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ISO RECOMMENDATION

R 760

DETERMINATION OF WATER BY THE KARL FISCHER METHOD

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BRIEF HISTORY

The ISO Recommendation R 760, *Determination of water by the Karl Fischer method,* was drawn up by Technical Committee ISO/TC 47, *Chemistry,* the Secretariat of which is held by the Ente Nazionale Italiano di Unificazione (UNI).

Work on this question led, in 1962, to the adoption of a Draft ISO Recommendation.

In November 1963, this Draft ISO Recommendation (No. 660) was circulated to all the ISO Member Bodies for enquiry. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

Australia	Hungary	Portugal
Austria	India	Romania
Belgium	Israel	Spain
Chile	Italy	U.A.R.
Colombia	Japan	United Kingdom
Czechoslovakia	Korea, Rep. of	U.S.S.R.
France	Netherlands	Yugoslavia
Germany	Poland	

Two Member Bodies opposed the approval of the Draft :

New Zealand
U.S.A.

The Draft ISO Recommendation was then submitted by correspondence to the ISO Council which decided, in June 1968, to accept it as an ISO RECOMMENDATION.

CONTENTS

ISO Recommendation R 760 June 1968

DETERMINATION OF WATER BY THE KARL FISCHER METHOD *

1. SCOPE

This ISO Recommendation describes a method suitable for the determination of free water or water of crystallisation in most solid or liquid chemical products, both organic and inorganic.

Precautions are necessary in certain cases and these are specified in the appropriate ISO Recommendations.

There are two methods of titration, depending on whether the end point is detected visually or electrometrically. The visual method can be used only with colourless solutions and when no electrometric apparatus is available; it is always a direct titration. The electrometric method on the other hand may involve either a direct titration or a back titration. The electrometric method, whether by direct titration or back titration, is the more accurate and for this reason it is recommended.

2. PRINCIPLE

Reaction of any water present with a solution of iodine and sulphur dioxide in a pyridine methanol mixture (Karl Fischer reagent), previously standardized by titration with an exactly known mass of water (see clauses 6.1, 7.1 and 8.1).

NOTE. – Methanol may be replaced by 2-methoxyethanol (ethylene glycol monomethyl ether). With this solvent a more constant titration volume is obtained and the reagent can be used with aldehydes and ketones, without using any special technique.**

3. REACTIONS***

$$H_2O + I_2 + SO_2 + 3C_5H_5N \longrightarrow 2C_5H_5N.HI + C_5H_5N.SO_3$$

$$C_5H_5N.SO_3 + ROH \longrightarrow C_5H_5NH.OSO_2OR$$

4. REAGENTS AND AUXILIARY MATERIALS

4.1 *Methanol,* analytical reagent grade not containing more than 0.05 % of water. If the reagent contains more than this quantity of water, dry it by distillation from magnesium turnings activated with iodine. Collect the distillate in a receiver protected from atmospheric moisture by means of a guard tube filled with anhydrous aluminium sodium silicate or activated silica gel (4.9).

4.2 *2-Methoxyethanol (ethylene glycol monomethyl ether),* not containing more than 0.05 % of water. If the reagent contains more than this quantity of water, dry it by distillation, rejecting the first portion of distillate which will contain the water.

* Fischer, Karl. *Angewandte Chemie* 1935, *48*, page 394.

** Peters, E.D. and Jungnikel, I.L. *Anal. Chem.* 1955, *27*, 450-53.

*** Smith, D.M. Bryant, W.N.D. and Mitchell Jr., J. *Journal of the American Chemical Society* 1939, *61*, page 2407.

4.3 *Pyridine,* not containing more than 0.05 % of water. If the reagent contains more than this quantity of water, dry it by distillation, rejecting the first portion of distillate which will contain the water.

4.4 *Sample solvent.* Either methanol (4.1), a mixture containing 4 parts of methanol (4.1) and 1 part of pyridine (4.3) (by volume) or (preferably for determinations with compounds containing carbonyl groups) a mixture containing 4 parts of 2-methoxyethanol (4.2) and 1 part of pyridine (4.3) (by volume). In special cases, other solvents may be recommended, for example, acetic acid, pyridine or a mixture containing 1 part of methanol (4.1) and 3 parts of chloroform (by volume).

