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

*Médecine traditionnelle chinoise — Dosage du dioxyde de soufre dans
les produits naturels par titrage*

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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).

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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*.

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.

Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration

1 Scope

This document specifies the determination method of sulfur dioxide in natural products used in traditional Chinese medicine, which includes the requirements of the device, chemicals, operational procedures and formula.

It is applicable to natural products of traditional Chinese medicine, including Chinese materia medica (whole medicinal materials) and decoction pieces derived from plants or animals.

It is not applicable to minerals used in traditional Chinese medicine.

2 Normative references

There are no normative references in this document.

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:

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

3.1

sulfur dioxide

toxic gas with a pungent, irritating odour, the chemical compound with the formula SO_2

3.2

sulfur dioxide residue

sulfur dioxide that remains in or on a natural product

Note 1 to entry: Sulfur dioxide residue is expressed as mg/kg.

3.3

maximum residue limit

MRL

highest level of sulfur dioxide that is permitted in or on a natural product

Note 1 to entry: Maximum residue limit is expressed as mg/kg.

3.4

acceptable daily intake

ADI

estimate of the amount of sulfur dioxide in natural products that can be safely consumed daily over a lifetime without adverse health effects

Note 1 to entry: ADI is expressed in milligrams of the sulfur dioxide, as it appears in the natural products, per kilograms of body mass per day (mg/kg/day).

3.5 acid-base titration

determination of the concentration of an acid or base by exactly neutralizing the acid or base with a base or acid of known concentration

4 Apparatus

- 4.1 **Glass sample**, cleaned thoroughly before use.
- 4.2 **Electronic balance**, with a minimum reading of 0,1 mg.
- 4.3 **Separatory funnel**, with a capacity of 50 ml or 100 ml.
- 4.4 **Round-bottom flask**, with a capacity of 500 ml or 1 000 ml.

5 Reagents

All reagents shall be of recognized chromatographic grade or analytical purity. Distilled water or water of equivalent purity or above, recently boiled, shall be used.

- 5.1 **Distilled water**.
- 5.2 **Bromophenol blue**, 1 g/l solution of bromophenol blue in ethanol (20 % volume fraction).
- 5.3 **Hydrochloric acid**, 6 mol/l solution of diluted hydrochloric acid. Dilute one volume of concentrated hydrochloric acid.
- 5.4 **Hydrogen peroxide**, 3 % volume fraction solution of diluted hydrogen peroxide, free from sulfate ions.
- 5.5 **Sodium hydroxide**, 0,1 mol/l solution of sodium hydroxide (standard volumetric solution).

6 Sampling and preservation of samples

6.1 Sampling

6.1.1 Laboratory samples

Raw material samples received by the laboratory shall be accompanied with complete information such as the source and time of collection of the samples. The samples for testing can include Chinese materia medica (whole medicinal materials) and decoction pieces derived from plants or animals.

6.1.2 Sample identification

When a sample is received, it shall be immediately assigned a unique label which will accompany it through all stages of the analysis to the reporting of the results. Samples shall be subject to the appropriate disposal review system and all records shall be accurately kept.

6.2 Sample preparation and preservation

6.2.1 Sample preparation

Before testing, the sample shall be dried and powdered. Samples shall be pre-treated as soon as possible and stored in a cool and dry place – if possible, in a refrigerator.

6.2.2 Sample preservation

If samples cannot be analysed immediately, they shall be stored in a cool and dry place, away from the direct sunlight, and analysed within a few days.

