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.

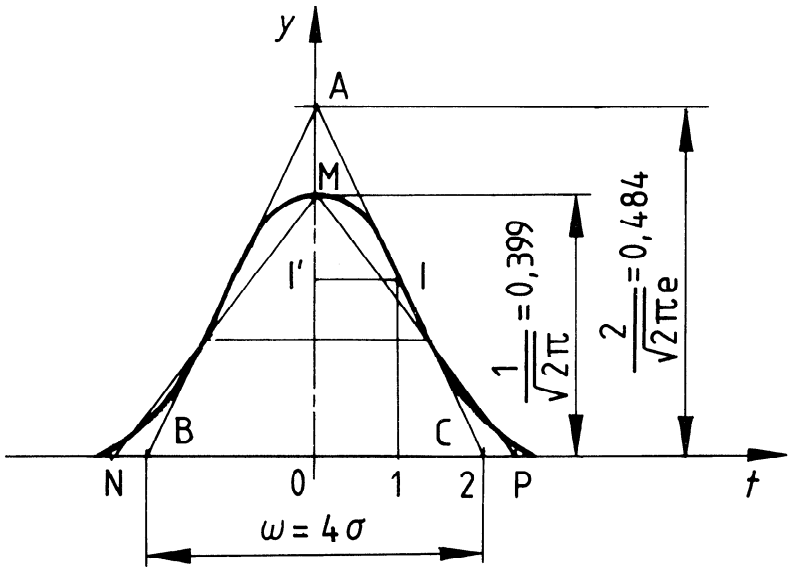

Figure 1

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).

