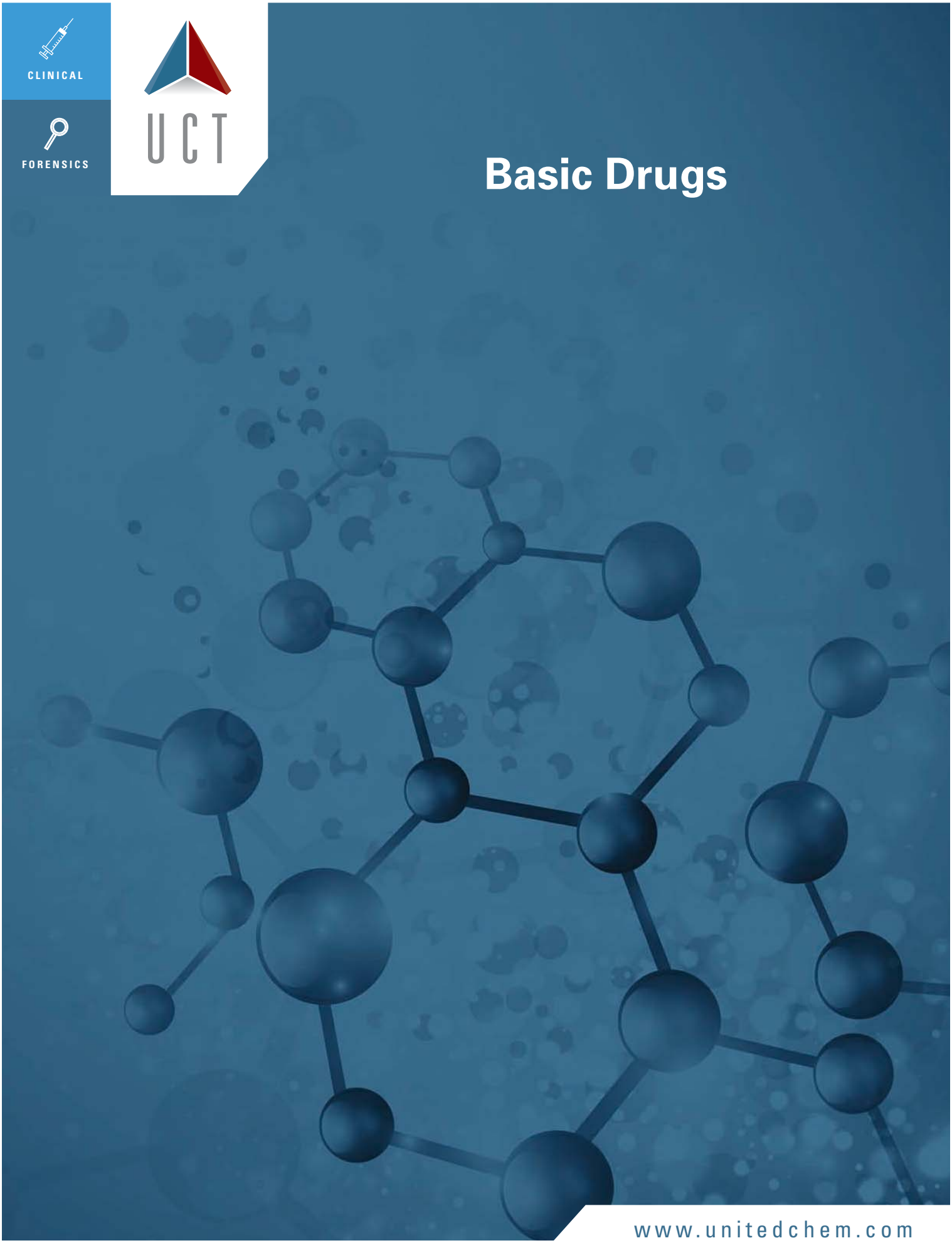
CLINICAL

FORENSICS

UCT

Basic Drugs



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ANTIDEPRESSANTS IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

CSDAU – CLEAN SCREEN® DAU

SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
   Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
   Mix/vortex and let stand for 5 minutes
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM phosphate buffer (pH 6.0)
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE ANTIDEPRESSANTS:**
   1 x 3 mL CH_2Cl_2/IPA/NH_4OH (78:20:2 v/v)
   Collect eluate at 1 to 2 mL/minute
   or
   1 x 3 mL Ethyl Acetate/ IPA/ NH_4OH (78:20:2 v/v)

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add Ethyl Acetate (pH 11-12)

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 20 µL.
   - **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

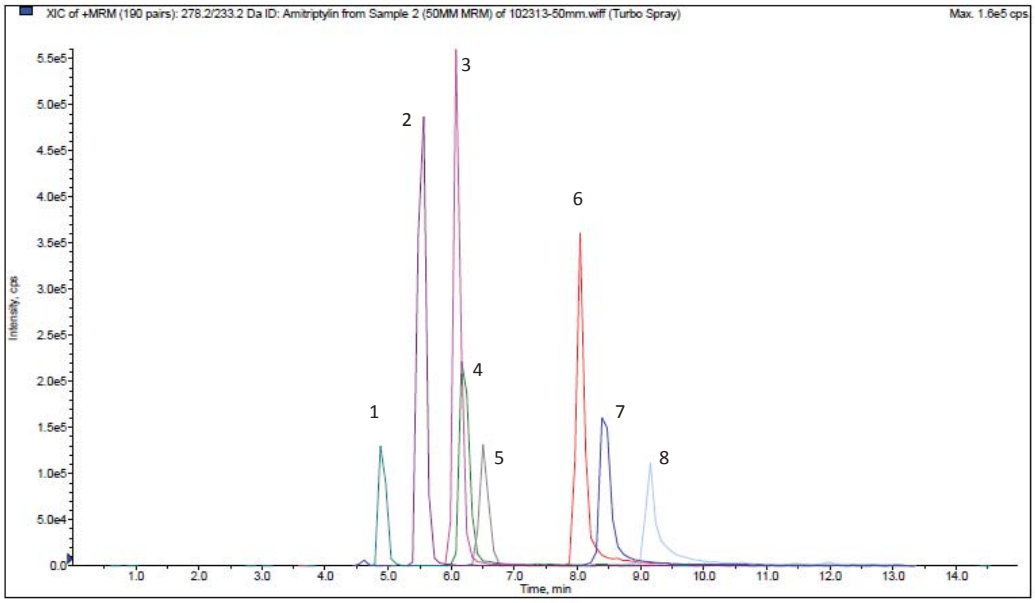
   Alternate Derivatization

   Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Venlafaxaine	278.2	260.2	4.90
2.Zolpidem	308.2	235.2	5.50
3.Trazadone	372.2	176.1	6.05
4.PCP	244.2	86.1	6.20
5.Quintiapine	384.2	253.1	6.50
6.Imipiramine	281.2	86.1	8.40
7.Amitriptyline	278.2	233.2	8.42
8.Sertraline	306.1	159	9.25

PARAMETERS

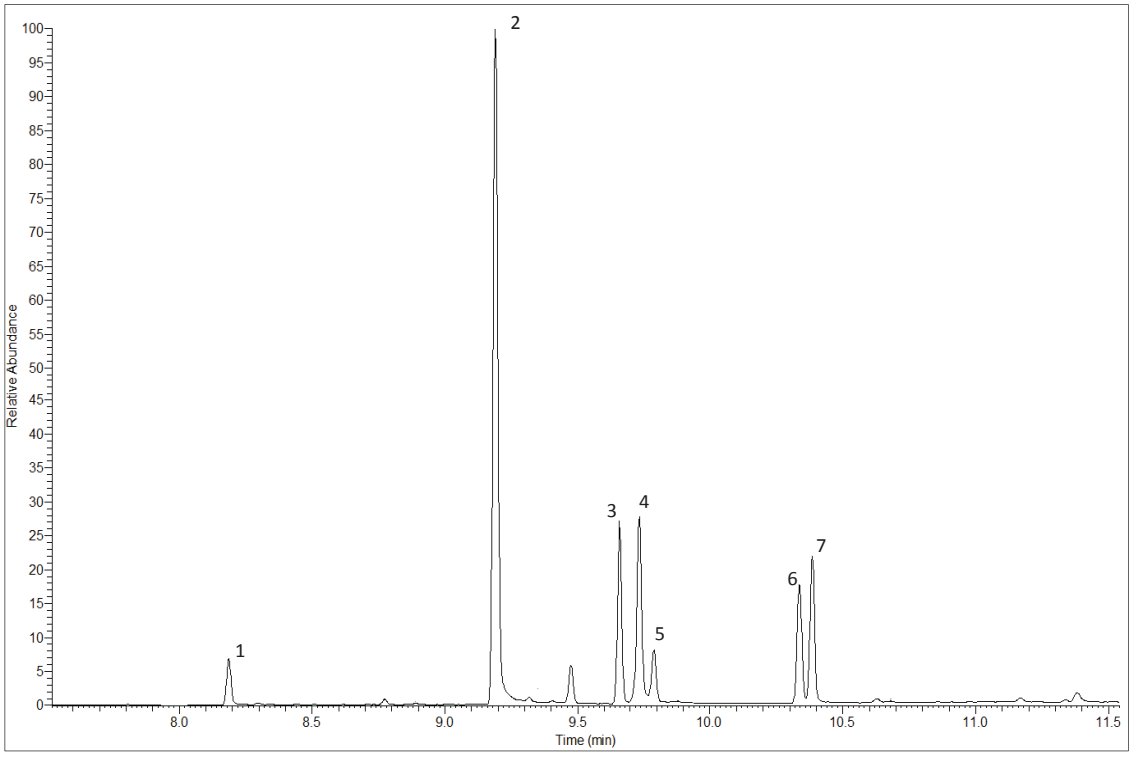
Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Injection Volume: 20 µL
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time minutes
1. **Fluoxetine**	309	91	104	8.19
2. **Venlafaxine**	134	179	202	9.19
3. **Amitriptyline**	115	203	202	9.66
4. **Nortryptyline**	189	202	115	9.72
5. **Imipramine**	193	280	234	9.77
6. **Sertraline**	274	262	159	10.34
7. **Citalopram**	324	208	238	10.39

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

GC capillary column: 30m x 0.25mm (0.25µm) TG-1MS

Injector: 1 µL Splitless, 250 °C

Oven temperature program: 70 °C (0.5) to 320 °C (25 °C/minute): hold (2 minutes)

Carrier gas: Carrier Gas: Helium (1.2 mL/minute)

MSD condition: Aux temperature: 280 ºC, MS Source: 350 ºC, MS Quad: 150 ºC

BASIC ANALYTES IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

CSDAU - CLEAN SCREEN® DAU SPE cartridge

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

SLDA50ID21-5UM – Selectra® DA HPLC Column, 50x2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
   Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
   Mix/vortex and let stand for 5 minutes
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet
   NOTE: See Hydrolysis step if required

Hydrolysis: To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C. (Hydroxylamine can be added to sample here if oxime derivative is preferred.) Allow sample to cool

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM phosphate buffer (pH 6.0)
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at full vacuum or pressure)

5. **ELUTE BASIC ANALYTES:**
   1 x 3 mL CH_2Cl_2:/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

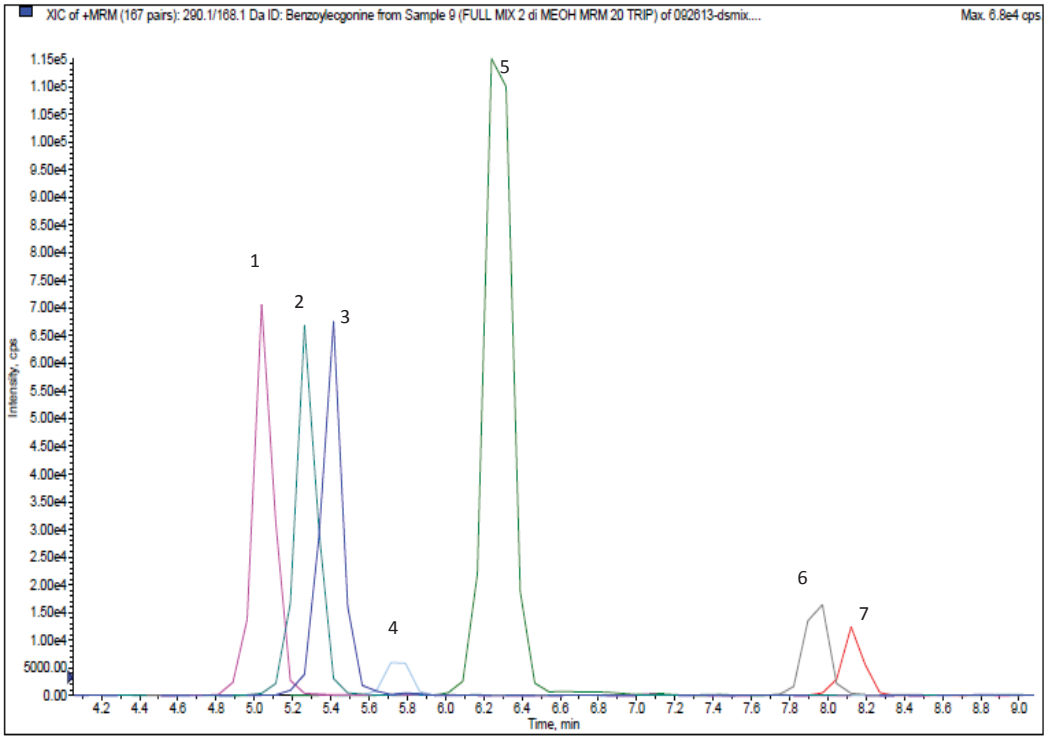
Alternate Derivatization

Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (LC-MS/MS):