7 Test method

7.1 Test procedure

- a) Introduce 150 ml of water into the flask (see [Figure 1](#), key 1), open the condenser pipe (key 3) and pass carbon dioxide through the whole system for 15 min at a rate of 100 ± 5 ml/min. To 10 ml of diluted hydrogen peroxide solution add 0,15 ml of a 1 g/l solution of bromophenol blue in ethanol (20 % volume fraction).
- b) Add 0,1 M sodium hydroxide until a violet-blue colour is obtained, without exceeding the end point.
- c) Place the solution in the test tube (key 4). Without interrupting the stream of carbon dioxide, remove the funnel (key 2) and introduce through the opening into the flask 25,0 g of the prepared sample, with the aid of 100 ml of water.
- d) Replace the funnel.
- e) Close the tap of the funnel and add 80 ml of dilute hydrochloric acid to the funnel.
- f) Open the tap of the funnel to allow the hydrochloric acid solution to flow into the flask, making sure that no sulfur dioxide escapes into the funnel by closing the tap before the last few millilitres of hydrochloric acid solution drain out.
- g) Boil for 1 h. Open the tap of the funnel and stop the flow of carbon dioxide as well as the heating and the cooling of the water.
- h) Transfer the contents of the test tube with the aid of a little water to a 200 ml wide-necked, conical flask.
- i) Heat on a water-bath for 15 min and allow to cool.
- j) Add 0,1 ml of a 1 g/l solution of bromophenol blue in ethanol (20 % volume fraction) and titrate with 0,1 M sodium hydroxide until the colour changes from yellow to violet-blue (V_1 ml).
- k) Carry out a blank titration (V_2 ml).

NOTE 1 An alternative test method which is validated to be the same as this method can be used (see [Table C.1](#)).

NOTE 2 [Annex B](#) describes the method of gas chromatography to determine sulfur dioxide in natural products.

NOTE 3 [Annex C](#) provides an analysis of the similarities and differences of acid-base titration in different countries.

7.2 Calculation

Calculate the content of sulfur dioxide in parts per million using the following formula:

$$32\,030 \times (V_1 - V_2) \times (n/m)$$

where

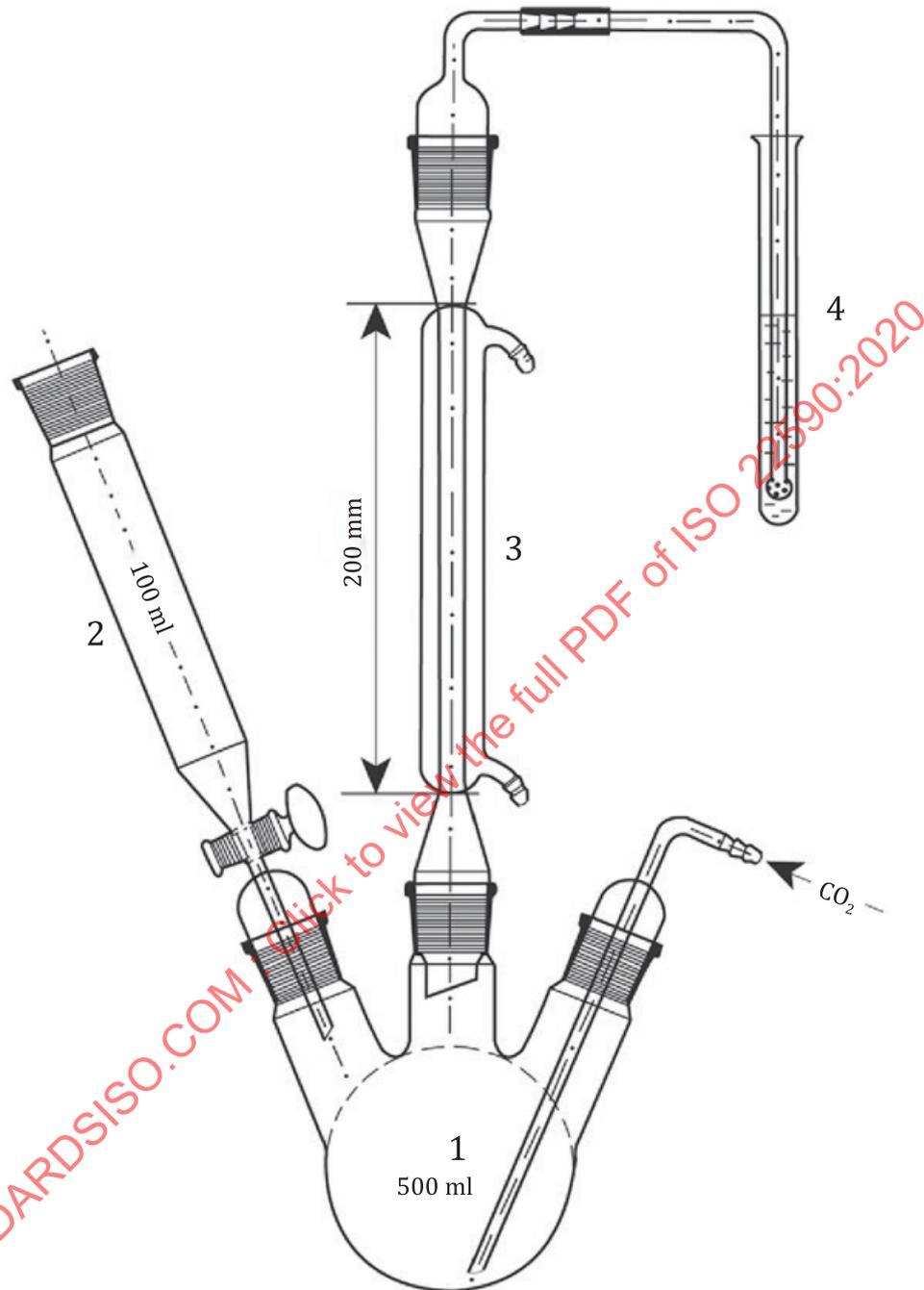
n is the molarity of the sodium hydroxide solution used as titrant;

