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**BHUTAN STANDARD**

**Pine Essential Oil**



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**BHUTAN STANDARDS BUREAU**

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Director General  
Bhutan Standards Bureau  
Rijug Lam  
Thimphu-11001  
Tel: 00975-2-325104/325401  
Fax: 00975-2-323712/328298  
Web: [www.bsb.gov.bt](http://www.bsb.gov.bt)  
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## FOREWORD

This Bhutan Standard for Pine Essential oil was developed by Bhutan Standards Bureau after the draft finalised the Pharmaceuticals and Traditional Medicines Technical Committee (TC 05) and approved by the Bhutan Standards Bureau Board (BSB Board) on ..... 2023.

This standard is subject to systematic review after five years to keep pace with the market trends, industrial and technological developments. Any suggestions and further information may be directed to the concerned Technical Committee.

## **INTRODUCTION**

*Pinus wallichiana*, commonly known as Bhutan pine, Himalayan pine or blue pine is an evergreen conifer in the Pinaceae family. *Pinus wallichiana* is native to the Himalayas from eastern Afghanistan to northern Burma and grows in mountain valleys at altitudes of 1800 - 4300 metres and can reach heights of 30 to 50 metres. It has long and dropping blue-gray needles and produces resinous and banana-shaped cones. The plant regenerates vigorously and colonises fallow grounds and scrubs. In Bhutan, *Pinus wallichiana* is an important timber tree that is often used in construction. The pine needles are traditionally used as a burnt offering in rituals, while the resin is for tar/turpentine and the wood as fuel. In olden days, locals used pine needles as filling for mattresses. It is believed that the needles provide protection against fleas and lice.

Pine needle essential oil is distilled from pine needles and the twigs of blue pine by steam distillation method. Pine needle essential oil has a fresh woody fragrance and has clarifying, uplifting and invigorating effect and is used in aromatherapy, hot stone baths and incense. Pine needle essential oil has various properties that may benefit health and wellness, such as antimicrobial, antiseptic, antifungal, anti-neuralgic, and anti-rheumatic properties. Pine essential oil has become a popular aroma in detergents and cosmetic products. Like any other essential oil, pine needle essential oil may also cause allergic reactions, skin irritation, or toxicity if used improperly.

As the pine needle oil distillation and production is being commercialised, there is a risk of adulteration and production of sub-standard essential oils. There is also the health concerns and toxicity associated with the improper use and poor quality essential oils. Therefore, it is important to assess and control the quality of essential oil production for the safety of consumers.

This standard contains basic requirements to assess and evaluate the quality and safety of pine essential oil. While this standard is intended to outline only the minimum requirements, the technical committee could not verify limits for some parameters prescribed herein due to the lack of testing capacity. However, this standard has been prepared in consultation with stakeholders to suit the intended purposes.

The national standard is developed to standardise and ensure the quality, safety and reliability of the product. The standard will also guide competent authorities in the certification of essential oil and hopes to facilitate trade and ensure product consistency. It is the responsibility and discretion of each individual or company to adopt or comply with this standard. The standard organisation or the technical committee shall not be liable for any untoward events, either health-related or material-related losses.





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# BHUTAN STANDARD

## Pine Essential Oil

### 1 Scope

This standard specifies essential characteristics of pine (*Pinus wallichiana*) essential oil from Bhutan to facilitate the assessment of its quality.

### 2 Normative References

There are no normative references for these documents.

### 3 Terms and Definition

For the purpose of this standard the following definitions shall apply:

#### 3.1 Adulteration

Adulteration is an act of intentionally debasing the originality of a product either by the admixture or substitution with inferior substances or by the removal of some valuable ingredients.

#### 3.2 Absolute density at 20°C

Ratio of the mass of a given volume of the oil at 20°C to the same volume. This quantity is expressed in grams per millilitre.

#### 3.3 Batch

Identified quantity of essential oil, assumed to have uniform characteristics, made up of one or more containers.

#### 3.4 Contaminants

Any biological/chemical/physical, or any other substances not intentionally added to the product, which may compromise the quality.

#### 3.5 Container

Recipient constituting the whole or part of the batch and containing the essential oil to be sampled.

#### 3.6 Delivery

Quantity of essential oil dispatched at a single time and forming the subject of a specific contract or dispatch document.

#### 3.7 Increment

Quantity of essential oil sampled at a single time at a point in the container to be sampled.

### **3.8 Optical rotation**

Angle, expressed in milliradians and/or degrees of angle, described by the polarization plane of luminous radiation whose wavelength is  $589.3 \text{ nm} \pm 0.3 \text{ nm}$ , corresponding to the D lines of sodium, when such light travels through a thickness of 100 mm of essential oil under given conditions of temperature.

When the determination is carried out on different thicknesses, the optical rotation value should be computed by reference to a thickness of 100 mm. Additionally, the measurements according to the Faraday magneto-optical principle are possible. The thickness of the sample is approximately 10 mm in that case.

Optical rotation of a solution of essential oil divided by the mass of essential oil in the unit of volume.

### **3.9 Pine essential oil**

Essential oil obtained by water or steam distillation of the freshly cut pine needles and the twigs of *Pinus wallichiana* of the Pinaceae family.

#### **3.10 Refractive index**

Ratio of the sine of the angle of incidence to the sine of the angle of refraction, when a ray of light of defined wavelength passes from air into the essential oil kept at a constant temperature.

#### **3.11 Relative density at 20°C**

Ratio of the mass of a given volume of the oil at 20°C to the mass of an equal volume of distilled water at 20°C.

#### **3.12 Suspended matters**

Insoluble extraneous matter found in the product. It may consist of but is not limited to dirt and miscellaneous debris, mineral matter, nitrogenous materials of animal or plant origin and carbohydrate substances such as vegetable fibres.

#### **3.13 Sample**

Quantity of essential oil obtained by mixing the different increments of a container. On the basis of the samples, the laboratory may conduct its own sampling plan in view of the analysis.

