

CCP-TP-197

Revision 1

CCP Determination of Hydrazine by High-Performance Liquid Chromatography (HPLC)

EFFECTIVE DATE: 09/15/2010

Larry Porter

PRINTED NAME

APPROVED FOR USE

RECORD OF REVISION

Revision Number	Date Approved	Description of Revision
0	09/07/2010	Initial issue.
1	09/15/2010	Editorial changes such as removing redundant “%” and changing “ <u>M</u> ” to “M”.

TABLE OF CONTENTS

1.0 PURPOSE..... 4
1.1 Scope..... 4
1.2 Discussion 4
2.0 REQUIREMENTS..... 6
2.1 References 6
2.2 Training Requirements..... 7
2.3 Equipment List 7
2.4 Sample Handling 12
2.5 Precautions and Limitations..... 13
2.6 Prerequisite Actions..... 14
2.7 Definitions 14
2.8 Quality Control Requirements..... 14
2.9 Calculations 15
3.0 RESPONSIBILITIES..... 20
3.1 HPLC Operator 20
4.0 PROCEDURE..... 21
4.1 Extracting Samples 21
4.2 Derivatizing Working Standards and Sample Extracts..... 23
4.3 Preparing Sample Extracts and Dilutions for Analysis 24
4.4 Setting up the Instrument..... 24
4.5 Determining the Retention Time (RT) Window 25
4.6 Performing Initial Calibration..... 26
4.7 Analyzing Samples 26
4.8 Reporting Data..... 28
4.9 Determining the Method Detection Limit (MDL) 29
4.10 Demonstrating Method Precision and Accuracy (P&A)..... 29
5.0 RECORDS..... 30

LIST OF TABLES

TABLE 1. WORKING CALIBRATION STANDARD PREPARATION 12

LIST OF FIGURES

Figure 1. Benzalazine Derivative Formation Reaction..... 5

LIST OF APPENDIXES

Appendix A – Acceptance Criteria and Required Corrective Actions 31

1.0 PURPOSE

This procedure provides instruction for determining hydrazine in homogenous solids and soil/gravel. Samples are extracted in an acid matrix and derivatized using benzaldehyde. Analysis is performed using high performance liquid chromatography (HPLC) with a photodiode array detector.

1.1 Scope

This method implements U.S. Environmental Protection Agency (EPA) SW-846 Method 8000B, *Determinative Chromatographic Separations*, and was developed from references listed in Section 2.1.

This procedure functions as an Idaho Cleanup Project (ICP) Use Type 2 document for performing operations within the ICP Analytical Laboratory facility.

1.2 Discussion

Hydrazine is extracted from the solid sample matrix using an acid extraction. The extract is derivatized with benzaldehyde and the benzalazine derivative is analyzed by HPLC. Steps in the procedure are discussed below.

1.2.1 Sample Extraction

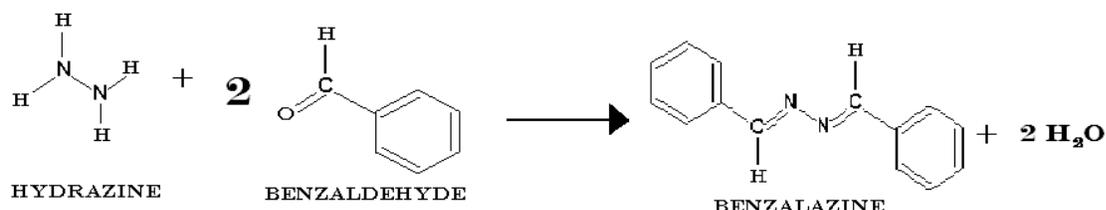
Hydrazine is extracted from the solid matrix using a 1:10 w/v mixture of sample in 0.1 M sulfuric acid (H_2SO_4). Hydrazine in an acid matrix is generally considered stable. Loss of analyte due to auto-oxidation or catalysis due to the presence of metal ions is minimal when the pH is low. The sample is extracted on a shaker for at least 30 minutes and the extract is then filtered.

1.2.2 Derivatization

Reaction of hydrazine in the sample with excess benzaldehyde forms a benzalazine derivative and water. In this reaction, benzaldehyde acts as an electrophile. The reaction occurs quickly because the resonance structure of the phenyl ring helps to stabilize the partial positive charge on the carbonyl carbon of benzaldehyde. Slightly acidic conditions also help stabilize the partial positive charge on the carbonyl carbon and improve reaction efficiency. The pH of the final solution should be between 2 and 4. If the reaction media is too acidic, the nitrogen on the hydrazine molecule can become protonated, thus deactivating it as a nucleophile (the unshared pair of electrons is no longer available). Benzalazine forms when the hydrazine molecule reacts with two benzaldehyde molecules. The rate of the initial reaction is dependant only on the concentration of

the reactants and the stability of the electrophile. The coupling of the second benzaldehyde to the intermediate should not vary much in rate from the first addition. The reaction rate is moderately fast; therefore, the single condensation hydrazine species should not be detected unless an insufficient amount of benzaldehyde is used. Figure 1, Benzalazine Derivative Formation Reaction, shows the benzalazine derivative formation reaction proceeds as follows:

Figure 1. Benzalazine Derivative Formation Reaction



The final reaction conditions have an excess of benzaldehyde as well as a sufficiently high concentration of methanol to dissolve any benzalazine formed by the reaction.