m is the mass of the substances being examined;

V_1 is the volume of depleted sodium hydroxide of the test;

V_2 is the volume of depleted sodium hydroxide of the blank.

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**Key**

- 1 three-neck round-bottom flask
- 2 funnel
- 3 condenser pipe
- 4 test tube

SOURCE 'Sulfur dioxide', 2.5.29, *European Pharmacopoeia*, 9th edition, used with the permission of the EDQM.

Figure 1 — Apparatus for the determination of sulfur dioxide

NOTE The limits of sulfur dioxide in different countries, regions and organizations and the calculated limits using target hazard quotients based on USEPA and WHO are shown in Annex A.

Annex A (informative)

Reference of national, regional and organizational limits of sulfur dioxide in food and natural products of traditional Chinese medicine and calculated limits using target hazard quotients based on USEPA and WHO

Different countries, regions and organizations give their own limits of sulfur dioxide in food and natural products of traditional Chinese medicine. [Table A.1](#) shows these limits in various types of food and herbal products. Also, some health authorities provide risk assessment methods [United States Environmental Protection Agency (USEPA)] and recommended sulfur dioxide intake per week [World Health Organization (WHO)]. According to this information and traditional Chinese medicine characteristics, the calculated limits using target hazard quotients (THQ) are listed with the summarized maximum limits from previous references in [Table A.1](#).

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Table A.1 — National, regional and organizational recommended limits of sulfur dioxide in food and natural traditional Chinese medicine materials and calculated limits using THQs based on USEPA and WHO

	Food and traditional Chinese medicine category	Maximum level of sulfur dioxide mg/kg
"Codex Alimentarius" (CXS)	Frozen fruit	500
	Dried fruit	1 000
	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	500
	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine or soybean sauce	100
	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter)	500
	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g. vegetable desserts and sauces, candied vegetables) other than seed purees and spreads (e.g. peanut butter)	300
	Fermented vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soybean products	500
	Flours	200
	Herbs and spices	150
	Seasonings and condiments	200
	Sauces and similar products	300
	Grape wines	350
	Wines (other than grape)	200

NOTE It is regarded as safe when THQ is lower than 1.

^a Calculations use THQs given by USEPA.

$$THQ = \frac{C \times EF \times ED \times IR}{BW \times AT \times ADI \times 1\,000}$$

where

C is the metal concentration in food (mg/kg);

EF is exposure frequency (days/year), i.e. 30 days/year;

ED is the exposure duration, i.e. 30 years;

IR is the ingestion or inhalation rate (g/day), i.e. 200 g/day;

BW is the average body mass (60 kg for adults);

AT is the average exposure time for non-carcinogens (365 days/year × 70 years);

ADI is the acceptable daily intake [0,7 mg/(kg·day)].