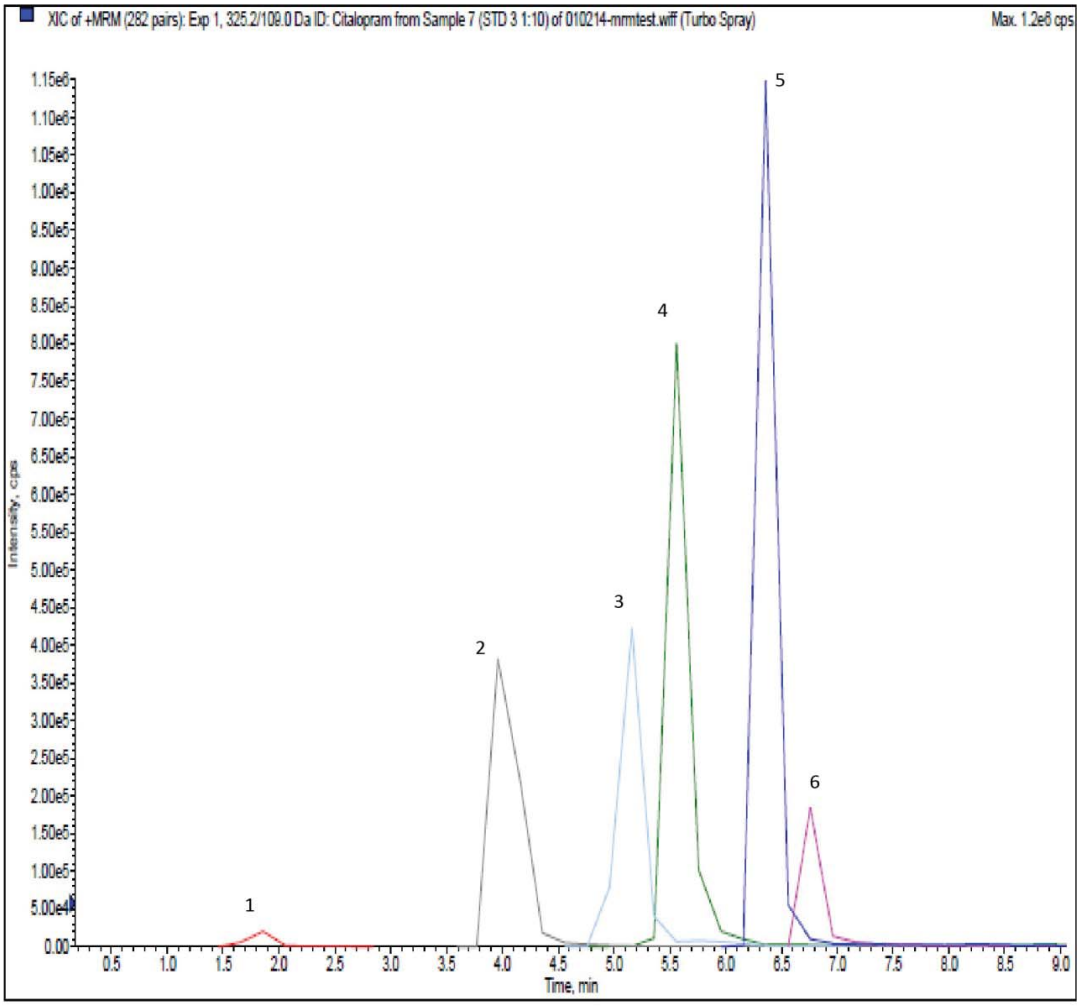
CHROMATOGRAMS

Basic Panel 1



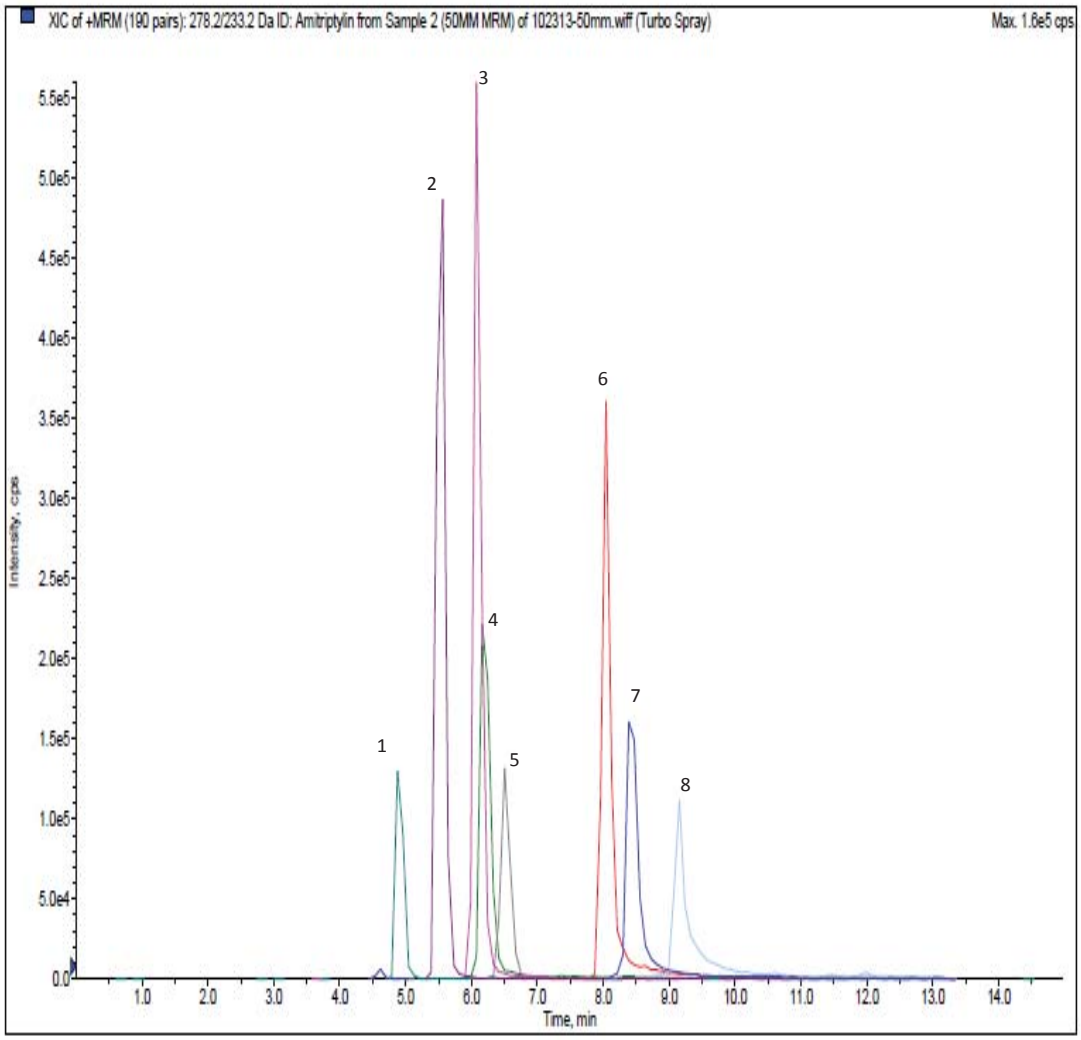
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Tapentadol	222.2	107.2	5.10
2.Tramadol	264.2	58.0	5.25
3.Benzoylecgonine	290.1	168.1	5.40
4.Meperidine	248.2	220.0	5.75
5.Cocaine	304.1	182.1	6.30
6.Fentanyl	337.2	188.2	7.90
7.Buprenorphine	468.3	396.3	8.15

Basic Panel 2



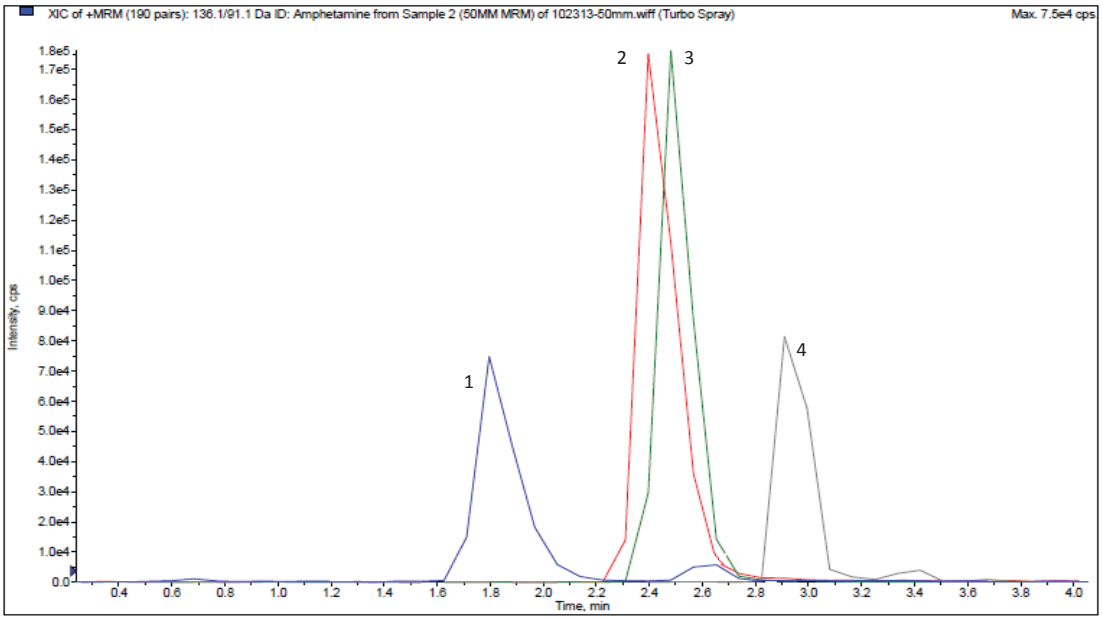
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 20 µL

L Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm

Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
SYMPATHOMIMETICS
TCA'S(7)
MDMA/MDA/MDEA
MEPERIDINE/NORMEPERIDINE
CYCLOBENZAPRINE
DIPHENHYDRAMINE
OPIATES(7)
PCP
FENTANYL/NORFENTANYL
CITALOPRAM
METHADONE/EDDP
COCAINE/BZE
SERTRALINE TRAMADOL/NORTRAM
CLONIDINE

BASIC ANALYTES IN BLOOD/URINE/SERUM BY LC-MS/MS OR GC-MS CLEAN SCREEN® XCEL I 96 WELLPLATE

Part #
WSH96EXE11 – CLEAN SCREEN XCEL® I 130 mg, 96 well plate
BETA-GLUC-10 – Selectrazyme® Beta-Glucuronidase
SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. PREPARE SAMPLE:

To 1-2 mL whole blood, plasma/ serum or urine add 500 µL 100mM phosphate buffer (pH 6.0)

Add appropriate volume and concentration of internal standard.

Note: See Hydrolysis step if required

Hydrolysis: To 1-2 mL of urine sample, add 500 µL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase. Optionally, add 500 µL of acetate buffer and 25 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C. (Hydroxylamine can be added to sample here if oxime derivative is preferred.)
Allow sample to cool
Do not adjust pH~ sample is ready to be added to the extraction plate.

2. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 1 mL 98% Methanol: 2% Acetic Acid
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. ELUTION

1 x 1 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. DRY ELUTE

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

Alternate Derivatization

Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and
react at 70 °C for 30 minutes; Cool and inject 1-2 µL

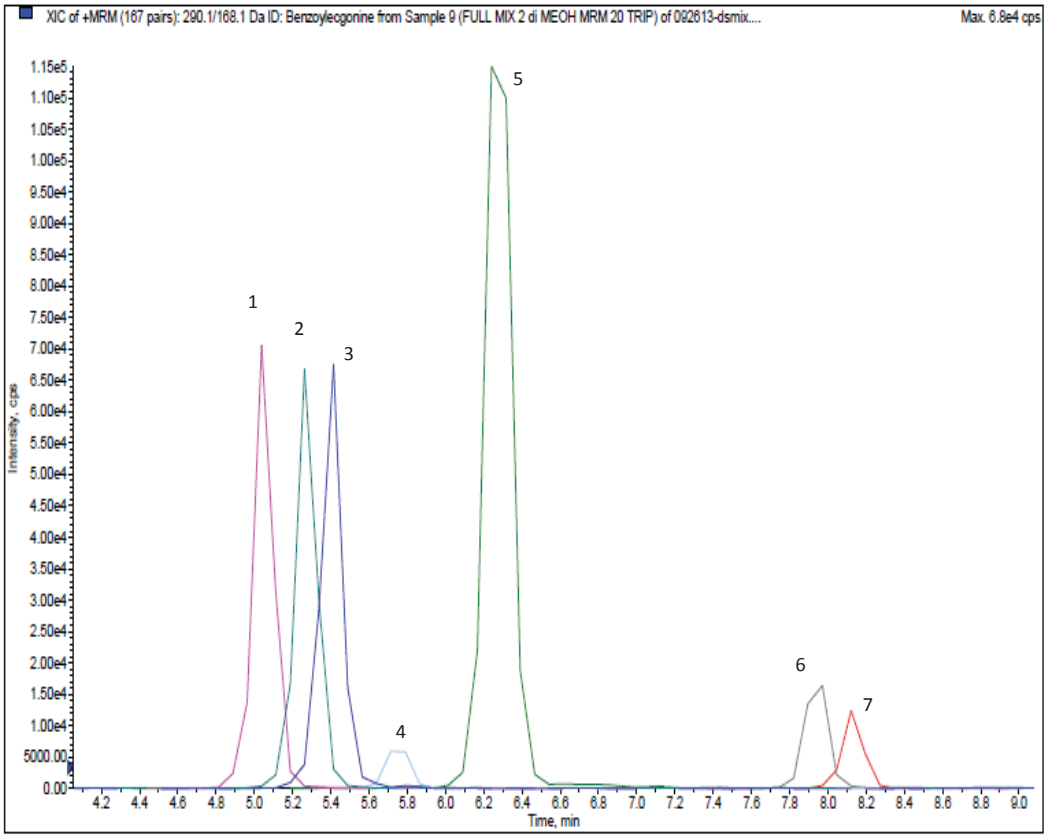
NOTES

(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.

INSTRUMENT CONDITIONS (LC-MS/MS):

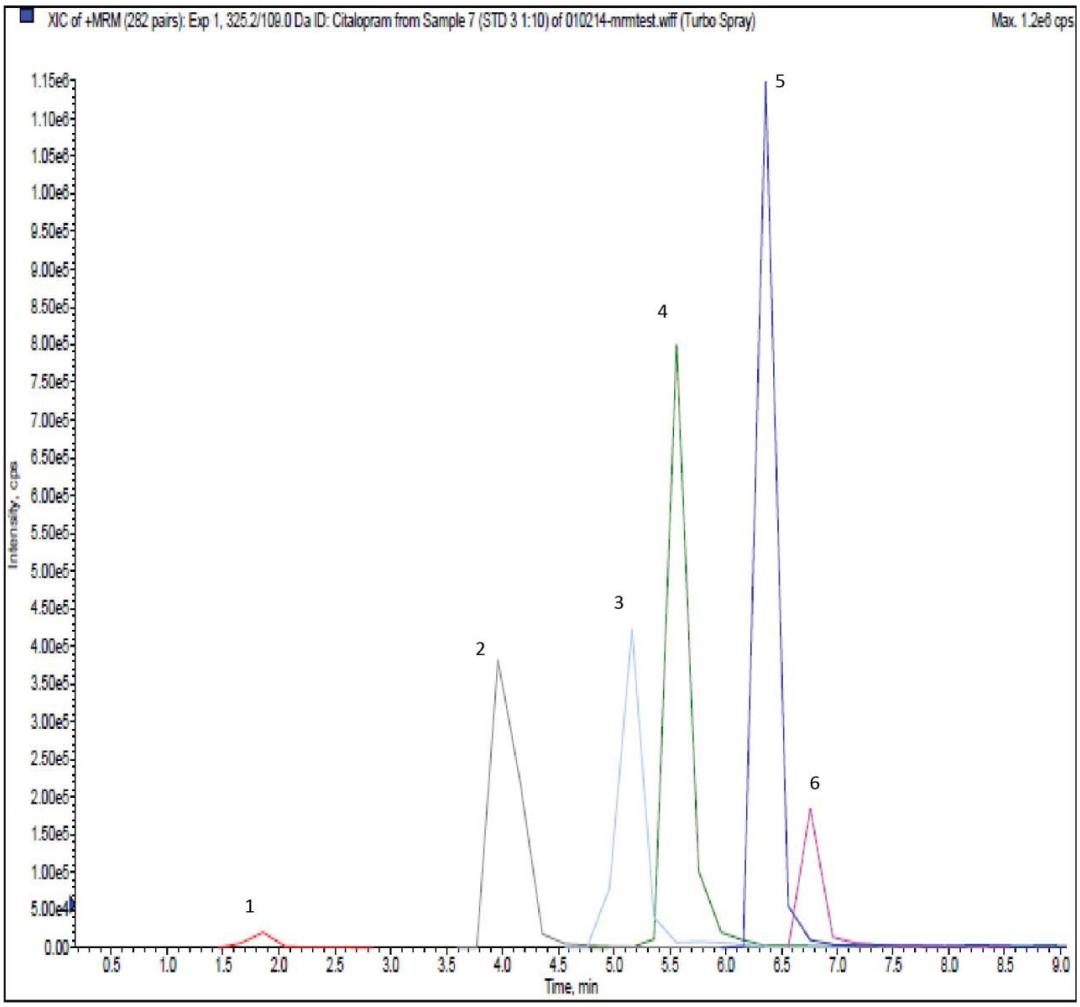
CHROMATOGRAMS

Basic Panel 1



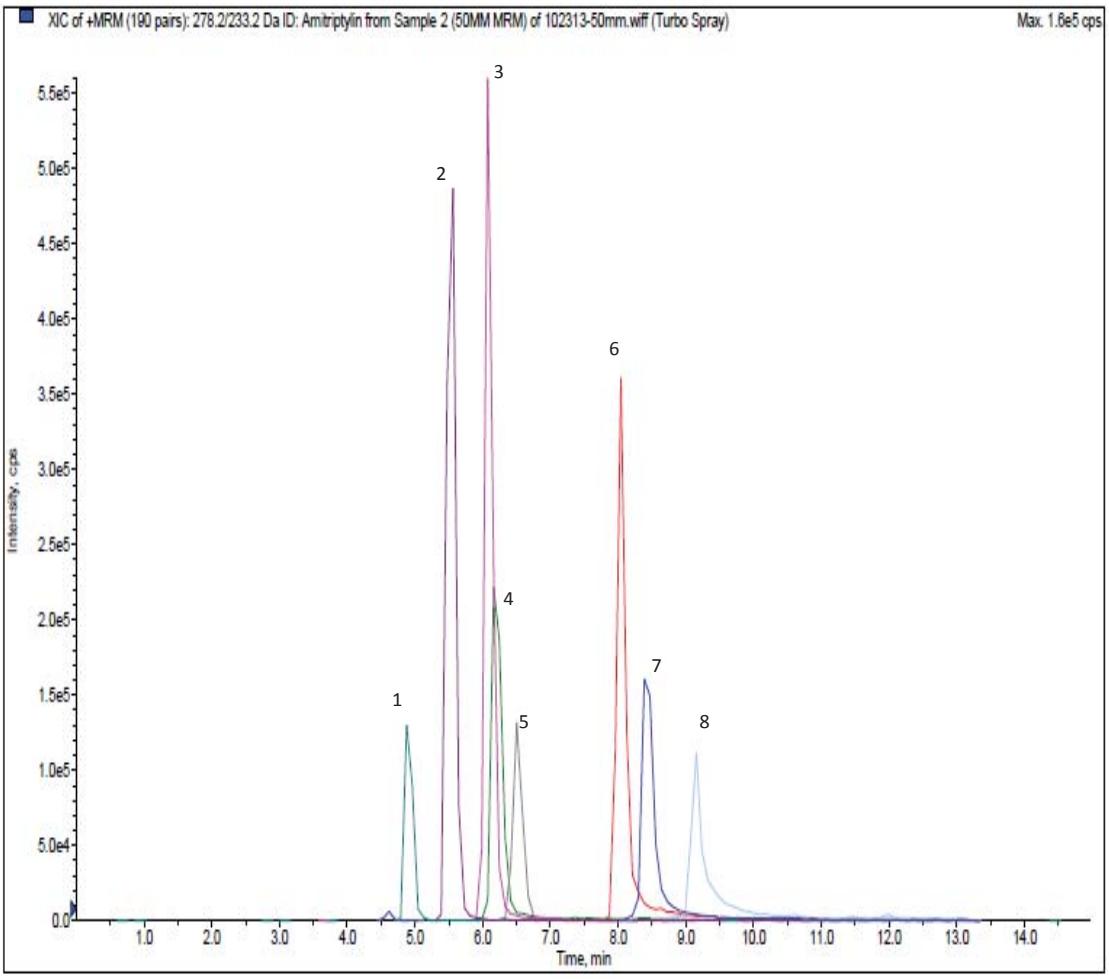
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylecgonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



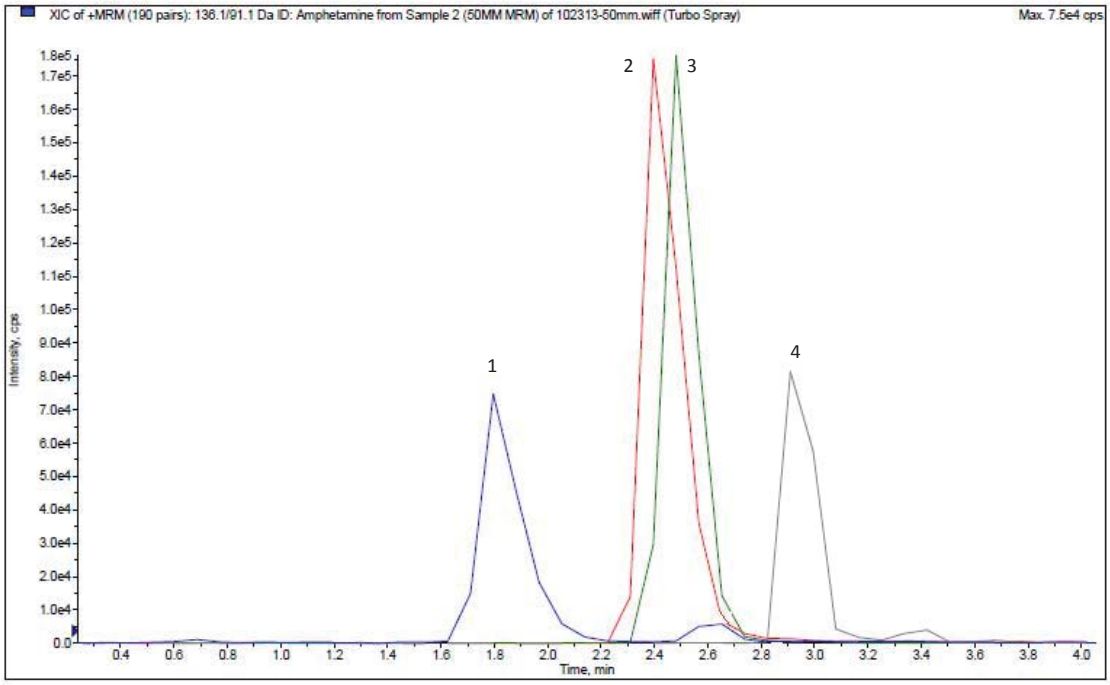
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Amphetamine	136.1	91.1	1.18
2. Methamphetamine	150.1	91.1	2.40
3. MDA	180.1	105.0	2.45
4. MDMA	194.1	105.1	2.95

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Injection Volume: 20 µL
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
SYMPATHOMIMETICS
TCA'S(7)
MDMA/MDA/MDEA
MEPERIDINE/NORMEPERIDINE
CYCLOBENZAPRINE
DIPHENHYDRAMINE
OPIATES(7)
PCP
FENTANYL/NORFENTANYL
CITALOPRAM
METHADONE/EDDP
COCAINE/BZE
SERTRALINE TRAMADOL/NORTRAM
CLONIDINE

BASIC ANALYTES IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN XCEL® I EXTRACTION COLUMN

Part #

CSXCE111 – CLEAN SCREEN XCEL® I 130 mg, 1 mL Tube

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

SLDA50ID21-5UM – Selectra® DA HPLC Column, 50 x 2.1 mm, 5 µm

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet
Note: See Hydrolysis step if required

Hydrolysis: To 1-2mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL Selectrazyme® β-glucuronidase. Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C.
(Hydroxylamine can be added to sample here if oxime derivative is preferred.)
Allow sample to cool

2. APPLY SAMPLE

Load sample directly to column without any preconditioning.
Pull sample through at a rate of 1-2 mL/ minute.
Dry column thoroughly under full vacuum or positive pressure for 1 minute.