1.2.3 High Performance Liquid Chromatography (HPLC)

HPLC is a form of liquid chromatography that separates compounds dissolved in solution based on their interaction with the mobile phase and the stationary phase. This method uses reverse phase HPLC, in which the stationary phase is relatively non-polar and the mobile phase is relatively polar; thus more non-polar analyte molecules will be retained longer on the column.

Basic components of an HPLC include: mobile phase reservoirs; a pump with a proportioning valve; an injection valve; an analytical column; a detector; and a data system. The pump delivers the mixed mobile phase consisting of 5:95 water/methanol into the chromatographic system. The analytical column used is a reverse phase C18 column (column packed with silica particles charged with hydrophobic C18 alkyl chains). The benzalazine derivative is detected with an optical detector measuring peak absorbance at 313 nm.

2.0 REQUIREMENTS

2.1 References

Baseline Documents

- CCP-PO-001, *CCP Transuranic Waste Characterization Quality Assurance Project Plan*
- CCP-QP-022, *CCP Software Quality Assurance Plan*
- EPA SW-846, *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods*, Third Edition, Method 8315A "Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)," U.S. Environmental Protection Agency, 1986
- OSHA Analytical Methods Manual; Vol. 1, Publ. #4542, U.S. Department of Labor, Occupational Safety and Health Administration; OSHA Salt Lake Technical Center: Salt Lake City, UT, 1990; Method 20: Hydrazine; American Conference of Governmental Industrial Hygienists (ACGIH): Cincinnati, OH
- Audrieth, L.F.; Ogg, B.A., *The Chemistry of Hydrazine*, John Wiley and Sons, Inc.: New York, 1951
- Elias, G.; Bauer, W.F.; J. Sep. Sci. 2006, 29, 460-464, *Hydrazine Determination in Sludge Samples by High Performance Liquid Chromatography*
- Leasure, C.S.; Miller, E.L.; *Measurement of Hydrazine Contamination in Soils, the Third Conference on the Environmental Chemistry of Hydrazine Fuels*, ESL-TR-87-74; January 1988

Referenced Documents

- ACLP-0.24, *Laboratory Spill Cleanup*
- ACLP-0.255, *Mechanical Pipetor Calibration Verification*
- ACLP-0.27, *Routine Handling of Corrosives*
- ACLP-0.40, *Analytical Laboratory Waste Management*
- CCP-QP-002, *CCP Training and Qualification Plan*

- CCP-QP-005, *CCP TRU Nonconforming Item Reporting and Control*
- CCP-QP-008, *CCP Records Management*
- CCP-TP-188, *CCP Analytical Data Recording, Review, and Reporting*
- JSA-2280, *CCP Determination of Hydrazine by High-Performance Liquid Chromatography (HPLC)*
- MCP-7, *Radiological Work Permit*
- MCP-3635, *Chemical Hygiene Plan*
- EPA SW-846, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, Third Edition, Method 8000B, "Determinative Chromatographic Separations," U.S. Environmental Protection Agency, 1986

2.2 Training Requirements

- 2.2.1 Personnel performing this procedure will be trained and qualified in accordance with CCP-QP-002, *CCP Training and Qualification Plan*, prior to performing this procedure.

2.3 Equipment List

2.3.1 Apparatus and Materials

- [A] High Performance Liquid Chromatograph (modular), Dionex Summit HPLC system or similar, equipped with:
- [A.1] Pumping system, gradient, with flow control capable of 1.00 mL/min, equipped with an integrated degassing system, Dionex P680 LPG or equivalent
 - [A.2] Photodiode array detector, 313 nm, Dionex PDA-100 Photodiode Array Detector or equivalent
 - [A.3] High pressure injection valve with 25- μ L loop
 - [A.4] Analytical column, 250 mm \times 4.6 mm I.D., 5- μ m particle size, reverse phase C-18 column, Supelcosil LC-18 or equivalent

- [A.5] Guard column, Supelcosil Supelguard or equivalent
- [A.6] Autosampler, Dionex ASI-100 or equivalent
- [A.7] Computer with data system
- [B] Orbital shaker, heated, capable of rotation between 50 and 250 rpm, New Brunswick Excella X24 or equivalent
- [C] Mechanical pipetors, 0.010–10-mL, with disposable tips, verified per ACLP-0.255, *Mechanical Pipetor Calibration Verification*
- [D] Pipets, glass, Class A, 1.00–50.00-mL
- [E] Volumetric flasks, glass, Class A, various sizes
- [F] Funnels, plastic
- [G] Analytical balance, minimum sensitivity of 0.1 mg and 100 g minimum capacity
- [H] Bottles, polyethylene, various sizes
- [I] Bottles, glass with plastic safety coating, 125-mL, with polytetrafluoroethylene (PTFE)-lined caps
- [J] Bottles, glass, with PTFE-lined caps, various sizes
- [K] Spatula
- [L] Syringes, disposable, 60-mL or other appropriate volume
- [M] Syringe filters (Nalgene™ or equivalent), 0.2- and 0.45- μ m pore size
- [N] Compressed air, Ultra High Purity (UHP) grade or better
- [O] Centrifuge, capable of 2500 rpm
- [P] Centrifuge tubes, plastic, 60-mL
- [Q] Beakers, glass or plastic, various sizes
- [R] Vials, glass, pre-cleaned with PTFE-lined caps, various sizes

