Quantitation and Confirmation of Alkaline Drugs

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

This procedure allows for quantitation and confirmation of amitriptyline, chlorpheniramine, chlorpromazine, citalopram, clomipramine, cyclobenzaprine, desipramine, dextromethorphan, doxylamine, diphenhydramine, doxepin, duloxetine, EDDP (methadone metabolite; 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), fentanyl, fluoxetine, imipramine, ketamine, meperidine, methadone, mirtazapine, nordoxepin, norfentanyl, norfluoxetine, normeperidine, norpropoxyphene, nortriptyline, paroxetine, PCP (phencyclidine), pheniramine, propoxyphene, propranolol, sertraline, tramadol, trazodone, trimipramine, venlafaxine, verapamil and zolpidem in blood. This procedure also allows for qualitative confirmation of brompheniramine, bupropion, clozapine, metoprolol, norsertraline, quetiapine and thioridazine in blood, as well as confirmation of all analytes in urine. It can also be used for quantitative analysis of additional alkaline drugs, as per instructions in the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) and *FBI Laboratory Practices for Validating Chemical Procedures*.

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 Specimens

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

5 Equipment/Materials/Reagents

a. 16 x 100 mm screw-top tubes with Teflon-lined caps

b. 12 x 75 mm culture tubes with polypropylene snap-tops

c. Acetonitrile (Optima grade or better)

d. Formic Acid (Puriss grade or better)

e. Hexane (UV grade or better)

f. Sodium hydroxide (ACS grade or better)

g. Water (Deionized, 18 MΩ)

h. Water (Optima or better grade)

i. 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.

j. Centrifuge

k. Evaporator w/ Nitrogen

l. Vortex mixer

m. 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.

n. Routine laboratory supplies, including disposable pipettes, wooden sticks, test tube racks, graduated cylinders, etc.

o. Liquid Chromatograph - Mass Spectrometer capable of 15,000 resolution

p. HPLC Column (Xterra C-18 MS, 3.0 x 150 mm, 3.5 µm dp; or equivalent)

q. Mobile Phase A (Acetonitrile 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 for 2 months.

r. Mobile Phase B (Water 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 2 months.

6 Standards and Controls

a. Standard Stock Solutions (0.1 mg/mL) of the following may be purchased from Cerilliant (Round Rock, TX), Lipomed, or an equivalent supplier. Typically, calibration material is purchased from Cerilliant and control material is purchased from Lipomed. Solutions may be in methanol or acetonitrile, and will be stored according to the manufacturer's recommendations. Stability is determined by the manufacturer.

Citalopram	EDDP
Normeperidine	Norsertraline

b. Standard Stock Solutions (1.0 mg/mL) of the following may be purchased from Cerilliant (Round Rock, TX), Lipomed, or an equivalent supplier. Typically, calibration material is purchased from Cerilliant and control material is purchased from Lipomed. Solutions may be in methanol or acetonitrile, and will be stored according to the manufacturer's recommendations. Stability is determined by the manufacturer.

Amitriptyline	Fentanyl	PCP
Brompheniramine	Fluoxetine	Pheniramine
Bupropion	Imipramine	Propoxyphene
Chlorpheniramine	Ketamine	Propranolol
Chlorpromazine	Meperidine	Quetiapine
Clomipramine	Methadone	Sertraline
Clozapine	Metoprolol	Thioridazine
Cyclobenzaprine	Mirtazapine	Tramadol
Desipramine	Nordoxepin	Trazodone
Dextromethorphan	Norfentanyl	Trimipramine
Diphenhydramine	Norfluoxetine	Venlafaxine
Doxepin	Norpropoxyphene	Verapamil
Doxylamine	Nortriptyline	Zolpidem
Duloxetine	Paroxetine	

c. Internal Standard Stock Solutions (0.1 mg/mL) of the following may be purchased from Cerilliant (Round Rock, TX) or an equivalent supplier. Solutions may be in methanol or acetonitrile, and will be stored according to the manufacturer's recommendations. Stability is determined by the manufacturer.

