Alkaline Drug Quantitation/Confirmation

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Alkaline Drug Quantitation/Confirmation

1 INTRODUCTION

This procedure is used to quantitate common alkaline drugs in blood. It is also used to confirm common alkaline drugs in both blood and urine.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☒ Quantitation	
Matrices	Blood, serum, plasma, urine	
Analytes	Quantitative	
	amitriptyline chlorpheniramine chlorpromazine citalopram clomipramine cyclobenzaprine desipramine dextromethorphan doxylamine diphenhydramine doxepin duloxetine EDDP fentanyl fluoxetine imipramine ketamine meperidine methadone	mirtazapine nordoxepin norfentanyl norfluoxetine normeperidine norpropoxyphene nortriptyline paroxetine PCP pheniramine propoxyphene propranolol sertraline tramadol trazodone trimipramine venlafaxine verapamil zolpidem
	Qualitative	
	brompheniramine bupropion clozapine metoprolol	norsertraline quetiapine thioridazine
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.	

3 PRINCIPLE

Specimens are mixed with internal standard(s), adjusted to alkaline pH, and extracted into hexane. The hexane is taken to dryness and reconstituted prior to analysis by liquid chromatography/Fourier transform mass spectrometry (LC/FTMS).

4 SPECIMEN CRITERIA

This procedure is validated for whole blood and urine. Typically, 2 x 0.5 mL samples are analyzed; samples suspected to be above the procedure's linear range may be diluted before extraction.

5 EQUIPMENT

5.1 Equipment

A. Centrifuge
B. Evaporator w/ Nitrogen
C. Vortex mixer
D. Routine laboratory supplies, including disposable pipettes, wooden sticks, test tube racks, graduated cylinders, etc.

5.1.1 Column

A. HPLC Column (Xterra C-18 MS, 3.0 x 150 mm, 3.5 µm dp; or equivalent)
B. Guard/Filter (matched to column)

5.2 Consumables

A. 16 x 100 mm screw-top tubes with Teflon-lined caps
B. 12 x 75 mm culture tubes with polypropylene snap-tops

5.3 Instruments

A. Thermo LTQ Orbitrap XL Hybrid Ion Trap/Fourier Transform Mass Spectrometer
B. Shimadzu HPLC

5.4 Software

Component	Software	Version
Operating System	Microsoft Windows	7 Pro SP 1 / XP Professional
Mass Spectrometer	Foundation	1.0.2 or higher
	Xcalibur	2.1.0 SP1 / 2.0.7
	LTQ Tune Plus	2.5.5
	Shimadzu LC Controller	5.4 / 6.5

5.5 Chemicals/Reagents

Storage/stability determined by manufacturer unless otherwise noted.

5.5.1 Purchased

A. Acetonitrile	Optima grade or better
B. Formic Acid	Puriss grade or better
C. Hexane	UV grade or better
D. Methanol	Optima grade or better
E. Sodium hydroxide	ACS grade or better
F. Water	Deionized, 18 MΩ
G. Water	Optima or better grade

5.5.2 Prepared

A. Mobile Phase 1 (Aqueous) Water with 0.1% Formic Acid
Combine 500 mL Optima grade water and 0.5 mL formic acid and mix well. Store in glass at room temperature. Stable 2 months.
B. Mobile Phase 2 (Organic) Acetonitrile with 0.1% Formic Acid
Combine 500 mL Optima grade acetonitrile and 0.5 mL formic acid and mix well. Store in glass at room temperature. Stable for 2 weeks.
C. 4% Sodium hydroxide
Dissolve 2 g sodium hydroxide in 50 mL deionized water. Store in plastic at room temperature. Stable for at least 6 months.
D. Methanol:Water (10:90 v:v)
Mix 5 mL methanol with 45 mL water (both Optima grade). Store in glass at room temperature. Stable 12 months.

5.6 Standards/Controls

A Level 4 document (*TOX425 Standards/Control Prep*) is available to assist with preparations. Solutions containing all or selected analytes may be prepared. The analytes are grouped into Panel A and B, denoting higher and lower dose drugs respectively.

Working Solutions and Internal Standard Solutions may be made in groupings or individually, depending on case needs.

The following drugs are validated at a lower concentration range than most of the drugs in the procedure, and therefore use Stock Solutions and Internal Standard Solutions at lower concentrations: cyclobenzaprine, fentanyl, norfentanyl, paroxetine, PCP and zolpidem. Brompheniramine is also in this group, but is validated for qualitative analysis only.

5.6.1 *Purchased*

5.6.1.1 *Internal Standards*

Cerilliant or an equivalent supplier.

