International Standard 6439

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Water quality — Determination of phenol index — 4-Aminoantipyrine spectrometric methods after distillation

Qualité de l'eau — Détermination de l'indice phénol — Méthode spectrométrique à l'amino-4-antipyrine après distillation

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 6439 was developed by Technical Committee ISO/TC 147, *Water quality*, and was circulated to the member bodies in February 1983.

It has been approved by the member bodies of the following countries :

Australia	Germany, F.R.	New Zealand
Austria	Hungary	Norway
Belgium	India	Poland
Canada	Iran	Romania
Chile	Iraq	South Africa, Rep. of
China	Italy	Spain
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Denmark	Korea, Dem. P. Rep. of	Switzerland
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Finland	Mexico	USSR
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No member body expressed disapproval of the document.

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Water quality — Determination of phenol index — 4-Aminoantipyrine spectrometric methods after distillation

0 Introduction

The term "phenol index" as used in this International Standard only includes phenols which react with 4-aminoantipyrine under the conditions specified to give coloured compounds.

In a water containing phenol itself, there will usually be associated with it other phenolic compounds whose sensitivity to the reagents used in the following methods may not necessarily be the same.

The percentage composition of the various phenolic compounds (3.1) present in a given test sample is unpredictable. It is obvious, therefore, that a standard containing a mixture of phenolic compounds cannot be made applicable to all test samples. For this reason, phenol (C_6H_5OH) has been selected as a standard, and any colour produced by the reaction of other phenolic compounds is measured as phenol and reported as the phenol index (3.2).

It is not possible to use the procedures specified in this International Standard to differentiate between different kinds of phenols. Some phenolic compounds with substituents such as alkyl, aryl and nitro in the *para* position do not produce colour with 4-aminoantipyrine. Phenolic compounds containing *para* substituents such as a carboxyl, halogen, hydroxyl, methoxyl or sulfonic acid, do produce colour with 4-aminoantipyrine. Hence the phenol index includes only those phenolic compounds which can be determined under specified conditions.

1 Scope and field of application

This International Standard specifies methods for determining the phenol index (3.2) in drinking waters, surface waters, brines (saline waters), domestic waters and industrial waste waters.

After a preliminary distillation, the test samples are analysed according to specific application as follows :

method A (direct colorimetric method) : this method is capable of measuring the phenol index in test samples that contain more than 0,10 mg/l in the aqueous phase (without chloroform extraction), using phenol as a standard;

method B (chloroform extraction method) : this method is capable of measuring the phenol index without dilution from about 0,002 to about 0,10 mg/l when the coloured end-product is extracted and concentrated in chloroform phase, using phenol as a standard.

NOTE — According to the results of a German interlaboratory trial using an almost identical method to method B, the lower limit of detection is 0,01 mg/l.

2 References

ISO 5667, *Water quality — Sampling —*

Part 1 : Guidance on the design of sampling programmes.

Part 2 : Guidance on sampling techniques.

Part 3 : Guidance on the preservation and handling of samples.[1)]

3 Definitions

For the purpose of this International Standard, the following definitions apply :

3.1 phenolic compounds : Hydroxy derivatives of benzene and its analogues.

3.2 phenol index : A number giving a concentration, expressed in milligrams of phenol per litre, of different phenolic compounds based on the degree of colour they produce with 4-aminoantipyrine according to the procedure given.

1) At present at the stage of draft.

4 Method A — Direct colorimetric method

4.1 Principle

Separation of phenolic compounds from impurities and preservative agents by distillation. The rate of volatilization of the phenolic compounds is gradual, so that the volume of the distillate must equal that of the test sample being distilled.

Reaction of the steam-distillable phenolic compounds with 4-aminoantipyrine at a pH of 10,0 ± 0,2 in the presence of potassium hexacyanoferrate(III) to form a coloured antipyrine dye.

Measurement of the absorbance of the dye at 510 nm. The phenol index is expressed as milligrams of phenol (C_6H_5OH) per litre.

The minimum detectable quantity is equivalent to 0,01 mg phenol when a 50 mm cell is used in the spectrometric measurement and 100 ml distillate are used in the determination.

4.2 Reagents

During the analysis, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

4.2.1 4-aminoantipyrine, 20 g/l solution.

