

# BHUTAN STANDARD Lemongrass Spray



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## **BHUTAN STANDARD**

Lemongrass Spray

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#### FOREWORD

This Bhutan Standard for Lemongrass spray is developed by Bhutan Standards Bureau after the draft finalised by the Sub-committee on Essential Oil (TC 05/ SC 02 and 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

Lemongrass spray is a product formulated with lemongrass essential oil as the main ingredient. Lemongrass oil is distilled from the fresh aerial parts of *Cymbopogon flexuosus* (Nees ex Steudel Hackel), a perennial grass in the family of Poaceae. Essential oil from *C. flexuosus* is one of the few Non-Wood Forest Products (NWFPs) that has market access in Europe and other niche export markets and local distillers/producers have decades of experience.

Lemongrass spray is primarily used as an air freshener to purify and refresh the air in the work environment and at home. The spray as a natural fragrance intends to create a natural, uplifting, and reinvigorating atmosphere. The product is also promoted as a natural disinfectant and insect repellent, especially against mosquitoes. According to the literature, lemongrass oil has antiseptic, antibacterial, antimicrobial, and nervine properties, and as such has a wide range of applications in aromatherapy, cosmetics, and perfumery.

Recognizing this potential, there are arrays of products developed using lemongrass oil in the market. Although most of the ingredients used in spray production are assessed for their quality, there are concerns of product contamination and/or adulteration and production of sub-standard quality that may pose health risks over prolonged exposure to the air spray. Therefore, it is important to assess and control the quality of spray production for the safety of consumers.

This standard contains basic requirements to assess and evaluate the quality and safety of air spray production. While this standard is intended to outline only the minimum requirements, the technical committee could not verify specific 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 standardize and ensure the quality, safety and reliability of the spray. It is the responsibility and discretion of each individual or company to adopt or comply with this standard. The standard organization or the technical committee shall not be liable for any untoward events, either health-related or material-related losses.

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#### Lemongrass Spray

#### 1. Scope

This standard specifies essential characteristics of lemongrass (*Cymbopogon flexuosus*) spray from Bhutan and prescribes physical and chemical methods to facilitate the assessment of its quality.

#### 2. Normative References

There are no normative references for this document.

#### 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 lemongrass spray, 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 lemongrass spray to be sampled.

#### 3.6 Delivery

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

#### 3.7 Increment

Quantity of lemongrass spray sampled at a single time at a point in the container to be sampled.

#### 3.8 Lemongrass spray

Lemongrass spray is a product formulated with lemongrass oil obtained by steam distillation of the fresh aerial parts of *Cymbopogon flexuosus* and other diluents such as alcohol, and distilled water.

#### 3.9 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.

#### 3.10 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.

#### 3.11 Solid impurities

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.

#### 3.12 Sample

Quantity of the product obtained by mixing the different increments of a container or number of products to be selected from the lot based on lot size.

#### 3.13 Optical rotation

Angle, expressed in milliradians and/or degrees of angle, described by the polarization plane of luminous radiation whose wavelength is 589.3 nm  $\pm$  0.3 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.

#### 4. Acronyms and Symbols

cm fid	: centimetre :Flame Ionisation Detector
g	: grams
ĞC	: Gas Chromatography
g/l	: gram/ litre
Μ	: mass
mg	: milligram
ml	: millilitre
mm	: millimetre
mrad	: milliradians
m/v	: mass by volume
nm	: nanometre
q.s.ad	: means 'as much as is sufficient'
v/v	: volume by volume
v/w	: volume by weight
w/w	: weight by weight

#### 5. Requirements

#### 5.1 Description

Lemongrass spray is a colourless mixture of clear pale yellow to yellowish brown lemongrass oil in alcohol and distilled water with a characteristic citral odour.

Lemongrass spray is a product formulated with lemongrass oil obtained by steam distillation of the fresh aerial parts of *Cymbopogon flexuosus* and diluents such as alcohol, and distilled water.

#### 5.2 Appearance

Lemongrass spray is a colourless clear mobile liquid free from sediments, suspended matters, and added adulterants.