Analyte	Conc (mg/mL)
Amitriptyline-d3	0.1
Chlorpheniramine-d6	0.1
Chlorpromazine-d3	0.1
Citalopram-d6	0.1
Clomipramine-d3	0.1
Cyclobenzaprine-d3	0.1
Desipramine-d3	0.1
Dextromethorphan-d3	0.1
Diphenhydramine-d3	0.1
Doxepin-d3	0.1
Doxylamine-d5	0.1
Duloxetine-d3	0.1
EDDP-d3	0.1
Fentanyl-d5	0.1
Fluoxetine-d6	0.1
Imipramine-d3	0.1
Ketamine-d4	0.1
Meperidine-d4	0.1
Methadone-d3	0.1
Norfentanyl-d5	0.1
Norfluoxetine-d6	0.1
Normeperidine-d4	0.1
Norpropoxyphene-d5	0.1
Nortriptyline-d3	0.1
Paroxetine-d6	0.1
PCP-d5	0.1
Pheniramine-d6	0.1
Propoxyphene-d5	0.1
Sertraline-d3	0.1
Tramadol-13C-d3	0.1
Trazodone-d6	0.1
Trimipramine-d3	0.1
Venlafaxine-d6	0.1
Zolpidem-d6	0.1

5.6.1.2 Calibration/Control

Cerilliant, Lipomed or an equivalent supplier.

Analyte	Panel	Type	Conc (mg/mL)
bupropion		Qual	1
clozapine		Qual	1
metoprolol		Qual	1
norsertraline		Qual	0.1
quetiapine		Qual	1
thioridazine		Qual	1
brompheniramine		Qual	1
amitriptyline	A	Quant	1
chlorpheniramine	A	Quant	1
chlorpromazine	A	Quant	1
citalopram	A	Quant	1
clomipramine	A	Quant	1
desipramine	A	Quant	1
dextromethorphan	A	Quant	1
diphenhydramine	A	Quant	1
doxepin	A	Quant	1
doxylamine	A	Quant	1
duloxetine	A	Quant	1
EDDP	A	Quant	1
fluoxetine	A	Quant	1
imipramine	A	Quant	1
ketamine	A	Quant	1
meperidine	A	Quant	1
methadone	A	Quant	1
mirtazapine	A	Quant	1
nordoxepin	A	Quant	1
norfluoxetine	A	Quant	1
normeperidine	A	Quant	1
norpropoxyphene	A	Quant	1
nortriptyline	A	Quant	1
pheniramine	A	Quant	1
propoxyphene	A	Quant	1
propranolol	A	Quant	1
sertraline	A	Quant	1
tramadol	A	Quant	1
trazodone	A	Quant	1

trimipramine	A	Quant	1
venlafaxine	A	Quant	1
verapamil	A	Quant	1
cyclobenzaprine	B	Quant	1
fentanyl	B	Quant	1
norfentanyl	B	Quant	1
paroxetine	B	Quant	1
PCP	B	Quant	1
zolpidem	B	Quant	1

5.6.1.3 Matrix

A. Negative Control Blood:
Purchased from Diagnostics Products Corporation, UTAK Laboratories, Inc., Cliniqa, or obtained in-house from a drug-free donor. Store refrigerated or frozen. Stability determined by manufacturer.
B. Negative Control Urine:
Purchased from Diagnostics Products Corporation, UTAK Laboratories, Inc., Cliniqa, or obtained in-house from a drug-free donor. Store refrigerated or frozen. Stability determined by manufacturer.

