Worklist: 2452 reviewed 6/8/18

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

Extraction Date: 6-5/18 Analyst: Anne Nord
Plate lot#: 0507680 Plate Expiration: 6-5-2018

Blank Blood Lot: 17J20718 Column: Agilent 120 EC-C18 (2.1x 100-4um)

LCMS-QQQ ID: 62340

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.
- □ 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.
- ✓ 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.
- \boxtimes 10. Wait 5 minutes.
- □ 11. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- □ 12. Add 900uL ethyl acetate.
- \boxtimes 13. Wait 5 minutes.
- □ 14. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- □ 16. Reconstitute in 100μL 20% MeOH and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- ☑ 1. Create batch and process data.Worklist path: 06062018 Batch Name: mdq pla
- \boxtimes 3. Integration linear and R² values \ge 0.98 for each analyte.
- □ 5. Did all QCs pass for each analyte?
 ② N _____ Add Control data to QC tracking spreadsheet.
- ⊠ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS:

Only ran panel 2a and evaluated amitriptyline, mirtazapine, and mitragynine

Mirturapine dropped 250 calibrature top of peak out off.

Toxicology AM method 28 panel 2 external prep information working solution 10000 ng/ml in meoh mirtazapine, amitriptyline, meperidine Stock solution 1mg/ml 100 ul each in 9700 ul meOH

Ppd 8/7/17 Exp: 8/7/18 lot 2718

by AMN

meperidine FE1	amitriptyline fn0:	mirtazapine fe0	Drug lot	
FE1191502 2/1/2020	fn07081401 9/1/2019	fe04201503 4/1/2020	expiration	

ppd 8/7/17 Exp 11/7/17	AM 28 control 50 ul working solution lot (2718) in 9950 ul blood lot (321632)
by AMN	g solution lot (2718) ii
Concentration 50 ng/ml each	n 9950 ul blood lot (321632)

ppd 6/5/18 Exp 8/7/18	ppd 12/5/17 Exp 5/7/18
by AMN	by AMN
Concentration 50 ng/ml each	Concentration 50 ng/ml each
blank blood lot (17J20718)	



Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time6/6/2018 9:27 AMAnalyst NameISP ToxReport Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

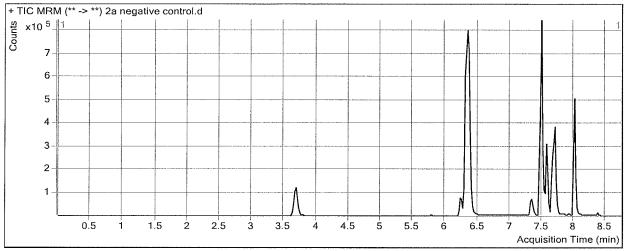
Analysis Info

Acq Time2018-06-05 18:56Data File2a negative control.dSample TypeSampleSample Name2a negative controlDilution1Acq MethodMDQ Panel 2a 3-12-17-3.m

Position P2-E6 **Sample Info**

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram





Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time6/6/2018 9:27 AMAnalyst NameISP ToxReport Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

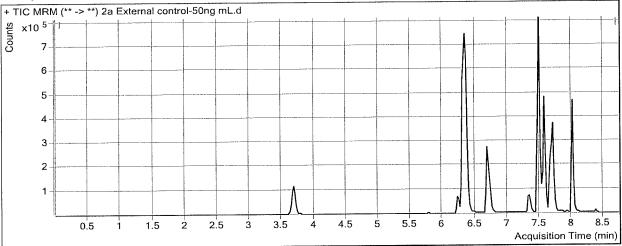
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Position P2-F6 **Sample Info**

Inj Vol -1 Comment AM 28 mirtazapine 50ng, amitriptyline 50 ng

Sample Chromatogram



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.704	475851	240674	1.9772	32.9489
Amitriptyline	Amitriptyline-D3	7.567	252133	120665	2.0895	39.8779



Batch Data Path

Last Calib Update

D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time Report Time 6/6/2018 9:27 AM

