
**Traditional Chinese medicine —
Quality and safety of raw materials
and finished products made with raw
materials —**

**Part 3:
Testing for contaminants**

*Médecine traditionnelle chinoise — Qualité et sécurité des matières
premières et des produits finis fabriqués à partir de matières
premières —*

Partie 3: Essais pour la recherche de contaminants



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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).

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).

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.

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

A list of all parts in the ISO 19609 series can be found on the ISO website.

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.

Introduction

The ISO 19609 series consists of four parts with different content, as shown in [Figure 1](#).

ISO 19609			
Part 1	Part 2	Part 3	Part 4
General	Identity	Testing for contaminants	Absence of unwanted compounds
Overview	Organoleptic	Microorganisms	Preservatives
Physical parameters	Sample preparation for chromatography	Aflatoxins	Radiation
	HPLC	Heavy metals	Toxic compounds
	TLC	Pesticides	

Figure 1 — Overview of the ISO 19609 series

To ensure the safety of herbal medicinal products, all raw materials and products must be analysed for possible contamination. In this field four main types of contaminants are controlled so that their absence or presence is lower than the relevant limit values defined by different target markets or nations.

Specific documents for heavy metals, pesticides, aflatoxins and microorganisms have been developed within the framework of ISO/TC 249.

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Traditional Chinese medicine — Quality and safety of raw materials and finished products made with raw materials —

Part 3: Testing for contaminants

1 Scope

This document specifies the sample preparation methods for the determination of contamination of natural products by heavy metals, pesticides, aflatoxins and microorganisms. It is applicable to raw materials and finished products used in traditional Chinese medicine.

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

ISO 19609-1:2021, *Traditional Chinese medicine — Quality and safety of raw materials and finished products made with raw materials — Part 1: General requirements*

ISO 22258, *Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography*

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

aflatoxin

naturally occurring poisonous, carcinogenic and mutagenic mycotoxin, produced by certain fungi (mainly *Aspergillus* species), commonly found on agricultural crops

3.2

compactate

irregular-shaped agglomerate obtained directly from the dried extract-by-compaction

[SOURCE: ISO 23419: 2021, 3.4, modified — Note to entry removed.]

3.3

heavy metal

metal with relatively high density as a natural constituent of the environment, commonly detected in air, water and soil

3.4

microbial contamination

presence of unintended bacteria, fungi, protozoa or viruses

Note 1 to entry: This can include extrinsic and/or intrinsic contamination.

[SOURCE: ISO 18362:2016, 3.17]

3.5

microorganism

entity of microscopic size, encompassing bacteria, fungi, protozoa and viruses

[SOURCE: ISO 11139:2018, 3.176]

3.6

pesticide

substance or mixture of substances intended to prevent, destroy, repel or mitigate any pest or weed

Note 1 to entry: Pesticides (plant protection products) approved for use in one country will not necessarily be approved in another country.

[SOURCE: ISO 18889:2019, 3.7, modified — Note to entry revised.]

4 Testing of contaminants

4.1 Determination of heavy metals in herbal medicines and products used in and as traditional Chinese medicine

4.1.1 General

The determination of heavy metals shall be done in accordance with ISO 18664.

4.1.2 Sample preparation

4.1.2.1 General

The quality and reliability of analytical results depend on both the analytical method and the sample preparation of the test sample. If a sample preparation is not appropriate, the analytical results can be slightly incorrect or completely wrong. Sample preparation methods depend on the constitution of the testing material. Typical established sample preparation techniques are listed in the following subclauses.

4.1.2.2 Sample size for the determination of heavy metals

Based on experiences with pharmaceuticals worldwide, and especially herbal-based phytopharmaceuticals, a typical amount of testing material is required. An appropriate sample size should be 250 mg of dry or dried material or equivalent.

4.1.2.3 Solid samples

The preparation of test samples shall be carried out in accordance with ISO 19609-1:2021, 6.2.

The typical size of test material should be under 1 mm in diameter. Different starting test materials can include the following:

- a) crude materials (sliced or uncut, fresh or dried);
- b) tablets, pills, dragees, capsules;
- c) granules, compactates and powders;
- d) other solid samples.

Crude materials, tablets, dragees and capsules shall be ground and sieved in accordance with ISO 19609-1.

Granules, compactates and powders, as well as other solid samples, shall be ground only if necessary and sieved in accordance with ISO 19609-1.

4.1.2.4 Semi-solid samples

Semi-solids are often emulsions of fatty constituents with waterish extracts or gelatinous gels based on polysugars such as alginates.

Creams, ointments, lotions and other semi-solid samples shall be homogenized in an appropriate way.

The dry mass as a percentage of sample material shall be estimated.

4.1.2.5 Liquid samples

Extracts, tinctures, juices and other liquid samples shall be homogenized in an appropriate way.

The dry mass as a percentage of sample material shall be estimated.