- [S] pH paper, full range (universal) or equivalent
- [T] Vials, autosampler, 2-mL, glass, PTFE-lined septa
- [U] Pipets, transfer, disposable glass
- [V] Microsyringes, glass, various sizes
- [W] Vortex mixer
- [X] Electrical tape or equivalent
- [Y] Duct tape or equivalent
- [Z] Stirrer, magnetic
- [AA] Stirring bars, magnetic, plastic coated
- [BB] Bags, plastic, zip-top, various sizes
- [CC] Mobile Phase Filter Degasser, MicroSolv catalog #58800-00, or equivalent
- [DD] Membrane Filters, Teflon or nylon, 47-mm diameter, 0.22- or 0.45- μm pore size

2.3.2 Reagents and Standards

[A] Reagents

Use Analytical Reagent Grade chemicals and organic-free American Society for Testing of Materials (ASTM) Type II conductivity water or better for preparation of all reagents, standards, and samples, unless otherwise specified.

NOTE

Prepared volumes may vary provided that constituent ratios are maintained.

[A.1] Benzaldehyde

[A.2] Sulfuric acid, 9 M. Prepare by adding 25 mL of concentrated sulfuric acid (H_2SO_4) to a 50-mL glass volumetric flask containing ~20 mL of water, diluting to volume with water, and mixing thoroughly.

- [A.3] Sulfuric acid, 0.1 M. Prepare by adding 5.5 mL of 9 M sulfuric acid (see Item [A.2]) to a 500-mL glass volumetric flask containing ~200 mL of water, diluting to volume with water, and mixing thoroughly.
- [A.4] Sodium hydroxide solution, 50% w/v. Purchase or prepare by dissolving 50 g sodium hydroxide (NaOH) in water and diluting to 100 mL with water.
- [A.5] Methanol, HPLC or purge-and-trap grade, or better.
- [A.6] Reagent sand. Purchase sea sand, Fisher catalog # S25 or equivalent. Bake the sand for a minimum of 4 hours at 400 °C, cool, and store in a tightly closed glass container.
- [A.7] Sodium borate stock solution, 0.05 M. Prepare by dissolving 9.53 g sodium borate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) in 500 mL of water.
- [A.8] Benzaldehyde solution, 1% v/v. Prepare weekly with use by aliquoting 0.500 mL of benzaldehyde (see Item [A.1]) into a 50-mL glass volumetric flask. Dilute to volume with methanol and mix thoroughly.
- [A.9] Sodium borate working solution, 0.01 M. Prepare by adding 20 mL of 0.05 M sodium borate stock solution (see Item [A.7]) to a 100-mL volumetric flask and diluting to volume with water.

[B] Standards

[B.1] Stock Standards

- (a) Hydrazine Calibration Stock Standard Solution (CalSt), 1000 $\mu\text{g}/\text{mL}$. Purchase a NIST-traceable manufacturer-certified solution in aqueous matrix. Use within the manufacturer-specified expiration date. Store at 4 ± 2 °C.
- (b) Hydrazine Initial Calibration Verification Stock Solution (ICVSt), 500 $\mu\text{g}/\text{mL}$. Purchase a NIST-traceable manufacturer-certified solution in aqueous matrix from a source independent from that of the CalSt. Use within the

manufacturer-specified expiration date. Store at 4 ± 2 °C.

[B.2] Intermediate Stock and Working Standards

Document the preparation of all intermediate and working standards in the HPLC Standard Preparation Logbook. Store all intermediate and working standards at 4 ± 2 °C.

- (a) Calibration Intermediate Stock Standard, 100 µg/mL. Prepare weekly with use by aliquoting 1.00 mL of the Hydrazine CalSt (see Item [B.1](a)) into a 10-mL glass volumetric flask, diluting to volume with water, and mixing thoroughly.
- (b) Working Calibration Standards. Prepare daily with use by aliquoting the volumes of 0.1 M H₂SO₄ (see Item [A.3]) and 100 µg/mL Calibration Intermediate Stock Standard (see Item [B.2](a)) specified below in Table 1, Working Calibration Standard Preparation, into glass vials with PTFE-lined caps, and mixing thoroughly.

