INTERNATIONAL STANDARD

ISO 19609-4

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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 —

Partie 4: Essais des conservateurs et composés indésirables

Reference number
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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 different parts with different content as shown in Figure 1.

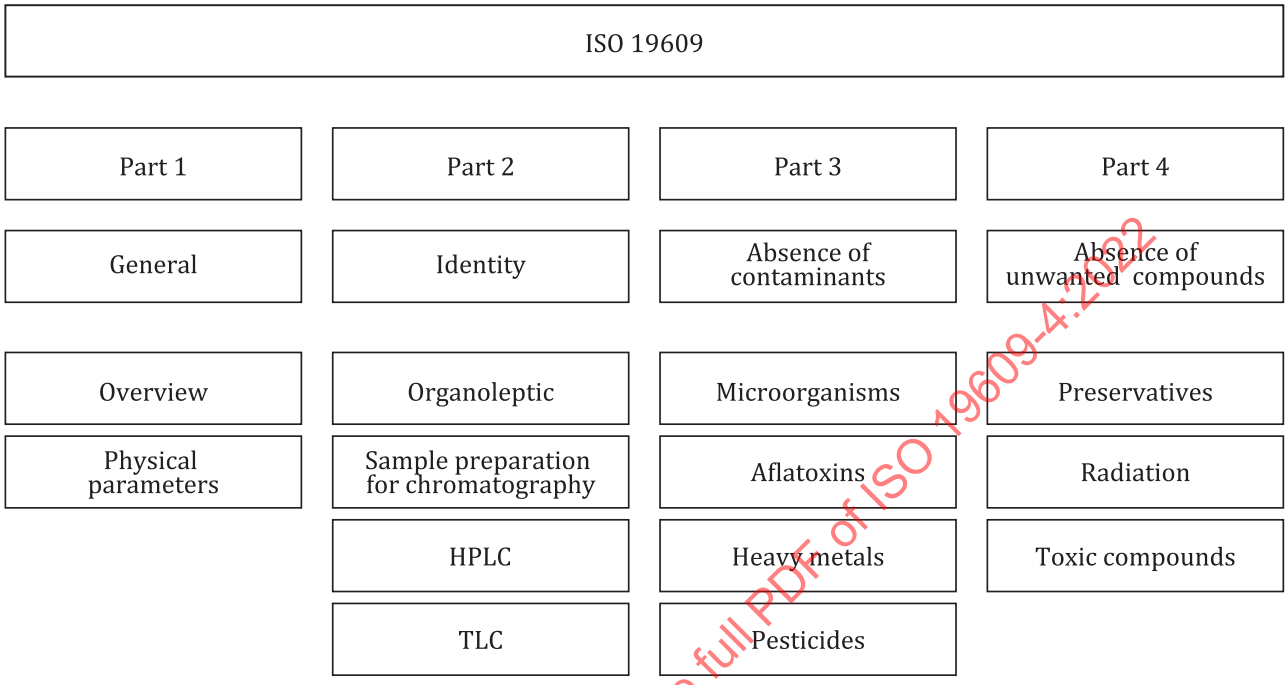


Figure 1 — Overview of the ISO 19609 series

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 oxidein 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 SO_2 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 etylene 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 (2l 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	4 × 4 mm
	X	Column	30 m × 0,32 mm Inner surface coated with 0,1 µm layer of poly(dimethyl) siloxan
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.

4.5 Determination of toxic compounds

4.5.1 General

In some cases, herbal materials with a known toxicity are used in traditional Chinese medicine. Toxins can be formed by degradation reactions, for example in processing and manufacturing steps.

4.5.2 Natural toxins

4.5.2.1 General

NOTE Herbal materials with known toxic constituents can be used for therapeutic applications if their critical limits are fulfilled or their contents are reduced by processing methods or specific extraction processes (e.g. lipophilic toxic compounds).

Typical materia medica with natural toxins are shown in Table 2.