Amitriptyline-d_3	Duloxetinie-d_3	Nortriptyline-d_3
Chlorpheniramine-d_6	EDDP-d_3	Paroxetine-d_6
Chlorpromazine-d_3	Fentanyl-d_5	PCP-d_5
Citalopram-d_6	Fluoxetine-d_6	Pheniramine-d_6
Clomipramine-d_3	Imipramine-d_3	Propoxyphene-d_5
Cyclobenzaprine-d_3	Ketamine-d_4	Sertraline-d_3
Desipramine-d_3	Meperidine-d_4	Tramadol-13C-d_3
Dextromethorphan-d_3	Methadone-d_3	Trazodone-d_6
Diphenhydramine-d_3	Norfentanyl-d_5	Trimipramine-d_3

Doxepin-d_3	Norfluoxetine-d_6	Venlafaxine-d_6
Doxylamine-d_5	Normeperidine-d_4	Zolpidem-d_6
	Norpropoxyphene-d_5	

d. High Calibration Working Solution (5.0 µg/mL)[1]:
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.

e. Low Calibration Working Solution (WS) (0.5 µg/mL)[1]:
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.

f. Low Dose Drugs Intermediate Calibration Solution (10 µg/mL)[1,2]:
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.

g. Low Dose Drugs High Calibration Working Solution (1.0 µg/mL)[1,2]:
Add 1.0 mL of the Low Dose Drugs 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.

h. Low Dose Drugs Low Calibration Working Solution (0.1 µg/mL)[1,2]:
Add 1.0 mL of the Low Dose Drugs 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.

[1] Working Solutions and Internal Standard Solutions may be made in groupings or individually, depending on case needs.
[2] 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.

Tables 1-2 show the amount of the Calibration Working Solutions to add to 0.5 mL of Negative Control Blood for calibrator preparation.

Table 1: Calibrator Preparation for amitriptyline, chlorpheniramine, chlorpromazine, citalopram, clomipramine, desipramine, dextromethorphan, diphenhydramine, doxepin, duloxetine, EDDP, fluoxetine, imipramine, ketamine, meperidine, methadone, mirtazapine, nordoxepin, norfluoxetine, normeperidine, norpropoxyphene, nortriptyline, pheniramine, propoxyphene, propranolol, sertraline, tramadol, trimipramine, venlafaxine and verapamil

Calibrator Level (ng/mL)	High Cal WS (5 µg/mL) Volume (µL)	Low Cal WS (0.5 µg/mL) Volume (µL)
50*	-	50
100	-	100
250	25	-
500	50	-
750	75	-
1000**	100	-

*****The 50 ng/mL calibrator will not be analyzed for mirtazapine and propranolol, as it is out of the linear range for these analytes.
**The 1000 ng/mL calibrator will not be analyzed for doxylamine and verapamil as it is out of the linear range for these analytes.

Table 2: Calibrator Preparation for cyclobenzaprine, fentanyl, norfentanyl, paroxetine, PCP and zolpidem

Calibrator Level (ng/mL)	Low Dose Drugs High Cal WS (1 µg/mL) Volume (µL)	Low Dose Drugs Low Cal WS (0.1 µg/mL) Volume (µL)
10	-	50
20	-	100
50	25	-
100	50	-
150	75	-
200	100	-

i. Internal Standard Working Solution (3 µg/mL)[1]:
Combine 0.3 mL of each Internal 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 two years.

j. Low Dose Drugs Internal Standard Working Solution (1 µg/mL)[1,2]:
Combine 0.1 mL of each Internal 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 two years.

k. High Control Working Solution (4 µg/mL)[1]:
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.

l. Low Control Working Solution (0.4 µg/mL)[1]:
Add 0.5 mL of the 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.

m. Low Dose Drugs High Control Working Solution (0.8 µg/mL)[1,2]:
Add 1.0 mL of the 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.

n. Low Dose Drugs Low Control Working Solution (0.2 µg/mL)[1,2]:
Add 0.25 mL of the 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.

o. 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. A Negative Control Blood sample will be extracted and analyzed with every blood assay.

p. 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. A Negative Control Urine sample will be extracted and analyzed with every urine assay.

