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Traditional Chinese medicine — *Schisandra chinensis* (Turcz.) Baill. seeds and seedlings

Médecine traditionnelle chinoise — Graines et plants de Schisandra chinensis (Turcz.) baill.

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Foreword

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This document was prepared by ISO/TC 249, *Traditional Chinese medicine*.

Traditional Chinese medicine — *Schisandra chinensis* (Turcz.) Baill. seeds and seedlings

1 Scope

This document specifies minimum requirements and test methods for *Schisandra chinensis* (Turcz.) Baill. seeds and seedlings.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

International Seed Testing Association (ISTA):2016, *International Rules for Seed Testing*

International Seed Testing Association (ISTA), *Working Sheets on Tetrazolium Testing*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
lot
specified quantity of seeds or seedlings that are physically and uniquely identifiable

3.2
primary sample
portion taken from the *lot* (3.1) during one single sampling action

3.3
composite sample
formed by combining and mixing all the *primary samples* (3.2) taken from the *lot* (3.1)

3.4
test sample
portion of the *composite sample* (3.3) to which one of the test required in this document is applied

Note 1 to entry: The *test samples* (3.4) may be packed in different materials meeting conditions for specific tests [e.g. moisture or *purity* (3.6)].

3.5
sealed
condition whereby a container which contains seeds is closed in such a way that it cannot be opened to get access to the seed and be closed again without either destroying the seal or leaving evidence of tampering

Note 1 to entry: This definition refers to the sealing of seed lots, as well as of seed samples.

3.6
purity
weight of pure seed fraction over the total weight of the *test sample* (3.4) in per cent

Note 1 to entry: The pure seed refers to the species stated by the applicant, or found to predominate in the test, and includes all botanical varieties and cultivars of that species.

3.7
1 000-seed weight
average weight of every 1 000 pure seeds of a *test sample* (3.4)

3.8
seed width
largest distance from the side of raphe to its opposite, in millimetre

Note 1 to entry: See Figure 1.

3.9
viability
index to show the potential ability of seed to germinate, or the capability of embryo to live

Note 1 to entry: It is expressed as the percentage of stained seeds in the *test sample* (3.4).

Note 2 to entry: It is estimated by the method of Topographical Tetrazolium Test.

3.10
stratification rate
percentage of stratified seed, determined by number, in the *test sample* (3.4)

Note 1 to entry: Stratified seed is the seed with embryos fully grown, endosperm volume expanded, seed coat longitudinally fissured through the raphe by after-ripening treatment (see Figure 1).

3.11
stem diameter
boundary of aboveground and belowground of the seedling

Note 1 to entry: See Figure 2.

3.12
height of stem
length of the stem from the root to the top hibernaculum

Note 1 to entry: See Figure 2.

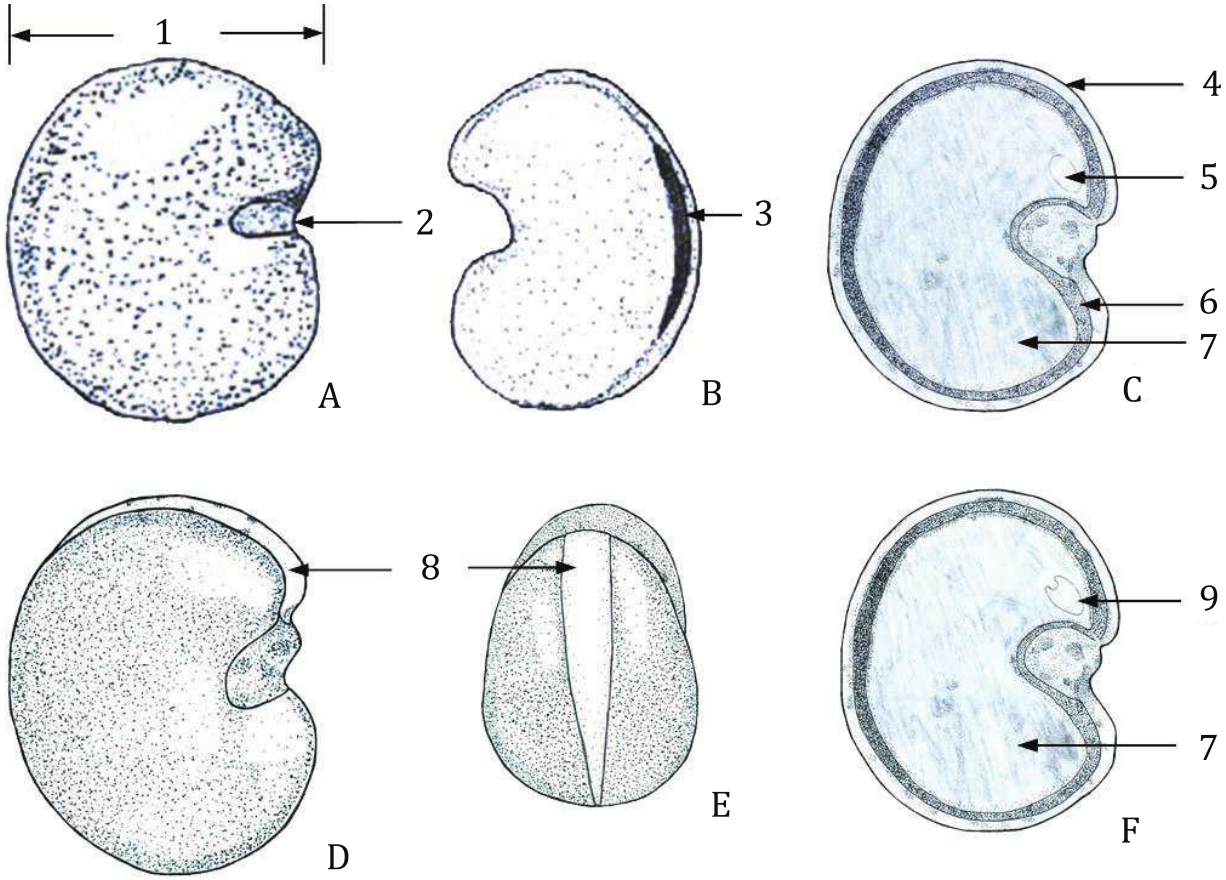
3.13
seedling weight
average weight of a single seedling

