འབྲུག་ དྲུང་ཛ་སྨན་གྱི་གནས་ཚད།

BHUTAN STANDARD

Lemongrass Essential Oil

EDITED

ICS 71.100.60

© Copyright 2023

BHUTAN STANDARDS BUREAU

The National Standards Body of Bhutan

THIMPHU 11001

……, 2023

Price group B

BTS XXX: 2023

འབྲུག་ དྲུང་ཧ་ཅིའི་སྐུམ་གྱི་གནས་ཚད།

BHUTAN STANDARD

Lemongrass Essential Oil

FDBTS

PDF disclaimer

This PDF may contain embedded typefaces. In accordance with Adobe's Licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The Bhutan Standards Bureau accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

FDBTS

COPYRIGHT PROTECTED DOCUMENT

© BSB 2023

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from BSB at the address below in the country of the requester.

Director General
Bhutan Standards Bureau
Rijug Lam
Thimphu-11001
Tel: 00975-2-325104/325401
Fax: 00975-2-323712/328298
Web: www.bsb.gov.bt
Published in Thimphu, Bhutan

Contents

FDBTS

FOREWORD

This Bhutan Standard for Lemongrass oil was developed by Bhutan Standards Bureau after the draft finalised by the Sub-committee on Essential Oil (TC 05/ SC02 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.

Final Draft Bhutan Standard

BTS XXX: 2023

INTRODUCTION

Lemongrass is a perennial grass in the family of Poaceae. They thrive in a wide range of soils with good drainage in sunny, warm, and humid conditions. Generally, lemongrass grows in clumps and can reach heights of up to 2 meters.

Although there are six known lemongrass species found in Bhutan, namely *Cymbopogon khasianus, C. flexuosus, C. pendulus, C. bhutanicus, C. munroi* and *C. jwarancusa,* the ***Cymbopogon flexuosus*** (Nees ex Steudel Hackel)**,** locally known as 'Sor Bang,' is the main species used for oil extraction.

Another species that grows in the same area is *C. distans* which is a form of *C. bhutanicus*. It produces oil rich in piperitone which has a pungent smell. As such, it is a minor source of commercial production. Locals refer to this plant as 'So-fe.'

In contrast to *C. distans* oil, the *C. flexuosus* essential oil has a consistently sweeter and more appealing aroma. The oil has a high *Citral*, the main biologically active constituent, which is a mixture of double-bound isomers. The E-isomer, or citral A, is also known as geranial and the Z-isomer is known as citral B or neral. Because of its potent antiseptic, antibacterial, antimicrobial, and nervine properties, it has a wide range of applications in aromatherapy, cosmetics, and perfumery. Because of these factors, it is the most economically valuable species used in commercial production.

The *C. flexuosus* grows in association with Chirpine (*Pinus roxhurghii,* Himalayan long needle pine) forests in dry, steep slopes and V-shaped inner valleys of Eastern Bhutan in four Dzongkhags, namely Mongar, Trashigang, Trashiyangtse, and Lhuentse. The plant occurs mostly in dry and warm sites that receive less precipitation and long hours of sunshine, and where soils have lower moisture and water-holding capacity.

As opposed to being cultivated for oil production in other countries, Bhutan is an exceptional case where lemongrass is collected from the wild. The collection, processing, and extraction of lemongrass essential oil *(Cymbopogon flexuosus)* from the wild (community-managed forests) has become a good source of income for rural communities in Eastern Districts. The oil is extracted using steam distillation methods.

The fact that the *C. flexuosus* is one of the few Non-Wood Forest Products (NWFPs) that has market access in the EU and other niche export markets; local distillers/producers have decades of experience, and the species grows in an estimated 50,000 hectares of Chirpine forest provide an immense opportunity for employment creation, revenue generation and convertible currency earning from the export market. However, the risk of adulteration and the production of sub-standard quality are the main challenges in realizing the potential.

The national standard is developed to standardize and ensure the quality, safety and reliability of the product. The standard will also guide competent authorities in the certification of lemongrass essential oil. In addition, it facilitates trade, improves systems, and ensures consistency.

This standard has been prepared in consultation with stakeholders to suit the intended purposes. 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.

