Cyanide and Azide Analysis in Biologicals and/or Foodstuffs

1 Introduction

Cyanide, a rapidly acting poison, has a high affinity for iron in the ferric state. As such, when cyanide is biologically absorbed, it readily reacts with the trivalent iron of cytochrome oxidase. Reactions with mitochondrial enzymes cause an inhibition of cellular respiration that can lead to hypoxia and rapid death. The minimum adult lethal dose has been estimated as 100 mg for hydrocyanic acid and 200 mg for potassium cyanide.

Industrial uses of cyanide salts (fumigants, insecticides and electroplating solutions) make this toxic anion available for suicidal purposes and criminal mischief, such as food tampering.

Sodium azide is the most commonly encountered toxic form of the azide moiety. An inorganic compound, this colorless salt has a diverse set of uses. It is used as the gas-forming component of some automotive air-bag systems and airplane escape chutes. Industrially, it is used as a precursor for other azide compounds, although applications are limited due to its inherent explosion hazard. It can also be used as a biochemical research tool and as a preservative in laboratory environments.

Similar to cyanide, azide inhibits cytochrome oxidase by irreversible binding to the heme cofactor. Sodium azide is acutely toxic, with estimated adult lethal dose at 700 mg.

2 Scope

This procedure allows for the screening, identification, and quantitation of cyanide in biologicals and foodstuffs. This procedure also allows for the qualitative identification of azide in blood and aqueous samples. This document applies to Chemistry Unit case working personnel who perform toxicology analyses.

3 Principle

This procedure for cyanide and azide is predicated on the condition that the analytes will be released as hydrogen cyanide gas (HCN) and hydrozoic acid, respectively, upon acidification of the biological and/or food matrix.

For cyanide, screening is performed by headspace (HS) gas chromatography with selective nitrogen-phosphorus detection (GC/NPD). Qualitative confirmational analysis of cyanide and screening/confirmation of azide is performed by headspace gas chromatography-mass spectrometry. Quantitative confirmation of any cyanide positive sample may be achieved by headspace gas chromatography with nitrogen-phosphorus detection.

4 Specimens

For cyanide, sample matrices can be comprised of biologicals (blood, tissue homogenate, or stomach contents) as well as common foodstuffs such as cakes, candy and beverages. For azide, blood and aqueous matrices are currently validated. Typically, 0.5 mL or 1.0 mL (or gram) of sample is used for these analyses.

5 Equipment/Materials/Reagents

Guidance for preparing reagents may be found in the *Preparation of Chemical Reagents* standard operating procedure (Tox 103).

a. Hewlett Packard Gas chromatograph equipped with an RT-QS-BOND (or equivalent) column, a nitrogen phosphorus detector and a headspace autosampler.

b. Hewlett Packard Gas chromatograph equipped with a DB-624 (or equivalent) column, a mass spectrometric detector and a headspace autosampler.

c. Volumetric flasks (100 and 200-mL)

d. Glass reagent bottles (100 - 500 mL capacity)

e. 10-mL and/or 20-mL disposable headspace vials and caps

f. 1.0 and 2.5 cc syringes with needles

g. Vortex mixer

h. Homogenizer and/or mortar and pestle

i. Potassium Cyanide (Reagent grade)

j. Sodium Azide (Reagent grade)

k. Acetonitrile (HPLC grade)

l. Deionized Water

m. 1-Butanol (ACS grade)

n. 5 N Sulfuric Acid:
To a 100-mL graduated cylinder containing 70 mL deionized water, slowly add 12.5 mL concentrated sulfuric acid. Mix well and bring to 90 mL with deionized water. Store in glass at room temperature. Stable 1 year.

o. 5 N Sodium Hydroxide:
To a 100-mL Nalgene volumetric flask, add 60 mL water and 20 g sodium hydroxide. Mix well to dissolve, and let cool. Bring to volume with deionized water. Store in Nalgene containers at room temperature. Stable 1 year.

p. 0.4% Sodium Hydroxide
To a 25-mL volumetric flask, add approximately 15 mL of deionized water. Add 0.1 g of sodium hydroxide. Dilute to the mark with deionized water and mix well. Store in Nalgene containers at room temperature. Stable for 1 year.

q. Routine laboratory supplies, including pH paper, disposable glass pipets, spatulas, test tube racks, graduated cylinders, etc.