## **4 Acronyms and Symbols**

cm	: centimetre
g	: grams
GC	: Gas Chromatography
g/l	: Gram per litre
KOH	: Potassium Hydroxide
M	: Mass

meq	: Milliequivalent
Mg	: Milligram
ml	: Millilitre
mm	: Millimetre
mrad	: Milliradians
M.S	: Mass spectra
m/v	: Mass by volume
mol/l	: Moles per litre
mmol	: Millimoles
nm	: Nanometre
v/v	: Volume by volume
v/w	: Volume by weight
w/w	: Weight by weight

## **5 Requirements**

### **5.1 Description**

Pine essential oil is a colourless to pale yellow liquid with fresh woody fragrance obtained by water or steam distillation of freshly cut pine needles and the twigs of *Pinus wallichiana* of the Pinaceae family.

### **5.2 Scientific classification**

Kingdom	: Plantae
Division	: Pinophyta
Class	: Pinopsida
Order	: Pinales
Family	: Pinaceae
Genus	: Pinus
Species	: wallichiana

### **5.3 Appearance**

Pine essential oil should be clear mobile liquid free from sediments, suspended matters, separated water and added adulterants.

### **5.4 Colour**

Pine essential oil should be colourless to pale yellow.

### **5.5 Odour**

Pine needle essential oil has a fresh woody fragrance.

### **5.6 Tests**

#### **5.6.1 Identification**

Analysis of the pine needle essential oil shall be carried out by gas chromatography described in Annex A. In the chromatogram obtained, the representative and characteristic components shown in Table 1 shall be identified. This constitutes the chromatographic profile of the pine essential oil.

**Table 1 — Chromatographic profile of the Pine oil**  
(Clause 5.6.1)

Component	Minimum %	Maximum %
$\alpha$ -pinene	14.8	21.0
$\beta$ -pinene	19.0	34.0
limonene	17.8	41.0
myrcene	1.60	12.3
NOTE: The chromatographic profile is normative		

#### 5.6.2 Relative density

The relative density of pine essential oil shall be a minimum of 0.858 and a maximum of 0.868 when determined at 20°C as per the method described in Annex C.

#### 5.6.3 Refractive index

The refractive index of pine essential oil shall be minimum of 1.474 and a maximum of 1.478 when determined at 20°C as per the method described in Annex D.

#### 5.6.4 Optical rotation

The optical rotation of pine essential oil shall be between -15 degrees and -7 degrees when determined at 20°C as per the method described in Annex E.

#### 5.6.5 Solubility

One volume of pine essential oil mixed with 10 volumes of ethanol (90% by volume) shall give a clear solution when determined at 20°C as per the methods described in Annex F.

#### 5.6.6 Flashpoint

The mean value is +42°C obtained with Pensky Martens equipment. The information on flashpoints is provided in Annex G.

#### 5.6.7 Acid Value

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The acid value of pine essential oil should be less than 1 as per the methods described in Annex H.

### **5.6.8 Peroxide value**

The peroxide value of pine essential oil should be less than 20 mmol/kg as per the methods described in Annex I.

## **6 Sampling**

Minimum volume of the test sample that allows each of the tests specified in this standard to be carried out at least once is 25 ml. However, the laboratory may conduct its own sampling plan in view of the analysis.

The general rules for a sampling of pine needle essential oils, in order to provide a laboratory with quantities that are suitable to be handled for expertise purposes is described in Annex J.

## **7 Packaging**

Pine essential oil must be packaged in an airtight container preferably glass, tin-lined, or aluminium, which by nature do not change the product and protect it from external attacks. The material shall be protected from light and stored in a cool and dry place.

In general, the materials of the container must be inert toward the packed product to avoid simultaneous damage to the material and the product.

## **8 Labelling or Marking**

The labelling materials shall be durable and affixed directly to the container to withstand the transport conditions and avoid tampering and subsequent use for other purposes.

The labelling shall include the following information, however not limited to:

- a) the name of the product/material,
- b) net weight or volume,
- c) batch number,
- d) manufacturing date,
- e) expiry date,
- f) full address of the manufacturer,
- g) storage conditions, and
- h) disclaimer or caution, if any

## **9 Storage**

Pine essential oil is a flammable liquid and should be stored in appropriate places. The container must be checked for any liquid or vapour leaks and stored in a cool and dry place, away from direct light and heat.

Annex A

(Normative)

Gas Chromatographic analysis of Pine essential oil

A.1 General

The gas chromatography (GC) method describes the general guidelines for the determination of the chromatographic profile of essential oil, as it is one of the specifications that enable assessment of the quality of pine essential oil. The GC evaluates relative proportions of essential oil content and does not determine the actual concentration of the components. The chromatographic conditions given here are for guidance only.

NOTE The principles of GC and the application of the technique are also described in international pharmacopoeias. For further guidance on chromatographic profiles, refer to ISO 11024-1 Part 1: Preparation of chromatographic profiles for presentation in standards and ISO 11024-2 Part 2: Utilization of chromatographic profiles of samples of essential oils.

A.2 Sample preparation and method

Dissolve a sample of the material in a suitable solvent, such as cyclohexane or petroleum ether. Inject the sample solution into the gas chromatograph, where the carrier gas carries it from one end of the column to the other. The constituents of the sample undergo distribution at different rates and ultimately separate from one another during its movement. As the separated constituents emerge from the end of the column one after another, the signals are detected by suitable means whose response is related to the amount of a specific component leaving the column.

A.3 Apparatus

Use any GC that is capable of being operated under conditions suitable for resolving the individual constituents into distinct peaks. The GC may be operated under the following chromatographic conditions:

Sample	Pine essential oil
Column	Fused-silica capillary column
Material	Stainless steel
Length	30 m
Orifice	0.25 mm
Stationary phase and solid support	Column coated with a phase 5 % phenyl, 95 % dimethylpolysiloxane, non-polar DB-5 column and then a polar Supelcowax 10 coated with a phase polyethylene glycol.

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Carrier Gas	Nitrogen at a constant flow of 0.9 ml/min.		
Conditions	Oven temperature programmed from 40 - 230°C at 2°C /min, injector and detector temperatures 240°C.		
Detector			
	Type	F.I.D	
	Temperature	240°C	

### A.4 Calculation

#### A.4.1 Area Measurements

The area of the peak is measured by multiplying the peak height times the width of the half-height since normal peaks approximate a triangle. The normal peak base is not taken since large deviations may be observed due to tailing or adsorption. When peaks are symmetrical and of reasonable width, this technique is fairly accurate and simple to use.

Other methods, such as triangulation, disc integrators, and electronic digital integrators, can be used for area measurements.