#### 5.3 Colour

Lemongrass spray is a colourless clear liquid.

#### 5.4 Odour

Lemongrass spray has a characteristic citral odour or pleasant lemony aroma.

#### 5.5 Composition

The percent composition of each of the ingredients in the lemongrass spray mixture must be clearly indicated. The main ingredients in the spray are but not limited to the following:

- a) Lemongrass oil
- b) Isopropyl alcohol or ethanol
- c) Distilled water, q.s.ad

#### 5.6 Tests

#### 5.6.A Identification

Analysis of the lemongrass oil in the spray 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 lemongrass oil in the spray.

#### Table 1 — Chromatographic profile of the lemongrass oil

Component	Minimum %	Maximum %
Limonene	0.2	3.5
6-Methyl-5-heptene-2-one	0.1	3.5
Caryophyllene	0.2	3.5
Neral	25.0	35.0
Geranial	35.0	47.0
Geranyl acetate	0.5	6.0
Geraniol	1.5	8.0
NOTE The chromatographic profile is r	normative	

(Clause 5.6.1)

#### 5.6.B Determination of citral content

Lemongrass oil in spray shall contain a minimum of 60 percent by volume citral when determined as per the method described in Annex B.

#### 5.6.C Relative density

The relative density of lemongrass oil in spray shall be a minimum of 0.881 and a maximum of 0.905 when determined at 20°C as per the method described in Annex D.

#### 5.6.D Refractive index

The refractive index of lemongrass oil in spray shall be minimum of 1.477 and a maximum of 1.489 when determined at 20°C as per the method described in Annex E.

#### 5.6.E Optical rotation

The optical rotation of lemongrass oil in spray shall be between -3 degrees and +1 when determined at 20°C as per the method described in Annex F.

#### 5.6.F Solubility

Lemongrass spray is very soluble in water.

#### 5.6.G Flashpoint

The mean value is +75°C (167°F) obtained with Pensky Martens equipment. The information on flashpoints is provided in Annex G.

#### 6. Sampling

The laboratory may conduct its own sampling plan in view of the analysis and tests specified in this standard.

The general rules for sampling, in order to provide a laboratory with quantities that are suitable to be handled for testing purposes, is described in Annex H.

#### 7. Manufacturing and packaging

Lemongrass spray must be manufactured applying good manufacturing practice and industrial hygiene practices by ensuring proper ventilation. One should observe good personal hygiene and not eat, drink or smoke while handling the product. Take precautions to avoid static discharges in the working area.

The product should be packaged in an airtight spray bottle preferably made of glass or other material that does not react with the product and protects it from light.

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 product package must also accompany the material safety data sheet.

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

- a) the name of the product/material,
- b) the percent composition,
- c) net weight or volume,
- d) the percentage of total citral content,
- e) batch number,
- f) manufacturing date,

- g) expiry date,
- h) full address of the manufacturer,
- i) storage conditions, and
- j) disclaimer, or caution if any.

#### 9. Storage and Handling

Lemongrass spray is a flammable liquid and should be stored and handled appropriately. The product should be stored in a tightly closed container, in a cool, dry and ventilated area away from heat sources and protected from light. The container must be checked for any liquid or vapour leaks and keep air contact to a minimum.

## Annex A

(Normative)

#### Gas Chromatographic analysis

#### A.1 General

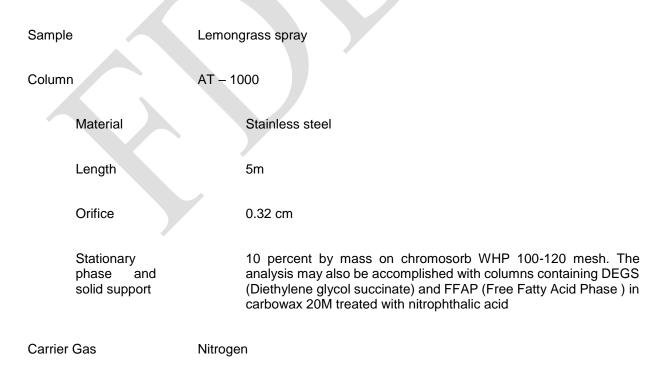
The gas chromatography (GC) method describes the general guidelines for the determination of the chromatographic profile of essential oil, as it is one of the specifications that enable assessment of the quality of lemongrass essential oil. The GC evaluates relative proportions of essential oil content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