6 Standards and Controls

a. Cyanide Stock Standard (0.2 mg/mL)[1]:
Prepared by adding 50 mg of potassium cyanide to a 100-mL volumetric flask containing 2 mL of 5 N NaOH. Dilute to volume with deionized water and mix thoroughly. Stable for at least one year.

b. Concentrated Cyanide Stock Standard (2 mg/mL; needed for quantitation only):
Prepared by adding 50 mg of potassium cyanide to a 10-mL volumetric flask containing 2 mL of 0.5 N NaOH. Dilute to volume with deionized water and mix thoroughly. Stable for at least one year.

c. Azide Stock Standard (1 mg/mL):
See the Safety Section prior to handling sodium azide. Using a plastic spatula, weigh out 10 mg of sodium azide into a tared plastic weigh boat. Add sufficient 0.4% sodium hydroxide solution to dissolve the powder. Pour the solution into a 10-mL volumetric flask, and dilute to the mark with 0.4% sodium hydroxide. Store in Nalgene containers at room temperature. Stable for 1 year.

d. Azide Working Standard (100 µg/mL)
See the Safety Section prior to handling sodium azide. Add approximately 5 mL of 0.4% Sodium Hydroxide to a 10-mL volumetric flask. Add 1 mL of the Azide Stock Standard (1 mg/mL). Dilute to the mark with 0.4% Sodium Hydroxide. Store in Nalgene containers at room temperature. Stable for 1 year.

[1] For quantitative analyses, separate stocks will be prepared for controls and calibrators.

e. Negative Control:
Deionized water, blood or an appropriate matrix blank, is used as a Negative Control. A Negative Control will be extracted and analyzed with every assay. Note that some materials are preserved with sodium azide, and would therefore be not be appropriate for use as an azide negative control. Verify the negative control matrix does not contain sodium azide prior to use.

f. 0.04% Acetonitrile (v/v) (Internal Standard):
Add 40 µL acetonitrile to about 90 mL deionized water in a 100-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store at room temperature in a tightly sealed glass or plastic container. Stable for 6 months.

g. 0.1% 1-butanol (v/v) (Internal Standard):
Prepared by dissolving 0.1 mL of 1-butanol in 50 mL of deionized water in a 100-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Stable for 6 months.

h. Aqueous Positive Control (1 µg/mL cyanide):
Prepared by adding 0.05 mL of the Cyanide Stock Standard to a 10-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store in plastic or glass. Stable at least one year. A Positive Control will be analyzed with every assay. When possible, the Positive Control will be matrix matched.

i. Aqueous Low Positive Control – Quantitative (3 µg/mL cyanide):
Prepared by adding 0.15 mL of the Cyanide Stock Standard to a 10-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store in plastic or glass. Stable at least one year. A Positive Control will be analyzed with every assay. When possible, the Positive Control will be matrix matched.

j. Aqueous Positive Control (10 µg/mL cyanide):
Prepared by adding 0.5 mL of the Cyanide Stock Standard to a 10-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store in plastic or glass. Stable at least one year. A Positive Control will be analyzed with every assay. When possible, the Positive Control will be matrix matched.

k. Aqueous High Positive Control - Quantitative (24 µg/mL cyanide):
Prepared by adding 1.2 mL of the Cyanide Stock Standard to a 10-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store in plastic or glass. Stable at least one year. A Positive Control will be analyzed with every assay. When possible, the Positive Control will be matrix matched.

l. Positive Control Blood (1 µg/mL cyanide):
Prepared by first diluting the Cyanide Stock Standard to a concentration of 10 µg/mL by

adding 5 mL of the Cyanide Stock Standard to a 100-mL volumetric flask and bringing to volume with deionized water. Add 0.1 mL of this 10 µg/mL solution to 0.9 mL of whole blood. Prepare fresh. A Positive Control will be extracted and analyzed with every assay. When possible, the Positive Control will be matrix matched. For quantitative assays, the Positive Control should be prepared in accordance with the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101).

