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R 1241

ESSENTIAL OILS

ESTIMATION OF FREE ALCOHOLS CONTENT

BY DETERMINATION OF ESTER VALUE AFTER ACETYLATION

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BRIEF HISTORY

The ISO Recommendation R 1241, *Essential oils – Estimation of free alcohols content by determination of ester value after acetylation*, was drawn up by Technical Committee ISO/TC 54, *Essential oils*, the Secretariat of which is held by the Repartição de Normalização (IGPAI).

Work on this question led to the adoption of Draft ISO Recommendation No. 1241, which was circulated to all the ISO Member Bodies for enquiry in May 1967. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

Australia	Israel	Sweden
Belgium	Italy	Thailand
Bulgaria	Japan	Turkey
Canada	Netherlands	U.A.R.
France	New Zealand	United Kingdom
Greece	Portugal	U.S.S.R.
India	Romania	
Iran	South Africa, Rep. of	

No Member Body opposed the approval of the Draft.

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

ISO Recommendation | R 1241 | July 1971

ESSENTIAL OILS

ESTIMATION OF FREE ALCOHOLS CONTENT

BY DETERMINATION OF ESTER VALUE AFTER ACETYLATION

1. SCOPE

This ISO Recommendation describes a method for the estimation of the free alcohols content of essential oils by the determination of the ester value after acetylation.

This method is applicable to essential oils other than those containing appreciable quantities of tertiary alcohols the acetylation of which would be incomplete; it is not applicable to essential oils containing appreciable quantities of phenols, lactones, aldehydes or enolysable ketones, which would also be subject to acetylation.

This method does not enable the content of free alcohols in oil of citronella to be calculated but may be used, with modifications, for the determination of total acetylisable constituents in this essential oil.

2. DEFINITION

Ester value after acetylation. The number of milligrammes of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of 1 g of the acetylated essential oil.

3. PRINCIPLE

Acetylation of the essential oil by acetic anhydride in the presence of sodium acetate. Isolation and drying of the acetylated essential oil. Determination of the ester value after acetylation. Calculation of the free alcohols content, taking into account the ester value of the original oil before acetylation.

4. REAGENTS

4.1 *Acetic anhydride*, 98 to 100 %, analytical reagent grade.

4.2 *Sodium acetate*, anhydrous, freshly fused and powdered.

4.3 *Sodium chloride*, saturated solution.

4.4 *Sodium carbonate/sodium chloride* solution, containing 20 g of anhydrous sodium carbonate per dm^3, saturated with sodium chloride.

4.5 *Magnesium sulphate*, anhydrous, neutral, freshly ignited and powdered. As an alternative, *sodium sulphate* can be used.

4.6 *Litmus paper*.

4.7 *Phenolphthalein*, solution of 2 g of phenolphthalein per dm^3 in 95 % (V/V) neutralized ethanol, at 20 °C.

4.8 *Potassium hydroxide*, 0.1 N solution in 95 % (V/V) ethanol.

5. APPARATUS

5.1 *Acetylation apparatus*, including a 100 cm^3 round-bottomed acetylation flask 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 inside diameter.

5.2 *Measuring cylinders* of 10 cm^3 capacity, graduated in 0.1 cm^3.

5.3 *Measuring cylinders* of 50 cm^3 capacity, graduated in 1 cm^3.

5.4 *Suitable heating device* for maintaining boiling without local overheating.

5.5 *Separating funnel*, with capacity of at least 250 cm^3.

5.6 *Saponification apparatus*, including an alkali-resistant glass flask, of capacity 100 to 200 cm^3, to which a glass tube acting as reflux condenser may be fitted, at least 1 m in length and of at least 10 mm inside diameter. Attach a curved carbon dioxide absorber tube to the condenser during the cooling.

5.7 *Burette*, of at least 20 cm^3 capacity, graduated in 0.1 cm^3.

6. SAMPLING

Carry out sampling in accordance with ISO Recommendation R 212, *Essential oils – Sampling.*

7. PROCEDURE

7.1 Preparation of test sample

Prepare the test sample in accordance with ISO Recommendation R 356, *Essential oils – Methods of test – Preparation of sample.*

7.2 Determination

Mix approximately 10 cm^3 of the essential oil, 10 cm^3 of acetic anhydride (4.1) and 2 g of anhydrous sodium acetate (4.2) in the flask of the acetylation apparatus (5.1). Add fragments of pumice-stone or porcelain and fit the flask with its reflux condenser.

Heat the flask by the heating device (5.4) and gently reflux the liquid for 2 hours or for the time given in the specification for the essential oil under examination.

At the end of this period, allow the liquid to cool; add 50 cm^3 of distilled water and heat at a temperature between 40 and 50 °C using the heating device (5.4) for 15 minutes, shaking frequently. Cool to room temperature, remove the reflux tube and transfer the liquid to a separating funnel (5.5); wash the flask twice with 10 cm^3 of distilled water and add these washings to the contents of the separating funnel. Wait until separation of the liquids is complete, then reject the aqueous layer.

Wash the oil layer by shaking successively with

(*a*) 50 cm^3 of sodium chloride solution (4.3),

(*b*) 50 cm^3 of sodium carbonate/sodium chloride solution (4.4),

(*c*) 50 cm^3 of sodium chloride solution (4.3), and

(*d*) 20 cm^3 of distilled water.

Shake the acetylated essential oil vigorously with the saturated solutions, and gently with the distilled water, which, if the washings have been properly conducted, will be neutral to litmus paper (4.6).

Run the oil layer into a dry tube and shake several times during 15 minutes with at least 3 g of anhydrous magnesium sulphate (4.5). Filter the dried oil. Repeat the contact and shaking with further portions of 3 g magnesium sulphate until the acetylated oil is free from water.

In the flask of the saponification apparatus (5.6), weigh to an accuracy of 0.5 mg about 2 g of the acetylated essential oil, and add 2 cm^3 of distilled water and 0.5 cm^3 of phenolphthalein solution (4.7).

Neutralize the liquid with the ethanolic potassium hydroxide solution (4.8), using the burette (5.7).

Determine the ester value following the procedure given in ISO Recommendation R 709, *Determination of ester value and calculation of ester content of essential oils*, but adding 50 cm^3 of 0.5 N ethanolic potassium hydroxide solution instead of 25 cm^3.