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

Part 4:

**Testing for preservatives and
unwanted compounds**

*Médecine traditionnelle chinoise — Qualité et sécurité des matières
premières et des produits finis fabriqués à partir de matières
premières — 9-4:2022*

Partie 4: Essais des conservateurs et composés indésirables



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Contents

| | Page |
|--|-----------|
| Foreword..... | iv |
| Introduction..... | v |
| 1 Scope..... | 1 |
| 2 Normative references..... | 1 |
| 3 Terms and definitions..... | 1 |
| 4 Testing for the absence of unwanted compounds and radiation..... | 2 |
| 4.1 General..... | 2 |
| 4.2 Overview..... | 2 |
| 4.3 Determination of preservatives..... | 2 |
| 4.3.1 General..... | 2 |
| 4.3.2 Determination of preservatives..... | 2 |
| 4.3.3 Declaration..... | 3 |
| 4.3.4 Analysis of sulfur and sulfur derivatives..... | 3 |
| 4.3.5 Analysis of ethylene oxide..... | 5 |
| 4.4 Determination of irradiated material..... | 6 |
| 4.4.1 General..... | 6 |
| 4.4.2 Analysis of irradiated traditional Chinese medicine using photostimulated luminescence..... | 6 |
| 4.5 Determination of toxic compounds..... | 7 |
| 4.5.1 General..... | 7 |
| 4.5.2 Natural toxins..... | 7 |
| 4.5.3 Toxins resulting from degradation reactions..... | 13 |
| Annex A (informative) Materia medica..... | 14 |
| Annex B (informative) Examples of typical chromatograms of <i>Ephedra herba</i>, <i>Strychni semen</i>, <i>Sophorae tonkinensis radix et rhizoma</i> and their toxic compounds..... | 15 |
| Bibliography..... | 22 |

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 different parts with different content as shown in [Figure 1](#).

| | | | |
|---------------------|---------------------------------------|-------------------------|-------------------------------|
| ISO 19609 | | | |
| Part 1 | Part 2 | Part 3 | Part 4 |
| General | Identity | Absence of 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

[ISO 19609-4:2022](#)

<https://standards.iteh.ai/catalog/standards/sist/724dbcedd-a6a4-41b3-bb35-5c01492f907b/iso-19609-4-2022>

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

Part 4: Testing for preservatives and unwanted compounds

1 Scope

This document specifies the testing of preservatives and unwanted compounds within a quality control framework for starting materials and finished products used in and as 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 22256, *Traditional Chinese medicine — Detection of irradiated natural products by photostimulated luminescence*

ISO 22590, *Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration*

ISO 23190, *Traditional Chinese medicine — Determination of aristolochic acids in natural products by high-performance liquid chromatography (HPLC)*

ISO 23956, *Traditional Chinese medicine — Determination of benzopyrene in processed natural products*

ISO 23962, *Traditional Chinese medicine — Processed *Aconitum carmichaelii* lateral root*

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

unwanted compound

Constituent of a product which is unsuitable or unsafe for the intended use of that product.

Note 1 to entry: Such compounds can be those added for preservation or which result from a degradation process. Toxic natural compounds can also be considered unwanted compounds.

3.2

preservative

component intended to prevent the growth of microorganisms in or on a product

[SOURCE: ISO 18369-1:2017, 3.1.11.7]

3.3

excipient

material that is present in a therapeutic product administered to a patient, other than the active substance(s)

[SOURCE: ISO/TS 20399-1:2018, 3.7, modified — Definition revised, example and note to entry removed.]

4 Testing for the absence of unwanted compounds and radiation

4.1 General

For the risk assessment of traditional Chinese medicine products, the presence and/or the amount of unwanted compounds shall be estimated.

4.2 Overview

These unwanted compounds and radiation can be categorized in three main groups:

- a) preservatives;
- b) radiation;
- c) toxic compounds:
 - natural toxins;
 - toxins resulting from degradation reactions;
- d) other additives.

4.3 Determination of preservatives

4.3.1 General

Preservatives are pharmaceutical excipients added to therapeutic products to extend their shelf life and prevent contamination with microorganisms such as bacteria and fungi. Their possible adverse effects include allergic reactions and irritation.

