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1.0 Purpose

This procedure defines how to make accurate stock standard solutions of organic and inorganic compounds in reagent water that are traceable to certified materials. Working standard solutions are then prepared by accurately diluting these stock solutions with reagent water.

2.0 Scope

The scope of this procedure includes preparation of standards ranging in concentrations from “0” (no) added carbon through 5.0 ppm of organic carbon in the stock solution. The working standards can be used to directly calibrate Ranges 1, 2, 3, 4 and 5 of the Total Organic Carbon Analyzer.

3.0 Authority and Responsibility

- 3.1 The Director of Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.
- 3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).
- 3.3 PA/QC personnel are responsible for the performance of this procedure.
- 3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.
- 3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Reagents

- Reagent Water

The reagent water used must be of the highest quality deionized, distilled or reverse osmosis water available, containing no more than 0.1 ppm C for low-level analysis.

Blank Water

Reagent water that has been treated and manipulated as if it were a standard but contains "0" (no) added carbon from known sources.

- Potassium Hydrogen Pthalate (KHP) C₈H₅KO₄

- Grade: NIST Standard
- Purity: 99.95% - 100.05%
- Molecular Weight: 204.22
- % Carbon 47.05
- BDP PN: 30428

- Sucrose C₁₂H₂₂O₁₁

- Grade: USP Reference Standard <11> or equivalent
- Molecular Weight: 342.30
- % Carbon: 42.10
- BDP PN: 30430

- 1, 4 - Benzoquinone C₆H₄O₂

- Grade: USP reference Standard <11> or equivalent
- Molecular Weight 108.09
- % Carbon 66.67
- BDP PN: 30005

- Glassware

All volumetric glassware and pipettes that are used in this procedure must be serialized, certified, and Class A quality. The surface must be as free of carbon as possible and be cleaned using **SOP 22918 - Glassware Cleaning for Trace TOC Analysis**. The glassware must be dedicated for TOC analysis to minimize carbon contamination.

Volumetric flasks are recommended for all concentration standards. The pipette volumes will range from 1 to 50 milliliter depending on the concentrations being prepared. The pipettes must be of the type that are calibrated "to deliver" their volume. Volumetric pipettes must be used for the large volumes.

- Analytical Balance, within calibration, accurate to 0.1 milligram.

5.0 Procedure

Label each flask with the name of the solution including the concentration, date prepared, expiration date, and initials of the preparer.

5.1 Preparation of Stock Calibration Solutions

5.1.1 Sucrose/H₂O 40 ppm C

5.1.1.1 If necessary, dry Sucrose crystals and store in a desiccator. If drying is needed, dry at 105°C for 2 hours in appropriate equipment prior to preparation of the stock solution.

5.1.1.2 Weigh 95 mg ± 4.0 mg of sucrose on a weigh boat and record the exact weight.

5.1.1.3 Transfer the sucrose to a 1000 milliliter volumetric flask which is half full of reagent water.

5.1.1.4 Squirt the weigh boat with reagent water, from a squirt bottle, until all of the crystals have been transferred to the flask.

5.1.1.5 Invert the flask to dissolve the sucrose. Sonicate if needed.

5.1.1.6 Fill the flask to the 1000 milliliter mark with reagent water.

5.1.1.7 Seal the container with a flask stopper of appropriate size and invert the flask 10 times to equilibrate the solution.

5.1.1.8 The 40 ppm C solution is good for 1 month and stored at 2°-8°C.

5.1.1.9 Record the preparation of the Stock Calibration Solutions in the reagent logbook, per **SOP 22702 - Solutions Used in BQC**.