Table 1. Working Calibration Standard Preparation

Working Standard	0.1 M H ₂ SO ₄ (mL)	100 µg/mL Calibration Intermediate Stock Standard (mL)
Calibration blank	8.00	0.000
0.5 µg/mL	7.960	0.040
1.0 µg/mL	7.920	0.080
2.5 µg/mL	7.800	0.200
5.0 µg/mL	7.600	0.400
10.0 µg/mL	7.200	0.800

NOTE

Concentrations of working calibration standards other than those listed may be used if they better approximate concentration expected in the samples.

- (c) ICV Working Standard, 5.00 µg/mL. Prepare daily with use by aliquoting 0.500 mL of the Hydrazine ICVSt (see Item 2.3.2[B.1](b)) into a 50-mL volumetric flask, diluting to volume using 0.1 M H₂SO₄ (see Item [A.3]), and mixing thoroughly.

2.4 Sample Handling

2.4.1 Store all samples and extracts at 4 ± 2 °C.

2.4.2 Extract samples within 14 days of collection.

2.4.3 Derivatize sample extracts within 28 days of extraction.

2.4.4 Analyze derivatized samples within 3 days of derivatization.

2.4.5 Analyze samples in analytical batches. An analytical batch consists of a suite of samples of similar matrix that is processed as a unit, using the same analytical method, within a specific time period. An analytical batch can contain up to 20 samples, excluding laboratory Quality Control (QC) samples, all of which must be received by the laboratory within 14 days of validated time of sample receipt (VTSR) of the first sample in the batch.

2.5 Precautions and Limitations

2.5.1 Chemical Hazards

- [A] Handle all chemicals per MCP-3635, *Chemical Hygiene Plan*.
- [B] Wear the following standard laboratory personal protective equipment (PPE) in the lab:
 - (a) Lab coat
 - (b) Safety glasses with side shields
 - (c) Substantial footwear
 - (d) Appropriate gloves
- [C] Change gloves immediately upon chemical contact.
- [D] Handle corrosives per ACLP-0.27, *Routine Handling of Corrosives*.
- [E] Handle all spills per ACLP-0.24, *Laboratory Spill Cleanup*.

2.5.2 Radiological Hazards

- [A] Perform all work under an applicable Radiation Work Permit (RWP) per MCP-7, *Radiological Work Permit*.
- [B] Use safety coated glass bottles while mixing sample extracts on the orbital shaker.

2.5.3 Physical Hazards

- [A] Use a holding device or passive shielding technique for recapping syringes.
- [B] Dispose of syringe needles in appropriate containers.

2.5.4 Waste Disposition and Pollution Prevention

- [A] Manage all waste generated per ACLP-0.40, *Analytical Laboratory Waste Management*.

2.6 Prerequisite Actions

2.6.1 None.

2.7 Definitions

2.7.1 None.

2.8 Quality Control Requirements

2.8.1 Method Performance Demonstration

[A] Method Detection Limit (MDL) Determination

[A.1] Determine the Method Detection Limit (MDL) per Section 4.9 initially before analyzing samples.

[A.2] Redetermine the MDL at least every 6 months with use.

[A.3] Redetermine the MDL whenever there is a change in the method that affects how the test is performed or when a change in instrumentation occurs that affects the sensitivity of the analysis (e.g., when the analytical column is changed).

[B] Precision and Accuracy (P&A) Demonstration

[B.1] Determine the Precision and Accuracy (P&A) per Section 4.10 initially before analyzing samples.

[B.2] Redetermine the P&A at least every 6 months with use.

[B.3] Redetermine the P&A whenever there is a change in the method that affects how the test is performed or when a change in instrumentation occurs that affects the sensitivity of the analysis (e.g., when the analytical column is changed).

2.8.2 Initial Calibration

[A] Perform an initial calibration daily with use per Section 4.6.

- [B] Calibrate using a minimum of five standards and a blank, with the concentration of at least one standard less than the program-required quantitation limit (PRQL).
- [C] Ensure that the calibration and initial calibration verifications meet acceptance criteria defined in Appendix A, Acceptance Criteria and Required Corrective Actions, before analyzing samples.

2.8.3 Analytical Batch and Daily Instrument QC Requirements

- [A] Analyze analytical batch and daily instrument QC samples as identified in Appendix A.

2.9 Calculations

2.9.1 Arithmetic Mean

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n}$$

where:

- \bar{x} = arithmetic mean
- x_i = i^{th} data point
- n = total number of data points.

2.9.2 Standard Deviation

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}}$$

where:

- s = standard deviation
- x_i = i^{th} data point
- \bar{x} = arithmetic mean
- n = total number of data points.

2.9.3 Least Squares Linear Regression

$$x = \frac{y - b}{m}$$

where:

x = target analyte concentration

y = instrument response

m = slope of instrument response (analyte amount per unit response)

b = y-intercept (zero-analyte response).

