

GENERAL INFORMATION

1. Aristolochic acid

Aristolochic acid, which occurs in plants of *Aristolochiaceae*, is suspected to cause renal damage. It is also reported to have oncogenicity (see References).

There will be no problem when crude drugs of the origin designated in the JP are used, but there may be differences in crude drug nomenclature between different countries, and it is known that crude drug preparations not meeting the specifications of the JP are circulating in some countries. Consequently, when crude drugs or their preparations are used, it is important that the materials should not include any plants containing aristolochic acid.

Crude drugs for which particular care is necessary are as follows:

Asiasarum root, Akebia stem, Sinomenium stem, and Saussurea root.

References:

Drug & Medical Device Safety Information (No.161) (July, 2000); New England Journal of Medicine (June 8, 2000)

An example of an assay method for aristolochic acid is as follows:

Assay—

1) Preparation of sample solution To 2.0 g of powdered material to be tested add 50 mL of a mixture of methanol and water (3:1), shake for 15 minutes (if an ultrasonicator is used, for 20 minutes), filter, and use the filtrate as the sample solution.

2) Preparation of standard solution Weigh exactly X mg¹⁾ of aristolochic acid, equivalent to 10 mg of aristolochic acid I, and dissolve in a mixture of methanol and water (3:1) to make exactly 200 mL. Pipet 2 mL of this solution, add a mixture of methanol and water (3:1) to make exactly 250 mL, and use this solution as the standard solution.

Note 1): X mg = $10 \times 100/F$, where F is the labeled amount (%) of aristolochic acid I.

3) Standard procedure and assessment of result Perform the test with exactly 10 μ L each of the sample solution and the standard solution, according to the following conditions, as directed under the Liquid chromatography. The sample is acceptable if the sample solution shows no peak at the retention time corresponding to aristolochic acid I from the standard solution. If the sample shows such a peak, repeat the test under different conditions: if the sample no longer shows a peak at a retention time that coincides with that of standard aristolochic acid under the new conditions, the sample is acceptable.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 400 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

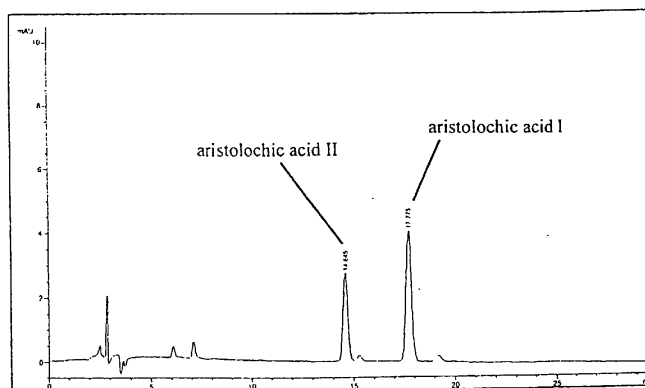
Column temperature: A constant temperature of between 25°C and 40°C.

Mobile phase: A mixture of 0.05 mol/L NaH_2PO_4 (H_3PO_4 2 mL)²⁾ and CH_3CN (11:9).

Flow rate: 1.0 mL/min

Note 2): To 7.8 g of sodium dihydrogenphosphate dihydrate and 2 mL of phosphoric acid add water to make 1000 mL.

4) Others The operating conditions may be changed within the limits described under the Liquid chromatography.
5) Chromatogram of aristolochic acid (reference substance) A chromatogram of aristolochic acid obtained under the operating conditions described in 3) Standard procedure and assessment of result is shown below:



2. Decision of Limit for Bacterial Endotoxins

The endotoxin limit for injections is to be decided as follows:

$$\text{Endotoxin limit} = K/M$$

where K is a minimum pyrogenic dose of endotoxin per kg body mass (EU/kg), and M is equal to the maximum dose of product per kg per hour.

M is expressed in mL/kg for products to be administered by volume, in mg/kg or mEq/kg for products to be administered by mass, and in Unit/kg for products to be administered by biological units. Depending on the administration route, values for K are set as in the following table.

Intended route of administration	K (EU/kg)
Intravenous	5.0
Intravenous, for radiopharmaceuticals	2.5
Intraspinal	0.2

Notes:

- 1) For products to be administered by mass or by units, the endotoxin limit should be decided based on the labeled amount of the principal drug.
- 2) Sixty kg should be used as the average body mass of an adult when calculating the maximum adult dose per kg.
- 3) The pediatric dose per kg body mass should be used when this is higher than the adult dose.
- 4) The *K* values for the intravenous route are applicable to drugs to be administered by any route other than those shown in the table.