4.3.2 Determination of preservatives

4.3.2.1 General

The determination and quantification should be done by specific and valid analytical procedures.

Preservatives in traditional Chinese medicine products are listed on the product ingredient list.

4.3.2.2 Sulfur and sulfur derivatives

Sulfur and sulfur derivatives include elemental sulfur and sulfites such as potassium sulfide and sodium sulfite, as well as sulfur dioxide and other sulfur-containing additives.

NOTE Sulfur dioxide or sulfite formed in aqueous solution has a preserving effect by irretrievably inhibiting metabolism and damage to the cell membranes from microorganisms as well as destroying the secondary and tertiary structure of microbial enzymes.

4.3.2.3 Ethylene oxide

Ethylene oxide gas kills bacteria, viruses and fungi, so it can be used for fumigation of heat-sensitive substances. Sterilization with ethylene oxide is a widespread process in the industrial manufacture of medical products, especially disposable products such as dressings, sutures or syringes and catheters.

NOTE The use of ethylene oxide in pharmaceuticals has been banned in the territory of the European pharmacopoeia since 1981 because it can produce toxic 2-chloroethanol.

4.3.3 Declaration

Preservatives shall be declared in all traditional Chinese medicine products and starting materials on the product label or in related documents such as a Certificate of Analysis (CoA).

4.3.4 Analysis of sulfur and sulfur derivatives

4.3.4.1 General

Based on the chemical inhomogeneity of the variety of preservatives, a few specific valid analytical methods shall be implemented.

NOTE Sulfur dioxide is a toxic gas and reacts immediately with water to sulfurous acid.

4.3.4.2 Elemental sulfur

NOTE Elemental sulfur is used as a protecting compound on herbal surfaces.

4.3.4.2.1 Sample preparation

In the case of declaration on the product documents or suspicion based on yellow powder on the surfaces, an analytical measurement shall be used.

An appropriate amount of test sample of minimum 50 g shall be used and washed with cold water.

The resulting suspension shall be separated from the test material and then filtered over an appropriate typical laboratory folded filter.

The resulting residue shall be dried.

4.3.4.2.2 Reagents

Reagents are not needed.

4.3.4.2.3 Apparatus

Test tube or melting pot: bunsen burner or other appropriate heating source with a minimum temperature of about 150 °C.

4.3.4.2.4 Analytical instrumentation

None.

4.3.4.2.5 Analytical procedure

The dried test residue shall be heated with air contact until melting under appropriate conditions.

4.3.4.2.6 Measurement and reporting

If the test sample contains elemental sulfur or other sulfur derivatives in the reaction with air, the colourless gas SO₂ with a characteristic sticky odour appears.

Other appropriate methods can be used.

4.3.4.3 Quantification of sulfur derivatives as sulfur dioxide

ISO 22590 shall be applied for the quantification of sulfur derivatives as sulfur dioxide.

Other appropriate valid methods can be used alongside ISO 22590.

4.3.4.4 Qualitative quick test for sulfur dioxide

4.3.4.4.1 General

For easy screening it is also possible to use a quick test with a lead acetate paper.

4.3.4.4.2 Sample preparation

The herbal test material shall be milled to < 1 mm diameter.

4.3.4.4.3 Reagents

Water for analysis (p.a.).

Zinc for analysis (p.a.).

Hydrochloric acid for analysis (p.a.).

Lead acetate solution for analysis (p.a.).

4.3.4.4.4 Apparatus

Test tube.

4.3.4.4.5 Analytical instrumentation

None.

4.3.4.4.6 Analytical procedure

Place about 0,5 g of zinc granules with 2 ml of 5 mol/l hydrochloric acid and add about 0,1 g of the test material (dried residue) in a test tube.

The hydrogen sulfide with typical odour is formed from the nascent hydrogen and the sulfur compound.

A commercial lead acetate paper is placed on the top of the test tube.

4.3.4.4.7 Measurement and reporting

If the paper turns dark (brown to black) this is a positive result in a reaction to lead sulfide (PbS) which only appears if sulfur dioxide is present in the test sample.

4.3.4.5 Other test methods

For the identification of sulfur dioxide, other appropriate analytical methods such as gas chromatography or typical commercial quick test kits can be used.