The regression factors m and b are calculated as follows:

$$m = \frac{n \sum_{i=1}^n x_i y_i - \left(\sum_{i=1}^n x_i \right) \left(\sum_{i=1}^n y_i \right)}{n \sum_{i=1}^n x_i^2 - \left(\sum_{i=1}^n x_i \right)^2} \quad b = \bar{y} - m\bar{x}.$$

x_i = target analyte concentration of i^{th} calibration point

y_i = instrument response of i^{th} calibration point

n = number of calibration points

\bar{y} = mean instrument response of n calibration points

\bar{x} = mean target analyte concentration of n calibration points

2.9.4 Correlation Coefficient (r^2)**NOTE**

The value for r^2 is calculated by squaring the calculated r value.

$$r = \frac{\sum_i ((C_i - \bar{C})(A_i - \bar{A}))}{\sqrt{\left(\sum_i (C_i - \bar{C})^2\right)\left(\sum_i (A_i - \bar{A})^2\right)}}$$

where:

C_i = concentration of analyte being injected

\bar{C} = concentration calculated from the regression line

A_i = total area response for the analyte

\bar{A} = area response from the regression line.

2.9.5 Sample Concentration in milligram per kilogram

$$\text{Concentration (mg/kg)} = \frac{C_x \times V_f \times D}{W_x} \times \frac{1000g}{kg} \times \frac{mg}{1000\mu g}$$

where:

C_x = calculated concentration ($\mu\text{g/mL}$) from the HPLC data station

V_f = final volume of derivatized sample (mL)

W_x = initial sample weight (g)

D = dilution factor.

2.9.6 Percent Recovery (%R)

[A] ICVs, Continuing Calibration Verifications (CCVs),
Laboratory Control Samples (LCSs), and P&A Standards

$$\%R = \frac{C_m}{C_t} \times 100$$

where:

C_m = measured concentration ($\mu\text{g/mL}$ or mg/kg) of the
standard

C_t = true (known) concentration ($\mu\text{g/mL}$ or mg/kg) of the
standard.

[B] Matrix Spike/Matrix Spike Duplicate (MS/MSD) Recovery

$$\%R = \frac{SSR - SR}{SA} \times 100$$

where:

SSR = measured concentration in the spiked sample (mg/kg)

SR = measured concentration in the unspiked sample
(mg/kg) ($SR=0$ if the analyte was not detected in the
unspiked sample)

SA = known concentration of the spike added (mg/kg).

2.9.7 Relative Percent Difference (RPD)

$$RPD = \frac{|S - D|}{\frac{(S + D)}{2}} \times 100 = \frac{|S - D|}{(S + D)} \times 200$$

where:

S = %R of the MS

D = %R of the MSD.

2.9.8 Percent Relative Standard Deviation (%RSD)

$$\%RSD = \frac{s}{\bar{x}} \times 100$$

where:

s = standard deviation (Equation 2.9.2) (mg/kg)

\bar{x} = mean of replicate analyses (Equation 2.9.1) (mg/kg).

2.9.9 Method Detection Limit (MDL)

$$MDL = t_{(n-1, \alpha=0.99)} \times s$$

where:

s = standard deviation (Equation 2.9.2) (mg/kg)

$t_{(n-1, \alpha=0.99)}$ = student-t statistic for a 99 percent confidence level (one tailed) and standard deviation estimate with n-1 degrees of freedom (n is the number of measurements).

n = number of replicate measurements obtained

t-Distribution values for n=7 to n=11

n	n-1	$t_{(n-1, 1-\alpha=0.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764

3.0 RESPONSIBILITIES

3.1 HPLC Operator

3.1.1 Performs this procedure.

4.0 PROCEDURE

4.1 Extracting Samples

4.1.1 Prepare the laboratory blank (LB).

- [A] Weigh 2.5 g of reagent sand (see Item 2.3.2[A.6]) into a 125-mL safety-coated glass bottle with a PTFE-lined cap.
- [B] Add 25 mL of 0.1 M H₂SO₄ (see Item 2.3.2[A.3]).
- [C] Label the LB as LB-LLLL-PP where “LLLL” is the Hydrazine Sample Preparation Logbook number and “PP” is the logbook page number.

4.1.2 Prepare the Laboratory Control Sample (LCS).

- [A] Weigh 2.5 g of reagent sand (see Item 2.3.2[A.6]) into a 125-mL safety-coated glass bottle with a PTFE-lined cap.
- [B] Add 25 mL of 0.1 M H₂SO₄ (see Item 2.3.2[A.3]).
- [C] Aliquot 250 µL of the 1000 µg/mL Hydrazine CalSt (see Item 2.3.2[B.1](a)) directly into the bottle.
- [D] Record the volume and identity of the solution added in the preparation logbook.

NOTE

An LCS spiking amount other than that specified may be used, provided that the actual volume used is recorded in the preparation log.

- [E] Label the LCS as LCS-LLLL-PP where “LLLL” is the Hydrazine Sample Preparation logbook number and “PP” is the logbook page number.

4.1.3 Aliquot each sample in the analytical batch.

NOTE

At least one MS and one MSD are required for each analytical batch.

- [A] Mix the sample thoroughly.