3. WASH

1 x 3 mL 98% Methanol: 2% Acetic Acid
Dry column thoroughly under full vacuum or positive pressure for a minimum of 5 minutes.

4. ELUTION

1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.
Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

5. DRY ELUTE

Evaporate fraction to complete dryness under stream of dry air or nitrogen at ~ 35 °C.

6. RECONSTITUTE / DERIVATIZE

- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 20 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

Alternate Derivatization

Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of derivatizing reagent and react at 70 °C for 30 minutes; Cool and inject 1-2 µL

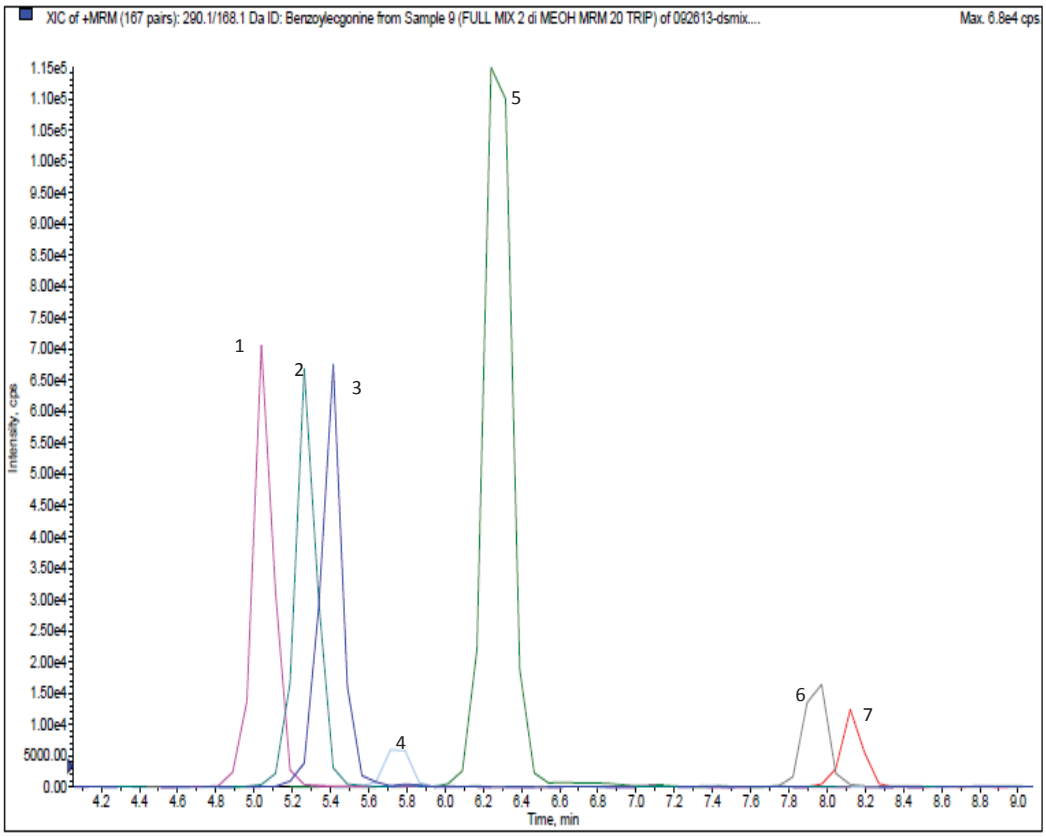
NOTES

(It is important to dry the column thoroughly to achieve the highest recovery of all compounds. Any residual moisture will slow down the drying of the elution solvents prior to derivatization for GC/MS analysis, if being used. Also, any residual moisture could reduce the reactivity of the derivatization agent resulting in low GC/MS sensitivity.

INSTRUMENT CONDITIONS (LC-MS/MS):

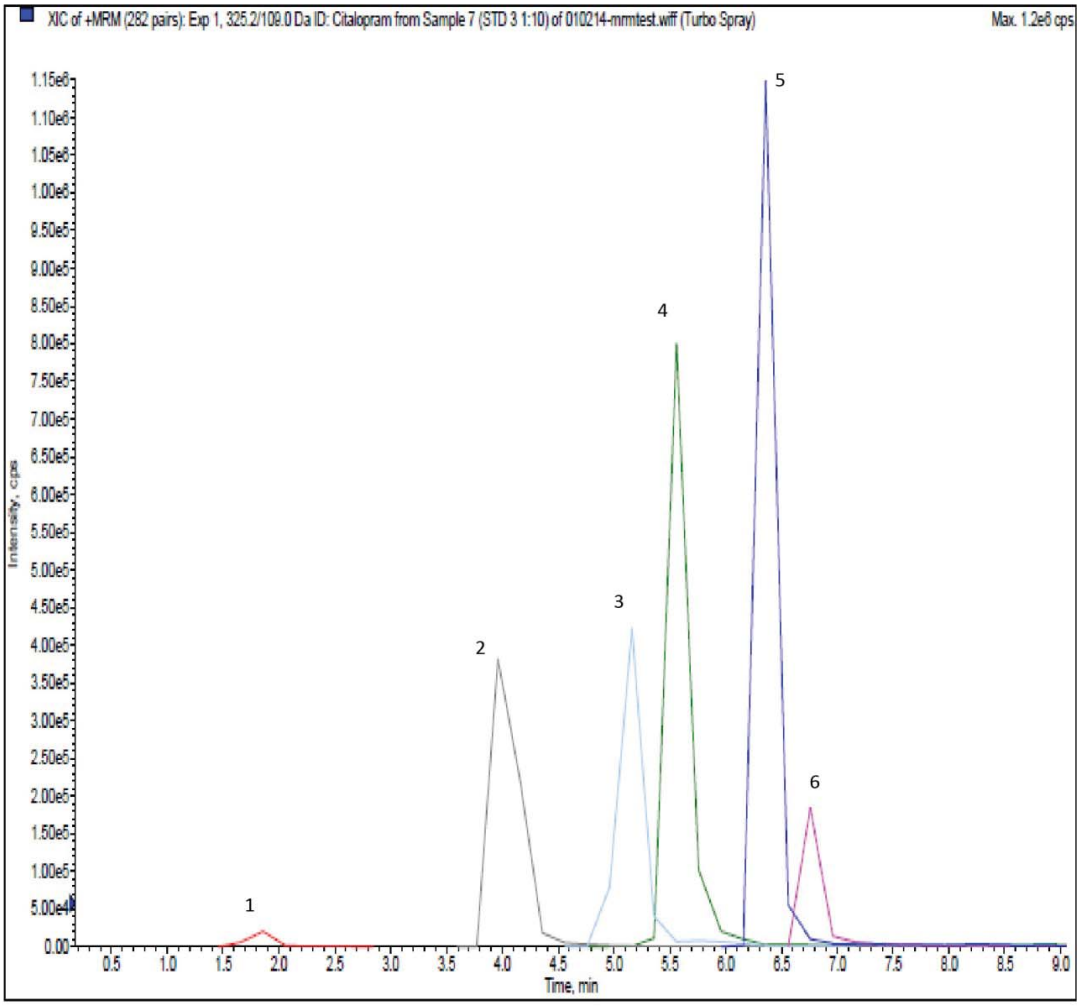
CHROMATOGRAMS

Basic Panel 1



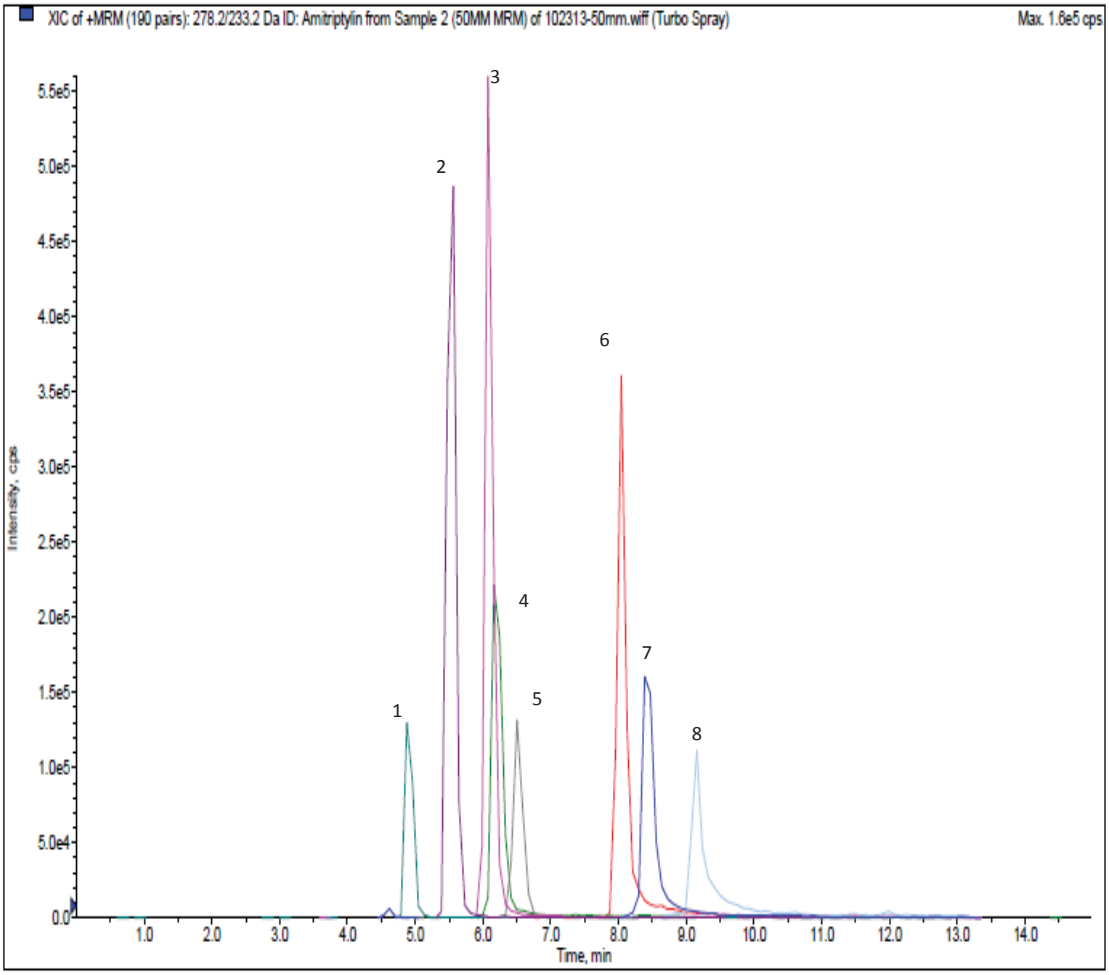
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Tapentadol	222.2	107.2	5.10
2. Tramadol	264.2	58.0	5.25
3. Benzoylecgonine	290.1	168.1	5.40
4. Meperidine	248.2	220.0	5.75
5. Cocaine	304.1	182.1	6.30
6. Fentanyl	337.2	188.2	7.90
7. Buprenorphine	468.3	396.3	8.15

Basic Panel 2



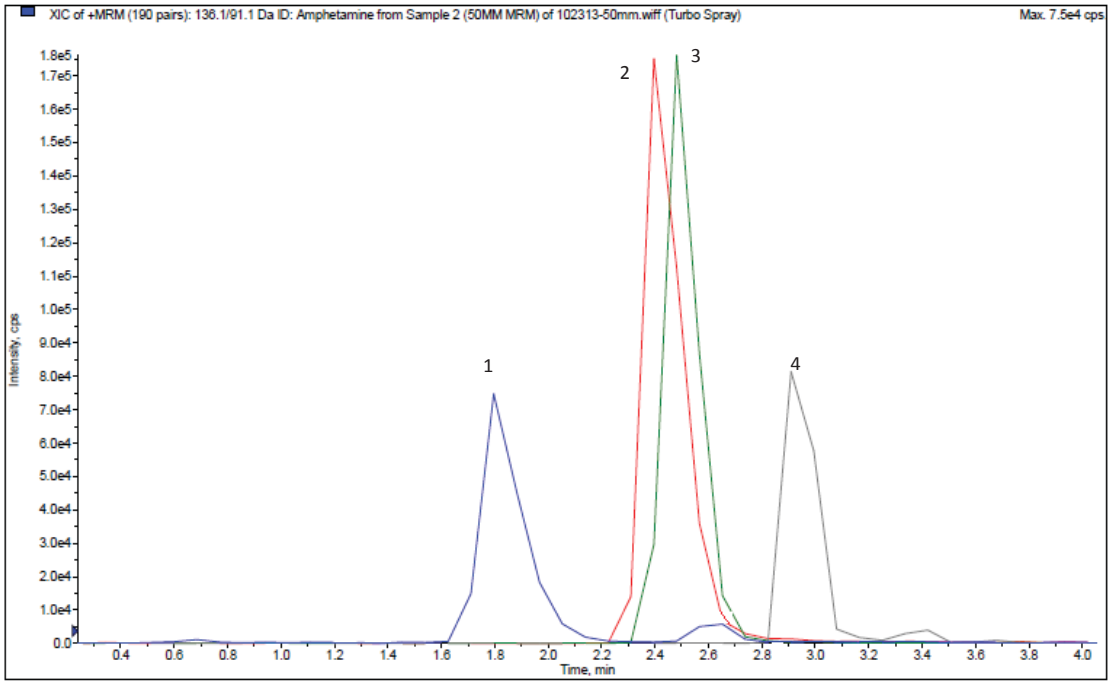
Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Clonidine	230.0	213.0	1.80
2. Ketamine	238.1	125.0	4.00
3. Mirtazepine	266.2	195.1	5.10
4. Clozapine	327.1	270.1	5.60
5. Citalopram	325.2	109.0	6.40
6. Norfluoxetine	296.2	134.2	6.80