4.5 *Karl Fischer reagent.* Place 670 ml of methanol (4.1) or 2-methoxyethanol (4.2) in a previously dried flask, coloured brown or painted black on the outside, fitted with a ground glass stopper and having a capacity slightly greater than 1 litre.

Add about 85 g of iodine. Stopper the flask and shake it occasionally until the iodine is completely dissolved. Then add approximately 270 ml of pyridine (4.3), stopper the flask again and mix thoroughly. Dissolve 65 g of sulphur dioxide in this solution, cooling to ensure that the temperature of the liquid does not exceed 20 °C.

NOTE. – The reaction being exothermix, it is necessary to cool the flask from the beginning and to maintain it at about 0 °C, for example, by immersing in an ice bath or in crushed solid carbon dioxide.

Replace the ground glass stopper by an attachment for introducing sulphur dioxide. This consists of a cork with a thermometer and an inlet glass tube 6 mm × 8 mm, reaching to within 10 mm of the bottom of the flask, and a small capillary tube for connecting to the atmosphere.

Place the whole assembly with the ice bath on a balance and weigh to the nearest 1 g. Connect the inlet tube to a siphon of sulphur dioxide by means of a flexible connection and a drying tube filled with anhydrous aluminium sodium silicate (4.9) as desiccant and gently open the tap on the siphon.

Adjust the rate of flow of sulphur dioxide so that all the gas is absorbed without the liquid showing any sign of rising in the inlet tube.

Maintain the equilibrium of the balance by gradually increasing the tare and ensure that the temperature of the liquid does not rise above 20 °C. Close the tap on the siphon as soon as the increase in mass reaches 65 g.

Immediately remove the flexible connection and reweigh the flask and its inlet attachment. The mass of dissolved sulphur dioxide should be between 60 and 70 g. A slight excess is not harmful.

Stopper the flask, mix the solution and leave for at least 24 hours before using it. In fact, as the result of imperfectly understood reactions which occur in the fresh reagent, the water equivalent of the reagent decreases rapidly to begin with and then much more slowly.

This water equivalent is between 3.5 and 4.5 mg/ml. It should be determined daily if methanol has been used, but may be done less frequently if 2-methoxyethanol has been used.

It is possible to prepare the Karl Fischer reagent with a lower water content by diluting the solution prepared as described above with the sample solvent (4.4).

Store the reagent out of the light and protected from atmospheric moisture. It should preferably be stored in a reagent bottle of brown or black-painted glass.

4.6 *Sodium tartrate,* crystalline, ($Na_2C_4H_4O_6.2H_2O$) or water.

4.7 *Water/methanol,* 10 mg/ml standard solution.

Using a microburette or a pipette, place 1 ml of water in a dry 100 ml one-mark volumetric flask, containing approximately 50 ml of methanol (4.1). Dilute to the mark with methanol (4.1) and mix (for standardization with this solution see Annex A, clause A.1 or A.2 depending on whether the visual method or the direct electrometric method is used).

4.8 *Water/methanol* solution, approximately 2 g/l.

Using a microburette or a pipette, place 1 ml of water into a perfectly dry 500 ml one-mark volumetric flask containing approximately 100 ml of methanol (4.1). Dilute to the mark with methanol (4.1) and mix (see the correspondence by volume of this solution with the Karl Fischer reagent in clause 8.2.2).

4.9 *Aluminium sodium silicate,* anhydrous, granules of diameter of 1.7 mm for use as a desiccant. These granules may be regenerated by washing with water and drying at 350 °C for at least 48 hours.

Or *activated silica gel.*

4.10 *Silicone base grease,* for lubricating the ground glass joints.

5. APPARATUS

5.1 **For direct titration** (by visual method or electrometric method).

5.1.1 A suitable form of apparatus for this method is given in Annex B, should no commerical apparatus be available. This standard apparatus comprises the parts described below.

5.1.1.1 *Automatic burette,* 25 ml capacity with a fine point, graduated in 0.05 ml and protected from atmospheric moisture by a guard tube filled with the desiccant (4.9).