#### A.4.2 Area Normalisation

For area normalization, the following formula can be used to calculate the percentage composition by measuring the area of each and dividing the individual areas by the total area:

$$\text{Percentage of component } i = \frac{\text{Area of component } i}{\text{Total area}} \times 100$$

Relative or indirect calibration method of internal standardization may be used if a pure appropriate internal standard is available.

**Annex B**  
(Normative)  
**Preparation of Test samples**

**B.1 General**

This annexure gives general guidance for the preparation of samples of essential oils to be submitted to a laboratory for analysis. It is applicable, in particular, to those essential oils that cannot be analysed directly; that is those which are solid or partially solid at room temperature or those which are cloudy due to the presence of water or suspended particles.

This method cannot be used for samples for the determination of water.

Filter the essential oil, if necessary liquefied by heating at a suitable temperature, after the addition of magnesium sulphate or sodium sulphate to eliminate water and the insoluble substances.

**B.2 Apparatus**

Usual laboratory apparatus and, in particular, the following are required:

- a. Oven
- b. Conical flasks
- c. Suitable filtration equipment

**B.3 Reagent**

Magnesium sulphate, recently desiccated and neutral or sodium sulphate, recently desiccated.

To desiccate the magnesium sulphate or sodium sulphate, heat to a constant mass at 180°C to 200°C (temperature taken in the continuously stirred material). Grind to a fine powder and keep in a dry flask with an airtight closure.

**B.4 Procedure**

**B.4.1 Essential oils which are solid or partially solid at ambient temperature**

Liquefy the essential oil by placing it in the oven maintained at the lowest temperature at which liquefaction may be obtained in less than 10 min. This temperature is usually about 10°C above the presumed freezing point. During this operation, especially in the case of essential oils containing aldehydes, avoid allowing air to enter the container holding the essential oil. To achieve this, loosen, but do not remove, the stopper. Pour the liquefied essential oil into a dry conical flask, previously warmed in the oven to the temperature indicated above, so that the flask is filled to not more than two-thirds of its capacity.

During all subsequent operations, the oil shall be kept at the lowest temperature at which it will remain liquid.

**B.4.2 Essential oils which are liquid at the ambient temperature**



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Transfer the essential oil to a dry conical flask at the same temperature, so that the flask is filled to not more than two-thirds of its capacity.

### **B.4.3 Treatment of the essential oil**

In the two cases indicated above, (B.4.1) or (B.4.2), add to the flask a mass of the dehydrating agent (magnesium sulphate or sodium sulphate) equal to about 15% of the mass of the essential oil. Shake vigorously from time to time over a period of at least 2 hours and filter the sample.

Verify the action of the dehydrating agent by adding about 5% of magnesium sulphate or sodium sulphate and wait for 2 hours before filtering.

The dehydrating agent should still be in a powdery form and the oil should be clear and limpid. In the first case (B.4.1), carry out the filtration in the oven at the appropriate temperature (see B.4.1), but do not keep the oil in the oven longer than necessary.

NOTE 1 These operations should immediately precede the analysis. If not, the filtered oil should be kept in a cool place protected from strong light, in a previously dried, well-filled container fitted with an airtight closure.

NOTE 2 In certain cases and where required, the metallic phenolates which colour the essential oil should be eliminated by agitation with citric or tartaric acid.

**Annex C**  
(Normative)  
**Determination of Relative Density at 20°C**

**C.1 General principle**

Equal volumes of the essential oil and water, at 20°C, are weighed successively in a pycnometer.

**C.2 Apparatus**

Ordinary laboratory apparatus and in particular the following are required:

- a. Glass pycnometer, of minimum nominal capacity of 5 ml.
- b. Water bath, capable of being maintained at  $20^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ .
- c. Standardized thermometer, graduated from 10°C to 30°C, with 0.2°C or 0.1°C divisions.
- d. Analytical balance, accurate to 0.001 g.

For routine controls and accurate measurement of the relative density, automatic electronic instruments that are available from the market may be used. However, in case of dispute, the reference method is the pycnometer method.

**C.3 Reagents**

Distilled water, freshly boiled and subsequently cooled to approximately 20°C.

**C.4 Sampling**

It is important that the laboratory receive a representative sample which has not been damaged or modified during transportation or storage. A recommended sampling method is given Annex J.

**C.5 Test sample**

Prepare the test samples according to the method in Annex B.

**C.6 Procedure**

**C.6.1 Preparation of pycnometer**

Carefully clean the pycnometer and then rinse it successively with, for example, ethanol and acetone, then dry the interior by means of a blower.

If necessary, wipe the outside with a dry cloth or filter paper.

When temperature equilibrium is reached between the balance case and the pycnometer, weigh the latter with its stopper, if any, to the nearest 1 mg.

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### C.6.2 Weighing of Distilled Water

Fill the pycnometer with distilled water.

Immerse the pycnometer in the water bath. After 30 min, adjust the water to the mark, if necessary. Insert the stopper, if any, and dry the outside as before with a dry cloth or filter paper.

When temperature equilibrium is reached between the balance room and the pycnometer, weigh the latter and its stopper, if any, to the nearest 1 mg.

### C.6.3 Weighing of essential oil

Empty the pycnometer, then wash it and dry it as specified in C.6.1.

Proceed as specified in C.6.2, replacing the water with the test sample prepared according to clause C.5.

### C.7 Expression of results

The relative density,  $\rho_{20}^{20}$ , is given by the following equation:

$$\frac{m_2 - m_0}{m_1 - m_0}$$

where,

$m_0$  is the mass, in grams, of the empty pycnometer determined in C.6.1;

$m_1$  is the mass, in grams, of the pycnometer filled with distilled water, determined according to C.6.2;

$m_2$  is the mass, in grams, of the pycnometer filled with the essential oil, determined according to C.6.3.

Express the result to three decimal places. In practice, no correction is made for the upthrust due to air.

If the absolute density of the essential oil is required, multiply the value obtained for the relative density by the absolute density of water at 20°C (i.e., 0.99823 g/ml).

### C.8 Test report

The test report shall state the method used; the result obtained; and if repeatability has been verified, the final result obtained.

It shall also mention any operating conditions as well as any circumstances that might have influenced the results. The test report shall include all details required for the complete identification of the sample

## Annex D (Normative)

### Determination of Refractive Index

#### D.1 Principle

Depending on the instrument being used, observe the limit of total reflection or take a direct measurement of the angle of refraction while maintaining the oil under isotropism and transparency conditions.

