**	
Injection Volume	2.5		
Acq. Date-Time	9/7/2019 12:16:51 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Levamisole	2.774	5068238	1.5844E+05	85.6	4.1422E+05	513527	1132.371 ng/ml
MDA	3.266	3459747	81529	26.3	1323.4	99723	963.146 ng/ml
MDMA	3.351	3970034	1.9573E+05	77.6	1.0144E+05	38420	997.189 ng/ml
Methocarbamol	4.459	502426	44293	92.4	4078.8	99723	537.566 ng/ml
Pseudoephedrine	2.668	11375145	766.24	16.8	2.4419E+05	513527	1015.042 ng/ml