5.6.2 *Prepared*

5.6.2.1 *Internal Standard Working Solution*

Analyte	Conc (mg/mL)	Panel	Aliquot (mL)	Diluent (mL)	Conc (ug/mL)
Amitriptyline-d3	0.1	A	0.3	10	3
Chlorpheniramine-d6	0.1	A	0.3	10	3
Chlorpromazine-d3	0.1	A	0.3	10	3
Citalopram-d6	0.1	A	0.3	10	3
Clomipramine-d3	0.1	A	0.3	10	3
Desipramine-d3	0.1	A	0.3	10	3
Dextromethorphan-d3	0.1	A	0.3	10	3
Diphenhydramine-d3	0.1	A	0.3	10	3
Doxepin-d3	0.1	A	0.3	10	3
Doxylamine-d5	0.1	A	0.3	10	3
Duloxetine-d3	0.1	A	0.3	10	3
EDDP-d3	0.1	A	0.3	10	3
Fluoxetine-d6	0.1	A	0.3	10	3
Imipramine-d3	0.1	A	0.3	10	3
Ketamine-d4	0.1	A	0.3	10	3
Meperidine-d4	0.1	A	0.3	10	3
Methadone-d3	0.1	A	0.3	10	3
Norfluoxetine-d6	0.1	A	0.3	10	3
Normeperidine-d4	0.1	A	0.3	10	3
Norpropoxyphene-d5	0.1	A	0.3	10	3
Nortriptyline-d3	0.1	A	0.3	10	3
Pheniramine-d6	0.1	A	0.3	10	3
Propoxyphene-d5	0.1	A	0.3	10	3
Sertraline-d3	0.1	A	0.3	10	3
Tramadol-13C-d3	0.1	A	0.3	10	3
Trazodone-d6	0.1	A	0.3	10	3
Trimipramine-d3	0.1	A	0.3	10	3
Venlafaxine-d6	0.1	A	0.3	10	3
Cyclobenzaprine-d3	0.1	B	0.1	10	1
Fentanyl-d5	0.1	B	0.1	10	1
Norfentanyl-d5	0.1	B	0.1	10	1
Paroxetine-d6	0.1	B	0.1	10	1
PCP-d5	0.1	B	0.1	10	1
Zolpidem-d6	0.1	B	0.1	10	1

Diluent	Deionized Water	Analytes may be selected individually or in groups depending upon batch needs.
Store	Frozen/Refrigerator	
Stable	At least two years	

5.6.2.2 LC/MS Performance Standard

Performance Mix
Dilute 0.010 mL of the Internal Standard Working Solution with 0.090 mL of Methanol:Water (10:90 v:v). Prepare fresh.

5.6.2.3 Calibration Prep

Reference Level 4 Document *TOX425 Standards/Control Prep for additional guidance.*

Panel A High Calibration Working Solution (5.0 µg/mL)
Combine 0.5 mL of each 0.1 mg/mL Standard Stock Solution and 0.05 mL of each 1.0 mg/mL Standard Stock Solution in a 10-mL volumetric flask and bring to the mark with deionized water. Store refrigerated or frozen in glass. Stable for at least one year.
Panel A Low Calibration Working Solution (WS) (0.5 µg/mL)
Add 1.0 mL of the High Calibration Working Solution to a 10-mL volumetric flask and bring to the mark with deionized water. Store refrigerated or frozen in glass. Stable for at least one year.
Panel B Intermediate Calibration Solution (10 µg/mL)
Combine 0.1 mL of each 1.0 mg/mL Standard Stock Solution in a 10-mL volumetric flask and bring to the mark with deionized water. Store refrigerated or frozen in glass. Stable for at least one year.
Panel B High Calibration WS (1.0 µg/mL)
Add 1.0 mL of the Panel B Intermediate Calibration Solution to a 10-mL volumetric flask and bring to the mark with deionized water. Store refrigerated or frozen in glass. Stable for at least one year.
Panel B Low Calibration WS (0.1 µg/mL)
Add 1.0 mL of the Panel B High Calibration Working Solution to a 10-mL volumetric flask and bring to the mark with deionized water. Store refrigerated or frozen in glass. Stable for at least one year.

Consult Section 11.2 for details on linear ranges for each analyte.

5.6.2.3.1 Calibration Scheme

Reference Level 4 Document *TOX425 Standards/Control Prep*

Panel	Calibrator Level (ng/mL)	High Calibration Solution Aliquot (uL)	Low Calibration Solution Aliquot (uL)
A	50		50
A	100		100
A	250	25	
A	500	50	
A	750	75	
A	1000	100	
B	10		50
B	20		100
B	50	25	
B	100	50	
B	150	75	
B	200	100	

5.6.2.4 Control Prep

Reference Level 4 Document *TOX425 Standards/Control Prep for additional guidance.*

Panel A High Control Working Solution (4 µg/mL)
Combine 0.4 mL of each 0.1 mg/mL Standard Stock Solution and 0.04 mL of each 1.0 mg/mL Standard Stock Solution in a 10-mL volumetric flask and bring to the mark with deionized water. Store refrigerated in glass. Stable for at least one year.
Panel A Low Control Working Solution (0.4 µg/mL)
Add 0.5 mL of the Panel A High Control Working Solution to a 5-mL volumetric flask and bring to the mark with deionized water. Store refrigerated in glass. Stable for at least one year.
Panel B High Control Working Solution (0.8 µg/mL)
Add 1.0 mL of the Panel A High Control Working Solution to a 5-mL volumetric flask and bring to the mark with deionized water. Store refrigerated in glass. Stable for at least one year.
Panel B Low Control Working Solution (0.2 µg/mL)
Add 0.25 mL of the Panel A High Control Working Solution to a 5-mL volumetric flask and bring to the mark with deionized water. Store refrigerated in glass. Stable for at least one year

5.6.2.4.1 Control Scheme

Reference Level 4 Document *TOX425 Standards/Control Prep* for additional guidance.

