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**Traditional Chinese medicine —  
Fermented *Cordyceps* powder**

*Médecine traditionnelle chinoise — Poudre de Cordyceps fermenté*

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ISO 4754:2022

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Published in Switzerland

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

*Cordyceps sinensis*, known as “冬虫夏草”, Chinese Pinyin *Dong Chong Xia Cao* (winter worm, summer plant), is a rare raw material used in traditional Chinese medicine. *Cordyceps sinensis* enjoys equal popularity with ginseng and velvet and ranks first among these three tonic medicines. The use of *Cordyceps sinensis* can be traced to AD 863, during the Tang Dynasty, in the Youyang Essays by Duan Chengshi. In recent years, the efficacy of *Cordyceps sinensis* has been further confirmed and its extensive and significant efficacy is acknowledged worldwide.

The bioactivities of *Cordyceps sinensis* include immunoregulation, anti-bacteria, anti-cancer, anti-oxidation, anti-aging, blood sugar control and fat reduction. Due to the specific environment required for the growth of *Cordyceps sinensis*, the resources of wild *Cordyceps sinensis* are limited. Increasing market demand has therefore resulted in high prices. Driven by increased interest, wild *Cordyceps sinensis* is facing extinction due to plunder digging, which impacts its natural ecological environment. The culture of *Cordyceps sinensis* has become a hot topic of research, with a focus on liquid fermentation technology. Fermented *Cordyceps* powder is manufactured with strain extracted from wild *Cordyceps sinensis* by low-temperature liquid fermentation, simulating the growth environment of wild *Cordyceps sinensis*. Quality control for fermented *Cordyceps* powder is complicated. There are many functional active substances in fermented *Cordyceps* powder, including polysaccharide compounds, alkaloids (Cordycepin), peptide compounds, sterols, terpenoids and other secondary metabolites. The content of these substances in fermented *Cordyceps sinensis* determines to a large extent the quality and efficacy of fermented *Cordyceps powder*. Traditional analytical methods vary and depend highly on experience. It is difficult to determine the quality of fermented *Cordyceps* powder with existing methods.

A guarantee of the quality of fermented *Cordyceps* powder is crucial to ensure the quality and safety of medicines and health products made from fermented *Cordyceps* powder. Exploring quality control methods of fermented *Cordyceps* powder and creating a standard combining traditional Chinese medicine characteristics and current technology will have profound influence on the industry of fermented *Cordyceps* powder.

As national implementation can differ, national standards bodies are invited to modify the values given in [4.5](#), [4.8](#) and [4.9](#). Reference values of national and regional limits of heavy metals, microbiological examination and aflatoxins are given in [Annex E](#).



# Traditional Chinese medicine — Fermented *Cordyceps* powder

## 1 Scope

This document specifies a set of requirements and a test method to control the quality of fermented *Cordyceps* powder, including test items such as identification, assay, water content, residue on ignition, microbial limit and heavy metals.

It is applicable to fermented *Cordyceps* powder which is produced by liquid fermentation with extracted strain of *Ophiocordyceps sinensis* (Berk.).

## 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.

ISO 1762, *Paper, board, pulps and cellulose nanomaterials — Determination of residue (ash content) on ignition at 525 °C*

ISO 6673, *Green coffee — Determination of loss in mass at 105 degrees C*

ISO 13903:2005, *Animal feeding stuffs — Determination of amino acids content*

ISO 18664, *Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine*

ISO 22283, *Traditional Chinese medicine — Determination of aflatoxins in natural products by LC-FLD*

ISO 22467, *Traditional Chinese medicine — Determination of microorganisms in natural products*

## 3 Terms and definitions

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

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

### 3.1

#### ***Ophiocordyceps sinensis* (Berk.)**

sac fungi parasite in bat moth larvae

### 3.2

#### **fermented *Cordyceps* powder**

dried powder of mycelium obtained by liquid fermentation of *Ophiocordyceps sinensis* (Berk.)

## 4 Recommendations and requirements

### 4.1 Characteristics

The powder should be grey to greyish yellow, slightly fishy and salty.

### 4.2 Thin-layer chromatography (TLC) identification

The test solution shall present the spots with the same position and colour as that of the reference powder solution and reference solution under sunlight and ultraviolet light.

### 4.3 High-performance liquid chromatography (HPLC) identification

The retention times of six peaks obtained with the test solution and the reference powder solution should be concordant with each other. The retention time of adenosine and uridine in the test solution should correspond to that of the reference solutions of adenosine and uridine, respectively.

### 4.4 Amino acid analyser identification

The retention time of the peaks in the test solution should be concordant with that of tyrosine, lysine, histidine and arginine in the reference solution.

