Evaluation Guideline for Sterile Quasi-Drugs

August 2009

Korea Food & Drug Administration

Biopharmaceuticals and Herbal Medicine Bureau

Cosmetics Review Division

This guideline describes the view of KFDA based on the experience up to now and scientific facts. The contents described in this guideline do not have legal effects, and they are the recommendations of KFDA except the ones regulated by laws or administrative rules, etc. This guideline may be amended if there are new scientific grounds. Therefore, if you have these new scientific facts, suggest your opinion to the KFDA.

※ If you have comments on this guideline, please contact Cosmetic Review Division, Biopharmaceuticals and Herbal Medicine Bureau in KFDA.

Telephone +82-43-719-3603, 3608, 3610, 3612

FAX +82-43-719-3600

Greetings

To protect wounds, etc. frequently occurring in everyday life, paper products such as a disposable Band-Aid, etc. are often used. According to the Pharmaceutical Affairs Act, fiber, rubber products or their similar agents used to treat, alleviate, handle or prevent diseases of humans or animals should be managed as quasi-drugs. The detailed range of quasi-drugs should be regulated by the notification of the Minister of Ministry of Health and Welfare. The quasi-drugs include masks, bandage, cotton ball, Band-Aid as well as sanitary pad. Some products are sterilized by sterilization process such as irradiation or ethylene oxide gas, etc.

When sterilizing products by irradiation, changes in physicochemical stability and biological activity of the products may occur after irradiation, and degraded products from irradiation may also occur. Therefore, the data proving the stability of the products should be submitted and approved by the KFDA. In addition, in terms of ethylene oxide sterilized products, residuals should be conducted to identify whether ethylene oxide gas or ethylene chlorohydrin, its degraded product, remains in the product.

The Biopharmaceuticals and Herbal Medicine Bureau, therefore, provides this guideline to standardize analytical methods such as stability test of irradiated products and residual test for ethylene oxide to help related companies and reviewers. This guideline presents standardized analytical methods and criteria for stability test of radiation sterilized products and ethylene oxide residual test as well as requirements for data submission.

I hope that this guideline will be helpful for quality evaluation of sterilized quasi-drugs, contributing to the development of the relevant industry and national health.

August 2009

Jeong-seok Lee

Head of Biopharmaceuticals and Herbal Medicine Bureau

Ministry of Food and Drug Safety

<Table of Contents>

I. Introduction

II. Stability Test of Radiation Sterilized Products

1. Definition of Terms
2. Test Criteria
3. Test Method
4. Submission of Test Data

III. Ethylene Gas Residual Test of Ethylene Oxide Sterilized Products

1. Test Criteria
2. Test Method
3. Submission of Test Data

IV. Reference

I. Introduction

1. This guideline is related to the review of sterile quasi-drugs submitted in relation to Article 14 Paragraph 7 of the Regulation on Pharmaceuticals Approval, Notification and Review KFDA Notification No. 2009-42 (June 30, 2009) for radioactive sterility and the Regulation on Standards and Analytical Method of Quasi-drugs KFDA Notification No. 2009-6 (February 23, 2009) for ethylene oxide sterility.

2. It is regulated that "when the final product is irradiated to sterilize as occasion demands, the condition (radioactivity level, time, etc.) shall be specified, and stability test data (3 lots) including comparisons of generation of degradation products which are irradiated and not irradiated shall also be attached," in Article 14 Paragraph 7 of the Regulation on Pharmaceuticals Approval, Notification and Review KFDA Notification No. 2009-42 (June 30, 2009).

3. In the Standard and Analytical Methods of Quasi-drugs, it is provided that "ethylene oxide (EO) gas residual test should be conducted for the products sterilized by ethylene oxide gas," in the Standard and Analytical Method of Tampons for Menstruation.

4. This guideline is to provide help in preparation of materials required for the review of radiation sterilization and ethylene oxide sterilization products among sterility process necessary for each product in manufacturing and importing quasi-drugs.

