Title: Exclusionary Drug Screening by Liquid Chromatography/Mass Spectrometry (LC/MS)

1.0 Introduction

A rapid high performance liquid chromatography/mass spectrometry (LC/MS) method using electrospray ionization (ESI) can be used to qualitatively screen biological specimens (e.g., blood, urine) for common drugs of abuse, as well as some other drugs. The amount of a detected analyte can be estimated by comparing the response (peak area) for an analyte to that for a corresponding internal standard. Positive findings can be confirmed by using either a second technique or a second aliquot of sample. Confirmation can also be achieved during quantitative analyses using appropriate procedures.

2.0 Scope

This procedure is limited to the qualitative screening of biological specimens for the presence of cocaine and metabolites, opioids, and benzodiazepines and other drugs (see list of target analytes and limits of detection).

3.0 Principle

Biological specimens are analyzed for the presence of drugs of abuse (DoA) and/or their metabolites by extraction using solid phase extraction (SPE) columns. Final extracts are analyzed by LC/MS using ESI and can involve a combination of selected reaction monitoring (SRM) and full scan analysis modes.

4.0 Specimens

This procedure uses biological fluid(s) such as blood, urine, serum, and/or plasma. Typically 0.5 mL of sample is consumed during the analysis but varying volumes may be used, as necessary. Dilution of samples due to limited specimen or due to suspicion of high drug or metabolite concentration is acceptable.

5.0 Equipment/Materials/Reagents

a. General laboratory glassware

b. Disposable borosilicate test tubes (e.g., 16 x 100 mm, round bottom, borosilicate glass with Teflon caps)

c. Vortex mixer

d. Sonicator

e. Automatic pipettes (with disposable tips)

f. Positive pressure solid phase extraction device – SPEWare Cerex, 48 sample (or equivalent)

g. Sample concentrator with nitrogen – SPEWare Cerex 48 heated (or equivalent)

h. Centrifuge

i. pH meter (or equivalent)

j. Trace B Extraction Columns – SPEWare (or equivalent)

k. Liquid Chromatograph/Mass Spectrometer (Shimadzu LCMS-8030, or equivalent)

l. HPLC column – Kinetex, phenyl hexyl, C18, 2.6 µm, 100Å, 50 mm x 2.1 mm (Phenomenex), or equivalent)

m. Pre-Column – SecurityGuard ULTRA Cartridge UHPLC Kinetex for 4.6mm ID Columns (Phenomenex or equivalent)

n. Autosample vials (LC/MS grade 1.8mL or equivalent)

o. Acetic acid, glacial ($CH_3COOH_{(l)}$, Reagent grade or equivalent)

p. Ammonium formate (NH_4CHOO, Reagent grade or equivalent)

q. β-Glucuronidase (p. vulgata ; Sigma or equivalent)

r. Formic acid (HCOOH, Reagent grade or equivalent)

s. Methanol (MeOH, Reagent grade, LC/MS Grade, or equivalent)

t. Morphine glucuronide (Alltech, Sigma, or equivalent)

u. Sodium acetate trihydrate ($NaCH_3COO{\cdot}3H_2O$, Reagent grade or equivalent)

v. Sodium bicarbonate ($NaHCO_3$; Reagent grade or equivalent)

w. Sodium carbonate (Na_2CO_3 ; Reagent grade or equivalent)

x. Water (H_2O, Millipore, Deionized (DIW) or equivalent)

y. Ammonium $Formate_{(aq)}$: ($NH_4CHOO_{(aq)}$; 5M ; 31.5% (w/v)): Can be prepared by dissolving 3.15 g of $NH_4CHOO_{(s)}$ in 10 mL of water. Stable for at least two (2) months in refrigerator.

z. Sodium $Acetate\ Buffer_{(aq)}$ ($NaCH_3COO$; pH~4.5): Can be prepared by combining 5.86 g of $NaCH_3COO{\cdot}3H_2O_{(s)}$ with 3.24 mL of $CH_3COOH_{(l)}$ in a 1 L volumetric cylinder and diluting to volume with water. Store in glass container at room temperature. Stable for at least one (1) year at room temperature. Check clarity and pH can be checked prior to use.

