10/13/2021

Worklist: 5285

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2021-2037		BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
C2021-2135		BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
C2021-2136		BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ
C2021-2211		BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ

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

Extraction Date 10/7/21 Analyst: Anne Nord
Plate lot#: 210609 (part IDP-122-2) Plate Expiration: 12/09/21

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: 21D52496 **Urine Blank lot:** 83121
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.
☒ 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/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 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: *Evaluated: Paroxetine, Amitriptyline, Nortriptyline, Maprotiline, Methocarbamol*
Blood only run
Curve limits:
Paroxetine 5-500

	1	2	3	4	5	6	7	8	9	10	11	12
A				IS + Cal. 1	IS + QC_1	2211-1						
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	2037-1							
G				IS + Cal. 7	2135-1							
H				IS + Cal. 8	2136-1							

c2021-

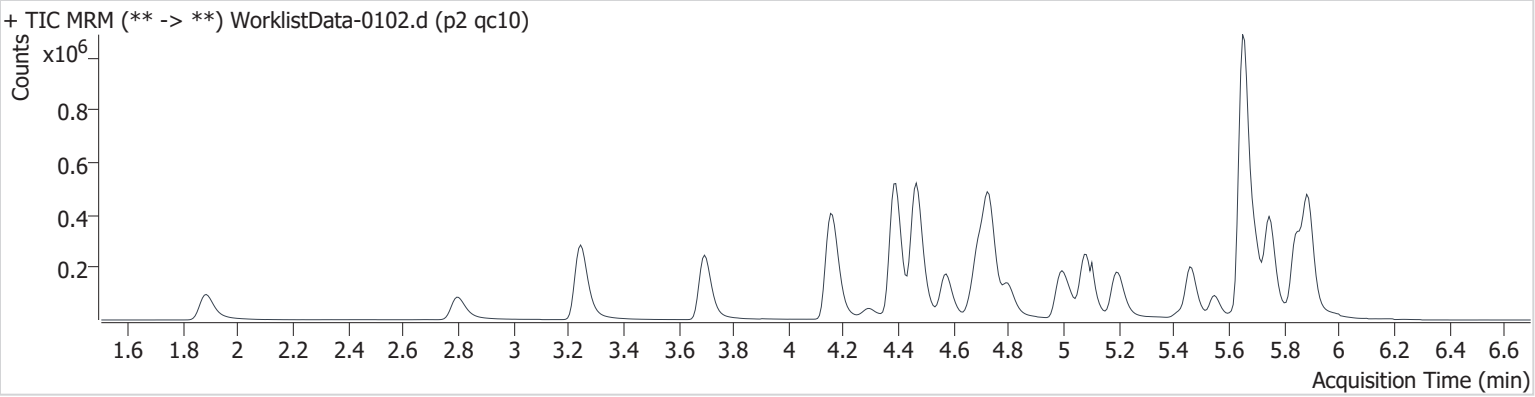
P2 plate map

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0102.d
Type	QC	**Sample**	p2 qc10
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-A5	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 9:38:41 AM		
Sample Info.			

Sample Chromatogram



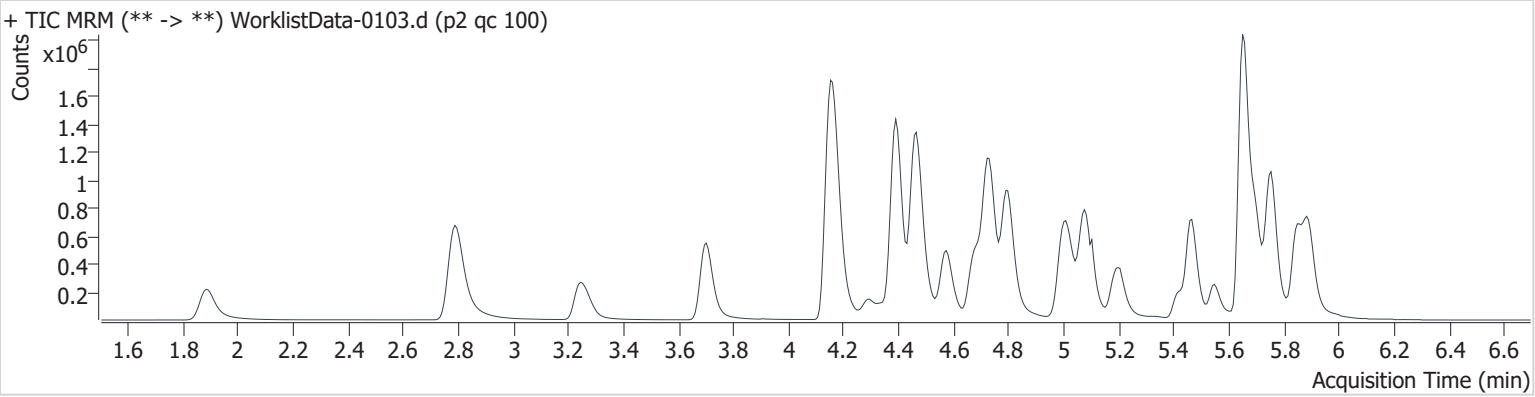
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	8808	222.3	34.9	21.0	35633	9.574	ng/ml
Maprotiline	5.729	10540	116.7	53.7	146.8	35633	10.001	ng/ml
methocarbamol	4.495	19866	360.9	85.2	332.9	115305	10.369	ng/ml
Nortriptyline	5.751	8177	48.4	39.2	75.2	29327	10.318	ng/ml
paroxetine	5.630	2597	26.1	63.5	113.8	35633	9.201	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0103.d
Type	QC	**Sample**	p2 qc 100
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-B5	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 9:49:32 AM		
Sample Info.			

Sample Chromatogram



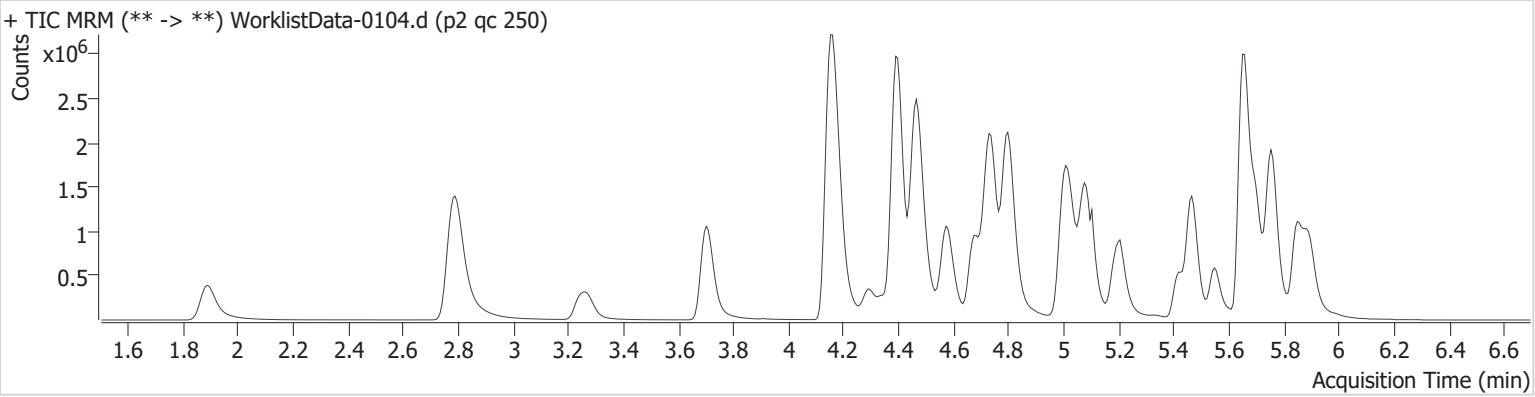
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	49878	1264.7	32.6	144.5	20470	105.557	ng/ml
Maprotiline	5.729	56228	1128.0	57.6	619.3	20470	104.591	ng/ml
methocarbamol	4.495	147816	7883.7	90.0	4674.8	95028	98.954	ng/ml
Nortriptyline	5.751	43114	4878.9	34.4	6569.5	15158	101.811	ng/ml
paroxetine	5.630	15977	161.5	49.0	4439.5	20470	98.048	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0104.d
Type	QC	**Sample**	p2 qc 250
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-C5	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 10:00:24 AM		
Sample Info.			