Control Level	Control Working Solution	Start (ug/mL)	Aliquot (uL)	Sample (mL)	Final (ng/ml)
Low A	Panel A Low	0.4	90	0.5	72
Low A*	Panel A High*	4	40	0.5	320
High A	Panel A High	4	85	0.5	680
* (mirtazepine and propranolol only)					
Low B	Panel B Low	0.2	60	0.5	24
High B	Panel B High	0.8	90	0.5	144

6 PROCEDURE

	Step	Note	Reference/Lot
	A. Samples (duplicate for quantitative exams)		
	1. To labeled 16 x 100 mm screw-top tubes add:		
☐	i. 0.5 mL of biological fluid		
	a. Samples may be diluted with deionized water to get on-scale		
	B. Controls		
☐	1. Prepare Negative Control(s)		
☐	2. Prepare Positive Control(s) (duplicate for quantitative exams)		
	i. Panel A		
☐	a. Low Control Working Solution		
☐	b. High Control Working Solution		
	ii. Panel B		
☐	a. Low Control Working Solution		
☐	b. High Control Working Solution		
	C. Calibrators		
	1. Panel A		
☐	i. Low Calibration Solution		
☐	ii. High Calibration Solution		
	2. Panel B		
☐	i. Low Calibration Solution		
☐	ii. High Calibration Solution		
	D. Internal Standard(s)		
☐	1. Add 50 µL of Internal Standard Working Solution		
	E. Adjust pH		
☐	1. Add 0.2 mL of 4% sodium hydroxide		
☐	2. Vortex		
	F. Extract		
☐	1. Add 2mL hexane to each tube (down side of tube)		
☐	2. Rotate for 20 minutes		

☐	3. Centrifuge 10 minutes at 3000 rpm		
	i. If emulsions develop, break up with wooden stick and recentrifuge		
☐	4. Transfer organic (top) layer to a 12 x 75 mm tube		
	G. Concentrate		
☐	1. Evaporate to dryness under nitrogen at 40°C (do not overdry)		
	H. Reconstitute		
☐	1. Add 100 µL of Methanol:Water (10:90) 2. Vortex		
☐	I. Instrumental Analysis 1. LC/MS: analyze 10 µL i. Analyze LC/MS Performance Standard prior to batch analysis		
	ii. Mobile Phase 1 (aqueous)		
	iii. Mobile Phase 2 (organic)		
	iv. LC Column		

7 ANALYTICAL PARAMETERS

7.1 Shimadzu HPLC

7.1.1 Gradient

Time (min)	Mobile Phase %		Flow Rate
	1-Aqueous	2-Organic	(mL/min)
0	90	10	0.3
5	90	10	0.3
20	10	90	0.3
30	10	90	0.3
31	90	10	0.3
36	90	10	0.3
37 (stop)	90	10	0.3

7.1.2 Conditions

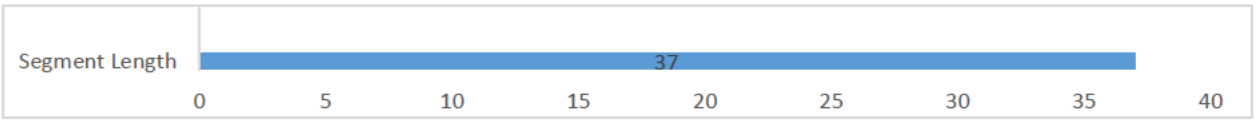
Column Heater (°C)	30
Autosampler (°C)	15
Run Time (min)	37

7.2 Thermo LTQ Orbitrap XL

7.2.1 Source

Mode	ESI
Polarity	(+)

7.2.2 Segment(s)



7.2.3 Scan Events

Event	Mode	Range (m/z)	Details			Analyzer	Resolution
1	Full Scan	200-500	Centroid			FTMS	15,000

8 DATA ANALYSIS

8.1 Decision Criteria

8.1.1 LC/MS Performance Standard

In addition to the performance checks specified in the LC/MS standard operating procedure, a performance standard mix is analyzed through the analytical column to monitor the performance of the column.

8.1.1.1 Chromatography

The analyte's molecular ion traces shall:

A. Have reasonable peak shape (varies by analyte)
B. Compare favorably to the previous analysis of the standard using the same Equipment
    1. Retention times ±5%
    2. Responses 50-200%

8.1.1.2 Mass Spectrometry

The analyte mass assignments shall be present.