Table 2 — Typical materia medica with natural toxins

Herbal material	Toxic constituents	Reference document
Aconiti kusnezoffii radix	Aconitines	ISO 23962
Aconiti lateralis radix praeparata		
Aconiti radix		
Aristolochia species	Aristolochic acids	ISO 23190
Ephedra species	Ephedrines	
Sophorae tonkinensis radix et rizoma	Matrines	
Strychni semen	Strychnine	
NOTE This list can be supplemented based on new scientific data.		

4.5.2.2 Toxic compound limits

Toxic compounds shall be defined in the monographs for raw materials. In this case, specific quantitative tests are described in detail and shall be done accordingly.

NOTE Limits are described in the individual monographs or regulated nationally.

4.5.2.3 Products made from or with processed *Aconitum carmichaelii* lateral root or other aconitum species

NOTE Aconitines are the typical toxic constituents of the aconitum species. Processing steps are the typical method of detoxification of these herbal materials. The biological activity is based on neurotoxic effects of aconitins. Typical symptoms are arrhythmias and bradycardia.

The determination of aconitines in this herbal material shall be done in accordance with ISO 23962.

Other appropriate valid methods can be applied alongside ISO 23962.

4.5.2.4 Products made from or with herbal materials potentially containing aristolochic acids

NOTE Aristolochic acids are typical constituents of the herbal family of *Aristolochiaceae*, such as the *Aristolochia* species and the *Asari* species, and are genotoxic, carcinogenic and nephrotoxic.

The determination of aristolochic acids in herbal material shall be done in accordance with ISO 23190. This method should also be applied for products made from or with such raw materials.

Other appropriate valid methods can be applied alongside ISO 23190.

4.5.2.5 Products made from or with the *Ephedra* species potentially containing ephedrines

NOTE Ephedrine and pseudoephedrine are typical isomeric constituents of the *Ephedra* species. These materials can also be used for the production of illegal drugs.

4.5.2.5.1 Sample preparation

0,5 g powdered drug or product shall be suspended in 20 ml 33 % methanol (aq.). The extraction shall be realized one time for 0,5 h in an appropriate ultrasonic bath and filtered. The resulting residue shall be extracted twice each with 20 ml 33 % methanol (aq.) and filtered. The resulting extracts shall be combined and filled to a final volume of 100 ml with 33 % methanol (aq.).

This resulting solution is the test solution.

Reference solution 1: 1 mg ephedrine hydrochloride is diluted in 20 ml 33 % methanol.

Reference solution 2: 1 mg pseudoephedrine hydrochloride is diluted in 20 ml 33 % methanol.

4.5.2.5.2 Reagents

Acetonitrile analytical grade (ACN).

Methanol analytical grade (MeOH).

Phosphoric acid 85 % analytical grade (H_3PO_4).

Water analytical grade.

Ephedrine hydrochloride CRS or appropriate reference material quality.

Pseudoephedrine hydrochloride CRS or appropriate reference material quality.

4.5.2.5.3 Apparatus

Analytical balance.

Milling apparatus.

Ultrasonic bath.

4.5.2.5.4 Analytical instrumentation and procedure

The quantification of ephedrine shall be done by high-performance liquid chromatography (HPLC) with an ultraviolet and visible light detector (UV vis) or a diode array detector (DAD) according to the conditions given in Table 3.

Table 3 — Conditions for liquid chromatographic HPLC analysis

Apparatus	HPLC apparatus isocratic or with gradient system and UV vis or DAD (low-pressure or high-pressure systems)		
Column	Type:	octadecyl reversed phase (RP) polymeric column 100 Å C18 5 µm	
	X	Pre-column	4 × 4 mm
	X	Column	250 mm × 4,0 mm or 4,6 mm
Detection	X	UV vis or diode-array detection	Fixed wavelength 207 nm
Flowrate	2,0 ml/min		
Temperature	25 ± 2 °C		
Record interval	20 min		
Inject volume	Typical 10 µl test solution and 10 µl reference solutions		

Table 3 *(continued)*

Mobile phases	A	ACN gradient grade	
	B	0,05 % aqu. phosphoric acid	
Gradient programme:			
Time (min)		% A	% B
0		4	96
20		4	96

Typical chromatograms are shown as Figures B.1 to B.3 in Annex B.

4.5.2.5.5 System suitability

The peaks for the marker constituents shall be baseline-separated from other signals of the test sample and the column efficiencies should not be less than 10 000 theoretical plates.