Analyst Name ISP Tox

6/6/2018 9:33 AM 6/6/2018 9:27 AM **Reporter Name** ISP Tox

Batch State

Processed

Analysis Info

Acq Time

2018-06-05 19:21

Data File

2a QC 10.d

Sample Type

QC

Sample Name

2a QC 10

Dilution

1

Acq Method

MDQ Panel 2a 3-12-17-3.m

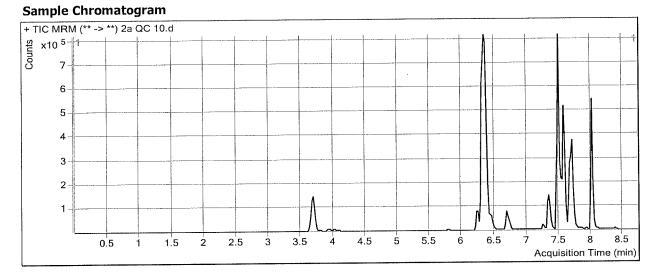
Position

P2-A6

Sample Info Comment

AM 28 Panel 2

Inj Vol



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.704	130144	388205	0.3352	7.7644
Mitragynine	Doxepin-D3	7,232	14689	388205	0.0378	8.6924
Amitriptyline	Amitriptyline-D3	7.567	116393	204290	0.5697	10.1972

Printed at: 9:34 AM on: 6/6/2018

D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin **Batch Data Path**

6/6/2018 9:27 AM **Analysis Time** 6/6/2018 9:33 AM **Report Time** 6/6/2018 9:27 AM **Last Calib Update**

Analyst Name ISP Tox Reporter Name ISP Tox Processed **Batch State**

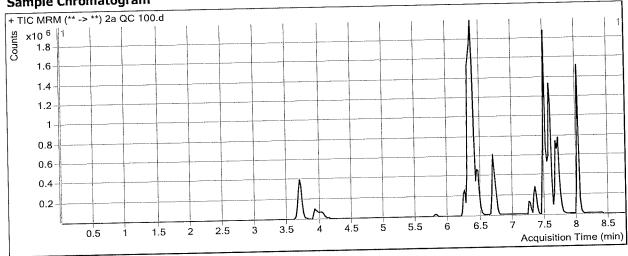
Analysis Info

2a QC 100.d Data File 2018-06-05 20:24 **Acq Time** Sample Name 2a QC 100 QC Sample Type

MDQ Panel 2a 3-12-17-3.m **Acq Method** 1 Dilution Sample Info P2-B6 Position

AM 28 Panel 2 Comment -1 Inj Vol

Sample Chromatogram



ResultsCompoundISTD CompoundRTMirtazapineDoxepin-D36.704MitragynineDoxepin-D37.232AmitriptylineAmitriptyline-D37.567	Response 1094601 117113 689761	ISTD Resp 255080 255080 132191	Resp Ratio 4,2912 0.4591 5.2179	Final Conc 68.4427 82.0349 100.9735
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Printed at: 9:34 AM on: 6/6/2018 Samples Report MDQ.xlsx

D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin **Batch Data Path**

Analyst Name ISP Tox **Analysis Time** 6/6/2018 9:27 AM

Report Time 6/6/2018 9:33 AM **Last Calib Update**

Reporter Name ISP Tox Processed **Batch State** 6/6/2018 9:27 AM

Analysis Info

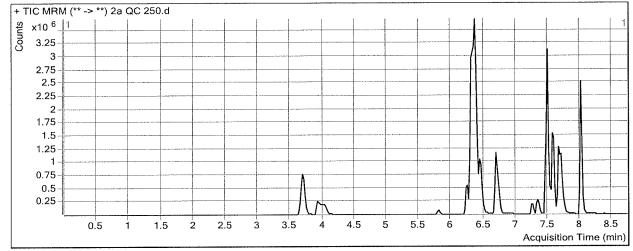
Acq Time 2018-06-05 20:37 Data File 2a QC 250.d Sample Name 2a QC 250 Sample Type QC

Acq Method MDQ Panel 2a 3-12-17-3.m Dilution 1

Sample Info P2-C6 **Position**

AM 28 Panel 2 Comment Inj Vol -1

Sample Chromatogram



Results	•					
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.704	2092434	122368	17.0995	264.9021
Mitragynine	Doxepin-D3	7.252	171178	122368	1.3989	245.6382
Amitriptyline	Amitriptyline-D3	7.567	464012	37776	12.2831	238.9537