Antidepressant Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Venlafaxaine	278.2	260.2	4.90
2. Zolpidem	308.2	235.2	5.50
3. Trazadone	372.2	176.1	6.05
4. PCP	244.2	86.1	6.20
5. Quintiapine	384.2	253.1	6.50
6. Imipiramine	281.2	86.1	8.40
7. Amitriptyline	278.2	233.2	8.42
8. Sertraline	306.1	159	9.25

Amphetamine Panel



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1.Amphetamine	136.1	91.1	1.18
2.Methamphetamine	150.1	91.1	2.40
3.MDA	180.1	105.0	2.45
4.MDMA	194.1	105.1	2.95

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Injection Volume: 20 µL
LC Column: Selectra® DA HPLC Column 50 x 2.1 mm 5 µm
Instrument: API 3200 Qtrap MS/MS with Shimadzu Prominence UFLC

Gradient:

Time	%A	%B
0.00	80	20
0.50	80	20
12.00	10	90
12.01	80	20
15.00	STOP	

REPRESENTATIVE ANALYTES EXTRACTED

AMPH/METHAMP
SYMPATHOMIMETICS
TCA'S(7)
MDMA/MDA/MDEA
MEPERIDINE/NORMEPERIDINE
CYCLOBENZAPRINE
DIPHENHYDRAMINE
OPIATES(7)
PCP
FENTANYL/NORFENTANYL
CITALOPRAM
METHADONE/EDDP
COCAINE/BZE
SERTRALINE TRAMADOL/NORTRAM
CLONIDINE

BETA BLOCKERS IN BLOOD OR URINE FOR GC/MS CONFIRMATIONS USING 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #:
ZSDAU020 without Tips
or
ZCDAU020 with CLEAN-THRU® Tips

1. **PREPARE SAMPLE:**
   To 1 mL of Acetate buffer (pH 4.5) add 1 mL of blood or urine. Add 2 mL of Acetate buffer (pH 4.5).
   Mix/vortex
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 3 mL 100 mM Acetate Buffer (pH 4.5).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/ minute.

4. **WASH COLUMN:**
   2 x 1 mL Acetone/ Methanol (1:1) aspirate.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE BETA BLOCKERS:**
   1 x 1 mL CH_2Cl_2/ IPA/NH4OH (78:20:2).
   Collect the eluate by gravity.
   NOTE: Prepare elution solvent fresh daily. Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

7. **DERIVATIZE:**
   Derivatization Solution: Methaneboronic acid at 5 mg/mL
   prepared in dry Ethyl Acetate (use molecular sieve).
   Store this solution at –20 °C (freezer conditions) until use.

   Reaction Mixture
   Add 100 µL of the Methaneboronic acid solution (see above).
   Mix/vortex.
   React 15 minutes at 70 °C. Remove from heat source to cool.

   NOTE: Do not evaporate this solution.

8. **ANALYSIS:**
   Inject 1 to 2 µL sample.

Reference:
Branum G, Sweeney S, Palmeri A, Haines L and Huber C
The Feasibility of the Detection and Quantitation of ß Adrenergic Blockers By Solid Phase Extraction and Subsequent Derivatization with Methaneboronic Acid. Journal of Analytical Toxicology 22: 135-141 (1998)

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM

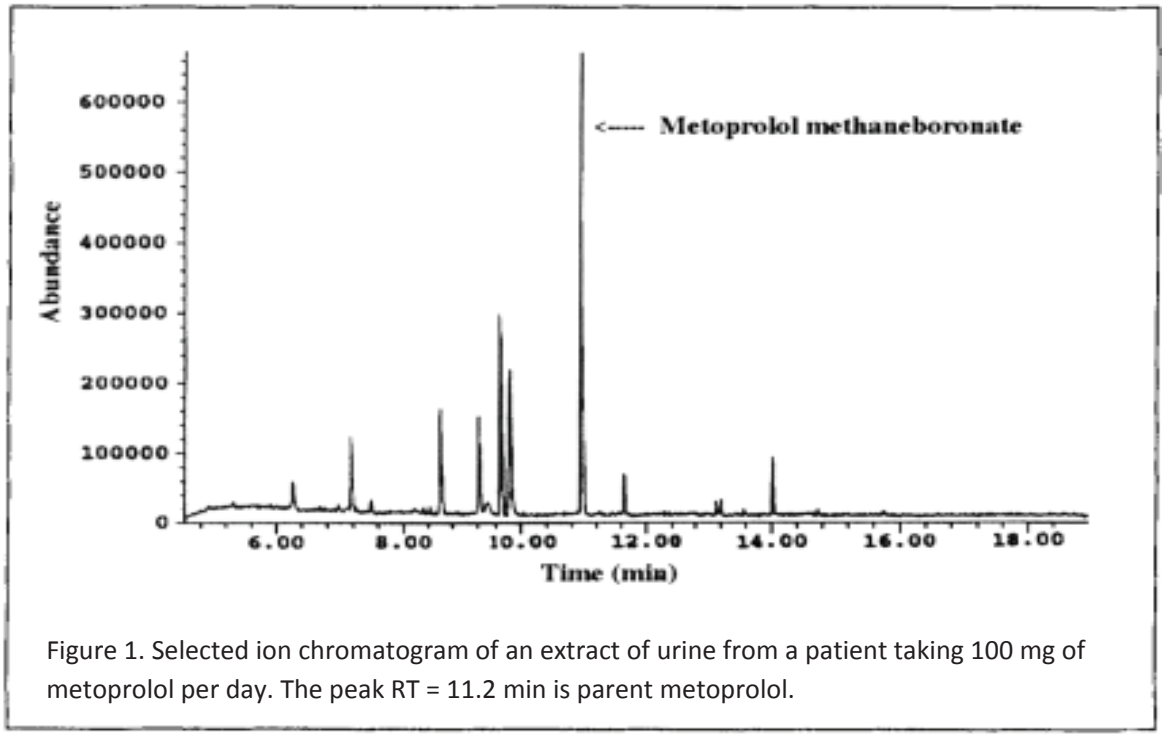


Figure 1. Selected ion chromatogram of an extract of urine from a patient taking 100 mg of metoprolol per day. The peak RT = 11.2 min is parent metoprolol.

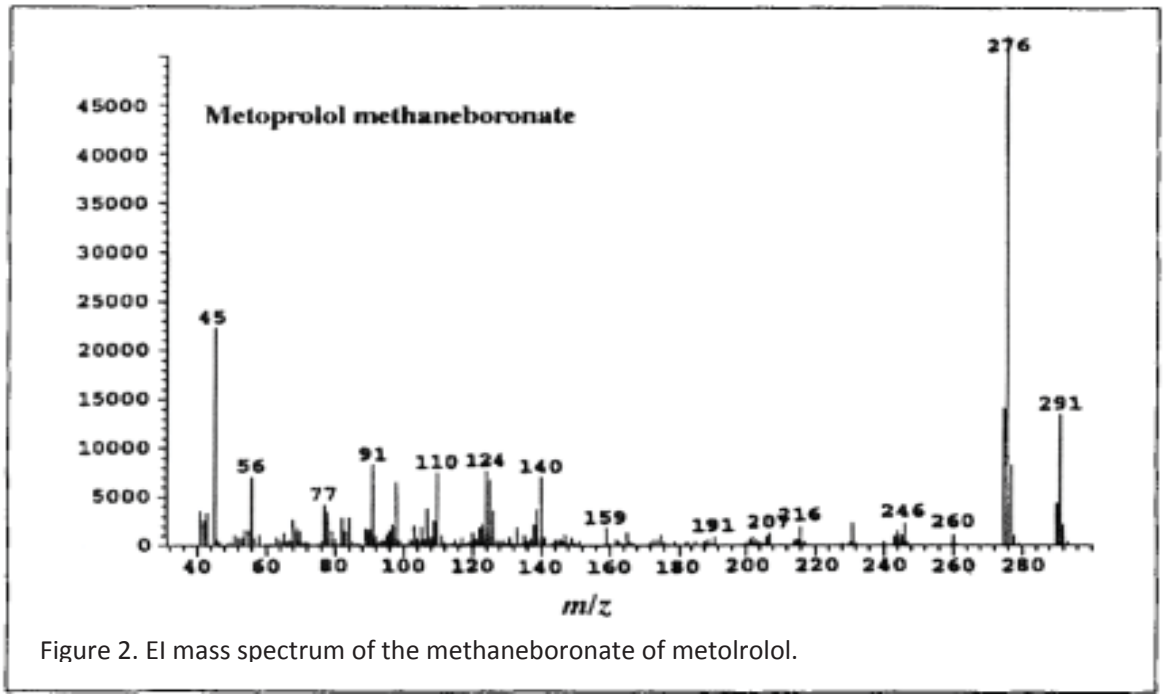


Figure 2. EI mass spectrum of the methaneboronate of metolrolol.

Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2
Acebutolol	246	299	360
Alprenolol	258	273	138
Atenolol	275	290	164
Betaxolol	316	331	246
Bisoprolol	230	334	349
Carteolol	301	316	218
Isoproteronol (IS)	202	244	259
Metoprolol	276	291	140
Propranolol	283	268	128
Soltalol	281	296	239

PARAMETERS

GC/MS: Agilent - 5971/ 5890 GC/MS System with 7683B ALS System

GC capillary column: Rtx-5sil MS 30m X 0.25mm, 0.25µm

Injector: 2µL Splitless, 250°C

Oven temperature program: 110°C for 1 min; 20°C/min to 170°C; 7°C/min to 225°C; 24°C/min to 290°C for 10 min

Carrier gas: Helium

BUPRENORPHINE AND NORBUPRENORPHINE IN BLOOD, PLASMA/SERUM, URINE, TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

CSDAU206 – CLEAN SCREEN® DAU 200 mg, 6 mL Tube

BETA-GLUC-10 – Selectrazyme® Beta-glucuronidase

SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS

1. **PREPARE SAMPLE:**

Blood: To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards.
Add 1 mL of blood, plasma/ serum, or 1 g (1:4) tissue homogenate.
Mix/vortex and let stand for 5 minutes
Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
Sample pH should be 6.0 ± 0.5.
Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
Centrifuge for 10 minutes at 2000 rpm and discard pellet

Urine: PREPARE ENZYME HYDROLYSIS OF GLUCURONIDES:
To 1-2 mL of urine sample, add 1 mL of acetate buffer (pH 5.0) containing 5,000 units/mL of Selectrazyme® β-glucuronidase.
Optionally, add 1 mL of acetate buffer and 25-50 µL of concentrated β-glucuronidase. Vortex and heat for 1-2 hours at 65 °C.
Allow sample to cool.
Do not adjust pH~ sample is ready to be added to the extraction column.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM Acetate buffer (pH 5.0)
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetate buffer (pH 5.0)
   1 x 3 mL CH_3OH
   Dry column (5-10 minutes at full vacuum or pressure)

5. **ELUTE BUPRENORPHINE/NORBUPRENORPHINE:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2 v/v)
   Collect eluate at 1 to 2 mL/minute

   NOTE: Prepare elution solvent daily
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12)