m. Positive Control Blood (10 µg/mL cyanide):
Add 0.05 mL of the Cyanide Stock Standard to 0.95 mL of whole blood. Prepare fresh. A Positive Control will be extracted and analyzed with every assay. When possible, the Positive Control will be matrix matched. For quantitative assays, the Positive Control should be prepared in accordance with the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101).

n. Low Positive Control Blood Spiking Solution – Quantitative (30 µg/mL cyanide):
Prepared by adding 1.5 mL of the Cyanide Stock Standard to a 10-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store in plastic or glass. Stable at least one year. On day of quantitative analysis, 0.05 mL of this Solution is added to 0.5 mL blood to represent a 3 µg/mL cyanide blood control.

o. High Positive Control Blood Spiking Solution – Quantitative (240 µg/mL cyanide):
Prepared by adding 1.2 mL of the Concentrated Cyanide Stock Standard to a 10-mL volumetric flask. Dilute to volume with deionized water and mix thoroughly. Store in plastic or glass. Stable at least one year. On day of quantitative analysis, 0.05 mL of this Solution is added to 0.5 mL blood to represent a 24 µg/mL cyanide blood control.

p. Low Positive Control (2 µg/mL Azide)
To 0.5mL of Negative Control Matrix, add 10µL of the Azide Working Standard, and vortex.

q. High Positive Control (20 µg/mL Azide)
To 0.5mL of Negative Control Matrix, add 100µL of the Azide Working Standard, and vortex.

This procedure may be used quantitatively for cyanide. A cyanide calibration graph is constructed from the aqueous cyanide calibrators per the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101). Although the Acetonitrile Internal Standard Solution is stable for 6 months, slow evaporation means that any given calibration curve is only valid for about 24 hours. Cyanide concentrations greater than the highest calibrator require dilution and re-quantitation for accurate quantitative results. Table 1 provides direction on the preparation of recommended calibrators for the quantitation of cyanide.

Table 1: Preparation of Recommended Calibrators for the GC/NPD Quant

Level (ug/mL)	Amount of 0.2 mg/mL stock diluted to 10 mL DI water (mL)
1	0.050
2.5	0.125
5	0.250
10	0.500
20	1.000
30	1.500

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.

Note: Sample preparation for all methods should be performed in a chemical fume hood.

8.1 Screening and Quantitation of Samples for Cyanide by GC/NPD

a. Thoroughly homogenize solid or bulky specimens to ensure a representative aliquot is sampled. Tissue, stomach contents and food specimens are mixed (1:1) with deionized water prior to blending. Liquid samples (blood and beverages) can be directly assayed. Measure and record the pH of all food specimens and stomach contents. Large quantities of inorganic cyanide salts may be indicated by an alkaline pH (>10).

b. Measure 0.5 mL of liquid sample or 1 gram of homogenate and 50 µL of 0.04% acetonitrile (Internal Standard) into a 10-mL headspace vial and cap. Process calibrators and controls similarly.

c. Using a 1.0 cc syringe, inject 0.5 mL of 5 N H_2SO_4 into the vial and thoroughly vortex the sample to uniformly distribute the acid.

d. Allow the sample to equilibrate at room temperature for 30 minutes.

e. Analyze headspace by GC/NPD using the instrumental parameters in Section 9.1.

f. Upon completion of the analysis, check and record the pH of the sample matrix in the

vial to ensure acidity (pH<2).

8.2 Confirmation of Samples for Cyanide, and Screening/Confirmation of Samples for Azide by Headspace GC/MS

a. Thoroughly homogenize solid or bulky specimens to ensure a representative aliquot is sampled. Tissue, stomach contents and food specimens are mixed (1:1) with deionized water prior to blending. Liquid samples (blood and beverages) can be directly assayed. Measure and record the pH of all food specimens and stomach contents. Large quantities of inorganic cyanide salts may be indicated by an alkaline pH (>10).

b. Measure 0.5 mL of liquid sample or 1 gram of homogenate and 50 µL of 0.1% 1-butanol (Internal Standard) into a 10-mL headspace vial and cap. Process controls similarly. (Alternatively, measure 1.0 mL of a liquid sample or 2 grams of a homogenate and 100 µL of 0.1% 1-butanol into a 20-mL headspace vial and cap.)