Printed at: 9:34 AM on: 6/6/2018 Samples Report MDQ.xlsx

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time6/6/2018 9:27 AMAnalyst NameISP ToxReport Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

Analysis Info

 Acq Time
 2018-06-05 20:50
 Data File
 2a QC 1000.d

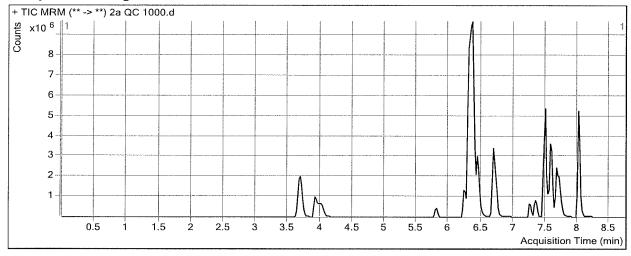
 Sample Type
 QC
 Sample Name
 2a QC 1000

Dilution 1 **Acq Method** MDQ Panel 2a 3-12-17-3.m

Position P2-D6 **Sample Info**

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.704	6851556	102840	66.6232	1024.5175
Mitragynine	Doxepin-D3	7.232	629517	102840	6.1213	1067.7708
Amitripty l ine	Amitriptyline-D3	7.567	1662981	36809	45.1792	881.3985

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Samples Report MDQ.xlsx

Printed at: 9:34 AM on: 6/6/2018

ISP Forensics Calibration Curve Report

Batch Data Path

D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

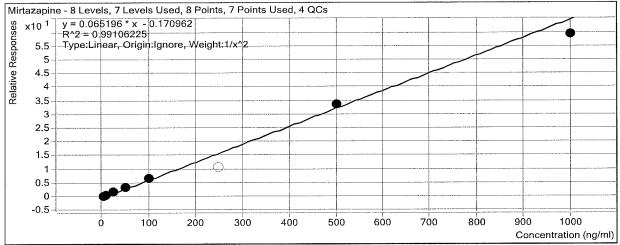
Last Calib Update 6/6/2018 9:27 AM **Analyst Name**

ISP TOX

Target Compound

Mirtazapine

Internal Standard Doxepin-D3



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
2a cal 1-5ng	1	\square	5	5.3	105.1
2a cal 2-10ng	2	\square	10	8.6	85.9
2a QC 10	2	\square	10	7.8	77.6
2a cal 3-25ng	3	☑	25	26.7	106.7
2a cal 4-50ng	4	\square	50	52.0	104.0
2a cal 5-100ng	5	\square	100	103.9	103.9
2a QC 100	5	\square	100	68.4	68.4
2a cal 6-250ng	6		250	163.6	65.4
2a QC 250	6		250	264.9	106.0
2a cal 7-500ng	7	\square	500	516.3	103.3
2a cal 8-1000ng	8	\square	1000	911.2	91.1
2a OC 1000	8	☑	1000	1024.5	102.5

Dropped 250 point in curve peak had a flat top. Dr



ISP Forensics Calibration Curve Report

Batch Data Path

D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

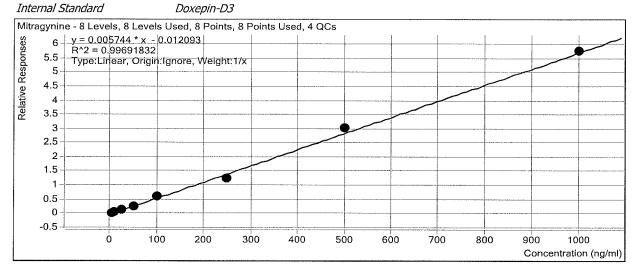
Last Calib Update 6/6/2018 9:27 AM

Analyst Name

ISP TOX

Target Compound

Mitragynine



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
2a cal 1-5ng	1	\square	5	5.8	116.1
2a cal 2-10ng	2	☑	10	9.4	94.4
2a QC 10	2	\square	10	8.7	86.9
2a cal 3-25ng	3	\square	25	24.2	96.9
2a cal 4-50ng	4	\square	50	47.9	95.8
2a cal 5-100ng	5	\square	100	103.4	103.4
2a QC 100	5	\square	100	82.0	82.0
2a cal 6-250ng	6	₫	250	219.5	87.8
2a QC 250	6	\square	250	245.6	98.3
2a cal 7-500ng	7	\square	500	526.0	105.2
2a cal 8-1000ng	8	☑	1000	1003.7	100.4
2a QC 1000	8	\square	1000	1067.8	106.8

