PHENOLICS (SPECTROPHOTOMETRIC, MANUAL 4-AAP WITH DISTILLATION)

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the analysis of ground water, drinking, surface, and saline waters, and domestic and industrial wastes.

1.2 The method is capable of measuring phenolic materials at the 5 ug/L level when the colored end product is extracted and concentrated in a solvent phase using phenol as a standard.

1.3 The method is capable of measuring phenolic materials that contain more than 50 ug/L in the aqueous phase (without solvent extraction) using phenol as a standard.

1.4 It is not possible to use this method to differentiate between different kinds of phenols.

2.0 SUMMARY OF METHOD

2.1 Phenolic materials react with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10 to form a stable reddish-brown antipyrine dye. The amount of color produced is a function of the concentration of phenolic material.

3.0 INTERFERENCES

3.1 For most samples a preliminary distillation is required to remove interfering materials.

3.2 Color response of phenolic materials with 4-aminoantipyrine is not the same for all compounds. Because phenolic-type wastes usually contain a variety of phenols, it is not possible to duplicate a mixture of phenols to be used as a standard. For this reason phenol has been selected as a standard and any color produced by the reaction of other phenolic compounds is reported as phenol. This value will represent the minimum concentration of phenolic compounds present in the sample.

3.3 Interferences from sulfur compounds are eliminated by acidifying the sample to a pH of <4 with H_2SO_4 and aerating briefly by stirring.

3.4 Oxidizing agents such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate. If chlorine is not removed, the phenolic compounds may be partially oxidized and the results may be low.

4.0 APPARATUS AND MATERIALS

4.1 Distillation apparatus: All glass, consisting of a 1-liter Pyrex distilling apparatus with Graham condenser.

4.2 pH meter.

4.3 Spectrophotometer: For use at 460 or 510 nm.

4.4 Funnels.

4.5 Filter paper.

4.6 Membrane filters.

4.7 Separatory funnels: 500- or 1,000-mL.

4.8 Nessler tubes: Short or long form.

5.0 REAGENTS

5.1 ASTM Type II water (ASTM D1193): Water should be monitored for impurities.

5.2 Sulfuric acid solution, H_2SO_4: Concentrated.

5.3 Buffer solution: Dissolve 16.9 g NH_4Cl in 143 mL concentrated NH_4OH and dilute to 250 mL with Type II water. Two mL of buffer should adjust 100 mL of distillate to pH 10.

5.4 Aminoantipyrine solution: Dissolve 2 g of 4-aminoantipyrine (4-AAP) in Type II water and dilute to 100 mL.

5.5 Potassium ferricyanide solution: Dissolve 8 g of $K_3Fe(CN)_6$ in Type II water and dilute to 100 mL.

5.6 Stock phenol solution: Dissolve 1.0 g phenol in freshly boiled and cooled Type II water and dilute to 1 liter (1 mL = 1 mg phenol).
NOTE: This solution is hydroscopic and toxic.

5.7 Working solution A: Dilute 10 mL stock phenol solution to 1 liter with Type II water (1 mL = 10 ug phenol).

5.8 Working solution B: Dilute 100 mL of working solution A to 1,000 mL with Type II water (1 mL = 1 ug phenol).

5.9 Chloroform.

5.10 Ferrous ammonium sulfate: Dissolve 1.1 g in 500 mL Type II water containing 1 mL concentrated H_2SO_4 and dilute to 1 liter with freshly boiled and cooled Type II water.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 Biological degradation is inhibited by the addition of H_2SO_4 to pH <4. Store at 4°C. The sample should be stable for 28 days.

7.0 PROCEDURE

7.1 Distillation:

7.1.1 Measure 500 mL of sample into a beaker. Lower the pH to approximately 4 with concentrated H_2SO_4 (1 mL/L), and transfer to the distillation apparatus.

7.1.2 Distill 450 mL of sample, stop the distillation, and when boiling ceases, add 50 mL of warm Type II water to the flask and resume distillation until 500 mL have been collected.