#### D.2 Apparatus

- a. Refractometer, allowing direct readings of refractive indices between 1.3000 and 1.7000 to be made with an accuracy of  $\pm 0.0002$ .
- b. Thermostat or apparatus for temperature maintenance, which ensures a circulation of water through the refractometer, thus keeping the instrument at the reference temperature to within  $\pm 0.2^{\circ}\text{C}$ .
- c. A light source, sodium light. Diffused daylight or light from an electric lamp may be used for refractometers fitted with an achromatic compensator.
- d. A plate of glass (optional), of known refractive index.

#### D.3 Reagents

Standard products, of refractometry grade, to adjust the refractometer, as follows.

- a. Distilled water, of refractive index 1.3330 at  $20^{\circ}\text{C}$ .
- b. p-Cymene, of refractive index 1.4906 at  $20^{\circ}\text{C}$ .
- c. Benzyl benzoate, of refractive index 1.5685 at  $20^{\circ}\text{C}$ .
- d. 1-Bromonaphthalene, of refractive index 1.6585 at  $20^{\circ}\text{C}$ .

#### D.4 Sampling

It is important that the laboratory receive a representative sample which has not been damaged or modified during transportation or storage. A recommended sampling method is given in Annex J.

#### D.5 Procedure

##### D.5.1 Preparation of test sample

Prepare the test samples according to the method in Annex B. The temperature of the test sample should be same temperature at which the measurements shall be made.

##### D.5.2 Regulation of the refractometer

D.5.2.1 Regulate the refractometer by measuring the refractive index of the standard products described in D.3 (a to d).

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D.5.2.2 Verify that the refractometer is maintained at the temperature at which the readings shall be made. This temperature shall not differ from the reference temperature by more than  $\pm 0.2^{\circ}\text{C}$  during the test.

The reference temperature is  $20^{\circ}\text{C}$ , except for those oils which are not liquid at this temperature, in which case a temperature of  $25^{\circ}\text{C}$  or  $30^{\circ}\text{C}$ , depending on the melting point of these essential oils, shall be used.

### D.6 Determination

Place the test sample, prepared according to D.5.1, in the refractometer. Wait until the temperature is stable and make the measurements.

### D.7 Calculation

The refractive index  $n_D^t$ , at the specified temperature  $t$ , is given by the equation:

$$n_D^t = n_D^{t'} + 0.0004(t' - t)$$

where

$n_D^{t'}$  is the reading taken at the working temperature  $t'$  at which the determination was actually made.

Express the result to four decimal places.

### D.8 Repeatability

The absolute difference between two independent single test results, obtained using the same method on an identical essential oil in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5% of cases be greater than  $\pm 0.0002$ .

### D.9 Test report

The test report shall state the sampling method used; the test method used; the test result obtained; and if repeatability has been checked, the final result obtained.

It shall also mention any operating conditions as well as any circumstances that might have influenced the results. The test report shall include all details required for the complete identification of the sample.

**Annex E**  
**(Normative)**  
**Determination of Optical Rotation**

**E.1 Principle**

This is the general method for determining the optical rotation of essential oils such as lemongrass oil. When dealing with solid oils, partially solid oils, oils that are highly viscous at room temperature, or highly coloured oils, this determination is carried out on a solution of the oil.

**E.2 Apparatus**

- a. **Polarimeter**, having a precision of at least  $\pm 0.5$  mrad ( $\pm 0.03^\circ$ ) and adjusted to give  $0^\circ$  and  $180^\circ$  with water.

The polarimeter shall be checked with a quartz plate of known optical rotation or, if that is unavailable, with an aqueous solution containing 26.00g of anhydrous pure saccharose per 100 ml of solution. The optical rotation of this solution shall be  $+604$  mrad ( $+34.62^\circ$ ) in a 200 mm layer, at a temperature of  $20^\circ\text{C}$ .

The instrument shall be under conditions of stability when in use, and non-electronic instruments shall be used in the dark.

- b. **The light source**, comprising any device giving the light of wavelength  $589.3\text{ nm} \pm 0.3\text{ nm}$ , preferably a sodium vapour lamp.
- c. **Polarimeter tubes**, usually  $100\text{ mm} \pm 0.5\text{ mm}$  long.

When testing slightly coloured samples of low optical rotation, tubes of length  $200\text{ mm} \pm 0.5\text{ mm}$  may be used. Tubes of length  $50\text{ mm} \pm 0.05\text{ mm}$  or  $10\text{ mm} \pm 0.05\text{ mm}$  or even less may be used, if necessary, for strongly coloured samples.

For determination at  $20^\circ\text{C}$  or at another specified temperature, use double-walled tubes, equipped with a thermometer to ensure water circulation at the required temperature.

For determination at ambient temperature, any type of tube may be used, although it is advisable to use the type described above in this case too.

- d. **Thermometer**, graduated in  $0.2^\circ\text{C}$  or  $0.1^\circ\text{C}$ , allowing determination of temperatures between  $10^\circ\text{C}$  and  $30^\circ\text{C}$ .
- e. **A thermostatically controlled device**, for maintaining the temperature of the sample at  $20^\circ\text{C} \pm 0.2^\circ\text{C}$  or any other specified temperature.

**E Reagents**

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Reagents shall be of analytical grade. Use distilled water or water of at least equivalent purity.

**Solvent** (only for essential oils that need to be tested in solution). Use preferably 95% ethanol by volume. It is advisable to check that the optical rotation of the solvent used is nil.

### E.4 Sampling

It is important that the laboratory receive a representative sample which has not been damaged or modified during transportation or storage. A recommended sampling method is given in Annex J.

### E.5 Procedure

#### E.5.1 Preparation of test sample

Prepare the test samples according to the method in Annex B.

When determining the specific rotation of essential oil in solution, prepare the oil solution in the appropriate solvent, at the concentration specified for the essential oil being analysed.

#### E.5.2 Determination

Switch on the light source and wait until full luminosity is obtained.

If necessary, bring the temperature of the test sample to  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$  or to another specified temperature, then pour the sample into the appropriate polarimeter tube, which should be at approximately the same temperature. Start water circulation under thermostatic control so as to keep the whole at the specified temperature ( $\pm 0.2^{\circ}\text{C}$ ) during the determination.