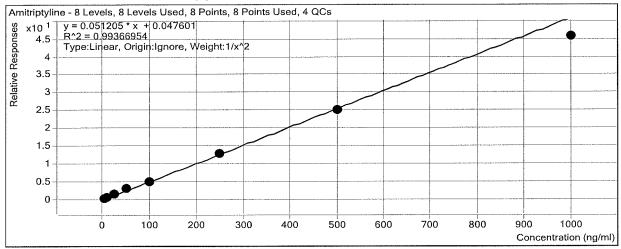


ISP Forensics Calibration Curve Report

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Last Calib Update 6/6/2018 9:27 AM Analyst Name ISP TOX

Target CompoundAmitriptylineInternal StandardAmitriptyline-D3



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
2a cal 1-5ng	1	\square	5	4.7	94.4
2a cal 2-10ng	2	\square	10	10.7	107.4
2a QC 10	2	\square	10	10.2	102.0
2a cal 3-25ng	3	\square	25	26.5	105.8
2a cal 4-50ng	4	\square	50	54.6	109.2
2a cal 5-100ng	5	\square	100	97.3	97.3
2a QC 100	5	\square	100	101.0	101.0
2a cal 6-250ng	6	\square	250	247.6	99.0
2a QC 250	6	\square	250	239.0	95.6
2a cal 7-500ng	7	\square	500	487.5	97.5
2a cal 8-1000ng	8	\square	1000	892.2	89.2
2a QC 1000	8	\square	1000	881.4	88.1



Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

 Analysis Time
 6/6/2018 9:27 AM
 Analyst Name
 ISP Tox

 Report Time
 6/6/2018 9:33 AM
 Reporter Name
 ISP Tox

 Last Calib Update
 6/6/2018 9:27 AM
 Batch State
 Processed

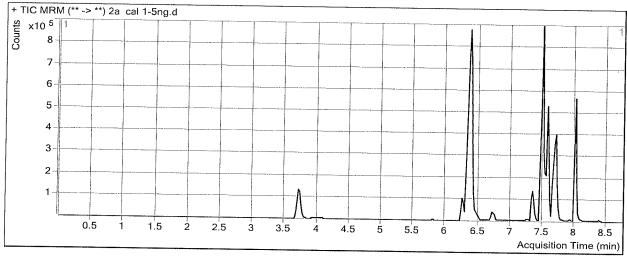
Analysis Info

Results

Acq Time2018-06-05 17:02Data File2a cal 1-5ng.dSample TypeCalibrationSample Name2a cal 1-5ngDilution1Acq MethodMDQ Panel 2a 3-12-17-3.mPositionP2-A5Sample Info

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Compound Mirtazapine	ISTD Compound Doxepin-D3	RT 6.724	Response	ISTD Resp	Resp Ratio	Final Conc
	•	0.724	68526	399038	0.1717	5.2563
Mitragynine	Doxepin-D3	7.252	8484	399038	0.0213	5.8065
Amitriptyline	Amitriptyline-D3	7.567	63113	218108	0.2894	4.7215

4

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time6/6/2018 9:27 AMAnalyst NameISP ToxReport Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

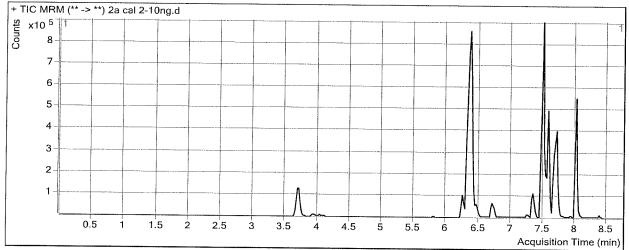
Analysis Info

Acq Time2018-06-05 17:14Data File2a cal 2-10ng.dSample TypeCalibrationSample Name2a cal 2-10ngDilution1Acq MethodMDQ Panel 2a 3-12-17-3.m