7.1.3 If the distillate is turbid, filter through a prewashed membrane filter.

7.2 Direct photometric method:

7.2.1 Using working solution A (5.6), prepare the following standards in 100-mL volumetric flasks:

Working Solution A (mL)	Concentration (ug/L)
0.0	0.0
0.5	50.0
1.0	100.0
2.0	200.0
5.0	500.0
8.0	800.0
10.0	1000.0

7.2.2 To 100 mL of distillate or to an aliquot diluted to 100 mL and/or standards, add 2 mL of buffer solution (5.2) and mix. The pH of the sample and standards should be 10 $\pm$ 0.2.

7.2.3 Add 2.0 mL aminoantipyrine solution (5.3) and mix.

7.2.4 Add 2.0 mL potassium ferricyanide solution (5.4) and mix.

7.2.5 After 15 min read absorbance at 510 nm.

7.3 Chloroform extraction method:

CAUTION: This method should be performed in a hood; chloroform is toxic.

7.3.1 Using working solution B (5.7), prepare the following standards. Standards may be prepared by pipetting the required volumes into the separatory funnels and diluting to 500 mL with Type II water:

Working Solution B (mL)	Concentration (ug/L)
0.0	0.0
3.0	6.0
5.0	10.0
10.0	20.0
20.0	40.0
25.0	50.0

7.3.2 Place 500 mL of distillate or an aliquot diluted to 500 mL in a separatory funnel. The sample should not contain more than 50 ug/L phenol.

7.3.3 To sample and standards add 10 mL of buffer solution (5.2) and mix. The pH should be 10 $\pm$ 0.2.

7.3.4 Add 3.0 mL aminoantipyrine solution (5.3) and mix.

7.3.5 Add 3.0 mL potassium ferricyanide solution (5.4) and mix.

7.3.6 After 3 min, extract with 25 mL of chloroform (5.9). Shake the separatory funnel at least 10 times, let $CHCl_3$ settle, shake again 10 times, and let chloroform settle again.

7.3.7 Filter chloroform extract through filter paper. Do not add more chloroform.

7.3.8 Read the absorbance of the samples and standards against the blank at 460 nm.

7.4 Calculation:

7.4.1 Prepare a standard curve by plotting the absorbance values of standards versus the corresponding phenol concentrations.

7.4.2 Obtain concentration value of sample directly from standard curve.

8.0 QUALITY CONTROL

8.1 All quality control data should be maintained and available for easy reference or inspection.

8.2 Calibration curves must be composed of a minimum of a blank and three standards. A calibration curve should be made for every hour of continuous sample analysis.

8.3 Dilute samples if they are more concentrated than the highest standard or if they fall on the plateau of a calibration curve.

8.4 Employ a minimum of one blank per sample batch to determine if contamination has occurred.

8.5 Verify calibration with an independently prepared check standard every 15 samples.

8.6 Run one spike duplicate sample for every 10 samples. A duplicate sample is a sample brought through the whole sample preparation and analytical process.