4.5.2.5.6 Assessment

The analytical data from the test sample shall be compared and calculated with the data produced with the reference solutions (see 4.5.2.5.1) made from authentic reference materials.

If the spectra and retention time of the peaks from the reference solution and the test solution are comparable, a calculation of the content of the toxic compounds can be realized by use of an appropriate calibration curve or at minimum a one-point calibration.

The content of the toxic compound shall be expressed as a value in mg/kg test material.

Other appropriate valid methods can be applied.

4.5.2.5.7 Validity

The validation of quantitative analysis of the toxic compounds is done with raw herbal material according to the international ICH guidelines for validation of test methods.

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

4.5.2.6 Products made from or with herbal materials potentially containing matrine and/or oxymatrine

NOTE Matrine and oxymatrine are typical constituents of *Sophorae tonkinensis radix et rhizoma* and other subspecies.

4.5.2.6.1 Sample preparation

0,5 g of powdered test material shall be weighed accurately into a stoppered conical flask with a 50-ml mixture of chloroform, methanol and concentrated ammonia (25 %) [40:10:1] (extraction solvent mixture). The flask shall be tightly stoppered, weighed and allowed to stand for 30 min (maceration). A single sonication step for 30 min and a filtration shall be done. The residue shall be extracted in an ultrasonic bath with 30 ml of the extraction solvent mixture once for 30 min and filtered again. The filtrates shall be combined, filled up to 100 ml and mixed well.

20 ml of this extract shall be concentrated in a vacuum at 40 °C to dryness, redissolved in a mixture of acetonitrile, isopropanol and 3 % H_3PO_4 (80:5:15) and transferred quantitatively to a 10-ml volumetric flask. The flask shall be filled up with the solvent mixture to volume.

This resulting solution is the test solution.

Reference solution 1: 0,5 mg matrine is diluted in 10 ml in a mixture of acetonitrile, isopropanol and 3 % H_3PO_4 (80:5:15).

Reference solution 2: 1,4 mg oxymatrine is diluted in 10 ml in a mixture of acetonitrile, isopropanol and 3 % H_3PO_4 (80:5:15).

4.5.2.6.2 Reagents

Acetonitrile analytical grade (ACN).

Methanol analytical grade (MeOH).

Chloroform analytical grade ($CHCl_3$).

Isopropanol analytical grade.

Concentrated ammonia (25 %) analytical grade (NH_3).

Phosphoric acid 85 % analytical grade (H_3PO_4).

Water analytical grade.

Matrine CRS or appropriate reference material quality.

Oxymatrine CRS or appropriate reference material quality.

4.5.2.6.3 Apparatus

Analytical balance.

Milling apparatus.

Ultrasonic bath.

4.5.2.6.4 Analytical instrumentation and procedure

The quantification of matrine shall be done by HPLC according to the conditions given in Table 4.

Table 4 — Conditions for liquid chromatographic HPLC analysis

Apparatus	HPLC apparatus isocratic or with gradient system and UV vis or DAD (low-pressure or high-pressure systems)		
Column	Type:	amino-bonded silica gel column 100 Å 5 µm	
	X	Pre-column	4 × 4 mm
	X	Column	250 mm × 4,0 mm or 4,6 mm
Detection	X	UV vis or diode-array detection	Fixed wavelength 220 nm
Flowrate	1,0 ml/min		
Temperature	25 ± 2 °C		
Record interval	25 min		
Inject volume	Typical 10 µl test solution and 10 µl reference solutions		
Mobile phases	A	ACN gradient grade/isopropanol/3 % aqu. phosphoric acid (80:5:15)	
	B	–	
Gradient programme:			
Time (min)		% A	% B
0		100	–
25		100	–

Typical chromatograms are shown as Figures B.7 to B.11 in Annex B.

4.5.2.6.5 System suitability

The peaks for the marker constituents shall be baseline-separated from other signals of the test sample and the column efficiencies should not be less than 10 000 theoretical plates.

4.5.2.6.6 Assessment

The analytical data from the test sample shall be compared and calculated with the data produced with the reference solutions (see 4.5.2.6.1) made from authentic reference materials.

