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

Médecine traditionnelle chinoise — Dosage des acides aristolochiques dans les produits naturels par chromatographie liquide haute performance (CLHP)

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Foreword

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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.

Introduction

Aristolochic acids, a class of chemical compounds with renal toxicity, carcinogenic and mutagenic toxicity, are widely distributed in over 350 species of plant from around the world, many of which have been used as natural products to treat gout, arthritis, rheumatism and acute inflammation of the skin; some species from North America have been used to treat snake bites. Clinical practice and research have confirmed that long-term use of natural products containing aristolochic acids can cause chronic renal failure and renal tubules, and natural products containing aristolochic acids have been prohibited and restricted to use in clinics in many counties. Aristolochic acid toxicity is of great concern worldwide.

Safety and efficacy are basic requirements for the use of natural medicines. Although many natural products containing aristolochic acids have been strictly controlled in clinics, some are still used as raw herbal materials or to produce manufactured products such as asarum, Kaempfer dutchmanspipe root, Herba Aristolochiae mollissimae, German birthwort, American snakeroot and Indian Aristolochia tagala. In addition, some prohibited plant medicines are easily confused or misused during manufacturing, which can cause large safety concerns in the application of natural products.

This document is beneficial for effectively supervising and reducing the toxic side effects of natural-medicine-derived products and ensuring their safety and efficacy in clinical use.

The high-performance liquid chromatography (HPLC) method is applied in organizations in such places as Europe, China, the United States of America, Japan and the Republic of Korea for the determination of aristolochic acid I, both qualitatively and quantitatively. The HPLC method is recommended internationally for the qualitative determination of aristolochic acid I in natural products.

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

1 Scope

This document specifies a method for using high-performance liquid chromatography (HPLC) to determine the presence of aristolochic acid I in natural products.

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.

World Health Organization, *Quality control methods for herbal materials*. World Health Organization, 2011

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
raw herbal material
medicinal part of medicinal plants after *preliminary processing* (3.2)

3.2
preliminary processing
physical technique of converting medicinal part of medicinal plants into *raw herbal material* (3.1)

3.3
extract
preparation containing the active ingredient of a substance from *raw herbal material* (3.1) in concentrated form based on the standardized production process and meeting certain quality standards

4 Abbreviated terms

DAD	diode array detector
HPLC	high-performance liquid chromatography
UV	ultraviolet absorption
LC-MS	liquid chromatography-mass spectrometry

5 Principle

The HPLC method is employed to determine the presence of aristolochic acid I. The HPLC system consists of a quaternary pump, continuous vacuum degasser, thermostated auto-sampler and column compartment coupled to a variable wavelength diode array detector (DAD).

The aristolochic acid I determinable by this method is shown in Table 1. The chemical structure of aristolochic acid I is given in Figure 1.

Table 1 — Aristolochic acid I determinable by this method

Analyte	Molecular formula	CA determinable S[a] No.	Molar mass
Aristolochic acid I	$C_{17}H_{11}NO_7$	313–67–7	341,27

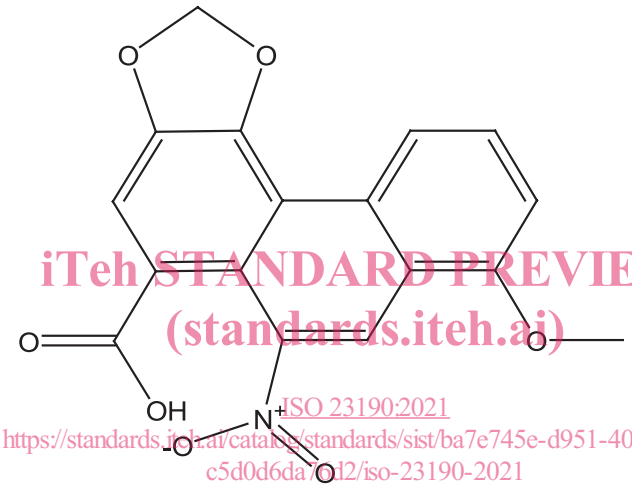


Figure 1 — Chemical structure of aristolochic acid I

6 Reagents

All reagents shall be of recognized chromatographic or analytical grade. The purity of the reagents used shall be checked by running a blank determination. The chromatogram obtained from the solvents shall have a baseline without noticeable peak that would interfere with those from the targeted aristolochic acid I for detection.

6.1 Aristolochic acid. The purity of aristolochic acid I should be more than 95 %.

6.2 Acetonitrile. The molecular formula of acetonitrile is CH_3CN, with a HPLC grade for HPLC and a LC-MS grade for LC –MS.

6.3 Phosphoric acid. The molecular formula of phosphoric acid is H_3PO_4, with a HPLC grade for HPLC.

6.4 Formic acid. The molecular formula of formic acid is HCOOH, with a LC-MS grade for LC –MS.

6.5 Water. The molecular formula of water is H_2O with a resistivity of 18,2 MΩ.

6.6 Methanol. The molecular formula of methanol is CH_3OH with a purity of analytical grade.

7 Apparatus

Use the usual laboratory apparatus and, in particular, the following.

7.1 HPLC, consisting of a high-pressure infusion pump, sampler and chromatographic column, DAD, integrator or data processing system.

7.2 Glassware. All glassware shall be thoroughly cleaned before use.

7.3 Grinder, used to smash the raw herbal materials.

7.4 Volumetric flasks, with a capacity of 2,0 ml and 10,0 ml.

7.5 Conical flask, with a capacity of 100 ml and a glass stopper.

8 Test method

8.1 General principle

The HPLC-DAD method is applied to detect the presence of aristolochic acid I. The parameters can vary depending on the particular condition of different instruments. The procedures of the HPLC-DAD method can be modified if the accuracy and precision are validated, such as a new analysis method with the same or higher accuracy and precision.