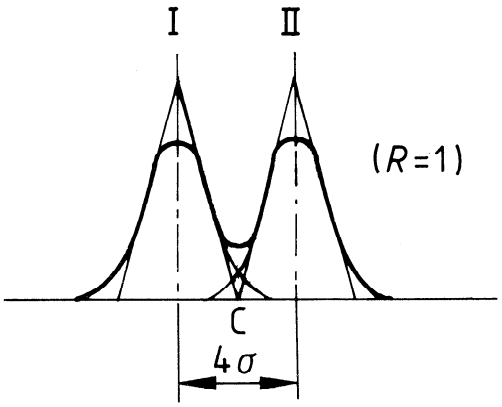

Figure 2

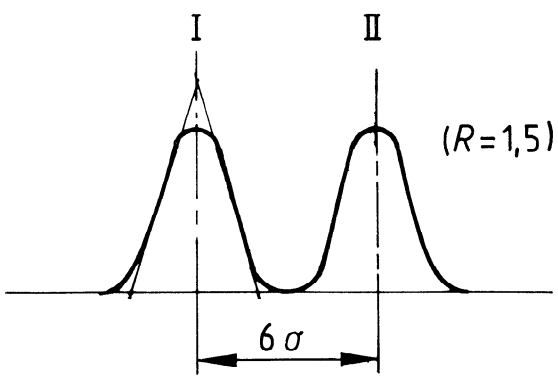

Figure 3

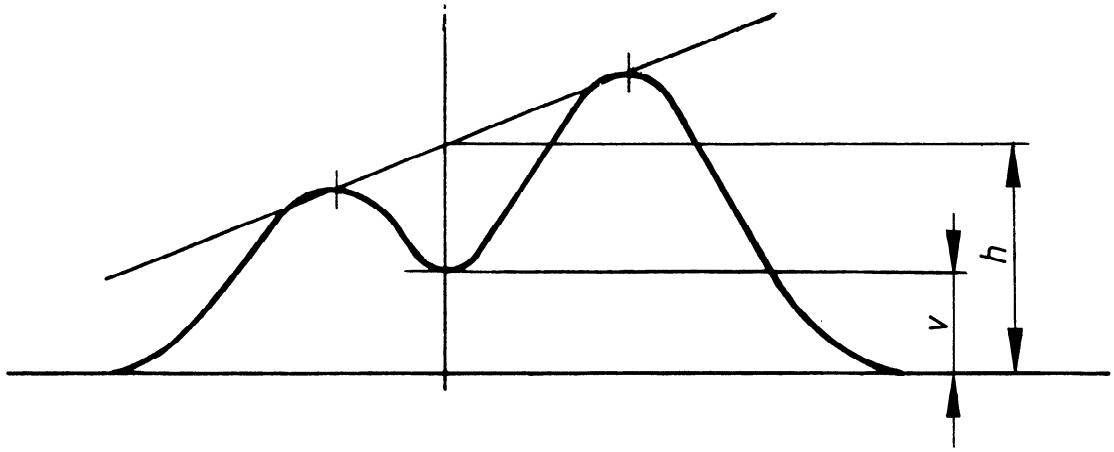

Figure 4

8.3.2 Determination of separation (see figure 4)

Draw a straight line connecting the tops of the peaks concerned. From the base line, draw a perpendicular through the minimum between the two peaks. Measure the distance, h, along this perpendicular, between the base line and the point of intersection with the straight line connecting the tops of the peaks concerned.

Measure also the distance, v, along the perpendicular from the baseline to the minimum between the two peaks.

Calculate the separation, p, expressed as a percentage, by means of the formula

$$p = \frac{100(h - v)}{h}$$

8.3.3 Checking the resolution at the programmed temperature

Use the following conditions:

— dimethylpolysiloxane or polyethylene glycol column;

— temperature programmed from 80 to 220 °C, at a rate of 2 or 3 °C/min.

The carrier gas flow rate shall allow elution of all the components of the test mixture (4.7) and the normal alkanes (4.6) required for measurement of their retention indexes before the end of the temperature programme.

8.3.3.1 Inject a suitable quantity of the test mixture (4.7).

On the chromatogram obtained:

a) in the case of dimethylpolysiloxane columns, the peaks of limonene and acetophenone shall have a separation of at least 95 % (see 8.3.2);

b) in the case of polyethylene glycol (M_r = 20 000) columns, the peaks of linalol and linalyl acetate shall have a separation of at least 95 % (see 8.3.2).

If another packing is specified in the relevant International Standard, the requirements will be specified therein.

8.3.3.2 Inject a suitable quantity of the test mixture (4.7) and calculate the retention indexes (see clause 9) of the constituents of the test mixture.

In the case of dimethylpolysiloxane columns, use alkanes C_{10} to C_{16}.

In the case of polyethylene glycol (M_r = 20 000) columns, use alkanes C_{11} to C_{24}.

The retention indexes so calculated indicate the polarity of the column in comparison to the various structural characteristics of the constituents analysed.

The results obtained on different columns with the same nominal packing are comparable if the retention indexes of the constituents of the test mixture only differ slightly on the different columns.[1)]

9 Determination of retention indexes

If it is necessary to determine the retention indexes, prepare a mixture of the test portion with normal alkanes including *n*-pentane. Choose the normal alkanes in accordance with the range of retention indexes expected. After stabilization of the temperature of the column, inject a suitable quantity of the mixture and proceed with the analysis under the conditions specified in 10.1.1.

Chromatogram "B" is thus obtained.

1) Limits for these differences will be specified later.

9.1 Measurement of retention indexes

Compare chromatograms "A" (see 10.1.1) and "B" (see clause 9) and note on chromatogram "B" the peaks corresponding to the normal alkanes.

On chromatogram "B", make the following measurements.

9.1.1 Isothermal conditions

9.1.1.1 If a detector with thermal conductivity is used, calculate the difference between the retention distances of the top of the peak under consideration and the top of the air peak. Let d'_x be this difference, in millimetres.

Calculate the difference between the retention distances of the top of the air peak and the top of the normal alkane peak appearing immediately before the peak considered. Let d'_n be this difference, in millimetres.

Calculate the difference between the retention distances of the top of the air peak and the top of the normal alkane peak appearing immediately after the peak considered. Let d'_{n+1} be this difference, in millimetres.