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

7. RECONSTITUTE / DERIVATIZE:

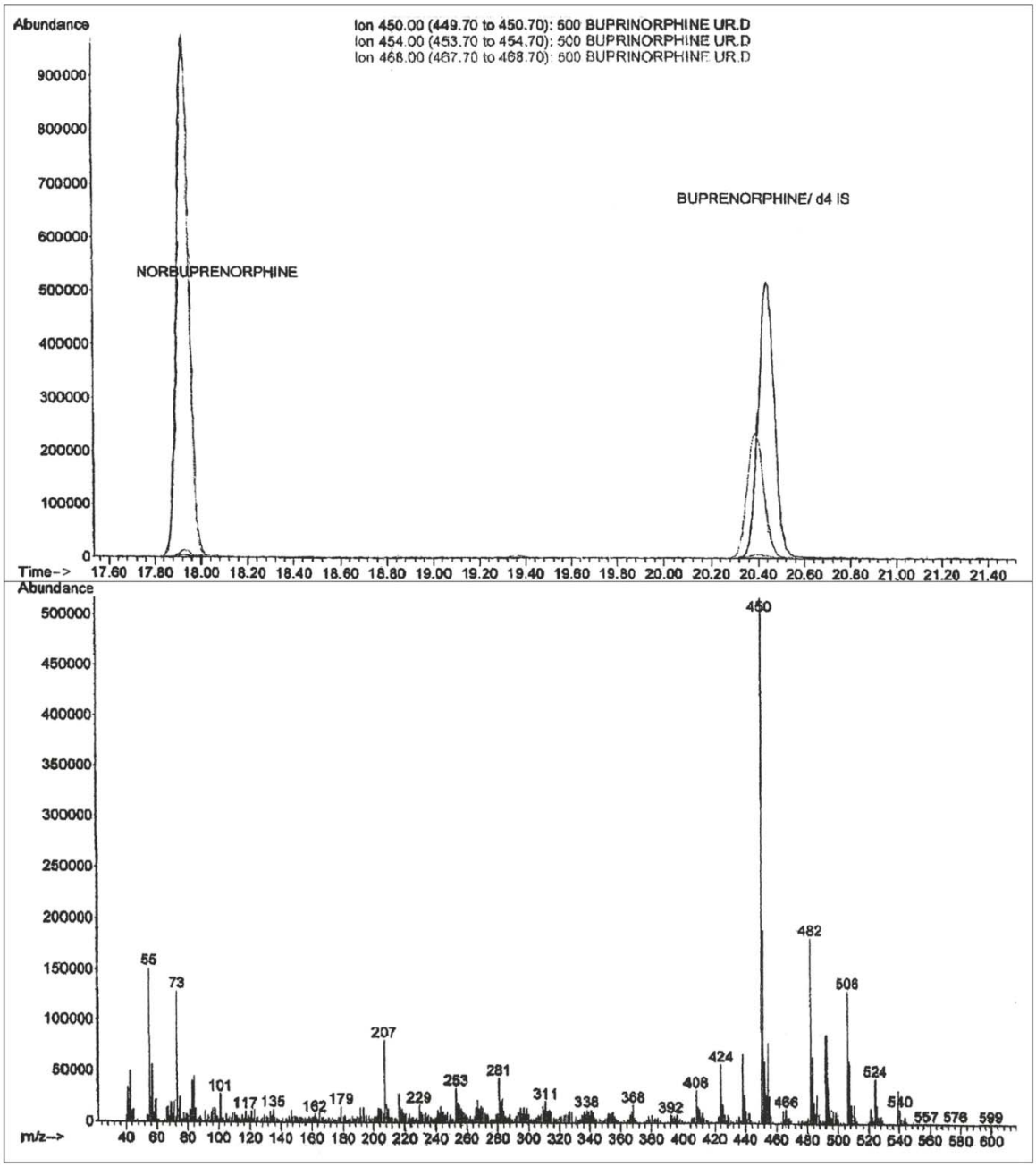
- **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
  Inject 10 µL.
- **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

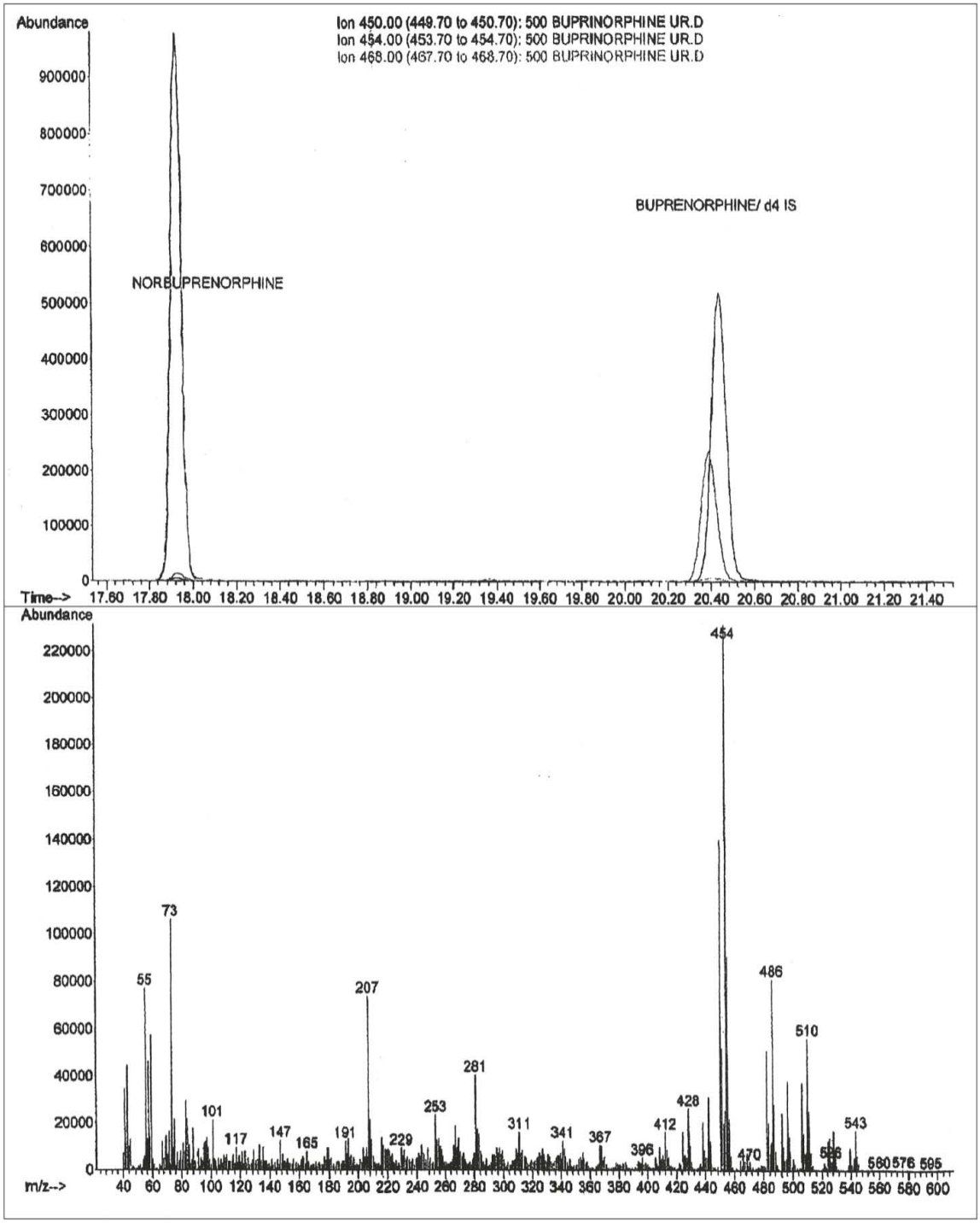
Alternate Derivatization

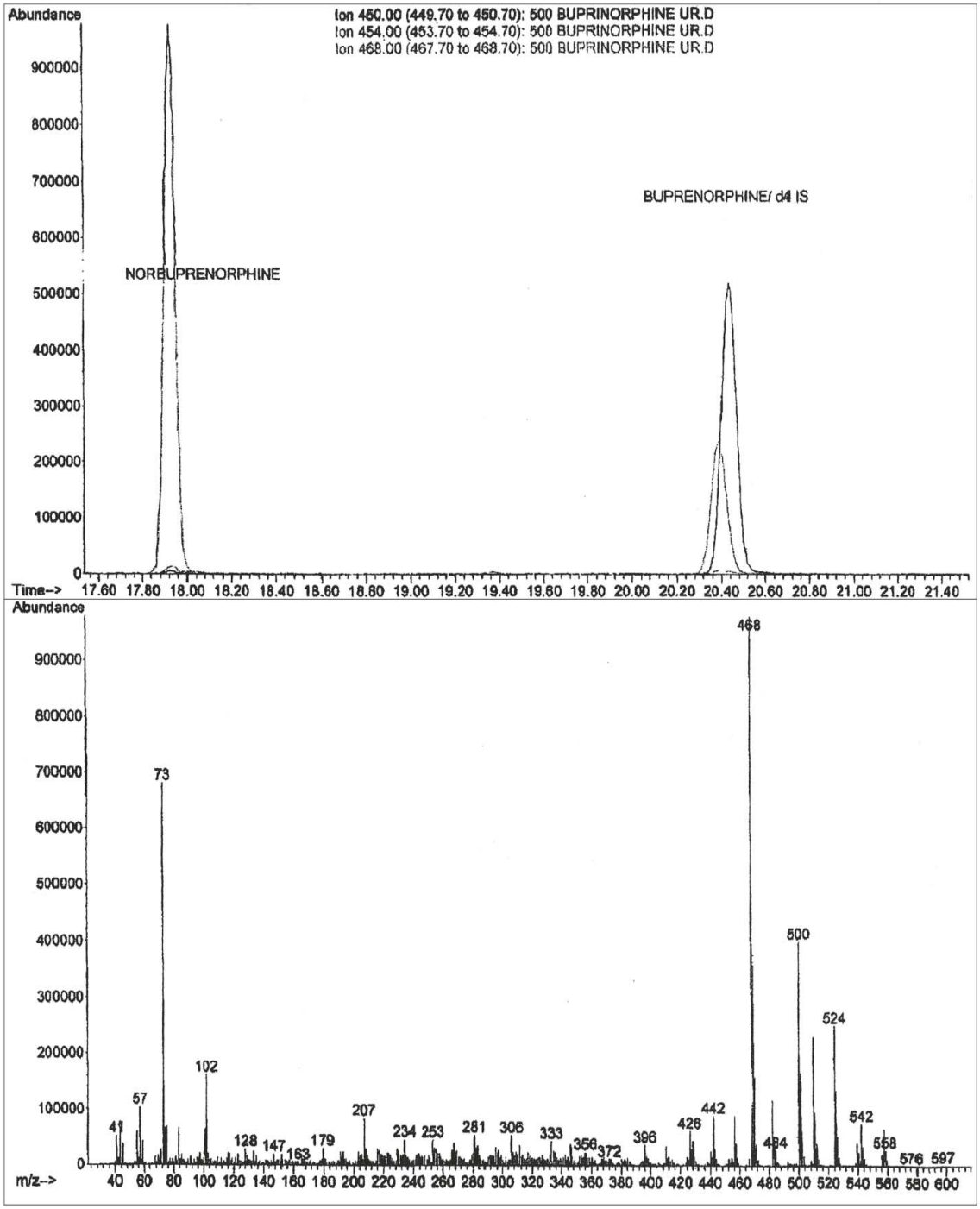
- Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of 50 µL BSTFA w/ 1% TMCS react at 70 °C for 30 minutes; Cool and inject 1-2 µL

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAMS




Abundance
Ion 450.00 (449.70 to 450.70): 500 BUPRINORPHINE UR.D
Ion 454.00 (453.70 to 454.70): 500 BUPRINORPHINE UR.D
Ion 468.00 (467.70 to 468.70): 500 BUPRINORPHINE UR.D
NORBUPRENORPHINE
BUPRENORPHINE/ d4 IS
Time-->
Abundance
m/z-->



Analyte	Primary Ion	Secondary Ion	Tertiary Ion
Buprenorphine-D_4-TMS	454	486	510
Buprenorphine-TMS	450	482	506
Norbuprenorphine-TMS	468	500	524
Norbuprenorphine-D_3-TMS	471	503	527

CLOZAPINE AND METABOLITES IN WHOLE BLOOD, SERUM/PLASMA AND URINE USING 200 mg CLEAN-UP® EXTRACTION COLUMN AND LC-MS/MS or HPLC-UV ANALYSIS

PART #:

CECNP123 – CLEAN-UP® CYANOPROPYL 200 mg, 3 mL Tube

1. **PREPARE SAMPLE:**

   To 1 mL of 100 mM prosphate buffer (pH 6.0) add internal standard.*

   Add 1 mL of blood, serum/plasma or urine. Add 2 mL of 100 mM phosphate buffer (pH 6.0).

   Mix/vortex Centrifuge as appropriate.

2. **CONDITION CLEAN-UP® CECNP123 EXTRACTION COLUMN:**

   1 x 1 mL Methanol.

   1 x 1 mL H_2O.

   1 x 0.5 mL 100 mM phosphate buffer (pH 6.0).

   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**

   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**

   1 x 1 mL D.I. H_2O

   1 x 0.5 mL 1% NH_4OH in D.I. H_2O.