9.0 METHOD PERFORMANCE

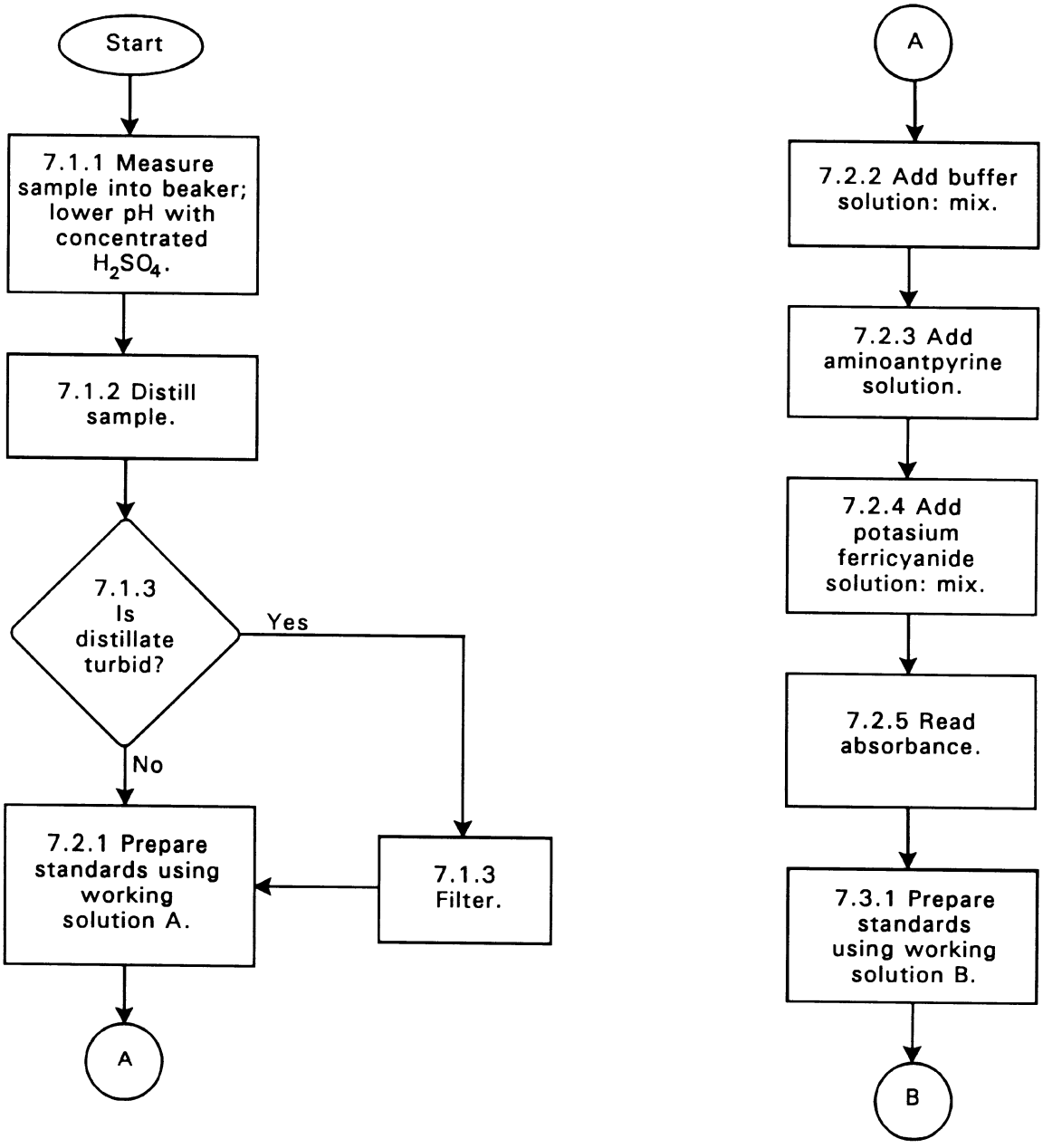
9.1 In a single laboratory using sewage samples at concentrations of 3.8, 15, 43, and and 89 ug/L, the standard deviations were $\pm$0.5, $\pm$0.6, $\pm$0.6, and $\pm$1.0 ug/L, respectively. At concentrations of 73, 146, 299, and 447 ug/L, the standard deviations were $\pm$1.0, $\pm$1.8, $\pm$4.2, and $\pm$5.3 ug/L, respectively.