Final Draft Bhutan Standard

འབྲུག་ དྲུང་ཌ་ཡི་སྒྲིག་གྲྭ་ གནས་ཚད།

BHUTAN STANDARD

Lemongrass Essential Oil

1 Scope

This standard specifies essential characteristics of lemongrass (*Cymbopogon flexuosus)* 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.

FDRTS

3.7 Increment

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

3.8 Lemongrass essential oil

Essential oil obtained by steam distillation of the fresh aerial parts of *Cymbopogon flexuosus* of the Poaceae family.

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 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.

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 ± 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 : centimetre
F.I.D : Flame Ionisation Detector

Final Draft Bhutan Standard

g	: grams
GC	: Gas Chromatography
g/l	: gram/ litre
M	: mass
Mg	: milligram
ml	: millilitre
mm	: millimetre
mrad	: milliradians
m/v	: mass by volume
nm	: nanometre
v/v	: volume by volume
v/w	: volume by weight
w/w	: weight by weight

5 Requirements

5.1 Description

Lemongrass essential oil is a clear pale yellow to yellowish brown liquid with a characteristic citral odour obtained by water or steam distillation of the freshly cut grass (aerial part) of the *Cymbopogan flexuosus* of the Poaceae family.

5.1 Scientific classification

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Liliopsida
Order	: Poales
Family	: Poaceae
Genus	: Cymbopogon
Species	: flexuosus

5.2 Appearance

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

5.3 Colour

Lemongrass oil should be pale yellow to yellowish brown.

5.4 Odour

Lemon grass oil has a characteristic citral odour.

5.5 Tests

5.5.1 Identification

Analysis of the lemongrass essenial oil shall be carried out by gas chromatography described in Annex A. In the chromatogram obtained, the representative and

FDBTS

characteristic components shown in Table 1 shall be identified. This constitutes the chromatographic profile of the lemongrass essential oil.

Table 1 — Chromatographic profile of the lemongrass oil
(Clause 5.5.1)

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 normative		

5.5.2 Determination of citral content

Lemongrass essential oil shall contain a minimum of 60 percent by volume carbonyl compound expressed as Citral when determined as per the method described in Annex B.

5.5.3 Relative density

The relative density of lemongrass essential oil 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.

5.5.4 Refractive index

Final Draft Bhutan Standard

The refractive index of lemongrass essential oil 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.

5.5.5 Optical rotation

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

5.5.6 Solubility

One volume of lemongrass essential oil mixed with three volumes of ethanol (70% by volume) shall give a clear solution when determined at 20°C as per the methods described in Annex G.

5.5.7 Flashpoint

The mean value is +85°C obtained with Pensky Martens equipment. The information on flashpoints is provided in Annex H.

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 lemongrass essential oils, in order to provide a laboratory with quantities that are suitable to be handled for expertise purposes is described in Annex I.

7 Packaging

Lemongrass 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:

a) the name of the product/material,
b) net weight or volume,
c) the percentage of total citral content
d) batch number,

FDRTS

e) manufacturing date,
f) expiry date,
g) full address of the manufacturer,
h) storage conditions, and
i) disclaimer or caution, if any

9 Storage

Lemongrass 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.

Final Draft Bhutan Standard

Annex A

(Normative)

Gas Chromatographic analysis of lemongrass 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 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.

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 typical chromatogram for lemongrass oil operated under the following chromatographic conditions is shown in Fig A.1.

Sample	Lemongrass essential Oil
Column	AT – 1000
Material	Stainless steel
Length	5 m
Orifice	0.32 cm
Stationary phase and solid support	10 percent by mass on chromosorb WHP 100-120 mesh. The analysis may also be accomplished with columns containing DEGS (Diethylene glycol succinate) and FFAP (Free Fatty Acid Phase) in carbowax 20M treated with nitrophthalic acid

FDIS

Carrier Gas	Nitrogen
Conditions	
Column temperature	190°C
Injection port temperature	250°C
Detector	
Type	F.I.D
Temperature	250°C