Fill the tube with the test sample, and ensure the absence of air bubbles.

Place the tube in the polarimeter and read the dextrorotatory (+) or laevorotatory (–) optical rotation of the oil on the scale of the instrument.

#### E.5.3 Number of determinations

Carry out at least three determinations with the same test sample.

Take as the result the mean of the values obtained for three measurements, provided that they do not differ by more than 1.4 mrad ( $0.08^{\circ}$ ).

### E.6 Expression of results

#### E.6.1 Calculation and formulae

##### a. Optical Rotation

The optical rotation, expressed in milliradians and/or degrees of angle, is given by the equation:

$$\alpha_{\square} = \frac{\square}{\square} \times 100$$

where

$A$  is the value of the angle of rotation in milliradians and/or degrees of angle;

$l$  is the length of the tube used, in millimetres.

Mark as positive (+) dextrorotatory optical rotations and as negative (–) laevorotatory ones.

When polarimeter tubes with double walls for water circulation are not available, it is necessary to apply appropriate correction factors according to the oils tested (for instance, for citrus oils and for some essential oils for which correction factors are known).

These correction factors should be given in the specifications of the oils in question.

**b. The optical rotation of oil in solution, the so-called “specific rotation”**

The specific rotation, expressed in milliradians and/or degrees of angle, is given by the equation:

$$[\alpha] = \frac{\alpha}{c \cdot l}$$

where

$\alpha$  is the optical rotation of the oil solution, calculated according to E.6.1 a;

$c$  is the concentration of the oil solution, in grams of oil per millilitre of solution.

**E.6.2 Precision**

The precision of the test method is  $\pm 3$  mrad ( $\pm 0.17^\circ$ ).

**E.7 Test report**

The test report shall state the sampling method used; the test method used; whether an oil in solution was used in the test, specifying the nature of the solvent and the concentration of the oil; the test result obtained; and if repeatability has been checked, the final result obtained.

It shall also mention any operating conditions as well as any circumstances that might have influenced the results. The test report shall include all details required for the complete identification of the sample.



**Annex F**  
**(Normative)**  
**Evaluation of Solubility in ethanol**

**F.1 Principle**

This annex specifies a method for the evaluation of the solubility or miscibility of essential oils with mixtures of ethanol and water of known ethanol content.

An ethanol solution of suitable concentration should be gradually added to an essential oil, at a temperature of 20°C. Evaluate the miscibility and possibly of opalescence.

**F.2 Classification of solubility or miscibility**

F.2.1 An essential oil is said to be miscible with  $V$  volumes or more of ethanol of a given concentration, at a temperature of 20°C, when the mixture of 1 volume of the oil in question with  $V$  volumes of that ethanol is clear and remains so after further gradual addition of ethanol of the same concentration up to a total of 20 volumes.

F.2.2 An essential oil is said to be miscible with  $V$  volumes of ethanol of a given concentration, at a temperature of 20°C, and to become cloudy when diluted in  $V'$  volumes, when the mixture of 1 volume of the oil in question with  $V$  volumes of the ethanol is clear and becomes cloudy after further gradual addition of  $(V' - V)$  volumes of ethanol of the same concentration and remains cloudy after further addition of the ethanol up to a total of 20 volumes.

F.2.3 An essential oil is said to be miscible with  $V$  volumes of ethanol of a given concentration, at a temperature of 20°C, and to become cloudy when diluted in  $V$  to  $V'$  volumes, when the mixture of 1 volume of the oil in question with  $V$  volumes of the ethanol is clear, becomes cloudy after further gradual addition of  $(V' - V)$  volumes of ethanol of the same concentration, and again becomes clear after further addition of  $(V'' - V')$  volumes of ethanol of the same concentration.

F.2.4 An essential oil is said to be miscible with opalescence when the mixture of the oil with ethanol of a given concentration (under the conditions as given in F.2.1, F.2.2 and F.2.3) shows an opalescence identical to the one of the standard solutions for opalescence, freshly prepared in accordance with the method given in F.4 (c).

NOTE The numerical values of  $V$ ,  $V'$  and  $V''$  are not more than 20.

**F.3 Apparatus**

Ordinary laboratory apparatus and, in particular, the following are required:

- a. Burette, of capacity 25 ml or 50 ml
- b. One-mark pipettes, capable of delivering 1 ml, or analytical balance, capable of weighing to the nearest 1 mg, as appropriate.
- c. Measuring cylinder or flask, of capacity 25 ml or 30 ml, provided with a stopper which is inert to either ethanol or essential oil to be examined.
- d. A device capable of maintaining a temperature of  $20^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ .
- e. Calibrated thermometer, graduated at  $0.2^{\circ}\text{C}$  or  $0.1^{\circ}\text{C}$ , allowing the temperature of the device to be checked.

**F.4 Reagents**

Use only reagents of recognized analytical quality and distilled water.

- a. Ethanol (95% volume fraction)
- b. Mixtures of ethanol and water.

Mixtures of ethanol and water with an ethanol content of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% and 95% (volume fraction) are normally used.

To prepare these mixtures, add distilled water to ethanol, following the directions given in Table 1, and check their concentrations with an alcoholmeter or a densimeter.

- c. Standard solution for opalescence

Add 0.5 ml of a silver nitrate solution, ( $\text{AgNO}_3$ ) = 0.1 mol/l, to 50 ml of sodium chloride solution, ( $\text{NaCl}$ ) = 0.0002 mol/l; then add 1 drop of concentrated nitric acid ( $\rho_{20} = 1.38 \text{ g/ml}$ ). Stir the solution and allow it to stand for 5 minutes. Keep away from direct light.

Prepare the solution freshly before use.

## F.5 Sampling

It is important that the laboratory receive a representative sample which has not been damaged or modified during transportation or storage. A recommended sampling method is given in Annex J.

## F.6 Preparation of test sample

Prepare the test samples according to the method in Annex B.

## F.7 Procedure

### F.7.1 Test Sample

With a pipette, introduce into the measuring cylinder or flask 1 ml of the oil. Place the cylinder and its contents in the device, maintained at a temperature of  $20^\circ\text{C} \pm 0.2^\circ\text{C}$ .

NOTE When the physical state of the essential oil does not permit the use of a pipette, weigh, to the nearest 1 mg,  $1 \text{ g} \pm 0.005 \text{ g}$  of essential oil. In this case, the definition and the results will be expressed in mass/volume.