4.1.3 Liberation or release of heavy metals with microwave digestion

4.1.3.1 General

A typical internationally recognized method for the release of heavy metals is microwave digestion.

4.1.3.2 Reagents

4.1.3.2.1 Distilled water (for trace analysis).

4.1.3.2.2 HNO₃ (conc.) (for trace analysis).

4.1.3.2.3 H₂O₂ (30 %) (for trace analysis).

4.1.3.3 Preparation method (microwave digestion) and materials

High-pressure PTFE vessels (microwave suitable) shall be used for microwave digestion.

The microwave apparatus should have an energy level of at least 600 W.

For the solubilization of heavy metals, reagents such as HNO₃ (conc.), H₂O₂ (30 %) and distilled water in an appropriate quality (for trace analysis) are required.

4.1.3.4 Microwave digestion process

4.1.3.4.1 General

A typical extraction of heavy metals can be done with or without preparation of residual ash in a process of oxidative acidic extraction.

Heavy metals in herbal materials and related products are not surface contaminants and are often associated with other constituents of the products. Therefore, an appropriate extraction process is required.

4.1.3.4.2 Sample preparation procedure

The procedure of extraction depends on the relative content of carbohydrates. Two established methods are described in this subclause. The user shall decide the appropriate method for the specific testing material.

- a) The sample to be analysed (250 mg or equivalent) should be added to a suitable vessel (5 ml volume).
- b) The appropriate extraction solution, A or B, should be added carefully.
- c) Close the vessel so it is airtight.
- d) Treat the vessel under the described conditions.
- e) Heat with a 600 W microwave for about 1 min.
- f) Allow to cool down to room temperature.
- g) Open the vessel carefully to reduce pressure.
- h) Treat again in a 600 W microwave for about 5 min.
- i) Allow to cool down to room temperature.
- j) Filter the solution using a suitable filter (hydrophilic paper filter).
- k) Transfer the sample into a suitable volumetric flask.
- l) Fill up the flask to 10 ml with distilled water.

For samples with low content of carbohydrates, add extraction solution A, consisting of 3 ml of HNO_3 (conc.) and 1 ml of H_2O_2 (30 %), following step b) of the sample preparation procedure.

For samples with higher content of carbohydrates, add extraction solution B, consisting of a mixture of 1,2 ml of HNO_3 (conc.), 0,4 ml of H_2O_2 (30 %) and 2,5 ml of distilled water, following step b) of the sample preparation procedure.

NOTE If the extraction solution A is applied to samples with higher contents of carbohydrates, an explosion can occur under microwave irradiation.

4.1.4 Sample solution

The resulting solution of the procedure described in [4.1.3](#) is the sample solution for the measurement according to [4.1.5](#).

4.1.5 Measurement of heavy metals

The measurement of heavy metals in herbal materials and related products should be done with the analytical procedure described in ISO 18664.

4.1.6 Method validity and reporting

Based on differences in raw materials or products and the matrix effects of complex mixtures (herbal-based materials), the validity of the measurement should be demonstrated for each testing material.

The validation procedures and parameters should be reported in a validation report according to Reference [7].

4.2 Determination of pesticides in herbal medicines and products used in and as traditional Chinese medicine

4.2.1 General

The determination of pesticides shall be done in accordance with ISO 22258.

Additionally, the following process for sample preparation should be followed.

4.2.2 Sample preparation

4.2.2.1 General

Pesticides occur frequently in herbal materials and related products, unless these are produced organically. These pesticides are often incorporated in the total herbal material and also occur in the products.

4.2.2.2 Sampling

Three representative samples equal in volume shall be taken from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take an appropriate amount from the mixture.

The samples shall be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples shall be stored in airtight containers at a temperature below 0 °C, protected from light.

The procedure for sample preparation shall be as described in [4.1.2.2](#) to [4.1.2.4](#).

4.2.3 Extraction of pesticides

4.2.3.1 General

Pesticides are not the only contaminants on the surface of herbal material, so an appropriate extraction process is required before measurement.

4.2.3.2 Reagents

Reagents of appropriate quality for the analysis of contaminants should be without residues of the analytes tested.

4.2.3.2.1 Acetone.

4.2.3.2.2 Toluene.

4.2.4 Extraction method

The following procedure for extraction of pesticides shall be followed:

- a) To 10 g of the milled sample add 100 ml of acetone (free from any contaminants).
- b) Allow to stand for 20 min.
- c) Optional: add suitable amounts of internal standards.
- d) Homogenize using a high-speed blender for 3 min.
- e) Filter and wash the filter cake with two quantities, each of 25 ml of acetone.
- f) Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40 °C until the solvent has almost completely evaporated.
- g) To the residue add a few millilitres of toluene (free from any contaminants) and heat again until the acetone is completely removed.
- h) Dissolve the residue in 8 ml of toluene.
- i) Filter through a filter membrane (0,45 µm).
- j) Rinse the flask and the filter with toluene and dilute to 10,0 ml with the same solvent.