   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE CLOZAPINE:**

   1 x 0.2 mL 1% NH_4OH in Methanol.

   Collect eluate at 1-2 mL /minute.

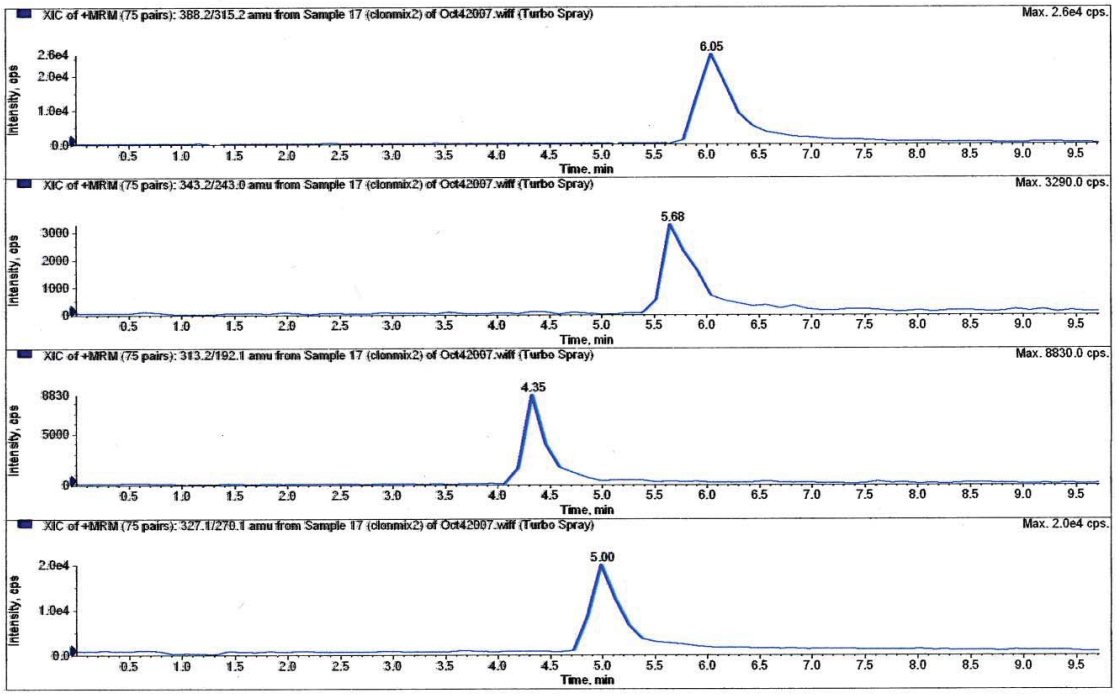
   Inject 5 µL (LC-MS/MS).

   Inject 20 µL (HPLC-UV).

INSTRUMENT CONDITIONS:

Column: 150 x 2.1 mm (3 µm) Zorbax: Agilent Technologies.
Mobile phase: Acetonitrile: 0.1% Formic Acid (33:67).
Flowrate: 0.35 mL/min.
Column Temperature: ambient.
Detector: API 2000 MS/MS.
HP1100 Diode-Array (230 nm).

Compound	MRM Transistion
Clozapine	327.1/270.1
Desmethylclozapine	313.1/192.1
Clozapine-N-oxide	343.1/243.1
*Flurazepam	388.1/315.2



GABAPENTIN/PREGABALIN/BACLOFEN IN BLOOD, PLASMA/SERUM BY LC-MS/MS OR GC-MS 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #
ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube
SBSTFA-1-1 – SELECTRA-SIL® BSTFA w/ 1% TMCS
Or
SMTBSTFA-1-1 – SELECTRA-SIL® MTBSTFA w/ 1% TBDMCS
SLDA100ID21-5UM – Selectra® DA HPLC Column, 100 x 2.1 mm, 5 µm

1. **PREPARE SAMPLE:**
   To 0.2-0.5 mL of sample add 1 mL of acetone dropwise while vortexing
   Add internal standards
   Mix/vortex and let stand for 5 minutes
   Transfer organic phase to clean tube
   Evaporate to dryness.
   Add 3 mL of 100 mM HCl
   Vortex mix and centrifuge as appropriate

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 1 mL 100 mM HCl
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL Ethyl Acetate
   1 x 3 mL Hexane
   Dry column (10 minutes at full vacuum or pressure)

5. **ELUTE GABAPENTIN/PREGABALIN/BACLOFEN:**
   1 x 3 mL CH_3OH containing 2% NH_4OH
   Collect eluate at 1 to 2 mL/minute

6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C

7. **RECONSTITUTE / DERIVATIZE:**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 50 µL of Ethyl Acetate and 50 µL of BSTFA w/1% TMCS;
     Cap and heat at 70 °C for 30 minutes;
     Remove and allow to cool.
     Inject 1-2 µL

Alternate Derivatization

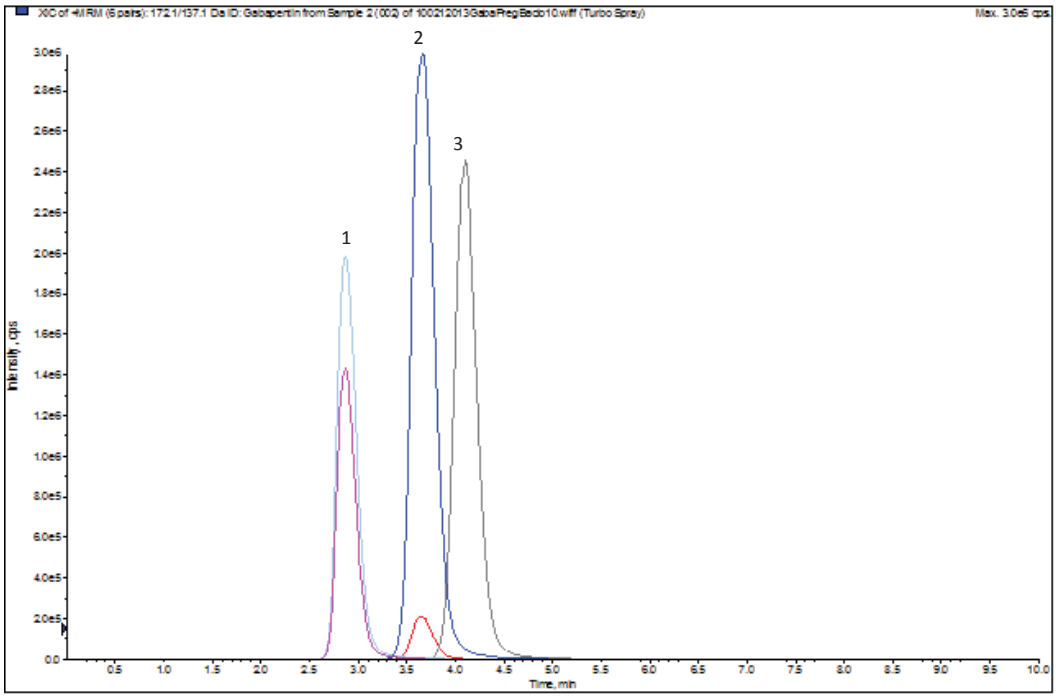
- 50 µL of Ethyl Acetate and 50 µL of MTBSTFA w/1% TBDMCS

GC-MS IONS

Compound	Primary	Secondary	Tertiary
Gabapentin-TMS	210	225	182
Gabapentin D_{10}-TMS	220	235	192

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM 1 SELECTRA® DA HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Pregabalin	160.1	97.1	2.96
2. Gabapentin	172.1	67.1	3.66
3. Baclofen	214.0	150.8	4.32

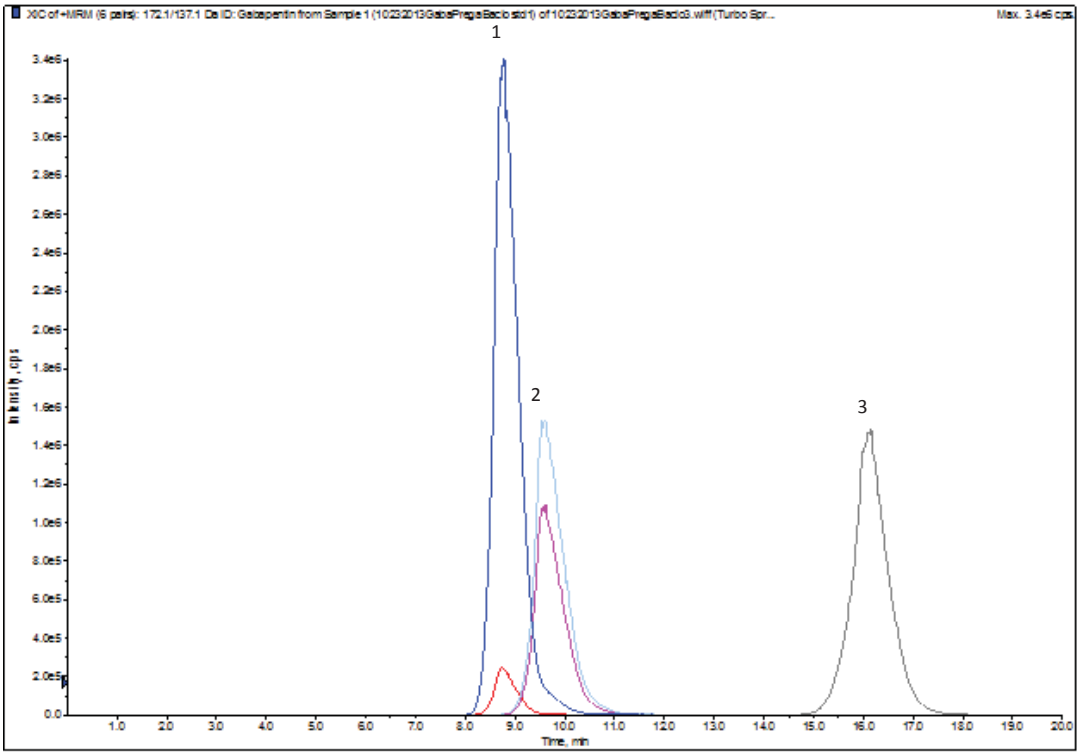
PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O
Mobile Phase B: 0.1% Formic Acid in Methanol
Flow Rate: 0.5 mL/minute
Polarity: Positive
Injection Volume: 10 µL
LC Column: Selectra® DA HPLC Column 100 x 2.1 mm 5 µm
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	85	15
11.00	STOP	

CHROMATOGRAM 2 SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Gabapentin	172.1	67.1	8.78
2. Pregabalin	160.1	97.1	9.56
3. Baclofen	214.0	150.8	16.10

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.5 mL/minute

Polarity: Positive

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 5 µm

Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	85	15
20.00	STOP	

NICOTINE, COTININE, AND ANABASINE IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE BY LC-MS/MS OR GC-MS CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

SLPFPP100ID21-3UM – Selectra® PFPP HPLC Column, 100 x 2.1 mm, 3 µm

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards
   Add 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
   Mix/vortex and let stand for 5 minutes
   Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 3 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at full vacuum or pressure

3. **APPLY SAMPLE:**
   Load at 1 to 2 mL/minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O.
   1 x 2 mL 200 mM HCl
   Dry column (5 minutes at full vacuum or pressure).
   1 x 3 mL Methanol
   Dry column (5 minutes at full vacuum or pressure).

5. **ELUTE NICOTINE, COTININE, ANABASINE:**
   1 x 3 mL CH_2Cl_2/ IPA/ NH_4OH (78:20:2)
   Collect eluate at 1 to 2 mL/minute.