c. Using a plastic syringe, inject 0.5 mL of 5 N H_2SO_4 into the vial and thoroughly vortex the sample to uniformly distribute the acid. (If using 20-mL headspace vials, add 1.0 mL of 5 N H_2SO_4.). Allow to equilibrate for 30 minutes at room temperature.

d. For cyanide analysis, analyze the headspace by GC/MS using the instrumental parameters in Section 9.2.

e. For azide analysis, analyze the headspace by GC/MS using the instrumental parameters in Section 9.3.

e. Upon completion of the analysis, check and record the pH of the sample matrix in the vial to ensure acidity (pH<2).

9 Instrumental Conditions

Appendix 1 contains an abbreviated list of instrumental conditions. Following are the instrumental parameters for the instruments used in this procedure:

9.1 For GC/NPD Analysis of Hydrogen Cyanide

9.1.1 Headspace Sampler Parameters

Syringe	2.5ml-HS	tray type	VT32-10/20
oven / syringe temp.	45°C / 55°C	Fill/injection volume	2500 / 250 µl
flush time	4.0 min	fill speed / strokes	500 µl /sec / 5
incubation time	5.0 min		
agitator speed	250 rpm	injection speed	1000 µl / sec
agitator on/off time	10 s / 1 s	Inj./Vial penetration	40 /25 mm

9.1.2 Gas Chromatograph and NPD Parameters

Oven Parameters		Inlet and Carrier Parameters		NPD Parameters	
temperature	110°C	inlet temperature	150°C	temperature	225°C
ramp	4 °C/min	purged packed inlet pressure	10.894 psi	makeup flow	3.5 mL/min
final temperature	130 °C	carrier gas	N_2	air flow	60 mL/min
final hold time	5 min	carrier mode	constant flow	hydrogen flow	3.0 mL/min
Column Parameters		carrier flow	1.5 mL/min	electrometer	on
type	RT-QS-BOND				
length	30 m				
internal diameter/film	320 µm /10 µm				

Hydrogen Cyanide should elute within 2 minutes under these conditions

9.2 For GC/MS Analysis of Hydrogen Cyanide

9.2.1 Headspace Sampler Parameters

Syringe	2.5ml-HS	tray type	VT32-10/20
oven / syringe temp.	45°C / 55°C	Fill/injection volume	2500 / 1000 µl
flush time	4.0 min	fill speed / strokes	500 µl /sec / 5
incubation time	5.0 min		
agitator speed	300 rpm	injection speed	1000 µl / sec
agitator on/off time	10 s / 1 s	Inj./Vial penetration	40 / 22 mm

9.2.2 Gas Chromatograph Parameters

Oven Parameters		Inlet and Carrier Parameters		Column Parameters	
temperature 1	60°C	inlet temperature	150°C	type	DB-624
hold 1	2 min	injection mode	split	length	30 m
ramp 1	50°C/min	carrier gas	ultrapure helium	internal diameter	0.25 mm
temperature 2	120°C	carrier mode	constant pressure	film thickness	1.4 µm
hold 2	2.5 min	carrier pressure	6.54 psi		
total run time	5.7 min	split ratio	10:1		

9.2.3 Mass Spectrometer Parameters

ionization mode	electron impact (+)	source temperature	230°C
solvent delay	1.5 min	transfer line temperature	260°C
scan mode	SIM	quadrupole temperature	150°C
group 1 (1.65 – 2.7 min)	m/z 12, 26, 27	multiplier offset	+200 V
group 2 (2.7 – 10.2 min)	m/z 31, 41, 56		

9.3 For GC/MS Analysis of Hydrozoic Acid

9.3.1 Headspace Sampler Parameters

Syringe	2.5 mL	Injection Volume	1000 µL
Syringe Temperaature	55°C	Injection Speed	1000 µL / s
Flush Time	240 s	Pullup Delay	0 s
Incubation Temperature	45°C	Fill Volume	2500 µL
Incubation Time	10.00 min	Fill Strokes	5
Agitator On/Off Time	10 s / 1 s	Fill Speed	500 µL / s
Agitator Speed	300 rpm	Pre/Post Inj. Delay	0/0 s
Inj./Vial Penetration	40 / 22 mm	Sample Tray Type	VT32-10/20

