

is generally used for heat-stable substances, such as glass, porcelain, and metal, as well as heat-stable products, such as mineral oils, fats and oils, powder samples, etc. This method is generally conducted in the way of direct heating by gas or electricity or circulating heated air. As a rule, one of the following conditions is used.

- 160 – 170°C for 120 minutes
- 170 – 180°C for 60 minutes
- 180 – 190°C for 30 minutes

2-2. Irradiation methods

(i) Radiation method

Microorganisms are killed by gamma-rays emitted from a radioisotope or electron beam and bremsstrahlung generated from an electron accelerator. This method is generally used for radiation-resistant substances such as glass, porcelain, metal, rubber, plastics, fiber, etc. The dose is decided according to the material properties, and the degree of contamination of the product to be sterilized. Special consideration is necessary of the possibility of qualitative change of the product after the application of the method.

(ii) Microwave method

Microorganisms are killed by the heat generated by direct microwave irradiation. This method is generally used for microwave-resistant products such as water, culture media, test solutions, etc. As a rule, microwave radiation with a wavelength of around 2450 ± 50 MHz is used.

2-3. Gas methods

Microorganisms are killed by a sterilizing gas. Suitable gases for killing microorganisms include ethylene oxide gas, formaldehyde gas, hydrogen peroxide gas, chlorine dioxide gas, etc. Temperature, humidity, the concentration of gas, and the exposure time differ in accordance with the species of gas used. As sterilizing gases are generally toxic to humans, full consideration is required of the environmental control for the use of gases and the concentration of residual gas. In some of the gas methods, it may be difficult to measure or estimate quantitatively the killing of microorganisms.

2-4. Filtration method

Microorganisms are removed by filtration with a suitable filtering device. This method is generally used for gas, water, or culture media and test solutions containing a substance that is water-soluble and unstable to heat. As a rule, a filter having a pore size of $0.22 \mu\text{m}$ or smaller is used for the sterilization. However, in this method, a filter with a pore size of $0.45 \mu\text{m}$ or smaller is permitted to be used.

4. Guideline for Residual Solvents, Residual Solvents Test, and Models for the Test in Monographs

1. Guideline for Residual Solvents

Refer to the Guideline for Residual Solvents in Pharmaceuticals (PAB/ELD Notification No.307; dated March 30, 1998).

The acceptable limits of residual solvents recommended in the Guideline were estimated to keep the safety of patients. The levels of residual solvents in pharmaceuticals should not exceed the limits, except for in a special case. Accordingly, pharmaceutical manufacturers should assure the

quality of their products by performing the test with the products according to the Residual Solvents Test, to keep the limits recommended in the Guideline.

2. Residual Solvents Test

Method—Perform the test as directed in the Residual Solvents Test under the General Tests, Processes and Apparatus.

International harmonization—The test may also be performed according to the Residual solvents in the EP or the Organic volatile impurities in the USP. Even in this case, Monographs should be described in the JP style.

3. Models for the Test in Monographs

The following are typical examples for the test in Monographs, but these do not necessarily imply that other suitable operating conditions can not be used. It is important to prepare (draft) monographs according to the Guideline for the Preparation of the Japanese Pharmacopoeia.

1) A model for test item, amounts of test sample and reference standard (reference substance), preparation of the sample solution and the standard solution, injection amount for gas chromatography, calculation formula, and preparation of the internal standard solution

Residual solvents (or name of the solvent)—Weigh accurately about 0.200 g of $\triangle\triangle\triangle$ (name of the substance to be tested), add exactly 5 mL of the internal standard solution to dissolve, add water to make exactly 20 mL, and use this solution as the sample solution. If necessary filter or centrifuge. Separately, weigh exactly 0.10 g of $\circ\circ\circ$ reference substance (name of the solvent), put in a vessel containing 50 mL of water, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and 20 mL of water, and use this solution as the standard solution. Perform the test with $1 \mu\text{L}$ each of the sample solution and the standard solution as directed (in the head-space method) under the Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of $\triangle\triangle\triangle$ (name of the substance to be tested) to that of the internal standard of each solution, respectively. The amount of $\triangle\triangle\triangle$ should be not more than $\times \times$ ppm.

$$\begin{aligned} & \text{Amount of } \circ\circ\circ \text{ } (\mu\text{g}) \\ & = \text{amount of } \circ\circ\circ \text{ reference substance } (\mu\text{g}) \times \frac{Q_T}{Q_S} \end{aligned}$$

Internal standard solution—A solution of $\triangle\triangle\triangle$ in $\nabla\nabla\nabla$ (name of solvent) (1 in 1000).