II. Stability Test for Radiation-Sterilized Products

Pharmaceuticals, etc. may be affected after irradiation in the manufacturing process regarding efficacy change, physicochemical stability, biological toxicity, etc. of the product. Thus, to receive approval for radiation-sterilized products, the following stability test materials should be submitted to identify whether physicochemical changes occur or degradation products are generated.

1. Definitions of Terms

① Sterilization: Refers to destroy or remove all microbes by giving physical and chemical stimuli. Therefore, sterilization which makes the target completely aseptic is distinguished from disinfection which makes the target reach nearly aseptic status.

② Acceleration test: Refers to a test to evaluate the effect on stability of pharmaceuticals, etc. in an accelerated condition at a short period.

2. Test Criteria (Acceleration Test)

1) Criteria

A. The items which are described in the standard and analysis method should be adequate to the criteria.

B. The dissolution test should be adequate for the following criteria.

① pH criteria: The difference of pH between the test and control solutions is not more than 1.5.

② Ultraviolet absorption spectrum (hereinafter UV) criteria: The absorbance at 220 ~ 241 nm is not more than 0.08, and the absorbance at 241 ~ 350 nm is not more than 0.05.

③ Cytotoxicity criteria: The concentration of test solution of which cell colony formation rate is 50% (IC_{50}(%))is not less than 90%. The result from other standard analytical methods is negative.

2) Selection of Lot

A. The same prescription, dosage form and packaging container as the product to be marketed should be used. However, lots not considered to affect the stability may be exceptable.

B. It is principle to test 3 lots and more.

3) Storage Condition: Temperature of 40 ± 2℃, relative humidity of 75 ± 5% or adequate relative humidity by temperature should be considered to set the temperature which is 15 ℃ higher than the designated storage temperature. However, for the semipermeable containers, temperature should be 40 ± 2℃, and relative humidity should be not more than 25%.

4) Test Period: Test for 6 months and more.

5) Measurement Period: At least 3 tests should be conducted including the beginning of the test at interval of 2 months from the date of test onset. If significant changes are observed in the acceleration test during the development stage, at least 4 tests should be conducted.

(Examples of significant changes)

A) Not adequate for the specifications

B) Change of content of 5% and more than the initial value occurs

C) The results of pH or dissolution test (pH, UV and cytotoxicity) are not adequate for the criteria, depending on dosage forms

D) The products are not adequate for the criteria in the appearance, physical property and functional tests

6) Test Items:

A. Regarding the irradiated and non-irradiated products, all items set in the standard and analytical methods should be the principle.

B. Regarding the irradiated and non-irradiated products, the comparison test of generation of degradation products should be conducted.

※ If degradation products can be compared through content test, etc., the generation of degradation products before and after irradiation may be proved by LC, GC, etc. However, in terms of quasi-drugs including cotton ball, gauze, etc., it is impossible to compare degradation products

with HPLC analysis, etc. for most quasi-products, pH and UV test materials of the eluates should be submitted. If content test can be conducted, cytotoxicity test results should be submitted if there are degradation products through ingredient analysis. In addition, if plastic is included such as pack gauze, etc., cytotoxicity test materials of the eluates should be submitted.

3. Test Methods

1) The items set in the standard and analytical method should be tested according to the test method of the approved criteria.

2) pH, UV and Cytotoxicity Test for Eluates

A. Manufacturing of Test and Control Solutions

With the irradiated product as the sample, cut it in a way that the sum of surface areas of both front and back sides should be 600 cm^2 to collect the cut sides. Cut them in an adequate size, wash them with water, and then dry them in the room temperature. Add these dried products in a container, add 200 mL water accurately, and close it with a stopper properly. Then, heat them with a high pressure steam sterilizer at 121℃ for 1 hour, take out the container, and leave it until the temperature reaches the room temperature. Use the extracted liquid as test solution. Control the non-irradiated product separately to product control solution. With the test and control solutions, conduct the following test.

B. Operation

(1) pH: Take 20 mL each of test and control solutions, add 1.0 mL of potassium chloride solution (1→1000) in them, measure pH of the 2 solutions, and calculate the difference.

