Standard Methods for the Examination of Water and Wastewater

5530 PHENOLS*#(1)

5530 A. Introduction

Phenols, defined as hydroxy derivatives of benzene and its condensed nuclei, may occur in domestic and industrial wastewaters, natural waters, and potable water supplies. Chlorination of such waters may produce odorous and objectionable-tasting chlorophenols. Phenol removal processes in water treatment include superchlorination, chlorine dioxide or chloramine treatment, ozonation, and activated carbon adsorption.

1. Selection of Method

The analytical procedures offered here use the 4-aminoantipyrine colorimetric method that determines phenol, ortho- and meta-substituted phenols, and, under proper pH conditions, those para-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl, or sulfonic acid group. The 4-aminoantipyrine method does not determine those para-substituted phenols where the substitution is an alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group. A typical example of these latter groups is paracresol, which may be present in certain industrial wastewaters and in polluted surface waters.

The 4-aminoantipyrine method is given in two forms: Method C, for extreme sensitivity, is adaptable for use in water samples containing less than 1 mg phenol/L. It concentrates the color in a nonaqueous solution. Method D retains the color in the aqueous solution. Because the relative amounts of various phenolic compounds in a given sample are unpredictable, it is not possible to provide a universal standard containing a mixture of phenols. For this reason, phenol (C_6H_5OH) itself has been selected as a standard for colorimetric procedures and any color produced by the reaction of other phenolic compounds is reported as phenol. Because substitution generally reduces response, this value represents the minimum concentration of phenolic compounds. A gas-liquid chromatographic procedure is included in Section 6420B and may be applied to samples or concentrates to quantify individual phenolic compounds.

2. Interferences

Interferences such as phenol-decomposing bacteria, oxidizing and reducing substances, and alkaline pH values are dealt with by acidification. Some highly contaminated wastewaters may require specialized techniques for eliminating interferences and for quantitative recovery of phenolic compounds.

Eliminate major interferences as follows (see Section 5530B for reagents):

Oxidizing agents, such as chlorine and those detected by the liberation of iodine on acidification in the presence of potassium iodide (KI)—Remove immediately after sampling by adding excess ferrous sulfate ($FeSO_4$). If oxidizing agents are not removed, the phenolic compounds will be oxidized partially.

Sulfur compounds—Remove by acidifying to pH 4.0 with H_3PO_4 and aerating briefly by

stirring. This eliminates the interference of hydrogen sulfide (H_2S) and sulfur dioxide (SO_2).

Oils and tars—Make an alkaline extraction by adjusting to pH 12 to 12.5 with NaOH pellets. Extract oil and tar from aqueous solution with 50 mL chloroform ($CHCl_3$). Discard oil- or tar-containing layer. Remove excess $CHCl_3$ in aqueous layer by warming on a water bath before proceeding with the distillation step.

3. Sampling

Sample in accordance with the instructions of Section 1060.

4. Preservation and Storage of Samples

Phenols in concentrations usually encountered in wastewaters are subject to biological and chemical oxidation. Preserve and store samples at 4°C or lower unless analyzed within 4 h after collection.

Acidify with 2 mL conc H_2SO_4/L.

Analyze preserved and stored samples within 28 d after collection.

5. Bibliography

ETTINGER, M.B., S. SCHOTT & C.C. RUCHHOFT. 1943. Preservation of phenol content in polluted river water samples previous to analysis. *J. Amer. Water Works Assoc.* 35:299.

CARTER, M.J. & M.T. HUSTON. 1978. Preservation of phenolic compounds in wastewaters. *Environ. Sci. Technol.* 12:309.

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5530 B. Cleanup Procedure

1. Principle

Phenols are distilled from nonvolatile impurities. Because the volatilization of phenols is gradual, the distillate volume must ultimately equal that of the original sample.

2. Apparatus

a. Distillation apparatus, all-glass, consisting of a 1-L borosilicate glass distilling apparatus with Graham condenser.*#(2)

b. pH meter.