Sample Chromatogram



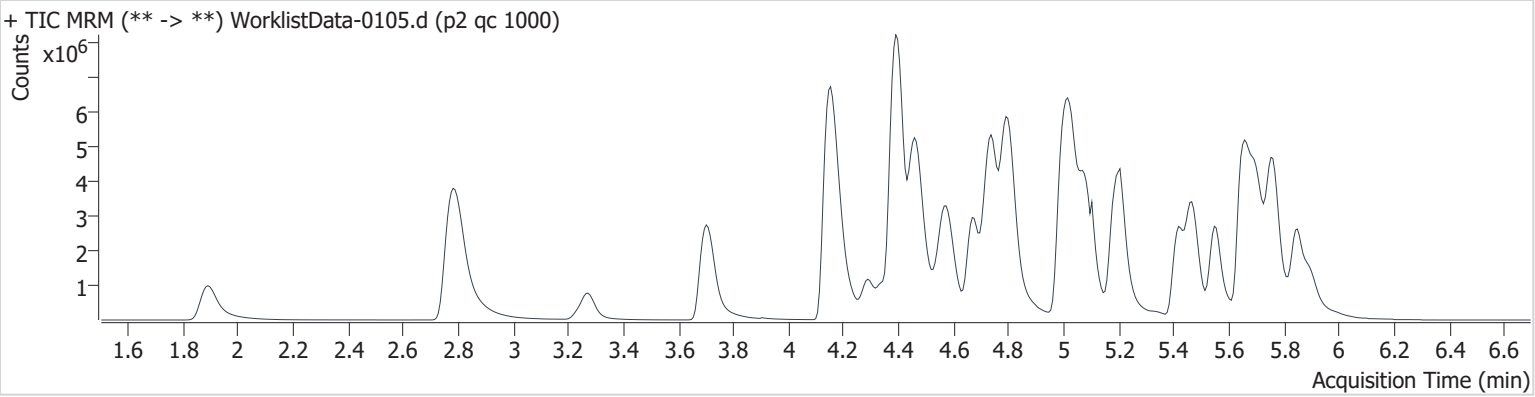
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	96615	1336.7	35.8	336.4	16452	256.189	ng/ml
Maprotiline	5.729	111590	1661.4	51.4	463.5	16452	260.352	ng/ml
methocarbamol	4.502	284577	10739.1	88.1	23601.4	77109	235.690	ng/ml
Nortriptyline	5.751	73760	1319.2	33.7	465.8	10469	251.653	ng/ml
paroxetine	5.630	27527	483.6	52.1	20298.7	16452	210.137	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0105.d
Type	QC	**Sample**	p2 qc 1000
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-D5	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 10:11:13 AM		
Sample Info.			

Sample Chromatogram



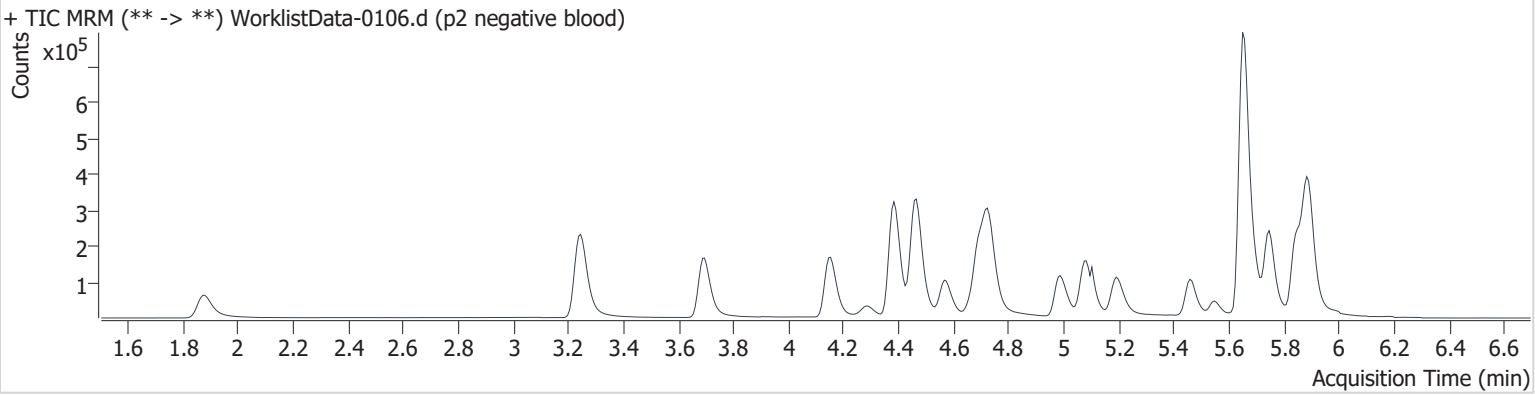
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.		
Amitriptyline	5.729	584810	1110.3	38.3	244.4	24387	1050.037	ng/ml	
Maprotiline	5.729	649802	4639.6	49.1	5003.7	24387	1026.914	ng/ml	
methocarbamol	4.502	549842	26909.1	89.6	46210.6	37852	929.630	ng/ml	
Nortriptyline	5.751	387509	1643.9	34.4	6736.9	14452	956.638	ng/ml	
~~paroxetine~~	~~5.630~~	~~189452~~	~~2324.3~~	~~53.3~~	~~78263.6~~	~~24387~~	~~975.512~~	~~ng/ml~~	OCR