4 Description

4.1 *Schisandra chinensis* seeds

In this document, *Schisandra chinensis* seed is the dehydrated seed of *Schisandra chinensis* (Turcz.) Baill. consisting of three basic parts: embryo, endosperm and seed coat, as shown in Figure 1.

NOTE Annex A provides information on how to identify a *Schisandra chinensis* seed.



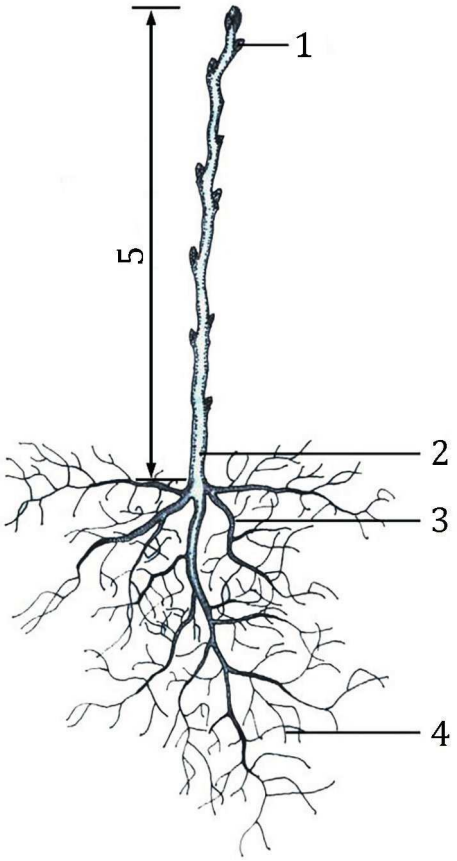
Key

1 seed width
2 hilum
3 raphe
4 seed coat
5 embryo
6 cavity of seed
7 endosperm
8 fissure of a stratified seed
9 embryo of a stratified seed
A seed
B kernel, without seed coat
C cross-section, longitudinally cut through the raphe
D front view of a stratified seed
E dorsal view of a stratified seed, shows the fissure
F section of the kernel of a stratified seed

Figure 1 — Structure of *Schisandra chinensis* seed

4.2 *Schisandra chinensis* seedlings

Schisandra chinensis seedlings consist of four parts: hibernaculum, stem, lateral root and fibrous root, as shown in Figure 2.



Key

1 hibernaculum
2 stem diameter
3 lateral root
4 fibrous root
5 height of stem

Figure 2 — Structure of *Schisandra chinensis* 1-year-old seedling

5 Requirements

5.1 General characteristics

The following requirements shall be achieved before separating the composite sample into test samples.

a) *Schisandra chinensis* seed shall be clean and free from foreign odours, with smooth surface and kidney shaped.

b) *Schisandra chinensis* seedling shall be healthy and intact.

c) Quarantine fungus (*Fusarium* spp. and *Alternaria* spp.) and nematodes shall not be detected.

