

physical strength of uncoated tablets upon exposure to mechanical shock or attrition.

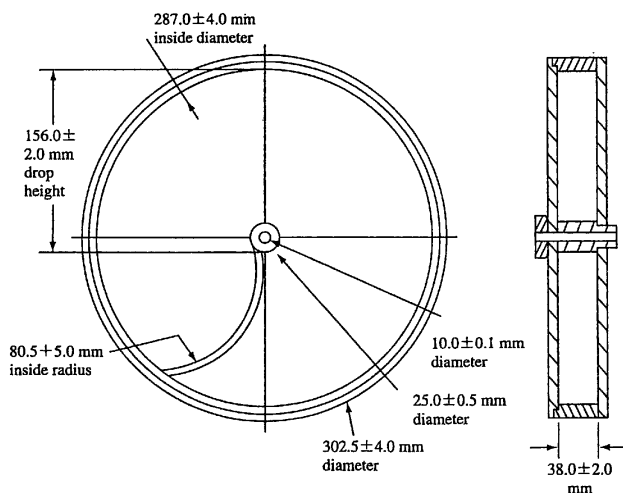
Apparatus

The apparatus consists of a drum and a motor. Use a drum with an inside diameter of about 287 mm and about 38 mm in depth, made of a transparent synthetic polymer with polished internal surfaces and not subject to static build-up, as illustrated in the figure. One side of the drum is removable.

Procedure

The drum is attached to the horizontal axis of a device that rotates at 24 – 26 revolutions per minute. For tablets weighing up to 650 mg each, take a sample consisting of the minimum number of tablets that makes a total mass of more than 6.5 g. For tablets weighing more than 650 mg each, take a sample of ten tablets. Dust should be carefully removed from the tablets prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum. Rotate the drum 100 times, and remove the tablets. Remove any loose dust from the tablets as before. If no tablets are cracked, split or broken, accurately weigh the tablets, and determine the friability (mass per cent of the lost mass with respect to the initial mass).

If the tablet size or shape results in irregular tumbling, adjust the drum base so that the base forms an angle of about 10° with the bench top and the tablets can fall freely when the drum is rotated. A drum with dual scooping supports for the running of two samples at the same time is also available. In the case of hygroscopic tablets, a humidity-controlled environment (relative humidity less than 40%) is required for testing.



15. Terminal Sterilization and Sterilization Indicators

Sterilization is a process whereby the killing or removal of all forms of viable microorganisms in substances is accomplished. It is achieved by terminal sterilization or a filtration method. For substances to which terminal sterilization can be applied, an appropriate sterilization method should be selected in accordance with the properties of the product, in-

cluding the packaging, after full consideration of the advantages and disadvantages of each sterilization method, from among the heat method, irradiation method and gas method. After installation of the sterilizer (including design and development of the sterilization process), validation is required to confirm that the sterilization process is properly performing its designed function, under conditions of loading and unloading of the product, on the basis of sufficient scientific evidence. After the process has been validated and the sterilization of the product commenced, the process must be controlled correctly, and qualification tests of the equipment and procedures must be performed regularly. The bioburden per product, prior to terminal sterilization, must be evaluated periodically or on the basis of batches. Refer to the ISO standard (ISO 11737-1) relevant to bioburden estimation. For a substance to which terminal sterilization can be applied, generally use sterilization conditions such that a sterility assurance level of less than 10^{-6} can be obtained. The propriety of the sterilization should be judged by employing an appropriate sterilization process control, with the use of a suitable sterilization indicator, and if necessary, based on the result of the sterility test. The filtration procedure is used for the sterilization of a liquid product, to which terminal sterilization can not be applied. Concerning the disinfection and/or sterilization necessary for processing equipment and areas of pharmaceutical products, and performing microbiological tests specified in the monographs, see Disinfection and Sterilization Methods.

1. Definitions

The definitions of the terms used in this text are as follows.

Terminal sterilization: A process whereby a product is sterilized in its final container or packaging, and which permits the measurement and evaluation of quantifiable microbial lethality.