q. Quantitative Positive Control Blood:
This is normally prepared in-house as per the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101), but may be purchased from an appropriate vendor as needed. A Quantitative Positive Control Blood sample will be extracted in duplicate with every quantitative assay. The Quantitative Positive Control Blood may be prepared for one or more analytes, depending on which analytes are being quantitated in a given analytical run. Quantitative Positive Controls will typically be prepared fresh from Control Working Solutions as described below:

1. Low Control (24 ng/mL) for cyclobenzaprine, fentanyl, norfentanyl, paroxetine, PCP and zolpidem: Add 0.06 mL of the Low Dose Drugs Low Control Working Solution to 0.5 mL of Negative Control Blood.

2. Low Control (72 ng/mL) for amitriptyline, chlorpheniramine, chlorpromazine, citalopram, clomipramine, desipramine, dextromethorphan, diphenhydramine, doxepin, duloxetine, EDDP, fluoxetine, imipramine, ketamine, meperidine, methadone, nordoxepin, norfluoxetine, normeperidine, norpropoxyphene, nortriptyline, pheniramine, propoxyphene, sertraline, tramadol, trimipramine, venlafaxine and verapamil: Add 0.09 mL of the Low Control Working Solution to 0.5 mL of Negative Control Blood.

3. Low Control (320 ng/mL) for mirtazapine and propranolol: Add 0.04 mL of the High Control Working Solution to 0.5 mL of Negative Control Blood.

4. High Control (144 ng/mL) for cyclobenzaprine, fentanyl, norfentanyl, paroxetine, PCP and zolpidem: Add 0.09 mL of the Low Dose Drugs High Control Working Solution to 0.5 mL of Negative Control Blood.

5. High Control (680 ng/mL) for amitriptyline, chlorpheniramine, chlorpromazine, citalopram, clomipramine, desipramine, dextromethorphan, diphenhydramine, doxepin, EDDP, fluoxetine, imipramine, ketamine, meperidine, methadone, mirtazapine, norfluoxetine, normeperidine, norpropoxyphene, nortriptyline, pheniramine, propoxyphene, propranolol, tramadol, trimipramine, venlafaxine and verapamil: Add 0.085 mL of the High Control Working Solution to 0.5 mL of Negative Control Blood.

r. Qualitative Positive Control Blood:
This is normally prepared in-house, but may be purchased from an appropriate vendor as needed. A Qualitative Positive Control Blood sample will be extracted and analyzed with every qualitative blood assay. The Qualitative Positive Control Blood may be prepared for one or more analytes, depending on which analytes are being confirmed in a given analytical run. Qualitative Positive Controls will typically be prepared fresh at any concentration above the assay's limit of detection from Control Working Solutions.

s. Positive Control Urine:
This is normally prepared in-house, but may be purchased from an appropriate vendor as needed. A Positive Control Urine sample will be extracted and analyzed with every urine assay. The Positive Control Urine may be prepared for one or more analytes, depending on which analytes are being confirmed in a given analytical run. Qualitative Positive Controls will typically be prepared fresh at any concentration above the assay's limit of detection from Control Working Solutions.

t. Column 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.

7 Sampling

Not applicable.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

a. To a properly labeled 16 x 100 mm screw-top tube, add 0.5 mL of specimen, calibrator or control. Prepare case specimens and positive controls in duplicate for quantitative analysis. Smaller volumes may be analyzed if required to ensure that the sample is within the linear range of the procedure. A ten-fold dilution using 0.05 mL specimen and 0.45 mL deionized water is recommended. Case samples and positive controls do not need to be prepared in duplicate for qualitative analysis.

b. Add 50 µL of the Internal Standard Working Solution(s) to each sample.

c. Add 0.2 mL of 4% sodium hydroxide to each sample and vortex briefly.

d. Add 2 mL of hexane down the inside of each tube and extract for 20 minutes on a rotator. Centrifuge 10 minutes at a minimum of 3000 rpm. Use a wooden stick to break up any emulsions that develop, and spin again.

e. Transfer organic (top) layer to a 12x75 mm culture tube.

f. Evaporate the hexane to dryness under a gentle stream of nitrogen at 40ºC. Do not overdry.

g. Reconstitute each sample in 0.1 mL Methanol:Water (10:90 v:v).

h. Analyze by LC/MS using the conditions below after verifying that the instrument is performing properly by analyzing the appropriate Column Performance Mix.