5.2 *Schisandra chinensis* seed

5.2.1 The moisture content shall not be greater than 14 %.

5.2.2 Seed purity shall not be less than 95 %.

5.2.3 Viability shall not be less than 80 %.

5.2.4 The seed width, 1 000-seed weight and stratification rate shall comply with the requirements in Table 1.

Table 1 — Grading standards of *Schisandra chinensis* seed

Grade	Seed width mm	1 000-seed weight g	Stratification rate %
First	≥4,0	≥26,0	≥55,0
Second	3,6 to <4,0	20,0 to <26,0	30,0 to <55,0
Unqualified	<3,6	<20,0	<30,0
For the First grade, the seeds, of which the seed width is not less than 4,0 mm, shall not be less than 95 %. Otherwise, it shall be judged to be the Second grade. For the Second grade, the seeds, of which the seed width is not less than 3,6 mm, shall not be less than 95 %. Otherwise, it shall be judged to be the Unqualified grade. NOTE The establishment of the above requirements is based on the seeds collected from different regions.			

5.3 *Schisandra chinensis* seedling

5.3.1 Lateral root and fibrous root shall be intact.

5.3.2 Hibernaculum of *Schisandra chinensis* seedling shall be intact.

5.3.3 Stem diameter, height of stem, seedling weight and number of lateral roots shall comply with the requirements in Table 2.

Table 2 — Grading standards of 1-year-old *Schisandra chinensis* seedling

Grade	Stem diameter cm	Height of stem cm	Seedling weight g	Number of lateral roots
First	≥0,45	≥20,0	≥9,0	≥5,0
Second	0,30 to <0,45	15,0 to <20,0	4,0 to <9,0	3,0 to <5,0
Unqualified	<0,30	<15,0	<4,0	<3,0
For the First grade, the seedlings, of which the stem diameter is not less than 0,45 cm, shall not be less than 95 %. Otherwise, it shall be judged to be the Second grade. For the Second grade, the seedlings, of which the stem diameter is not less than 0,30 cm, shall not be less than 95 %. Otherwise, it shall be judged to be the Unqualified grade. NOTE The establishment of the above requirements is based on the seedlings collected from different regions.				

6 Sampling

6.1 Seed sampling

Sampling shall be carried out in accordance with ISTA, *International Rules for Seed Testing*:2016, Chapter 2. Maximum weight of lot and minimum weight of sample are specified in Table 3.

Table 3 — Maximum weight of seed lot and minimum weight of sample

Maximum weight of seed lot kg	Minimum weight of sample g		
	Composite sample	Test sample for purity analysis	Test sample for other test
2 000	600	100	200
NOTE The establishment of the above requirements is based on the seeds collected from different regions.			

6.2 Seedling sampling

The maximum seedling lot shall be 300 000 seedlings and the minimum composite samples shall be 100 seedlings.

7 Test methods

7.1 1 000-seed weight

Determination of the 1 000-seed weight shall be in accordance with weight determination method specified in ISTA, *International Rules for Seed Testing*.

7.2 Purity

Determination of the seed purity shall be in accordance with the purity analysis of ISTA, *International Rules for Seed Testing*.

7.3 Seed width

Take 100 to 150 intact seeds in duplicate from the composite sample by quartering method. The seed width shall be measured with a vernier calliper in millimetres (to two decimal places). If the difference between measured values of the duplicate samples is less than 5 %, the seed width of the seed lot shall be the arithmetic mean of the two measured values; otherwise, it shall be re-measured.

7.4 Seed viability

Determination of the seed viability shall be in accordance with ISTA, *International Rules for Seed Testing* and ISTA, *Working Sheets on Tetrazolium Testing*.

7.5 Stratification rate

Take duplicate samples of after-ripening treated seeds by quartering method, each containing 100 to 150 seeds. Calculate the number of seeds with seed coat longitudinally fissured through the raphe by stratification. Formula (1) shall be used to express the stratification rate of a seed lot.

$$M(\%) = \frac{A_{\mathrm{m}}}{A} \times 100 \tag{1}$$

where

M is stratification rate (%);

A_m is the number of stratified seeds;

A is the number of seeds in the test sample.

If the difference between measured maturities of the duplicate samples is less than 5 %, the maturity of the seed lot shall be the arithmetic mean of two measured values; otherwise, it shall be re-estimated.

7.6 Moisture content

Determination of the mass fraction of moisture shall be in accordance with the high constant temperature method specified in ISTA, *International Rules for Seed Testing.*

7.7 Fungus testing

7.7.1 Preparation of seed

Take 100 seeds into a sterilized flask and add 20 ml sterilized water and shake for 8 min. Centrifuge the suspension at 4 000 r/min for 20 min. Re-suspend the residue with 2 ml of sterilized water. Introduce five spots, each spot of 100 µl of the final suspension on the blotter or filter paper in a plate for incubation.