Product: A generic term used to describe raw materials, intermediate products, and finished products, to be sterilized.

Bioburden: Numbers and types of viable microorganisms in a product to be sterilized.

Sterility assurance level (SAL): Probability of a viable microorganism being present in a product unit after exposure to the proper sterilization process, expressed as 10^{-n} .

Integrity test: A non-destructive test which is used to predict the functional performance of a filter instead of the microorganism challenge test.

D value: The value which shows the exposure time (decimal reduction time) or absorbed dose (decimal reduction dose) required to cause a 1-logarithm or 90% reduction in the population of test microorganisms under stated exposure conditions.

Sterilization indicator: Indicators used to monitor the sterilization process, or as an index of sterility, including biological indicators (BI), chemical indicators (CI), dosimeters and the like.

2. Sterilization

2-1. Heat Method

In the heat method, microorganisms are killed by heating.

(i) Moist heat method

Microorganisms are killed in saturated steam under pressure. In this method, factors which may affect the sterilization include temperature, steam pressure and exposure time.

Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, steam pressure and exposure time, and they should be included in the specifications of the sterilizer.

(ii) Dry-heat method

Microorganisms are killed in dry heated air. This method is usually conducted in a batch-type dry heat sterilizer or a tunnel-type dry heat sterilizer. In this method, factors which may affect the sterilization include temperature and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature and exposure time, and they should be included in the specifications of the sterilizer.

2-2. Irradiation method

Microorganisms are directly killed by ionizing radiation, or by the heat generated by microwave radiation.

(i) Radiation method

Ionizing radiations which may be used are gamma rays emitted from a radioisotope such as cobalt 60, an electron beam and bremsstrahlung (X rays) generated from an electron accelerator. Although any procedure can be applied to thermally unstable products with no radioactivity residue, it is necessary to consider the possibility of material degradation. Although a 25 kGy dose is traditionally used as a sterilization dose, there are some ways to calculate the dose as follows: the bioburden of the substance to be sterilized is measured and the sterilization dose is calculated based on the mean bioburden and the standard resistance distribution (Method 1 in ISO 11137), the dose is calculated from the fraction positive information from a sterility test in which representative product samples are exposed to a substerilizing dose (Method 2 in ISO 11137), or the dose is calculated based on the bioburden and D value of the most resistant microorganisms (Log method) (see 5-3). In the case of the radiation sterilization procedure, factors which may affect the sterilization include dose (absorbed dose) and exposure time. Therefore, in gamma ray sterilization process control, it is required to determine the dose (the absorbed dose) at appropriate intervals and to monitor continuously the exposure time in terms of the operating parameters (the conveyer speed, the cycle time). The dose control mechanism should be included in the specifications of the sterilizer. In the case of electron beam or bremsstrahlung irradiation, it is required to monitor the acceleration voltage, the beam current and beam scanning width besides the above-mentioned items.

(ii) Microwave method

Microorganisms are killed by the heat generated by microwave radiation, usually at the frequency of 2450 ± 50 MHz. This method is applied to liquids or water-rich products in sealed containers. Since a glass or plastic container may be destroyed or deformed due to the rise of the inner pressure, the containers must be certified to be able to withstand the heat and the inner pressure generated during microwave sterilization. Leakage of electromagnetic radiation must be at a sufficiently low level to cause no harm to humans and no interference with radio communications and the like. In this method, factors which may affect the sterilization include temperature, processing time and microwave output power. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, time and the microwave output power, and they should be included in the specifications of the sterilizer.

2-3. Gas method

Ethylene oxide (EO) is widely used as a sterilization gas. Since EO gas has an explosive nature, a 10 – 30% mixture with carbon dioxide is commonly used. Also, as EO gas is a strong alkylating agent, it can not be applied to the products which are likely to react with or absorb it. Furthermore, because EO gas is toxic, the residual concentration of EO gas and other secondarily generated toxic gases in products sterilized with EO gas must be reduced to less than the safe levels thereof by means of aeration and the like before the product is shipped. In this method, factors which may affect the sterilization include temperature, gas concentration (pressure), humidity and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, gas concentration (pressure), humidity and exposure time, and they should be included in the specifications of the sterilizer.