2) Models for operating conditions for a head-space sample injection device

Operating conditions (1) for the head-space sample injection device—

Equilibration temperature for inside vial	A constant temperature of about 80°C
Equilibration time for inside vial	60 minutes
Transfer-line temperature	A constant temperature of about 85°C
Carrier gas	Nitrogen
Pressurisation time	30 seconds
Injection volume of sample	1.0 mL

Operating conditions (2) for the head-space sample injection device—

Equilibration temperature for inside vial	A constant temperature of about 105°C
Equilibration time for inside vial	45 minutes
Transfer-line temperature	A constant temperature of about 110°C
Carrier gas	Nitrogen
Pressurisation time	30 seconds
Injection volume of sample	1.0 mL

Operating conditions (3) for the head-space sample injection device—

Equilibration temperature for inside vial	A constant temperature of about 80°C
Equilibration time for inside vial	45 minutes
Transfer-line temperature	A constant temperature of about 105°C
Carrier gas	Nitrogen
Pressurisation time	30 seconds
Injection volume of sample	1.0 mL

3) Models for operating conditions and system suitability

In the operating conditions, generally, items required for the test such as detector, column, column temperature, carrier gas, flow rate, and time span of measurement should be specified, and in the system suitability, items such as test for required detectability, system performance, and system repeatability should be specified.

The following are several models for the operating conditions and the system suitability:

Operating conditions—

Detector: Specify in the following manner: "Hydrogen flame-ionization detector".

Column: Specify the inside diameter, length, material of column, name of stationary phase liquid and thickness of stationary phase in the following manner: "Coat the inside wall of a fused silica tube, 0.3 mm in inside diameter and 30 m in length, to 0.25 μm thickness with polyethylene glycol 20M." Describe the inside diameter and length of the column, and thickness or particle size of the stationary phase based on the data obtained for validation of the test method.

Column temperature: Specify in the following manner: "A constant temperature of about \times °C." or "Maintain at 40°C for 20 minutes, then increase to 240°C at 10°C per minute, and keep at 240°C for 20 minutes."

Carrier gas: Specify in the following manner: "Helium".

Flow rate: Specify in the following manner: "Adjust the flow rate so that the retention time of $\blacktriangle\blacktriangle\blacktriangle$ is about \times minutes." or "35 cm/second". Describe the flow rate based on the data obtained for validation of the test method.

Time span of measurement: About \times times as long as the retention time of $\triangle\triangle\triangle$ after the air peak.

System suitability—

Test for required detectability: Specify in the following manner: "Measure exactly \times mL of the standard solution, and add $\square\square\square$ to make exactly \times mL. Confirm that the peak area of $\square\square\square$ obtained from \times μL of this solution is equivalent to \times to $\times\times\%$ of that of $\square\square\square$ obtained from the standard solution". This item should be specified when, in Purity, the amount of an impurity is controlled by comparing the area of a specific peak from the sample solution

with that of the peak of $\times\times\times$ from the standard solution and the system repeatability alone is not sufficient to check the system suitability.

System performance: Specify in the following manner: "Dissolve \times g of $\square\square\square$ and \times g of $\triangle\triangle\triangle$ in \times mL of $\circ\circ\circ$. When the procedure is run with \times μL of this solution under the above operating conditions, $\square\square\square$ and $\triangle\triangle\triangle$ are eluted in this order with the separation coefficient between these peaks being not less than \times , and the number of theoretical plate and the symmetry coefficient of the peak of $\square\square\square$ are not less than $\times\times$ steps and not more than $\times\times\times$, respectively." This item should be specified in all of the test methods. Generally, the order of elution and the resolution, and in case of need (such as when the peak is asymmetrical) the symmetry coefficient, should be specified. The order of elution and the resolution may be replaced by the separation coefficient and the number of theoretical plates. When no suitable reference substance is available to check separation, the number of theoretical plates and the symmetry coefficient of the peak of the substance to be tested may be specified.

System repeatability: Specify in the following manner: "When the test is repeated \times times with \times μL of the solution for the system suitability test under the above operating conditions, the relative standard deviation of the peak areas of $\triangle\triangle\triangle$ is not more than $\times\%$." The system repeatability should be specified in any case, except in a qualitative test.