5.1.1.2 *Titration vessel,* effective capacity of 100 ml, connected to the tap of the burette (5.1.1.1) by a ground glass joint and having two side tubes, one permitting the introduction of platinum electrodes if the electrometric method is used and the other fitted with a "vaccine cap" to permit the introduction of liquid test samples by means of a syringe without opening the vessel.

5.1.1.3 *Platinum electrodes* (Fig. 1 and 2, Annex B) welded to a glass tube enabling them to be introduced into the bottom of the titration vessel (5.1.1.2) and joined to two copper wires which in turn connect to the device for the electrometric detections of the end point (5.1.1.7) (these are omitted in the case of the visual method).

5.1.1.4 *Electromagnetic stirrer,* operating at 150 to 300 revolutions per minute with a mild steel bar coated with glass or PTFE, fixed on a base of adjustable height.

5.1.1.5 *Reagent bottle for the Karl Fischer reagent* (capacity approximately 3 litres) of brown or black-painted glass in which the filling tube of the automatic burette is immersed through the ground glass stopper.

5.1.1.6 *Rubber bulb* connected to an air dryer filled with the desiccant (4.9) for the admission of dry air under pressure into the reagent bottle (5.1.1.5) in order to fill the burette (5.1.1.1).

5.1.1.7 *Device* for the electrometric detection of the end point shown diagramatically in Figure 3, Annex B (to be omitted in the case of the visual method).

5.1.2 *Medical syringes,* of suitable capacity and calibrated.

5.1.3 *A small glass tube,* closed at one end and fitted at the other with a rubber stopper, used for weighing and introducing into the titration vessel, for example, the crystalline sodium tartrate (approximately 250 mg) used to standardize the Karl Fischer reagent, or possibly test samples of solid products.

NOTE. – All glassware used should previously be dried at 130 °C in an oven, then placed in a desiccator containing the desiccant (4.9).

5.2 For the electrometric back titration method

5.2.1 A suitable form of apparatus for this method is given in Annex C, should no commercial apparatus be available.

This standard apparatus comprises the parts described below.

5.2.1.1 *Two automatic burettes,* 25 ml capacity with fine points, connected directly to their filling containers, one of brown or black-painted glass for the Karl Fischer reagent (4.5) and the other for the standard water/methanol solution (4.8).

5.2.1.2 *Titration vessel,* effective capacity of 100 ml, connected to the burette by means of ground glass joints and having two side tubes, one permitting the introduction of platinum electrodes and the other fitted with a "vaccine cap" to permit introduction of liquid test samples by means of a syringe without opening the vessel.

5.2.1.3 *Drying tube,* connecting the filling containers of the burettes and the stopper of the titration vessel by a closed circuit.

5.2.1.4 *Platinum electrodes* (Fig. 4 and 5, Annex C) welded to a glass tube enabling them to be introduced into the bottom of the titration vessel (5.1.1.2) and joined to two copper wires which in turn connect to the device for the electrometric detection of the end point (5.2.1.6).

5.2.1.5 *Electromagnetic stirrer,* operating at 150 to 300 revolutions per minute, with a mild steel bar coated with glass or PTFE, fixed on a base of adjustable height.

5.2.1.6 *Device* for the electrometric detection of the end point shown diagrammatically in Figure 3, Annex B.

5.2.2 *Medical syringes,* of suitable capacity, of which the volume is calibrated.

5.2.3 *A small glass tube,* closed at one end and fitted at the other with a rubber stopper, used for weighing and introducing into the titration vessel, for example, the mass of crystalline sodium tartrate (approximately 250 mg) used to standardize the Karl Fischer reagent or possibly test samples of solid products.

NOTE. – All glassware used should previously be dried at 130 °C in an oven and then placed in a desiccator containing the desiccant (4.9).

6. VISUAL METHOD

6.1 Principle for the detection of the end point

Development of colour in the test portion by addition of the first drop of excess Karl Fischer reagent, coloured with iodine which gradually becomes colourless on addition to the test portion containing the water to be determined.

6.2 Procedure

6.2.1 *Standardization of Karl Fischer reagent*

6.2.1.1 With the apparatus assembled as shown in Annex B and with the joints lubricated with the grease (4.10), introduce by means of a syringe (5.1.2) 25 ml of methanol (4.1) into the titration vessel (5.1.1.2) through the "vaccine cap".

