INTERNATIONAL STANDARD

ISO 1241

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Essential oils — Determination of ester values, before and after acetylation, and evaluation of the contents of free and total alcohols

Huiles essentielles — Détermination de l'indice d'ester, avant et après acétylation, et évaluation de la teneur en alcools libres et en alcools totaux

Reference number
ISO 1241:1996(E)

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 1241 was prepared by Technical Committee ISO/TC 54, *Essential oils.*

This second edition cancels and replaces the first edition (ISO 1241:1980), which has been technically revised.

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet central@isocs.iso.ch
X.400 c=ch; a=400net; p=iso; o=isocs; s=central

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Essential oils — Determination of ester values, before and after acetylation, and evaluation of the contents of free and total alcohols

1 SCOPE

This International Standard specifies a method to evaluate the contents of free alcohols and total alcohols in essential oils by determination of ester values before and after acetylation by acetic anhydride in the presence of sodium acetate.

The method is not applicable to essential oils containing appreciable quantities of tertiary alcohols (for example linalol and terpineols), which would not be completely acetylated.

NOTE: For these essential oils the method given in ISO 3794[1)] should be used.

The method is not applicable to essential oils containing appreciable quantities of phenols, lactones, aldehydes or enolysable ketones, which would be acetylated in addition to free alcohols.

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 212:1973, *Essential oils — Sampling.*

ISO 356:1996, *Essential oils — Preparation of test sample.*

ISO 385-1:1984, *Laboratory glassware — Burettes — Part 1: General requirements.*

ISO 709:1980, *Essential oils — Determination of ester value.*

1) ISO 3794:1976, *Essential oils (containing tertiary alcohols) - Estimation of free alcohols content by determination of ester value after acetylation.*

3 DEFINITIONS

For the purposes of this International Standard, the following definitions apply.

3.1 ester value: Number of milligrams of potassium hydroxide which are required to neutralize the acids liberated by the hydrolysis of the esters contained in 1 g of the oil.

3.2 ester value after acetylation: Number of milligrams of potassium hydroxide which are required to neutralize the acids liberated by the hydrolysis of the esters contained in 1 g of the acetylated oil.

4 PRINCIPLE

4.1 Determination of ester value in accordance with ISO 709.

4.2 Acetylation of the essential oil by acetic anhydride in the presence of sodium acetate. Isolation and drying of the acetylated oil, and determination of its ester value in accordance with ISO 709.

4.3 Calculation of the free, combined and total alcohol contents from the ester values before and after acetylation.

5 REAGENTS

Use only reagents of recognized analytical grade and distilled water or water of equivalent purity.

5.1 Reagents for determination of ester value

5.1.1 Ethanol, 95 % (*V*/*V*) at 20°C, freshly neutralized by the potassium hydroxide solution (5.1.2) in the presence of the coloured indicator (5.1.4) used for the determination.

5.1.2 Potassium hydroxide, standard ethanolic solution, c(KOH) = 0,5 mol/l.

5.1.3 Hydrochloric acid, standard solution, c(HCl) = 0,5 mol/l.

5.1.4 Coloured indicator: phenophthalein, 2 g/l solution in ethanol 95 % (*V*/*V*) or, if the essential oil contains phenolic groups, **phenol red**, 0,4 g/l solution in ethanol 20 % (*V*/*V*).

5.2 Reagents for acetylation

5.2.1 Acetic anhydride, not less than 98 %.

5.2.2 Sodium acetate, anhydrous, freshly melted and powdered.

5.2.3 Sodium chloride, saturated solution.

5.2.4 Sodium carbonate/sodium chloride, 20 g/l solution of anhydrous sodium carbonate saturated with sodium chloride.

5.2.5 Magnesium sulfate, anhydrous, or **sodium sulfate,** anhydrous, freshly dried and powdered.

5.2.6 Litmus paper.

6 APPARATUS

Usual laboratory apparatus and, in particular, the following.

6.1 Acetylation apparatus, including an acetylation flask of capacity 100 ml to 250 ml, with a ground glass neck, provided with a glass tube to act as a reflux condenser, at least 1 m in length and of at least 10 mm internal diameter.

The flask and the condenser shall be carefully dried before use.