Dissolve 2,0 g of 4-aminoantipyrine ($C_{11}H_{13}N_3O$) in water and dilute to 100 ml.

Prepare this reagent just before use.

4.2.2 Ammonium chloride, 20 g/l solution.

Dissolve 20 g of ammonium chloride (NH_4Cl) in water and dilute to 1 000 ml.

4.2.3 Ammonium hydroxide, ϱ = 0,90 g/ml.

4.2.4 Potassium sodium tartrate[1], buffer solution, pH = 10.

Dissolve 34 g ammonium chloride (NH_4Cl), 200 g potassium sodium tartrate ($NaKC_4H_4O_6$) and 15 ml ammonium hydroxide (4.2.3) in 700 ml water and dilute to 1 000 ml. Adjust the pH to 10 with ammonium hydroxide.

4.2.5 Copper (II) sulfate, pentahydrate ($CuSO_4 \cdot 5H_2O$).

4.2.6 Copper (II) sulfate, 100 g/l solution.

Dissolve 190 g of copper (II) sulfate pentahydrate (4.2.5) in water and dilute to 1 000 ml.

4.2.7 Hydrochloric acid, ϱ = 1,19 g/ml.

4.2.8 Methyl orange, indicator.

Dissolve 0,5 g methyl orange in water and dilute to 1 000 ml.

4.2.9 Phenol, stock solution, 1,00 g/l.

CAUTION — Phenol should not be allowed to come into contact with the skin.

Dissolve 1,00 g phenol in freshly boiled and cooled water, in a 1 000 ml volumetric flask and make up to the mark with the same water.

This solution should be used within 30 days of preparation.

NOTE — Phenol must not be liquid or discoloured. Checking the phenol concentration by titration may be necessary according to the procedure described in the annex.

4.2.10 Phenol, standard solution corresponding to 0,01 g C_6H_5OH per litre.

Dilute 10,0 ml of the phenol stock solution (4.2.9) to 1 000 ml with freshly boiled and cooled water in a 1 000 ml volumetric flask.

1 ml of this standard solution contains 0,01 mg of C_6H_5OH.

Prepare this solution on the day of use.

4.2.11 Phenol, standard solution corresponding to 0,001 g C_6H_5OH per litre.

Dilute 50 ml of the phenol standard solution (4.2.10) to 500 ml with freshly boiled and cooled water in a 500 ml volumetric flask.

1 ml of this standard solution contains 0,001 mg of C_6H_5OH.

Prepare this solution within 2 h of use.

4.2.12 Phosphoric acid, ϱ = 1,70 g/ml.

4.2.13 Phosphoric acid, solution 1 + 9.

Mix 1 part by volume phosphoric acid (4.2.12) with 9 parts by volume of water.

4.2.14 Potassium hexacyanoferrate(III),[2] 80 g/l solution.

Dissolve 8,0 g of potassium hexacyanoferrate(III) $\{K_3[Fe(CN)_6]\}$ in water and dilute to 100 ml. Filter if necessary.

Prepare this solution within 1 week of use.

1) Systematic nomenclature : potassium sodium 2,3-dihydroxybutanedioate.

2) Trivial name : potassium ferricyanide.

4.2.15 Sodium sulfate, Na_2SO_4, anhydrous and granular.

4.2.16 Special reagents for turbid distillates.

4.2.16.1 Sulfuric acid, 0,5 mol/l solution.

4.2.16.2 Sodium chloride.

4.2.16.3 Sodium hydroxide, 2,5 mol/l solution.

Dissolve 10 g NaOH in 100 ml water.

4.2.16.4 Chloroform.

4.3 Apparatus

4.3.1 Distillation apparatus, all glass, consisting of a 1 litre borosilicate glass distilling apparatus with Graham condenser or equivalent.

4.3.2 pH meter, and suitable electrodes.

4.3.3 Spectrometer, with selectors for continuous or discontinuous variation, suitable for use at 510 nm and accommodating a cell that gives a path length of 10 to 100 mm shall be used. The size of the cell used will depend on the absorbance of the coloured solutions being measured and the characteristics of the spectrometer. In general, if the absorbances are greater than 1,0 with a certain cell, the next smaller size cell should be used.