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

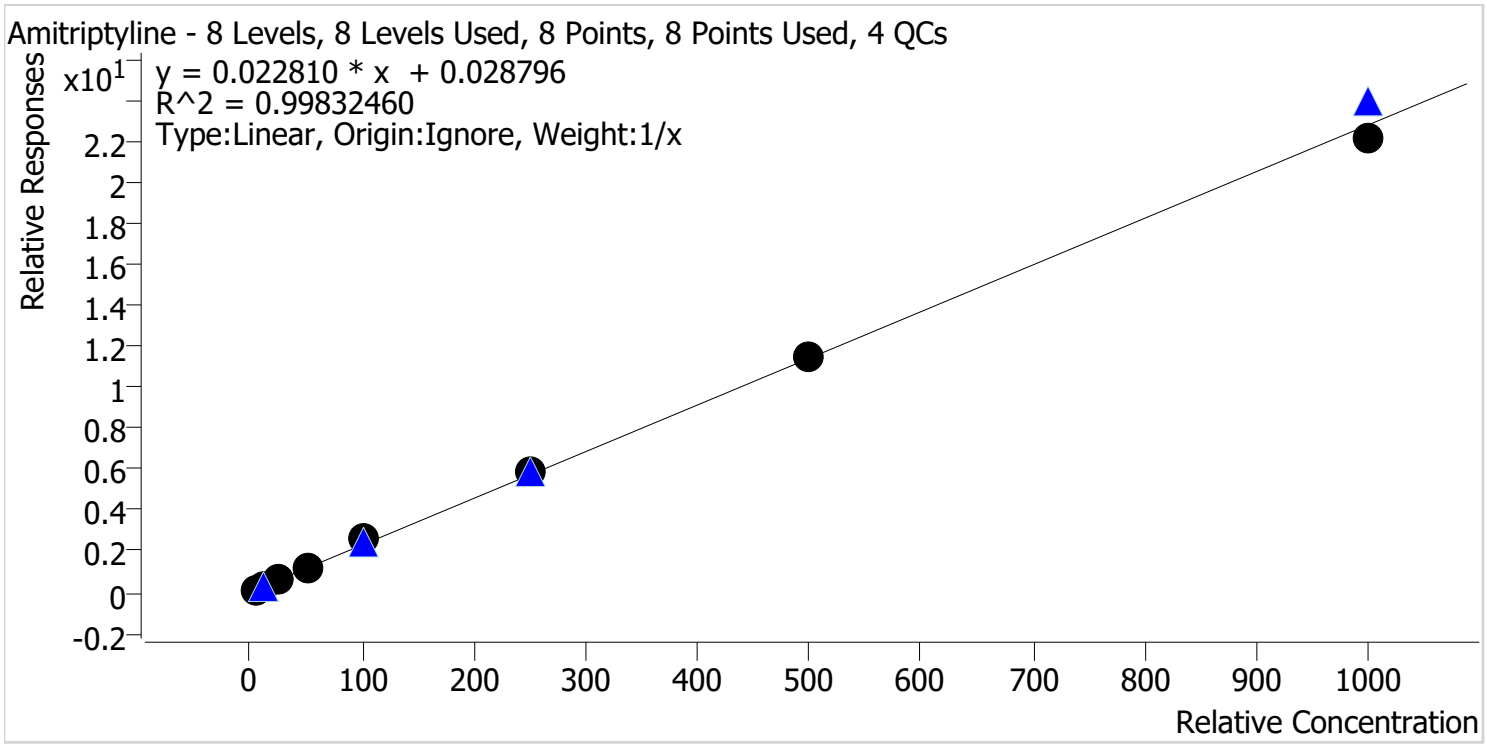
Instrument	69679	**Data File**	WorklistData-0106.d
Type	Sample	**Sample**	p2 negative blood
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-E5	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 10:32:52 AM		
Sample Info.			

Sample Chromatogram



Compound Calibration Report

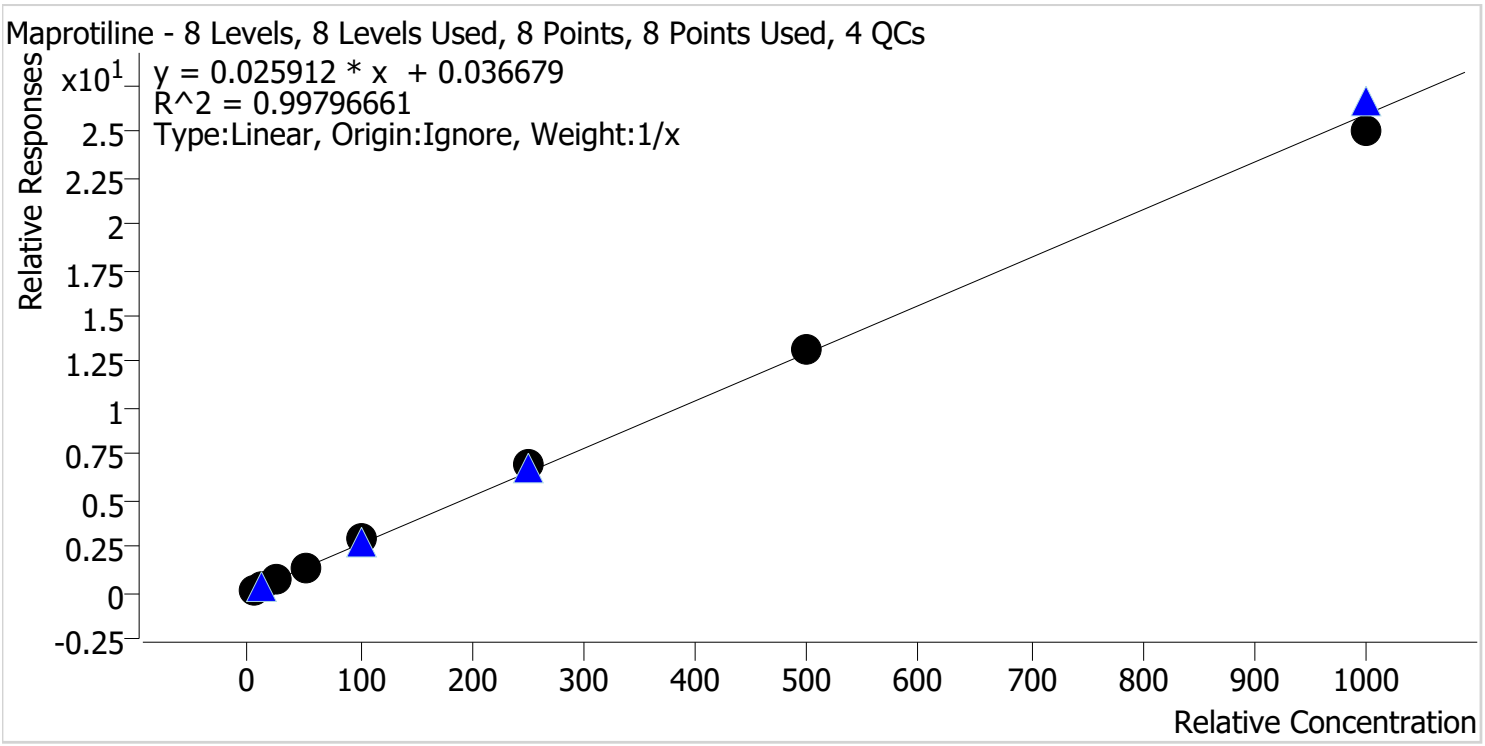
Batch results	D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Last Cal. Update	10/12/2021 10:36 AM
Analyst Name	ISP\datastor
Analyte	Amitriptyline
Internal Standard	Amitriptyline-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✔	5.0	4.1	82.9
p2 cal 2 mdq	2	✔	10.0	9.7	96.9
p2 cal 3 mdq	3	✔	25.0	25.4	101.5
p2 cal 4 mdq	4	✔	50.0	52.3	104.7
p2 cal 5 mdq	5	✔	100.0	111.8	111.8
p2 cal 6 mdq	6	✔	250.0	260.4	104.2
p2 cal 7 mdq	7	✔	500.0	505.1	101.0
p2 cal 8 mdq	8	✔	1000.0	971.1	97.1

