Solid Phase Extraction of Alkaline Drugs from Biological Fluids

Table of Contents

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1 INTRODUCTION

This procedure detects common alkaline drugs.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☐ Quantitation
Matrices	Blood, serum, plasma, urine, vitreous humor, or a prepared tissue homogenate
Analytes	Variety of alkaline drugs
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

Biological specimens are qualitatively assayed for basic drugs. Specimens are mixed with internal standards, adjusted to a slightly acidic pH, and extracted using solid phase extraction cartridges. Basic drugs are eluted using a mixed solvent system of methylene chloride, isopropanol, and ammonium hydroxide. The eluent is taken to dryness and reconstituted prior to analysis by LC/MS(ESI) (liquid chromatography/mass spectrometry)(electrospray ionization).

4 SPECIMEN CRITERIA

This procedure uses a biological fluid such as: blood, serum, plasma, urine, vitreous humor, or a prepared tissue homogenate (1:1). One mL of blood, other fluid, or tissue homogenate is used for screening; reduced aliquots may be appropriate for confirmatory exams. This procedure may also be used to screen food samples for alkaline drugs, providing that appropriate controls are simultaneously analyzed. A 1 g sample of a food homogenate (1:1) is suggested for analysis.

5 EQUIPMENT

5.1 Equipment

A. Centrifuge
B. Evaporator w/ Nitrogen
C. Vortex mixer
D. SPE Vacuum or Positive Pressure Manifold
E. Desiccator
F. Pipettors (various ranges)
G. Volumetric glassware

5.1.1 Columns

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

5.2 Consumables

A. 16 x 100 mm screw-top tubes with Teflon insert caps
B. 13 x 100 mm culture tubes with polypropylene snap-tops
C. 12 x 75 mm culture tubes with polypropylene snap-tops
D. Clean Screen DAU SPE cartridges (regular flow) - 200 milligrams
E. Mid-range pH paper
F. Automatic Liquid Sampling (ALS) vials, 12x32mm

5.3 Instruments

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

5.4 Software

5.4.1 Thermo LTQ Orbitrap XL

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

5.5.1 Purchased

A. Acetic acid, glacial	
B. Acetonitrile	≥ LC/MS grade, Optima grade
C. Ammonium hydroxide, concentrated	ACS grade
D. Formic Acid	≥ LC/MS grade
E. Isopropanol	≥ HPLC grade
F. Methanol	≥ GC^2 grade, HPLC, Optima grade
G. Methylene chloride	≥ HPLC grade
H. Sodium phosphate dibasic heptahydrate	
I. Sodium phosphate monobasic monohydrate	
J. Water	≥ 18mΩ, HPLC, Optima, or UPLC grade

5.5.2 Prepared

A. Mobile Phase 1 (Water with 0.1% Formic Acid)(Aqueous)	
	Combine 500 mL water (Optima) and 0.5 mL formic acid and mix well. Store in glass at room temperature. Stable 2 weeks.
B. Mobile Phase 2 (Acetonitrile with 0.1% Formic Acid)(Organic)	
	Combine 500 mL acetonitrile (Optima) and 0.5 mL formic acid and mix well. Store in glass at room temperature. Stable for 1 month.
C. 100 mM Phosphate Buffer (pH 6.0)	
	To a 500 mL volumetric flask, add 400 mL deionized water, 6.1 g sodium phosphate monobasic monohydrate, and 1.6 g sodium phosphate dibasic heptahydrate. Mix well to dissolve. Verify 5.8<pH<6.1. Bring to volume with deionized water. Store refrigerated in glass. Stable 2 months.
D. Elution Solvent (Methylene Chloride/Isopropanol/Ammonium Hydroxide (78/20/2))	
	Combine 20 mL isopropanol with 2 mL concentrated ammonium hydroxide and mix well. Add 78 mL methylene chloride and mix well. Store in glass at room temperature. Prepare fresh daily.
E. 100 mM Acetic Acid	
	To a 100-mL graduated cylinder, add 80 mL deionized water and 0.5 mL glacial acetic acid. Mix well and bring to 85 mL with deionized water. Store in glass at room temperature. Stable 6 months.

5.6 Standards/Controls

5.6.1 Purchased

Storage/stability determined by manufacturer unless otherwise noted.

A. Negative Control:	
	Purchased from Cliniqa, Dynatek or an equivalent approved supplier, or prepared in-house from an appropriate blank specimen. Store refrigerated or obtain fresh. Stability determined by manufacturer.

B. Benzodiazepine Mix-8 Stock Standard (250 µg/mL)

1. Alprazolam
2. Clonazepam
3. Diazepam
4. Flunitrazepam
5. Lorazepam
6. Nitrazepam
7. Oxazepam
8. Temazepam

Purchased from Cerilliant Corporation or equivalent, typically Product # B-033-1ML

C. Amine Mix-6 Stock Standard (250 µg/mL)

1. Amphetamine
2. Methamphetamine
3. Phentermine
4. Methylenedioxyamphetamine (MDA)
5. Methylenedioxymethamphetamine (MDMA)
6. Methylenedioxyethylamphetamine (MDEA)

Purchased from Cerilliant Corporation or equivalent, typically Product # A-050-1ML

D. Internal Standard Components (100 µg/mL)

1. d3-methadone
2. d5-fentanyl
3. d3-codeine
4. d3-benzoylecgonine
5. d5-amphetamine
6. d5-methamphetamine
7. d3-morphine
8. d3-hydrocodone
9. d6-chlorpheniramine
10. d4-7-aminoclonazepam
11. d5-diazepam
12. d5-alprazolam

Purchased from Cerilliant Corporation or equivalent.