(2) UV: Test the test and control solutions according to the ultraviolet-visible spectrophotometry, and measure the maximum absorbance at wavelength of 220 ~ 240 nm and 241 ~ 350 nm.

(3) Cytotoxicity: This experiment is to examine the effects of the irradiated products on cytolysis (cell necrosis) by eluates, cell

growth inhibition and other effects on cells. When producing test solutions, it is necessary to be cautious that contamination should not occur from microbes and other foreign matters. In addition to this test method, a standard test method equivalent to or better than this method can be used. However, if there is a suspect on the test results, the final judgment should be made through the method regulated in this method.

① Cell line: The cell line should be L929 cell (ATCC CCL1). Conduct subculture of this cell in Eagle minimum essential medium with fetal bovine serum. Until the cell layer covers 80% or more of plate, incubate it in CO_2 incubator with the concentration of CO_2 5 ± 1% and temperature 36 ~ 38℃ for 24 hours and longer. When observing the cell culture with a microscope, identify whether it has uniform and constant cell layers. However, a different cell line may be used if the form of cell colony and reproducibility of the results are almost same as the described cell line, after testing the above items in advance.

② Media: Use Eagle minimum essential medium. Melt the following substances in 1000 mL water, sterilize them in high pressure steam sterilization for 20 minutes at 121℃, and then add sodium bicarbonate solution 22 mL and glutamine solution 10 mL which were sterilized separately. Here, add fetal bovine serum to make 10 v/v%.

Sodium chloride	6.80 g	Potassium chloride	400 mg
Sodium dihydrogen phosphate (anhydrous)	115 mg	Magnesium sulfate (anhydrous)	93.5 mg
Calcium chloride (anhydrous)	200 mg	Glucose	1.00 g
L-Arginine hydrochloride	126 mg	L-Cysteine Monohydrochloride (monohydrate)	31.4 mg
L-Tyrosine	36.0 mg	L-Histidine Monohydrochloride (monohydrate)	24.0 mg
L-Isoleucine	52.0 mg	L-Leucine	52.0 mg
L-Lysine hydrochloride	73.0 mg	L-Methionine	15.0 mg
L-Phenylalanine	32.0 mg	L-Threonine	48.0 mg
L-tryptophan	10.0 mg	L-Valine	46.0 mg

Succinic acid	75.0 mg	Sodium succinate (hexahydrate)	100 mg
Choline bitartrate	1.8 mg	Folic acid	1.0 mg
Myo-inositol	2.0 mg	Nicotine acid amide	1.0 mg
D-Calcium pantothenate	1.0 mg	Pyridoxal hydrochloride	1.0 mg
Riboflavin	0.1 mg	Thiamine hydrochloride	1.0 mg
Biotin	0.02 mg	Phenol red	6.0 mg

C. Reagents

① Sodium bicarbonate solution: Dissolve 10 g of sodium bicarbonate solution to make 100 mL, sterilize it airtight in high pressure steam sterilization at 121℃ for 20 minutes or filter it through a membrane filter with diameter not more than 0.22 ㎛ to sterilize.

② Glutamine solution: Melt L-glutamine 2.92 g in the water to make 100 mL, and filter it through a membrane filter with diameter not more than 0.22 ㎛ to sterilize.

③ Sodium chloride buffer: Dissolve potassium chloride 0.20 g, monobasic potassium phosphate 0.20 g, sodium chloride 8.00 g, and dibasic sodium phosphate (anhydrous) 1.15 g in the water to make 1000 mL. Sterilize it in high pressure steam sterilization at 121℃ for 20 minutes.

④ Trypsin solution: Dissolve trypsin 0.5 g, ethylene diaminetetraacetic acid 0.2 g in phosphate buffer to make 1000 mL, and filter it through a membrane filter with diameter not more than 0.22 ㎛ to sterilize.

⑤ Diluted formaldehyde solution: Dilute formaldehyde solution 10 times with water.

