tor, and calculate the amount of organic carbon in the sample using a data processor or a recorder.

For the instrument where the removal of inorganic carbon is performed in the instrument, first inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, according to the test procedure specified for the instrument used. Then, remove inorganic carbon by adding the acid for removing inorganic carbon to the sample in the decomposition device, followed by bubbling the gas for removing inorganic carbon into the sample. Decompose organic carbon, detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon using a data processor or a recorder.

61. Test Methods for Plastic Containers

Test methods for plastic containers may be used for designing and quality assurance of plastic containers. Not all tests described here will be necessary in any phases for any containers. On the other hand, the set does not include sufficient number and kinds of tests needed for any design verification and quality assurance of any containers. Additional tests may be considered if necessary.

1. Combustion Tests

1.1 Residue on ignition

Weigh accurately about 5 g of cut pieces of the container and perform the test according to the Residue on Ignition.

1.2 Heavy metals

Place an appropriate amount of cut pieces of the container in a porcelain crucible, and perform the test according to Method 2 of the Heavy Metals Limit Test. Prepare the control solution with 2.0 mL of Standard Lead Solution.

1.3 Lead

Method 1: Place 2.0 g of cut pieces of a container in a crucible of platinum or quartz, moisten with 2 mL of sulfuric acid, heat slowly to dryness, then heat to combustion at between 450°C and 500°C. Repeat this procedure, if necessary. After cooling, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, evaporate to dryness on a water bath, then add 1 to 5 mL of hydrochloric acid, and warm to dissolve. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter if insoluble matter remains. To the obtained filtrate add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonia TS until the color of the solution changes from yellow to green. Then add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter if necessary, and use the layer as the sample solution. Separately, to 2.0 mL of Standard Lead Solution add water to make exactly 10 mL. To 1.0 mL of this solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and the standard solution according to Atomic Absorption Spectrophotometry under the following conditions, and determine the concentration of lead in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air
Lamp: Lead hollow-cathode lamp
Wavelength: 283.3 nm

Method 2: Cut a container into pieces smaller than 5-mm square, take 2.0 g of the pieces into a glass beaker, add 50 mL of 2-butanone and 0.1 mL of nitric acid, and warm to dissolve. To this solution add 96 mL of methanol gradually to precipitate a resinous substance, and filter by suction. Wash the beaker and the resinous substance with 12 mL of methanol followed by 12 mL of water, combine the washings and the filtrate, and concentrate to about 10 mL under reduced pressure. Transfer into a separator, add 10 mL of ethyl acetate and 10 mL of water, shake vigorously, and allow to stand to separate the water layer. Evaporate the water layer to dryness, add 5 mL of hydrochloric acid to the residue, and warm to dissolve. Then add 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter (G3) if insoluble matter remains. To the solution so obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and then add ammonia TS until the color of the solution changes from yellow to green. Further add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter the layer if necessary, and use the layer as the sample solution. Separately, pipet 5 mL of Standard Lead Solution, add water to make exactly 50 mL, and to 2.0 mL of this solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and the standard solution according to Atomic Absorption Spectrophotometry under the conditions described in Method 1, and determine the concentration of lead in the sample solution.

1.4 Cadmium

Method 1: To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in Method 1 under 1.3, and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in Method 1 under 1.3 and the standard solution according to Atomic Absorption Spectrophotometry under the following conditions, and determine the concentration of cadmium in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air
Lamp: Cadmium hollow-cathode lamp

Wavelength: 228.8 nm

Method 2: To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in Method 2 under 1.3, and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in Method 2 under 1.3 and the standard solution according to Atomic Absorption Spectrophotometry under the conditions described in Method 1, and determine the concentration of cadmium in the sample solution.