E. Positive Control Components (1.0 mg/mL)

1. Amitriptyline
2. Amphetamine
3. Cocaine
4. Diphenhydramine
5. Hydrocodone
6. Methamphetamine
7. Nordiazepam
8. Tramadol
9. Venlafaxine

Purchased from Cerilliant Corporation or equivalent.

5.6.2 Prepared

5.6.2.1 TOX203 Internal Standard Working Solution:

Analytes (100 µg/mL)	Volume added (mL)	25 mL volumetric flask	Resulting concentration when 50 µL is added to 1.0 mL matrix (ng/mL)
d_5-fentanyl	0.125	QS to 25 mL with methanol	25
d_3-benzoylecgonine	0.125		25
d_5-methamphetamine	0.125		25
d_3-morphine	0.125		25
d_5-alprazolam	0.125		25
d_3-methadone	1.000		200
d_3-codeine	0.125		25
d_5-amphetamine	0.125		25
d_3-hydrocodone	0.125		25
d_6-chlorpheniramine	0.125		25
d_4-7-aminoclonazepam	0.125		25
d_5-diazepam	0.125		25

This mixture is stored refrigerated in glass. Stable for at least one year.

5.6.2.2 TOX203 Control Working Solution (5 µg/mL):

Analytes (1 mg/mL)	Volume added (mL)	50 mL volumetric flask
Amitriptyline	0.25	QS to 50 mL with methanol
Amphetamine	0.25	
Cocaine	0.25	
Diphenhydramine	0.25	
Hydrocodone	0.25	
Methamphetamine	0.25	
Nordiazepam	0.25	
Tramadol	0.25	
Venlafaxine	0.25	

This mixture is stored refrigerated in glass. Stable for at least one year.

5.6.2.3 Positive Control (200 ng/mL each analyte)

Solution	Added Volume (mL)	Diluted to (mL) Negative Control
TOX203 Control Working Solution	0.040	1

Prepared day of use. Other alkaline drugs and/or metabolites may be added to an aliquot of Negative Control to prepare controls for specific needs.

Note: When drugs are indicated in an alkaline drug screen that are not present in the Positive Control, a sample of the reference material (typically ~100 µg/mL for GC/MS or ~1-5 µg/mL for LC/MS) may be analyzed instrumentally without extraction to verify the retention time and spectra of the analyte in the unknown sample. For confirmatory analysis, matrix matched positive controls will be extracted alongside the unknown samples when available.

5.6.2.4 Benzodiazepine Mix-8 Stock Standard Working Solution (5 ug/mL)

Solution	Added Volume (mL)	Diluted to (mL) Acetonitrile
Benzodiazepine Mix-8 Stock Standard	1	50

Store <0°C in glass or plastic. Stable for at least 2 years.

5.6.2.5 Amine Mixture-6 Stock Standard Working Solution (5 µg/mL)

Solution	Added Volume (mL)	Diluted to (mL) Methanol
AmineMix-6 Stock Standard	1	50

Store <0°C in glass or plastic. Stable for at least 2 years.

5.6.2.6 LC/MS Performance Standard (0.83µg/mL)

Solution	Added Volume (mL)
Benzodiazepine Mix-8 Stock Standard Working Solution	0.025
Amine Mixture-6 Stock Standard Working Solution	0.025
Water	0.100

Prepare fresh or store mixture under refrigerated conditions. Stable for at least 2 weeks.

6 PROCEDURE

	Step	Note	Reference/Lot
	A. Samples		
	1. To labeled 16 x 100 mm tubes add:		
☐	i. 1 mL of biological fluid		
☐	ii. 1 g of a prepared tissue homogenate		
	B. Controls		
☐	1. Prepare Negative Control(s)		
☐	2. Prepare Positive Control(s) i. Add 40 µL Positive Control Working Solution to Negative Control(s)		
	C. Internal Standard(s)		
☐	1. Add 50 µL of TOX203 Internal Standard Working Solution		
☐	i. Tissue specimens: add 100 µL		
	D. Buffer		
☐	1. Add 4 mL of 100mM phosphate buffer		
☐	2. Vortex		
☐	3. Check pH: 6 ± 0.5		
☐	**E. Centrifuge at ~3000 rpm for 15 minutes**		
☐	**F. Transfer supernatant to 16 x 100 mm tube**		
☐	**G. QS to 5 mL with deionized water**		
	H. Extract (SPE, sorbent should not be dried until step 4)		
	1. Condition cartridges (1 mL/min)		
☐	i. Add 3 mL methanol		
☐	ii. Add 3 mL deionized water		
☐	iii. Add 1 mL 100mM phosphate buffer		
☐	2. Load samples (1 mL/min)		
	3. Wash cartridges (1 mL/min)		
☐	i. Add 3 mL of deionized water		
☐	ii. Add 1 mL 100mM acetic acid		
☐	iii. Add 3 mL of methanol		
☐	4. Dry cartridge under full vacuum for 3 minutes		

	5. Elute (1 mL/min)		
☐	i. Add 3 mL Elution Solvent		
☐	ii. Collect eluent in 12 x 75 mm tubes		
☐	6. Evaporate to dryness under nitrogen at 40°C.		
	I. Reconstitute		
☐	1. Add 50 µL of methanol to 12 x 75 mm tubes		
☐	2. Vortex and transfer to ALS vial.		
☐	3. Add 100 µL of water to ALS vial.		
☐	**J. 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

Instrumental conditions may be modified to account for particular target analytes. Any modifications will be recorded in case notes.