- [B] Weigh 2.5 ± 0.2 g of sample into a 125-mL safety-coated glass bottle with a PTFE-lined cap and record the weight to at least 0.01 g in the preparation logbook.
- [C] **IF** the sample is selected for MS and MSD preparation, **THEN** weigh two additional 2.5 ± 0.2 g sample aliquots into separate 125 mL safety-coated glass bottles with PTFE-lined caps.
- [C.1] Record the identifiers for the spike and spike duplicate as the lab sample identification (ID) plus the suffix "MS" for the spike and "MSD" for the spike duplicate.
- [D] Add 25 mL of 0.1 M H₂SO₄ (see Item 2.3.2[A.3]).

- 4.1.4 Aliquot 250 μ L of the 1000 μ g/mL Hydrazine CalSt (see Item 2.3.2[B.1](a)) directly into both the MS and MSD bottles, and record the volume and identity of the solutions added.
- 4.1.5 Ensure the bottles are tightly capped and then tape around the bottle lid using electrical tape.
- 4.1.6 Seal each bottle in two plastic zip-top bags and tape the top of the outer bag with duct tape.
- 4.1.7 Place the samples in an orbital shaker and rotate at ~200 rpm for at least 30 minutes.
- [A] **IF** the ambient room temperature is <25 °C, **THEN** set the temperature on the orbital shaker to 25 °C.
- 4.1.8 Transfer the contents of the bottle to a 50-mL volumetric flask.

NOTE

Quantitative transfer of the undissolved solids to the volumetric flasks is not required or desirable.

- [A] Use a plastic funnel and water rinses to transfer the leachate from the bottle to the volumetric flask.
- [B] Rinse the bottle walls and undissolved solids thoroughly.
- [C] Dilute the sample in the volumetric flask to volume with water and mix thoroughly.

- 4.1.9 Allow the solution to stand in order to separate undissolved solids.
- 4.1.10 **IF** the undissolved solids do not adequately separate,
THEN transfer the solution to a centrifuge tube and centrifuge at
~2500 rpm for ~10 minutes.
- 4.1.11 Filter the leachate into a 125-mL glass bottle with a PTFE-lined cap
using a syringe filter.
- 4.1.12 Evaluate the pH of the sample extract by placing a few drops of the
sample extract on a piece of pH paper.

NOTE

Sample extract pH between 2 and 4 is required for derivatization.

[A] **IF** the pH <2,
THEN adjust the pH of the sample extract by adding
50% NaOH (see Item 2.3.2[A.4]) drop-wise until the pH is
between 2 and 4.

[B] **IF** the pH >4,
THEN adjust the pH of the sample extract by adding
9 M H₂SO₄ (see Item 2.3.2[A.2]) drop-wise until the pH is
between 2 and 4.

4.1.13 Store the extracts at 4 ± 2 °C until derivatization.

4.2 Derivatizing Working Standards and Sample Extracts

4.2.1 Transfer 2 mL of each working standard and each sample extract
into 20-mL pre-cleaned glass vials.

4.2.2 Add 2 mL of 1% benzaldehyde solution (see Item 2.3.2[A.8]) to
each vial.

4.2.3 Add 2 mL of 0.01 M sodium borate working solution (see
Item 2.3.2[A.9]) to each vial.

4.2.4 Add 1 mL of methanol (see Item 2.3.2[A.5]) to each vial.

4.2.5 Cap and mix each vial for ~10 seconds using a vortex mixer.

4.2.6 Allow the mixed solutions to sit at room temperature for at least
30 minutes.

4.2.7 Store the derivatized solutions at 4 ± 2 °C until analysis.

4.3 Preparing Sample Extracts and Dilutions for Analysis

4.3.1 **IF** sample extract dilutions are necessary based on screening results, known concentrations, or process knowledge, **THEN** prepare extract dilutions using 0.1 M H₂SO₄ (see Item 2.3.2[A.3]) as the diluent.

[A] Dilute sample extracts exceeding the calibration range such that the hydrazine concentration is brought into the upper half of the calibration range.

[B] Derivatize the diluted sample extract per Section 4.2.

4.3.2 Transfer ~1 mL of all derivatized sample solutions, QC samples and working standards to be analyzed into 2-mL autosampler vials.

4.3.3 Cap the vials.

4.4 Setting up the Instrument

NOTE

This section provides recommended HPLC operating parameters. Operating parameters may be optimized when necessary. Actual operating parameters used for sample analysis match those used for the associated calibration.

4.4.1 Set up the HPLC per the manufacturer instructions with the following operating parameters:

NOTE

The mobile phase can be either pre-mixed or mixed by the instrument.

[A] Mobile phase 5:95 water/methanol

[B] Flow rate 1 mL/min

[C] Detector Ultraviolet, 313 nm

[D] Injection volume 25 µL.

4.4.2 Ensure mobile phase reagents have been filtered/degassed using the Mobile Phase Filter Degasser.

4.4.3 Ensure sufficient mobile phase is available for the analytical run.

- 4.4.4 Ensure the column oven is set at 30 °C and allow the oven to warm to operating temperature.
- 4.4.5 Turn on the photodiode array detector lamp and allow the lamp to warm for at least 30 minutes.
- 4.4.6 Equilibrate the system by pumping mobile phase until a stable baseline is obtained.
- [A] **IF** the system background is unstable,
THEN consult supervision for guidance.