5.1.2 1, 4 - Benzoquinone/H₂O 40 ppm C 1000 milliliter

(Stock System Suitability/Calibration Solution routine analysis)

5.1.2.1 Accurately weigh 60 mg ± 3 mg of 1, 4 - Benzoquinone on a weigh boat and record the exact gross weight.

- 5.1.2.2 Transfer the 1, 4 - Benzoquinone to a clean, dedicated 1000 milliliter volumetric flask which is half full of reagent water.
- 5.1.2.3 Squirt the weigh boat with reagent water from a squirt bottle until all of the crystals have been transferred to the flask.
- 5.1.2.4 Swirl, and invert the flask to dissolve the 1, 4 - Benzoquinone. If required, sonicate to dissolve.
- 5.1.2.5 Fill the flask to the 1000 milliliter mark with reagent water.
- 5.1.2.6 Seal the container with a flask stopper of appropriate size and invert the flask 10 times to equilibrate the solution.
- 5.1.2.7 This 40 ppm C solution is good for 1 month and stored at 2°-8°C.
- 5.1.2.8 Record the preparation in the reagent logbook, per **SOP 22702 - Solutions Used in BQC**.

5.2 Preparation of Working Calibration Solutions

5.2.1 Preparation of the 5 ppm C WCS

- 5.2.1.1 Fill a 200-milliliter volumetric flask approximately half full with reagent water.
- 5.2.1.2 Transfer 25 mL of stock calibration solution into the flask with volumetric pipettes.
- 5.2.1.3 Swirl the flask a minimum of 10 seconds to mix.
- 5.2.1.4 Fill the flask to the 200-milliliter mark with reagent water.
- 5.2.1.5 Seal the container with a flask stopper of appropriate size and invert the flask 10 times to equilibrate the solution.
- 5.2.1.6 This is a 5 ppm C solution.
- 5.2.1.7 This 5 ppm C solution is good for 1 week and is to be stored at 2°-8°C.
- 5.2.1.8 Record preparation on Form 22919-01.

5.2.2 Preparation of 2 ppm C WCS

- 5.2.2.1 Fill a 500-milliliter volumetric flask approximately half full with reagent water.
- 5.2.2.2 Transfer 25 mL of the stock calibration solution into the flask with a volumetric pipette.
- 5.2.2.3 Swirl the flask a minimum of 10 seconds to mix.
- 5.2.2.4 Fill the flask to the 500-milliliter mark with reagent water.
- 5.2.2.5 Seal the container with a flask stopper of appropriate size and invert the flask 10 times to equilibrate solution.
- 5.2.2.6 This is a 2 ppm C solution.

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- 5.2.2.7 This 2 ppm C solution is good for 1 week and is to be stored at 2°-8°C.
- 5.2.2.8 Record preparation on Form 22919-01.
- 5.2.3 Preparation of 1 ppm C WCS
 - 5.2.3.1 Fill a 2.0-liter volumetric flask approximately half full with reagent water.
 - 5.2.3.2 Transfer 50 mL of the stock calibration solution with a volumetric pipette.
 - 5.2.3.3 Swirl the flask a minimum of 10 seconds to mix.
 - 5.2.3.4 Fill the flask to 2.0-liter mark.
 - 5.2.3.5 Seal the container with a flask stopper of appropriate size and invert flask 10 times to equilibrate solution.
 - 5.2.3.6 This is a 1 ppm C solution.
 - 5.2.3.7 This 1 ppm C solution is good for 1 week and is to be stored at 2°-8°C.
 - 5.2.3.8 Record preparation on Form 22919-01.
- 5.2.4 Preparation of a 0.5 ppm C WCS
 - 5.2.4.1 Fill a 2.0-liter volumetric flask approximately half full with reagent water.
 - 5.2.4.2 Transfer 25 mL of the stock calibration solution with a volumetric pipette.
 - 5.2.4.3 Swirl the flask a minimum of 10 seconds to mix.
 - 5.2.4.4 Fill the flask to the 2.0-liter mark.
 - 5.2.4.5 Seal the container with a flask stopper of appropriate size and invert the flask 10 times to equilibrate solution.
 - 5.2.4.6 This is a 0.5 ppm C solution.
 - 5.2.4.7 This 0.5 ppm C solution is good for 1 week and is to be stored at 2°-8°C.
 - 5.2.4.8 Record preparation on Form 22919-01.
- 5.2.5 Preparation of a 0.2 ppm C WCS.
 - 5.2.5.1 Fill a 2.0-liter volumetric flask approximately half full with reagent water.
 - 5.2.5.2 Transfer 10 mL of the stock calibration solution with a volumetric pipette.
 - 5.2.5.3 Swirl the flask a minimum of 10 seconds to mix.
 - 5.2.5.4 Fill the flask to the 2.0-liter mark.
 - 5.2.5.5 Seal the container with a flask stopper of appropriate size and invert the flask 10 times to equilibrate solution.
 - 5.2.5.6 This is a 0.2 ppm C solution.