3. Filtration method

Microorganisms are removed by using a sterilizing filter made of an appropriate material. However, this method is not intended for microorganisms smaller than bacteria. Generally, a sterilizing filter challenged with more than 10^7 microorganisms of a strain of *Brevundimonas diminuta* (ATCC 19146, IFO 14213, JCM 2428), cultured under the appropriate conditions, per square centimeter of effective filter area should provide a sterile effluent. In this method, factors which may affect the sterilization include pressure, flow rate, filter unit characteristics and the like. In routine filtration process control, it is required to perform integrity tests of the sterilizing filter after each filtration process (also prior to the filtration process, if necessary).

4. Sterilization Indicators

4-1. Biological indicator (BI)

A BI is prepared from specific microorganisms resistant to the specified sterilization process and is used to develop and/or validate a sterilization process. The dry type BI is classified into two kinds. In one, bacterial spores are added to a carrier such as filter paper, glass or plastic and then the carriers are dried and packaged. In the other, bacterial spores are added to representative units of the product to be sterilized or to simulated products. Packaging materials of the BI should show good heat penetration in dry heat sterilization and good gas or steam penetration in ethylene oxide and moist heat sterilizations. It should be confirmed that any carrier does not affect the D value of the spores. In the case of a liquid product, the spores may be suspended in the same solution as the product or in a solution showing an equivalent effect in the sterilization of biological indicator. However, when the spores are suspended in liquid, it is necessary to ensure that the resistance characteristics of the spores are not affected due to germination.

Typical examples of biological indicator

Sterilization	Representative microorganisms*	Strain name
Moist heat method	<i>Bacillus stearothermophilus</i>	ATCC 7953, IFO 13737, JCM 9488 ATCC 12980, IFO 12550, JCM 2501
Dry heat method	<i>Bacillus subtilis</i>	ATCC 9372, IFO 13721
Gas method	<i>Bacillus subtilis</i>	ATCC 9372, IFO 13721

* In addition to these microorganisms, other microorganisms with the greatest resistance to the sterilization procedure concerned, found in the bioburden, can be used as the biological indicator.

4-1-1. D value of BI

Methods for determination of the D value include the survival curve method and the fraction negative method (Stumbo, Murphy & Cochran procedure, Limited Spearman-Kärber procedure and the like). In using marketed BIs, it is usually unnecessary to determine the D value before use if the D value indicated on the label has been determined by a standardized biological indicator evaluation resistometer (BIER) under strictly prescribed conditions in accordance with ISO 11138-1. It is acceptable that the D value indicated on the label shows a scattering of not more than ± 30 seconds.

4-1-2. Setting up procedure of BI

(i) In the case of dry materials

A Dry type BI is placed at predetermined cold spots in the product to be sterilized or a suitable product showing an equivalent effect in the sterilization. The BIs are usually primary packaged in the same way as the product, including a secondary packaging, if applicable.

(ii) In the case of wet materials

Spores are suspended as the BI in the same solution as the product or in an appropriate similar solution, and should be placed at cold spots in the sterilizer.

4-1-3. Culture conditions of BI

Soybean casein digest medium is generally used. General culture conditions are at 55 – 60°C for 7 days in the case of *B. stearothermophilus* and at 30 – 35°C for 7 days in the case of *B. subtilis*.

4-2. Chemical indicator (CI)

CI is an indicator which shows a color change of a substance applied to a paper slip, etc. as a result of physical and/or chemical change due to exposure to heat, gas or radiation. The CI can be classified into three types. The first is employed to identify whether or not sterilization has already been implemented, the second is employed to control the sterilization process (for example, its color changes after sterilization for a sufficient time), and the third is the Bowie & Dick type used to evaluate the effectiveness of air removal during the pre-vacuum phase of the pre-vacuum sterilization cycle.