8.1.2 Batch Acceptance

A. Negative Control
No target analytes are detected.
B. Positive Control
Qualitative: Target analytes are detected.
Quantitative: Within ±20% of the target value
C. Internal Standards for Controls
The controls meet the recovery criteria from 8.1.3.1

8.1.3 Unknown Sample Acceptance

8.1.3.1 Internal Standard Recovery

The internal standards are detected.

8.1.4 Unknown Sample Compound Identification

In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard or extracted Positive Control.

8.1.4.1 Chromatography

The peak of interest will show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample will compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs.

Additionally, the following two criteria should be met.

8.1.4.1.1 LC Retention Time

The retention time of the peak will be within 5% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, Calibrator, or extracted Positive Control.

8.1.4.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio will exceed 3. Further, the baseline signal for the peak of interest will be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or solvent blank injected just prior to the sample.

8.1.4.2 Mass Spectrometry

The mass spectrum of the analyte of interest will compare favorably to a reference standard, extracted calibrator, or an extracted Positive Control. See the Guidelines for Comparison of Mass Spectra (TOX-104) for further guidance.

8.2 Analyte Identification Criteria

Approximate retention times and expected M+1 m/z values are listed below:

Analyte	Panel	Type	Expected RT (min)	Target: M+1	Target: Cl Isotope	Matched IS	IS: M+1	IS: Cl Isotope
amitriptyline	A	Quant	15.21	278.190		Amitriptyline-d3	281.209	
chlorpheniramine	A	Quant	12.74	275.131	277.128	Chlorpheniramine-d6	281.169	283.166
chlorpromazine	A	Quant	15.56	319.103	321.100	Chlorpromazine-d3	322.122	324.119
citalopram	A	Quant	14.47	325.171		Citalopram-d6	331.209	
clomipramine	A	Quant	15.76	315.162	317.160	Clomipramine-d3	318.181	320.179
cyclobenzaprine	B	Quant	14.98	276.175		Cyclobenzaprine-d3	279.194	
desipramine	A	Quant	14.86	267.186		Desipramine-d3	270.204	
dextromethorphan	A	Quant	14.02	272.201		Dextromethorphan-d3	275.220	
doxylamine	A	Quant	8.03	271.180		Doxylamine-d5	276.212	
diphenhydramine	A	Quant	14.36	256.170		Diphenhydramine-d3	259.189	
doxepin	A	Quant	14.39	280.170		Doxepin-d3	283.188	
duloxetine	A	Quant	15.15	298.128		Duloxetine-d3	301.145	
EDDP	A	Quant	14.99	278.190		EDDP-d3	281.209	
fentanyl	B	Quant	14.07	337.227		Fentanyl-d5	342.259	
fluoxetine	A	Quant	15.49	310.141		Fluoxetine-d6	316.179	
imipramine	A	Quant	15.01	281.201		Imipramine-d3	284.220	
ketamine	A	Quant	11.30	238.099	240.097	Ketamine-d4	242.124	244.142
meperidine	A	Quant	12.87	248.165		Meperidine-d4	252.190	
methadone	A	Quant	15.50	310.217		Methadone-d3	313.235	
mirtazapine	A	Quant	11.53	266.165		Norfentanyl-d5	238.196	
nordoxepin	A	Quant	14.26	266.154		Desipramine-d3	270.204	
norfentanyl	B	Quant	11.52	233.165		Norfentanyl-d5	238.196	
norfluoxetine	A	Quant	15.30	296.126		Norfluoxetine-d6	302.163	
normeperidine	A	Quant	12.81	234.149		Normeperidine-d4	238.174	
norpropoxyphene	A	Quant	19.10	326.211		Norpropoxyphene-d5	331.243	

nortriptyline	A	Quant	15.06	264.175		Nortriptyline-d3	267.194	
paroxetine	B	Quant	14.80	330.150		Paroxetine-d6	336.188	
PCP	B	Quant	13.69	244.206		PCP-d5	249.237	
pheniramine	A	Quant	8.63	241.170		Pheniramine-d6	247.208	
propoxyphene	A	Quant	15.38	340.227		Propoxyphene-d5	345.258	
propranolol	A	Quant	13.77	260.165		Desipramine-d3	270.204	
sertraline	A	Quant	15.57	306.081	308.078	Setraline-d3	311.097	
					310.075			
tramadol	A	Quant	12.14	264.196		Tramadol-13C-d3	268.218	
trazodone	A	Quant	13.38	372.159	374.156	Trazodone-d6	378.196	380.193
trimipramine	A	Quant	15.37	295.217		Trimipramine-d3	298.236	
venlafaxine	A	Quant	13.13	278.211		Venlafaxine-d6	284.249	
verapamil	A	Quant	15.17	455.290		Imipramine-d3	284.220	
zolpidem	B	Quant	12.84	308.176		Zolpidem-d6	314.215	
brompheniramine	B	Qual	12.99	319.080	321.078			
bupropion	A	Qual	13.15	240.115				
clozapine	A	Qual	13.02	327.137	329.134			
metoprolol	A	Qual	12.06	268.191				
norsertraline	A	Qual	15.41	292.065	294.062			
					296.060			
quetiapine	A	Qual	13.63	384.174				
thioridazine	A	Qual	16.20	371.161				