3. Reagents

Prepare all reagents with distilled water free of phenols and chlorine.

a. Phosphoric acid solution, H_3PO_4, 1 + 9: Dilute 10 mL 85% H_3PO_4 to 100 mL with water.

b. Methyl orange indicator solution.

c. Special reagents for turbid distillates:

1) *Sulfuric acid,* H_2SO_4, 1*N*.

2) *Sodium chloride,* NaCl.

3) *Chloroform,* $CHCl_3$, or *methylene chloride,* CH_2Cl_2.

4) *Sodium hydroxide,* NaOH, 2.5*N:* Dilute 41.7 mL 6*N* NaOH to 100 mL or dissolve 10 g NaOH pellets in 100 mL water.

4. Procedure

a. Measure 500 mL sample into a beaker, adjust pH to approximately 4.0 with H_3PO_4 solution using methyl orange indicator or a pH meter, and transfer to distillation apparatus. Use a 500-mL graduated cylinder as a receiver. Omit adding H_3PO_4 and adjust pH to 4.0 with 2.5*N* NaOH if sample was preserved as described in 5530A.4.

b. Distill 450 mL, stop distillation and, when boiling ceases, add 50 mL warm water to distilling flask. Continue distillation until a total of 500 mL has been collected.

c. One distillation should purify the sample adequately. Occasionally, however, the distillate is turbid. If so, acidify with H_3PO_4 solution and distill as described in ¶ 4*b*. If second distillate is still turbid, use extraction process described in ¶ 4*d* before distilling sample.

d. Treatment when second distillate is turbid: Extract a 500-mL portion of original sample as follows: Add 4 drops methyl orange indicator and make acidic to methyl orange with 1*N* H_2SO_4. Transfer to a separatory funnel and add 150 g NaCl. Shake with five successive portions of $CHCl_3$, using 40 mL in the first portion and 25 mL in each successive portion. Transfer $CHCl_3$ layer to a second separatory funnel and shake with three successive portions of 2.5*N* NaOH solution, using 4.0 mL in the first portion and 3.0 mL in each of the next two portions. Combine alkaline extracts, heat on a water bath until $CHCl_3$ has been removed, cool, and dilute to 500 mL with distilled water. Proceed with distillation as described in ¶s 4*a* and b.

NOTE: CH_2Cl_2 may be used instead of $CHCl_3$, especially if an emulsion forms when the $CHCl_3$ solution is extracted with NaOH.

5530 C. Chloroform Extraction Method

1. General Discussion

a. Principle: Steam-distillable phenols react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is extracted from aqueous solution with $CHCl_3$ and the absorbance is measured at 460 nm. This method covers the phenol concentration range from 1.0 µg/L to over 250 µg/L with a sensitivity of 1 µg/L.

b. Interference: All interferences are eliminated or reduced to a minimum if the sample is preserved, stored, and distilled in accordance with the foregoing instructions.

c. Minimum detectable quantity: The minimum detectable quantity for clean samples containing no interferences is 0.5 μg phenol when a 25-mL $CHCl_3$ extraction with a 5-cm cell or a 50-mL $CHCl_3$ extraction with a 10-cm cell is used in the photometric measurement. This quantity is equivalent to 1 μg phenol/L in 500 mL distillate.

2. Apparatus

a. Photometric equipment: A spectrophotometer for use at 460 nm equipped with absorption cells providing light paths of 1 to 10 cm, depending on the absorbances of the colored solutions and the individual characteristics of the photometer.

b. Filter funnels: Buchner type with fritted disk.*#(3)

c. Filter paper: Alternatively use an appropriate 11-cm filter paper for filtering $CHCl_3$ extracts instead of the Buchner-type funnels and anhydrous Na_2SO_4.

d. pH meter.

e. Separatory funnels, 1000-mL, Squibb form, with ground-glass stoppers and TFE stopcocks. At least eight are required.