   NOTE: Prepare elution solvent daily.
   Add IPA/ NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

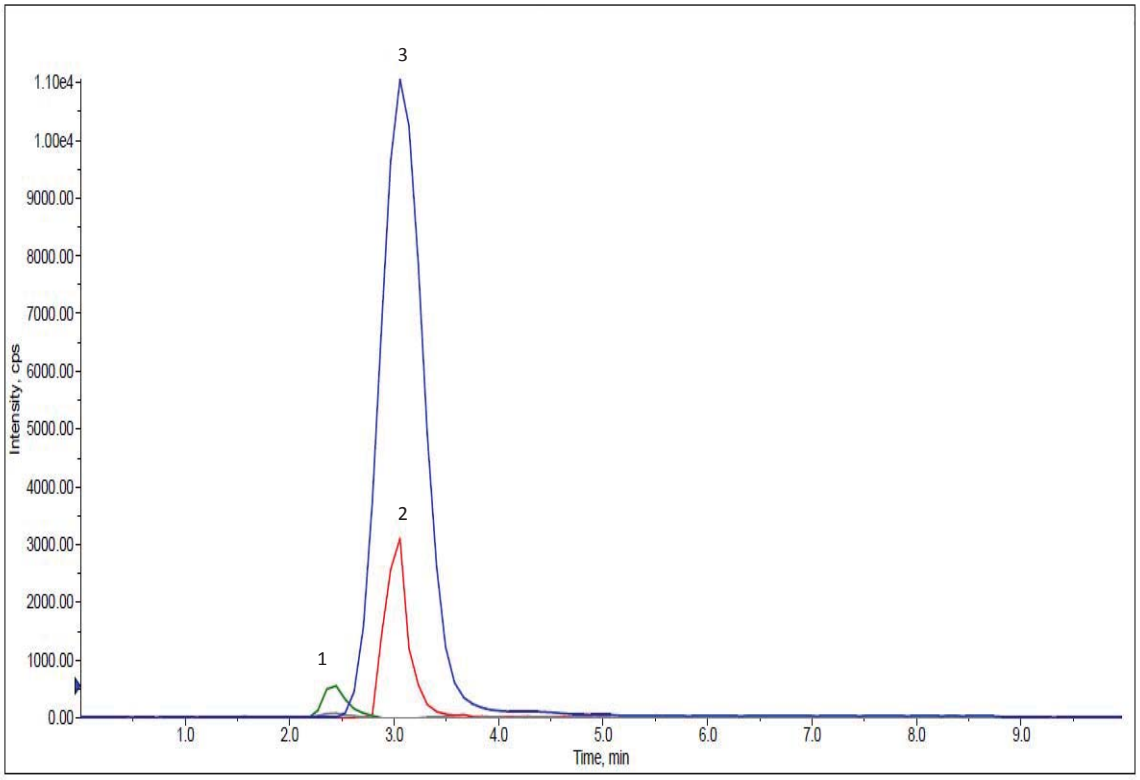
6. **DRY ELUATE:**
   Evaporate to dryness at < 40 °C.

7. **RECONSTITUTE / DERIVATIZE**

   - **LC-MS/MS:** Reconstitute sample in 100 µL of mobile phase
     Inject 10 µL.
   - **GC-MS**: Dissolve residue in 100 µL of Ethyl Acetate

INSTRUMENT CONDITIONS (LC-MS/MS):

CHROMATOGRAM SELECTRA® PFPP HPLC COLUMN



Analyte	MRM Transitions		Relative Retention Time (min)
	Q1	Q3	
1. Cotinine	117.2	80.1	2.42
Cotinine D_3	180.2	101.2	-
2. Nicotine D_4	167.2	136.1	3.03
3. Nicotine	163.2	132.2	3.06

PARAMETERS

Mobile Phase A: 0.1% Formic Acid in D.I. H_2O

Mobile Phase B: 0.1% Formic Acid in Methanol

Flow Rate: 0.3 mL/minute

Polarity: Positive

Reconstitute: 100 µL

Injection Volume: 10 µL

LC Column: Selectra® PFPP HPLC Column 100 x 2.1 mm 3 µm

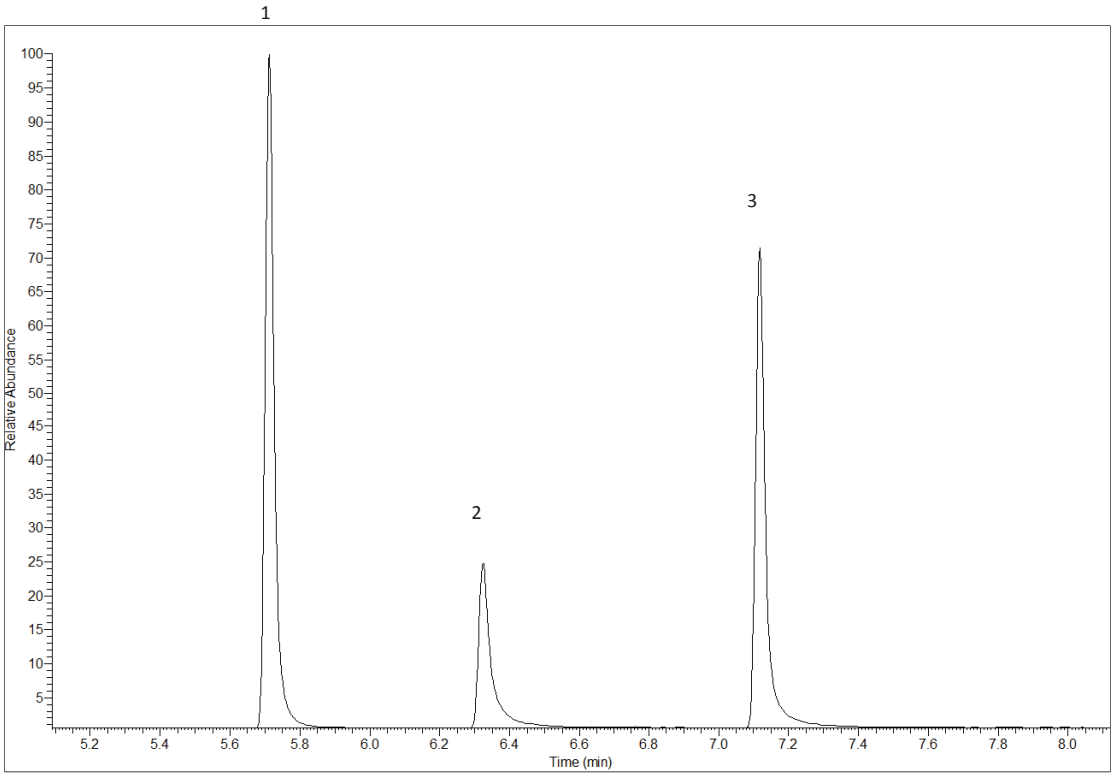
Instrument: API 4000 Qtrap MS/MS with Agilent 1200 Binary Pump SL

Isocratic:

Time	%A	%B
0.00	80	20
10.00	STOP	

INSTRUMENT CONDITIONS (GC-MS):

CHROMATOGRAM



Analyte	Quantify Ion	Qualifier Ion 1	Qualifier Ion 2	Relative Retention Time (min)
1. Nicotine	84	133	162	5.71
Nicotine D_4	88	137	166	-
2. Anabasine	84	105	133	6.32
3. Cotinine	98	119	176	7.12
Cotinine D_4	101	122	179	-

PARAMETERS

GC/MS: Thermo ISQ Trace 1300

GC capillary column: 30 m x 0.25 mm (0.25 µm) TG-1MS

Injector: 1 µL Splitless, 250 °C

Oven temperature program: 50 °C (0.5) to 320 °C (30 °C/ minute): hold (5 minutes)

Carrier gas: Helium (1.2 mL/ minute)

MSD condition: Aux temperature: 280 ºC, MS Source: 300 ºC, MS Quad: 150 ºC

PAROXETINE IN BLOOD, PLASMA/ SERUM AND URINE. LC-MSMS CONFIRMATIONS USING 200 mg CLEAN SCREEN® DAU EXTRACTION COLUMN

Part #:

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10 mL Tube

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standards*.
   Add 1 mL whole blood, Serum/Plasma or Urine. Add 2 mL of 100 mM phosphate buffer (pH 6.0).
   Vortex and centrifuge as appropriate.

2. **CONDITION COLUMN:**
   1 x 3 mL CH_3OH
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM phosphate buffer (pH 6.0).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL D.I. H_2O
   1 x 3 mL 100 mM acetic acid
   1 x 3 mL CH_3OH
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE PAROXETINE:**
   1 x 3 mL Ethyl Acetate: Acetonitrile: NH_4OH (78:20:2)
   Collect eluate at 1-2 mL / minute.

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40 °C
   Dissolve residue in 100 µL Methanol.

INSTRUMENT CONDITIONS:

Column: 50 x 2.1 mm (3 µm) Selectra® Phenyl (UCT, LLC)

Mobile phase:

Time	Acetonitrile	0.1% Formic Acid aq
0	10	90
15	50	50
16	10	90
20	10	10

Flow rate: 0.35 mL/ minute

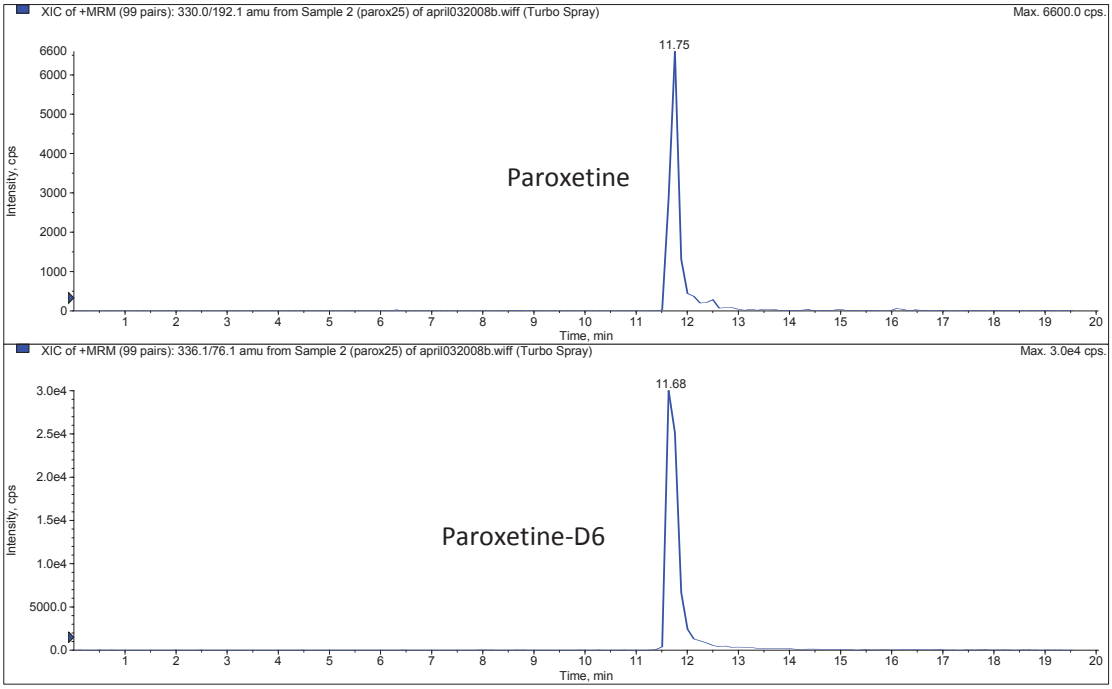
Injection Volume: 5 µL

Column Temperature: ambient

Detector: API 2000 MS/MS.

Compound	MRM Transition
Paroxetine	330.0 / 190.1
Paroxetine-D6	336.0 / 76.1

CHROMATOGRAM :



QUETIAPINE IN BLOOD, PLASMA/SERUM, URINE AND TISSUE USING: 200 mg CLEAN SCREEN® EXTRACTION COLUMN

PART #:

ZSDAU020 – CLEAN SCREEN® DAU 200 mg, 10mL Tube

1. **PREPARE SAMPLE:**
   To 1 mL of 100 mM phosphate buffer (pH 6.0) add internal standard.* Add 1 mL blood, plasma/serum, urine or 1 g (1:4) tissue homogenate
   Add 2 mL of 100 mM phosphate buffer (pH 6.0).
   Sample pH should be 6.0 ± 0.5.
   Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.
   Centrifuge as appropriate.

2. **CONDITION CLEAN SCREEN® EXTRACTION COLUMN:**
   1 x 3 mL CH_3OH.
   1 x 3 mL D.I. H_2O.
   1 x 3 mL 100 mM phosphate buffer (pH 6.0).
   Note: aspirate at < 3 inches Hg to prevent sorbent drying out.

3. **APPLY SAMPLE:**
   Load sample at 1-2 mL / minute.

4. **WASH COLUMN:**
   1 x 3 mL 100 mM phosphate buffer (pH 6).
   1 x 3 mL 1.0 M acetic acid.
   1 x 3 mL CH_3OH.
   Dry column (5 minutes at > 10 inches Hg).
   1 x 3 mL of Hexane.
   Dry column (5 minutes at > 10 inches Hg).

5. **ELUTE QUETIAPINE:**
   1 x 3 mL Ethyl Acetate/ Acetonitrile/ NH_4OH (78:20: 2 v/v).
   Collect eluate at 1-2 mL /minute.
   NOTE: Prepare elution solvent daily

6. **EVAPORATION:**
   Evaporate eluates under a gentle stream of nitrogen < 40 °C.

7. **Reconsititue sample in 100 µL 0.1% trifluoroacetic acid (aq).**
   Inject 50 µL.

INSTRUMENT CONDITIONS:

Column: C_{18} 150 x 4.6 mm (3 µm) Zorbax (Agilent Technologies).

Mobile phase: Acetonitrile: 0.1% Trifluoroacetic acid (25: 75).

Flowrate: 1 mL / min.

Column Temperature: 35 ˚C.

Detector: Diode Array (250 nm).

Chromatogram:

Quetiapine
Quinidine (internal standard)