International Standard 7359

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Essential oils — Analysis by gas chromatography on packed columns — General method

Huiles essentielles — Analyse par chromatographie en phase gazeuse sur colonne remplie — Méthode générale

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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. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 7359 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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Essential oils — Analysis by gas chromatography on packed columns — General method

0 Introduction

Since the description of methods of analysis by gas chromatography is very long, it is considered useful to establish general methods on the one hand, giving detailed information on all the recurrent parameters, apparatus, products, methods, formulae, etc. and, on the other hand, standards with short details on the determination of specific constituents in the essential oils, giving only those operating conditions specific to the pertinent determination.

These short-version standards will either refer to the present International Standard for gas chromatographic analyses on packed columns or to ISO 7609 for analyses on capillary columns.

1 Scope and field of application

This International Standard specifies a general method for the analysis of essential oils by gas chromatography on packed columns for the purpose of determining the content of a specific constituent and/or searching for a characteristic profile.

2 References

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

ISO 7609, *Essential oils — Analysis by gas chromatography on capillary columns — General method.*

3 Principle

Analysis by gas chromatography under specified conditions of a small quantity of essential oil on a column packed with appropriate material.

If required, identification of the different constituents from their retention indexes.

Quantitative determination of specific constituents by measurement of peak areas.

4 Reagents and products

During the analysis, unless otherwise specified, use only reagents of recognized analytical grade, and freshly distilled products.

4.1 Carrier gas: hydrogen [1], helium or nitrogen, according to the type of detector used. If detectors are used which require carrier gases other than those mentioned, the carrier gas shall be specified.

4.1.1 Auxiliary gases: any gases suitable for the detector used.

4.2 Product for checking the chemical inertness of the column: linalyl acetate, of purity at least 98 %.

4.3 Products for checking the efficiency of the column. [2]

4.3.1 Linalol, of purity at least 99 % determined by chromatography.

4.3.2 Methane, of purity at least 99 % determined by chromatography.

4.4 Reference substance, corresponding to the constituent to be determined or detected. The reference substance will be indicated in each relevant International Standard.

4.5 Internal standard.

The internal standard will be specified in each relevant International Standard; it should elute as near as possible to the constituent to be determined and should not superimpose on the peaks of any of the constituents of the essential oil.

4.6 Normal alkanes, of purity at least 95 % determined by chromatography. The range of normal alkanes to be used in a specific International Standard depends on the retention indexes of the constituents involved under the test conditions.

NOTE — Normal alkanes are used only when it is required to determine the retention indexes.

1) Strict observance of safety regulations is essential when using this gas.

2) Other products may be used to check the efficiency of the column; they will be specified in each relevant International Standard.

4.7 Test mixture.

Prepare a mixture containing approximately equal proportions of:

- limonene,
- acetophenone,
- linalol,
- linalyl acetate,
- naphthalene,
- cinnamic alcohol.

All these reagents shall be of purity at least 95 % determined by chromatography.

NOTE — Other products may be used; they will be specified in each relevant International Standard.

5 Apparatus

5.1 Chromatograph, equipped with a suitable detector and a temperature programmer. The injection and detection systems shall be fitted with devices for independent control of their respective temperatures.

5.2 Column, made of an inert material (for example glass or stainless steel), of internal diameter between 2 and 4 mm, and preferably of length 2 to 4 m.

The support shall be as inert as possible, for example silanized and acid-washed celite. It is necessary to use a particular particle size, which will be specified in the relevant International Standard.

The nature of the stationary phase will be specified in each relevant International Standard. At present, the most frequently used stationary phases are non-polar phases, such as dimethyl polysiloxanes, and polar phases such as polyethylene glycol. The ratio of the stationary phase to the support is expressed in grams of stationary phase per 100 g of support.

The composition of the column packing will be specified in each relevant International Standard.

NOTE — If a column packing has been used not using a separate stationary phase, this packing shall be suitably characterized.

5.3 Recorder and **integrator,** the performances of which shall be compatible with the rest of the apparatus.

6 Preparation of test sample

See ISO 356.

If the test sample to be injected has to undergo special preparation, this will be indicated in the relevant International Standard.

7 Operating conditions

7.1 Temperatures

The temperatures of the oven, the injection system and the detector will be specified in each relevant International Standard.

7.2 Carrier gas flow rate

Regulate the flow rate so as to obtain the necessary efficiency (see 8.2).

7.3 Auxiliary gases flow rate

Refer to the manufacturer's instructions to obtain the optimum response from the detector.

8 Column performance

8.1 Chemical inertness test

Inject a quantity of linalyl acetate under the test conditions (see 7.1).

One peak only shall be obtained (within the defined limit of purity).