If the spectra and retention time of the peaks from the reference solution and the test solution are comparable, a calculation of the content of the toxic compounds can be realized by use of an appropriate calibration curve or at minimum a one-point calibration.

The content of the toxic compound shall be expressed as a value in mg/kg test material.

Other appropriate valid methods can be applied.

4.5.2.6.7 Validity

The validation of quantitative analysis of the toxic compounds is done with raw herbal material according to the international ICH guidelines for validation of test methods.

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

4.5.2.7 Products made from or with herbal materials potentially containing strychnine

NOTE Strychnine is a typical constituent of *Strychnos nux-vomica*.

4.5.2.7.1 Sample preparation

0,5 g of powdered test material shall be weighed accurately into an appropriate flask with 3 ml 1 mol sodium hydroxide solution and allowed to stand for 30 min (maceration). 20 ml chloroform shall be added, the flask stoppered and weighed and a single sonication step for 30 min and a filtration shall be done. The cooled residue shall be weighed again and the loss of solvent shall be filled up with chloroform and mixed well. The resulting extract shall be filtered with a folded paper filter with about 1 g non-aqueous sodium sulfate to volume. 3 ml of this filtrate shall be filled up with methanol to 10 ml.

This resulting solution is the test solution.

Reference solution 1: dilute 1 mg strychnine in 10 ml methanol.

4.5.2.7.2 Reagents

Acetonitrile analytical grade (ACN).

Methanol analytical grade (MeOH).

Chloroform analytical grade ($CHCl_3$).

Phosphoric acid 85 % analytical grade (H_3PO_4).

Sodium sulfatenon aqueous (Na_2SO_4).

Sodium hydroxide (NaOH).

Triethylamine analytical grade.

Potassium dihydrogen phosphate (KH_2PO_4).

Water analytical grade.

Strychnine CRS or appropriate reference material quality.

4.5.2.7.3 Apparatus

Analytical balance.

Milling apparatus.

Ultrasonic bath.

4.5.2.7.4 Analytical instrumentation and procedure

The quantification of strychnine shall be done by HPLC according to the conditions given in Table 5.

Table 5 — Conditions for liquid chromatographic HPLC analysis

Apparatus	HPLC apparatus isocratic or with gradient system and UV vis or DAD (low-pressure or high-pressure systems)		
Column	Type:	octadecyl RP-phase column 100 Å C18 5 µm	
	X	Pre-column	4 × 4 mm
	X	Column	250 mm × 4,0 mm or 4,6 mm
Detection	X	UV vis or diode-array detection	Fixed wavelength 260 nm
Flowrate	1,0 ml/min		
Temperature	25 ± 2 °C		
Record interval	25 min		
Inject volume	Typical 10 µl test solution and 10 µl reference solutions		
Mobile phase A	6,8 g potassium dihydrogen phosphate (KH_2PO_4)/1 l water, ACN gradient grade, triethylamine (45:5:1) with a pH value of 3,2 realized by the addition of phosphoric acid		
Gradient programme:			
Time (min)		% A	% B
0		100	–
25		100	–

Typical chromatograms are shown as Figures B.4 to B.6 in Annex B.

4.5.2.7.5 System suitability

The peak for the marker constituent shall be baseline-separated from other signals of the test sample and the column efficiency should not be less than 10 000 theoretical plates.

4.5.2.7.6 Assessment

The analytical data from the test sample shall be compared and calculated with the data produced with the reference solution (see 4.5.2.7.1) made from authentic reference material.

If the spectra and retention time of the peak from the reference solution and the test solution are comparable, a calculation of the content of the toxic compound can be realized by use of an appropriate calibration curve or at minimum a one-point calibration.

The content of the toxic compound shall be expressed as a value in mg/kg test material.

Other appropriate valid methods can be applied.

4.5.2.7.7 Validity

The validation of quantitative analysis of the toxic compound is done with raw herbal material according to the international ICH guidelines for validation of test methods.