### F.7.2 Determination of Solubility

Using the burette, add a mixture of ethanol and water of known concentration, which has previously been brought to a temperature of  $20^\circ\text{C} \pm 0.2^\circ\text{C}$ , in increments of 0.1 ml until complete miscibility occurs, shaking vigorously after each addition. When the mixture is perfectly clear, record the volume of the water/ethanol mixture added.

Continue adding the mixture of ethanol and water in increments of 0.1 ml, up to a total of 20 ml, and shake after each addition. If the mixture becomes cloudy or opalescent before the total addition is completed, record the volume added at the point where cloudiness or opalescence appears and, if applicable, the volume at which one or the other disappears.

If a clear mixture is not obtained after 20 ml of solvent has been added, repeat with the next higher concentration of the mixture of ethanol and water given in Table 1.

### F.7.3 Opalescence

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If a clear mixture cannot be obtained but an opalescent mixture is obtained, compare its opalescence with that of the standard solution, as detailed in F.8.2.

### F.8 Expression of results

#### F.8.1 Miscibility

The miscibility of the essential oil with ethanol of concentration  $Q$  (see Table 1), at a temperature of 20°C, is expressed as follows:

##### a) Case F.2.1

1 volume of essential oil in  $V$  volumes of ethanol of concentration  $Q$ ;

##### b) Case F.2.2

1 volume of essential oil in  $V$  volumes of ethanol of concentration  $Q$  with cloudiness from  $V$  volumes of ethanol of the same concentration;

##### c) Case F.2.3

1 volume of essential oil in  $V$  volumes of ethanol of concentration  $Q$  with cloudiness appearing between  $V$  and  $V'$  volumes of ethanol of the same concentration;

where

$V$  is the volume, in millilitres, of ethanol of concentration  $Q$  needed to obtain a clear solution;

$V$  is the volume, in millilitres, of ethanol of concentration  $Q$  needed to produce cloudiness, following the clearness, if it occurs;

$V'$  is the volume, in millilitres, of ethanol of the same concentration  $Q$  at which cloudiness disappears, if it occurs.

Express the values of  $V$ ,  $V$  and  $V'$  numerically to one decimal place.

#### F.8.2 Opalescence

If only opalescence occurs (see F.2.4), report whether the opalescence is "greater than", "equal to" or "less than" that of the standard solution (F.4c).

### F.9 Test report

The test report shall state the method used; the concentration  $Q$  of the ethanol used; and the result obtained.

It shall also mention any operating conditions as well as any circumstances that might have influenced the results. The test report shall include all details required for the complete identification of the sample.

Table 1 — Preparation of the mixtures of ethanol and water

Dilution: ml of ethanol in 100 ml of a mixture, to the nearest 0.1%	Volume of distilled water at 20°C to be added to 100 ml of ethanol (95% volume fraction), at the same temperature $\pm$ 0.1°C, for preparation of the corresponding dilutions.	Mass of ethanol (95% volume fraction)	Mass of water to be added	Values of the relative density and apparent density	
				1)	2)
				$\rho_{20}^{20}$ $\pm 0.0001$	$\rho_{20}$ $\pm 0.00001$ g/ml
Q % (volume fraction)	ml	g	g		
50	95.76	45.9	54.1	0.9318	0.93014
55	77.90	51.1	48.9	0.9216	0.91996
60	62.92	56.4	43.6	0.9108	0.90911
65	50.15	61.8	38.2	0.8993	0.89765
70	39.12	67.5	32.5	0.8872	0.88556
75	29.47	73.4	26.6	0.8744	0.87279
80	20.94	79.5	20.5	0.8608	0.85927
85	13.31	85.9	14.1	0.8604	0.84485
90	6.40	92.7	7.3	0.8307	0.82818
95	0.0	100.0	0.0	0.8129	0.81138
1) Reference: Swiss Federal Bureau of Weights and Measurements. 2) Reference: International Organization of Legal Metrology.					

**Annex G**  
(Informative)

**Flashpoint**

**G.1 General information**

The information on flashpoints of the essential oil which are mostly flammable is required for safety purposes by companies such as transport and insurance.

Given that there is a wide variation in the chemical composition of oil, the sample volume needed and the availability of different equipment, it is difficult to recommend a single apparatus for standardization purposes.

The equipment with which the provided flashpoint value was obtained should be specified.

**G.2 Flashpoint of the pine essential oil**

The mean value is +42°C.

NOTE Further guidance on flashpoint determination can be found on ISO Technical report ISO/TR 11018:1997 Essential oils – General guidance on the determination of flashpoint.

**Annex H**  
(Normative)

## Determination of Acid value by titration methods

### H.1 Principle

Two titration methods for determining the acid value in essential oils involve neutralization of the free acids with a titrated ethanolic potassium hydroxide solution. However, the methods are not applicable to essential oils containing lactones.

### H.2 Reagents

The following reagents of analytical grade and distilled water should be used:

- a. Ethanol 96% (volume fraction) at 20°C, freshly neutralized before each series of measurements with the potassium hydroxide solution, in the presence of the coloured indicator used for the determination in the case of manual titration.
- b. Standard ethanolic potassium hydroxide solution previously titrated at  $C_{KOH} = 0.05$  mol/l, 0.1 mol/l or 0.5 mol/l and checked before each series of measurements.

The choice of concentration  $C_{KOH}$  depends on the capacity of the burette used, the test portion and the target acid value in order to tend towards an optimal volume of ethanolic potassium hydroxide solution  $V_{KOH}$ .

NOTE  $V_{KOH}$  is optimal when the equivalent volume is at least equal to half the capacity of the burette used.

For information, examples of optimized analysis conditions are presented in Table H.1.

**Table H.1 — Examples of optimized analysis conditions**

Target acid value	Example of essential oils	Theoretical concentration $C_{KOH}$ mol/l	Approximate test portions g
Maximum 1.2	<i>Lavandula angustifolia</i>	0.05	2
Maximum 4.0	<i>Pogostemon cablin</i>	0.05	1
Minimum 15.0	<i>Cinnamomum aromaticum</i> , China type	0.10	1
30.0 to 60.0	<i>Vetiveria zizanioides</i> , Brazil type	0.50	1

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- c. Coloured indicator used for manual titration.
  - i. Phenolphthalein or thymolphthalein 2 g/l solution in neutralized ethanol 96% (volume fraction); or if the essential oil contains phenolic groups:
  - ii. Phenol red 0.4 g/l solution in ethanol 20% (volume fraction).