Compound Calibration Report

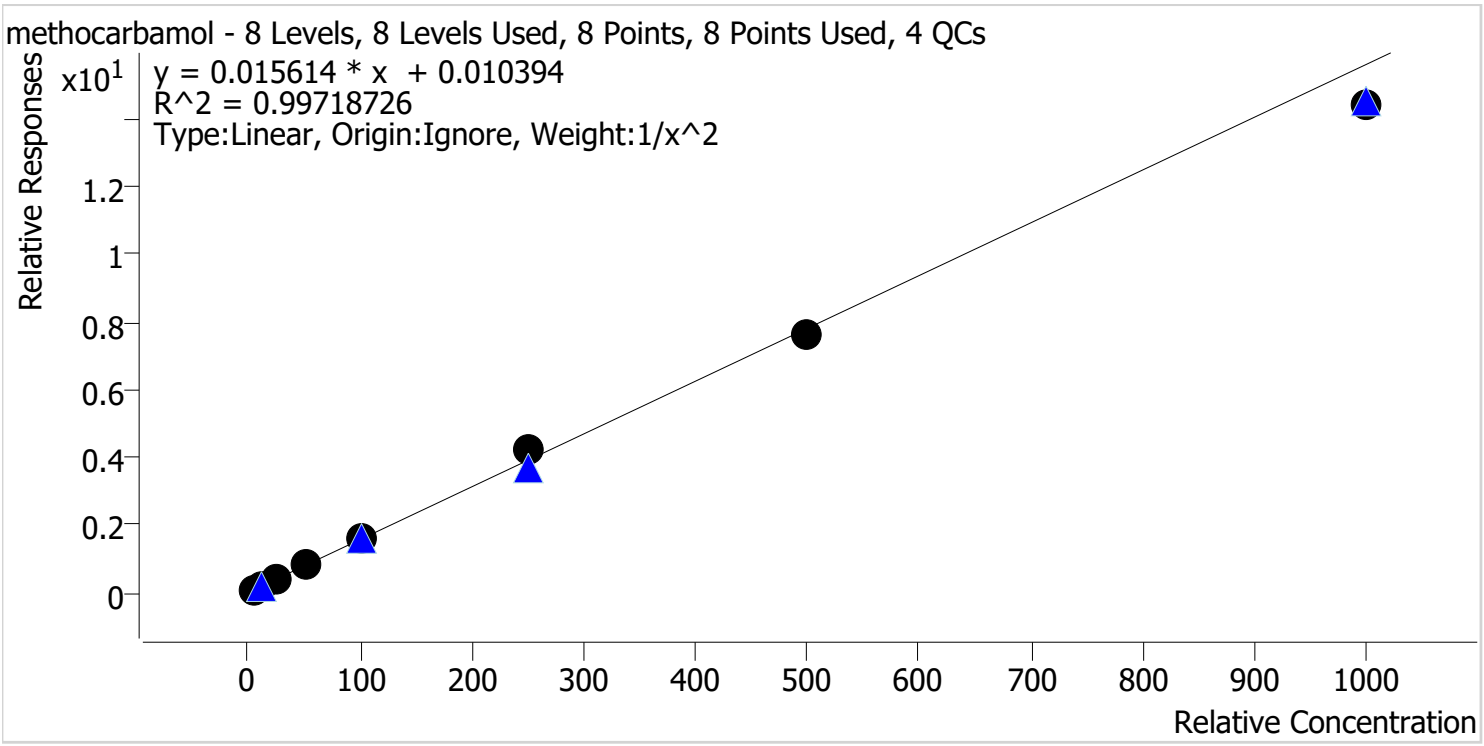
Batch results	D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Last Cal. Update	10/12/2021 10:36 AM
Analyst Name	ISP\datastor
Analyte	Maprotiline
Internal Standard	Amitriptyline-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✔	5.0	4.1	81.4
p2 cal 2 mdq	2	✔	10.0	9.7	97.4
p2 cal 3 mdq	3	✔	25.0	25.8	103.3
p2 cal 4 mdq	4	✔	50.0	51.5	103.1
p2 cal 5 mdq	5	✔	100.0	110.8	110.8
p2 cal 6 mdq	6	✔	250.0	265.0	106.0
p2 cal 7 mdq	7	✔	500.0	507.4	101.5
p2 cal 8 mdq	8	✔	1000.0	965.7	96.6

Compound Calibration Report

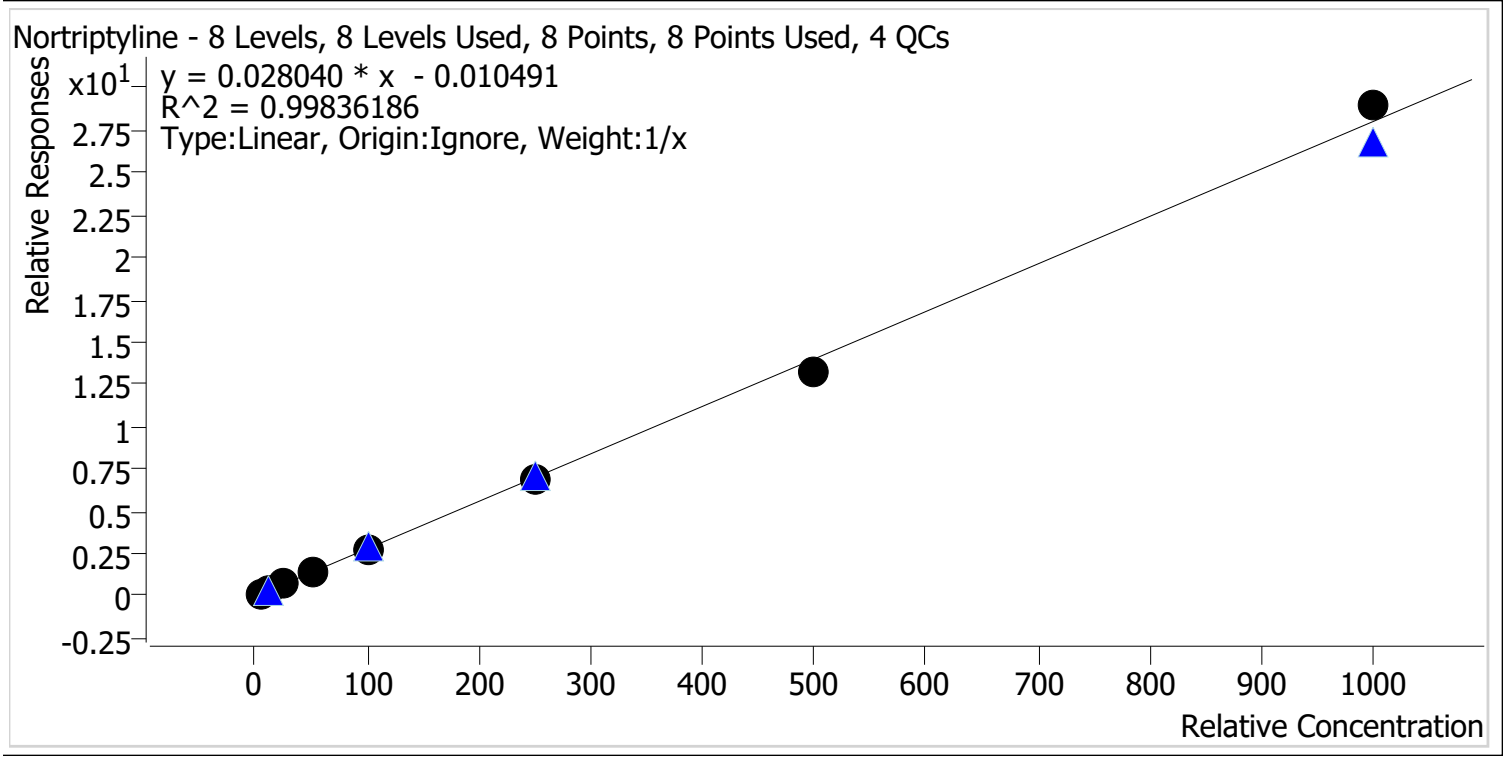
Batch results	D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Last Cal. Update	10/12/2021 10:36 AM
Analyst Name	ISP\datastor
Analyte	methocarbamol
Internal Standard	Flunitrazepam-D7