7.7.2 Preparation of seedling

Take 500 ml sterilized water into a sterilized flask. Take 10 to 20 seedlings. Immerse the seedlings into the flask and shake for 8 min one by one. Centrifuge the suspension at 4 000 r/min for 20 min. Re-suspend the residue with 2 ml of sterilized water. Introduce five spots, each spot of 100 µl of the final suspension on the blotter or filter paper in a plate for incubation.

7.7.3 Test of *Alternaria* spp.

Alternaria spp. testing shall be in accordance with ISTA, *International Rules for Seed Testing*:2016, method 7-001a.

7.7.4 Test of *Fusarium* spp.

Fusarium spp. testing shall be in accordance with ISTA, *International Rules for Seed Testing*:2016, method 7-009.

7.8 Nematode testing

Take 100 to 150 seedlings. Inspect nematode knot or lesion with the naked eye. Check the root knot or root lesion nematode for nematode under microscope.

7.9 Stem diameter

Take triplicate samples randomly, each containing 20 to 30 seedlings. Using the vernier calliper, measure the diameter 1 cm above the roots.

The percentage of seedlings not meeting the minimum weight requirement of the grade shall not be more than 5 %. Otherwise, it shall be judged to be of inferior grade and be tested accordingly.

7.10 Height of stem

Take triplicate samples randomly, each containing 20 to 30 seedlings. Measure the length of completely mature stems from root base to the top of the hibernaculum.

7.11 Seedling weight

Take triplicate samples randomly, each containing 20 to 30 seedlings. Measure the average weight of single seedlings.

7.12 Number of lateral roots

Take triplicate samples randomly, each containing 20 to 30 seedlings. Count the number of roots per seedling and find the arithmetic mean.

8 Test report

For each test method, the test report shall specify the following:

a) all information necessary for the complete identification of the sample, including the production sites and origins of the seed;

b) the sampling method used;

c) the test method used;

d) the test result(s) obtained;

e) all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

f) any unusual features (anomalies) observed during the test;

g) the date of the test.

9 Packaging, storage and transportation

The packaging shall be strong enough to withstand normal handling, storage and transportation. The packaging shall not transmit any odour or flavour to the product and shall not contain substances which may damage the product or constitute a health risk.

The temperature of seed storage should not be higher than 15 °C and the relative humidity should not be higher than 65 %.

The temperature of dormant seedling storage should be suitable for inhibition of seedling germination.

The roots of seedlings should be in humid condition and be prevented from loss of water during long distance delivery.

10 Marking

The following items shall be marked or labelled on the packages.

a) Grade of the product in accordance with 5.2 and 5.3.

b) All quality features, indicated in 5.2 and 5.3, determined in accordance with methods specified in Clause 7.

c) The maximum weight of lot and that of samples specified in Table 3.

d) Country of origin and province/state of the seed.

e) Expiry date of the seed.

f) Items required by regulatory body of destination country.

Annex A
(informative)

Identification of the *Schisandra chinensis* seed

A.1 Morphology identification

There is significantly U-shape hilum in the *Schisandra chinensis* seed, and the seed has reniform shape, yellowish-brown and lustrous seed coat.

NOTE The seed of *Schisandra sphenanthera* is in long elliptic shape, with brown seed coat, and the hilum of *Schisandra sphenanthera* is in oblique V-shape.

A.2 Chemical identification by thin-layer chromatography (TLC)

A.2.1 Test solution

Take 1,0 g pulverized *Schisandra chinensis* seed into a round bottom flask. Add 20 ml of methanol and reflux in a water bath for 30 min. Evaporate the solution to dryness and re-dissolve the residue with 1 ml of methanol.

A.2.2 Reference solution

Dissolve approximately 1 mg of schisandrin and 1 mg of γ-schisandrin in 5 ml of methanol.

A.2.3 Procedure

Spot 2 µl of the extract and the reference on a TLC plate of silica gel (F_{254}). Develop the plate with a mixture of toluene and ethyl acetate in the ratio of 7:3, to a distance of about 7 cm and air-dry the plate. Visualize the plate by using ultraviolet light at 254 nm (Detection A) or chemical process using dilute sulfur acid to the plate and heating at 105 °C for 2 min (Detection B).

A.2.4 Reporting results

The results of *Schisandra chinensis* seed identification using the Detection A method should be reported as Table A.1:

Table A.1 — Report form of Detection A method

Top of the plate	
γ-schisandrin: a quenching zone	A quenching zone (γ-schisandrin)
	A weak quenching zone
Schisandrin: a quenching zone	A quenching zone (schisandrin)
Reference solution	**Test solution**

Results of Detection B method should be reported as Table A.2:

Table A.2 — Report form of Detection B method

Top of the plate	
γ-schisandrin: a brown zone	A brown zone (γ-schisandrin)
	A faint zone
Schisandrin: an intense, brownish-green zone	An intense, brownish-green zone (schisandrin)
Reference solution	**Test solution**

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