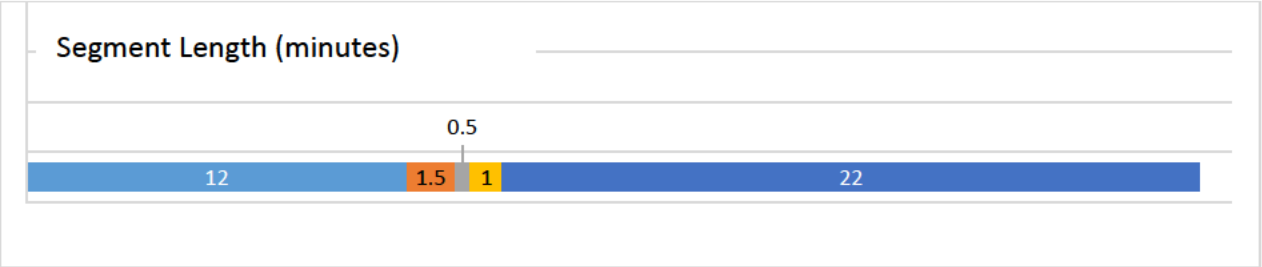
7.1 Shimadzu HPLC

7.1.1 Gradient/Conditions

Time (min)	Mobile Phase %		Flow Rate		
	1-Aqueous	2-Organic	(mL/min)	Column Heater (^{O}C)	30
0	90	10	0.3	Autosampler (^{O}C)	15
5	90	10	0.3	Run Time (min)	37
20	10	90	0.3		
30	10	90	0.3		
31	90	10	0.3		
37	90	10	0.3		

7.2 Thermo LTQ-XL Orbitrap

A. Source Mode: ESI (+)
B. Different MS/MS parameters (i.e., higher collision energy or MS^3) may be used to target specific drugs and metabolites as long as the same parameters are used for all controls and case samples and the method is recorded in the case notes.



Event	Mode	Range (m/z)	Analyzer	Resolution
1	Full Scan	100-650	FTMS	15000
2-4	Full Scan MS/MS	Data Dependent Product Ion (Segment Specific Precursor/Exclusion List from event #1; tables 11.1.2/7.2.1)	ITMS	unit
5	Full Scan	100-650	FTMS	15000
6-7	Full Scan MS/MS	Data Dependent Product Ion (Segment Specific Precursor/Exclusion List from event #5; tables 11.1.2/7.2.1)	ITMS	unit

Events may be modified for targeted analysis as required.

7.2.1 Data Dependent Settings

7.2.1.1 Reject Mass List Event #1

m/z	Analyte
141.14	D5-amphetamine
142.15	D5-13C-amphetamine
155.16	D5-Methamphetamine
156.16	D5-13C-Methamphetamine
281.17	D6-chlorpheniramine
282.17	D6-13C-chlorpheniramine
283.17	D6-37Cl-chlorpheniramine
289.16	D3-Morphine
290.10	D4-7-aminoclonazepam
290.17	D3-13C-Morphine
291.10	D4-13C-7-aminoclonazepam
293.16	D3-BZE
294.16	D3-13C-BZE
303.18	D3-codeine/hydrocodone
304.18	D3-13C-codeine/hydrocodone
312.23	D2-methadone
313.24	D3-methadone
314.12	D5-Alprazolam

314.24	D3-13C-methadone
315.12	D5-13C-Alprazolam
315.24	D3-13C2-methadone
316.12	D5-37Cl-Alprazolam
341.25	D4-Fentanyl
342.26	D5-Fentanyl
343.26	D5-13C-Fentanyl
344.26	D5-13C2-Fentanyl
391.28	Dioctyl-pthalate

7.2.1.2 Reject Mass List Event #5

m/z	Analyte
290.11	D5-diazepam
291.11	D5-13C-diazepam
292.11	D5-37Cl-diazepam
312.23	D2-methadone
313.24	D3-methadone
314.12	D5-Alprazolam
314.24	D3-13C-methadone
315.12	D5-13C-Alprazolam
315.24	D3-13C2-methadone
316.12	D5-37Cl-Alprazolam
342.26	D5-Fentanyl
343.26	D5-13C-Fentanyl
391.28	Dioctyl-pthalate

8 DATA ANALYSIS

8.1 Decision Criteria

8.1.1 LC/MS Performance Standard Decision Criteria

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 ±0.6 min
    2. Responses 50-200%

8.1.1.2 Mass Spectrometry

The analyte mass assignments shall be present:

Analyte	Unit.Exact Mass (±0.005 m/z)
Clonazepam	316.048
Nitrazepam	282.087
Flunitrazepam	314.094
Alprazolam	309.090
Diazepam	285.079
Amphetamine	136.112
Methamphetamine	150.128
Phentermine	150.128
MDA	180.102
MDMA	194.118
MDEA	208.133

8.1.2 Batch Acceptance

A. Negative Control
   No target analytes are detected (target analytes may vary by batch)

B. Positive Control
   The target analytes are detected.

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

Internal Standard
d3-methadone
d5-fentanyl
d3-codeine
d3-benzoylecgonine
d5-amphetamine
d5-methamphetamine
d3-morphine
d3-hydrocodone
d6-chlorpheniramine
d4-7-aminoclonazepam
d5-diazepam
d5-alprazolam

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% or ±0.6 min (whichever is greater) of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, an extracted Positive Control, or an appropriate deuterated analog.

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 standard operating procedure (TOX-104) for further guidance.