aa. Sodium $Bicarbonate_{(aq)}$ (0.1M ; 0.84% (w/v) ; pH~8): Can be prepared by dissolving 4.2 g of $NaHCO_{3(s)}$ in water within a 500 mL volumetric flask and diluting to volume with water. Store

in glass container at room temperature. Store in glass container at room temperature. Stable for at least one (1) year at room temperature. Check clarity and pH can be checked prior to use.

bb. Sodium Carbonate$_{(aq)}$ (0.1M ; 1.1% (w/v) ; pH~11): Can be prepared by dissolving 5.3 g of $Na_2CO_{3(s)}$ in water within a 500 mL volumetric flask and diluting to volume with water. Store in glass container at room temperature. Stable for at least one (1) year at room temperature. Check clarity and pH can be checked prior to use.

cc. Bicarbonate/Carbonate Buffer$_{(aq)}$ (pH~9): Can be prepared by transferring 0.1M $NaHCO_{3(s)}$ solution into a beaker and checking pH (should be pH~8). Adjust the pH to 9 (+/-0.2) using the 0.1M $Na_2CO_{3(aq)}$ solution. Store in glass container at room temperature. Stable for at least one (1) year at room temperature. Check clarity and pH can be checked prior to use.

dd. Solid Phase Extraction Elution Solution (e.g., 50 mL):
{Dichloromethane (CH_2Cl_2) : IPA : NH_4OH (80 : 18 : 2)}.
Can be prepared by adding 9 mL of IPA to 1 mL of $NH_4OH_{(l)}$ within a 50 mL volumetric cylinder. To this mixture add 40 mL of CH_2Cl_2 and mix. This solution will be prepared fresh.

Note: The volume should be adjusted to account for number of samples that are to be extracted (2 mL are needed for each extraction sample)

ee. MeOH$_{(aq)}$ (20% (v/v)): Can be prepared by adding 80 mL of water and 20 mL of MeOH to a 100 mL volumetric cylinder and mixing. Stable for at least one (1) month at room temperature, but can be prepared fresh. Check clarity prior to use.

ff. Hydrolysis Sodium Acetate Buffer$_{(aq)}$ (0.1 M ; $NaCH_3COO$; pH~5): Can be prepared by combining first making a 1M Sodium Acetate Buffer Solution: 42.9 g of $NaCH_3COO{\cdot}3H_2O_{(s)}$ with 400 mL of water. Add 10.4 mL of $CH_3COOH_{(l)}$ in a 500 mL volumetric cylinder and diluting to volume with water. Store in glass container at room temperature. Stable for at least one (1) year at room temperature. Check clarity and pH can be checked prior to use. Use 1M acetic acid to adjust pH to 5.0 (+/- 0.1). The 0.1M Sodium Acetate Buffer Solution can be made by diluting 20 mL of the 1M Sodium Acetate Buffer Solution in 200 mL water.

gg. β-Glucuronidase (in 0.1M $NaCH_3COO$ buffer solution ; pH~5): Prepare daily for use. The concentration of the enzyme buffer solution should be 5000 Units/mL. The volume of enzyme solution per [0.5 mL] urine specimen should be 0.5 mL. Each 0.5 mL urine sample requires 2500 enzyme F-Units to ensure adequate enzymatic hydrolysis. A slight excess of solution should be made to ensure enough is available for all of the samples.
Note: β-Glucuronidase has different activities based on the lot that is used. See enzyme activity information on individual bottles (i.e., Units/gram of material).

Example: Ten (10) urine specimens (0.5 mL each) that need hydrolyzing means there will need to be 5 mL of enzyme solution total. If the activity of the β-Glucuronidase is 1,439,000 Units/g, then the amount of β-G$_{(s)}$ needed is:

[(5 mL) x (5000 β-G Units/mL) x (1 g β-G/1,439,000 β-G Unit) x 1000 mg/g] = 17.4 mg
Dissolve 17.4 mg $\beta\text{-G}_{(s)}$ in 5 mL 0.1M $NaCH_3COO$ buffer solution (pH~5).

hh. Mobile Phase A – 0.01 %(v/v) $HCOOH_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$:
Can be prepared by mixing 0.5 mL of 5M ammonium formate with 50 µL of formic acid in a 500 mL volumetric cylinder, diluting to volume with water, and mixing well. Store in glass at room temperature. Stable for one (1) week. {Note: 5mM $NH_4CHOO_{(aq)}$ is equivalent to 0.0032 % (w/v) $NH_4CHOO_{(aq)}$.}

ii. Mobile Phase B – (MeOH or CH_3OH). Store in glass at room temperature – stable indefinitely.

