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PHYTOPHARMACEUTICAL ASPECT OF FREEZE DRIED WATER EXTRACT FROM TONGKAT ALI ROOTS - SPECIFICATION

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Department of Chemistry, Malaysia
Department of Standards Malaysia
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The Technical Committee on Herbs which supervised the development of this Malaysian Standard consists of representatives from the following organisations:

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Department of Agriculture Malaysia
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Institute for Medical Research
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TPM Biotek Sdn Bhd
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Universiti Sains Malaysia
Universiti Teknologi Malaysia (Chemical Engineering Pilot Plant)

FOREWORD

This Malaysian Standard was developed by the Working Group on Phytopharmaceutical Aspect of Herbs under the authority of the Industry Standards Committee on Food and Food Products.

This Malaysian Standard is based on Malaysian Herbal Monograph, 1999 (FRIM) and findings by reliable research institutions.

Compliance with a Malaysian Standard does not of itself confer immunity from legal obligations.

PHYTOPHARMACEUTICAL ASPECT OF FREEZE DRIED WATER EXTRACT FROM TONGKAT ALI ROOTS - SPECIFICATION

1 Scope

This Malaysian Standard prescribes the commercial quality requirements of freeze dried water extract derived from dried root (Radix) of *Eurycoma longifolia* Jack (Simaroubaceae). This herb is also known as "tongkat ali".

2 Normative references

The following normative references are indispensable for the application of this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the normative references (including any amendments) applies.

MS 1860, *Herbs and herbal products - Vocabulary*

3 Terms and definitions

For the purposes of this standard, the following terms and definitions given in MS 1860 and the following apply.

3.1 Extract

A concentrated form of the herbal preparation obtained by processing the crude herb with an appropriate solvent, by maceration, fractionation, infusion, supercritical fluid extraction or any other means of extraction.

3.2 Standardised extract

Extracts can be standardised to different degree or level of requirement, either:

a) chemically standardised, which means extracts shall contain consistent level of marker compound(s) qualitative and quantitatively; or

b) biologically standardised, which means extracts shall expressed consistent pharmacological effect on certain biological method; or

c) chemically and biologically guaranteed, which means guaranteed potency extracts.

4 Water extraction

Flow chart of the commercial water extraction of Radix Eurycomae is as specified in Annex A.

5 Drying

Recommended method of drying for Radix Eurycomae extract is freeze drying (See Annex A).

6 Formulation

6.1 Any suitable excipient such as maltodextrin, dextrose and lactose can be used.

6.2 Finished products shall be in the form of oral dosage, but not limited to soluble powder, capsule and tablet.

7 Standardisation

7.1 Freeze dried water extract shall be standardised to the following requirements:

Table 1. Specification of freeze dried water extract of Radix Eurycomae

Marker compound	Amount (% w/v)
Eurycomanone	0.80 - 1.50
Total polysaccharides	> 30
Total protein	> 20
Total glycosaponin	> 40

7.2 Results of analysis and expiry date shall be recorded on Certificate of Analysis (COA) of every batch of production.

7.3 Recommended method of analysis for qualitative and quantitative determination of marker compounds are as prescribed in Annex B.

7.4 pH of freeze dried water extracts of Radix Eurycomae shall be 6.47 $\pm$ 0.3.

7.5 Standardised extracts shall be free from any chemical adulterants.

7.6 Generally, for healthy people, the recommended daily consumption of freeze dried water extract of Radix Eurycomae is 50 mg to 200 mg.

8 Stability of extract

8.1 Shelf life can be determined using the method prescribed in Annex C.

8.2 Estimated shelf life for freeze dried water extracts of Radix Eurycomae is two years at room temperature (22 °C to 25 °C) in air-tight container.

9 Application

Extracts may be used as food ingredients and shall comply with food regulations.

Annex B
(normative)

Qualitative and quantitative determination of marker compounds

B1 Eurycomanone estimation using high pressure liquid chromatography (HPLC) (on water extract Radix Eurycomae)

B1.1 Chemical and reagents: Standard sample of Eurycomanone (Chromadex) was required. Solvents used are HPLC grade acetonitrile and water and ortho-phosphoric acid.

B1.2 HPLC analysis was performed using a Waters 600 gradient pump coupled to a Waters 996 Photodiode-array detector. Separation was achieved using Ascentris™ RP-Amide 5 µm column with dimension of 250 x 4.6 mm using a 20 minute water-acetonitrile-*ortho*-phosphoric acid gradient system.