### H.3 Apparatus

#### Apparatus for manual titration

- a. Ordinary laboratory glassware, adapted to the kind of titration to be carried out (determination of the acid value alone or determination of the acid value followed by the determination of the ester value).

If ester value is determined with the same test portion, use a flask with a capacity of 100 ml to 250 ml and follow the specifications regarding the saponification device.

- b. Measuring cylinder of 5 ml capacity.
- c. Burette of capacity 2 ml or 5 ml, graduated in 0.01 ml.
- d. Analytical balance of precision 0.001 g.

#### Apparatus for automatic titration

- a. Titrator
- b. Analytical balance of precision 0.001 g.

### H.4 Sampling

It is important that the laboratory receive a representative sample which has not been damaged or modified during transportation or storage. A recommended sampling method is given in Annex J.

### H.5 Preparation of test sample

Prepare the test samples according to the method in Annex B.

### H.6 Procedure

#### Manual titration

##### H.6.1 Test portion

Weigh to the nearest 0.001 g a test portion of the sample of 1 g minimum, depending on the target acid value and  $CKOH$  concentration, in order to tend towards an optimal volume of ethanolic potassium hydroxide solution  $V_{KOH}$  if possible.

##### H.6.2 Determination

Introduce the test portion into suitable glassware and add 5 ml of neutralized ethanol. Add no more than five drops of coloured indicator (phenolphthalein or thymolphthalein solution, or phenol red solution), depending on the case. Then titrate the liquid with potassium hydroxide solution until a persistent colour change is achieved. The volume  $V_{KOH}$  of potassium hydroxide used is noted. The flask and its contents may be reserved in case of determination of the ester value.

The colour shifts observed according to the different indicators used are:

- colourless to pink with phenolphthalein;
- colourless to blue with thymolphthalein;
- yellow-orange to red with phenol red.

### **Automatic titration**

In a titrator cup, weigh the appropriate test sample to the nearest 0.001 g and record its mass and identity in the sequence creation. Add approximately 50 ml of neutralized ethanol 96% (v/v). To confirm the result, check the curve shape and equilibrium point suggested by the software. An alternative equilibrium point can be selected if deemed more significant. If the curve profile is incomplete or does not resemble an acid-base assay, it may indicate an incomplete assay or titration error. In such cases, repeat the analysis with new conditions, (e.g. test portion,  $C_{KOH}$ ).

## **H.7 Calculation**

The acid value,  $A_v$ , is given by Formula:

$$A_v = 56.11 \times C_{KOH} \times V_{KOH} / m$$

where

$C_{KOH}$  is the concentration, in moles per litre, of potassium hydroxide solution used;

$V_{KOH}$  is the volume, in millilitres, of potassium hydroxide solution used;

$m$  is the mass, in grams, of the test portion.

In the case of automatic titration, the neutralization of ethanol and titration of potassium hydroxide can take place simultaneously. In this case, an additional factor can be added to the above formula.

## **H.8 Precision**

### **H.8.1 Repeatability**

When conducting tests using this method, the difference between two independent test results carried out by the same operator, in the same laboratory, using the same apparatus, and with the same essential oil, within a short timeframe, should not exceed 0.05 in absolute terms or 2.5% in relative terms, considering the highest value obtained among the results.



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### **H.8.2 Reproducibility**

The difference between two individual test results obtained by means of this method, with the same essential oil tested in different laboratories and by different operators using different apparatus, shall not be greater than 0.1 in absolute terms or not greater than 5 % in relative terms, considering the highest value obtained among the results.

### **H.9 Test report**

The test report shall state the sampling method used; the test method used; whether an oil in solution was used in the test, specifying the nature of the solvent and the concentration of the oil; the test result obtained; and if repeatability has been checked, the final result obtained.

It shall also mention any operating conditions as well as any circumstances that might have influenced the results. The test report shall include all details required for the complete identification of the sample.

**Annex I**  
(Normative)

**Determination of Peroxide value**

**I.1 Principle**

The peroxide value is the measure of the oxidation present in an essential oil. The method is a redox titration, specifically of the iodometry type. Iodide ions in the essential oil oxidises when they react with peroxides, producing iodine that is then titrated with thiosulfate. This titration can be done using either volumetric or potentiometric methods.

Potentiometric titration is recommended for essential oils that are highly colored and may have difficulty showing a clear end point with colored indicators, such as vetiver essential oil.

**I.2 Reagents**

During the analysis, only reagents of recognized analytical grade and reverse osmosis or distilled or deionized water should be used.

- a. Trichloromethane, 99% (volume/fraction) or cyclohexane 99.5% (volume/fraction) for laboratories with restrictions on the use of chloroform.
- b. Glacial acetic acid 99.5% (volume/fraction). Degassed with a fresh pure and dry inert gas (carbon dioxide or nitrogen).
- c. Potassium iodide saturated solution in deionised water, freshly prepared. The solution has to be kept protected from light.
- d. Sodium thiosulfate solution, 0.01 mol/l (0.01 N) or 0.1 mol/l (0.1 N).
- e. Coloured standard indicator, starch solution 1% (volume/fraction). It is not necessary for potentiometric titration.

**I.3 Apparatus**

Usual laboratory apparatus and, in particular, the following.

- a. Balance  $\pm 1$  mg.
- b. Erlenmeyer flask of capacity 250 ml.
- c. Shaker.
- d. Pipettes of capacity 1 ml, 10 ml, graduated in 0.1 ml.
- e. Burette of capacity 10 ml graduated in 0.05 ml.
- f. Test tubes of capacity 50 ml, 100 ml.
- g. Potentiometer.

**I.4 Sampling**

It is important that the laboratory receive a representative sample which has not been damaged or modified during transportation or storage. A recommended sampling method is given in Annex J.

**I.5 Preparation of test sample**

Prepare the test samples according to the method in Annex B.

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The test sample for determining the peroxide value should be taken first, and the peroxide value should be calculated immediately. Homogenize the sample, ideally without heating or aeration. Avoid direct sun exposure. Carefully heat solid samples to 10 °C above their melting point. Samples with visible impurities shall be filtered.