9.1.1.2 If a flame ionisation detector is used, calculate the difference between the retention distances of the top of the peak considered and the top of the methane peak. Let d'_x be this difference, in millimetres.

Calculate the difference between the retention distances of the top of the methane peak and the top of the normal alkane peak appearing immediately before the peak considered. Let d'_n be this difference, in millimetres.

Calculate the difference between the retention distances of the top of the methane peak and the top of the normal alkane peak appearing immediately after the peak considered. Let d'_{n+1} be this difference, in millimetres.

9.1.2 Procedure using linear temperature programming from injection

Measure the distance on the base line between the top of the peak the retention index of which is to be determined and the top of the normal alkane peak (n carbon atoms) appearing immediately before the peak considered. Let Λ_x be this distance, in millimetres.

Measure the distance on the base line between the tops of the neighbouring normal alkane peaks (that with n carbon atoms and that with $n + 1$ carbon atoms appearing immediately after the peak considered). Let Δ_y be this distance, in millimetres.

9.2 Calculation of retention index

9.2.1 Isothermal conditions

Calculate the retention index, I, by means of the formula

$$I = 100 \frac{\log d'_x - \log d'_n}{\log d'_{n+1} - \log d'_n} + 100\,n$$

where

d'_x is the distance, in millimetres, between the top of the peak the retention index of which is to be calculated and the top of the air peak (or methane peak) (see 9.1.1);

d'_n is the distance, in millimetres, between the top of the peak of the normal alkane with n carbon atoms and the top of the air peak (or methane peak) (see 9.1.1);

d'_{n+1} is the distance, in millimetres, between the top of the peak of the normal alkane with $(n + 1)$ carbon atoms and that of the air peak (or methane peak) (see 9.1.1).

NOTE — The formula is only valid if $d'_{n+1} > d'_x > d'_n$.

9.2.2 Procedure using linear temperature programming from injection

This calculation is valid only for constituents having retention times which are included in the linear zone of the temperature programme.

Calculate the retention index, I, by means of the formula

$$I = 100 \frac{\Delta_x}{\Delta_y} + 100\,n$$

where

Δ_x is the distance, in millimetres, between the top of the peak the retention index of which is to be calculated and the top of the peak of the normal alkane with n carbon atoms (see 9.1.2);

Δ_y is the distance, in millimetres, between the top of the peak of the normal alkane with n carbon atoms and the top of the peak of the normal alkane with $(n + 1)$ carbon atoms (see 9.1.2).

NOTE — If different temperature programmes have been carried out, it is not possible to calculate the retention index.

10 Methods of determination

10.1 General conditions

10.1.1 Chromatogram of the essential oil

Record the chromatogram as specified in the relevant International Standard.

The temperature and flow conditions shall be the same as those used for testing the efficiency of the column (see 8.2);

For the determination of certain specific constituents, the relevant International Standard may specify the use of isothermal conditions at a specified temperature. In such cases, the flow rate shall be controlled so that the separation obtained is that specified in the relevant International Standard.

After stabilization of the temperature of the column, inject a suitable quantity of the test portion.

Chromatogram "A" is thus obtained.

10.2 Internal standard method

Record the chromatogram of the essential oil and that of the internal standard (4.5) under the same operating conditions. Check on the chromatograms that the products to be determined are separated from the other constituents of the essential oil and that the internal standard does not interfere with any constituent of the essential oil.

10.2.1 Determination of response factor

If, for quantitative determinations, the response factor of a constituent relative to the internal standard has to be determined, weigh appropriate quantities of the internal standard (4.5) and of the reference substance (4.4) such that the corresponding peak areas will be approximately equal.

If a solvent has to be used, it will be specified in the relevant International Standard.

After stabilization of the temperature of the column, inject a suitable quantity of this mixture and carry out the analysis under the conditions specified in 10.1.1.

Chromatogram "F" is thus obtained.