Switch on the electromagnetic stirrer (5.1.1.4). Add the Karl Fischer reagent (4.5) from the automatic burette (5.1.1.1) until a brown colour is obtained.

6.2.1.2 In the small tube (5.1.3) weigh 250 mg of sodium tartrate (4.6) to the nearest 0.1 mg. Place this in the titration vessel very quickly, removing the "vaccine cap" for a few seconds. Weigh the small tube (5.1.3) empty, so as to determine by difference the mass of crystalline sodium tartrate (4.6) used.*

Titrate the known quantity of water introduced in this way with the Karl Fischer reagent (4.5) to be standardized, until the same end point is reached, and note the volume of reagent used.

6.2.2 *Test determination*

Empty the titration vessel (5.1.1.2) by means of the emptying tap. Place in it 25 ml of methanol (4.1) or other solvent (4.3 or 4.4) or any other suitable volume indicated in the procedure for the product to be analysed, using a syringe (5.1.2) passing through the "vaccine cap". Switch on the electromagnetic stirrer (5.1.1.4). Add Karl Fischer reagent (4.5) from the automatic burette (5.1.1.1) until a brown colour is obtained.

Introduce the required amount of the test portion by means of a syringe in the case of a liquid, or weighed to the nearest 0.1 mg in the small glass tube (5.1.3) in the case of a solid powder. Titrate with the Karl Fischer reagent (4.5) until the same end point is reached.

NOTE. – It is advisable to use a quantity of test portion the water content of which corresponds to a volume of Karl Fischer reagent that can be measured with sufficient accuracy. If necessary increase in proportion the quantities of methanol and test portion used and then use a titration vessel of suitable capacity.

6.3 Expression of results

6.3.1 Water equivalent T of the Karl Fischer reagent (4.5)

$$\text{mg } H_2O/\text{ml} = T = \frac{M_1}{a} \text{ or } \frac{M_2}{a}$$

where

a is the volume, in millilitres, of Karl Fischer reagent (4.5) used;

M_1 is the mass, in milligrammes, of water used (see clause 6.2.1.2);

M_2 is the mass, in milligrammes, of sodium tartrate (4.6) introduced multiplied by 0.1566.

6.3.2 Water content of the sample

$$H_2O\ \%\ (m/m) = \frac{b \times T}{E \times 10} \text{ or } \frac{b \times T}{V \times \rho \times 10}$$

where

b is the volume, in millilitres, of Karl Fischer reagent (4.5) used for the test;

E is the mass, in grammes, of the test portion (for solid products);

V is the volume, in millilitres, of the test portion (for liquid products);

T is the water equivalent, in milligrammes per millilitre, of Karl Fischer reagent (see clause 6.3.1);

ρ is the density of the sample, in grammes per millilitre, at 20 °C (for liquid products only).

* For standardization with the water/methanol standard solution (4.7) see Annex A, clause A.1. Standardization may also be effected by introducing a mass of pure water of approximately 40 mg from a dropping bottle, weighed before and after introduction into the titration vessel.

7. DIRECT ELECTROMETRIC TITRATION

7.1 Principle of the detection of the end point

Indication of the end point of the titration by the depolarization of the cathode accompanied by a sudden increase in current intensity (which is shown by a suitable electrical device), the two platinum electrodes being immersed in the solution and subjected to a potential difference, but while water is present in the solution the polarization of the cathode opposes the passage of a current.

7.2 Procedure

7.2.1 *Standardization of Karl Fischer reagent*

7.2.1.1 Assemble the apparatus as shown in Figure 1, Annex B, lubricating the joints with the grease (4.10). Introduce by means of a syringe (5.1.2) 25 ml of methanol (4.1) into the titration vessel (5.1.1.2) through the "vaccine cap". Switch on the electromagnetic stirrer (5.1.1.4) and close the circuit of the device for the electrometric detection of the end point (5.1.1.7).

Adjust the apparatus so that a voltage of 1 to 2 V is applied to the electrodes and the galvanometer shows a low current, usually a few microamperes. Add the Karl Fischer reagent (4.5) until the galvanometer shows a sudden increase in current of about 10 to 20 μA, which remains stable for at least 1 minute.