8.3 Calculations

Quantitation is performed by constructing a multi-point calibration curve based on the ratio of the area for the M+1 peak for each analyte to its internal standard. The chlorine isotope is added to the M+1 peak of both the analyte and the internal standard before ratioing, if applicable. Ion traces are drawn at a 0.005 m/z mass tolerance. See the *Quality Control for Toxicology Examinations* (TOX-101) for acceptable practices in calculating quantitative results.

8.3.1 Calibration

Model	Linear
Weighting	1/x

Refer to TOX-101 for further guidance.

8.3.2 Software

Quantitative and qualitative calculations may be performed by one or more of the following software packages:

A. Thermo Xcalibur
    1. QualBrowser
    2. QuanBrowser
    3. Tracefinder
B. Microsoft
    1. Excel

9 REPORTING

9.1 Measurement Uncertainty

Refer to CHEM-100 and TOX-101.

10 CORRECTIVE MEASURES

Refer to TOX-101 for guidance on action steps in the event of a quality control failure.

11 PERFORMANCE CHARACTERISTICS

11.1 LOD

Analyte	Panel	Type	LOD-Blood (ng/ml)	LOD-Urine (ng/mL)
amitriptyline	A	Quant	10	5
chlorpheniramine	A	Quant	10	5
chlorpromazine	A	Quant	10	5
citalopram	A	Quant	10	5
clomipramine	A	Quant	10	5
cyclobenzaprine	B	Quant	1	1
desipramine	A	Quant	10	5
dextromethorphan	A	Quant	10	5
diphenhydramine	A	Quant	25	5
doxepin	A	Quant	10	5
doxylamine	A	Quant	10	5
duloxetine	A	Quant	25	10
EDDP	A	Quant	10	5
fentanyl	B	Quant	1	1
fluoxetine	A	Quant	10	5
imipramine	A	Quant	10	5
ketamine	A	Quant	10	5
meperidine	A	Quant	10	5
methadone	A	Quant	10	5
mirtazapine	A	Quant	10	5
nordoxepin	A	Quant	25	5
norfentanyl	B	Quant	5	5
norfluoxetine	A	Quant	25	5
normeperidine	A	Quant	10	5
norpropoxyphene	A	Quant	10	5
nortriptyline	A	Quant	25	5
paroxetine	B	Quant	5	1
PCP	B	Quant	1	5
pheniramine	A	Quant	10	10
propoxyphene	A	Quant	10	5
propranolol	A	Quant	10	5
sertraline	A	Quant	10	5
tramadol	A	Quant	10	5
trazodone	A	Quant	10	5
trimipramine	A	Quant	10	5
venlafaxine	A	Quant	10	5

verapamil	A	Quant	10	5
zolpidem	B	Quant	1	1
brompheniramine	B	Qual	1	1
bupropion	A	Qual	5	5
clozapine	A	Qual	10	5
metoprolol	A	Qual	10	5
norsertraline	A	Qual	50	5
quetiapine	A	Qual	25	5
thioridazine	A	Qual	10	10

11.2 LOQ–Linear Range

The LOQ is set as the lowest calibrator. Values in (ng/mL).

Analyte	Panel	Type	Low/LOQ	High
amitriptyline	A	Quant	50	1000
chlorpheniramine	A	Quant	50	1000
chlorpromazine	A	Quant	50	1000
citalopram	A	Quant	50	1000
clomipramine	A	Quant	50	1000
cyclobenzaprine	B	Quant	10	200
desipramine	A	Quant	50	1000
dextromethorphan	A	Quant	50	1000
doxylamine	A	Quant	50	750
diphenhydramine	A	Quant	50	1000
doxepin	A	Quant	50	1000
duloxetine	A	Quant	50	1000
EDDP	A	Quant	50	1000
fentanyl	B	Quant	10	200
fluoxetine	A	Quant	50	1000
imipramine	A	Quant	50	1000
ketamine	A	Quant	50	1000
meperidine	A	Quant	50	1000
methadone	A	Quant	50	1000
mirtazapine	A	Quant	100	1000
nordoxepin	A	Quant	50	1000
norfentanyl	B	Quant	10	200
norfluoxetine	A	Quant	50	1000
normeperidine	A	Quant	50	1000
norpropoxyphene	A	Quant	50	1000
nortriptyline	A	Quant	50	1000
paroxetine	B	Quant	10	200
PCP	B	Quant	10	200