8.1.4.2.1 Screening

To screen high resolution LC/MS data for a wide number of analytes, M+1 ions for dozens of analytes may be traced at a mass tolerance of ±5 mmu.

9 REPORTING

9.1 Cocaine (LC/MS)

To report cocaine qualitatively based upon this method, the area of the M+H peak for cocaine must be greater than or equal to 5% of the M+H peak for benzoylecgonine (from the same sample).

9.2 Reporting Cut-offs

A. College of American Pathologists (CAP) T Series and FTC Series

When analyzing CAP T-Series or FTC specimens, if all decision criteria for an analyte of interest are met, but the concentration of 6-acetylmorphine, buprenorphine, fentanyl, norfentanyl, and/or norbuprenorphine is estimated to be below 5 ng/mL (or 15 ng/mL for all other alkaline drugs) in two independent analyses, the analyte will not be reported. Note: the second analysis may be a repeat of this procedure or via another validated procedure. A Positive Control at the Cut-off Level is recommended for the second analysis.

10 CORRECTIVE MEASURES

Refer to Quality Control for Toxicology Examinations (TOX-101) for guidance on action steps in the event of a quality control failure.

10.1 LC/MS Performance Standard

A retention time shift of >0.6 minutes or poor peak shape may be an indicator for a frit change.

11 PERFORMANCE CHARACTERISTICS

11.1 LOD

While the limit of detection varies depending upon analyte and matrix, this assay readily detects a wide variety of alkaline drugs and metabolites in blood and urine specimens at concentrations of approximately 5 ng/mL.

11.1.1 Alphabetical Sort

Analytes Sorted Alphabetically			Blood	Urine	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
25B-NBOME	380.086	14.95	5	5	OK
25C-NBOME	336.136	14.78	5	5	OK
25H-NBOME	302.175	14.10	5	5	OK
25I-NBF	416.052	14.92	5	5	OK
25I-NBMD	442.051	15.00	5	5	OK
25I-NBOH	414.056	14.61	5	5	OK
25I-NBOME	428.072	15.15	5	5	OK
3-methoxyPCP	274.217	13.80	5	5	OK
6-AM	328.154	7.66	5	5	BP
7-aminoclonazepam	286.074	11.19	5	5	OK
7-aminoflunitrazepam	284.119	12.51	5	5	OK
acetylfentanyl	323.212	13.14	5	5	OK
alfentanyl	417.261	13.60	5	5	OK
alpha-PVP	232.170	12.29	5	5	OK
alprazolam	309.090	16.55	5	5	OK
amitriptyline	278.190	14.95	5	5	OK
amlodipine	409.152	14.72	25	25	OK
amoxapine	314.105	14.08	5	5	OK
amphetamine	136.112	6.05	25	25	BP
aripiprazole	448.155	14.84	25	25	OK
asenapine	286.099	14.34	5	10	I, MI
atenolol	267.170	3.44	5	10	SP
atomoxetine	256.170	14.50	5	5	OK

atropine	290.175	11.16	5	5	OK
baclofen	214.063	8.04	NS	NS	I
benzocaine	166.086	15.59	5	I	I
benzoylecgonine	290.139	11.70	5	5	OK
benztropine	308.201	15.04	5	5	OK
brexpiprazole	434.190	14.32	5	10	OK
bromazepam	316.008	14.70	5	10	OK
brompheniramine	319.080	12.83	5	5	OK
bupivacaine	289.227	13.28	5	5	I
buprenorphine	468.311	14.14	5	5	OK
bupropion	240.115	12.98	5	5	OK
buspirone	386.255	13.23	5	5	OK
butylone	222.112	9.85	5	5	BP
butyrylfentanyl	351.243	14.27	5	5	OK
BZP (benzylpiperazine)	177.139	2.25	5	10	SP
carbinoxamine	291.126	12.62	5	5	OK
carfentanil	395.233	14.19	5	5	OK
cetirizine	389.163	15.26	10	5	I
chlordiazepoxide	300.090	13.02	5	5	OK
chlorpheniramine	275.131	12.52	5	5	OK
chlorpromazine	319.103	15.26	5	5	OK
citalopram	325.171	14.15	5	5	OK
clobazam	301.075	18.36	NS	NS	NS
clomipramine	315.162	15.45	5	5	OK
clonazepam	316.048	16.77	NS	NS	NS
clonidine	230.025	5.20	5	5	BP
clozapine	327.137	12.94	5	5	OK
cocaethylene	318.170	13.34	5	5	OK
cocaine	304.154	12.62	5	5	OK
codeine	300.159	5.10	5	5	BP
cotinine	177.102	2.28	5	I	I

cyclobenzaprine	276.175	14.77	5	5	OK
cyproheptadine	288.175	14.74	5	5	OK
desalkylflurazepam	289.054	17.10	5	5	OK
desipramine	267.186	14.64	5	5	OK
desloratadine	311.131	12.11	25	25	I
desmethylcitalopram	311.155	14.04	5	5	OK
desmethylclomipramine	301.147	15.32	5	5	OK
desmethylclozapine	313.121	12.44	10	10	OK
desmethylcyclobenzaprine	262.159	14.65	5	5	OK
desmethyldoxepin	266.154	14.10	5	5	OK
desmethylflunitrazepam	300.078	16.34	NS	NS	NS
desmethyltapentadol	208.170	12.20	5	5	OK
desmethyltrimipramine	281.201	14.97	5	5	OK
desmethylvenlafaxine	264.196	11.38	5	5	OK
dextromethorphan	272.201	13.70	5	5	OK
dextrorphan	258.185	11.84	5	5	OK
diazepam	285.079	18.14	5	5	OK
dihydrocodeine	302.175	4.67	5	5	OK
diltiazem	415.169	14.22	5	5	OK
diphenhydramine	256.170	14.00	5	5	OK
donepezil	380.222	13.61	5	5	OK
doxepin	280.170	14.21	5	5	OK
doxylamine	271.180	6.00	5	5	BP
duloxetine	298.126	14.84	5	10	OK
EDDP	278.190	14.46	5	5	OK
EEE	214.144	2.26	25	25	BP
EME	200.128	2.10	5	5	BP
estazolam	295.075	16.30	5	5	OK
etizolam	343.077	17.11	5	5	OK
fentanyl	337.227	13.80	5	5	OK
fexofenadine	502.295	14.87	5	5	OK