9 Instrumental Conditions

Appendix 2 contains an abbreviated version of the instrumental conditions in this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

9.1 Liquid Chromatograph Parameters

Mobile Phase Compositions	Flow Parameters			Column Parameters	
A: Acetonitrile with 0.1% Formic Acid	total flow	0.3 mL/min		type	C-18 MS
	time (min)	%A	%B	length	15 cm
B: Water with 0.1% Formic Acid	0	10	90	internal diameter	3.0 mm
	5	10	90	particle size	3.5 µm
	20	90	10	temperature	30°C
	30	90	10		
	31	10	90		
	36	10	90		
	37	10	90		
	total time	37 min			

9.2 Mass Spectral Parameters

ionization mode	electrospray (+)
scan mode	full scan, centroid
scan range	200 - 500 AMU
resolution	15,000
All source parameters are set through the instrument tuning process. See the Instrument Operations and Support Submit SOP Manual for details.	

10 Decision Criteria

10.1 Batch Acceptance Criteria

No analytes of interest should be detected in the Negative Control. For this purpose, analytes of interest are defined as those analytes that will be reported for this batch.

All intended analytes should be present in the Positive Control. Each Quantitative Positive Control will quantitate within ±20% of the target value. See the Guidelines for Toxicological Quantitations standard operating procedure (Tox 101) for more information.

10.2 Sample Acceptance Criteria

10.2.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. Ion peaks are typically extracted at ±5 mmu. In order to be determined acceptable, a chromatographic peak in an unknown sample should 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.

10.2.1.1 Retention Time

The retention time of the peak should be within ±5% of the retention time (relative or absolute, as appropriate) obtained from injection of an extracted Positive Control or extracted calibrator.

Approximate expected retention times are listed in Table 3 below for reference.

Table 3: Approximate expected retention times

Analyte	RT (min)	Analyte	RT (min)
doxylamine	8.03	doxepin	14.39
pheniramine	8.63	citalopram	14.47
ketamine	11.30	paroxetine	14.80
norfentanyl	11.52	desipramine	14.86
mirtazepine	11.53	cyclobenzaprine	14.98
metoprolol	12.06	EDDP	14.99
tramadol	12.14	imipramine	15.01
chlorpheniramine	12.74	nortriptyline	15.06
normeperidine	12.81	duloxetine	15.15
zolpidem	12.84	verapamil	15.17
meperidine	12.87	amitriptyline	15.21
brompheniramine	12.99	norfluoxetine	15.30
clozapine	13.02	trimipramine	15.37
venlafaxine	13.13	propoxyphene	15.38
bupropion	13.15	norsertraline	15.41
trazodone	13.38	fluoxetine	15.49
quetiapine	13.63	methadone	15.50
phencyclidine	13.69	chlorpromazine	15.56
propranolol	13.77	sertraline	15.57
dextromethorphan	14.02	clomipramine	15.76
fentanyl	14.07	thioridazine	16.20
nordoxepin	14.26	norpropoxyphene	19.10
diphenhydramine	14.36		

10.2.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Note: nonsensical signal to noise values may result from high resolution mass spectral data. Further, the baseline signal for the peak of interest should be at least ten-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.

10.2.2 Mass Spectrometry

The M+1 for the analyte of interest should match those in Tables 4 and 5 within ±5 mmu.

M+1 ions for the drugs that have been quantitatively validated are listed in Table 5. M+1 for drugs that have been validated qualitatively only are listed in Table 4 below.

Table 4: M + 1 ions for analytes

Analyte	M+1 (+ Br or Cl isotope, when applicable)
Brompheniramine	319.080, 321.078
Bupropion	240.115
Clozapine	327.137, 329.134
Metoprolol	268.191
Norsertraline	292.065, 294.062, 296.060
Quetiapine	384.174
Thioridazine	371.161

11 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 5 mmu mass tolerance. 1/x weighting is used for all analytes. See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

Table 5 lists the M+1 and internal standard (IS) for each quantitatively validated analyte in this procedure.