6.2 Measuring cylinders, of capacity 10 ml, graduated in 0,1 ml.

6.3 Measuring cylinders, of capacity 50 ml, graduated in 1 ml.

6.4 Water bath, capable of being maintained at between 40 °C and 50 °C.

6.5 Suitable heating device, for boiling acetic anhydride without local overheating.

6.6 Separating funnel, of capacity at least 250 ml.

6.7 Saponification apparatus, including an alkali-resistant glass flask, of capacity 100 ml to 250 ml, to which can be fitted a reflux condenser, at least 1 m in length and of at least 10 mm iinternal diameter.

6.8 Burette, of capacity 50 ml, graduated in 0,1 ml, conforming to the requirements of ISO 385-1.

6.9 Analytical balance.

7 SAMPLING

Sampling shall be carried out in accordance with ISO 212.

8 PROCEDURE

8.1 Preparation of test sample

The test sample shall be prepared in accordance with ISO 356.

8.2 Determination of ester value before acetylation

Determine the ester value before acetylation in accordance with the method given inISO 709.

8.3 Acetylation

Mix approximately 10 ml of the test sample (8.1), 10 ml of the acetic anhydride (5.2.1) and 2 g of the anhydrous sodium acetate (5.2.2) in the acetylation flask (6.1).

Add fragments of pumice stone or porcelain and fit the reflux condenser to the flask.

Heat the flask by means of the heating device (6.5) and maintain the liquid at moderate boiling for 2 h or for the time given in the International Standard for the essential oil being analysed.

At the end of this period, allow the liquid to cool. Add 50 ml of distilled water and heat on the water bath (6.4), set at between 40 °C and 50 °C, for 15 min, shaking frequently. Allow to cool to room temperature, remove the reflux tube and transfer the liquid to the separating funnel (6.6). Wash the flask twice with 10 ml of water and collect the washing in the separating funnel. Wait until separation of the phase is complete, then draw off and reject the aqueous phase.

Wash the oil by shaking successively with

a) 50 ml of the sodium chloride solution (5.2.3),
b) 50 ml of the sodium carbonate/sodium chloride solution (5.2.4),
c) 50 ml of the sodium chloride solution (5.2.3), and
d) 20ml of water.

If the washing have been properly conducted, they will be neutral to the litmus paper (5.2.6). Run the oil phase into a dry tube and shake several times over 15 min with at least 3 g of the magnesium or sodium sulfate (5.2.5). Filter. Repeat the contact and shaking with further 3 g portions of magnesium or sodium sulfate until the acetylated oil is free of water.

8.4 Determination of ester value after acetylation

Determine the ester value of the acetylated oil (8.3), in accordance with the requirements of ISO 709, by using approximately 2 g, weighed to the nearest 0,05 g, of the acetylated oil and 50 ml of the potassium hydroxide solution (5.1.2).

9 EXPRESSION OF RESULTS

9.1 The ester value after acetylation is given by the formula:

$$E_2 = \frac{28{,}05}{m}\left(V'_0 - V'_1\right)$$

9.2 The free alcohols content, expressed as a percentage by mass with respect to a given alcohol, is given by the formula:

$$\frac{M_r(E_2 - E_1)}{561\text{-}0{,}42E_2}$$

NOTE: This formula takes into account the increase in mass of the test portion during acetylation.

9.3 The combined alcohols content, expressed as a percentage by mass, with respect to a given alcohol, is given by the formula:

$$\frac{M_r \times E_1}{561}$$

9.4 The total alcohols content, expressed as a percentage by mass with respect to a given alcohol, is obtained by adding the two percentages obtained in 9.2 and 9.3.

In the above formulae:

m is the mass, in grams, of the test sample of the acetylated oil;

V'_0 is the volume, in millilitres, of the hydrochloric acid solution (5.1.3) used for the blank test;

V'_1 is the volume, in millilitres, of the hydrochloric acid solution (5.1.3) used for the determination of the ester value after acetylation ;

M_r is the relative molecular mass of the alcohol used to express results conventionally and which is given in the International Standard for the essential oil being analysed;

E_1 is the ester value of the oil before acetylation (8.2) calculated in accordance with ISO 709;

E_2 is the ester value of the oil after acetylation (8.4), calculated in accordance with ISO 709.

10 TEST REPORT

The test report shall state the method used and the results obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any details that may have influenced the results.

The test report shall include all details required for the complete identification of the sample.

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