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

4.5.3 Toxins resulting from degradation reactions

4.5.3.1 General

Under specific conditions in a manufacturing process or processing steps, new non-natural toxic compounds can be formed. A typical example is acrylamide by reaction of reducing carbohydrates with fats. Another toxic compound called benzopyrene as a polycyclic aromatic hydrocarbon can be formed by incomplete combustions of organic matter at temperatures higher than 200 °C.

4.5.3.2 Benzopyrene

The determination of benzopyrene in processed natural products shall be done in accordance with ISO 23956.

NOTE Benzopyrenes are carcinogenic compounds.

4.5.3.3 Typical materia medica with restrictions of use

Annex A, Table A.1 includes a list of typical materia medica with restrictions of use.

Annex A
(informative)

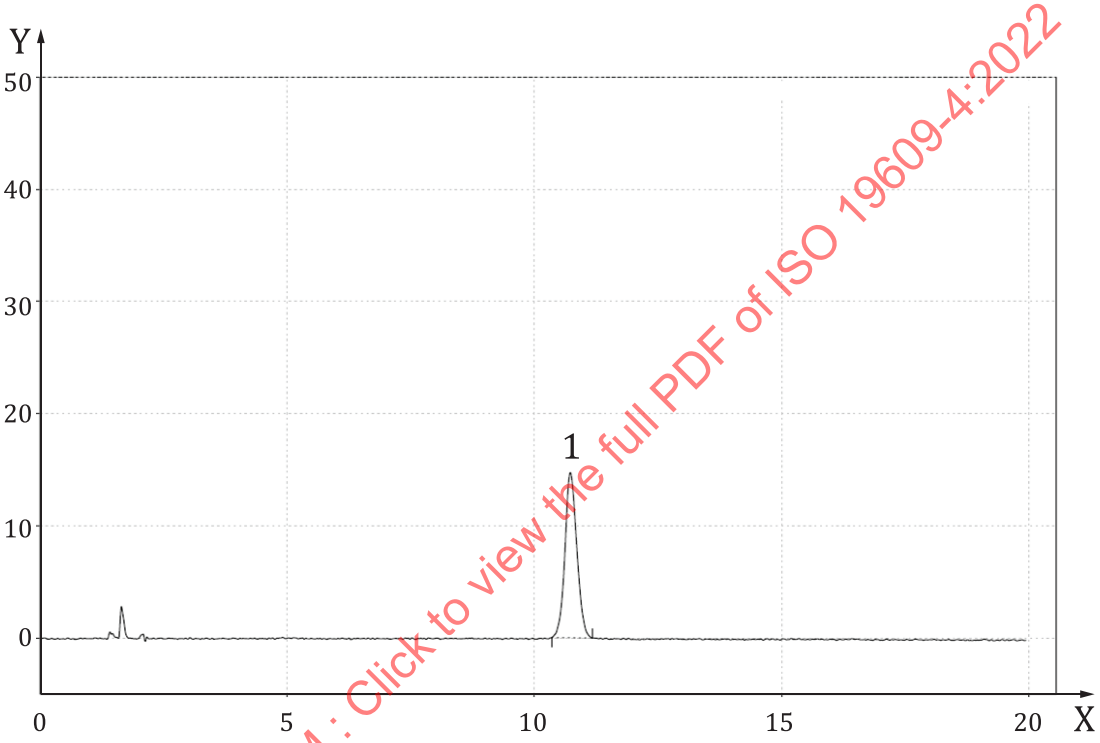
Materia medica

Table A.1 — List of typical materia medica with restrictions of use

Herbal material	Application	Contraindication
Arisaematis rhizoma	For topical application only	–
Crotonis fructus	For topical application only	Incompatible with *Pharbitidis semen* Contraindicated for pregnant patients
Daturae flos	Dosage limited	Contraindicated for pregnant patients and those suffering from exterior contractions, phlegm-heat, wheezing and coughing, glaucoma, hypertension or tachycardia
Euphorbiae ebracteolatae radix	For topical application only	Incompatible with *Lithargyum*
Euphorbiae semen	For topical application only, dosage limited in pills and powders	Contraindicated for pregnant patients
Hyoscyami semen	Dosage limited	Contraindicated for patients suffering from heart disease or glaucoma, or pregnant patients
Kansui radix	Unprocessed for topical applications only, processed dosage limited	Contraindicated for pregnant patients, incompatible with *Glycyrrhizae radix et rhizoma*
Pinelliae rhizoma	Unprocessed for topical applications only, processed dosage limited	Incompatible with *Aconiti* species
Rhododendri mollis flos	For topical applications only, or dosage limited only if extracted in wine or liqueur	Overdosage or prolonged administration is inadvisable, use with caution in patients with weak constitution and in pregnant patients
Typhonii rhizoma	Unprocessed for topical applications only, oral use only in processed form	Contraindicated for pregnant patients