4.4 Sampling and samples

Sampling of different kinds of waters should be carried out in accordance with ISO 5667/1 to 3, observing the following additional precautions. Samples shall be collected in glass bottles.

Phenolic compounds in water are subject to both chemical and biochemical oxidation. Therefore, unless the samples are analysed within 4 h of collection, they shall be preserved when collected, using the following procedure :

a) acidify the samples to a pH of approximately 4,0 with phosphoric acid (4.2.13). Use methyl orange (4.2.8) or a pH meter (4.3.2) to check the pH;

b) inhibit biochemical oxidation of phenolic compounds in the sample by adding 1,0 g of copper (II) sulfate (4.2.5) per litre of the sample;

c) store the sample in the cold (5 to 10 °C), and analyse the preserved samples within 24 h of collection.

4.5 Preliminary distillation

The use of copper(II) sulfate as described in 4.5.1 during distillation of an acidic sample permits the formation of copper(II) sulfide without subsequent decomposition to hydrogen sulfide. The acidic solution also prevents the precipitation of copper(II) hydroxide, which acts as an oxidizing agent towards phenolic compounds.

4.5.1 Measure 500 ml of the sample into a beaker. If the sample was not preserved with copper (II) sulfate [4.4.2 b)], add 5 ml of copper (II) sulfate solution (4.2.6), and adjust the pH of the solution to between 1 and 2 with phosphoric acid (4.2.13). Transfer the mixture to the distillation apparatus (4.3.1). Use a 500 ml graduated cylinder as receiver.

Distil 400 ml of the sample. Stop the distillation and, when boiling ceases, add 100 ml of water to the distillation flask. Continue the distillation until a total of 500 ml has been collected.

NOTE — It is also possible to distil smaller quantities.

4.5.2 If the distillate is turbid, a second distillation may prove helpful. Acidify the turbid distillate with phosphoric acid (4.2.13), add 5 ml of copper (II) sulfate solution (4.2.6) and then repeat the distillation described in 4.5.1. The second distillation usually eliminates the turbidity. However, if the second distillate is also turbid, extract another sample as described in 4.5.3.

4.5.3 Extract as quickly as possible a 500 ml aliquot of the laboratory sample as follows.

Add 4 drops methyl orange (4.2.8) and sufficient sulfuric acid (4.2.16.1) to make the solution acidic. Transfer to a separating funnel and add 150 g sodium chloride (4.2.16.2). Shake with five increments of chloroform, starting with 40 ml and adding four subsequent volumes of 25 ml. Place the chloroform layer in a second separating funnel and shake with three increments of sodium hydroxide (4.2.16.3), starting with 4,0 ml and adding two subsequent volumes of 3,0 ml. Combine the alkaline extracts, heat on a water-bath until the chloroform has been removed, then cool and dilute to 500 ml with water. Proceed with the distillation as described in 4.5.1.

NOTE — In some cases, in waste waters with high concentrations of phenolic compounds, a rise in temperature occurs during extraction.

4.6 Procedure

4.6.1 Test portion

Place 100 ml of the distillate, or a suitable aliquot which contains not more than the equivalent of 0,5 mg phenol diluted to 100 ml, in a 250 ml beaker. If the sample is known to contain more than the equivalent of 0,5 mg phenol, a smaller aliquot shall be used. Trial and error tests may be necessary to determine the volume of a suitable aliquot. Practically, the smallest aliquot that contains not more than the equivalent of 0,5 mg phenol should be 10 ml. The distillate and all solutions used shall be at room temperature.

4.6.2 Blank test

Carry out a blank test in parallel with the determination, replacing the test portion with 100 ml water.

4.6.3 Preparation of the calibration graph

4.6.3.1 Preparation of the set of calibration solutions

Prepare a set of calibration solutions, in seven 500 ml one-mark volumetric flasks, containing 0; 25; 50; 100; 150; 200; and 250 ml of phenol standard solution (4.2.10). Make up to the mark with water. All solutions used shall be at room temperature. The set of calibration solutions shall be treated according to 4.5.1.

4.6.3.2 Formation of the absorbing compound

Develop colour in the set of calibration solutions according to the procedure described in 4.6.4.

4.6.3.3 Spectrometric measurements

After 15 min, transfer the solutions to absorption cells and measure the absorbance of each calibration solution at 510 nm using water in the reference cell.