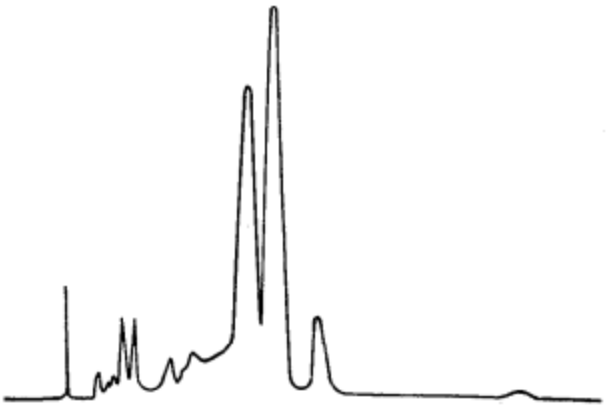


Fig A.1: Lemongrass essential oil chromatogram (extracted from IS 327)

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.

Final Draft Bhutan Standard

A.4.2 Area Normalization

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:

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

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

FDBTS

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 essential 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

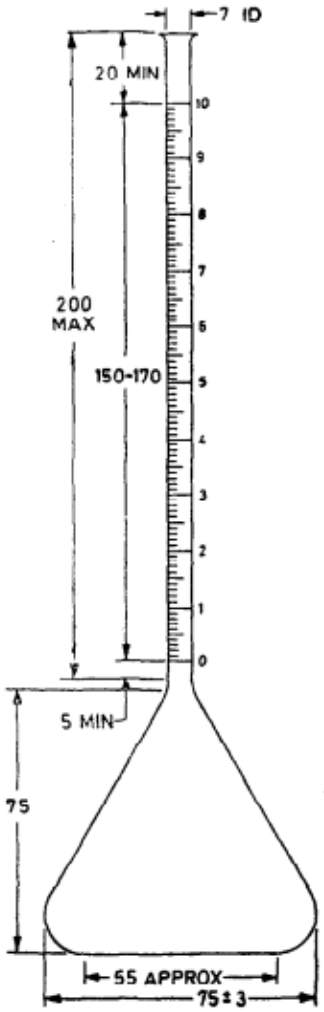
a. Measure 75 ml of Sodium Bisulphite solution using a graduated cylinder and introduce it into a cassia flask (Fig B.1).
b. Pipette exactly 10 ml of the material/sample into the flask and shake thoroughly.
c. Immerse the flask in a boiling water bath and shake repeatedly to dissolve the solid addition 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 shaking the material thoroughly with a small amount (about 1 percent) of powdered tartaric acid and filtering it.

First Draft Bhutan Standard

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:

$$M = \square \times \frac{\square\square\square\square\square\square\square\square\square \; \square\square\square\square\square\square\square\square \; \square\square \; \square\square\square\square\square\square\square\square\square}{\square\square\square\square\square\square\square\square\square \; \square\square\square\square\square\square\square\square \; \square\square \; \square\square\square\square\square\square\square\square\square}$$



All dimensions in millimetres.

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 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.

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 180ºC to 200ºC (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

C.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 10ºC 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.

C.4.2 Essential oils which are liquid at the ambient temperature

Final Draft Bhutan Standard

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.

C.4.3 Treatment of the essential oil

In the two cases indicated above, (C.4.1) or (C.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 (C.4.1), carry out the filtration in the oven at the appropriate temperature (see C.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.

FDBTS

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°C ± 0.2°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**.**

D.3 Reagents

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

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 pycnometer and then rinse it successively with, for example, ethanol and acetone, then dry the interior by means of 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

Final Draft Bhutan Standard

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, $\square_{20}^{20}$, is given by the following equation:

$$\frac{\square_2 - \square_0}{\square_1 - \square_0}$$

where,

$\square_0$ 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.

FDBTS

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 ± 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 ± 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 I.

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).

Final Draft Bhutan Standard

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 ± 0.2°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 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.

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 ± 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.

FDBTS

Annex F
(Normative)

Determination of Optical Rotation

F.1 Principle

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

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 ± 0.05 mm or 10 mm ± 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°C ± 0.2°C or any other specified temperature.

F.3 Reagents

Final Draft Bhutan Standard

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 I.

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°C ± 1°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 (± 0.2°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:

$$\alpha = \frac{}{} \times 100$$

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:

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

where

α is the optical rotation of the oil solution, calculated according to F.6.1 a;

$\square$ 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 ± 3 mrad (± 0.17°).