1.5 Tin

Cut a container into pieces smaller than 5-mm square, place 5.0 g of the pieces in a Kjeldahl flask, add 30 mL of a mixture of sulfuric acid and nitric acid (1:1), and decompose by gentle heating in a muffle furnace, occasionally adding dropwise a mixture of sulfuric acid and nitric acid (1:1) until the content changes to a clear, light brown solution. Then heat until the color of the solution changes to a clear, light yellow, and heat to slowly concentrate to practical dryness. After cooling, dissolve the residue in 5 mL of hydrochloric acid by warming, and after cooling, add water to make exactly 10 mL. Pipet 5 mL of this solution into a 25-mL volumetric flask (A). Transfer the remaining solution to a 25-mL beaker (B) by washing out with 10 mL of water, add 2 drops of bromocresol green TS, neutralize with diluted ammonia solution (28) (1 in 2), and measure the volume consumed for neutralization as *a* mL. To the volumetric flask, A, add potassium permanganate TS dropwise until a slight pale red color develops, and add a small amount of L-ascorbic acid to decolorize. Add 1.5 mL of 1 mol/L hydrochloric acid TS, 5 mL of a solution of citric acid monohydrate (1 in 10), *a* mL of diluted ammonia solution (28) (1 in 2), 2.5 mL of polyvinyl alcohol TS, 5.0 mL of phenylfluorone-ethanol TS and water to make 25 mL. Shake well, then allow to stand for about 20 minutes, and use this solution as the sample solution. Separately, pipet 1.0 mL of Standard Tin Solution, add 5 mL of water, add potassium permanganate TS dropwise until a slight pale red color develops, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and the standard solution according to Spectrophotometry at 510 nm, using water as the blank.

2. Extractable substances

Cut the container at homogeneous regions of low curvature and preferably the same thickness, gather pieces to make a total surface area of about 1200 cm^2 when the thickness is 0.5 mm or less, or about 600 cm^2 when the thickness is greater than 0.5 mm, and subdivide in general into strips approximately 0.5 cm in width and 5 cm in length. Wash them with water, and dry at room temperature. Place these strips in a 300-mL hard glass vessel, add exactly 200 mL of water, and seal the opening with a suitable stopper. After heating the vessel in an autoclave at 121°C for 1 hour, take out the vessel, allow to stand until the temperature falls to room temperature, and use the content as the test solution.

For containers made of composite plastics, the extraction may be performed by filling a labeled volume of water in the container. In this case, it is necessary to record the volume of water used and the inside area of the container.

When containers are deformed at 121°C, the extraction may be performed at the highest temperature which does not cause deformation among the following conditions: at 70 ± 2°C for 24 ± 2 hours, at 50 ± 2°C for 72 ± 2 hours or at 37 ± 1°C for 72 ± 2 hours.

Prepare the blank solution with water in the same manner. For containers made of composite plastics, water is used as the blank solution.

Perform the following tests with the test solution and the blank solution:

(i) Foaming test: Place 5 mL of the test solution in a glass-stoppered test tube about 15 mm in inside diameter and about 200 mm in length, shake vigorously for 3 minutes, and measure the time needed for almost complete disappearance of the foam thus generated.

(ii) pH: To 20 mL each of the test solution and the blank solution add 1.0 mL of a solution of potassium chloride (1 in 1000), and obtain the difference in the reading of pH between these solutions.

(iii) Potassium permanganate-reducing substances: Place 20 mL of the test solution in a glass-stoppered, conical flask, add 20.0 mL of 0.002 mol/L potassium permanganate VS and 1 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper tightly, shake, then allow to stand for 10 minutes, and titrate with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the test in the same manner, using 20.0 mL of the blank solution, and obtain the difference of the consumption of 0.002 mol/L potassium permanganate VS between these solutions.

(iv) UV spectrum: Read the maximum absorbances between 220 nm and 240 nm and between 241 nm and 350 nm of the test solution against the blank solution as directed under Spectrophotometry.

(v) Residue on evaporation: Evaporate 20 mL of the test solution on a water bath to dryness, and weigh the residue after drying at 105°C for 1 hour.