pheniramine	A	Quant	50	1000
propoxyphene	A	Quant	50	1000
propranolol	A	Quant	100	1000
sertraline	A	Quant	50	1000
tramadol	A	Quant	50	1000
trazodone	A	Quant	50	1000
trimipramine	A	Quant	50	1000
venlafaxine	A	Quant	50	1000
verapamil	A	Quant	50	750
zolpidem	B	Quant	10	200
brompheniramine	B	Qual		
bupropion	A	Qual		
clozapine	A	Qual		
metoprolol	A	Qual		
norsertraline	A	Qual		
quetiapine	A	Qual		
thioridazine	A	Qual		

11.3 Bias

N=15 for most analytes. Specifics available in validation records.

Analyte	Low	Medium	High
amitriptyline	2.72%	3.96%	3.68%
chlorpheniramine	2.03%	5.49%	4.59%
chlorpromazine	2.06%	6.22%	3.21%
citalopram	-2.51%	2.41%	3.82%
clomipramine	-1.69%	1.06%	1.88%
cyclobenzaprine	-13.14%	-10.35%	-10.70%
desipramine	-0.20%	5.83%	5.78%
dextromethorphan	-6.34%	-4.28%	-2.97%
diphenhydramine	4.92%	5.40%	7.34%
doxepin	1.63%	3.93%	4.40%
doxylamine	-10.27%	4.28%	-6.72%
duloxetine	-11.34%	-7.25%	-7.51%
EDDP	-1.15%	5.45%	5.93%
fentanyl	-1.74%	2.13%	0.84%
fluoxetine	-0.27%	5.19%	4.52%
imipramine	0.17%	6.32%	+5.38%
ketamine	0.56%	5.68%	2.08%
meperidine	-2.07%	1.85%	2.61%
methadone	-0.94%	3.42%	+3.72%
mirtazapine	0.66%	2.32%	5.51%

nordoxepin	-6.39%	-7.38%	-7.73%
norfentanyl	-7.01%	-2.12%	-3.01%
norfluoxetine	2.54%	6.43%	7.14%
normeperidine	-1.77%	-2.86%	-0.20%
norpropoxyphene	-1.48%	6.29%	2.45%
nortriptyline	1.28%	3.12%	2.87%
paroxetine	-9.06%	0.41%	-3.54%
PCP	-3.14%	0.67%	-1.52%
pheniramine	-3.96%	3.13%	+1.89%
propoxyphene	-5.51%	-1.93%	-2.03%
propranolol	-0.21%	-2.16%	+3.43 %
sertraline	-17.81%	-12.52%	-9.18%
tramadol	4.02%	7.81%	4.99%
trazodone	-8.94%	-6.27%	-6.53%
trimipramine	-1.14%	6.53%	4.85%
venlafaxine	-2.10%	-1.34%	-1.89%
verapamil	-7.28%	-5.12%	-10.67%
zolpidem	-0.57%	0.51%	-0.83%

11.4 Precision

11.4.1 Repeatability

N=15 for most analytes. Specifics available in validation records.

Analyte	Low	Medium	High
amitriptyline	1.31%	1.22%	2.30%
chlorpheniramine	1.34%	1.69%	3.00%
chlorpromazine	2.89%	0.62%	5.00%
citalopram	1.58%	0.48%	3.73%
clomipramine	2.76%	1.80%	1.95%
cyclobenzaprine	2.02%	1.44%	1.80%
desipramine	5.68%	2.91%	3.07%
dextromethorphan	1.18%	2.24%	0.61%
diphenhydramine	1.66%	1.48%	1.81%
doxepin	0.98%	0.71%	1.88%
doxylamine	3.99%	7.37%	2.22%
duloxetine	1.87%	1.45%	0.74%
EDDP	8.60%	3.74%	3.76%
fentanyl	3.50%	1.15%	2.60%
fluoxetine	1.14%	1.00%	1.46%
imipramine	5.12%	3.07%	2.51%