flubromazolam	371.030	16.58	5	5	OK
flunitrazepam	314.094	17.35	NS	NS	NS
fluoxetine	310.141	15.18	5	5	OK
fluphenazine	438.182	15.15	5	10	OK
flurazepam	388.159	13.94	5	5	OK
fluvoxamine	319.163	14.77	5	5	OK
furanylfentanyl	375.207	14.00	5	5	OK
gabapentin	172.133	5.29	Unk*	Unk*	*Unk
guaifenesin	199.096	12.75	NS	NS	NS
haloperidol	376.149	14.33	5	5	OK
hydrocodone	300.159	8.17	5	5	BP
hydromorphone	286.144	3.55	5	5	SP
hydroxyzine	375.183	14.77	5	5	OK
iloperidone	427.203	14.36	5	5	OK
imipramine	281.201	14.76	5	5	OK
ketamine	238.099	11.16	5	5	OK
lacosamide	251.139	12.79	NS	NS	NS
lamotrigine	256.015	11.89	5	5	OK
lidocaine	235.180	10.37	5	5	OK
loperamide	477.230	15.77	5	5	OK
loratadine	383.152	15.93	5	5	I
lorazepam	321.019	16.58	NS	NS	NS
lormetazepam	335.035	17.75	NS	NS	NS
loxapine	328.121	14.31	5	10	I
LSD	324.207	12.97	5	5	SP
lurasidone	493.263	15.62	5	5	OK
maprotyline	278.190	14.86	5	5	OK
MBDB	208.133	11.50	5	5	OK
MDA	180.102	7.13	5	5	BP
MDEA	208.133	10.72	5	5	OK
MDMA	194.118	8.44	5	5	BP

MDPV	276.159	12.57	5	5	OK
meclizine	391.194	16.63	5	5	OK
medazepam	271.100	13.88	5	5	OK
meperidine	248.165	12.69	5	5	OK
mephedrone	178.123	10.30	5	5	OK
mescaline	212.128	6.12	5	5	BP
mesoridazine	387.156	13.79	5	5	OK
metaxolone	222.112	16.81	NS	NS	NS
methadone	310.217	15.02	5	5	OK
methamphetamine	150.128	7.35	5	5	BP
methocarbamol	242.102	13.37	NS	NS	NS
methoxetamine	248.165	11.92	5	5	OK
methylone	208.099	5.63	5	5	BP
methylphenidate	234.149	12.10	5	5	OK
metoclopramide	300.147	11.60	5	5	OK
metoprolol	268.194	12.00	5	5	OK
midazolam	326.085	13.81	5	5	OK
mirtazapine	266.165	11.34	5	5	OK
molindone	277.191	11.95	NS	NS	NS
morphine	286.144	2.48	5	5	SP
MT45	349.264	14.90	5	5	BP
naloxone	328.154	4.89	5	5	BP
N-desmethyltramadol	250.180	12.08	5	5	OK
nicotine	163.123	2.06	5	I	I
nifedipine	347.124	18.13	NS	NS	NS
nitrazepam	282.087	16.26	5	5	OK
norbuprenorphine	414.264	12.99	5	5	OK
norchlorcyclizine	287.131	14.76	25	25	I
norchlordiazepoxide	286.074	12.88	5	5	OK
norcodeine	286.144	4.69	5	5	BP
nordiazepam	271.063	16.60	5	5	OK

norfentanyl	233.165	11.37	5	5	OK
norfluoxetine	296.126	15.02	5	5	OK
norhydrocodone	286.144	7.62	5	10	BP
norketamine	224.084	10.83	5	5	OK
normeperidine	234.149	12.64	5	5	OK
normorphine	272.128	2.46	25	25	I
noroxycodone	302.139	6.43	5	5	BP
norpheniramine	227.154	5.63	5	5	BP
norpropoxyphene	326.211	14.79	5	5	OK
norquetiapine	296.122	13.12	5	5	OK
norsertraline	292.065	15.10	25	25	OK
nortriptyline	264.175	14.83	5	5	OK
norverapamil	441.275	14.75	5	5	OK
O-desmethyltramadol	250.180	7.65	5	5	BP
OH-alprazolam	325.085	15.86	5	5	OK
OH-bupropion	256.110	12.00	5	5	OK
OH-midazolam	342.080	13.76	5	5	I
OH-quetiapine	400.169	8.62	10	10	BP
OH-risperidone	427.214	12.71	5	5	OK
OH-triazolam	359.046	15.90	NS	NS	NS
olanzapine	313.148	5.13	100	NS	BP
orphenadrine	270.185	14.50	5	5	OK
oxycodone	316.154	6.79	5	5	BP
oxymorphone	302.139	2.48	5	5	SP
paroxetine	330.150	14.58	5	5	OK
pentylone	236.128	12.00	5	5	OK
perphenazine	404.156	14.66	NS	NS	NS
phenazepam	348.972	17.81	100	NS	NS
phenethylamine	122.096	4.23	5	5	BP
pheniramine	241.170	6.52	5	5	BP
phentermine	150.128	9.10	5	5	BP