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✔	5.0	4.9	97.6
p2 cal 2 mdq	2	✔	10.0	10.4	104.0
p2 cal 3 mdq	3	✔	25.0	25.4	101.6
p2 cal 4 mdq	4	✔	50.0	49.5	99.0
p2 cal 5 mdq	5	✔	100.0	101.1	101.1
p2 cal 6 mdq	6	✔	250.0	268.4	107.4
p2 cal 7 mdq	7	✔	500.0	484.8	97.0
p2 cal 8 mdq	8	✔	1000.0	923.3	92.3

Compound Calibration Report

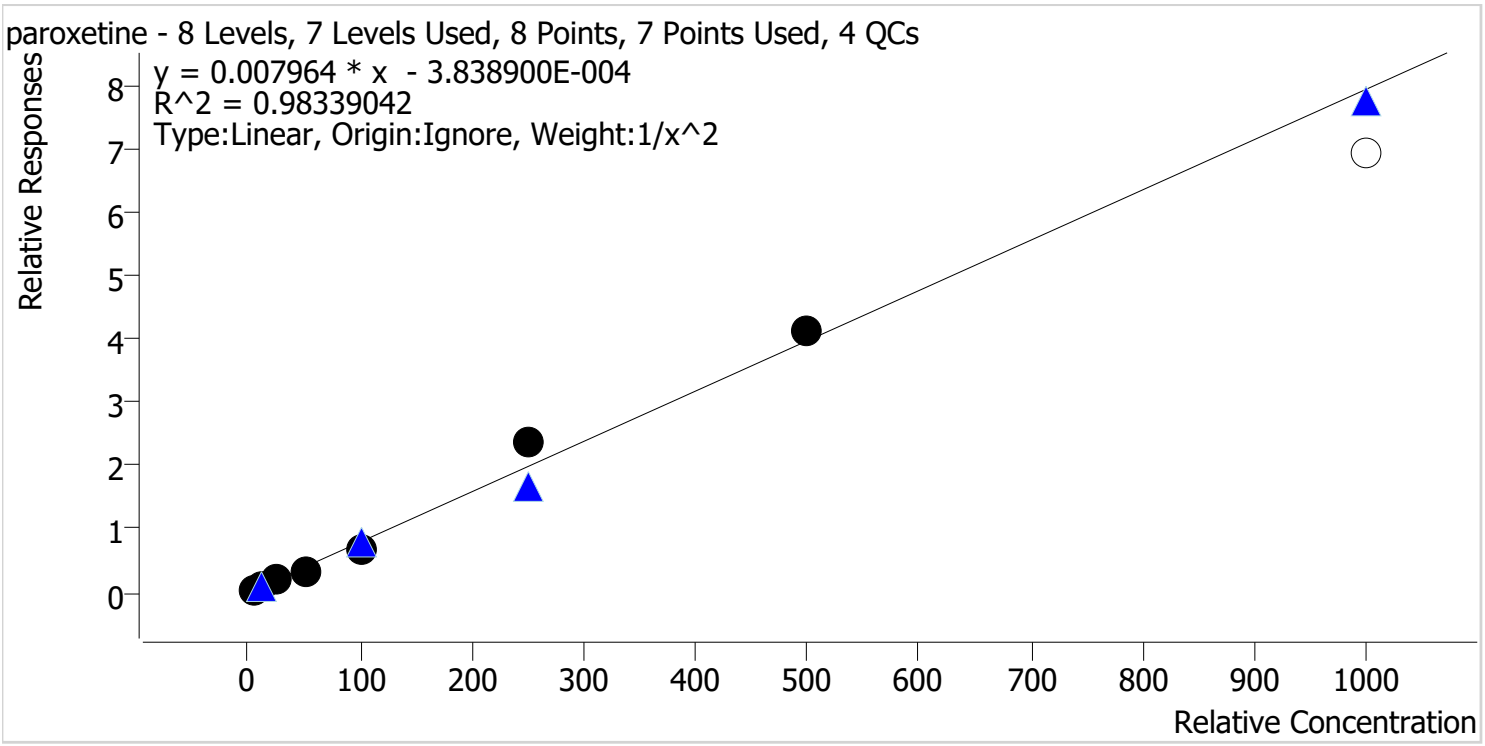
Batch results	D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Last Cal. Update	10/12/2021 10:36 AM
Analyst Name	ISP\datastor
Analyte	Nortriptyline
Internal Standard	Nortriptyline-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✔	5.0	5.4	107.4
p2 cal 2 mdq	2	✔	10.0	10.6	105.5
p2 cal 3 mdq	3	✔	25.0	24.5	98.0
p2 cal 4 mdq	4	✔	50.0	47.2	94.3
p2 cal 5 mdq	5	✔	100.0	97.2	97.2
p2 cal 6 mdq	6	✔	250.0	248.7	99.5
p2 cal 7 mdq	7	✔	500.0	472.9	94.6
p2 cal 8 mdq	8	✔	1000.0	1033.5	103.4

Compound Calibration Report

Batch results	D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Last Cal. Update	10/12/2021 10:36 AM
Analyst Name	ISP\datastor
Analyte	paroxetine
Internal Standard	Amitriptyline-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 cal 1 mdq	1	✔	5.0	5.2	104.1
p2 cal 2 mdq	2	✔	10.0	9.3	92.6
p2 cal 3 mdq	3	✔	25.0	26.2	104.8
p2 cal 4 mdq	4	✔	50.0	44.3	88.7
p2 cal 5 mdq	5	✔	100.0	87.9	87.9
p2 cal 6 mdq	6	✔	250.0	296.3	118.5
p2 cal 7 mdq	7	✔	500.0	517.0	103.4
p2 cal 8 mdq	8	✗	1000.0	872.0	87.2