4.5 Determining the Retention Time (RT) Window

NOTE

Retention time (RT) window determination is performed initially and then as needed based on instrument maintenance or QC results.

- 4.5.1 Analyze a derivatized working standard (e.g., ICV Working Standard, see Item 2.3.2[B.2](c)) at least three times during a period of at least 72 hours.
- 4.5.2 Calculate the mean absolute RT (Equation 2.9.1) and standard deviation (Equation 2.9.2) of the hydrazine peak.
- 4.5.3 Establish the RT window for hydrazine as $\pm 3\sigma$ around the mean absolute RT.
- [A] **IF** $\sigma < 0.05$ minutes,
THEN use $\sigma = 0.05$ minutes as the default to establish the RT window.
- [B] Set the RT window in the data system to be no greater than the established RT window.
- 4.5.4 **IF** changes in instrument operating conditions or maintenance (e.g., column change) affect the RT of hydrazine,
THEN redetermine the RT window.

4.6 Performing Initial Calibration

NOTE

Initial calibration is performed daily with use.

- 4.6.1 Inject the derivatized Working Calibration Standards, starting with the calibration blank and increasing in concentration to the highest concentration standard.
 - 4.6.2 Establish a linear regression calibration for hydrazine (see Equation 2.9.3).
 - 4.6.3 Evaluate the initial calibration against the acceptance criteria defined in Appendix A and take corrective actions indicated as necessary.
 - 4.6.4 Establish the daily RT window by re-centering the initial RT window around the RT of the mid-point calibration standard.
 - 4.6.5 Analyze the ICV working standard (see Item 2.3.2[B.2](c)).
 - 4.6.6 Evaluate the ICV against the acceptance criteria defined in Appendix A, and take corrective actions indicated as necessary.
 - 4.6.7 Analyze the calibration blank solution as the initial calibration blank (ICB) verification.
 - 4.6.8 Evaluate the ICB against the acceptance criteria defined in Appendix A, and take corrective actions indicated as necessary.
- 4.7 Analyzing Samples
- 4.7.1 Inject samples and QC samples, ensuring that every 10 injections are bracketed by a CCV/Continuing Calibration Blank Verification (CCB) pair.

NOTE

The ICV and ICB may function as the first CCV/CCB. The ICV solution or midrange calibration standard may be used as the CCV. The ICB solution or calibration blank may be used as the CCB.

NOTE

It is recommended that the LB and the LCS be analyzed first.

- 4.7.2 Compare the results obtained for each QC sample to the acceptance criteria in Appendix A.
- [A] **IF** the results obtained meet the acceptance criteria in Appendix A,
THEN continue with the next analysis in the run sequence.
- [B] **IF** the results obtained do **NOT** meet acceptance criteria in Appendix A,
THEN complete the specified corrective action.
- 4.7.3 **IF** a benzaldehyde artifact peak is **NOT** present in the sample chromatogram,
THEN prepare a dilution of the extract per Section 4.3 and analyze per Section 4.7.
- 4.7.4 **IF** the measured sample concentration exceeds the calibration range,
THEN prepare a dilution of the extract per Section 4.3 and analyze per Section 4.7.
- 4.7.5 **IF** a sample saturates the detector or exceeds the calibration range by a factor of 10 or greater,
THEN analyze a cleaning blank to check for system contamination.
- [A] **IF** the cleaning blank meets the acceptance criterion for CCBs in Appendix A,
THEN continue with sample analysis.
- [B] **IF** the cleaning blank does **NOT** meet the acceptance criterion for CCBs in Appendix A,
THEN decontaminate the system and demonstrate a passing cleaning blank before continuing with sample analysis.

4.7.6 **IF** the daily retention time alone is insufficient to identify hydrazine due to poor resolution or coeluting interferences, **THEN** accurately measure 5 mL of the sample extract into a clean, glass vial.

[A] Spike the sample extract using 0.125 mL of the 100 µg/mL Calibration Intermediate Stock Standard (see Item 2.3.2[B.2](a)).

[B] Cap the vial and mix.

[C] Re-derivatize and prepare the spiked sample extract for analysis per Sections 4.2 and 4.3.

[D] Analyze the spiked extract per Section 4.7 to confirm the identity of the hydrazine peak.

4.7.7 Examine the full chromatogram.

[A] Evaluate the peak integration, and accept or edit as necessary.

[B] **IF** manual integration is performed, **THEN** document the manual integration and its justification.

4.8 Reporting Data

4.8.1 Calculate the sample results to two significant figures in units of milligram per kilogram using Equation 2.9.5.

4.8.2 Report sample results per CCP-TP-188, *CCP Analytical Data Recording, Review, and Reporting*.

[A] Report sample concentrations measured as less than the MDL as “MDL U,” corrected for any sample dilution.

[B] Report sample concentrations between the MDL and the PRQL (100 mg/kg) as the concentration flagged with a “J” qualifier.

[C] Use other data qualifier flags as required by CCP-TP-188.