4.6.3.4 Plotting the graph

Plot the absorbances against the corresponding masses, in milligrams, of phenol.

4.6.4 Determination

Add 5 ml buffer solution (4.2.4) to each test portion (4.6.1), or add 5 ml of ammonium chloride solution (4.2.2) to each, adjust the pH to 10 ± 0,2 with ammonium hydroxide (4.2.3). Add 2,0 ml of 4-aminoantipyrine solution (4.2.1), mix immediately, then add 2,0 ml potassium hexacyanoferrate(III) solution (4.2.14) and again mix immediately.

After 15 min, measure the absorbance of each solution in a cell (see 4.3.3) at the wavelength of maximum absorbance (approximately 510 nm) using water in the reference cell. By reference to the calibration graph (4.6.3.4) calculate the mass, in milligrams, of phenol equivalent to the phenolic compounds in the test portion, after making allowance for the blank (4.6.2).

4.7 Expression of results

The phenol index, expressed in milligrams per litre, is given by the formula

$$\frac{m}{V_0} \times 1\,000$$

where

m is the mass, in milligrams, of phenol equivalent to the phenolic compounds in the test portion;

V_0 is the volume, in millilitres, of the test portion.

4.8 Interferences

Common interferences that may occur in waters are phenol-decomposing bacteria, oxidizing and reducing substances, and strongly alkaline conditions of the sample. Biological degradation is inhibited by the addition of copper (II) sulfate [4.4 b)] to the sample. Acidification with phosphoric acid [4.4 a)] assures the presence of the copper (II) ion and eliminates any chemical changes resulting from the presence of strong alkaline conditions. Treatment procedures required prior to the analysis for removal of interfering compounds may result in the unavoidable elimination or loss of certain types of phenolic compound.

Consequently, some highly contaminated waste waters may require specialized techniques for elimination of interferences and for quantitative recovery of the phenolic compounds.

A few methods for eliminating certain interferences are suggested.

4.8.1 Oxidizing agents

If the sample smells of chlorine, or if iodine is liberated from potassium iodide on acidification of the sample, the oxidizing agents so indicated should be removed immediately after sampling.

Iron(II) sulfate solution or ascorbic acid solution should be added to destroy all of the oxidizing substances. Excess iron(II) sulfate or ascorbic acid do not interfere since they are removed in the distillation procedure.

4.8.2 Oils and tar

If the sample contains oil or tar, some phenolic compounds may be dissolved in these materials. An alkaline extraction, in the absence of copper (II) sulfate, may be used to eliminate the tar or oil. Adjust the pH of the sample to between 12 and 12,5 with sodium hydroxide (4.2.16.3) to avoid extraction of phenolic compounds. Extract the mixture with carbon tetrachloride as quickly as possible. Discard the carbon tetrachloride layer. Remove any carbon tetrachloride remaining in the aqueous portion of the sample, for example by gentle heating and adjust the pH to 4,0 (see 4.4).

4.8.3 Sulfur compounds

Compounds that liberate hydrogen sulfide on acidification may interfere with the determination of the phenol index. Treatment of the acidified sample with copper (II) sulfate usually eliminates such interference. Add a sufficient quantity of copper (II) sulfate solution (4.2.6) to give a light blue colour to the sample or until no more copper (II) sulfide precipitate is formed, then acidify the sample with phosphoric acid (4.2.12) until just acid to methyl orange (4.2.8).

4.8.4 Reducing agents

In the presence of reducing agents, add an excess of potassium hexacyanoferrate (III).

4.8.5 Amines

Under the specified reaction conditions some amines will be determined as phenols, thus resulting in values which are too high. This interference may be minimized by distillation at below pH 0,5.

5 Method B — Chloroform extraction method

5.1 Principle

Separation of phenolic compounds from impurities and preservative agents by distillation. The rate of volatilization of the phenolic compounds is gradual, so that the volume of the distillate must equal that of the test sample being distilled.

Reaction of the steam-distillable phenolic compounds with 4-aminoantipyrine at a pH of 10,0 ± 0,2 in the presence of potassium hexacyanoferrate(III) to form a coloured antipyrine dye.