### 4.5 Heavy metals

The contents of heavy metals such as arsenic, lead, cadmium and mercury shall be determined.

### 4.6 Loss on drying

Loss on drying should not be more than 6,0 %. [ISO 4754:2022](https://standards.iteh.ai/catalog/standards/sist/3208b8ba-fc1d-4a9a-9aa8-e5a66a1ff3fb/iso-4754-2022)

### 4.7 Residue on ignition

Residue on ignition should not be more than 7,0 %.

### 4.8 Microbial limit

Total aerobic microbial count (TAMC), total combined yeast and mould count (TYMC) and *Escherichia coli* (*E. coli*) shall be determined.

### 4.9 Aflatoxins

The contents of aflatoxins such as aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and the sum of Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), Aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), Aflatoxin G<sub>1</sub> (AFG<sub>1</sub>) and Aflatoxin G<sub>2</sub> (AFG<sub>2</sub>) shall be determined.

### 4.10 Content of total amino acids

Total amino acids should not be less than 30,0 %.

### 4.11 Content of mannitol

Mannitol (C<sub>6</sub>H<sub>14</sub>O<sub>6</sub>) should not be less than 7,0 %.

### 4.12 Content of adenosine

Adenosine (C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>) should not be less than 0,08 %.



## 5 Test method

### 5.1 Characteristics

Take an appropriate amount of the sample on clean white paper, observe at a distance of 25 cm to 30 cm under adequate lighting, smell and taste.

### 5.2 TLC identification

See [Annex A](#) for additional information.

### 5.3 HPLC identification

See [Annex B](#) for additional information.

If TLC and HPLC identification are not sufficient to distinguish similar products, the reference test method specified in [Annex C](#) should be applied.

### 5.4 Amino acid analyser identification

The testing method specified in ISO 13903 shall be applied.

### 5.5 Determination of heavy metals

The testing method specified in ISO 18664 shall be applied.

### 5.6 Determination of loss on drying

The testing method specified in ISO 6673 shall be applied.

### 5.7 Determination of residue on ignition

The testing method specified in ISO 1762 shall be applied.

### 5.8 Microorganism examination

The testing method specified in ISO 22467 shall be applied.

### 5.9 Determination of aflatoxin

The testing method specified in ISO 22283 shall be applied.

### 5.10 Determination of total amino acids

The testing method of total amino acids specified in ISO 13903:2005, 2.2 shall be applied.

### 5.11 Determination of mannitol

Accurately weigh 1 g of the sample into a 150-ml round-bottomed flask, add precisely 100 ml of ethanol, weigh, heat under reflux for 2h, cool, replenish the weight loss with ethanol, mix well and filter. Measure 5 ml of successive filtrate into an iodine flask, add 50 ml of sodium (potassium) periodate solution, heat in a water bath for 15 min, cool, add 10 ml of potassium iodide TS, stopper tightly and allow to stand for 5 min. Titrate with 0,05 mol/l sodium thiosulfate VS, towards the end of titration add starch IS and continue to titrate until the blue colour disappears. 1 ml of 0,05 mol/l sodium thiosulfate is equivalent to 0,910 9 mg of mannitol ( $C_6H_{14}O_6$ ).

## 5.12 Determination of adenosine

See [Annex D](#) for additional information.

## 6 Test report

The test report shall include the following:

- a) the sample;
- b) a reference to this document, i.e. ISO 4754:2022;
- c) the method used;
- d) the result(s), including a reference to the clause which explains how the results were calculated;
- e) any deviations from the procedure or unusual features observed during the test;
- f) the date of the test.

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## Annex A (informative)

### Test method for identification by thin layer chromatography (TLC)

#### A.1 Preparation of test solution

Take 0,5 g of the sample, add 10 ml of methanol, ultrasonicate for 1 h, filter and use the filtrate as the test solution.

#### A.2 Preparation of reference powder solution

Take 0,5 g of fermented *Cordyceps* reference powder, add 10 ml of methanol, ultrasonicate treat for 1 h, filter and use the filtrate as the reference powder solution.

#### A.3 Preparation of reference solution

Dissolve ergosterol chemical reference substance (CRS) in methanol to produce a solution containing 0,4 mg per ml as the reference solution.

#### A.4 Identification by TLC

Use silica gel G as the coating substance and a mixture of petroleum ether (60 °C to 90 °C), ethyl acetate and formic acid in the ratio of 5:1:0,1 (volume fraction) as the mobile phase. Apply separately 10 µl of each of the three solutions to the plate. After developing and removal of the plate, dry in air. Spray with 10 % solution of sulphuric acid in ethanol, heat at 105 °C until the spots clear and observe under sunlight and ultraviolet light (365 nm). A typical TLC chromatogram is shown in [Figure A.1](#).