3. Disinfection and Sterilization Methods

Disinfection and Sterilization Methods are applied to kill microorganisms in processing equipment/utensils and areas used for drug manufacturing, as well as to perform microbiological tests specified in the monographs, and so differ from "Terminal Sterilization" and "Filtration Method" described in "Terminal Sterilization and Sterilization Indicators". The killing effect on microorganisms or the estimated level of sterility assurance is greatly variable, so the conditions for disinfection and sterilization treatment must be chosen appropriately for each application. Generally, the following methods are to be used singly or in combination after appropriate optimization of operation procedures and conditions, in accordance with the kind and the degree of the contaminating microorganisms and the nature of the item to which the methods are applied.

The validation of sterilization in accordance with Terminal Sterilization and Sterilization Indicators is required when the methods are applied to the manufacturing processes of drug products.

1. Disinfection methods

These methods are used to reduce the number of living microorganisms, but do not always remove or kill all microorganisms present. Generally, disinfection is classified into chemical disinfection with the use of chemical drugs (disinfectants) and physical disinfection with the use of moist heat, ultraviolet light, and other agents.

1-1. Chemical disinfection

Microorganisms are killed with chemical drugs. The killing effect and mechanisms of a chemical drug differ depending on the type, applied concentration, action temperature, and action time of the chemical drug used, the degree of contamination on the object to be disinfected, and the species and state (e.g., vegetative bacteria or spore bacteria) of microorganisms.

Therefore, in applying the method, full consideration is required of the sterility and permissible storage period of the prepared chemical drug, the possibility of resistance of microorganisms at the site of application, and the effect of residual chemical drug on the product. In selecting a suitable chemical drug, the following items should be considered in relation to the intended use.

- 1) The antimicrobial spectrum
- 2) Action time for killing microorganisms
- 3) Action durability
- 4) Effect of the presence of proteins

- 5) Influence on the human body
- 6) Solubility in water
- 7) Influence on the object to be disinfected
- 8) Odor
- 9) Convenience of use
- 10) Easy disposability
- 11) Influence on the environment at disposal
- 12) Frequency of occurrence of resistance

1-2. Physical disinfection

Microorganisms are killed without a chemical drug.

(i) Steam flow method

Microorganisms are killed by direct application of steam. This method is used for a product which may be denatured by the moist heat method. As a rule, the product is kept in flowing steam at 100°C for 30 - 60 minutes.

(ii) Boiling method

Microorganisms are killed by putting the object in boiling water. This method is used for a product which may be denatured by the moist heat method. As a rule, the product is put in boiling water for 15 minutes or more.

(iii) Intermittent method

Microorganisms are killed by heating for 30 - 60 minutes repeatedly, three to five times, once a day in water at 80 - 100°C or in steam. This method is used for a product which may be denatured by the moist heat method. There is another method called the low temperature intermittent method with repeated heating at 60 - 80°C. During the intermission periods between heating or warming, a suitable temperature for the growth of microorganisms of 20°C or higher, must be maintained.

(iv) Ultraviolet method

As a rule, microorganisms are killed by irradiation with ultraviolet rays at a wavelength of around 254 nm. This method is used for products which are resistant to ultraviolet rays, such as smooth-surfaced articles, facilities, and equipment, or water and air. This method does not suffer from the occurrence of resistance, which is observed in chemical disinfection, and shows a killing effect on bacteria, fungi, and viruses. It must be taken into consideration that direct ultraviolet irradiation of the human body can injure the eyes and skin.

2. Sterilization methods

2-1. Heating methods

In these methods, the heating time before the temperature or pressure reaches the prescribed value differs according to the properties of the product, the size of the container, and the conditions. The duration of heating in conducting these methods is counted from the time when all the parts of the product have reached the prescribed temperature.

(i) Moist heat method

Microorganisms are killed in saturated steam at a suitable temperature and pressure. This method is generally used for heat-stable substances, such as glass, porcelain, metal, rubber, plastics, paper, and fiber, as well as heat-stable liquids, such as water, culture media, reagents, test solutions, liquid samples, etc. As a rule, one of the following conditions is used.

- 115 - 118°C for 30 minutes
- 121 - 124°C for 15 minutes
- 126 - 129°C for 10 minutes

(ii) Dry-heat method

Microorganisms are killed in dry-heated air. This method