4-3. Dosimeter

In the radiation (gamma-ray) method, the sterilization effect depends on the absorbed radiation dose, so the sterilization process control is mainly performed by measuring the dose. A dosimeter is installed at a position corresponding to the minimum dose region of an exposed container or a position where the dose is in a known relation to that in the above region. Measurement should be done for each radiation batch. If there are many containers in the same batch, dosimeters should be employed so that more than one dosimeter is always installed at the effective radiation section of the irradiation chamber. It should be noted that dosimeters may be affected by environmental conditions (temperature, humidity, ultraviolet light, time until reading, etc.) before and during irradiation. Practical dosimeters for gamma-ray and bremsstrahlung sterilization include the dyed polymethylmethacrylate dosimeter, clear polymethylmethacrylate dosimeter, ceric-cerous sulfate dosimeter, alanine-EPR dosimeter and the like. A dosimeter

for gamma radiation can not generally be used for sterilization process control with an electron beam of less than 3 MeV energy. Dosimeters for electron beam sterilization include the cellulose acetate dosimeter, radiochromic film dosimeter and the like. A practical dosimeter must be calibrated against an appropriate national or international standard dosimetry system.

5. Determination of sterilization conditions using microorganism as an indicator

Taking account of the characteristics upon the sterilization concerned, bioburden, etc. of a product to be sterilized, chose a suitable method from the followings and determine the conditions.

5-1. Half-cycle method

In this method, a sterilization time of twice as long as that required to inactivate all of 10^6 counts of BI placed in the product is used, regardless of bioburden count in the product being sterilized or the resistance of the objective microorganisms to the sterilization.

5-2. Overkill method

In this method, a sterilization condition giving a sterility assurance level of not more than 10^{-6} counts is used, regardless of bioburden count in the product being sterilized or the resistance of the objective microorganisms to the sterilization. Generally, a sterilization condition providing 12 logarithmic reduction (12D) of a known count of BI of more than 1.0 D value is used.

5-3. Combination of BI and bioburden

Generally, a count of mean bioburden added three times of its standard deviation obtained by an extensive bioburden estimation is considered as the maximum bioburden count, and the sterilization time (or radiation dose) is calculated with the bioburden count based on an objective sterility assurance level. When this procedure is used, it is required to determine the resistance of the bioburden to the sterilization as well as the bioburden count in the product being sterilized. If a more resistant microorganism than the BI spore is found in the bioburden estimation, it should be used as the BI.

$$\text{Sterilization time (or radiation dose)} = D \times \log \frac{N_0}{N}$$

D: D value of the BI

N: Sterility assurance level

N_0 : Maximum bioburden count in the product

5-4. Absolute bioburden method

The sterilization conditions are determined by employing the D value of the most resistant microorganism found in the product or environment by the resistant estimation and being based on the bioburden count in the product. Generally, a count of mean bioburden added three times of its standard deviation obtained by an extensive bioburden estimation is employed as the bioburden count. When this procedure is used, it is required to make frequent counting and resistance determination of microorganisms in daily bioburden estimation.

References

- 1) ISO 11134 Industrial moist heat sterilization
- 2) ISO 11135 Ethylene oxide sterilization
- 3) ISO 11137 Radiation sterilization
- 4) ISO 11138 Biological indicators

- 5) ISO 11140 Chemical indicators
- 6) ISO 11737 Microbiological methods
Part 1: Estimation of population of micro-organisms on products

16. Validation of Analytical Procedures

The validation of an analytical procedure is the process of confirming that the analytical procedure employed for a test of pharmaceuticals is suitable for its intended use. In other words, the validation of an analytical procedure requires us to demonstrate scientifically that risks in decision by testing caused by errors from analytical steps are acceptably small. The performance of an analytical procedure is established by various kinds of validation characteristics. The validity of a proposed analytical procedure can be shown by demonstrating experimentally that the validation characteristics of the analytical procedure satisfy the standards set up according to the acceptable limits of testing.