*Examples of operating conditions and system suitability**Test conditions (1)**Operating conditions—*

Detector: Hydrogen flame-ionization detector

Column: Coat the inside wall of a fused silica tube, 0.53 mm in inside diameter and 30 m in length, to 3 μm thickness with 6% cyanopropylphenyl-methyl silicon polymer for gas chromatography. Use a guard column if necessary.

Column temperature: Maintain at 40°C for 20 minutes, then increase to 240°C at 10°C per minute if necessary, and keep at 240°C for 20 minutes.

Injection port temperature: A constant temperature of about 140°C

Detector temperature: A constant temperature of about 250°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: 1:5

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

*Test conditions (2)**Operating conditions—*

Detector: Hydrogen flame-ionization detector

Column: Coat the inside wall of a fused silica tube, 0.53 mm in inside diameter and 30 m in length, to 5 μm thickness with 5% phenyl-methyl silicon polymer for gas chro-

matography. If necessary use a guard column prepared by coating the inside wall of a fused silica tube, 0.53 mm in inside diameter and 5 m in length, to 5 μm thickness with 5% phenyl-methyl silicon polymer for gas chromatography.

Column temperature: Maintain at 35°C for 5 minutes, then increase to 175°C at 8°C per minute, further increase to 260°C at 35°C per minute if necessary, and keep at 260°C for 16 minutes.

Injection port temperature: A constant temperature of about 70°C

Detector temperature: A constant temperature of about 260°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: Splitless

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

Test conditions (3)

Operating conditions—

Detector: Hydrogen flame-ionization detector

Column: Coat the inside wall of a fused silica tube, 0.32 mm in inside diameter and 30 m in length, to 0.25 μm thickness with polyethylene glycol 20M for gas chromatography. Use a guard column if necessary.

Column temperature: Maintain at 50°C for 20 minutes, then increase to 165°C at 6°C per minute if necessary, and keep at 165°C for 20 minutes.

Injection port temperature: A constant temperature of about 140°C

Detector temperature: A constant temperature of about 250°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: 1:5

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

5. International Harmonization Implemented in the Japanese Pharmacopoeia Fourteenth Edition

Items for which harmonization has been agreed among

the European Pharmacopoeia, the United States Pharmacopoeia and the Japanese Pharmacopoeia are implemented in the Japanese Pharmacopoeia Fourteenth Edition (JP 14). They are shown in the table below. The column headed Harmonized items shows the harmonized items written in the Pharmacopoeial Harmonization Agreement Document, and the column headed JP 14 shows the items as they appear in JP 14. In the Remarks column, notes on any differences between JP 14 and the agreement are shown as occasion demands.

Harmonized items	JP 14	Remarks
Bacterial Endotoxin Test	Bacterial Endotoxin Test	
Apparatus	Apparatus	
Preparation of Standard Endotoxin Stock solution	Preparation of Standard Endotoxin Stock solution	
Preparation of Standard Endotoxin solution	Preparation of Standard Endotoxin solution	
Preparation of sample solutions	Preparation of sample solutions	
Determination of Maximum Valid Dilution	Determination of Maximum Valid Dilution	
Gel-clot technique	Gel-clot technique	
(1) Preparatory testing	(1) Preparatory testing	
(2) Limit test	(2) Limit test	
(3) Assay	(3) Assay	
Photometric techniques	Photometric techniques	
(1) Turbidimetric technique	(1) Turbidimetric technique	
(2) Chromogenic technique	(2) Chromogenic technique	
(3) Preparatory testing	(3) Preparatory testing	
(4) Assay	(4) Assay	
Reagents, Test Solutions	Reagents, Test Solutions	
Amebocyte lysate	Lysate reagent	
Lysate TS	Lysate TS	
Water for bacterial endotoxins test (BET)	Water for bacterial endotoxins test	

Note: The method for decision of the limit for bacterial endotoxins was agreed between the three pharmacopoeias, but in the Decision of Limit for Bacterial Endotoxins under the General Information in JP 14, the maximum adult dose is calculated based on an average body mass of an adult of 60 kg.

6. Media Fill Test

The media fill test (MFT) is one of the processing validations employed to evaluate the propriety of the aseptic processing of pharmaceutical products using sterile media, etc. instead of actual products. Therefore, media fill tests should be conducted with the manipulations normally performed in actual processing, e.g. filling and closing operation, operating environment, processing operation, number of personnel involved, etc., and conducted under processing conditions that include "worst case" conditions. Refer to GMP (1), WHO/GMP for pharmaceutical products (2), and ISO 13408 (3), etc. for necessary information to conduct this test.