phenycyclidine	244.206	13.48	5	5	OK
phenylephrine	168.102	2.41	5	5	SP
phenylpropanolamine	152.107	3.83	25	25	OK
PMA	166.123	7.94	5	5	BP
PMMA	180.138	9.54	5	5	BP
prazepam	325.111	20.23	5	5	OK
pregabalin	160.133	5.25	NS	NS	NS
procainamide	236.176	2.48	5	5	SP
prochlorperazine	374.145	14.90	25	25	BP
promethazine	285.142	14.40	5	5	OK
propoxyphene	340.227	14.94	5	5	OK
propranolol	260.165	13.60	5	10	OK
protriptyline	264.175	14.67	5	5	OK
pseudo/ephedrine	166.123	4.70	5	5	BP
psilocin	205.134	4.45	NS	NS	NS
psilocybin	285.100	3.24	NS	NS	NS
quetiapine	384.174	13.48	5	5	OK
quinine/quinidine	325.191	9.46	I	I	I
ranitidine	315.149	3.66	10	10	SP
risperidone	411.219	12.68	5	5	OK
ropinirole	261.196	11.08	5	5	OK
scopolamine	304.154	6.88	5	5	BP
sertraline	306.081	15.24	5	5	OK
strychnine	335.175	10.84	5	5	OK
suvorexant	451.164	20.74	5	5	OK
tapentadol	222.185	12.31	5	5	OK
temazepam	301.074	17.40	NS	NS	NS
tetrahydrozoline	201.139	9.87	5	5	BP
tetrazepam	289.111	16.37	5	5	OK
TFMPP	231.110	13.15	5	5	OK
thioridazine	371.161	15.80	100	100	OK

tizanidine	254.026	4.51	5	5	OK
tramadol	264.196	11.99	5	5	OK
trazodone	372.159	13.30	5	5	OK
triazolam	343.051	16.79	5	5	OK
triflupromazine	408.172	15.44	5	10	OK
trimipramine	295.217	15.08	5	5	OK
triprolidine	279.186	12.91	5	5	BP
U47700	329.118	13.72	5	5	SP
venlafaxine	278.211	13.04	5	5	OK
verapamil	455.290	14.86	5	5	OK
W18	422.094	20.04	NS	NS	NS
zaleplon	306.135	16.08	NS	NS	NS
ziprasidone	413.120	13.82	25	100	OK
zolpidem	308.176	12.79	5	5	OK
zolpidem metabolite	338.150	11.19	5	5	OK
zopiclone	389.112	12.00	10	5	OK
Not suitable for analysis by this method		NS			
LOD above tested values; PC at 5 µg/mL will work		Unk*			
Peak shape is broad; less than ideal		BP			
Compound known to give split peaks		SP			
Compound validated with no issues		OK			
Matrix Interference		I			

11.1.2 Retention Time Sort

Analytes Sorted by Retention Time			**Blood**	**Urine**	
Analyte	**M+1**	**RT (min)**	**LOD**	**LOD**	**Notes**
nicotine	163.123	2.06	5	I	I

EME	200.128	2.10	5	5	BP
BZP (benzylpiperazine)	177.139	2.25	5	10	SP
EEE	214.144	2.26	25	25	BP
cotinine	177.102	2.28	5	I	I
phenylephrine	168.102	2.41	5	5	SP
normorphine	272.128	2.46	25	25	I
morphine	286.144	2.48	5	5	SP
oxymorphone	302.139	2.48	5	5	SP
procainamide	236.176	2.48	5	5	SP
psilocybin	285.100	3.24	NS	NS	NS
atenolol	267.170	3.44	5	10	SP
hydromorphone	286.144	3.55	5	5	SP
ranitidine	315.149	3.66	10	10	SP
phenylpropanolamine	152.107	3.83	25	25	OK
phenethylamine	122.096	4.23	5	5	BP
psilocin	205.134	4.45	NS	NS	NS
tizanidine	254.026	4.51	5	5	OK
dihydrocodeine	302.175	4.67	5	5	OK
norcodeine	286.144	4.69	5	5	BP
pseudo/ephedrine	166.123	4.70	5	5	BP
naloxone	328.154	4.89	5	5	BP
codeine	300.159	5.10	5	5	BP
olanzapine	313.148	5.13	100	NS	BP
clonidine	230.025	5.20	5	5	BP
pregabalin	160.133	5.25	NS	NS	NS
gabapentin	172.133	5.29	*Unk	*Unk	*Unk
methylone	208.099	5.63	5	5	BP
norpheniramine	227.154	5.63	5	5	BP
doxylamine	271.180	6.00	5	5	BP
amphetamine	136.112	6.05	25	25	BP
mescaline	212.128	6.12	5	5	BP