When an analytical procedure is to be newly carried in the Japanese Pharmacopoeia, when a test carried in the Japanese Pharmacopoeia is to be revised, and when the test carried in the Japanese Pharmacopoeia is to be replaced with a new test according to regulations in general notices, analytical procedures employed for these tests should be validated according to this document.

Required data for analytical procedures to be carried in the Japanese Pharmacopoeia

(1) Outline

This section should provide a brief explanation of the principle of a proposed analytical procedure, identify the necessity of the analytical procedure and its advantage compared with other procedures, and summarize the validation. When an analytical procedure is revised, the limitation of the current analytical procedure and the advantage offered by the new analytical procedure should be described.

(2) Analytical procedure

This section should contain a complete description of the analytical procedure to enable skilled persons to evaluate correctly the analytical procedure and replicate it if necessary. Analytical procedures include all important operating procedures for performing analyses, the preparation of standard samples, reagents and test solutions, precautions, procedures to verify system suitability (e.g. the verification of the separating performance of a chromatographic system), formulas to obtain results, the number of replications and so forth. Any instruments and apparatus that are not stated in the Japanese Pharmacopoeia should be described in detail. The physical, chemical or biological characteristics of any new reference standards should be clarified and their testing methods should be established.

(3) Data showing the validity of analytical procedures

This section should provide complete data showing the validity of the analytical procedures. This includes the experimental designs to determine the validation characteristics, experimental data, calculation results and results of hypothesis tests.

Validation characteristics

The definition of typical validation characteristics to be as-

essed in validation of analytical procedures and examples of assessing procedures are given below.

The terminology and definitions of the validation characteristics may possibly vary depending upon the fields to which analytical procedures are applied. The terminology and definitions shown in this document are established for the purpose of the Japanese Pharmacopoeia. Typical methods for assessing the validation characteristics are shown in the item of assessment. Various kinds of methods to determine the validation characteristics have been proposed and any methods that are widely accepted will be accepted for the present purpose. However, since values of the validation characteristics may possibly depend upon methods of determination, it is required to present the methods of determining the validation characteristics, the data and calculation methods in sufficient detail.

Although robustness is not listed as a validation characteristic, it should be considered during the development of analytical procedures. Studying the robustness may help to improve analytical procedures and to establish appropriate analytical conditions including precautions.

(1) Accuracy/Trueness

Definition: The accuracy is a measure of the bias of observed values obtained by an analytical procedure. The accuracy is expressed as the difference between the average value obtained from a large series of observed values and the true value.

Assessment: The estimate of accuracy of an analytical method is expressed as the difference between the total mean of observed values obtained during investigation of the reproducibility and the true value. The theoretical value is used as the true value (e.g., in the case of titration methods, etc.). When there is no theoretical value or it is difficult to obtain a theoretical value even though it exists, a certified value or a consensus value may be used as the true value. When an analytical procedure for a drug product is considered, the observed value of the standard solution of the drug substance may be used as the consensus value.

It may be inferred from specificity data that an analytical procedure is unbiased.

The estimate of accuracy and a 95% confidence interval of the accuracy should be calculated using the standard error based on the reproducibility (intermediate precision). It should be confirmed that the confidence interval includes zero or that the upper or lower confidence limits are within the range of the accuracy required of the analytical procedure.

(2) Precision

Definition: The precision is a measure of the closeness of agreement between observed values obtained independently from multiple samplings of a homogenous sample and is expressed as the variance, standard deviation or relative standard deviation (coefficient of variation) of observed values.

The precision should be considered at three levels with different repetition conditions; repeatability, intermediate precision and reproducibility.

(i) Repeatability/Intra-assay precision

The repeatability expresses the precision of observed values obtained from multiple samplings of a homogenous sample over a short time interval within a laboratory, by the same analyst, using the same apparatus and instruments, lots of reagents and so forth (repeatability conditions).