B1.3 HPLC analysis can be performed using equivalent system provided the results obtained are comparable.

B1.4 The mobile phase is started at 90 %, 0.05 % phosphoric acid (Solvent A) : 10 % acetonitrile (Solvent B) to 75 % (A) : 25 % (B) for the first 10 min. The mobile phase was then transitioned from 75 % (A) : 25 % (B) to 90 % (A) : 10 % (B) for the remaining 10 min of the run. The wavelength used is set at 254 nm.

Table B1. The ratio of mobile phase between phosphoric acid (Solvent A) and acetonitrile (Solvent B)

No.	Time (minutes)	Flow rate	Percentage A, %	Percentage B, %
1	0	1	90	10
2	10	1.8	75	25
3	20	1	90	10

B2 Protein estimation by Lowry's method

B2.1 Principle

The blue colour developed due to the reduction of the phosphomolybdic components in the Folin - Ciocalteau reagent by the amino acids, tyrosine & tryptophan present in the protein. Plus the colour developed by the Biuret reaction of the protein with the alkaline cupric tartarate is measured in the Lowry's method.

B2.2 Materials

B2.2.1 2 % sodium carbonate in 0. 1N sodium hydroxide (Reagent A)

Weigh 2.0 g sodium hydroxide (NaOH) pellet, dissolve in distilled water and make up to 500 ml with distilled water to give 0.1 N NaOH. Weigh 5.0 g sodium carbonate (Na_2CO_3), dissolved in 0.1 N NaOH and make up to volume in 250 ml volumetric flask.

B2.4 Calculation

Determine the amount of protein in the sample using the standard calibration curve of concentration versus intensity.

B3 Total polysaccharide

B3.1 Principle

The sample is treated with 80 % alcohol to remove sugars and then starch is extracted with 25 % hydrochloric acid. In hot acidic medium starch is hydrolysed to glucose and dehydrated to hydroxymethyl furfural. This compound forms a green coloured product with Anthrone reagent.

B3.2 Materials

B3.2.1 Anthrone reagent

Dissolve 200 mg analytical grade Anthrone in 100 ml of ice cold 95 % sulphuric acid.

B3.2.2 Ethanol

80 % ethanol analytical grade.

B3.2.3 Hydrocloric acid

25 % hydrochloric acid analytical grade (HCl).

B3.2.4 Glucose stock solution

Weigh accurately 100 mg glucose anhydrous analytical grade, dissolve in distilled water and make up to volume in 100 ml volumetric flask to give 1000 ppm stock solution.

B3.2.5 Glucose working standard

Pipette out 10 ml from glucose stock solution and make up to volume with distilled water in 100 ml volumetric flask to give 100 ppm concentration.

B3.3 Method

B3.3.1 Dissolve 0.2 g of the sample in warm water bath (40 °C) 80 % ethanol (7 ml) in a centrifuge tube to remove sugars. Mix and centrifuge at 2700 rpm for 10 minutes and retain the residue. Repeat washings with 80 % hot ethanol (mix, centrifuge and retain residue) for several times until the washing does not give colour with Anthrone reagent.

B3.3.2 Dry the residue well over a hot water bath (80 °C).

B3.3.3 Add 5.0 ml of distilled water and 5.0 ml of 25 % HCl.

B3.3.4 Extract at 0 °C for 20 min. Centrifuge at 2700 rpm for 10 minutes and collect the supernatant.

B3.3.5 Repeat the extraction using fresh 25 % HCl. Centrifuge and pour the supernatant and make up to 100 ml with distilled water.

B3.3.6 Pipette out 0.1 ml of the supernatant from B3.3.5 into test tube and make up the volume to 1 ml with distilled water.

B3.3.7 Prepare standards by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the glucose working standards B3.2.5 and make up the volume to 1 ml in each test tube with distilled water.

B3.3.8 Use 1 ml of distilled water as blank.

B3.3.9 Add four ml of Anthrone reagent to each test tube of blank, sample and standards.

B3.3.10 Heat for 8 min in a boiling water.

B3.3.11 Cool rapidly and read the intensity of the colour produced using a spectrophotometer at 630 nm.