noroxycodone	302.139	6.43	5	5	BP
pheniramine	241.170	6.52	5	5	BP
oxycodone	316.154	6.79	5	5	BP
scopolamine	304.154	6.88	5	5	BP
MDA	180.102	7.13	5	5	BP
methamphetamine	150.128	7.35	5	5	BP
norhydrocodone	286.144	7.62	5	10	BP
O-desmethyltramadol	250.180	7.65	5	5	BP
6-AM	328.154	7.66	5	5	BP
PMA	166.123	7.94	5	5	BP
baclofen	214.063	8.04	NS	NS	I
hydrocodone	300.159	8.17	5	5	BP
MDMA	194.118	8.44	5	5	BP
OH-quetiapine	400.169	8.62	10	10	BP
phentermine	150.128	9.10	5	5	BP
quinine/quinidine	325.191	9.46	I	I	I
PMMA	180.138	9.54	5	5	BP
butylone	222.112	9.85	5	5	BP
tetrahydrozoline	201.139	9.87	5	5	BP
mephedrone	178.123	10.30	5	5	OK
lidocaine	235.180	10.37	5	5	OK
MDEA	208.133	10.72	5	5	OK
norketamine	224.084	10.83	5	5	OK
strychnine	335.175	10.84	5	5	OK
ropinirole	261.196	11.08	5	5	OK
atropine	290.175	11.16	5	5	OK
ketamine	238.099	11.16	5	5	OK
7-aminoclonazepam	286.074	11.19	5	5	OK
zolpidem metabolite	338.150	11.19	5	5	OK
mirtazapine	266.165	11.34	5	5	OK
norfentanyl	233.165	11.37	5	5	OK

desmethylvenlafaxine	264.196	11.38	5	5	OK
MBDB	208.133	11.50	5	5	OK
metoclopramide	300.147	11.60	5	5	OK
benzoylecgonine	290.139	11.70	5	5	OK
dextrorphan	258.185	11.84	5	5	OK
lamotrigine	256.015	11.89	5	5	OK
methoxetamine	248.165	11.92	5	5	OK
molindone	277.191	11.95	NS	NS	NS
tramadol	264.196	11.99	5	5	OK
metoprolol	268.194	12.00	5	5	OK
OH-bupropion	256.110	12.00	5	5	OK
pentylone	236.128	12.00	5	5	OK
zopiclone	389.112	12.00	10	5	OK
N-desmethyltramadol	250.180	12.08	5	5	OK
methylphenidate	234.149	12.10	5	5	OK
desloratadine	311.131	12.11	25	25	I
desmethyltapentadol	208.170	12.20	5	5	OK
alpha-PVP	232.170	12.29	5	5	OK
tapentadol	222.185	12.31	5	5	OK
desmethylclozapine	313.121	12.44	10	10	OK
7-aminoflunitrazepam	284.119	12.51	5	5	OK
chlorpheniramine	275.131	12.52	5	5	OK
MDPV	276.159	12.57	5	5	OK
carbinoxamine	291.126	12.62	5	5	OK
cocaine	304.154	12.62	5	5	OK
normeperidine	234.149	12.64	5	5	OK
risperidone	411.219	12.68	5	5	OK
meperidine	248.165	12.69	5	5	OK
OH-risperidone	427.214	12.71	5	5	OK
guaifenesin	199.096	12.75	NS	NS	NS
lacosamide	251.139	12.79	NS	NS	NS

zolpidem	308.176	12.79	5	5	OK
brompheniramine	319.080	12.83	5	5	OK
norchlordiazepoxide	286.074	12.88	5	5	OK
triprolidine	279.186	12.91	5	5	BP
clozapine	327.137	12.94	5	5	OK
LSD	324.207	12.97	5	5	SP
bupropion	240.115	12.98	5	5	OK
norbuprenorphine	414.264	12.99	5	5	OK
chlordiazepoxide	300.090	13.02	5	5	OK
venlafaxine	278.211	13.04	5	5	OK
norquetiapine	296.122	13.12	5	5	OK
acetylfentanyl	323.212	13.14	5	5	OK
TFMPP	231.110	13.15	5	5	OK
buspirone	386.255	13.23	5	5	OK
bupivacaine	289.227	13.28	5	5	I
trazodone	372.159	13.30	5	5	OK
cocaethylene	318.170	13.34	5	5	OK
methocarbamol	242.102	13.37	NS	NS	NS
phenycyclidine	244.206	13.48	5	5	OK
quetiapine	384.174	13.48	5	5	OK
alfentanyl	417.261	13.60	5	5	OK
propranolol	260.165	13.60	5	10	OK
donepezil	380.222	13.61	5	5	OK
dextromethorphan	272.200	13.70	5	5	OK
U47700	329.118	13.72	5	5	SP
OH-midazolam	342.080	13.76	5	5	I
mesoridazine	387.156	13.79	5	5	OK
3-methoxyPCP	274.217	13.80	5	5	OK
fentanyl	337.227	13.80	5	5	OK
midazolam	326.085	13.81	5	5	OK
ziprasidone	413.120	13.82	25	100	OK

medazepam	271.100	13.88	5	5	OK
flurazepam	388.159	13.94	5	5	OK
diphenhydramine	256.170	14.00	5	5	OK
furanylfentanyl	375.207	14.00	5	5	OK
desmethylcitalopram	311.155	14.04	5	5	OK
amoxapine	314.105	14.08	5	5	OK
25H-NBOME	302.175	14.10	5	5	OK
desmethyldoxepin	266.154	14.10	5	5	OK
buprenorphine	468.311	14.14	5	5	OK
citalopram	325.171	14.15	5	5	OK
carfentanil	395.233	14.19	5	5	OK
doxepin	280.170	14.21	5	5	OK
diltiazem	415.169	14.22	5	5	OK
butyrylfentanyl	351.243	14.27	5	5	OK
loxapine	328.121	14.31	5	10	I
brexpiprazole	434.190	14.32	5	10	OK
haloperidol	376.149	14.33	5	5	OK
asenapine	286.099	14.34	5	10	I, MI
iloperidone	427.203	14.36	5	5	OK
promethazine	285.142	14.40	5	5	OK
EDDP	278.190	14.46	5	5	OK
atomoxetine	256.170	14.50	5	5	OK
orphenadrine	270.185	14.50	5	5	OK
paroxetine	330.150	14.58	5	5	OK
25I-NBOH	414.056	14.61	5	5	OK
desipramine	267.186	14.64	5	5	OK
desmethylcyclobenzaprine	262.159	14.65	5	5	OK
perphenazine	404.156	14.66	NS	NS	NS
protriptyline	264.175	14.67	5	5	OK
bromazepam	316.008	14.70	5	10	OK
amlodipine	409.152	14.72	25	25	OK