6.0 Standards and Controls

Negative Control Solution: See below.

Positive Control Solution: Can be purchased (e.g., UTAK Labs) or generated in-house (e.g., spiked blank blood). Store frozen, refrigerated, or obtain fresh. If purchased, the stability should be determined by manufacturer. A positive control blood should be extracted and analyzed with each blood assay or batch.

Internal Standard Components: Purchased from Cerilliant or other suitable vendors (e.g., 0.1 mg/mL solutions or neat solid). Storage and stability is determined by manufacturer(s). Additional or alternative deuterated compounds may be used.

- Ethinamate (or Amobarbital-D_5)
- Diazepam-D_5

a. Internal Standard (I.S.) Working Solution:
((Ethinamate + Diazepam-D_5) ; 500 ng/mL each), or
((Amobarbital-D_5 + Diazepam-D_5) ; 500 ng/mL each)

Can be prepared by combining the listed volumes of each I.S. component into a 100 mL volumetric flask and bringing to volume with MeOH. Store refrigerated in glass and stable for at least one (1) year.

Drug	Initial Concentration	Volume	Final Concentration (in 100 mL flask)
Ethinamate	100 µg/mL	500 µL	500 ng/mL
Amobarbital-D_5	100 µg/mL	500 µL	500 ng/mL
Diazepam-D_5	100 µg/mL	500 µL	500 ng/mL

b. Mophine-3-β-glucuronide or Morphine-6-β-glucuronide (0.1 mg/mL):
Purchased from Cerilliant or another suitable vendor as 0.1 mg/mL solutions. Storage and stability determined by manufacturer.

c. Morphine Glucuronide Control Working Solution (2.5 µg/mL):
Can be prepared by diluting 250 µL of the Morphine-β-glucuronide Stock Solution with 1:1 methanol:water to a final volume of 10 mL. Store in glass in a freezer. Stable for 6 months.

d. Hydrolysis Control Urine (~75 ng/mL morphine; ~125 ng/mL morphine glucuronide):
Can be prepared by adding 25 µL of the Morphine Glucuronide Control Working Solution (2.5 µg/mL) to 500 µL Negative Control Urine on the day of analysis.

e. Procedure Performance Standard Mix:
((Amobarbital-D_5 + Diazepam-D_5) ; 10 ng/mL each)
This is optional and can be used to evaluate the instrument in addition to the performance check solution specific for the LC/MS instrument.
Can be prepared by diluting the I.S. Working Solution 1:50 with water to make a 10 ng/mL final concentration. This can be done by mixing 10 µL of the I.S. Working Solution and combining it with 500 µL of deionized water, place into an autosample vial, and cap. Prepare fresh on day of use.

f. Negative Control Blood:
Purchased (e.g., Diagnostics Products Corporation, UTAK Labs), donated (e.g., American Red Cross), or in-house negative control blood (e.g., from a proven blank specimen). Store frozen, refrigerated, or obtain fresh. If purchased or donated, stability should be determined by manufacturer. A negative control blood should be extracted and analyzed with each blood assay or batch.

g. Negative Control [Other Matrix]:
Synthetic or in-house negative control [other matrix]. Store refrigerated or obtain fresh. If purchased, stability should be determined by manufacturer. A negative control [other matrix] should be extracted and analyzed with each appropriate assay or batch.

h. Positive Control:
If prepared in-house, can be prepared by adding 250 µL of UTAK DAU Level 2 solution to 250 µL blank matrix (e.g., blood. urine) and mixing. If a non-routine matrix is analyzed, then dilute with 250 µL of appropriate material. The resulting 0.5 mL positive control sample will contain (at least) the following analytes: Methylenedioxymethamphetamine (MDMA), Temazepam, Benzoylecgonine (BE), Methadone, Methaqualone, Codeine, Phencyclidine (PCP), Nortriptyline. Prepare fresh and mix adequately.

7.0 Calibration

This procedure is qualitative only.

8.0 Sampling

Not applicable.