3. Test for fine particles

Rinse thoroughly with water the inside and outside of containers to be used for the tests, fill the container with the labeled volume of 0.9 w/v% sodium chloride solution, adjust so that the amount of air in the container is about 50 mL per 500 mL of the labeled volume, stopper tightly, and heat at 121°C for 25 minutes in an autoclave. After allowing to cool for 2 hours, take out the container, and then allow to stand at ordinary temperature for about 24 hours. If the containers are deformed at 121°C, employ a suitable temperature-time combination as directed under Extractable substances. Clean the outside of the container, mix by turning upside-down 5 or 6 times, insert immediately a clean needle for filterless infusion into the container through the rubber closure of the container, take the effluent while mixing gently in a clean container for measurement, and use as the test solution. Perform the test with the solution according to the following fine particle test, and count the numbers of fine particles with diameters of 5 - 10 μm, 10 - 25 μm and larger than 25 μm in 1.0 mL of the test solution.

Fine particle test—Counting of the fine particles must be performed in dustless, clean facilities or apparatus, using a light-shielded automatic fine particle counter. The sensor of the counter to be used must be able to count fine particles of 1.5 μm or more in diameter. The volume to be measured is 10 mL. Adjust the counter before measurement. For calibra-

tion of the diameter and number of particles, the standard particles for calibration of the light-shielded automatic fine particle counter should be used in suspension in water or 0.9 w/v% sodium chloride solution.

Count five times the numbers of particles with diameters of 5 - 10 μm, 10 - 25 μm and more than 25 μm while stirring the test solution, and calculate the average particle numbers of four counts, excluding the first, as the number of particles in 1.0 mL of the test solution.

Note: Water and 0.9 w/v% sodium chloride solution to be used for the tests should not contain more than 0.5 particles of 5 - 10 μm in size per 1.0 mL.

4. Transparency test

Method 1: This can be applied to containers which have a smooth and not embossed surface and rather low curvature. Cut the container at homogeneous regions of low curvature and preferably the same thickness to make 5 pieces of about 0.9 × 4 cm in size, immerse each piece in water filled in a cell for determination of the ultraviolet spectrum, and determine the transmittance at 450 nm using a cell filled with water as a blank.

Method 2: Sensory test—This can be applied to containers which have a rough or embossed surface. It can also be applied to testing of the transparency of containers in case where the turbidity of their pharmaceutical contents must be checked.

Reagents

Hydrazine Sulfate $N_2H_6SO_4$ [K-8992, Hydrazinium Sulfate, Special class]

Test solutions

Hexamethylenetetramine TS Dissolve 2.5 g of hexamethylenetetramine in 25 mL of water.

Hydrazinium sulfate TS Dissolve 1.0 g of hydrazinium sulfate in water to make 100 mL.

Formadin stock suspension To 25 mL of hexamethylenetetramine TS add 25 mL of hydrazinium sulfate TS, mix, and use after standing at 25 ± 3°C for 24 hours. This suspension is stable for about 2 months after preparation, provided it is stored in a glass bottle free from inside surface defects. Mix well before use.

Standard suspension: Dilute 15 mL of the formadin stock suspension with water to make 1000 mL. Prepare before use and use within 24 hours.

Reference suspension: Dilute 50 mL of the standard suspension with water to make 100 mL.

Tests

(i) Method 2A (with control) Take 2 containers to be tested, and place in one of them the labeled volume of the reference suspension and in the other, the same volume of water. Show the two containers to five subjects, separately, who do not know which one is which, ask which one seems to be more turbid, and calculate the rate of correct answers.

(ii) Method 2B (without control) Take 6 numbered containers to be tested, and place in three of them the labeled volume of the reference suspension and in the others, the same volume of water. Show each one of the containers at random order to five subjects, separately, who do not know which one is which, ask if it is turbid or not, and calculate the percentage of the answer that it is turbid (100 X/15, X: number of containers judged as "being turbid") in each group.

5. Water vapor permeability test

Method 1 : This test method is applicable to containers for aqueous injection. Fill the container with the labeled volume of water. After closing it hermetically, accurately weigh the container and record the value. Store the container at 65 ± 5% relative humidity and a temperature of 20 ± 2°C for 14 days, and then accurately weigh the container again and record the value. Calculate the mass loss during storage.