Hence, using ADI provided by the WHO we arrive at a risk assessment which approaches 750 mg/kg.

Table A.1 (continued)

	Food and traditional Chinese medicine category	Maximum level of sulfur dioxide mg/kg
China	Herbs including Dioscoreae rhizoma (山药), Achyranthis bidentatae radix (牛膝), Puerariae thomsonii radix (粉葛), Gastrodiae rhizome (天麻), Asparagi radix (天冬), Trichosanthis radix (天花粉), Bletillae rhizome (白及), Paeoniae radix alba (白芍), Atractylodis macrocephalae rhizome (白术), and Codonopsis radix (党参)	400
China	Herbs other than the 10 herbs with the maximum level of 400 mg/kg (listed above) are not included	150
Japan	Herbs	50
Korea	Herbs	30
WHO/FAO	Herbs	150
US	Herbs	50
Calculating MRLs ^a		750
Recommended limits		400

NOTE It is regarded as safe when THQ is lower than 1.

^a Calculations use THQs given by USEPA.

$$THQ = \frac{C \times EF \times ED \times IR}{BW \times AT \times ADI \times 1\,000}$$

where

C is the metal concentration in food (mg/kg);

EF is exposure frequency (days/year), i.e. 30 days/year;

ED is the exposure duration, i.e. 30 years;

IR is the ingestion or inhalation rate (g/day), i.e. 200 g/day;

BW is the average body mass (60 kg for adults);

AT is the average exposure time for non-carcinogens (365 days/year × 70 years);

ADI is the acceptable daily intake [0,7 mg/(kg day)].

Hence, using ADI provided by the WHO we arrive at a risk assessment which approaches 750 mg/kg.

Annex B (informative)

Gas chromatography — Determination of sulfur dioxide in natural traditional Chinese medicine materials

B.1 General

Gas chromatography is a chemical analytical method commonly used in traditional Chinese medicine. The method has high sensitivity and accuracy and can also be used to detect sulfur dioxide residue in natural products used in traditional Chinese medicine.

This document provides another determination method of sulfur dioxide for reference.

B.2 Reagents

- B.2.1 **Solid paraffin**, with a melting point of 52 °C to 56 °C.
- B.2.2 **Helium** carrier gas, with a flow rate of 2 ml/min.
- B.2.3 **Sodium sulfite**, of analytical purity.
- B.2.4 **Mannitol**, with a mass fraction of 0,5 %.
- B.2.5 **Ethylenediaminetetraacetic acid disodium salt**, with a mass fraction of 0,1 %.
- B.2.6 **Hydrochloric acid**, with a concentration of 2 mol/l.

B.3 Equipment

- B.3.1 **Headspace bottle**, with a capacity of 20 ml.
- B.3.2 **Thermal conductivity detector (TCD)**, of detector temperature 250 °C.
- B.3.3 **Column**, GS-GasPro bonded silica porous layer open-tube column (length 30 m, internal diameter 0,32 mm) or equivalent column.

B.4 Procedure

Accurately weigh 1,0 g of NaCl and 1,0 g of paraffin wax and place into the headspace bottle. Add 2 ml of 2 mol/l HCl to the bottle and place the bottle into a water bath at 60 °C. When the paraffin wax melts, take the bottle out of the water bath and re-solidify the paraffin wax at room temperature. Accurately weigh 0,2 g of sample powder and place onto the re-solidified paraffin wax, add 100 µl of 0,5 % mannitol and 0,1 % EDTA mixed solution and seal the bottle.

Inject 1 ml of the headspace gas containing the test sample and the reference solution into gas chromatography. The content of SO_3^{2-} is quantified by the standard curve method, where the result is multiplied by 0,507 9 to calculate the content of SO_2 .

Annex C
(informative)

**Analysis of the similarities and differences of acid-base titration
in different countries**

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