B3.4 Calculation

Determine the glucose content using the standard calibration curve. Multiply the value by a factor 0.9 to arrive at the starch content.

B4 Glycosaponins in extracts (Gravimetry method)

B4.1 Principle

The glycosaponin is extracted from sample with methanol. Acetone is added drop wisely to completely precipitate the glycosaponin.

B4.2 Materials

B4.2.1 Chemicals and solvents

Methanol and acetone used are of analytical grade.

B4.3 Method

B4.3.1 Weigh 1 g of extract and dissolve in 50 ml of methanol. Reflux on warm water bath (50 °C) for half-hour and filter. Collect the filtrate.

B4.3.2 Re-reflux the residue twice in 50 ml methanol.

B4.3.3 Concentrate total methanolic extract to 10 ml using rotary evaporator.

B4.3.4 Add dropwise 10 ml of methanolic extract concentrate (B4.3.3) to 50 ml of acetone in a pre-weighed beaker to precipitate the saponins.

B4.3.5 Dry the precipitate on a warm water bath (50 °C) before drying in oven at 100 °C to constant weight.

B4.4 Calculation

$$\text{Glycosaponins} = \frac{\text{weight of beaker - weight of empty beaker}}{\text{sample weight}} \times 100$$

Annex C
(normative)

Stability testing of herbal substances and products

C1 Introduction

C1.1 The purpose of the stability study is to establish, based on testing a minimum of three batches of the herbal substance and evaluating the stability information (including, as appropriate, results of the physical, chemical, biological, and microbiological tests), a re-test period applicable to all future batches of the herbal substance manufactured under similar circumstances. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout the assigned re-test period.

C1.2 The data may show so little degradation and so little variability that it is apparent from looking at the data that the requested re-test period will be granted. Under these circumstances, it is normally unnecessary to go through the formal statistical analysis; providing a justification for the omission should be sufficient.

C1.3 An approach for analysing the data on a quantitative attribute that is expected to change with time is to determine the time at which the 95 % one-sided confidence limit for the mean curve intersects the acceptance criterion. If analysis shows that the batch-to-batch variability is small, it is advantageous to combine the data into one overall estimate. This can be done by first applying appropriate statistical tests (e.g., p values for level of significance of rejection of more than 0.25) to the slopes of the regression lines and zero time intercepts for the individual batches. If it is inappropriate to combine data from several batches, the overall re-test period should be based on the minimum time a batch can be expected to remain within acceptance criteria.

C1.4 The nature of any degradation relationship will determine whether the data should be transformed for linear regression analysis. Usually the relationship can be represented by a linear, quadratic, or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit of the data on all batches and combined batches (where appropriate) to the assumed degradation line or curve.

C1.5 Limited extrapolation of the real time data from the long term storage condition beyond the observed range to extend the re-test period can be undertaken at approval time, if justified. This justification should be based on what is known about the mechanism of degradation, the results of testing under accelerated conditions, the goodness of fit of any mathematical model, batch size, existence of supporting stability data, etc. However, this extrapolation assumes that the same degradation relationship will continue to apply beyond the observed data.

C1.6 Any evaluation should cover not only the assay, but also the levels of degradation products and other appropriate attributes.

C2 Storage conditions

C2.1 In general, a herbal substance should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use.

C2.2 The long term testing should cover a minimum of 12 months duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed re-test period. Additional data accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and, if appropriate, from the intermediate storage condition can be used to evaluate the effect of short term excursions outside the label storage conditions (such as might occur during shipping).

C2.3 Long term, accelerated, and, where appropriate, intermediate storage conditions for herbal substances are detailed in the sections below. The general case applies if the herbal substance is not specifically covered by a subsequent section. Alternative storage conditions can be used if justified.

C3 General case

Table C3. Storage condition for stability studies

Stability Study	Storage condition
Real time	30 °C ± 2 °C/75 % RH ± 5 % RH
Accelerated	40 °C ± 2 °C/75 % RH ± 5 % RH

The choice of test conditions defined in the table is based on the ASEAN Guideline on Stability Study of Drug Product (2005). Alternative storage conditions should be used if justified or mandated by the country. For the accelerated studies, a 6-month study is recommended. The real time testing will be continued for a sufficient time to cover shelf-life at appropriate test periods. Frequency of testing should be sufficient to establish the stability profile of the herbal drug preparations/herbal medicinal products.

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Acknowledgements

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