Method 2 : This test method is provided for evaluating moisture permeability of containers for hygroscopic drugs. Unless otherwise specified, perform the test according to the following procedure.

Desiccant—Place a quantity of calcium chloride for water determination in a shallow container, taking care to exclude any fine powder, then dry at 110°C for 1 hour, and cool in a desiccator.

Procedure—Select 12 containers, clean their surfaces with a dry cloth, and close and open each container 30 times in the same manner. Ten among the 12 containers are used as "test containers" and the remaining two, as "control containers". A torque for closing screw-capped containers is specified in the table. Add desiccant to 10 of the containers, designated test containers, filling each to within 13 mm of the closure if the container volume is 20 mL or more, or filling each to two-thirds of capacity if the container volume is less than 20 mL. If the interior of the container is more than 63 mm in depth, an inert filler or spacer may be placed in the bottom to minimize the total mass of the container and desiccant; the layer of desiccant in such a container shall be not less than 5 cm in depth. Close each container immediately after adding desiccant, applying the torque designated in the table. To each of the control containers, add a sufficient number of glass beads to attain a mass approximately equal to that of each of the test containers, and close, applying the torque designated in the table. Record the mass of the individual containers so prepared to the nearest 0.1 mg if the container volume is less than 20 mL, to the nearest 1 mg if the container volume is 20 mL or more but less than 200 mL, or to the nearest 10 mg if the container volume is 200 mL or more, and store the containers at 75 ± 3% relative humidity and a temperature of 20 ± 2°C. After 14 days, record the mass of the individual containers in the same manner. Completely fill 5 empty containers with water or a noncompressible, free-flowing solid such as fine glass beads, to the level indicated by the closure surface when in place. Transfer the contents of each to a graduated cylinder, and determine the average container volume, in mL. Calculate the rate of moisture permeability, in mg per day per liter, by use of the formula:

$$(1000/14\,V)\,[(T_f - T_i) - (C_f - C_i)]$$

V: average volume (mL)

$T_f - T_i$: the difference between the final and initial masses of each test container (mg)

$C_f - C_i$: the average of the differences between the final and initial masses of the two controls (mg)

Table Torque Applicable to Screw-Type Container

Closure Diameter (mm)	Torque (N·cm)
8	59
10	60
13	88
15	59–98
18	78–118
20	88–137
22	98–157
24	118–206
28	137–235
30	147–265
33	167–284
38	196–294
43	196–304
48	216–343
53	235–402
58	265–451
63	284–490
66	294–510
70	314–569
83	363–735
86	451–735
89	451–794
100	510–794
110	510–794
120	618–1069
132	677–1069

6. Leakage test

Fill a container with a solution of fluorescein sodium (1 in 1000), stopper tightly, place filter papers on and under the container, and apply a pressure of 6.9 N (0.7 kg)/cm^2 at 20°C for 10 minutes. Judge the leakiness by observing the color of the paper.

7. Cytotoxicity test

The following test methods are designed to detect cytotoxic substances in plastic materials by evaluating the cytotoxicity of the culture medium extracts from plastic containers for pharmaceutical products. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable.

Cell lines

The recommended cell lines are L929 (American Type Culture Collection-ATCC CCL1) and V79 (Health Science Research Resources Bank-JCRB0603). In addition, other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 and V79 cells.

Culture medium

Eagle's minimum essential medium prepared as follows shall be used. Dissolve the chemicals listed below in 1000 mL of water. Sterilize the solution by autoclaving at 121°C for 20 minutes. Cool the solution to room temperature, and add 22 mL of sterilized sodium hydrogen carbonate TS and 10 mL of sterilized glutamine TS. To the resultant solution add fetal calf serum (FCS) to make 10 vol% FCS in the medium.

sodium chloride	6.80 g
potassium chloride	400 mg
sodium dihydrogenphosphate (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 hydrochloride monohydrate	31.4 mg
L-tyrosine	36.0 mg
L-histidine hydrochloride monohydrate	42.0 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
succinic acid (hexahydrate)	100 mg
choline bitartrate	1.8 mg
folic acid	1.0 mg
myo-inositol	2.0 mg
nicotinamide	1.0 mg
calcium D-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

Reagents

(i) Sodium hydrogen carbonate TS: Dissolve 10 g of sodium hydrogen carbonate in water to make 100 mL. Sterilize the solution either by autoclaving in a well-sealed container at 121°C for 20 minutes or by filtration through a membrane filter with a nominal pore diameter of 0.22 μm or less.