Annex B
(informative)

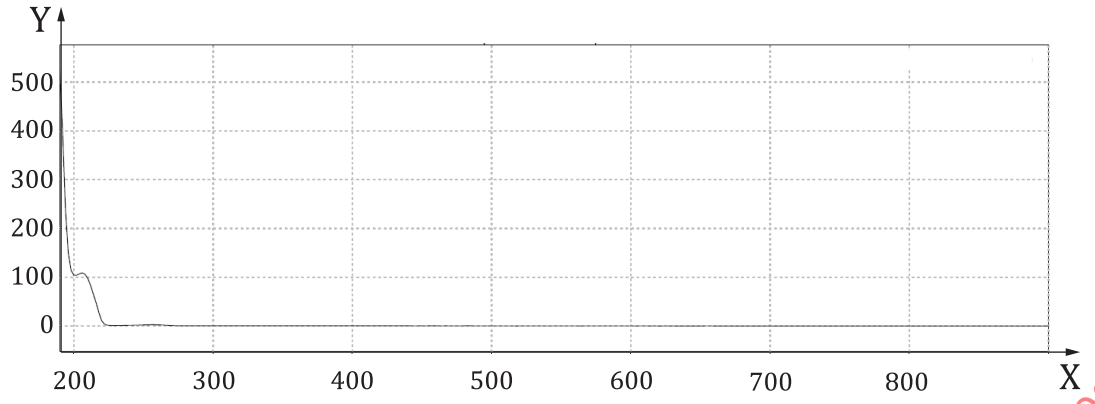
Examples of typical chromatograms of *Ephedra herba, Strychni semen, Sophorae tonkinensis radix et rhizoma* and their toxic compounds



