Miscellaneous Operating Procedure

I. **TITLE**: Method for analyzing AQUI-S with GIBBS reagent

II. **PURPOSE**: Describe procedures for preparing AQUI-S standards and unknown water samples for analysis of AQUI-S.

III. **MATERIALS**

A. Dose-verification Materials
1. HEPES buffer (n[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid])
   a. 2.383 g
2. GIBBS reagent (2,6-Dichloroquinone-4-Chlorimide)
   a. 0.025 g
3. Spectrophotometric grade ethyl alcohol
   a. 100 mL
4. 1,000 mL graduated cylinder
5. 25 mL volumetric flask (1) and stopper
6. 100 mL volumetric flasks (7) and stoppers
7. 250 volumetric flasks (1) and stopper
8. 2,000 mL volumetric flask (1) and stopper
9. 125 mL Erlenmeyer flask or equivalent
10. 250 mL Erlenmeyer flask or equivalent
11. 1,000 mL nalgene-type bottle (1) or equivalent
12. Volumetric auto-pipetters and tips to deliver 0.5, 1.0, 2.0, and 4.0 mL
13. 5 or 10 mL volumetric pipette (1) and pipette bulb (1)
14. 14 reagent tubes and caps for 6-point calibration standards and 20 mg/L standard
15. reagent tubes and caps for unknowns
16. Smaller (1) and larger funnel (1)
17. 25 mL graduated cylinder (1) or equivalent
18. Parafilm or equivalent
19. Scissors
20. Disposable pipettes (5)
21. Vortex
22. Squirt bottle (1)
23. AQUI-S pre-weighed into a 50 - 400 mL plastic bottle with cap
    a. 2.00 g

B. Sample Collection and Analysis Materials
1. Spectrophotometer
2. Cuvettes (>40)
3. Cuvette racks (1–3)
4. Kimwipes
5. 50 - 400 mL plastic bottles with caps (>10)

IV. PROCEDURES:

A. General considerations:

1. Volumetric flasks, volumetric pipettes, and automatic pipetters may be used as appropriate in the following procedures to ensure accuracy of volumes of liquids measured.

2. If prepared solutions are not used immediately after preparation, they must be stored in labeled containers with covers (i.e., Parafilm™, caps, or stoppers).

3. Prepare standards using the same water (i.e., source water) that will be used to prepare working solutions of AQUI-S.

B. Reagents:

1. HEPES Buffer 10 mM, pH 7.5

   Make up a stock solution of 10 mM HEPES by weighing out **2.383 g** of HEPES and dissolving it in approximately 200 - 600 mL of source water. Mix thoroughly and bring up to final 1,000 mL volume with source water. Label and cover bottle for later use.

2. GIBBS reagent

   Weigh out **0.025 g** of GIBBS reagent and transfer to a 25 mL volumetric flask. Dissolve the reagent in 25 mL ethanol (spectrophotometric grade). This reagent must be prepared daily.

C. Spectrophotometer:

1. Method

a. Standard curve

A standard curve will be prepared each time unknown samples are analyzed. To make a standard curve:

Prepare a stock solution - Please refer to SOP MISC 231. Weigh out 2.0 g of AQUI-S in a suitable container, dilute with source water, and mix to disperse the dose. Transfer the dose into a 2,000 mL volumetric flask partially filled with source water. Rinse the container repeatedly with source water, each time transferring the rinse to the volumetric flask, until the total dose has been transferred to the volumetric flask. Add additional source water to the 2,000 mL mark. Cover or stopper the volumetric flask and mix the contents thoroughly.

Prepare the standard curve dilutions - The standard curve will consist of five concentrations in addition to the blank: 5, 10, 20, 40, and 80 mg/L of AQUI-S. The following standards will be prepared in 100 mL volumetric flasks as follows:

Standard concentration (mg/L)	Stock solution (mL)	Source water (mL)	Final volume (mL)
0	0.0	Up to 100 mL	100
5	0.5	Up to 100 mL	100
10	1.0	Up to 100 mL	100
20	2.0	Up to 100 mL	100
40	4.0	Up to 100 mL	100
80	8.0	Up to 100 mL	100

Prepare the colorimetric reaction reagent (i.e., HEPES-GIBBS reagent) - Use a volumetric pipette to transfer 12.5 mL of prepared GIBBS reagent (see Section B) in a 250 mL volumetric flask. Add 10 mM HEPES buffer up to the 250 mL mark. Seal the flask with a stopper, and mix the contents thoroughly.

Prepare the reagent tubes - Use a auto-pipetter to add 2 mL HEPES-GIBBS reagent and 1 mL of each of the prepared standards to the reagent tube with the corresponding label. Briefly vortex each reagent tube after addition of the prepared standards. Seal reagent tubes with caps. After all standards have been added to the appropriate reagent tube, wait at least 30 min, but no more than 60 min, before reading the absorbance of the standards on a spectrophotometer at a wavelength of 352 nm. A third '0' concentration will be used as the sample blank. Data will be recorded on an appropriate data form. Note - prepare and analyze standards and unknowns at the same time.

2. Determination of concentration of AQUI-S in study samples

a. General considerations

All samples will be run in duplicate.

Label reagent tubes for unknown water samples (Note: for pivotal field efficacy trials and target animal safety trials, label reagent tubes to ensure blinding of the analyst). Add 2 mL of the prepared HEPES-GIBBS reagent to each tube. Use an auto-pipetter to add 1 mL each of the sample collected from the AQUI-S working solution to the reagent tube with the corresponding label. Briefly vortex each reagent after addition of the sample. Seal reagent tubes with caps. After the addition of the sample to the reagent, wait at least 30 min, but no more than 60 min, before reading the absorbance of the standards on a spectrophotometer at a wavelength of 352 nm. For each set of readings, a freshly prepared standard curve will be included in the set.

b. Preparation of the blank and the control standard

The blank used to zero the spectrophotometer will be one of the three 0 mg/L AQUI-S standards.

To prepare the control standard, transfer 2.0 mL of the original AQUI-S stock solution (as prepared in the Methods section 1a) to a 100 mL volumetric flask and fill the 100 mL mark with source water to make a 20 mg/L control standard. Add 1.0 mL of the 20 mg/L control standard to 2.0 mL of the HEPES-GIBBS colorimetric reagent.

c. Determination of AQUI-S concentrations of study samples

After determining the absorbances of the 6-point calibration curve standards, the 20 mg/L control standard, and the study samples, the duplicate study sample absorbance readings will be averaged and the concentration of the sample will be calculated by using the standard curve and recorded on an appropriate form. Calculations may be performed as time permits, but will be completed before the study is completed. Study samples may be discarded after all samples have been measured.

Use the following information to calculate the concentration of AQUI-S in unknown samples using coefficient values generated from a linear regression of the standard curve:

Using either SYSTAT or SigmaStat, run a linear regression using absorbance readings from the AQUI-S standards; make sure that the slope of the line is positive and that the R^2 value adequately demonstrates linearity:

Dependent variable: Measured concentration of AQUI-S standards

Independent variable: Absorbance reading for standards

Based on coefficient values generated from the linear regression, use the following equation ($y = mx + b$) to calculate the concentration of AQUI-S in study samples.

AQUI-S concentration = constant coefficient + (coefficient of the independent variable x absorbance reading of the study sample).

Example:

Constant coefficient = -181.686

Coefficient of independent variable = 727.289

Absorbance reading = 0.278

AQUI-S concentration = -181.686 + (727.289 x 0.278) = 20.5 mg/L

APPROVED BY: _____ **DATE** _____
Management

APPROVED BY: _____ **DATE** _____
Study Director