⑥ Diluted Giemsa test solution: Dilute giemsa test solution about 50 times with diluted solution, and filter it through a filter paper to remove insoluble matters. Make the test solution at use.

※ Giemsa test solution: Add azur II-eosin 3 g and azur II 0.8 g in glycerin 250 g, rise temperature at 60℃ to melt it, cool down, and then add methanol 250 g to mix well. Leave it for 24 hours and then filter. Cover it with a stopper and store it.

⑦ Diluted solution: Dissolve potassium dihydrogen phosphate 4.54 g and anhydrous sodium dihydrogen phosphate 4.75 g in the water to make 1000 mL.

D. Instrument and Equipment

① Pipette: Pasteur pipette, mess pipette and tipped micropipette

② Corkscrew glass bottle: 50 ~ 1000 mL

③ Plastic sterile centrifuge tube: 15 mL and 50 mL

④ Plastic sterile culture flask: 25 cm^2 or 75 cm^2

⑤ Plastic sterile culture plate (24 columns)

⑥ Microscope: Inverted and stereoscopic microscope

⑦ CO_2 Incubator: Maintain CO_2 concentration 5% and temperature 37℃

⑧ Positive control culture medium: Select the one appropriate for the characteristics of the product such as polyurethane film containing zinc diethyldithiocarbamate

⑨ Negative control culture medium: Select the one appropriate for the characteristics of the product such as polyethylene film, etc.

E. Operation

(1) Preparation of cell suspension: Remove the culture medium from the plastic sterile culture flask in which cells have been incubated, add an adequate amount of phosphate buffer still, tilt the flask twice or 3 times slowly to wash the cell layer, and then throw away the phosphate buffer. Add trypsin solution in a degree that the cell layers should not be exposed, cover the flask with a stopper, put the flask in the CO_2 incubator, and leave it for 1 ~ 2 minutes. Take the flask out of the incubator, and observe the peeled status through a microscope. Add an adequate amount of medium, take it with Pasteur pipette still, and peel the cell from the wall of the flask completely. Transfer this solution in the plastic sterile centrifuge tube, and centrifuge it at 800 ~ 1000 rotations per 1 minute for 2 ~ 5 minutes. Discard the supernatant liquid, add a certain amount of new media, take it with Pasteur pipette still to make uniform cell suspension, and measure the cell

concentration using a counting chamber.

(2) Cell Toxicity Test: Dilute the cell suspension with medium to make cell concentration 10^5 ea/mL. Dispense 0.5mL each of this solution in each column of the plasticsterile culture plate. Station the culture plate in the CO_2 incubator for 4 ~ 6 hours to attach the cells in the bottom panel of the plate. Discard media in each column in the culture plate, and add test solution, control solution or new medium 0.5mL with various concentrations which were prepared before in each column. Use 4 columns for each condition of the solution. Add the culture plate in the CO_2 incubator immediately again and culture for a fixed period. The culture period should be 7 ~ 9 days for L929 cells. After the culture finishes, discard test solution, etc. from the culture plate, add an adequate amount of diluted formal dehydesolution, and leave it for about 30 minutes to fix the cells. Discard the diluted formal dehydesolution from each column, and add an adequate amount of diluted giemsatest solution. After identifying that cell colony is well stained, discard the diluted giemsatest solution, and count the number of cell colony in each column. Calculate the average of the cell colony of the test solution in each concentration, and divide the values by the mean of the number of cell colony in only with medium to calculate the cell colony formation rate(%) of the test and control solutions. Take the test solution concentration (%) in the log arithmic axis in the semilo garithmic graph paper, and take the cell colony formation rate in an other axis. Plot the result obtained from the graph to obtain growth inhibition curve. From this curve, read the test solution concentration[IC_{50}(%)] which reaches 50% of the cell colony formation rate. If necessary, test the positive or negative control culture media to identify the sensitivity or reproducibility of the test.

4. Submission of Test Materials

The test materials should be the ones that were tested according to the

validated test method by an experienced and responsible operator in an institute with sufficient facilities in accordance with the test criteria of this guideline. The following contents must be included.