Key

X min
Y mAU
1 DL-ephedrine

Figure B.1 — Typical chromatogram of DL-ephedrine standard

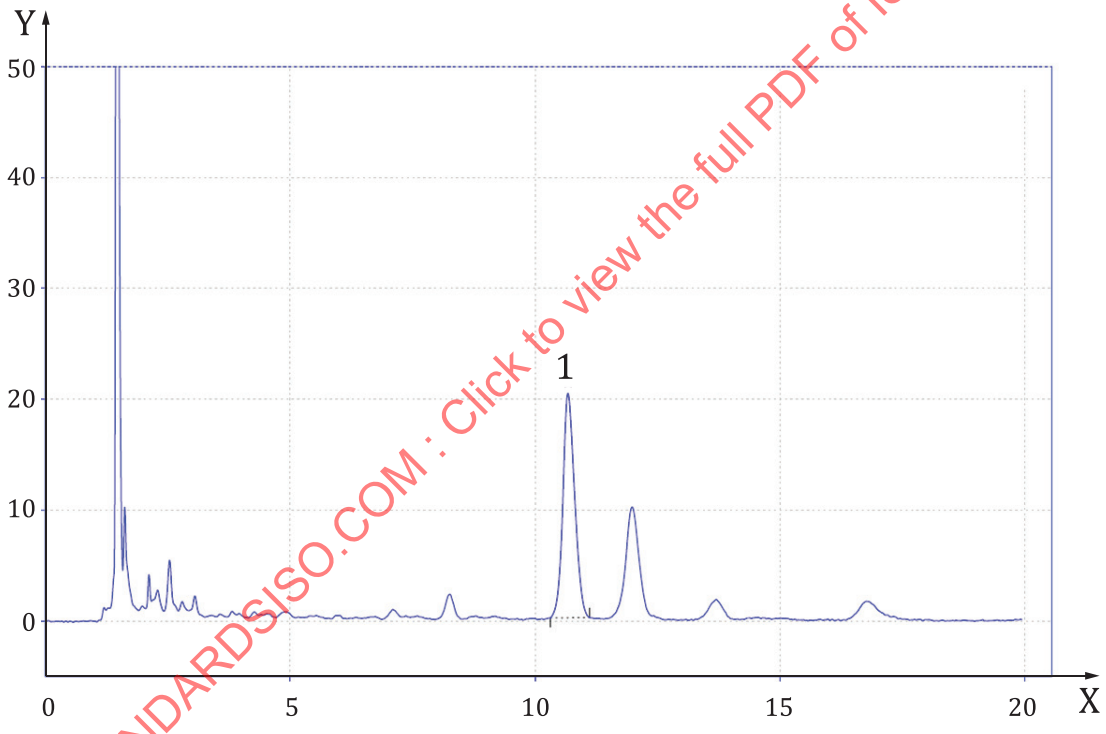


Key

X min

Y mAU

Figure B.2 — Typical UV-vis spectrum of DL-ephedrine



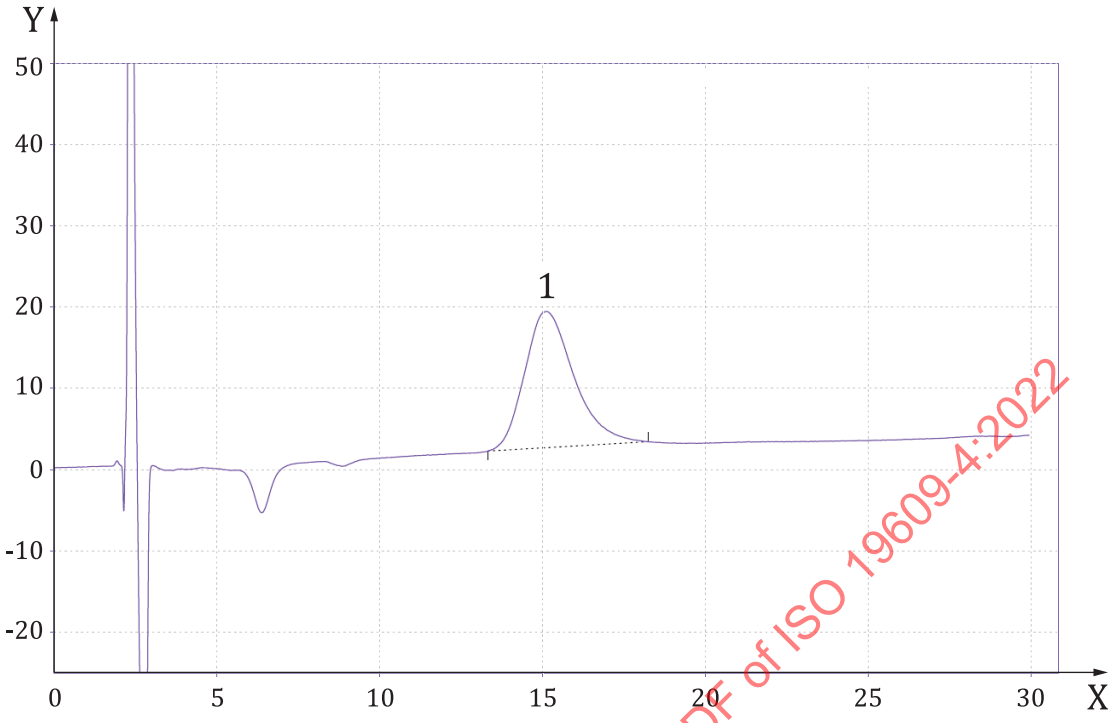
Key

X min

Y mAU

1 DL-ephedrine

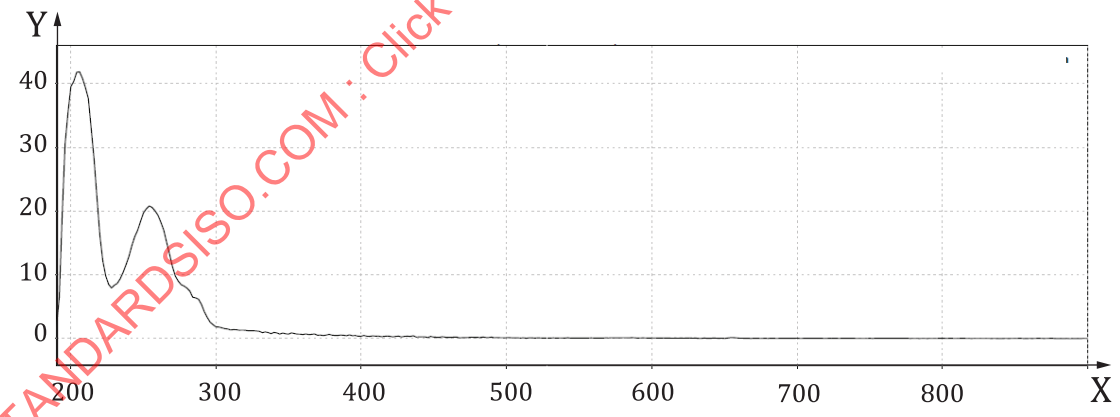