Some products may include less than 5 g of extracted essential oil or could contain over 30 meq of active oxygen per kilogram of essential oil. The user should pick a smaller sample mass in these circumstances.

### I.6 Procedure

#### I.6.1 Test portion

10 mm of the essential oil to be titrated should be added to an Erlenmeyer flask.

#### I.6.2 Determination

20 ml of trichloromethane or cyclohexane, 30 ml of glacial acetic acid, and 1 ml of potassium iodide, saturated solution should be added to a test tube, and if volumetric titration is being done, 2 drops of starch solution should also be added.

Shake for approximately 1 min. The dissolution acquires an orangey colour. Add about 100 ml of distilled water.

Titrate with sodium thiosulfate solution. Use 0.1 mol/l when the expected peroxide value are over 20 mmol/l. Use 0.01 mol/l sodium thiosulfate solution when the expected peroxide value are less than 20 mmol/l. The end point is obtained when the dissolution becomes white.

Perform a blank titration in the same circumstances. Consumption of sodium thiosulfate solution should not exceed 0.5 ml of 0.01 mol/l.

#### I.6.3 Automated Potentiometric titration

If automated equipment for titration is used, consider the following:

- Use amber beaker glasses to prevent the formation of iodine while the sample is still in the tray if system uses an autosampler.
- If addition of potassium iodide is automated, prepare 70 % (volume/fraction) solution in distilled water instead of saturated solution, fill an opaque bottle, and replace it every week. The addition of potassium iodide in I.6.2 should be 10 ml instead of 1 ml.

### I.7 Expression of results

#### I.7.1 Calculation

The peroxide value,  $I_p$ , in mmol/l, is given by Formula:

$$I_p = (V_1 - V_0) \times (N_{\text{titrant}}) \times 50 \quad (1)$$

where

$V_1$  is the volume, in millilitres, of titrant sodium thiosulphate, used in the main test;

$V_0$  is the volume, in millilitres, of titrant sodium thiosulphate, used in the blank test;

$N_{\text{titrant}}$  is the concentration of titrant, sodium thiosulphate, 0.1 N.

### **I.7.2 Conversion from mmol/l to meq/kg**

The conversion from mmol/l to meq/kg is calculated by Formula:

$$\text{meq/kg} = \text{mmol/l} \times 2/\text{density (kg/l)}$$

## **I.8 Precision**

### **I.8.1 Repeatability**

The absolute difference between two separate test results obtained from the same essential oil using the same method, by the same operator, in the same laboratory, with the same equipment, and within a short timeframe, did not exceed 5% in absolute values or 0.1 in relative values.

### **I.8.2 Reproducibility**

The absolute difference between two individual tests conducted using the same method on the same essential oil, but in different laboratories with different operators and equipment, did not exceed 10% in absolute values or 0.3 in relative values.

**Annex J**  
**(Normative)**  
**Sampling**

**J.1 Principle**

The organoleptic, physical and chemical characteristics of batches of essential oils are determined by means of an examination of the samples.

This annex describes the general rules for the sampling of essential oils, in order to provide a laboratory with quantities that are suitable to be handled for expertise purposes.

In the presence of a high content of water or other foreign bodies, this method may only be applicable to the “essential oil” fraction free from water and impurities.

**J.2 Apparatus**

The sampling devices and the related instruments shall be made of materials which do not affect the sampled essential oil.

The type of apparatus required for sampling should be adapted to the volume to be sampled: e.g. cylindrical probes, pipettes, and bottom sampler.

**J.3 Sampling**

**J.3.1 Inspection**

The inspection concerns the physical condition of the delivery, the integrity of the containers, the state of the guarantee systems (lead seals, crown caps, etc.), the designation and the contractual inscriptions.

On opening, conserve the guarantee systems.

**J.3.2 Shaking**

Prior to any sampling, shake the essential oil using means suited to both the volume and the shape of the recipient.

Those essential oils that are known to crystallize or to thicken should be slowly warmed to a suitable temperature to dissolve crystals or crystalline mass, before shaking. This action shall not alter the composition of the essential oil.

**J.3.3 Sampling method**

All sampling operations shall be performed immediately after an appropriate shaking.

Take sample three increments per container at a single time, as follows:

- take the first increment from the section corresponding to 20% of the container height;
- take the second between 40% and 60% of the container height;

– take the third at over 90% of the container height.

Gather together the three equal part increments and mix them. After shaking, take 30 ml, which constitutes the sample.

The number of samples per container for the laboratory shall be equal to the number of parts concerned plus a reference sample.

## **J.4 Packaging and labelling of laboratory samples**

### **J.4.1 Packaging**

Use glass or inert material bottles which protect the essential oil against the light.

Pack the samples in clean, dry recipients.

The nature of the recipient shall not alter the essential oil.

Leave a headspace of 2 ml between the essential oil and the stopper to allow for expansion. This space shall not be too great in order to limit possible oxidation due to the air.

Close the recipients using crown tops or new stoppers which do not have any reaction on the product.

Close each sample by means of a guarantee system such that it is inaccessible without breaking the seal.

Ascertain the air tightness.

### **J.4.2 Marking**

The label shall be attached to each of the samples and shall bear indications enabling the traceability of the product, for example,

- the sampling date;
- the nature of the product: goods and origin;
- the name of the supplier;
- the batch number;
- the serial number of the sample out of the total number of containers.

The information on the label shall be marked in indelible ink.

### **J.4.3 Conservation**

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Store the samples intended for the laboratory, protected from light, at a temperature which guarantees their quality.

### **J.4.4 Dispatch**

The packaging shall meet the requirements of the postal services or of the other bodies involved in the transport of the sample within the relevant country (countries).

### **J.5 Sampling report**

The sampling report shall indicate:

- the identification of the supplier;
- the product identification marks;
- the origin;
- the batch number;
- the quantity represented in grams, kilograms or tons;
- the nature and the number of containers;
- the presence or absence of the guarantee systems;
- the date and time of sampling;
- the name, signature and function of the person who carried out the sampling.

The sampling report shall give the physical condition of the sampled essential oil. It shall also indicate the technique employed, if different from that described in this annex, as well as all circumstances which may have influenced the sampling.

A satisfactory sampling operation therefore needs to provide, for analysis, samples representative of the batches from which they originate without modification of the original composition.

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