7.2.1.2 In the small glass tube (5.1.3) weigh 250 mg of crystalline sodium tartrate (4.6) to the nearest 0.1 mg. Place this in the titration vessel (5.2.1.2) very quickly, removing the "vaccine cap" for a few seconds. Weigh the small glass tube (5.1.3) empty, so as to determine, by difference, the mass of crystalline sodium tartrate (4.6) used.*

Titrate the known quantity of water introduced in this way with the Karl Fischer reagent (4.5) to be standardized, until the same deflection of the pointer of the galvanometer is reached and remains stable for at least 1 minute. Note the volume of reagent used.

7.2.2 *Test determination*

Empty the titration vessel (5.2.1.2) by means of the emptying tap. Place in it 25 ml of methanol (4.1) or other solvent (4.3 or 4.4), or any other suitable volume indicated in the procedure for the product to be analysed, using a syringe (5.1.2) passing through the "vaccine cap". Switch on the electromagnetic stirrer (5.1.1.4). Add Karl Fischer reagent (4.5), proceeding as described in clause 7.2.1 until there is a sudden and constant deflection lasting for at least 1 minute. Then introduce the required amount of test portion taken by means of a syringe (5.2.2) in the case of a liquid or weighed to the nearest 0.1 mg in a small weighing tube (5.1.3) in the case of a solid powder. Titrate with Karl Fischer reagent (4.5) using the same electrometric procedure for detecting the end point of the reaction.

Note the volume of Karl Fischer reagent (4.5) for the determination.

NOTE. – It is advisable to use a quantity of test portion the water content of which corresponds to a volume of Karl Fischer reagent that can be measured with sufficient accuracy. If necessary, increase in proportion the quantities of solvent and test sample used and then use a titration vessel of suitable capacity.

* For standardization with the water/methanol standard solution (4.7), see Annex A, clause A.2. Standardization may also be effected by introducing a mass of pure water of approximately 40 mg from a dropping bottle, weighed before and after introduction into the titration vessel.

7.3 Expression of results

7.3.1 Water equivalent T of the Karl Fischer reagent (4.5)

$$\text{mg } H_2O/\text{ml} = T = \frac{M_1}{a} \text{ or } \frac{M_2}{a}$$

where

a is the volume, in millilitres, of Karl Fischer reagent (4.5) used;

M_1 is the mass, in milligrammes, of water used (see clause 7.2.1.2);

M_2 is the mass, in milligrammes, of sodium tartrate (4.6) introduced multiplied by 0.1566.

7.3.2 Water content of the sample

$$H_2O \text{ \% (m/m)} = \frac{b \times T}{E \times 10} \text{ or } \frac{b \times T}{V \times \rho \times 10}$$

where

b is the volume, in millilitres, of Karl Fischer reagent (4.5) used for the test;

E is the mass, in grammes, of the test portion (for solid products);

V is the volume, in millilitres, of the test portion (for liquid products);

T is the water equivalent, in milligrammes per millilitre, of the Karl Fischer reagent (see clause 7.3.1);

ρ is the density of the sample, in grammes per millilitre, at 20 °C (for liquid products only).

8. ELECTROMETRIC BACK TITRATION

8.1 Principle for the detection of the end point

Addition of an excess of Karl Fischer reagent which is then back titrated with a water/methanol standard solution. Indication of the end point of the titration by the polarization of the cathode accompanied by the sudden interruption of the current (which is shown by a suitable electrical device) the electrodes being subjected to a very slight potential difference but sufficient to cause a large deflection of the galvanometer pointer at the start of the back titration.

8.2 Procedure

8.2.1 *Standardization of Karl Fischer reagent*

8.2.1.1 Assemble the apparatus as shown in Annex C lubricating the joints with the grease (4.10). Place in the titration vessel (5.2.1.2) sufficient Karl Fischer reagent (4.5) from one of the automatic burettes (5.2.1.1) to cover the electrodes (5.2.1.4). Switch on the electromagnetic stirrer (5.2.1.5) and the circuit of the device for the electrical detection of the end point (5.2.1.6). Allow the water/methanol standard solution (4.8) to flow from the second automatic burette (5.2.1.1) until the pointer of the galvanometer moves suddenly to zero.