1) The statement regarding facilities including name and address of the test institute, main instruments used in the test, etc.
2) Names of principal investigator and sub-investigators
3) Product name, amount of drug substances including additives, container and package type, manufactured date, production quantity and lot No.
4) Study Report

e.g.) Cytotoxicity Test of Eluates

① Test date and duration
② Description on sample
③ Cell line
④ Media
⑤ Test method
⑥ Negative, positive, and control group
⑦ Observation statement, test results and analysis
⑧ Other relevant data required for the evaluation of the results

5) Overall comments of the principal investigator on the test results

III. Ethylene Gas Residual Test of Ethylene Oxide-Sterilized Products

With regard to the tampon sterilized by ethylene oxide gas, the following ethylene oxide and ethylene chlorohydrin residual test should be set in the standard and analytical method.

1. Test Criteria

When tested with the product sterilized by ethylene oxide gas, ethylene oxide (EO) and ethylene chlorohydrin (ECH) should be not more than 25 ppm, respectively.

2. Test Method

Take 1 sample, cut narrowly, and add it in a flask with a stopper. Then, add accurate 50 mL of ethanol, cover the stopper, and shake it in the water bath at 70℃ for 2 hours to extract. After the extraction, cool it down at 20℃, filter it, and the filtrate should be the test solution. Take an accurate amount of EO standard bulk separately, add ethanol, and dilute the EO concentration to contain 1.0 ppm ~ 30.0 ppm. Then, this should be EO standard solution Operate ECH in the same way, and the solution should be the ECH standard solution. With 2 ㎕ each of test and standard solutions (EO and ECH), test them according to gas chromatography under each condition, and calculate the residual of EO and ECH of the test solution using the calibration curve obtained from the peak of the standard solution.

(Operation condition of EO)

Detector: Hydrogen flame ion detector

Column: In the stainless steel tube with inner diameter approx. 3 mm, length approx. fill by polyethylene glycol 6000 as a filler dressed by silane-treated chromosorb W of 60 ~ 80 ㎛ at 10% ratio.

Column Temp.: Constant temperature around 60℃

Inlet Temp.: Constant temperature around 120℃

Detection Temp.: Constant temperature around 120℃

Transporting Gas: Nitrogen

Flow rate: 60mL/min

(Operation Condition of ECH)

Detector: Hydrogen flame ion detector

Column: In the stainless steel tube with inner diameter approx. 3 mm, length approx. fill by polyethylene glycol 6000 as a filler dressed by silane-treated chromosorb W of 60 ~ 80 ㎛ at 10% ratio.

Column Temp.: Constant temperature around 150℃

Inlet Temp.: Constant temperature around 215℃

Detection Temp.: Constant temperature around 215℃

Transporting Gas: Nitrogen

Flow rate: 60mL/min

3. Submission of Test Materials

The test materials should be the ones that were tested according to the validated test method by an experienced and responsible operator in an institute with sufficient facilities in accordance with the test criteria of this guideline. The following contents must be included.

1) The statements regarding facilities including name and address of the test institute, main instruments used in the test, etc.
2) Names of principal investigator and sub-investigators
3) Product name, manufactured date and lot No.
4) Test date
5) Test method
6) Test result and analysis

IV. Reference

1. Regulation on Pharmaceuticals, etc. Approval, Notification and Review (KFDA Notification No. 2009-42, June 30, 2009).
2. Interpretation Regarding Review of Safety and Efficacy of Pharmaceuticals, etc. in Regulation on Pharmaceuticals, etc. Approval, Notification and Review (December 2008)
3. Safety Test Criteria of Pharmaceuticals (KFDA Notification 2007-14, March 19, 2007)
4. ISO 10993 Biological evaluation of medical device-part 5: In vitro methods (Tests for cytotoxicity: in vitro methods)
5. Standards and Analytical Methods of Quasi-drugs (KFDA Notification 2009-6, February 23, 2009)