Analyte	M+1 (+ Cl isotope, when applicable)	IS	M + 1 for IS (+ Cl isotope)
Amitriptyline	278.190	Amitriptyline-d3	281.209
Chlorpheniramine	275.131 + 277.128	Chlorpheniramine-d6	281.169 + 283.166
Chlorpromazine	319.103 + 321.100	Chlorpromazine-d3	322.122 + 324.119
Citalopram	325.171	Citalopram-d6	331.209
Clomipramine	315.162 + 317.160	Clomipramine-d3	318.181 + 320.179
Cyclobenzaprine	276.175	Cyclobenzaprine-d3	279.194
Desipramine	267.186	Desipramine-d3	270.204
Dextromethorphan	272.201	Dextromethorphan-d3	275.220
Diphenhydramine	256.170	Diphenhydramine-d3	259.189
Doxepin	280.170	Doxepin-d3	283.188
Doxylamine	271.180	Doxylamine-d5	276.212
Duloxetine	298.128	Duloxetine-d3	301.145
EDDP	278.190	EDDP-d3	281.209
Fentanyl	337.227	Fentanyl-d5	342.259
Fluoxetine	310.141	Fluoxetine-d6	316.179
Imipramine	281.201	Imipramine-d3	284.220
Ketamine	238.099 + 240.097	Ketamine-d4	242.124 + 244.142
Meperidine	248.165	Meperidine-d4	252.190
Methadone	310.217	Methadone-d3	313.235
Mirtazapine	266.165	Norfentanyl-d5	238.196
Nordoxepin	266.154	Desipramine-d3	270.204
Norfentanyl	233.165	Norfentanyl-d5	238.196
Norfluoxetine	296.126	Norfluoxetine-d6	302.163
Normeperidine	234.149	Normeperidine-d4	238.174
Norpropoxyphene	326.211	Norpropoxyphene-d5	331.243
Nortriptyline	264.175	Nortriptyline-d3	267.194
Paroxetine	330.150	Paroxetine-d6	336.188
PCP	244.206	PCP-d5	249.237
Pheniramine	241.170	Pheniramine-d6	247.208
Propoxyphene	340.227	Propoxyphene-d5	345.258
Propranolol	260.165	Desipramine-d3	270.204
Sertraline	306.081+ 308.078 + 310.075	Setraline-d3	311.097
Tramadol	264.196	Tramadol-13C-d3	268.218
Trazodone	372.159, 374.156	Trazodone-d6	378.196, 380.193
Trimipramine	295.217	Trimipramine-d3	298.236
Venlafaxine	278.211	Venlafaxine-d6	284.249
Verapamil	455.290	Imipramine-d3	284.220
Zolpidem	308.176	Zolpidem-d6	314.215

12 Measurement Uncertainty

The critical sources of measurement uncertainty in this procedure include:

- historical random uncertainty of repeated measurements
- accuracy of the pipette used to deliver the sample
- accuracy of the pipette used to deliver the calibrators
- uncertainty in the concentration of the calibration standards
- precision of the delivery of internal standard

When quantitative results are included in an FBI Laboratory report, the measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements* standard operating procedure (CUQA 13). Information used to derive uncertainty measurements will be tracked in an electronic database.

13 Limitations

a. Linear range using 1/x weighting and a linear calibration model:

Analyte	Range (ng/mL)	Analyte	Range (ng/mL)
Amitriptyline	50-1000	Mirtazapine	100-1000
Chlorpheniramine	50-1000	Nordoxepin	50-1000
Chlorpromazine	50-1000	Norfentanyl	10-200
Citalopram	50-1000	Norfluoxetine	50-1000
Clomipramine	50-1000	Normeperidine	50-1000
Cyclobenzaprine	10 - 200	Norpropoxyphene	50-1000
Desipramine	50-1000	Nortriptyline	50-1000
Dextromethorphan	50-1000	Paroxetine	10-200
Diphenhydramine	50-1000	PCP	10-200
Doxepin	50-1000	Pheniramine	50-1000
Doxylamine	50-750	Propoxyphene	50-1000
Duloxetine	50-1000	Propranolol	100-1000
EDDP	50-1000	Sertraline	50-1000
Fentanyl	10-200	Tramadol	50-1000
Fluoxetine	50-1000	Trazodone	50-1000
Imipramine	50-1000	Trimipramine	50-1000
Ketamine	50-1000	Venlafaxine	50-1000
Meperidine	50-1000	Verapamil	50-750
Methadone	50-1000	Zolpidem	10-200

b. Limit of Detection:

Analyte	LOD (ng/mL) Blood / Urine	Analyte	LOD (ng/mL) Blood / Urine
Amitriptyline	10 / 5	Metoprolol	10 / 5
Brompheniramine	1 / 1	Mirtazapine	10 / 5
Bupropion	5 / 5	Nordoxepin	25 / 5
Chlorpheniramine	10 / 5	Norfentanyl	5 / 5
Chlorpromazine	10 / 5	Norfluoxetine	25 / 5
Citalopram	10 / 5	Normeperidine	10 / 5
Clomipramine	10 / 5	Norpropoxyphene	10 / 5
Clozapine	10 / 5	Norsertraline	50 / 5
Cyclobenzaprine	1 / 1	Nortriptyline	25 / 5
Desipramine	10 / 5	Paroxetine	5 / 1
Dextromethorphan	10 / 5	PCP	1 / 5
Diphenhydramine	25 / 5	Pheniramine	10 / 10
Doxepin	10 / 5	Propoxyphene	10 / 5
Doxylamine	10 / 5	Propranolol	10 /5
Duloxetine	25 / 10	Quetiapine	25 / 5
EDDP	10 / 5	Sertraline	10 / 5
Fentanyl	1 / 1	Thioridazine	10 / 10
Fluoxetine	10 / 5	Tramadol	10 / 5
Imipramine	10 / 5	Trazodone	10 / 5
Ketamine	10 / 5	Trimipramine	10 / 5
Meperidine	10 / 5	Venlafaxine	10 / 5
Methadone	10 / 5	Verapamil	10 / 5
		Zolpidem	1 / 1

c. Bias (n=15):

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% (n = 14)	+5.93%
Fentanyl	-1.74% (n = 12)	+2.13% (n = 12)	+0.84% (n = 12)

Analyte	Low	Medium	High
Fluoxetine	-0.27%	+5.19%	+4.52%
Imipramine	+0.17%	+6.32% (n = 14)	+5.38%
Ketamine	+0.56%	+5.68%	+2.08%
Meperidine	-2.07%	+1.85%	+2.61%
Methadone	-0.94%	+3.42% (n = 14)	+3.72%
Mirtazapine	+0.66%	+2.32%	+5.51% (n = 13)
Nordoxepin	-6.39%	-7.38%	-7.73%
Norfentanyl	-7.01% (n = 12)	-2.12% (n = 12)	-3.01% (n = 11)
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% (n =12)	+0.41% (n =12)	-3.54% (n = 11)
PCP	-3.14% (n = 11)	+0.67% (n = 12)	-1.52% (n = 12)
Pheniramine	-3.96%	+3.13% (n = 14)	+1.89%
Propoxyphene	-5.51%	-1.93%	-2.03%
Propranolol	-0.21% (n = 12)	-2.16% (n =11)	+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% (n = 12)	-0.83% (n = 14)

d. Repeatability (n=15):

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% (n = 14)	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% (n = 14)	3.76%
Fentanyl	3.50% (n = 12)	1.15% (n = 12)	2.60% (n = 12)
Fluoxetine	1.14%	1.00%	1.46%

