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Essential oils — Analysis by high performance liquid chromatography — General method

Huiles essentielles — Analyse par chromatographie liquide sous pression — Méthode générale

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.

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 8432 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.

Essential oils — Analysis by high performance liquid chromatography — General method

1 Scope and field of application

This International Standard specifies a general method for the analysis of essential oils by high performance liquid chromatography for the purpose of determining the content of a specific compound and/or searching for a characteristic profile.

2 Reference

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

3 Principle

Liquid chromatography is a method of separation based on the phenomena of adsorption, partition, ion exchange and/or exclusion. It enables a small quantity of aromatic essential oil or other raw materials for the perfume industry to be analysed on a chromatographic column with an appropriate packing and under appropriate conditions, the possible identification of the different constituents and the quantitative determination of specific compounds by measuring the area or height of their peaks.

4 Reagents and materials

4.1 Reference substance, corresponding to the compound to be determined or detected. The reference substance will be indicated in each relevant International Standard. The reference substance shall have been recently purified.

4.2 Internal standard or **external standard.**

The internal standard or external standard will be specified in each relevant International Standard; it shall elute as closely as possible to the compound to be determined and its peak shall not superpose on that of any compound in the essential oil which is detectable by the detection system used.

4.3 Mobile phase.

The composition of the mobile phase may remain constant during operation (isocratic elution) or change according to a specified programme (gradient of elution). It will be specified in each relevant International Standard.

4.4 Elution solvent.

The nature and quality of solvents will be chosen according to the sample to be analysed and according to the nature of the column and detector used. The solutions shall be free from solid particles smaller than 0,5 µm.

4.5 Gas.

If the pumping system requires the use of a gas, it shall be inert. Helium, nitrogen or argon may be used.

5 Apparatus

5.1 Separation system.

5.1.1 Chromatograph.

5.1.2 Pumping system or **any other system,** which enables a constant or programmed flow rate to be obtained and maintained.

5.1.3 Solvent supply system.

5.1.4 Solvent degassing system.

5.1.5 Suitable detector system, which enables the quantities of compounds present in the sample to be determined.

5.2 Recorder, and (optional) **integrator,** whose performance is compatible with the rest of the apparatus.

5.3 Column, made of an inert material (for example glass, stainless steel) having mechanical properties which enable the pressure to be withstood.

The nature and the particle size of the stationary phase will be specified in each relevant International Standard.

The temperature of the column may be stabilized or programmed.

6 Preparation of the 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 Procedure

7.1 Temperature

If the temperature is different from ambient, it will be specified in each relevant International Standard.

7.2 Elution solvent flow rate

Regulate the flow rate so as to obtain the separation of constituents to be determined.

8 Column performance

8.1 Chemical inertness test

Ensure that the products to be identified are not damaged.

8.2 Column efficiency

Determine the number of effective plates (N) from the internal standard peak specified in each relevant International Standard and using the solvents also specified, at an isothermal temperature, and under isocratic conditions from the equation

$$N = 5,54 \left(\frac{d_r}{b_{0,5}} \right)^2$$

where

d_r is the distance, in millimetres, measured on the baseline between the injection point and the falling edge of the peak being studied;

$b_{0,5}$ is the width, in millimetres, at half of the peak height.

8.3 Resolution

Calculate the resolution factor, R, of two neighbouring peaks I and II, from the equation

$$R = 2 \frac{d_r(II) - d_r(I)}{b_{0,5}(I) + b_{0,5}(II)}$$

where

$d_r(I)$ is the retention distance of peak I;

$d_r(II)$ is the retention distance of peak II;

$b_{0,5}(I)$ is the width, in millimetres, at half of peak I height;

$b_{0,5}(II)$ is the width, in millimetres, at half of peak II height.

(See the figure.)

9 Methods of determination

9.1 General conditions

Record the chromatogram as specified in the relevant International Standard.

For the determination of certain specific constituents, the relevant International Standard may specify isocratic operation or an operation by elution gradient.

In such cases, the flow rate shall be regulated so that the separation obtained is that specified in the relevant International Standard.

After stabilization of the operation conditions, inject a suitable quantity of the test portion.

Chromatogram ''A'' is thus obtained.