9.3.2 Gas Chromatograph Parameters

Oven Parameters		Inlet and Carrier Parameters		Column Parameters	
temp 1	40°C (1 min)	inlet temperature	150°C	type	DB-624
temp 2	110°C @ 15°C/min (0 min)	injection mode	split	length	30 m
temp 3	250 °C @ 35°C/min (4 min)	carrier gas	ultrapure helium	internal diameter	0.25 mm
Eq. Time	0.2 min	carrier mode	constant pressure	film thickness	1.4 µm
Run Time	14.167 min	carrier pressure	5.463 psi	split ratio	10:1

9.3.3 Mass Spectrometer Parameters

ionization mode	electron impact (+)	source temperature	230°C
solvent delay	1.9 min	transfer line temperature	260°C
scan mode	SIM	quadrupole temperature	150°C
group 1	m/z 15, 29, 43, 45, 58	multiplier offset	+200 V

10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. 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 calibrator or extracted positive control. In most cases, all of the below should be met in order to identify cyanide or azide within a biological specimen.

10.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. 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.

10.1.1 Retention Time

The retention time of the peak should be within ±2% of the retention time (relative or absolute) obtained from injection of a calibrator or extracted Positive Control of cyanide.

10.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak 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 injected just prior to that sample.

10.2 Mass Spectrometry

The mass spectrum of the analyte of interest should match that of 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.

10.3 Batch Acceptance

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

Each of the analytes in the Positive Control should be detected. High and Low Positive Controls should fall within ±20% of the target value. See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for further guidance.

11 Calculations

See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

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

13.1 Cyanide Analysis

a. Linearity: 1 – 30 µg/mL with 1/x weighting

b. Limit of Detection: GC/NPD: 0.65 µg/mL (water)
0.50 µg/ml (blood)
GC/MS: 1.0 µg/mL

c. Lower Limit of Quantitation: 1.0 µg/mL in both blood and water

d. Accuracy:

	3 µg/mL	8 µg/mL	24 µg/mL
% Bias (water)	+4.91%	-1.43%	+0.04%
%Bias (blood)	-2.53%	-8.24%	+1.83%

e. Precision:

	3 µg/mL	8 µg/mL	24 µg/mL
Repeatability (water)	2.35%	1.82%	4.53%
Repeatability (blood)	7.11%	4.91%	9.10%
Intermediate Precision (water)	6.51%	9.03%	4.53%
Intermediate Precision (blood)	9.96%	6.73%	9.10%

13.2 Azide Analysis

a. Limit of Detection: GC/MS: 2 µg/mL in blood and aqueous matrices

13.3 Interferences

Azide will interfere with the NPD quantitation of cyanide, but is chromatographically resolved from cyanide on the GC/MS, and so will not cause a false positive result. However, positive cyanide results must be confirmed by GC/MS analysis. Grossly decomposed or putrefied samples may affect both detection and quantitation limits.

13.4 Reporting of Results

As this procedure generates hydrogen cyanide and hydrozoic acid which are then detected by GC/NPD or GC/MS, reported positive results will include language stating the limitations of this technique. Suggested language includes, but is not limited to:

> *The identification of hydrogen cyanide in the Item 1 blood sample is indicative of exposure to a cyanide containing compound. Various cyanide compounds will produce hydrogen cyanide under acidic conditions, including sodium cyanide and potassium cyanide.*
>
> *The identification of hydrozoic acid in the Item 1 blood sample is indicative of exposure to an azide containing compound. Various azide compounds will produce hydrozoic acid under acidic conditions, including sodium azide and lead azide.*

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Acid liberates hydrogen cyanide gas and care must be taken to isolate acid solutions from cyanide sources.