8.2 Caution and safety considerations

Due to the toxicity of aristolochic acid I, necessary precautions shall be taken in all operations. The solutions and the apparatus used in the test should not pollute the environment.

8.3 Stock solution of standard

Dissolve 2 mg of aristolochic acid I in methanol or 80 % methanol and store at 4 °C.

8.4 HPLC condition

8.4.1 Chromatographic system and system suitability

a) Column

   Stationary phase: octadecylsilyl bonded silica gel as chromatography column or equivalent.

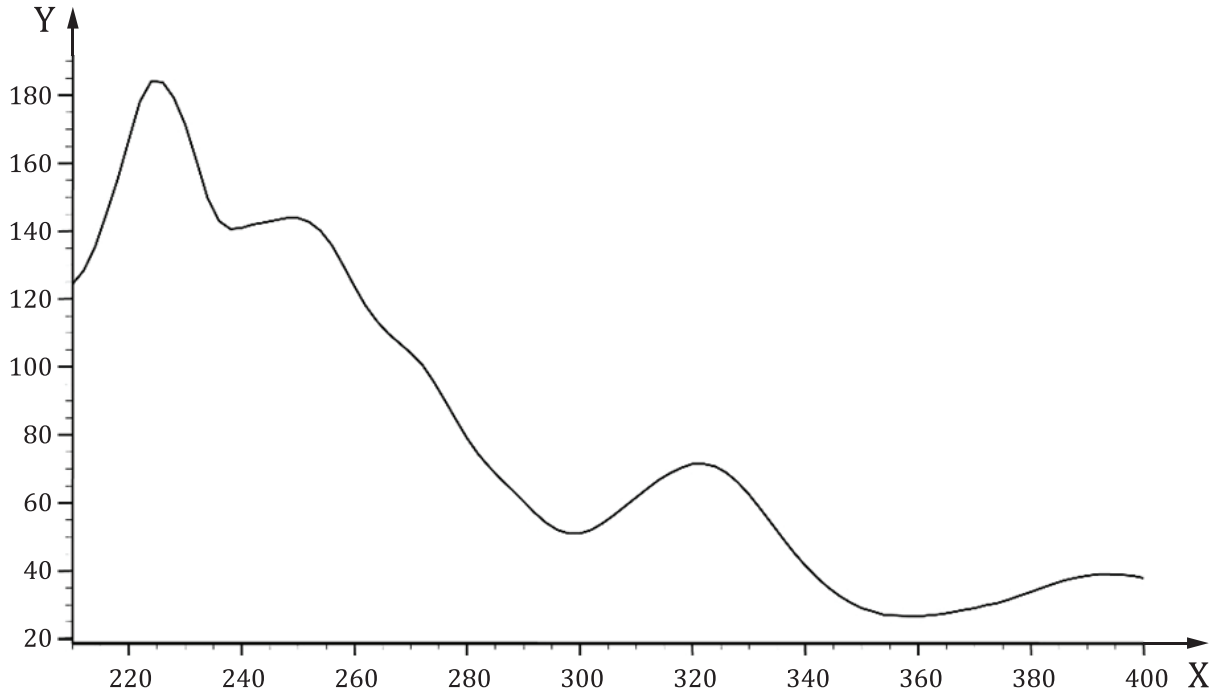
b) Mobile phase

   — Mobile phase A: acetonitrile.

   — Mobile phase B: 0,05 % phosphoric acid solution

c) Wavelength of the detector: 254 nm.

d) The ultraviolet absorption (UV) spectrum of aristolochic acid I is shown in Figure 2.



Key

X absorption wavelength, nm

Y response value of electrical signal, mAU

Figure 2 — UV spectrum of aristolochic acid I

See Annex A for typical HPLC-DAD chromatographic conditions.

8.4.2 Standard solution

Dilute the stock solution of aristolochic acid I with methanol or 80 % methanol to prepare the standard solution of aristolochic acid I (0,25 ug/ml).

8.4.3 Test solution I

Weigh 100 g of raw herbal materials to grind and pass it through an 80-mesh or finer sieve. Weigh accurately 0,5 g of the powdered raw herbal material in a conical flask with stopper. Add accurately 25 ml of 70 % methanol or 80 % methanol and weigh. Ultrasonicate (powder, 250 W; frequency, 40 kHz) for 40 minutes, cool, weigh again, make up the loss of weight with 70 % methanol or 80 % methanol, mix well and filter through a membrane filter (normal pore size 0,45 μm) using the successive filtrate as the test solution.

8.4.4 Test solution II

Grind approximately 10 g to 100 g of the extract, bolus, powder, pellet and tablet to fine powder, weigh an appropriate amount (equivalent to 0,5 g of aristolochic acid-containing raw herbal material) in a conical flask with stopper. Add accurately an appropriate amount of 70 % methanol or 80 % methanol with a ratio of material to liquid of 50:1. Ultrasonicate (powder, 250 W; frequency, 40 kHz) for 40 minutes, cool and then filter. Evaporate the filtrate to dryness, dissolve the residue in an appropriate amount of 70 % methanol or 80 % methanol to prepare a solution containing 20 mg aristolochic acid-containing raw herbal material per ml and filter through a membrane filter (normal pore size 0,45 μm) using the successive filtrate as the test solution.