9.2 Internal standard method

Record the chromatogram of the essential oil and that of the internal standard (4.2), under the same operating conditions.
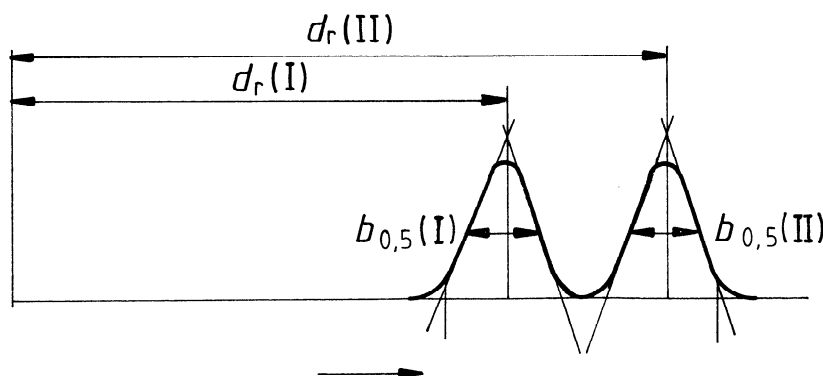

Figure — Calculation of the resolution factor

Check on the chromatogram that the products to be determined are separated from the constituents of the essential oil, and that the internal standard does not interfere with any constituent of the essential oil.

9.2.1 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 (4.2) and that of the reference substance (4.1) such that the corresponding peak areas will be approximately equal.

The solvent to be used for dilutions will be specified in the relevant International Standard.

After stabilization of the whole system, inject a suitable quantity of this mixture and carry out the analysis under conditions specified in 9.1. Chromatogram "B" is thus obtained.

Calculate the response factor, K, by means of the equation

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

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.

9.2.2 Determination

If the relevant International Standard specifies the use of an internal standard, prepare a mixture by weighing, to the nearest 0,1 mg, appropriate quantities of the essential oil and of the internal standard. Choose the amount of internal standard so that the peak area of the compound to be determined and that of the internal standard will be approximately equal.

After stabilization of the whole system, inject a suitable quantity of the mixture and carry out the analysis under the conditions specified in 9.1. Chromatogram "C" is thus obtained.

9.3 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 compound to be determined, and Y is a compound giving a neighbouring peak on the chromatogram "D" obtained.

Then prepare, by weighing to the nearest 0,1 mg, a mixture of m g of the test sample and m_X g of the reference substance (4.1) corresponding to the compound X to be determined.

Inject an appropriate quantity of this mixture. Chromatogram "E" is thus obtained.

9.4 External standard method

If it is not possible to use the methods specified in 9.2 and 9.3, use the external standard method.

This method is only applicable if the apparatus has an injection system which enables identical volumes to be injected (e.g. loop valve).

Prepare solutions of increasing concentrations of the reference substance. Inject successively the same quantity of each of these different solutions. Plot a graph of peak areas against concentration of reference substance in the different solutions. A plot which gives peak area of reference substance according to the concentration in the different solutions is thus obtained.

Inject the same quantity of the essential oil where "Z" is the compound to be determined. Measure the area of the peak given by "Z". From the area given by "Z", read off the "Z" concentration from the graph.

10 Expression of results

10.1 Internal standard

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

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

where

A_X is the peak area, in integrator units, corresponding to the compound to be determined;

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

m_X 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 compound to be determined relative to the internal standard.

10.2 Addition method

Calculate the content, c_X, expressed as a percentage by mass, of the compound 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 (4.1);

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

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

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in which

A_X is the peak area corresponding to the compound X on chromatogram "D";

A_Y is the peak area, corresponding to the compound Y close to X on chromatogram "D";

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

in which

A'_X is the peak area corresponding to the compound X on chromatogram "E";

A'_Y is the peak area corresponding to the compound Y close to X on chromatogram "E".

10.3 Results and repeatabilty

Take as the result for the response factor K and the content c_X of the compound to be determined the mean values of several (at least three) determinations carried out on the same test sample. These different values should not differ from their means by more than a certain percentage. This percentage and the number of determinations will be specified in the relevant International Standards.

11 Test report

The test report shall include the following information:

a) the apparatus system;

b) reference to this International Standard;

c) the characteristics of the column (material, dimensions, packing, stationary phase);

d) the characteristics of the detector (optional) and the operating conditions;

e) the characteristics of the mobile phase (flow rate and nature);

f) identification of the test sample (quantity injected and final dilution);

g) results obtained.

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