Position P2-B5 Sample Info

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.724	112925	290243	0.3891	8.5900
Mitragynine	Doxepin-D3	7.232	12234	290243	0.0422	9.4435
Amitriptyline	Amitriptyline-D3	7.567	83437	139604	0.5977	10.7425

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

 Analysis Time
 6/6/2018 9:27 AM
 Analyst Name
 ISP Tox

 Report Time
 6/6/2018 9:33 AM
 Reporter Name
 ISP Tox

 Last Calib Update
 6/6/2018 9:27 AM
 Batch State
 Processed

Analysis Info

Results

 Acq Time
 2018-06-05 17:27
 Data File
 2a cal 3-25ng.d

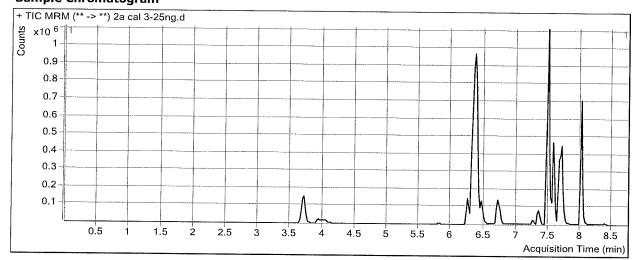
 Sample Type
 Calibration
 Sample Name
 2a cal 3-25ng

 Dilution
 1
 Acq Method
 MDQ Panel 2a 3-12-17-3,m

Position P2-C5 Sample Info

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.724	246715	157336	1.5681	26.6741
Mitragynine	Doxepin-D3	7.252	19985	157336	0.1270	24,2191
Amitriptyline	Amitriptyline-D3	7.567	64159	45754	1.4023	26.4557

Samples Report MDQ.xlsx

Printed at: 9:34 AM on: 6/6/2018

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time 6/6/2018 9:27 AM Analyst Name ISP Tox Report Time 6/6/2018 9:33 AM Reporter Name ISP Tox

6/6/2018 9:27 AM

Reporter Name ISP Tox
Batch State Processed

Analysis Info

Last Calib Update

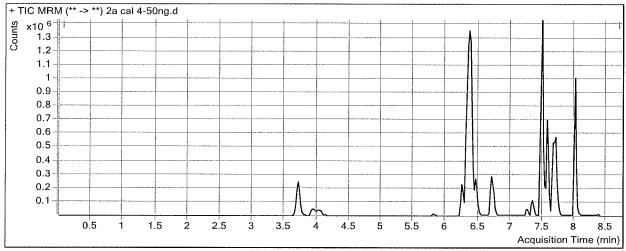
Acq Time2018-06-05 17:40Data File2a cal 4-50ng.dSample TypeCalibrationSample Name2a cal 4-50ng

Dilution 1 **Acq Method** MDQ Panel 2a 3-12-17-3.m

Position P2-D5 **Sample Info**

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.724	515595	160121	3.2200	52.0124
Mitragynine	Doxepin-D3	7.252	42104	160121	0.2629	47.8824
Amitriptyline	Amitriptyline-D3	7.567	134463	47274	2.8444	54.6193

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Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time 6/6/2018 9:27 AM Analyst Name ISP Tox

Report Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

Analysis Info

 Acq Time
 2018-06-05 17:52
 Data File
 2a cal 5-100ng.d

 Sample Type
 Calibration
 Sample Name
 2a cal 5-100ng

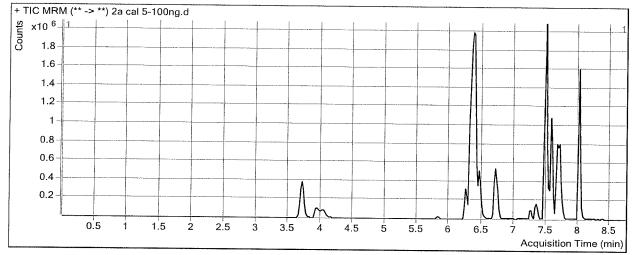
 Dilution
 1
 Acq Method
 MDO Panel 2a 3-12-17-24