cyproheptadine	288.175	14.74	5	5	OK
norverapamil	441.275	14.75	5	5	OK
imipramine	281.201	14.76	5	5	OK
norchlorcyclizine	287.131	14.76	25	25	I
cyclobenzaprine	276.175	14.77	5	5	OK
fluvoxamine	319.163	14.77	5	5	OK
hydroxyzine	375.183	14.77	5	5	OK
25C-NBOME	336.136	14.78	5	5	OK
norpropoxyphene	326.211	14.79	5	5	OK
nortriptyline	264.175	14.83	5	5	OK
aripiprazole	448.155	14.84	25	25	OK
duloxetine	298.126	14.84	5	10	OK
maprotyline	278.190	14.86	5	5	OK
verapamil	455.290	14.86	5	5	OK
fexofenadine	502.295	14.87	5	5	OK
MT45	349.264	14.90	5	5	BP
prochlorperazine	374.145	14.90	25	25	BP
25I-NBF	416.052	14.92	5	5	OK
propoxyphene	340.227	14.94	5	5	OK
25B-NBOME	380.086	14.95	5	5	OK
amitriptyline	278.190	14.95	5	5	OK
desmethyltrimipramine	281.201	14.97	5	5	OK
25I-NBMD	442.051	15.00	5	5	OK
methadone	310.217	15.02	5	5	OK
norfluoxetine	296.126	15.02	5	5	OK
benztropine	308.201	15.04	5	5	OK
trimipramine	295.217	15.08	5	5	OK
norsertraline	292.065	15.10	25	25	OK
25I-NBOME	428.072	15.15	5	5	OK
fluphenazine	438.182	15.15	5	10	OK
fluoxetine	310.141	15.18	5	5	OK

sertraline	306.081	15.24	5	5	OK
cetirizine	389.163	15.26	10	5	I
chlorpromazine	319.103	15.26	5	5	OK
desmethylclomipramine	301.147	15.32	5	5	OK
triflupromazine	408.172	15.44	5	10	OK
clomipramine	315.162	15.45	5	5	OK
benzocaine	166.086	15.59	5	I	I
lurasidone	493.263	15.62	5	5	OK
loperamide	477.230	15.77	5	5	OK
thioridazine	371.161	15.80	100	100	OK
OH-alprazolam	325.085	15.86	5	5	OK
OH-triazolam	359.046	15.90	NS	NS	NS
loratadine	383.152	15.93	5	5	I
zaleplon	306.135	16.08	NS	NS	NS
nitrazepam	282.087	16.26	5	5	OK
estazolam	295.075	16.30	5	5	OK
desmethylflunitrazepam	300.078	16.34	NS	NS	NS
tetrazepam	289.111	16.37	5	5	OK
alprazolam	309.090	16.55	5	5	OK
flubromazolam	371.030	16.58	5	5	OK
lorazepam	321.019	16.58	NS	NS	NS
nordiazepam	271.063	16.60	5	5	OK
meclizine	391.194	16.63	5	5	OK
clonazepam	316.048	16.77	NS	NS	NS
triazolam	343.051	16.79	5	5	OK
metaxolone	222.112	16.81	NS	NS	NS
desalkylflurazepam	289.054	17.10	5	5	OK
etizolam	343.077	17.11	5	5	OK
flunitrazepam	314.094	17.35	NS	NS	NS
temazepam	301.074	17.40	NS	NS	NS
lormetazepam	335.035	17.75	NS	NS	NS

phenazepam	348.972	17.81	100	NS	NS
nifedipine	347.124	18.13	NS	NS	NS
diazepam	285.079	18.14	5	5	OK
clobazam	301.075	18.36	NS	NS	NS
W18	422.094	20.04	NS	NS	NS
prazepam	325.111	20.23	5	5	OK
suvorexant	451.164	20.74	5	5	OK
Not suitable for analysis by this method		NS			
Peak shape is broad; less than ideal		BP			
Compound known to give split peaks		SP			
Compound validated with no issues		OK			
Matrix Interference		I			

11.2 Carry Over

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

12 LIMITATIONS

A. In-Source Fragmentation

Adjustment of the source parameters may be necessary to avoid excessive ESI source fragmentation for the following analytes:

1. Diphenhydramine
2. Doxylamine
3. Norsertraline

B. Interferences:

None known. Grossly decomposed or putrefied samples, as well as samples that have been embalmed, may affect detection limits.

C. Not Detected

Although present in the LC/MS check mix, oxazepam, lorazepam, and temazepam are not reliably detected by this procedure.

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
10	02/11/2022	Complete document reformat. Removed all GC/MS references. 1-Simplified introductory statement 2-Reformat of scope statement 3,4-Minor text changes 5-Complete reformat of Equipment 8-Simplified reformat of Decision Criteria 11-Moved LOD information to this section