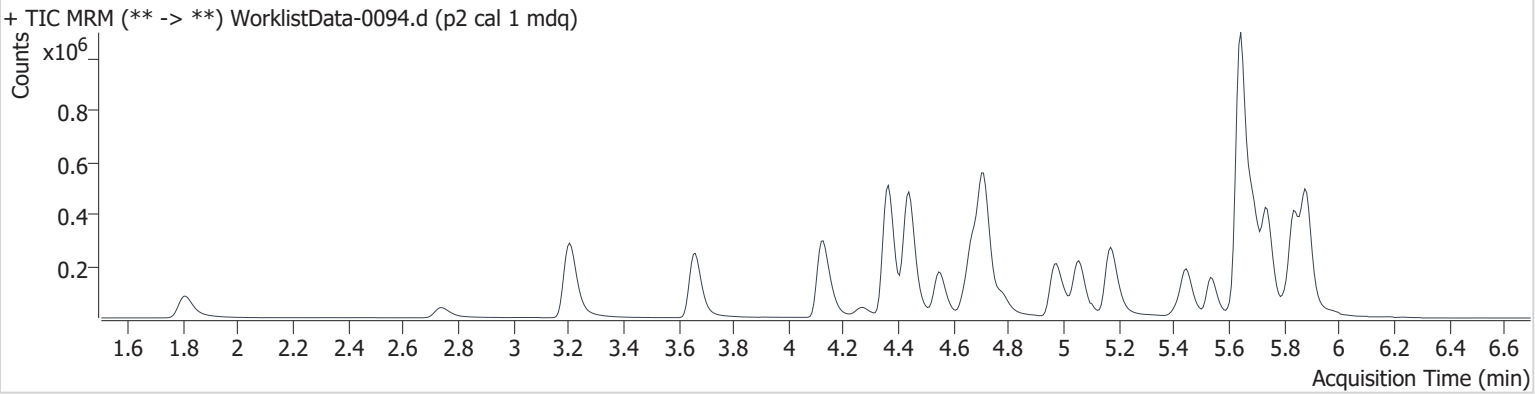
cal 8 dropped due being outside linerity range.

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0094.d
Type	Cal	**Sample**	p2 cal 1 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-A4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/7/2021 11:59:51 PM		
Sample Info.			

Sample Chromatogram



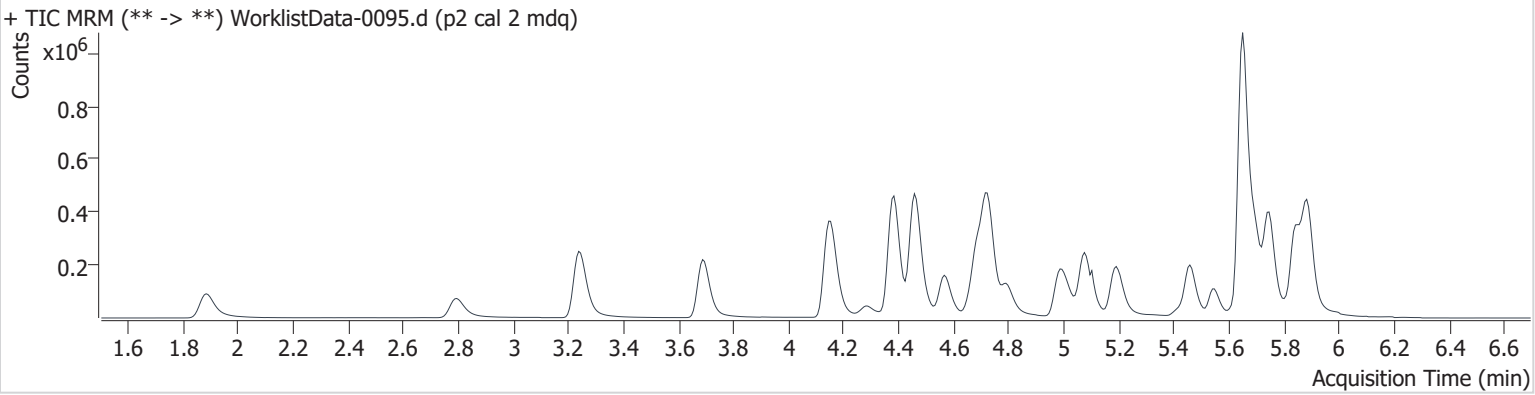
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.723	14412	500.0	32.3	39.5	116792	4.147	ng/ml
Maprotiline	5.723	16601	257.8	57.5	109.0	116792	4.070	ng/ml
methocarbamol	4.468	10081	185.9	84.5	150.9	116382	4.881	ng/ml
Nortriptyline	5.744	14316	1183.1	33.8	283.3	102158	5.372	ng/ml
paroxetine	5.623	4795	124.4	58.5	1161.2	116792	5.203	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0095.d
Type	Cal	**Sample**	p2 cal 2 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-B4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 8:12:03 AM		
Sample Info.			

Sample Chromatogram



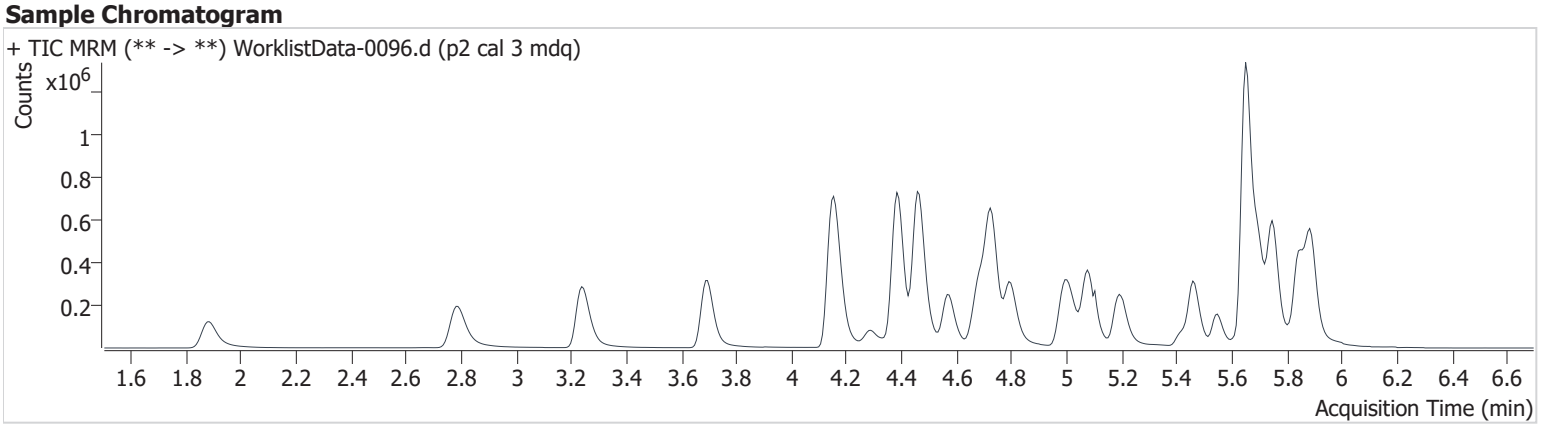
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	14541	275.0	32.7	21.1	58224	9.686	ng/ml
Maprotiline	5.729	16823	89.8	58.5	394.9	58224	9.735	ng/ml
methocarbamol	4.488	18647	462.7	87.2	129.2	107893	10.403	ng/ml
Nortriptyline	5.751	14373	9320.7	32.6	718.0	50364	10.552	ng/ml
paroxetine	5.630	4272	36.1	55.3	1542.9	58224	9.261	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0096.d
Type	Cal	**Sample**	p2 cal 3 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-C4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 8:22:53 AM		
Sample Info.			