8.2 Column efficiency

Determine the column efficiency from the linalol peak at an isothermal temperature of 130 °C. Determine the number of effective plates N, which should be at least 3 000, by either of the following formulae:

Formula No. 1: (See figure 1.)

$$N = 16\left(\frac{d'_r}{\omega}\right)^2$$

Formula No. 2:

$$N = 5{,}54\left(\frac{d'_r}{b}\right)^2$$

where

d'_r is the reduced retention distance, expressed in length units (retention distance of the linalol peak minus the retention distance of the air peak or the methane peak at 130 °C, comparable to the air peak);

ω is the distance, expressed in the same length units as the retention distance, between the two points of intersection of the base line with the two tangents at the points of inflection of the linalol peak;

b is the width, in millimetres, of the peak of the specified compound (linalol) at half of the peak height.

The chart speed of the recorder shall be such that ω is at least 10 mm, in order to obtain adequate precision.

The chart speed of the recorder shall be such that b is at least 5 mm, in order to obtain adequate precision.

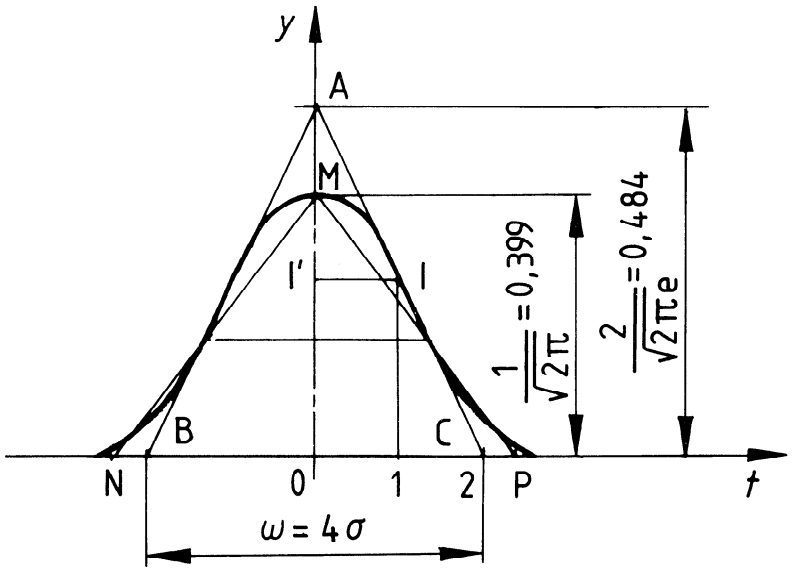

Figure 1

8.3 Resolution and separation

In order to determine resolution and/or separation, inject a suitable quantity of test mixture (4.7) under the conditions of test.

8.3.1 Determination of resolution (see figure 2)

Calculate the resolution factor R of two neighbouring peaks I and II, by means of the formula

$$R = 2\frac{d_{r(II)} - d_{r(I)}}{\omega_{(I)} + \omega_{(II)}}$$

where

$d_{r(I)}$ is the retention distance of peak I;

$d_{r(II)}$ is the retention distance of peak II;

$\omega_{(I)}$ is the width of the base of peak I;

$\omega_{(II)}$ is the width of the base of peak II.

If $\omega_{(I)} \approx \omega_{(II)}$, calculate R by means of the formula

$$R = \frac{d_{r(II)} - d_{r(I)}}{\omega} = \frac{d_{r(II)} - d_{r(I)}}{4\sigma}$$

where σ is the standard deviation (see figure 1).

If the distance between the two peaks, $d_{r(II)} - d_{r(I)}$, is equal to 4σ, the resolution factor $R = 1$.

If the two peaks are not completely resolved, the tangents to the points of inflection of the two peaks meet at point C. For the resolution to be total, the distance between the peaks shall be equal to

$$d_{r(II)} - d_{r(I)} = 6\sigma$$

from which $R = 1{,}5$ (see figure 3).

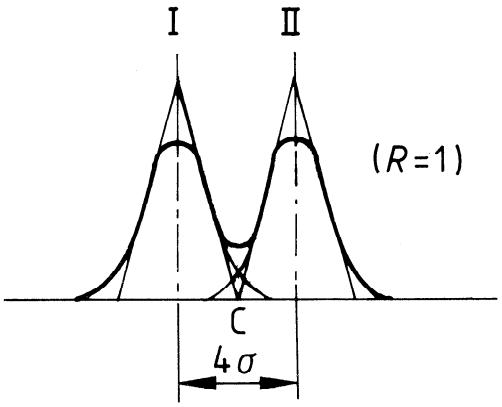

Figure 2

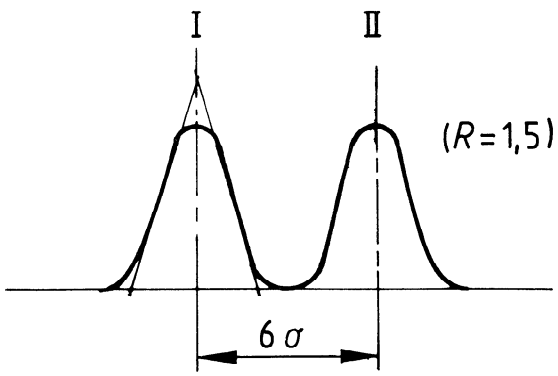

Figure 3