Calculate the response factor K of the constituent relative to the internal standard by means of the formula

$$K = \frac{A_E \times m_R}{A_R \times m_E}$$

where

A_R is the peak area, in integrator units, corresponding to the reference substance the response factor of which is to be calculated;

A_E is the peak area, in integrator units, corresponding to the internal standard;

m_R is the mass, in milligrams, of the reference substance;

m_E is the mass, in milligrams, of the internal standard.

10.2.2 Determination

If the relevant International Standard specifies the use of an internal standard, prepare a mixture by weighing, to the nearest 0,001 g, appropriate quantities of the essential oil and of the internal standard. Choose the amount of internal standard so that the peak area of the constituent to be determined and that of the internal standard will be approximately equal.

After stabilization of the temperature of the column, inject a suitable quantity of the mixture and carry out the analysis under the conditions specified in 10.1.1.

Chromatogram "C" is thus obtained.

10.3 Addition method

If it is not possible to use the internal standard method for a particular determination, use the addition method.

For this, inject a suitable quantity of the essential oil in which X is the constituent to be determined and Y is a constituent giving a peak close to X on the chromatogram "D" obtained.

Then prepare, by weighing to the nearest 0,001 g, a mixture of m grams of the essential oil and m_R grams of the reference substance (4.4) corresponding to the constituent X to be determined.

Inject this mixture.

Chromatogram "E" is thus obtained.

10.4 Internal normalization method

This method is not a true method of determination, but allows only a rough estimation of the relative concentrations of different constituents eluted from a mixture by comparison of their peak areas, but not their determination as percentages by mass.

11 Expression of results

11.1 Internal standard method

Calculate the content c_X, expressed as a percentage by mass, of the constituent to be determined, by means of the formula

$$\frac{A_X \times m_E \times K}{A_E \times m} \times 100$$

where

A_X is the peak area, in integrator units, corresponding to the constituent to be determined (see 10.2.2);

A_E is the peak area, in integrator units, corresponding to the internal standard (see 10.2.2);

m is the mass, in milligrams, of the essential oil;

m_E is the mass, in milligrams, of the internal standard;

K is the response factor for the constituent to be determined relative to the internal standard (see 10.2.1).

11.2 Addition method

Calculate the content c_X, expressed as a percentage by mass, of the constituent to be determined, by means of the formula

$$\frac{m_R}{m} \times \frac{r}{r' - r} \times 100 \qquad (r' > r)$$

where

m_R is the mass, in grams, of the reference substance (4.4);

m is the mass, in grams, of the essential oil;

and

$$r = \frac{A_X}{A_Y}$$

A_X being the peak area corresponding to constituent X on chromatogram "D" (see 10.3);

A_Y being the peak area, corresponding to a constituent Y close to X on chromatogram "D";

and

$$r' = \frac{A'_X}{A'_Y}$$

A'_X being the peak area corresponding to constituent X on chromatogram "E" (see 10.3);

A'_Y being the peak area corresponding to a constituent Y close to X on chromatogram "E".

11.3 Internal normalization method

When the test portion is completely volatile (essential oil without residue) under the test conditions and the chromatogram obtained does not present too many small peaks, calculate the content c_X, expressed as a percentage, of the constituent to be determined by means of the formula:

$$\frac{A_X}{\sum A} \times 100$$

where

A_X is the peak area, in integrator units, of the constituent to be determined;

$\sum A$ is the sum, in integrator units, of all peak areas.

11.4 Results and repeatability

Take as the results for the response factor K and the content c_X of the constituent to be determined the mean values of several (at least three) determinations carried out on the same sample. The values used, for the calculation should not differ from the means by more than a certain percentage (in general ±2,5 %). This percentage and the number of determinations will be specified in the different methods or relevant International Standards.

12 Test report

The test report shall include the following information:

a) identification of the sample;

b) reference to this International Standard;

c) the type of apparatus used;

d) the characteristics of the column (material, packing, temperature);

e) the characteristics of the injection system (type and temperature);

f) the characteristics of the detector (type and temperature);

g) the carrier gas and flow rate;

h) the characteristics of the recorder (maximum signal height, chart speed, full-scale response time);

i) the results obtained.