#### A.2 Sample preparation and method

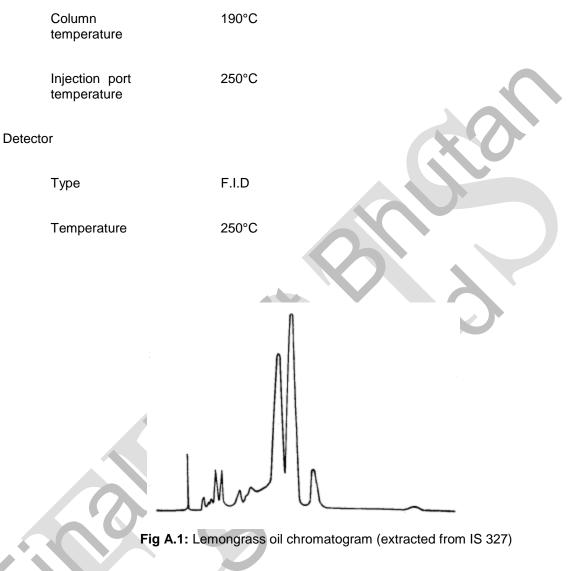
Dissolve a sample of the material in a suitable solvent, such as cyclohexane or petroleum ether. Inject the sample solution into the gas chromatograph, where the carrier gas carries it from one end of the column to the other. The constituents of the sample undergo distribution at different rates and 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 typical chromatogram for lemongrass oil operated under the following chromatographic conditions is shown in Fig A.1.



#### Conditions



#### 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 normalisation, 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:



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

## Annex B

(Normative)

#### **Determination of Citral Content**

#### B.1 General

Two methods, namely, the hydroxylamine method and sodium bisulphite methods are prescribed for determining citral content in lemongrass oil. While any one of these may be adopted for determining citral content, this annexe describes the procedure for the sodium bisulphite method.

When reporting an analytical result, it is important to record and indicate the method used.

#### **B.2** Apparatus

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

- a. Graduated cylinder
- b. Cassia flask
- c. Pipette
- d. Water bath

#### **B.3 Reagents**

Freshly prepared Sodium Bisulphite Solution, 35 percent (m/v).

#### **B.4 Procedure**

- a. Measure 75 ml of Sodium Bisulphite solution using a graduated cylinder and introduce it into a cassia flask (Fig B.1).
- b. Pipette exactly 10 ml of the material/sample into the flask and shake thoroughly.
- c. Immerse the flask in a boiling water bath and shake repeatedly to dissolve the solid addition compound completely into the solution.
- d. Further, add 25 ml of the bisulphite solution and shake the flask repeatedly for one-half to one hour to ensure a complete reaction of the carbonyl compound with the bisulphite solution.
- e. Allow the cassia flask to stand undisturbed in boiling water for 10 minutes to permit the unreacted material to rise to the surface.
- f. Add a sufficient volume of sodium bisulphite solution to raise the residual material to the neck of the flask.
- g. Gently tap the flask and rotate it rapidly to raise droplets of materials adhering to the sides of the flask to the neck.
- h. Cool the flask to room temperature and measure the volume of the residual material.
- i. If the precipitates at the surface where material and aqueous layers meet renders exact reading difficult, add a few drops of water in such a way that the water runs down along the inside of the flask neck so that it may remain temporarily on top of the bisulphite solution and give a sharp separation of the material and aqueous layers.
- j. If the material contains heavy metals, these should be removed before by shaking the material thoroughly with a small amount (about 1 percent) of powdered tartaric acid and filtering it.

Citral content, percent by volume = 10(10 - v); where v = volume in ml of residual material.