4.3.5 Analysis of ethylene oxide

4.3.5.1 General

The measurement of ethylene oxide shall be done by the proposed analytical method with headspace gas chromatography (GC) as described in [Table 1](#).

4.3.5.2 Sample preparation

Weigh 1,0 g of the test material into a 10-ml headspace vial, dilute or suspend in 3,0 ml of water, then close the vial and mix to obtain a homogeneous solution or suspension. Allow to stand at 70 °C for 45 minutes. This resulting mixture shall be used as the test sample.

Reference solution 1: weigh 1,0 g of the test material into a 10-ml headspace vial and dilute or suspend in 2,5 ml of water. Add 0,5 ml of a reference solution of ethylene oxide (21 µg/ml), then close the vial and mix to obtain a homogeneous solution or suspension. Allow to stand at 70 °C for 45 minutes. This resulting mixture shall be used as reference solution 1.

NOTE Reference solutions can be produced or purchased from qualified sources.

Reference solution 2: Add 0,5 ml of a reference solution of ethylene oxide (2 µg/ml), 0,1 ml of a freshly prepared (10 mg/l) solution of acetaldehyde and 0,1 ml of water into a 10 ml headspace vial, close the vial and mix to obtain a homogeneous solution or suspension. Allow to stand at 70 °C for 45 minutes. This resulting mixture shall be used as reference solution 2.

4.3.5.3 Reagents

Ethylene oxide for analysis (p.a.).

Acetaldehyde for analysis (p.a.).

Water for analysis (p.a.).

Helium for GC, or nitrogen for GC.

Hydrogen and oxygen for GC with flame ionization detector (FID).

4.3.5.4 Apparatus

Analytical balance.

Laboratory oven.

4.3.5.5 Analytical instrumentation

Table 1 — Conditions for gas chromatographic headspace GC analysis

| Apparatus | Gas chromatographic headspace apparatus (headspace GC) with flame ionization detector (FID) | |
|------------|---|----------------------------------|
| Column | Type: | Capillary glass or quartz column |
| | X | Pre-column |
| | X | Column |
| Detection | X | Flame ionization detector |
| Flowrate | Helium or nitrogen as carrier gas with a linear velocity of about 20 cm/s | |
| Splitratio | 1:20 | |

Table 1 (continued)

| Apparatus | Gas chromatographic headspace apparatus (headspace GC) with flame ionization detector (FID) |
|--|---|
| Temperature | Equilibrate 70 °C for 45 minutes |
| | Transfer-line temperature 75 °C |
| | Injection port temperature 150 °C |
| | Detector temperature 250 °C |
| Time headspace | Pressurization time 1 min, injection time 12 s |
| Record interval | 38 min |
| Injection | A suitable volume, e.g. 1 ml of gaseous phases |
| Temperature gradient programme: | |
| 50 °C for 5 min | |
| Raise temperature with a rate of 5 °C/min to 180 °C | |
| Raise temperature with a rate of 30 °C/min to 230 °C | |
| 230 °C for 5 min | |

4.3.5.6 System suitability

The resolution of the peaks of acetaldehyde and ethylene oxide shall be at least 2,0.

The relative standard deviation of ethylene oxide of three values shall be not greater than 15 %.

4.3.5.7 Assessment

All tests shall be carried out three times.

The analytical data from the test sample shall be compared and calculated with the data produced with the reference solutions (see 4.3.4.3.2) made from authentic reference materials.

The content of ethylene oxide shall be calculated in parts per million.

4.3.5.8 Validity

The validation of quantitative analysis of unwanted compounds shall be done with raw herbal material according to ICH Q2(R1).^[4]

The validity of complex mixtures such as finished products should be demonstrated.

Other appropriate validation methods can be applied, such as in ISO 10993-7.

4.4 Determination of irradiated material

4.4.1 General

Radiation in herbal products is subject to national regulations. The World Trade Organization (WTO) established a consensus which allows this for food under strict declaration.^[5]

NOTE Most countries have no regulations for radiation in pharmaceuticals.

4.4.2 Analysis of irradiated traditional Chinese medicine using photostimulated luminescence

ISO 22256 shall be used for the analysis of irradiated material.