9.0 Procedure

The Appendix contains an abbreviated version of this procedure. This form may be used at the bench by analysts who perform the procedure. If SPE cartridges are in a rack and are not removed during the extraction process and are always in a fixed position, then labeling of SPE cartridges is optional, regardless of what is written in the following steps or in the Appendix. However, the vials which collect the final eluent must be properly labeled.

9.1. Specimen Preparation:

9.1.1. Add 0.5 mL of each sample to a properly labeled test tube.

9.1.2. Add 100 µL of the I.S. Working Solution into each sample (50 ng each)

9.2. Urine Specimens Only (De-conjugate):
Note: Approval from Lead Examiner or Deputy Director needs to be obtained if urine specimens are to be hydrolyzed.

9.2.1. Add 250 µL of β–Glucuronidase in 0.1 M acetate buffer (pH 5.0) to hydrolyze any conjugated drugs within the urine.

9.2.2. Heat for 3 hours at 60 ˚C and then cool specimens to room temperature.

9.3. Add 1 mL of the Sodium Acetate buffer (pH 4.5) to each specimen.

9.4. Add 500 µL of water – cap and vortex-mix samples.

9.5. Sonicate for at least 15 minutes.

9.6. Centrifuge all samples (e.g., 8 minutes at ~5200 RPM)

9.7. Label Trace-B extraction [SPE] columns appropriately

9.8. Place labeled Trace-B extraction columns in the SPE column rack in the appropriate order. Position plastic waste tray labeled “Methanol” underneath SPE column rack.

9.9. Condition each column sequentially with 1 mL MeOH, then drain (≈3 psi) to Solvent “Hazardous Waste” stream

9.10. Remove plastic waste tray labeled “Methanol” and replace with plastic tray labeled “Biohazardous/Buffers”

9.11. Add 1 mL of water to each SPE column, then drain (≈ 3 psi) to "Non-Hazardous" regulated waste stream.

9.12. Individually transfer samples to the center of their respective [properly labeled] SPE column. Avoid splashing and/or transferring any sediment found at the bottom of the tube.

9.13. To each SPE column:

9.13.1. Wash with 1 mL of Bicarbonate buffer (pH 9.0), then drain (≈ 3 psi) to non-hazardous regulated waste stream.

9.13.2. Wash with 1 mL water, then drain (≈ 3 psi) to non-hazardous regulated waste stream.

9.14. Dry the columns for 10 minutes using maximum pressure (e.g., between 60-80 psi).

9.15. During this 10 minute window (or earlier) label autosampler vials and place in the appropriate position in the SPE collection rack underneath the corresponding SPE column. Prepare the elution solvent.

9.16. The elution solvent ratio is 80:18:2 CH_2Cl_2:IPA:NH_4OH.
(Approximately 2 mL will be needed for each tube).

9.17. After 10 minutes, replace plastic waste tray with the SPE collection rack containing appropriately labeled autosample vials. Ensure that each vial is placed under their corresponding SPE column.

9.18. Elute columns with two (2) 1 mL aliquots of the Elution Solution and eluent are collected into appropriate autosample vials. Set flow at 2-4 mL/min for optimal recovery.

9.19. Remove top SPE column rack and transfer collection rack from SPE manifold to sample concentrator.

9.20. Evaporate all eluents to dryness at <40 ˚C.

9.21. Reconstitute each extract sample with 520 µL of mobile phase (i.e., 20% $MeOH_{(aq)}$).

9.22. Analyze extract solutions by LC/MS

10.0 Instrumental Parameters

The following are the typical operating parameters for the instrument used in this procedure. The instrument conditions may be modified to adjust or improve the procedure. The appendix contains an abbreviated version of the procedure and this checklist can be used by analysts.

HPLC Parameters:

Mobile Phase A: 0.01 %(v/v) $HCOOH_{(aq)}$ and 5mM $NH_4CHOO_{(aq)}$
Mobile Phase B: $MeOH_{(l)}$ (neat)
HPLC Column: Kinetex, 2.6 µm Phenyl-Hexyl, 100 Å, 50 mm x 4.6 cm (Phenomenex)
Column Temperature: 40 °C
Constant Flow: 0.5 mL/min.
Autosampler Temperature: 15 °C
Injection Volume: 10 µL
Needle Wash: 500 µL before and after aspiration
Equilibration Time: 4 min.

