INTERNATIONAL STANDARD

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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

Reference number
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 6439 was prepared by Technical Committee ISO/TC 147, *Water quality*.

This second edition cancels and replaces the first edition (ISO 6439 : 1984), of which it constitutes a minor revision.

Annex A forms an integral part of this International Standard.

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
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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.

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

1 Scope

This International Standard specifies methods for determining the phenol index (3.2) in drinking waters, surface waters and 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 mg/l to about 0,10 mg/l when the coloured end-product is extracted and concentrated in chloroform phase, using phenol as a standard.

NOTES

1 The limits of detection achievable with both methods are insufficient for checking compliance with the limits given in the Directive 80/778/EEC for drinking water.

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

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5667-1 : 1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes.*

ISO 5667-2 : 1982, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

ISO 5667-3 : 1985, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples.*

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.

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 of phenol when a 50 mm cell is used in the spectrometric measurement and 100 ml of 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.

If red particles remain, the solution cannot be used again.

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 of ammonium chloride (NH_4Cl) and 200 g of potassium sodium tartrate ($NaKC_4H_4O_6$) in 700 ml of water. Add 150 ml of ammonium hydroxide (4.2.3) and dilute to 1 000 ml with water.

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

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 is stable for about 1 week.

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

4.2.10 Phenol, standard solution corresponding to 0,01 g of 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 of 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 of 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.

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 of NaOH in 100 ml of water.

4.2.16.4 Chloroform.

WARNING — Chloroform is toxic and a suspected carcinogen. Do not breathe vapour. Avoid contact with skin and eyes.

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.

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

2) Trivial name : potassium ferricyanide.

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 mm 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, ISO 5667-2 and ISO 5667-3, 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 °C 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 of 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 of sodium chloride (4.2.16.2). Shake with five separate portions of chloroform, starting with a volume of 40 ml, and then with four volumes of 25 ml. Separate the chloroform layer after each extraction and combine the chloroform extracts in a second separating funnel. Shake with three separate portions of sodium hydroxide solution (4.2.16.3), starting with a volume of 4,0 ml and then with two volumes of 3,0 ml. Separate the sodium hydroxide solution after each extraction. 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 of 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 of 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 of 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 of 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 ml; 25 ml; 50 ml; 100 ml; 150 ml; 200 ml; 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

Allow the absorbing compound to form 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 of 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 and 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 of 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).

Estimate the phenol index of the test portion by reference to the calibration graph and to the absorbance obtained with the solution of sample.

4.7 Expression of results

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

Estimate the phenol index of the test portion by reference to the calibration graph and to the absorbance obtained with the solution of sample.

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

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 as follows.

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.

Ascorbic acid solution should be added immediately after sampling to destroy all of the oxidizing substances. Excess ascorbic acid does not interfere since it is 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 of phenol when the test portion is extracted with 25 ml of chloroform and measured in a 50 mm cell, or extracted with 50 ml of chloroform and measured in a 100 mm cell. The minimum detectable phenol index is 0,002 mg/l in 500 ml of 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 in 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 of 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 of 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 of 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 ml; 1 ml; 2 ml; 5 ml; 10 ml; 20 ml; 30 ml; 40 ml; 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

Allow the absorbing compound to form 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 absorbing compound to form for 15 min.

Add exactly 25 ml of chloroform (4.2.16.4) to each separating funnel if a 10 mm 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 of sodium sulfate (4.2.15), or through a 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.

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).

1) See the note to clause 1.