ketamine	1.58%	2.68%	1.88%
meperidine	0.95%	1.73%	0.61%
methadone	5.53%	3.29%	3.00%
mirtazapine	19.81%	20.29%	7.93%
nordoxepin	12.24%	4.85%	8.60%
norfentanyl	2.25%	2.94%	3.04%
norfluoxetine	1.44%	2.23%	0.74%
normeperidine	1.56%	0.50%	1.76%
norpropoxyphene	6.33%	2.02%	2.51%
nortriptyline	1.25%	0.87%	1.42%
paroxetine	2.46%	1.79%	5.93%
PCP	3.99%	0.86%	2.31%
pheniramine	7.64%	2.80%	2.57%
propoxyphene	2.57%	2.28%	2.25%
propranolol	16.84%	7.01%	11.41%
sertraline	1.56%	1.79%	1.59%
tramadol	2.77%	2.19%	1.40%
trazodone	1.58%	1.50%	1.02%
trimipramine	5.41%	2.77%	2.47%
venlafaxine	1.12%	1.00%	2.03%
verapamil	17.63%	17.36%	18.13%
zolpidem	3.06%	1.25%	2.22%

11.4.2 Intermediate Precision

N=15 for most analytes. Specifics available in validation records.

Analyte	Low	Medium	High
amitriptyline	1.40%	1.92%	2.44%
chlorpheniramine	2.75%	2.58%	3.11%
chlorpromazine	3.06%	0.66%	5.00%
citalopram	2.81%	0.95%	3.93%
clomipramine	3.16%	2.21%	2.08%
cyclobenzaprine	2.56%	1.78%	3.35%
desipramine	6.41%	2.91%	3.07%
dextromethorphan	2.46%	2.43%	0.78%
diphenhydramine	3.38%	1.65%	1.81%
doxepin	2.35%	1.81%	2.19%
doxylamine	4.67%	8.26%	3.67%
duloxetine	2.87%	1.45%	1.07%
EDDP	9.95%	3.74%	3.88%
fentanyl	5.43%	4.73%	2.60%

fluoxetine	4.10%	1.69%	1.68%
imipramine	5.22%	3.07%	2.62%
ketamine	2.59%	2.68%	1.88%
meperidine	3.19%	1.73%	1.31%
methadone	5.76%	3.29%	3.08%
mirtazapine	19.81%	20.29%	9.62%
nordoxepin	13.70%	7.39%	8.92%
norfentanyl	3.15%	3.27%	3.04%
norfluoxetine	5.26%	2.23%	1.00%
normeperidine	1.93%	1.98%	2.48%
norpropoxyphene	13.29%	5.62%	4.45%
nortriptyline	1.90%	1.53%	1.81%
paroxetine	3.66%	4.20%	5.93%
PCP	5.72%	3.78%	2.42%
pheniramine	8.02%	5.21%	4.52%
propoxyphene	5.11%	2.87%	2.25%
propranolol	16.84%	9.52%	12.59%
sertraline	2.42%	2.60%	1.90%
tramadol	5.30%	2.62%	1.41%
trazodone	2.14%	1.85%	1.16%
trimipramine	5.41%	2.77%	2.64%
venlafaxine	2.30%	1.35%	2.03%
verapamil	18.51%	17.36%	32.96%
zolpidem	5.75%	2.98%	2.44%

11.5 Carryover

High analyte concentrations in samples may carryover into subsequent samples. Analysts should investigate evidence for carryover if high sample analytes loads are encountered.

For extracted negative control samples analyzed immediately following extracted 700 ng/ml positive control samples, no analyte showed signal greater than 2% of that seen in the positive control.

12 LIMITATIONS

12.1 General

Grossly decomposed or putrefied samples may affect both detection and quantitation limits.

Doxylamine elutes very early in the analysis time, so data should be interpreted with care due to possible sample related matrix effects.

12.2 Interferences

The following drug pairs cannot be quantitated or identified if they are present in the same sample because they elute within 0.3 min and their exact masses are within 0.05 m/z:

Amitriptyline	EDDP
Methadone	Propoxyphene
Imipramine	EDDP
Imipramine	Amitriptyline
Desipramine	Nortriptyline
Nortriptyline	propoxyphene

12.3 Processed Sample Stability

Six of eight compounds tested show no problems with processed sample stability after 8 days of refrigerated storage. However, thioridazine may degrade in prepared extracts, and negative results should be repeated if the extracted samples cannot be analyzed within the first 24 hours of extraction. Doxylamine controls will be monitored closely if the extracted samples cannot be analyzed within the first 24 hours of extraction.

13 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 Revision History

Revision	Issued	Changes
05	02/11/2022	Document reformat. Updated title. Differentiated "higher/lower dose" drugs with Panel A/B throughout. 2 - Revised scope statement 5 - Reorganization of Equipment. Referenced Level 4 document to aid solution prep. 6 – Updated procedure format 7 – Updated Instrument parameter format 8 – Updated decision criteria (multiple); combined identification criteria into one table