Dilution 1 Acq Method MDQ Panel 2a 3-12-17-3.m

Position P2-E5 Sample Info

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.724	964673	146151	6.6005	103,8637
Mitragynine	Doxepin-D3	7.252	85043	146151	0.5819	103,4067
Amitriptyline	Amitriptyline-D3	7.567	245261	48746	5.0314	97.3315

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time6/6/2018 9:27 AMAnalyst NameISP ToxReport Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

Analysis Info

 Acq Time
 2018-06-05 18:05
 Data File
 2a cal 6-250ng.d

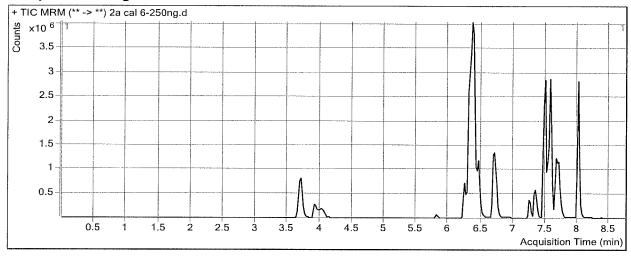
 Sample Type
 Calibration
 Sample Name
 2a cal 6-250ng

Dilution 1 **Acq Method** MDQ Panel 2a 3-12-17-3.m

PositionP2-F5Sample Info

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.724	2763506	263347	10.4938	163.5803
Mitragynine	Doxepin-D3	7.232	328904	263347	1.2489	219.5342
Amitriptyline	Amitriptyline-D3	7.567	1811964	142388	12.7255	247.5935

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time6/6/2018 9:27 AMAnalyst NameISP ToxReport Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

Analysis Info

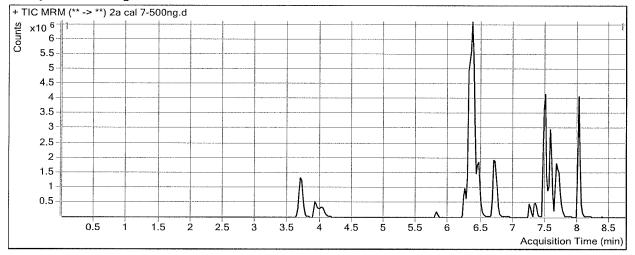
Acq Time2018-06-05 18:18Data File2a cal 7-500ng.dSample TypeCalibrationSample Name2a cal 7-500ng

Dilution 1 **Acq Method** MDQ Panel 2a 3-12-17-3.m

Position P2-G5 **Sample Info**

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.724	4067661	121456	33.4908	516.3186
Mitragynine	Doxepin-D3	7.232	365518	121456	3.0095	526.0265
Amitriptyline	Amitriptyline-D3	7.567	1087629	43491	25.0080	487.4641

Batch Data Path D:\2018 Data\06062018 mdq\QuantResults\mdq p2a.batch.bin

Analysis Time6/6/2018 9:27 AMAnalyst NameISP ToxReport Time6/6/2018 9:33 AMReporter NameISP ToxLast Calib Update6/6/2018 9:27 AMBatch StateProcessed

Analysis Info

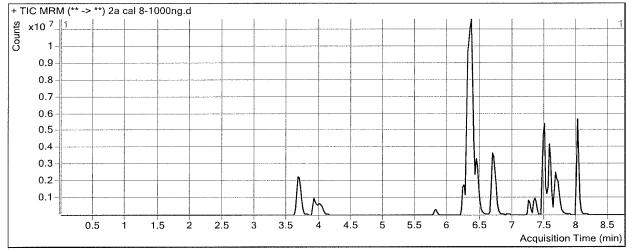
Acq Time2018-06-05 18:30Data File2a cal 8-1000ng.dSample TypeCalibrationSample Name2a cal 8-1000ng

Dilution 1 **Acq Method** MDQ Panel 2a 3-12-17-3.m

Position P2-H5 **Sample Info**

Inj Vol -1 Comment AM 28 Panel 2

Sample Chromatogram



Results	ì
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Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
Mirtazapine	Doxepin-D3	6.704	7954308	134277	59.2382	911.2436
Mitragynine	Doxepin-D3	7.232	772516	134277	5.7532	1003.6811
Amitriptyline	Amitriptyline-D3	7 . 567	1998923	43709	45.7327	892.2087