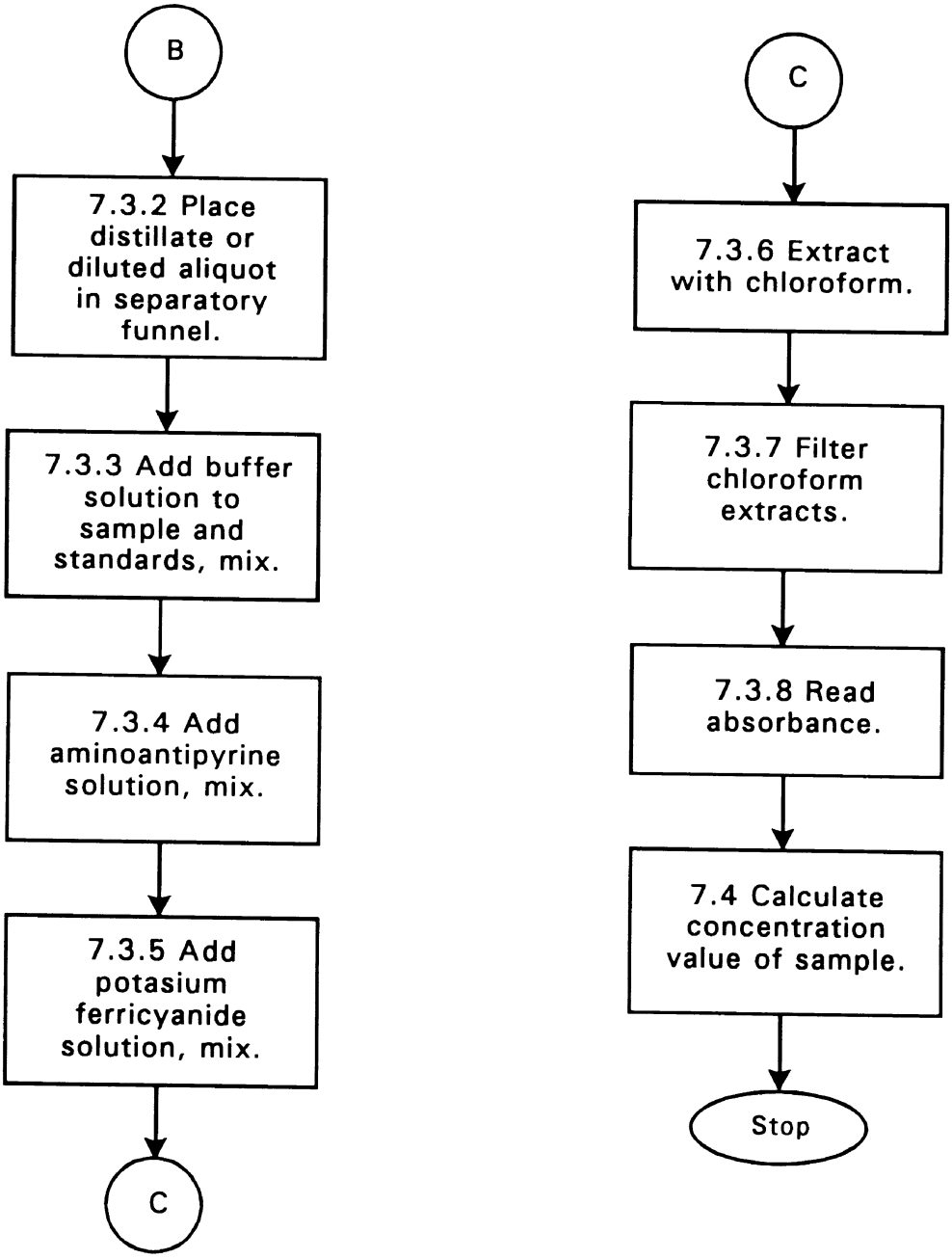
9.2 In a single laboratory using sewage samples at concentrations of 5.3 and 82 ug/L, the recoveries were 78% and 98%,respectively. At concentrations of 168 and 489 ug/L, the recoveries were 97% and 98%, respectively.

10.0 REFERENCES

1. Annual Book of ASTM Standards, Part 31, "Water," Standard D1783-70, p. 553 (1976).

2. Standard Methods for the Examination of Water and Wastewater, 14th ed., pp. 574-581, Method 510 through 510C (1975).


Start
7.1.1 Measure sample into beaker; lower pH with concentrated H_2SO_4.
7.1.2 Distill sample.
7.1.3 Is distillate turbid?
Yes
No
7.1.3 Filter.
7.2.1 Prepare standards using working solution A.
A
A
7.2.2 Add buffer solution: mix.
7.2.3 Add aminoantpyrine solution.
7.2.4 Add potasium ferricyanide solution: mix.
7.2.5 Read absorbance.
7.3.1 Prepare standards using working solution B.
B


B
7.3.2 Place distillate or diluted aliquot in separatory funnel.
7.3.3 Add buffer solution to sample and standards, mix.
7.3.4 Add aminoantipyrine solution, mix.
7.3.5 Add potasium ferricyanide solution, mix.
C
C
7.3.6 Extract with chloroform.
7.3.7 Filter chloroform extracts.
7.3.8 Read absorbance.
7.4 Calculate concentration value of sample.
Stop