Standard Methods for the Examination of Water and Wastewater

5510 AQUATIC HUMIC SUBSTANCES*#(1)

5510 A. Introduction

1. General Discussion

Aquatic humic substances (AHS) are heterogeneous, yellow to black, organic materials that include most of the naturally occurring dissolved organic matter in water. Aquatic humic substances have been shown to produce trihalomethanes (THMs) on chlorination and to affect the transport and fate of other organic and inorganic species through partition/adsorption, catalytic, and photolytic reactions.

Humic substances, the major fraction of soil organic matter, are mixtures; their chemical composition is poorly understood. They have been classified into three fractions based on water ‘‘solubility’’†#(2): humin is the fraction not soluble in water at any pH value; humic acid is not soluble under acidic conditions (pH < 2) but becomes soluble at higher pH; and fulvic acid is soluble at all pH conditions.

AHS have the solubility characteristics of fulvic acids but they should not be referred to as such unless they have been fractionated by precipitation at pH < 2. Avoid using the terms ‘‘humic acid’’ and ‘‘tannic acid’’ to describe AHS because they represent other classifications of natural organic materials.

The heterogeneity of AHS requires an operational definition. Isolation by the methods included herein most likely will be incomplete and compounds that are not AHS may be isolated incidentally. Users of these methods are cautioned in the interpretation of results; the bibliography suggests several sources for more information.

Measurement of AHS begins by separation of the sample into dissolved (containing AHS) and particulate organic carbon fractions. Although there is no distinct size that separates these two groups, 0.45 μm is used as the compromise between acceptable flow rate and rejection of small colloidal materials. Low-pressure liquid chromatography serves to concentrate these materials and to isolate them from interfering substances. AHS are quantified by measuring dissolved organic carbon (DOC), Method 5310.

2. Selection of Method

Concentration/isolation of AHS may be achieved by sorption on the nonpolar resin XAD-8 (Method Section 5510C) or by anion-exchange on diethylaminoethyl (DEAE) cellulose (Method Section 5510B). In a collaborative study with seven laboratories using deionized water fortified with about 10 mg AHS/L (previously isolated with XAD), the DEAE method gave better recoveries. Nevertheless, the XAD method has been used extensively; refer to the discussions of interferences and minimum detectable concentrations to assist in method selection. Both methods require further quality control development.

3. Bibliography

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5510 B. Diethylaminoethyl (DEAE) Method

1. General Discussion

a. Principle: AHS are concentrated by column chromatography on diethylaminoethyl (DEAE) cellulose and measured as dissolved organic carbon (DOC). AHS are weak organic acids that bind to anion-exchange materials, such as DEAE cellulose, at neutral pH values. The method is based on the assumption that AHS are the major dissolved organic acids present.

b. Interferences: Any carbonaceous nonhumic materials that are concentrated and isolated by the chromatographic method will interfere (false positive response). Substances that have been shown to interfere include fatty acids, phenols, surfactants, proteinaceous materials, and DOC leached from cellulose.

c. Minimum detectable concentration: Estimated limit of detection is 1.1 mg/L using a 50-mL water sample. The detection limit can be decreased by increasing sample volume. The major limitation is blank contamination.

d. Standard substance: Eliminate documentation of false negatives by analyses of a sample of known humic concentration at regular intervals (at least once per batch of samples).

2. Apparatus

a. Membrane filtration apparatus: Use an all-glass filtering device and 0.45-µm silver membrane filters. Consult manufacturer's specifications for filter details. Do not use filters that sorb AHS or are contaminated with detergents and other organic material.

b. Glass column, approximately 1 × 20 cm with silanized glass wool.

c. Dye-impregnated paper or strips for approximate pH measurements.

d. Organic carbon analyzer capable of measuring concentrations as low as 0.1 mg/L (see Section 5310).