Figure B.3 — Typical chromatogram of *Ephedra Herba*



Key

X min
Y mAU
1 strychnine

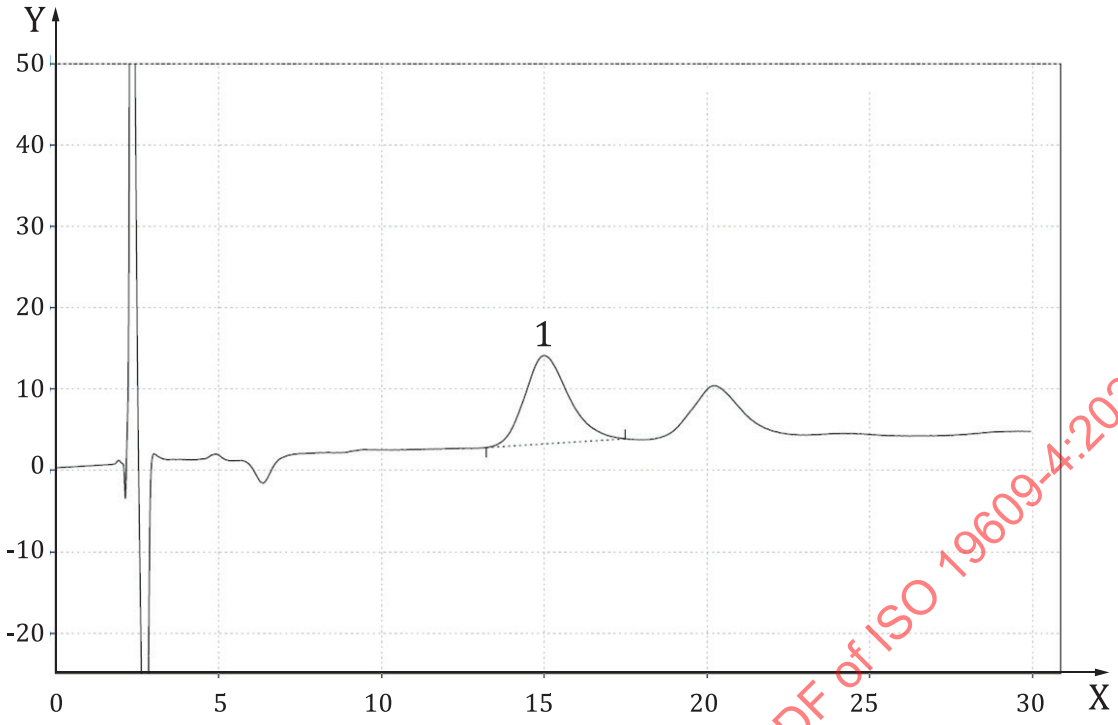
Figure B.4 — Typical chromatogram of strychnine standard



Key

X min
Y mAU

Figure B.5 — Typical UV-vis spectrum of strychnine



Key

X min
Y mAU
1 strychnine

Figure B.6 — Typical chromatogram of *Strychnos nux-vomica*