3. Reagents

Prepare all reagents with distilled water free of phenols and chlorine.

a. Stock phenol solution: Dissolve 100 mg phenol in freshly boiled and cooled distilled water and dilute to 100 mL. CAUTION—*Toxic; handle with extreme care.* Ordinarily this direct weighing yields a standard solution; if extreme accuracy is required, standardize as follows:

1) To 100 mL water in a 500-mL glass-stoppered conical flask, add 50.0 mL stock phenol solution and 10.0 mL bromate-bromide solution. Immediately add 5 mL conc HCl and swirl gently. If brown color of free bromine does not persist, add 10.0-mL portions of bromate-bromide solution until it does. Keep flask stoppered and let stand for 10 min; then add approximately 1 g KI. Usually four 10-mL portions of bromate-bromide solution are required if the stock phenol solution contains 1000 mg phenol/L.

2) Prepare a blank in exactly the same manner, using distilled water and 10.0 mL bromate-bromide solution. Titrate blank and sample with 0.025*M* sodium thiosulfate, using starch solution indicator.

3) Calculate the concentration of phenol solution as follows:

$$\text{mg phenol/L} = 7.842\,[(A \times B) - C]$$

where:

A = mL thiosulfate for blank,
B = mL bromate-bromide solution used for sample divided by 10, and
C = mL thiosulfate used for sample.

b. Intermediate phenol solution: Dilute 1.00 mL stock phenol solution in freshly boiled and cooled distilled water to 100 mL; 1 mL = 10.0 µg phenol. Prepare daily.

c. Standard phenol solution: Dilute 50.0 mL intermediate phenol solution to 500 mL with freshly boiled and cooled distilled water; 1 mL = 1.0 µg phenol. Prepare within 2 h of use.

d. Bromate-bromide solution: Dissolve 2.784 g anhydrous $KBrO_3$ in water, add 10 g KBr crystals, dissolve, and dilute to 1000 mL.

e. Hydrochloric acid, HCl, conc.

f. Standard sodium thiosulfate titrant, 0.025*M:* See Section 4500-O.C.2*e*.

g. Starch solution: See Section 4500-O.C.2*d*.

h. Ammonium hydroxide, NH_4OH, 0.5*N*: Dilute 35 mL fresh, conc NH_4OH to 1 L with water.

i. Phosphate buffer solution: Dissolve 104.5 g K_2HPO_4 and 72.3 g KH_2PO_4 in water and dilute to 1 L. The pH should be 6.8.

j. 4-Aminoantipyrine solution: Dissolve 2.0 g 4-aminoantipyrine in water and dilute to 100 mL. Prepare daily.

k. Potassium ferricyanide solution: Dissolve 8.0 g $K_3Fe(CN)_6$ in water and dilute to 100 mL. Filter if necessary. Store in a brown glass bottle. Prepare fresh weekly.

l. Chloroform, $CHCl_3$.

m. Sodium sulfate, anhydrous Na_2SO_4, granular.

n. Potassium iodide, KI, crystals.

4. Procedure

Ordinarily, use Procedure *a*; however, Procedure *b* may be used for infrequent analyses.

a. Place 500 mL distillate, or a suitable portion containing not more than 50 µg phenol, diluted to 500 mL, in a 1-L beaker. Prepare a 500-mL distilled water blank and a series of 500-mL phenol standards containing 5, 10, 20, 30, 40, and 50 µg phenol.

Treat sample, blank, and standards as follows: Add 12.0 mL 0.5*N* NH_4OH and *immediately* adjust pH to 7.9 ± 0.1 with phosphate buffer. Under some circumstances, a higher pH may be required.†#(4) About 10 mL phosphate buffer are required. Transfer to a 1-L separatory funnel, add 3.0 mL aminoantipyrine solution, mix well, add 3.0 mL $K_3Fe(CN)_6$ solution, mix well, and let color develop for 15 min. The solution should be clear and light yellow.

Extract immediately with $CHCl_3$, using 25 mL for 1- to 5-cm cells and 50 mL for a 10-cm cell. Shake separatory funnel at least 10 times, let $CHCl_3$ settle, shake again 10 times, and let $CHCl_3$ settle again. Filter each $CHCl_3$ extract through filter paper or fritted glass funnels containing a 5-g layer of anhydrous Na_2SO_4. Collect dried extracts in clean cells for absorbance measurements; do not add more $CHCl_3$ or wash filter papers or funnels with

$CHCl_3$.