Sample Chromatogram



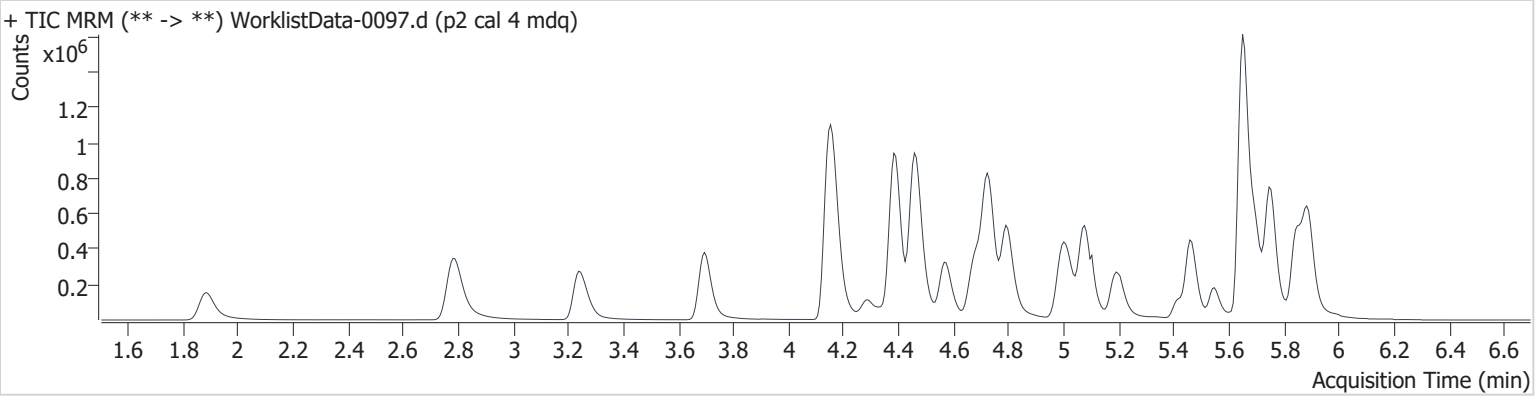
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	35920	427.6	33.7	64.5	59139	25.365	ng/ml
Maprotiline	5.729	41756	677.9	60.4	310.5	59139	25.833	ng/ml
methocarbamol	4.495	46884	1662.3	87.8	5536.3	115156	25.409	ng/ml
Nortriptyline	5.751	34532	31519.9	35.7	187.0	51024	24.511	ng/ml
paroxetine	5.630	12319	633.4	50.8	57.8	59139	26.204	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0097.d
Type	Cal	**Sample**	p2 cal 4 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-D4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 8:33:42 AM		
Sample Info.			

Sample Chromatogram



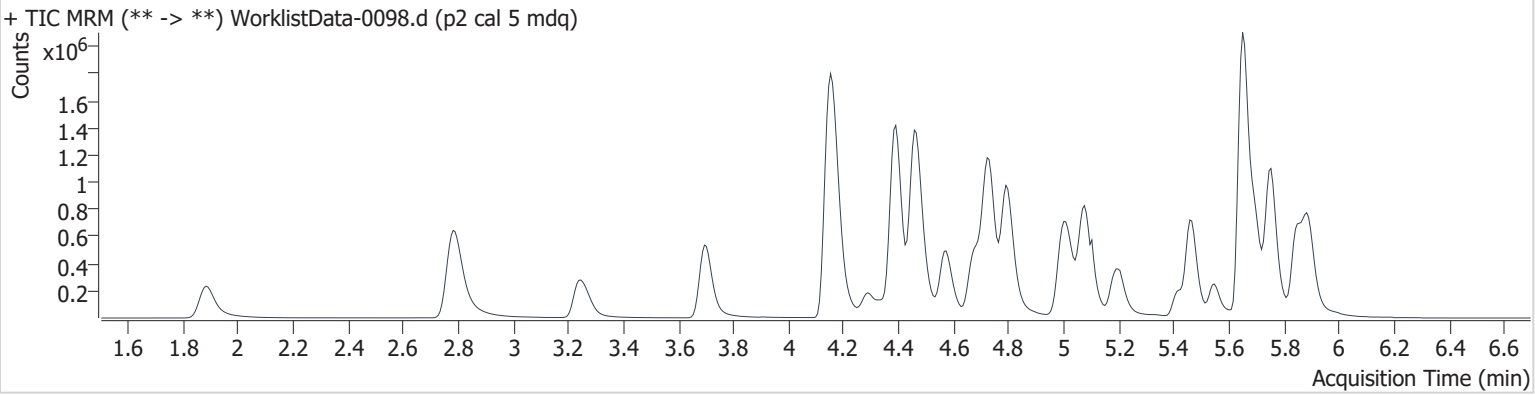
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	32489	888.7	31.1	34.4	26576	52.330	ng/ml
Maprotiline	5.729	36470	295.2	56.8	124.2	26576	51.544	ng/ml
methocarbamol	4.495	85545	1198.3	88.0	822.4	109213	49.498	ng/ml
Nortriptyline	5.751	27003	19691.5	34.3	860.0	20578	47.173	ng/ml
paroxetine	5.630	9375	249.9	56.0	178.7	26576	44.341	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0098.d
Type	Cal	**Sample**	p2 cal 5 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-E4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 8:44:32 AM		
Sample Info.			

Sample Chromatogram



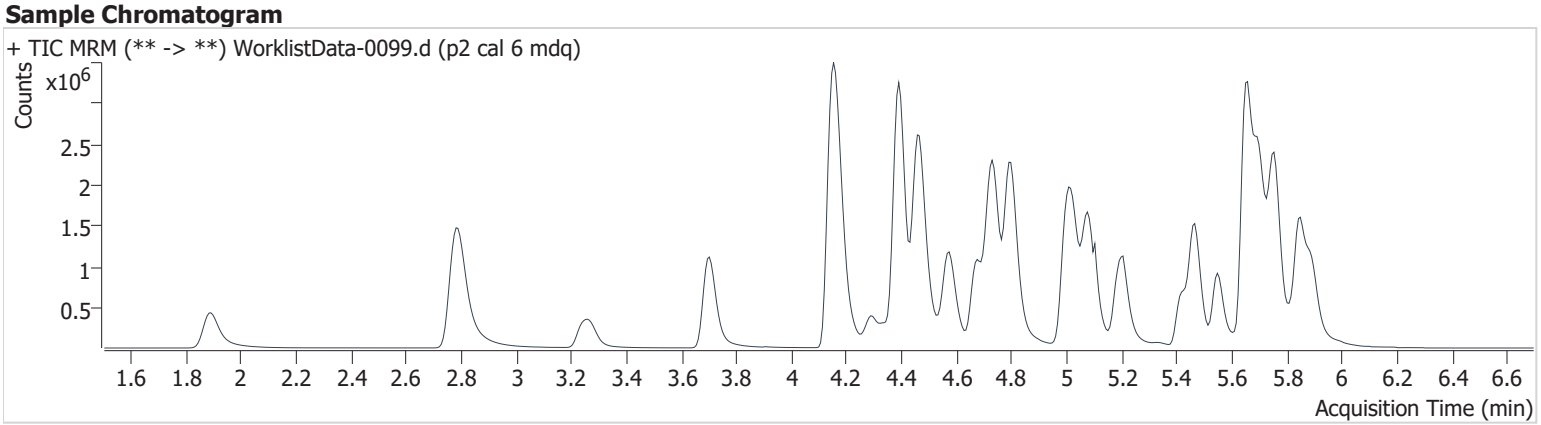
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	38571	6113.4	32.3	229.2	14962	111.754	ng/ml
Maprotiline	5.729	43497	680.3	54.7	512.3	14962	110.783	ng/ml
methocarbamol	4.495	156843	1483.7	88.2	9785.0	98748	101.056	ng/ml
Nortriptyline	5.751	30949	36708.5	33.7	1450.8	11396	97.231	ng/ml
paroxetine	5.630	10467	197.5	54.7	3790.3	14962	87.892	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0099.d
Type	Cal	**Sample**	p2 cal 6 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-F4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 8:55:22 AM		
Sample Info.			