4.2.5 Sample solution

The solution obtained in 4.2. shall be used as the sample solution for further analysis.

4.2.6 Measurement of pesticides

The measurement of pesticides in herbal materials and related products shall be done in accordance with ISO 22258.

4.2.7 Method validity and reporting

Based on differences in raw materials or products and the matrix effects of complex mixtures (herbal-based materials), the validity of the measurement should be demonstrated for each testing material.

The validation procedures and parameters should be reported in a validation report according to Reference [7].

4.3 Determination of aflatoxins in herbal medicines and products used in and as traditional Chinese medicine

4.3.1 General

The determination of aflatoxins should be done in accordance with ISO 22283.

Additionally, the following process for sample preparation should be followed.

NOTE Aflatoxins are naturally occurring mycotoxins produced mainly by the aspergillus species. Favourable conditions for the production of aflatoxins are high moisture content, high temperature and a carbon source such as herbal materials.

At least 13 types of aflatoxins are produced in nature. Aflatoxin B1 is considered to be the most toxic.

4.3.2 Sample preparation

4.3.2.1 General

Aflatoxins occur frequently in herbal materials and related products in root or bulbous material as well as fruits and nuts. A high content of fats or fatty oils increases the risk of contamination, for example nuts show a high contamination risk if stored at a high humidity.

4.3.2.2 Sampling

A minimum of two representative samples equal in volume shall be taken out of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an appropriate amount.

The samples shall be analysed immediately to avoid possible degradation of the residues. If this is not possible the samples shall be stored in airtight containers at a temperature below 0 °C, protected from light.

The procedure of sample preparation shall be as described in [4.1.2.2](#) to [4.1.2.4](#).

4.3.3 Extraction of aflatoxins

4.3.3.1 General

Aflatoxins are not the only contaminants on the surface of herbal material. In some cases, fungi grow within herbal organs and produce contaminants, leading to related products also containing these contaminants. Washing processes cannot reduce the contamination of aflatoxins.

WARNING — Aflatoxins are toxic in very small amounts.

NOTE Direct measurement without a concentration process is not possible.

4.3.3.2 Reagents

4.3.3.2.1 Distilled water

4.3.3.2.2 Methanol for analysis (p.a.)

4.3.3.3 Extraction method

The following procedure for extraction of aflatoxins shall be followed:

- a) Use 5 g of the samples.
- b) Add 100 ml of 70 % volume fraction aqueous methanol.
- c) Sonicate the mixture for 30 min.
- d) Filter the mixture through a folded filter paper.
- e) Pipette 10 ml of the filtrate and add 70 ml of water.

For semi-solid materials, an appropriate defatting method shall be applied.

For liquid samples, the water content should be considered and appropriate amounts of methanol should be added.

The amount of semi-solid and liquid samples should be an equivalent of 5 g dry mass.

Other appropriate and valid methods can be used.

4.3.4 Sample solution

The solution in [4.3.3.3](#) can be used as sample solution for enrichment on affinity columns before further analysis.

4.3.5 Measurement of aflatoxins

The enrichment and measurement of aflatoxins in herbal materials and related products shall be done in accordance with ISO 22283.

4.3.6 Method validity and reporting

Based on differences in raw materials or products and the matrix effects of complex mixtures (herbal-based materials), the validity of the measurement should be demonstrated for each testing material.

The validation procedures and parameters should be reported in a validation report according to Reference [7].

4.4 Determination of microbial contamination in herbal medicines and products used in and as traditional Chinese medicine

4.4.1 General

The determination of microbial contamination shall be done in accordance with ISO 22467.

Additionally, the following process for sample preparation should be followed.

NOTE 1 Microorganisms can cause a great number of problems in humans. Natural materials and related products are typically contaminated with microorganisms during growing and harvesting. Washing procedures can reduce the amount of microorganisms, which typically occur only on the surface of the herbal raw materials. Additionally, specific decontamination processes according to national regulations can be used for the reduction of microbial contamination.

NOTE 2 The presence of certain microorganisms in non-sterile preparations has the potential to reduce or even deactivate the therapeutic activity of the product and can have adverse effects on the health of patients.

NOTE 3 Different methods of reduction of microbial contamination are used worldwide. In some countries, certain methods, such as the use of sulfur dioxide or other sulfur derivatives, as well as ethylene oxide or irradiation, are not permitted. Details are given in ISO 19609-4.¹⁾

4.4.2 Sample preparation

4.4.2.1 General

Microorganisms often occur in herbal materials and related products. A concentration process is not necessary. Microbial limits are defined nationally.

4.4.2.2 Sampling

A minimum of three representative samples equal in volume shall be taken out of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an appropriate amount.

The samples shall be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples shall be stored in airtight containers at a temperature between 4 °C and 8 °C, protected from light.

1) Under preparation. Stage at the time of publication: ISO/FDIS 19609-4:2022.