Read absorbance of sample and standards against the blank at 460 nm. Plot absorbance against micrograms phenol concentra tion. Construct a separate calibration curve for each photometer and check each curve periodically to insure reproducibility.

b. For infrequent analyses prepare only one standard phenol solution. Prepare 500 mL standard phenol solution of a strength approximately equal to the phenolic content of that portion of original sample used for final analysis. Also prepare a 500-mL distilled water blank.

Continue as described in ¶ a, above, but measure absorbances of sample and standard phenol solution against the blank at 460 nm.

5. Calculation

a. For Procedure *a:*

$$\mu g \text{ phenol/L} = \frac{A}{B} \times 1000$$

where:

A = µg phenol in sample, from calibration curve, and
B = mL original sample.

b. For Procedure *b,* calculate the phenol content of the original sample:

$$\mu g \text{ phenol/L} = \frac{C \times D \times 1000}{E \times B}$$

where:

C = µg standard phenol solution,
D = absorbance reading of sample,
E = absorbance of standard phenol solution, and
B = mL original sample.

6. Precision and Bias

Because the ‘‘phenol’’ value is based on C_6H_5OH, this method yields only an approximation and represents the minimum amount of phenols present. This is true because the phenolic reactivity to 4-aminoantipyrine varies with the types of phenols present.

In a study of 40 refinery wastewaters analyzed in duplicate at concentrations from 0.02 to 6.4 mg/L the average relative standard deviation was ± 12%. Data are not available for precision at lower concentrations.

7. Bibliography

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cresols. *Ind. Eng. Chem.*, Anal. Ed. 3:67.

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5530 D. Direct Photometric Method

1. General Discussion

a. Principle: Steam-distillable phenolic compounds react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is kept in aqueous solution and the absorbance is measured at 500 nm.

b. Interference: Interferences are eliminated or reduced to a minimum by using the distillate from the preliminary distillation procedure.

c. Minimum detectable quantity: This method has less sensitivity than Method C. The minimum detectable quantity is 10 µg phenol when a 5-cm cell and 100 mL distillate are used.

2. Apparatus

a. Photometric equipment: Spectrophotometer equipped with absorption cells providing

light paths of 1 to 5 cm for use at 500 nm.

b. pH meter.

3. Reagents

See Section 5530C.3.

4. Procedure

Place 100 mL distillate, or a portion containing not more than 0.5 mg phenol diluted to 100 mL, in a 250-mL beaker. Prepare a 100-mL distilled water blank and a series of 100-mL phenol standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg phenol. Treat sample, blank, and standards as follows: Add 2.5 mL 0.5*N* NH_4OH solution and immediately adjust to pH 7.9 ± 0.1 with phosphate buffer. Add 1.0 mL 4-aminoantipyrine solution, mix well, add 1.0 mL $K_3Fe(CN)_6$ solution, and mix well.

After 15 min, transfer to cells and read absorbance of sample and standards against the blank at 500 nm.

5. Calculation

a. Use of calibration curve: Estimate sample phenol content from photometric readings by using a calibration curve constructed as directed in Section 5530C.4*a*.

$$\text{mg phenol/L} = \frac{A}{B} \times 1000$$

where:

A = mg phenol in sample, from calibration curve, and
B = mL original sample.

b. Use of single phenol standard:

$$\text{mg phenol/L} = \frac{C \times D \times 1000}{E \times B}$$

where:

C = mg standard phenol solution,
D = absorbance of sample, and
E = absorbance of standard phenol solution.

6. Precision and Bias

Precision and bias data are not available.

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Endnotes

1 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
2 (Popup - Footnote)
* Corning No. 3360 or equivalent.
3 (Popup - Footnote)
* 15-mL Corning No. 36060 or equivalent.
4 (Popup - Footnote)
† For NPDES permit analyses, pH 10 ± 0.1 is required.