Gradient Program: Initial 20% B Flow 0.5 mL/min

Time (min.)	**Mobile Phase A [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$] (%)**	**Mobile Phase B $MeOH_{(neat)}$ (%)**
Initial	95	5
2.50	55	45
4.50	55	45
5.50	5	95
7.50	5	95
7.51	95	5
9.00	STOP	STOP

Mass Spectrometer Parameters:

Ionization Source	Electrospray	Polarity	Positive Ion
Ion Spray Potential	-3.5 kV	Heat Block	500 °C
Nebulizer Gas	Nitrogen	Nebulizer	2 L/min
Drying Gas	Nitrogen	Drying Gas	15 L/min.
Scan Type	MRM (&/or Full Scan)	Resolution	Unit
Desolvation Line	300 °C		

11.0 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), fragmentation pattern and qualified ion ratios (mass spectrometric characteristics), and other characteristics are used as the basis for detection and identification. In most cases all of the criteria below should be met in order to identify the appropriate drugs within biological specimens.

11.1. Chromatography

All chromatographic peaks for the analytes of interest should show good chromatographic characteristics, with reasonable peak shape, width, and resolution. For low concentrations of an analyte (<5 ng/mL), there may be transitions that are not optimal. In order to be determined as acceptable, a chromatographic peak in an unknown sample should compare favorably to the same analyte's chromatographic peak in a known sample which has been analyzed on the same system and in the same, or subsequent, analytical timeframe. Additionally, the following two criteria should be met:

11.1.1. Retention Time

The retention time of the peak should be within ±2 % of the retention time (relative or absolute) obtained from injection of a reference standard or positive control.

If analyzed, the retention times of the analytes within the Procedural Performance Solution should be within ±2 % of their previously analyzed times. Minor changes in mobile phase percentage may account for slight retention time shifts.

11.1.2. Signal-to-Noise

To justify the existence of a peak, it's baseline signal to noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10 fold greater than that for any observed peak at a similar retention time in a negative control or blank sample injected prior to that sample.

11.2. Mass Spectrometry

In order for the MS to be considered in good operating condition, the correct mass assignments for each of the analytes in the performance standard should be present. Independent MS/MS experiments are conducted for each analyte. Ion ratios should compare favorably to ion ratios from reference standards or an extracted positive control at a comparable concentration. Generally, ion ratios are within the following limits:

Expected [Set] Ion Ratio	Allowance
>50 %	20%
20% - 50%	25%
<20%	30%

11.3. Batch Acceptance:

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

In this analysis, the internal standards are considered the positive control for each injection. Each of the internal standards should be detected in each sample.

If applicable – for urine batches, morphine should be detected in the hydrolysis control.

11.4. Unknown Sample Compound Detection:

This procedure is not used for identification, but only screening purposes.

12. Calibration

Not applicable.

13. Uncertainty

Not applicable.

14. Limitation

14.1. Limits of Detection (LOD)

This information should be found in validation paperwork.

14.2. Specificity

No known interferences. However, this procedure will be used as a screen only and all forensically significant positive findings will be confirmed by a second technique, unless alternative situations apply (e.g., Memorandum of Understanding (MOU) or directive from customer)).

Note: A second technique could include LC/MS using a separate MS/MS experiment.

15. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use, especially. When casework samples are being processed/analyzed brown paper (or other similar barrier) should be placed in between the surface and the specimens.

16. References

Screening and Confirmation of Benzodiazepines in Blood by Electrospray LCMSMS. West Chester County Department of Laboratories and Research: Division of Forensic Toxicology.

Solid-Phase Extraction Method for Trace B Columns. SPEWARE: Baldwin Park, CA.

A Comparison of the Validity of Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry Analysis of Urine Samples for Morphine, Codeine, 6-Acetylmorphine, and Benzoylecgonine; Peter R. Stout, Nichole D. Bynum, John M. Mitchell, Michael R. Baylor and Jeri D. Ropero-Miller; J.Anal Toxicol (2009) 33 (8): 398-408

Exclusionary Drug Screen by UPLC-ESI-FTMS, FBI Laboratory.

Benzodiazepine Quant in Blood LCMSMS procedure, Toxicology Unit, Division of Scientific Services.

Processing of Opioids by LCMSMS procedure, Toxicology Unit, Division of Scientific Services.