Extraction of this dye from aqueous solution with chloroform and measurement of the absorbance at 460 nm. The phenol index is expressed as milligrams of phenol per litre.

For the spectrometric measurement, the minimum detectable quantity is equivalent to 0,005 mg phenol when the test portion is extracted with 25 ml chloroform and measured in a 50 mm cell, or extracted with 50 ml chloroform and measured in a 100 mm cell. The minimum detectable phenol index is 0,002 mg/l in 500 ml distillate.[1)]

5.2 Reagents

See 4.2.

5.3 Apparatus

See 4.3, with the following modification and addition :

5.3.1 Spectrometer, as 4.3.3, but suitable for use at 460 nm.

5.3.2 Büchner funnel, with coarse fritted disc or phase separator filter.

5.4 Sampling and samples

See 4.4.

5.5 Procedure

5.5.1 Test portion

Place 500 ml of the distillate, or a suitable aliquot which contains not more than the equivalent of 0,05 mg phenol diluted to 500 ml, in a 1 litre beaker. Trial and error tests may be necessary to determine the volume of a suitable aliquot. Practically, the smallest aliquot that contains not more than the equivalent of 0,05 mg phenol should be 50 ml. The distillate and all solutions used shall be at room temperature.

5.5.2 Blank test

Carry out a blank test in parallel with the determination, replacing the test portion with 500 ml water.

5.5.3 Preparation of the calibration graph

5.5.3.1 Preparation of the set of calibration solutions

Prepare a set of calibration solutions, in nine 500 ml one-mark volumetric flasks, containing 0; 1; 2; 5; 10; 20; 30; 40; and 50 ml of phenol standard solution (4.2.11). Make up to the mark with water. All solutions used shall be at room temperature. The set of calibration solutions shall be treated according to 4.5.1.

5.5.3.2 Formation of the absorbing compound

Develop colour in the set of calibration solutions according to the procedure described in 5.5.4.

5.5.3.3 Spectrometric measurements

Measure the absorbance of each calibration solution at 460 nm using chloroform in the reference cell.

5.5.3.4 Plotting the graph

Plot the absorbances against the corresponding masses, in milligrams, of phenol.

5.5.4 Determination

Add 20 ml of buffer solution (4.2.4) to each test portion (5.5.1) and adjust the pH to 10 ± 0,2 with ammonium hydroxide (4.2.3) if necessary. Transfer each mixture to a 1 litre separating funnel. Add 3,0 ml of 4-aminoantipyrine solution (4.2.1), mix immediately, then add 3,0 ml of potassium hexacyanoferrate(III) solution (4.2.14), and again mix immediately. Allow the colour to develop for 15 min.

Add exactly 25 ml of chloroform (4.2.16.4) to each separating funnel if a 10 to 50 mm cell is to be used in the spectrometer. Add 50 ml if a 100 mm cell is to be used. Shake the separating funnel vigorously for 1 min, and allow the phases to separate.

Filter each of the chloroform extracts through separate Büchner funnels (5.3.2) containing 5 g sodium sulfate (4.2.15), or phase separator filter or any other system that will remove traces of water, directly into 25 ml measuring flasks. Bring to volume with chloroform. Use 50 ml flasks if 10 mm cells are used. The measurement should be performed within 1 h.

1) See the note to clause 1.

Using the chloroform, adjust the spectrometer to zero absorbance at 460 nm. Measure the absorbance of the blank and the sample extracts, at the same wavelength. By reference to the calibration graph (5.5.3.4), calculate the mass, in milligrams, of phenol equivalent to the phenolic compounds in the test portion after making allowance for the blank (5.5.2).

5.6 Expression of results

The phenol index, expressed in milligrams per litre, is given by the formula

$$m \times \frac{1000}{V_0}$$

where

m is the mass, in milligrams, of phenol equivalent to the phenolic compounds in the test portion;

V_0 is the volume, in millilitres, of the test portion.

5.7 Interferences

See 4.8.

6 Test report

The test report shall include the following information :

a) a reference to this International Standard;

b) complete identification of the sample;

c) the phenol index, expressed in milligrams per litre;

d) the method used;

e) the preparation of the test portion;

f) any unusual features noted during the determination;

g) any deviation from the procedures specified in this International Standard, or regarded as optional.