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.

Final Draft Bhutan Standard

Annex G
(Normative)

Evaluation of Solubility in ethanol

G.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.

G.2 Classification of solubility or miscibility

G.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.

G.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.

G.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.

G.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 G.2.1, G.2.2 and G.2.3) shows an opalescence identical to the one of the standard solutions for opalescence, freshly prepared in accordance with the method given in G.4 (c).

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

G.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°C ± 0.2°C.

FDIS

e. Calibrated thermometer, graduated at 0.2°C or 0.1°C, allowing the temperature of the device to be checked.

G.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 G.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, (□□□□$_3$) = 0.1 mol/l, to 50 ml of sodium chloride solution, (NaCl) = 0.0002 mol/l; then add 1 drop of concentrated nitric acid (□$_{20}$ = 1.38 g/ml). Stir the solution and allow it to stand for 5 minutes. Keep away from direct light.

   Prepare the solution freshly before use.

G.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 I.

G.6 Preparation of test sample

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

G.7 Procedure

G.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°C ± 0.2°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 g ± 0.005 g of essential oil. In this case, the definition and the results will be expressed in mass/volume.

G.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°C ± 0.2°C, in increments of 0.1 ml until complete miscibility occurs, shaking

Final Draft Bhutan Standard

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.

G.7.3 Opalescence

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 G.8.2.

G.8 Expression of results

G.8.1 Miscibility

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

a) Case G.2.1

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

b) Case G.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 G.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.

G.8.2 Opalescence

FDBTS

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

G.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 G.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 ± 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)
Q % (volume fraction)	ml	g	g	d_{20}^{20} ±0.0001	ρ_{20} ±0.00001 g/ml
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.

Final Draft Bhutan Standard

BTS XXX: 2023

FDBTS

Annex H
(Informative)

Flashpoint

H.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 standardisation purposes.

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

H.2 Flashpoint of the essential oil of lemongrass

The mean value is +85 °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.

Final Draft Bhutan Standard

Annex I
(Normative)

Sampling

I.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.

I.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.

I.3 Sampling

I.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.

I.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.

I.3.3 Sampling method

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

FDBTS

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.

I.4 Packaging and labelling of laboratory samples

I.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.

I.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.

Final Draft Bhutan Standard

I.4.3 Conservation

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

I.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).

I.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.

FDBTS

10 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

Final Draft Bhutan Standard

SUB-COMMITTEE ESSENTIAL OIL STANDARDS (TC 05/SC 02)

Organization	*Representative(s)*
Menjong Sorig Pharmaceuticals Corporation Ltd., Thimphu	Mr Sherab Tenzin (Chairperson)
Competition and Consumer Affairs Authority, MoICE	Mr Gopal Pradhan
M/S Bio Bhutan, Thimphu, Bhutan	Mr Ugyen
M/S Bhutan Natural Products	Mr Chencho Wangdi
Health Service and Quality Assurance Division, Ministry of Health	Mr Kuenzang Dorji

Member Secretary
Ms Cheten Zangmo
Standardization Division
Bhutan Standards Bureau

FDBTS

PHARMACEUTICAL AND TRADITIONAL MEDICINES TECHNICAL COMMITTEE (TC05)

Organization	*Representative(s)*
Menjong Sorig Pharmaceuticals Corporation Ltd., Thimphu	Mr Sherab Tenzin (Chairperson)
Bhutan Food and Drug Authority	Mr Thinley Zangpo
Competition and Consumer Affairs Authority, MoICE	Mr Gopal Pradhan
Department of Medical Products, NMS	Mr Jangchhup Peljor
Health Service and Quality Assurance Division, Ministry of Health	Mr Kuenzang Dorji
Royal Centre for Disease Control, MoH	Mr Amin Ngawang Tashi
National Biodiversity Centre, MoAL	Ms. Jamyang Choden
Bhutan Standards Bureau	Mr Karma Wangdi Offtg. Director General (Ex-officio member)

Member Secretary
Ms Cheten Zangmo
Standardization Division
Bhutan Standards Bureau

Final Draft Bhutan Standard