*e. Buchner funnel and filter paper.**#(3)

3. Reagents

a. Water, DOC-free: Preferably use activated-carbon-filtered, redistilled water.

b. DEAE cellulose, exchange capacity 0.22–1.0 meq/g.†#(4) Do not use high-exchange-capacity cellulose, which may decrease recovery of AHS. Take care not to overload low-exchange-capacity cellulose.

c. Hydrochloric acid, HCl, 0.1*N:* Add 8.3 mL conc HCl to 1000 mL water.

d. Hydrochloric acid, HCl, 0.5*N:* Add 41.5 mL conc HCl to 1000 mL water.

e. Sodium hydroxide, NaOH, 0.1*N:* Dissolve 4.0 g NaOH in 1000 mL water.

f. Sodium hydroxide, NaOH, 0.5*N:* Dissolve 20 g NaOH in 1000 mL water.

g. DOC standards: See Section 5310.

h. Potassium chloride, KCl, 0.01*N:* Add 0.75 g KCl to 1000 mL water.

i. Phosphoric acid, H_3PO_4, conc.

4. Procedure

a. Sample concentration and preservation: AHS are sensitive to biodegradatio n and photodegradation. Collect and store samples in organic-free glass containers. Filter at least duplicate portions through a 0.45-µm silver membrane filter as soon after collection as possible. Store samples in the dark at 4°C.

Use care to avoid overloading chromatographic columns and losing AHS. A rough guideline for sample volume selection is as follows:

Sample DOC *mg/L*	Sample Volume *mL*
0–2	250
2–10	50
10–50	25

b. Preparation of DEAE cellulose: Add 70 g DEAE cellulose to 1000 mL 0.5*N* HCl and stir gently for 1 h. Rinse cellulose with water in a Buchner funnel until funnel effluent pH is about 4. Resuspend DEAE in 1000 mL 0.5*N* NaOH and stir for 1 h. Rinse in a Buchner funnel with water until pH is about 6. Remove fines by suspending the treated DEAE in a 1000-mL graduated cylinder filled with water. Let mixture stand undisturbed for 1 h, then decant and discard the supernatant. Repeat removal of fines. Filter remaining DEAE using a Buchner funnel and store in a refrigerated glass container. Avoid prolonged storage, which may lead to microbial contamination.

c. Chromatography: Add 10 mL water to about 1 g DEAE to make a slurry. Carefully pipet enough into a 1- × 20-cm column fitted with a small (0.5-cm) glass-wool plug to make a 1-cm-deep column bed. Avoid getting DEAE on the sides of the column. Carefully place

another 0.5-cm glass-wool plug on top of the bed. Rinse column with 50 mL 0.01*N* KCl (adjusted to pH 6 with 0.1*N* HCl or NaOH) just before sample concentration.

Adjust sample to pH 6 and pass it through the column at a flow rate of about 2 mL/min. Rinse with 5 mL water (pH 6). Elute AHS by adding about 3 mL 0.1*N* NaOH to the top of the column. Start collecting column effluent when it appears colored. (This will occur after about 1 mL has passed out of the column). Collect eluate in a graduated, conical test tube until it becomes colorless (about 2 mL). Acidify with conc H_3PO_4 to a pH of 2 or less (about 2 to 3 drops) and remove dissolved carbon dioxide (inorganic carbon) by purging with nitrogen for 10 min. Avoid exposure of alkaline samples to air (i.e. acidify immediately) to minimize contamination with CO_2. Determine volume and DOC of acidified eluate.

Process two portions of water and a second portion of sample by the same procedure. Pack a fresh column of DEAE for each sample and each control (DEAE cannot be reused).

5. Calculation

Calculate the concentration of AHS as:

$$\text{AHS, mg DOC/L} = [(A - B) \times C]/D$$

where:

A = average DOC concentration of the two sample NaOH eluates, mg C/L,
B = average DOC concentration of the two control NaOH eluates, mg C/L,
C = volume of eluate, L, and
D = volume of sample, L.

Multiplication of AHS, mg DOC/L, by 2 converts concentration to AHS, mg/L, if it is assumed that AHS contain 50% carbon. This will be the minimum concentration of AHS because recoveries are less than 100%.

6. Precision and Bias

For seven single-operator analyses, the relative standard deviation of triplicate samples (about 10 mg/L as AHS) ranged from 2.5 to 14.4% with an average of 4.9% (n = 7).

For seven single-operator analyses, recoveries ranged from 59.3 to 97.3% with an average of 77.4% and a relative standard deviation of 18.1%.