Analyte	Low	Medium	High
Imipramine	5.12%	3.07% (n = 14)	2.51%
Ketamine	1.58%	2.68%	1.88%
Meperidine	0.95%	1.73%	0.61%
Methadone	5.53%	3.29% (n = 14)	3.00%
Mirtazapine	19.81%	20.29%	7.93% (n = 13)
Nordoxepin	12.24%	4.85%	8.60%
Norfentanyl	2.25% (n =12)	2.94% (n =12)	3.04% (n =11)
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% (n = 12)	1.79% (n = 12)	5.93% (n = 11)
PCP	3.99% (n = 11)	0.86% (n = 12)	2.31% (n = 12)
Pheniramine	7.64%	2.80% (n = 14)	2.57%
Propoxyphene	2.57%	2.28%	2.25%
Propranolol	16.84% (n = 12)	7.01% (n =11)	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% (n = 14)	2.47%
Venlafaxine	1.12%	1.00%	2.03%
Verapamil	17.63%	17.36%	18.13%
Zolpidem	3.06%	1.25% (n = 12)	2.22% (n = 14)

e. Intermediate Precision (n=15):

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 % (n = 14)	3.88%
Fentanyl	5.43% (n = 12)	4.73 (n = 12)	2.60% (n = 12)
Fluoxetine	4.10%	1.69%	1.68%
Imipramine	5.22%	3.07% (n = 14)	2.62%

Analyte	Low	Medium	High
Ketamine	2.59%	2.68%	1.88%
Meperidine	3.19%	1.73%	1.31%
Methadone	5.76%	3.29% (n = 14)	3.08%
Mirtazapine	19.81%	20.29%	9.62%
Nordoxepin	13.70%	7.39%	8.92%
Norfentanyl	3.15% (n =12)	3.27% (n =12)	3.04% (n =11)
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% (n =12)	4.20% (n =12)	5.93% (n =11)
PCP	5.72% (n = 11)	3.78% (n = 12)	2.42% (n = 12)
Pheniramine	8.02%	5.21% (n = 14)	4.52%
Propoxyphene	5.11%	2.87%	2.25%
Propranolol	16.84% (n =12)	9.52% (n =11)	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% (n = 14)	2.64%
Venlafaxine	2.30%	1.35%	2.03%
Verapamil	18.51%	17.36%	32.96%
Zolpidem	5.75%	2.98% (n = 12)	2.44% (n = 14)

f. 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 amu: amitriptyline and EDDP, methadone and propoxyphene, imipramine and EDDP, imipramine and amitriptyline, desipramine and nortriptyline, and nortriptyline and propoxyphene. Grossly decomposed or putrefied samples may affect both detection and quantitation limits.

g. Carryover: 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.

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

i. 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.

14 Safety

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

15 References

Guidelines for Toxicological Quantitations (Tox 101); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

FBI Laboratory Practices for Validating Chemical Procedures; FBI Laboratory Operations Manual.

Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements (CUQA 13); FBI Laboratory Chemistry Unit Quality Assurance and Operations Manual.

Guidelines for Comparison of Mass Spectra (Tox 104); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
3	04/11/13	Added 8 new internal standards, adding doxylamine and trazodone to the list of quantitative analytes, and updating quantitations for dextromethorphan, duloxetine, citalopram, sertraline, venlafaxine and cyclobenzaprine. Subsequently, the following sections were updated: 2, 6c, 6q2, 6q3, 7.1, Table 4, Table 5, 14a, 14c, 14d, and 14e. A limitation for doxylamine was added in Section 14h. Added line for pipette documentation on bench sheet (Appendix 1).
4	10/01/14	In Sections 2 and 15, updated reference to Division validation procedure. In Sections 5 and 15, removed reference to Tox 103 and added preparation instructuons that were not included to Section 5. In Sections 6.a-b., specified Lipomed as a second source for standard material. Removed Section 7 (Calibration), moved calibrator preparation information to Section 6, and renumbered subsequent sections. Added batch acceptance criteria in Section 10.1 and renumbered subsequent sections. Corrected a typo in Table 3. Deleted Section 10.3 since the information is covered elsewhere. Corrected a typo in Table 5. Added processed sample stability in Section 13.i. Reformatted Appendix 2 to include all pertinent instrumental parameters.

Approval

Redacted - Signatures on File

Appendix 1: Abbreviated version of the Quantitation and Confirmation of Alkaline Drugs Procedure for bench use.

Redacted - Form on File

Appendix 2: Abbreviated version of the Instrumental Parameters for bench use.

Redacted - Form on File