Sample Chromatogram



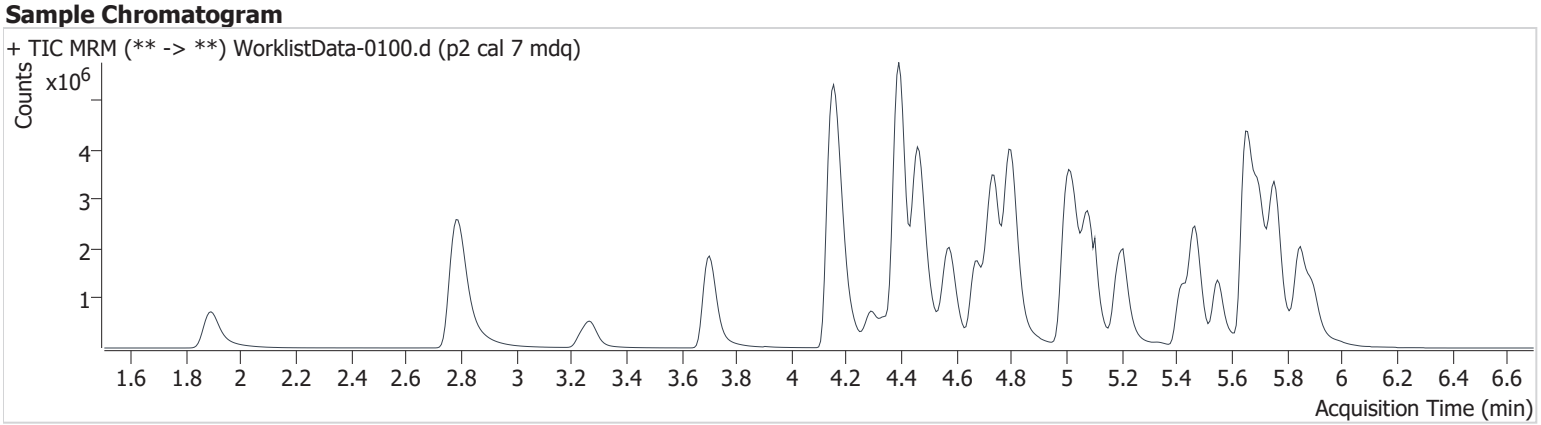
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	353665	13117.6	37.6	519.6	59249	260.422	ng/ml
Maprotiline	5.729	409009	2147.4	55.2	1328.1	59249	264.999	ng/ml
methocarbamol	4.495	305429	5041.5	89.8	74530.2	72700	268.393	ng/ml
Nortriptyline	5.751	319202	566.9	34.5	878.6	45838	248.727	ng/ml
paroxetine	5.630	139806	10904.5	52.0	40101.7	59249	296.335	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0100.d
Type	Cal	**Sample**	p2 cal 7 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-G4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 9:06:11 AM		
Sample Info.			

Sample Chromatogram



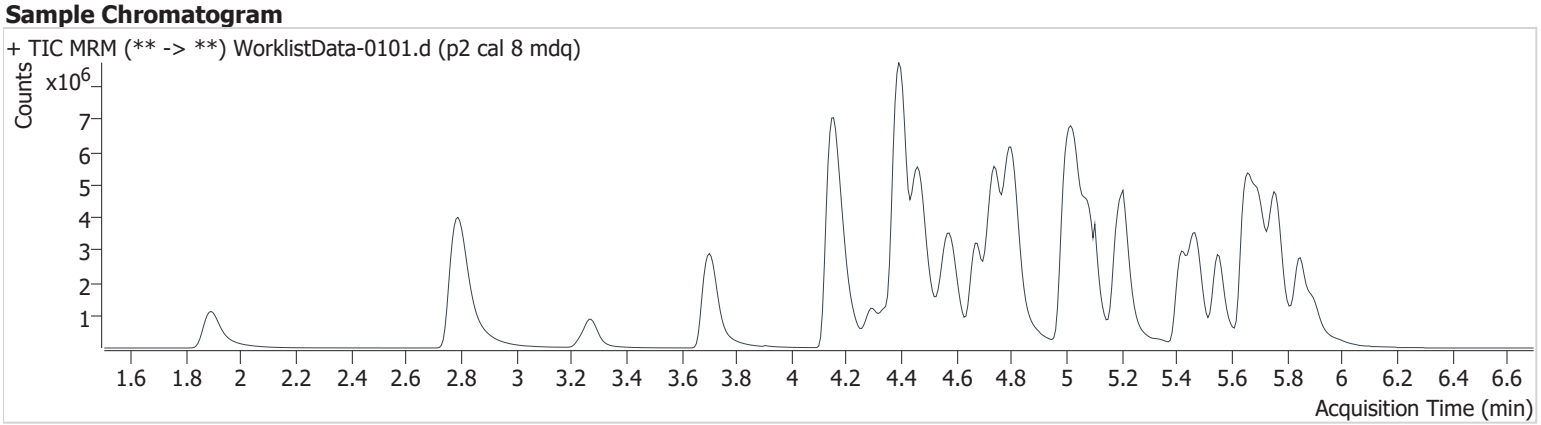
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	410838	20551.4	37.3	1644.6	35566	505.149	ng/ml
Maprotiline	5.729	468895	1501.4	52.1	660.0	35566	507.385	ng/ml
methocarbamol	4.502	467653	25119.0	90.1	29295.8	61688	484.844	ng/ml
Nortriptyline	5.751	321271	42769.2	34.9	7046.5	24248	472.896	ng/ml
paroxetine	5.630	146432	9853.0	52.5	71299.9	35566	517.023	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\mdqp2.batch.bin
Calibration Last Update 10/12/2021 10:36:34 AM

Instrument	69679	**Data File**	WorklistData-0101.d
Type	Cal	**Sample**	p2 cal 8 mdq
Acq. Method	mdqp2 1-21-21.m	**Operator**	Anne Nord
Sample Position	P2-H4	**Comment**	
Injection Volume	5		
Acq. Date-Time	10/8/2021 9:17:01 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Amitriptyline	5.729	617925	20013.4	39.0	3419.8	27858	971.146	ng/ml
Maprotiline	5.729	698078	3507.5	46.4	27581.0	27858	965.650	ng/ml
methocarbamol	4.502	571267	345.3	90.6	24052.2	39596	923.318	ng/ml
Nortriptyline	5.751	407183	26854.3	35.3	4070.1	14056	1033.538	ng/ml
paroxetine	5.630	193462	2436.9	52.8	3136.8	27858	872.036	ng/ml