5510 C. XAD Method

1. General Discussion

a. Principle: AHS are concentrated by column chromatography on XAD resin and measured as dissolved organic carbon (DOC). Acidification of AHS decreases polarity, allowing partition into the nonpolar XAD matrix. The method is based on the assumption that AHS are the major dissolved organic acids present.

b. Interferences: Any carbonaceous nonhumic materials that are concentrated and

isolated by the chromatographic method will interfere. This includes fatty acids, phenols, surfactants, proteinaceous materials, and DOC leached from the resin, chromatography pump, or tubing.

c. Minimum detectable concentration: Estimated limit of detection is 1.4 mg/L using a 50-mL water sample. The detection limit can be decreased by increasing sample volume. The major limitation is blank contaminations.

2. Apparatus

See Section 5510B.2*a*, c, and *d*. In addition, the following are required:

a. Glass column, 0.2 × 25 cm with silanized glass wool.

b. Pump, with inert internal parts and tubing, capable of flow rates of 0.2 to 1.0 mL/min.*#(5)

c. TFE tubing, 0.2 cm ID.

d. Extraction apparatus, Soxhlet.

3. Reagents

In addition to reagents *a*, *c*, *e*, *g*, and *i* of Section 5510B :

a. XAD resin,†#(6) approximately 250-µm size.

b. Hexane.

c. Methanol.

d. Acetonitrile.

4. Procedure

a. Sample collection and preservation: See Section 5510B.4*a*.

b. Preparation of XAD resin: Clean resin by successive washing with 0.1*N* NaOH for 5 d. Extract resin sequentially in a Soxhlet extractor with hexane, methanol, acetonitrile, and methanol, for 24 h each. Pack clean resin into a 0.2- × 25-cm glass column that has a 2-mm length of glass wool in one end. After filling, cap column with another 2-mm length of glass wool.

Wet dry column with methanol. When the air has been displaced, pump distilled water through the column until the effluent concentration of DOC decreases to 0.5 mg/L (approximately 20 bed volumes).

c. Chromatography: Preclean column with three cycles of 0.1*N* NaOH and 0.1*N* HCl just before pumping sample into column. Leave column saturated with 0.1*N* HCl. Acidify sample to pH 2.0 with concentrated HCl, and pump it onto the column at rate of 1.0 mL/min. Save column effluent for DOC analysis. Significant concentrations of DOC in the effluent can indicate that the column was overloaded and that a smaller sample volume should be used. Colored organic acids adsorb to the top of the column. Back-elute (reverse flow) the column with 0.1*N* NaOH at 0.2 mL/min and collect eluate in a graduated, conical test tube until it becomes colorless (about 2 mL). Acidify with conc H_3PO_4 to a pH of 2 or less (about 2 to 3 drops) and remove dissolved carbon dioxide (inorganic carbon) by purging with nitrogen for

10 min. Avoid exposure of alkaline samples to air (i.e. acidify immediately) to minimize contamination with CO_2. Determine volume and DOC of acidified column effluent.

After eluting and collecting AHS from the column with back-elution using 0.1*N* NaOH, continue rinsing with about 20 bedvolumes of the basic solution. Rinse with water for about 20 bed volumes. Repeat the triplicate acid/base column precleaning procedure described above, then reuse the column to analyze a replicate sample. Process two portions of water by the same procedure to serve as controls.

The XAD column may be reused to analyze subsequent samples and controls if the triplicate acid/base precleaning procedure is repeated immediately before analysis of each replicate. Replace the column if recovery is poor or the resin becomes discolored.

5. Calculation

Calculate the concentration of AHS as given in Section 5510B.5.

6. Precision and Bias

For seven single-operator analyses, the relative standard deviation of triplicate samples (about 10 mg/L as AHS) ranged from 0.9 to 20.7% with an average of 5.4% (n = 7).

For seven single-operator analyses, recoveries ranged from 15.1 to 71.0% with an average of 51.6% and a relative standard deviation of 35.1%.

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Endnotes

1 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
2 (Popup - Footnote)
† ‘‘Solubility’’ is here used as a general description of whether or not the material can be uniformly dispersed in an aqueous phase rather than as an expression of equilibrium between a pure solute and its aqueous solution.
3 (Popup - Footnote)
* Whatman No. 1 or equivalent.
4 (Popup - Footnote)
† Whatman pre-swollen microgranular DE 52 or DE 51, or equivalent.
5 (Popup - Footnote)
* Pump parts may be of stainless steel or TFE.
6 (Popup - Footnote)
† XAD-7 or equivalent.