8.2.1.2 In the small glass tube (5.2.3) weigh approximately 250 mg of crystalline sodium tartrate (4.6) to the nearest 0.1 mg. Place this in the titration vessel (5.2.1.2) very quickly, removing the "vaccine cap" for a few seconds. Weigh the small glass tube (5.2.3) empty, so as to determine by difference the mass of crystalline sodium tartrate (4.6) used.

8.2.2 *Correspondence between the Karl Fischer reagent and the standard solution of water in methanol*

Partially empty the titration vessel (5.2.1.2) leaving the electrodes submerged in the liquid neutralized as described in clause 8.2.1.

Add 20 ml of Karl Fischer reagent (4.5), measured in the first automatic burette (5.2.1.1), and titrate with the standard water/methanol solution (4.8) contained in the second automatic burette, until the pointer of the galvanometer moves suddenly to zero. Note the volume of this solution (4.8) used.

8.2.3 *Test determination*

Empty the titration vessel (5.2.1.2) by means of the emptying tap. Place in it 25 ml of methanol (4.1) or any other appropriate volume indicated in the procedure for the product to be analysed, using a syringe (5.2.2) passing through the "vaccine cap". Switch on electromagnetic stirrer (5.2.1.5).

Add a slight excess (approximately 2 ml of Karl Fischer reagent) (4.5) and then add the water/methanol standard solution (4.8) until the pointer of the galvanometer moves suddenly to zero. Introduce the required amount of test portion by means of a syringe (5.2.2) in the case of a liquid or weighed to the nearest 0.1 mg in a small glass tube (5.2.3) in the case of a solid powder.

Add a known excess volume of Karl Fischer reagent (4.5) stopping when the solution becomes brown in colour. Wait for 30 seconds and back titrate this excess with the water/methanol standard solution (4.8) until the pointer of the galvanometer moves suddenly to zero.

NOTE. – It is advisable to use a quantity of test portion the water content of which corresponds to a volume of Karl Fischer reagent that can be measured with sufficient accuracy. If necessary, increase in proportion the quantities of methanol and test sample used and then use a titration vessel of suitable capacity.

8.3 Expression of results

8.3.1 Water equivalent T of the Karl Fischer reagent (4.5)

$$\text{mg } H_2O/\text{ml} = T = \frac{M_1}{a - a' \times \frac{20}{a''}} \text{ or } \frac{M_2}{a - a' \times \frac{20}{a''}}$$

where

a is the volume, in millilitres, of Karl Fischer reagent (4.5) used in clause 8.2.1.2;

a' is the volume, in millilitres, of water/methanol standard solution (4.8) used in clause 8.2.1.2 for the back titration;

a'' is the volume, in millilitres, of water/methanol standard solution (4.8) used in clause 8.2.2 (correspondence with the Karl Fischer reagent);

M_1 is the mass, in milligrammes, of water used in clause 8.2.1.2;

M_2 is the mass, in milligrammes, of crystalline sodium tartrate (4.6) multiplied by 0.1566.

8.3.2 Water content of the sample introduced

$$H_2O\ \%\ (m/m) = (b - b' \times \frac{20}{a''}) \times \frac{T}{E \times 10}$$

$$\text{or } (b - b' \times \frac{20}{a''}) \times \frac{T}{V \times \rho \times 10}$$

where

- b is the volume, in millilitres, of Karl Fischer reagent (4.5) used in clause 8.2.3;
- b' is the volume, in millilitres, of water/methanol standard solution (4.8) used in clause 8.2.3 for the back titration;
- E is the mass, in grammes, of the test portion (for solid products),
- V is the volume, in millilitres, of the test portion (for liquid products);
- T is the water equivalent, in milligrammes per millilitre, of the Karl Fischer reagent;
- ρ is the density of the sample at 20 °C, in grammes per millilitre (for liquid products only).

9. TEST REPORT

Give the following particulars :

(*a*) the reference of the method used;

(*b*) the results and the method of expression used;

(*c*) any unusual features noted during the determination;

(*d*) any operation not laid down in this ISO Recommendation or regarded as optional.