5.2.5.7 This 0.2 ppm C solution is good for 1 week and is to be stored at 2°-8°C.

5.2.5.8 Record preparation on Form 22919-01.

5.2.6 Preparation of a 0.0 ppm C WCS

5.2.6.1 Fill a 2.0-liter volumetric flask with reagent water.

5.2.6.2 This 0.0 ppm C solution is good for 5 days at 2°-8 °C.

NOTE: A 40 ppm C stock solution of 1.4 BQ (5.3) can be made and used in place of Sucrose in preparation of Working Calibration Solutions (WCS).

NOTE: If different sized flasks are to be used, adjust the weights or volumes added accordingly to achieve the required concentration.

5.3 Preparation of USP <643> Total Organic Carbon “Standard Solution” and “System Suitability Solution.”

Both of these solutions contain 0.50 mg C/L of their respective compounds.

5.3.1 Standard Solution from Sucrose stock (0.5 ppm c)

5.3.1.1 Solution as prepared in 5.2.4

5.3.2 System Suitability Solution for 1, 4 - Benzoquinone

5.3.2.1 Solution as prepared in 5.2.4.

5.4 Cap and store the stock and working calibration solutions in a 2° - 8°C refrigerator; however, they need to be brought to room temperature prior to use.

6.0 Calculations

6.1 Stock Calibration Solutions

Use the following calculation to determine the carbon concentration of the stock solutions.

$$\text{mg C/L} = \frac{[\text{gross weight (mg)} - \text{tare weight (mg)}] \cdot \% \text{ carbon (C)}}{\text{Final volume (L)}}$$

(ppm C)

where % (C) = decimal fraction of carbon in the reference material,

KHP = 0.4705

1,4 Benzoquinone = 0.6667

Sucrose = 0.4210

6.2 Working Calibration Solutions

Use the following calculation to determine the carbon concentration added to the reagent water of the working calibration solutions.

$$\begin{aligned} \text{mg C/L} &= \frac{\text{stock conc. (mg C/L)} \cdot \text{transfer volume (mL)}}{\text{final volume (L)} \cdot 1000} \\ (\text{ppm C}) &= \end{aligned}$$

7.0 Documentation

- 7.1 Record the calibration standards in the logbook kept in the BQC laboratory, per **SOP 22702 - Solutions Used in Process Analytics**.

8.0 Definitions

- 8.1 **Stock Calibration Solutions**– A concentrated solution of a known amount of organic compound in water H₂O made from a certified reference material. Ideally, the final concentration of the stock solution is at least 100 times more concentrated than the background carbon present in the reagent water used to make this standard.
- 8.2 **Working Calibration Solutions**– A quantitative spike of a stock calibration solution into reagent water. These solutions and the amount of carbon added to the reagent water are to be used to develop the calibration curve for the TOC Analyzers. The working solutions are also used to test the analyzer for performance and system suitability as per USP <643> on TOC.

9.0 References and Related Documents

- 9.1 **SOP 22918** *Glassware Cleaning for Trace TOC Analysis*
- 9.2 **SOP 22702** *Solutions Used in Process Analytics*
- 9.3 Phoenix 8000 User Manual, Part Number 14-7045-074.
- 9.4 USP Method <643> Total Organic Carbon.
- 9.5 **Form 22919-01** *Working Calibration Standards Preparation*

10.0 Change Summary