4.9 Determining the Method Detection Limit (MDL)

NOTE

The frequency for MDL determination is specified in Section 2.8.1[A].

- 4.9.1 Determine the MDL from preparation and analysis of a minimum of seven replicate standards containing the hydrazine in concentrations 2-10× the expected MDL.
- 4.9.2 Prepare and analyze the MDL standards using the Calibration Intermediate Stock Standard (see Item 2.3.2[B.2](a)) as the spiking solution, per instructions in Sections 4.1– 4.4, 4.6, and 4.7.
- 4.9.3 Determine the MDL per Equation 2.9.9.
- 4.9.4 **IF** the MDL does **NOT** meet the acceptance criteria defined in Appendix A,
THEN complete the specified corrective action.

4.10 Demonstrating Method Precision and Accuracy (P&A)

NOTE

The frequency of P&A demonstration is specified in Section 2.8.1[B].

- 4.10.1 Determine precision and accuracy (P&A) from preparation and analysis of a minimum of seven replicate standards containing hydrazine in quantifiable concentrations less than or equal to the PRQL.
- 4.10.2 Prepare and analyze the P&A standards using the Calibration Intermediate Stock Standard (see Item 2.3.2[B.2](a)) as the spiking solution, per instructions in Sections 4.1–4.4, 4.6, and 4.7.
- 4.10.3 Calculate the mean %R per Equations 2.9.6[A] and 2.9.1 to establish the accuracy of the method.
- 4.10.4 Calculate the %RSD per Equation 2.9.8 to determine the method precision.
- 4.10.5 **IF** the P&A demonstration does **NOT** meet the acceptance criteria defined in Appendix A,
THEN complete the specified corrective action.

5.0 RECORDS

5.1 Records generated during the performance of this procedure are maintained as quality assurance (QA) records in accordance with CCP-QP-008, *CCP Records Management*. The records are the following:

5.1.1 QA Nonpermanent

[A] RT Window Determination Files

[B] MDL Determination File

[C] P&A Demonstration File

5.2 The following records generated during the performance of this procedure will be compiled into the Supporting Data Package, in accordance with CCP-TP-188.

5.2.1 QA Nonpermanent

[A] Copy of applicable pages of the HPLC Standard Preparation Logbook

[B] Copy of applicable pages of the Hydrazine Sample Preparation Logbook

[C] Copy of applicable pages of HPLC run logbook

[D] Instrument Printouts/Raw Data

Appendix A – Acceptance Criteria and Required Corrective Actions

QC Parameter	Minimum Frequency	Acceptance Criteria	Corrective Actions ^a
Calibration (ICAL)	Daily with use	<ul style="list-style-type: none"> • Five standards (minimum) and a blank • Correlation coefficient ≥ 0.990 • RTs within RT window • Acceptable ICV and ICB 	Troubleshoot as necessary and repeat calibration. Do not proceed with the analysis until the criteria are met.
ICV	Daily after ICAL	<ul style="list-style-type: none"> • %R = 85–115 • RT within daily RT window 	Reprepare and reanalyze ICV. If acceptance criteria still not met, recalibrate.
ICB	Daily after ICV	Less than or equal to solution-equivalent of program-required MDL (10 mg/kg)	Reprepare and reanalyze ICB. If acceptance criterion still not met, recalibrate.
CCV	Every 10 analytical sample injections, and at end of run	<ul style="list-style-type: none"> • %R = 85–115 • RT within daily RT window 	Reprepare and reanalyze CCV. If acceptance criteria still not met, recalibrate and reanalyze all analytical samples since the last compliant CCV.
CCB	After every CCV	Less than or equal to solution equivalent of program-required MDL (10 mg/kg)	Reprepare and reanalyze CCB. If acceptance criterion still not met, recalibrate and reanalyze all analytical samples since the last compliant CCB.
LCS	One per analytical batch	%R = 60–150	Reanalyze the LCS. If the LCS still does not meet acceptance criterion, reprocess and reanalyze all associated samples.

Appendix A – Acceptance Criteria and Required Corrective Actions (Continued)

QC Parameter	Minimum Frequency	Acceptance Criteria	Corrective Actions ^a
LB	One per analytical batch	≤10 mg/kg (program-required MDL)	Reanalyze the LB. If the LB still does not meet acceptance criterion, reprocess and reanalyze all associated samples.
MS/MSD	One pair per analytical batch	%R = 60–150 RPD ≤50	Qualify sample results per CCP-TP-188.
MDL	Initially and every 6 months thereafter with use	≤10 mg/kg (program-required MDL)	Identify and correct problem and repeat MDL determination.
P&A Demonstration	Initially and every 6 months thereafter with use	%R = 60–150 %RSD ≤50	Identify and correct problem, repeat P&A demonstration.
<p>a. Initiate a nonconformance report (NCR) per CCP-QP-005, <i>CCP TRU Nonconforming Item Reporting and Control</i>, when final reported QC samples do not meet the acceptance criteria and the exceedance is not matrix-related. Flag associated sample results with a “Z” qualifier.</p>			