Citral content may be also expressed as percent by mass (M) if the relative density of the original material and the carbonyl compound is known at the same temperature, using the following formula:

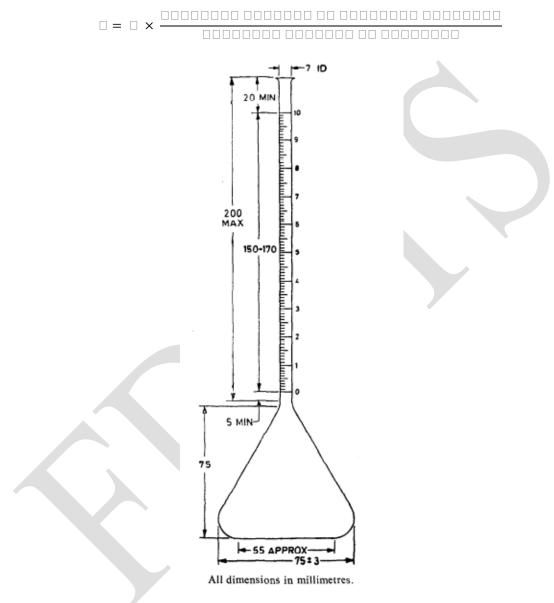


Fig B.1: Cassia Flask (extracted from IS 327)

#### Annex C (Normative)

#### **Preparation of Test samples**

#### C.1 General

This annexure gives general guidance for the preparation of samples to be submitted to a laboratory for analysis.

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

Filter the product after the addition of magnesium sulphate or sodium sulphate to eliminate water and the insoluble substances.

#### C.2 Apparatus

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

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

#### C.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.

#### C.4 Procedure

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

Add to the flask a mass of the dehydrating agent (magnesium sulphate or sodium sulphate) equal to about 15% of the mass of the product. Shake vigorously from time to time for 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 product should be clear and limpid.

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

#### Annex D

(Normative)

#### Determination of Relative Density at 20°C

#### **D.1 General principle**

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

#### **D.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  $20^{\circ}C \pm 0.2^{\circ}C$ .
- c. Standardised 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.

#### **D.3 Reagents**

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

#### D.4 Sampling

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

#### D.5 Test sample

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

#### **D.6 Procedure**

D.6.1 Preparation of pycnometer

Carefully clean the pychometer and then rinse it successively with, for example, ethanol and acetone, then dry the interior using 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.

D.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.

D.6.3 Weighing of essential oil

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

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

#### **D.7 Expression of results**

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

#### where

 $\Box_0$  is the mass, in grams, of the empty pycnometer determined in D.6.1;

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

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

#### D.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 E

(Normative)

#### **Determination of Refractive Index**

#### E.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.

#### **E.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  $\pm 0.2$ °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.

#### **E.3 Reagents**

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

- a. Distilled water, of refractive index 1.3330 at 20°C.
- b. p-Cymene, of refractive index 1.4906 at 20°C.
- C. Benzyl benzoate, of refractive index 1.5685 at 20°C.
- d. 1-Bromonaphthalene, of refractive index 1.6585 at 20°C.

#### 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 H.

#### E.5 Procedure

E.5.1 Preparation of test sample

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

E.5.2 Regulation of the refractometer

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

E.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  $\pm 0.2^{\circ}$ C during the test.

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

#### **E.6 Determination**

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

#### **E.7 Calculation**

The refractive index  $\Box_{\Box}^{\Box}$ , at the specified temperature *t*, is given by the equation:

$$\Box_{\Box}^{\Box} = \Box_{\Box}^{\Box'} + 0.0004(\Box' - \Box)$$

where

 $\Box_{\Box}^{\Box'}$  is the reading taken at the working temperature *t*' at which the determination was actually made.

Express the result to four decimal places.

#### E.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 not be greater than  $\pm 0.0002$ .

#### E.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 F

#### (Normative)

#### **Determination of Optical Rotation**

#### F.1 Principle

This is the general method for determining the optical rotation of essential oils. 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.