Similarly, acid also liberates the highly toxic hydrozoic acid, which then constitutes an inhalational hazard. Additionally, sodium azide in its powder form constitutes an explosion hazard. Handling of the powder is performed with plastic spatulas and plastic weigh boats. All

manipulations of the solid material must take place within a functioning fume hood. For storage of the powder and the solutions, keep azide materials segregated away from acids and metals. For disposal, do not discard sodium azide down the sink or mix with acidic waste. Collect all sodium azide waste as a separate hazardous waste stream. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 Precautionary Statement

Postmortem blood specimens may contain endogenous cyanogenic sources that render uncertain the toxicological significance of amounts less than 0.2 µg/mL. Also, it is not uncommon to find trace amounts of cyanide in the blood of smokers.

16 References

Charles C. Thomas. *Handbook of Emergency Toxicology*. *Cyanides* 3rd Edition. Springfield, IL. pp. 219-220 (1977).

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Rapid Quantitation of Cyanide in Blood by Gas Chromatography. *Journal of Analytical Toxicology*. 7:213-215. (September/October 1983).

Paper Strip Screening Method for Detection of Cyanide in Blood Using CYANTESMO Test Paper. *The American Journal of Forensic Medicine and Pathology*. 13:81-84 (1992).

Specific Determination of Cyanide in Blood by Headspace Gas Chromatography. *Journal of Analytical Toxicology* 18:205-207 (July/August 1994).

Disposition of Toxic Drugs and Chemicals in Man. Cyanide. 4th edition. Chemical Toxicology Institute, Foster City, CA . pp. 200 – 203 (1995).

Courtroom Toxicology. Cyanide; Volume 4. Matthew Bender and Company, Inc., San Francisco, CA. 1997.

Guidelines for Toxicological Quantitations (Tox 101)*;* FBI Laboratory Chemistry Unit - Toxicology Subunit SOP 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
2	09/19/12	Updated Sections 5 and 10.1 to reflect new chromatography column for NPD analysis. Added 1.0 cc syringes to Sections 5 and 9.2. Updated standard and control preparation in Section 6. Removed option to quantitate by GC/MS in Sections 7 and 9.2. Updated NPD curve in Section 9.2 and limitations in Section 14 based on new quantitative validation. Removed 20-mL vial option in Section 9.2. Updated Decision Criteria for chromatographic analysis in 11.1. Added a note about azide as a possible interference on the NPD in Section 14. Added Appendix 2 (instrumental parameters.)
3	02/09/18	Updated Scope language. Removed "reasonable degree of scientific certainty" language from Section 11.2. Updated approval lines. Updated Title, Introduction, and Scope to include azide description. Removed "necessitate" phrase from Introduction with regards to cyanide analysis. Removed all references to CYANTESMO, Merckoquant test kits, and Chloramine T derivatization in multiple sections. Updated Principle (3) section to better describe workflow. Updated Specimens (4) to remove references to the test strips and derivatization procedure. Removed materials and equipment related to deleted procedures in Section 5. Updated Section 6c to describe azide negative controls. *Updated Section 15 (Safety) to include cautionary statements about azide handling and disposal. Added 0.4% sodium hydroxide preparation to Section 5 (p) and renumbered. Added azide stock preparations in 6, c-d, and renumbered. Added azide blood controls to Section 6, p-q. Removed Calibration (formerly Section 7), and combined with Section 6, adding some information for azide analysis. Deleted former section 8.1 (Commercial test kits). Section 9: removed references to discontinued procedures and added azide procedure. Updated 9.1.1 to clarify method parameters. Removed footers. Section 12: renamed to Measurement Uncertainty. Broke Section 13 out into 13.1 Cyanide and 13.2 Azide sections, changed "ppm" to "µg/mL" for consistency, added statement requiring GC/MS analysis for confirmation of cyanide. Added 13.4 reporting language statements under Limitations section. Section 5: removed reference to TOX103, and updated items n and o. Also removed TOX103 reference in Section 16. Added 10.3: Batch Acceptance Criteria. Matched language in 8.1 and 8.2 for solid/bulky samples. Updated 10 to include azide.

Approval

Redacted - Signatures on File

Toxicology Technical Lead: Date: 02/08/2018

Chemistry Unit Chief: Date: 02/08/2018

QA Approval

Quality Manager: Date: 02/08/2018

Appendix 1: Abbreviated version of the Cyanide Procedure for bench use.

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