Appendix:
Bench Method:
Exclusionary Drug Screening by Liquid Chromatography/Mass Spectrometry (LC/MS)

The following is an abbreviated version of this procedure and a pdf-version can be used.
This form may be used at the bench level by the analyst performing the procedure.

Negative Control [drug-free] Blood/[other matrix]	**Lot:**
Positive Control Blood/[other matrix]	**Lot:**

Procedure:

	For Blood Specimens	
Add 0.5 mL standard/control/sample into properly labeled 16 x 100 mm test tubes		
Add 100 µL of I.S. Working Sol'n to each sample (50 ng each drug):		
	Ethinamate	**Lot:**
	Amobarbital-D_5 (500 ng/mL)	**Lot:**
	Diazepam-D_5 (500 ng/mL)	**Lot:**
Deconjugate urine specimens (approval needed):		
	Enzyme	**Lot:**
	Hydrolysis Sodium Acetate Buffer solution (pH~5) – heat for 3 hours at 60 ˚C	**Lot:**
Add 1 mL of Sodium Acetate $Buffer_{(aq)}$ (pH 4.5) to each test tube.		**Lot:**
Add 500 µL of water to each test tube, cap and vortex-mix samples, sonicate at least 15 minutes. Centrifuge.		
Label Trace-B extraction [SPE] columns appropriately.		**Lot:**
Place labeled Trace-B extraction columns in the SPE column rack in the appropriate order.		
Add 1 mL MeOH (to condition SPE), then drain (≈3 psi)		**Lot:**
Add 1 mL of water (to wash each SPE column), then drain (≈ 3 psi)		**Lot:**
Individually transfer samples to the center of their respective [properly labeled] SPE column. Avoid sediment / splashing. Allow to migrate through SPE column. Apply small pressure, if needed.		
To each SPE column:		
	Wash with 1 mL of Bicarbonate buffer (pH 9.0), then drain (≈ 3 psi)	**Lot:**
	Wash with 1 mL water, then drain (≈ 3 psi)	**Lot:**
Dry the columns for 10 minutes using maximum pressure (e.g., between 60-80 psi)		
During this 10 minute window (or earlier) label autosample vials, place in SPE rack. Prepare the elution solvent.		
The elution solvent ratio is 80:18:2 CH_2Cl_2:IPA:NH_4OH. (Approximately 2 mL per each tube).		
	Methylene Chloride (CH_2Cl_2)	**Lot:**
	Isopropyl Alcohol (2-Propanol ; IPA)	**Lot:**
	Ammonium Hydroxide (NH_4OH)	**Lot:**
After 10 minutes, replace plastic waste tray with SPE rack containing autosample vials. Ensure vials are properly placed.		
Elute columns with two (2), 1 mL aliquots of the Elution Solution Set flow at 2-4 mL/min.		**Lot:**
Transfer collection rack from SPE manifold to sample concentrator and evaporate to dryness.		
Reconstitute each extract sample with 520 µL of mobile phase (i.e., 20% $MeOH_{(aq)}$)		**Lot:**
Analyze by LC/MS/MS		

Appendix (Cont'd):

Specimen Procedure (Cont'd)

Analyze by LC/MS/MS:

Instrument: Shimadzu 8030	Instrument Identifier:
HPLC Column: Phenomenex ; Kinetex Phenyl-Hexyl ; 100 Å ; 2.6 µm ; 50 mm x 2.1 mm):	Lot:
Mobile Phase A: [0.01 %(v/v) $HCOOH_{(aq)}$ + 5mM $NH_4CHOO_{(aq)}$]	Lot:
Mobile Phase B: MeOH (CH_3OH) – 100%	Lot:

Gradient Program:
Initial 5% B Flow 0.5 mL/min

Time (min.)	%B
Initial	5
2.50	45
4.50	45
5.50	95
7.50	95
7.51	5
9.00	STOP

Mass Spectrometer Parameters

Ion Source	Electrospray
Spray Voltage	3.5 kV
Polarity	Positive
Nebulizer Gas	Nitrogen (2 L/min.)
Drying Gas	Nitrogen (15 L/min.)
Scan Type	MRM (&/or Full Scan)
Heat Block	500 °C
Resolution	Unit

Rev. #	History
1	New procedure for blood or other appropriate matrices.