#### F.2 Apparatus

a. **Polarimeter**, having a precision of at least ± 0.5 mrad (± 0.03°) and adjusted to give 0° and 180° 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°) in a 200 mm layer, at a temperature of 20°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 nm + 0.3 nm, preferably a sodium vapour lamp.
- C. Polarimeter tubes, usually 100 mm ± 0.5 mm long.

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

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

#### F.3 Reagents

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.

#### F.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 H.

#### F.5 Procedure

#### F.5.1 Preparation of test sample

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

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.

#### F.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  $20^{\circ}C \pm 1^{\circ}C$  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 ( $\pm 0.2^{\circ}C$ ) 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.

#### F.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°).

#### F.6 Expression of results

F.6.1 Calculation and formulae

#### a. Optical Rotation

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

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

where

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

*I* 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:

 $[\alpha] = \frac{\Box \Box}{\Box}$ 

where

 $\alpha_{\Box}^{\Box}$  is the optical rotation of the oil solution, calculated according to F.6.1 a;

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

#### F.6.2 Precision

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

#### F.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 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 lemongrass spray

The mean value is +75°C.

#### Annex H (Normative)

#### Sampling

#### H.1 Principle

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

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

#### **H.2 Apparatus**

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

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

#### H.3 Sampling

#### **H.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.

#### H.3.2 Shaking

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

#### H.3.3 Sampling method

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

- a. In a single consignment, all packages belonging to the same batch shall be grouped together and each group shall constitute a lot.
- b. For ascertaining the conformity of the material to the requirements of the specification, samples shall be tested from each lot separately.
- c. The number of containers to be sampled or taken from the lot depends on the size of the lot and shall be in accordance with column (1) and (2) of Table 1. From each selected package approximately equal number or quantity shall be taken from each packet so as to constitute the required sample size.

- d. The required number of packages from each selected lot and the required quantity from each selected packet shall be chosen at random.
- e. From the sample size, take sample three increments per container at a single time, in accordance with column (3) of Table 1.
- f. Gather together the three equal part increments, pool and mix them thoroughly. After shaking, take 30 ml, which constitutes the sample.
- g. The number of samples per container for the laboratory shall be equal to the number of parts concerned plus a reference sample.

#### Table 1: Scale of sampling

No. of Packages in the lot (N) (1)	No. of Packages/bottles to be selected (n) (2)	Incremental sampling per bottle (3)
Up to 3	each container	• take the first increment from the section corresponding to
4 - 50	3	<ul> <li>20% of the container height;</li> <li>take the second between 40% and 60% of the</li> </ul>
51 - 150	4	<ul><li>container height;</li><li>take the third at over 90% of</li></ul>
151 - 300	5	the container height.
301 - 500	6	
501 and above		

#### (Clause H.3.3)

#### H.4 Packaging and labelling of laboratory samples

#### H.4.1 Packaging

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

Pack the samples in clean, dry recipients.

The nature of the recipient shall not alter the product specification.

Leave a headspace of 2 ml between the product 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.

#### H.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.

#### **H.4.3 Conservation**

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

#### H.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).

#### **H.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 product. 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.

#### Bibliography

- 1] ISO 4718- Oil of Lemongrass
- 2] ISO/TR 210- Essential oils General rules for packaging, conditioning and storage
- 3] ISO/TR 211- Essential oils General rules for labelling and marking of containers
- 4] ISO 212, Essential oils Sampling
- 5] ISO 279, Essential oils Determination of relative density at 20 °C
- 6] ISO 280, Essential oils Determination of refractive index
- 7] ISO 592, Essential oils Determination of optical rotation
- 8] ISO 875, Essential oils Evaluation of miscibility in ethanol
- **9]** ISO 11024-1, Essential oils General guidance on chromatographic profiles Part 1: Preparation of chromatographic profiles for presentation in standards
- **10]** ISO 11024-2, Essential oils General guidance on chromatographic profiles Part 2: Utilization of chromatographic profiles of samples of essential oils
- 11] ISO/TR 11018:1997, Essential oils General guidance on the determination of flashpoint
- 12] IS 327, Oil of Lemongrass Specification

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