(ii) Glutamine TS: Dissolve 2.92 g of L-glutamine in water to make 100 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 μm.

(iii) Phosphate buffer solution (PBS): Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogenphosphate, 8.00 g of sodium chloride, and 1.15 g of disodium hydrogenphosphate (anhydrous) in water to make 1000 mL. Sterilize the solution by autoclaving at 121°C for 20 minutes.

(iv) Trypsin TS: Dissolve 0.5 g of trypsin and 0.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in phosphate buffer solution to make 1000 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 μm.

(v) Formaldehyde solution: Dilute formaldehyde solution with water by a factor of ten.

(vi) Giemsa's stain solution: Dilute a commercially available Giemsa's test solution with the diluent by a factor of fifty. Prepare before use.

(vii) Diluent: Dissolve 4.54 g of potassium dihydrogenphosphate and 4.75 g of disodium hydrogen phosphate (anhydrous) in water to make 1000 mL.

Devices and instruments

It is recommended to use the following devices and instruments for testing.

(i) Pipets : Pasteur pipet, pipet for partial delivery, measuring pipet for partial delivery, and dispenser with microtip.
(ii) Screw-capped glass bottles : 50 – 1000 mL volume.
(iii) Sterile disposable centrifuge tubes: 15 and 50 mL volume.
(iv) Sterile disposable tissue culture flasks with a flat growth area of approximately 25 or 75 cm^2.
(v) Sterile disposable multiple well plates (24 wells)
(vi) Inverted microscope and stereomicroscope
(vii) Humidified incubator—maintain the conditions as follows: temperature, 37°C; CO_2 gas concentration, 5%.

Control materials and substances

(i) Negative control material: polyethylene film
(ii) Positive control material (A): polyurethane film containing 0.1% zinc diethyldithiocarbamate
(iii) Positive control material (B): polyurethane film containing 0.25% zinc dibutyldithiocarbamate
(iv) Control substances: zinc diethyldithiocarbamate (reagent grade) and zinc dibutyldithiocarbamate (reagent grade)

Test procedure

(i) Sample preparation: When the material of the container consists of a single homogeneous layer, subdivide the cut pieces of a container into pieces of the size of approximately 2 × 15 mm and subject the pieces to the test. When the material of the container has multiple layers, such as laminated and coated materials, prepare cut pieces with a surface area of one side of 2.5 cm^2 and subject the pieces to the test without subdividing them into smaller pieces.

(ii) Preparation of test solutions: Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable centrifuge tube. Cap the bottle or tube loosely and cover the cap with clean aluminum foil. Sterilize the bottle or tube by autoclaving at 121°C for 20 minutes. When the material of the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used. In the case of EO sterilization, sufficient aeration should be achieved to avoid an additional toxic effect of residual EO in the test results. To the bottle or tube add the culture medium in a proportion of 10 mL to one gram or 1 mL to 2.5 cm^2 (one side) of the sample, loosely cap the bottle or tube, and allow to stand in a humidified incubator for 24 hours. Transfer the culture medium extract, which is designated 100% test solution, to a sterilized screw-capped glass bottle or a sterile disposable centrifuge tube. Dilute the 100% test solution with culture medium using a dilution factor of two to prepare serial dilutions having extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.13% and so on.

(iii) Preparation of cell suspension: Remove the culture medium from the maintained cell culture flask and rinse the cells with an appropriate volume of PBS by gentle rotation of the flask two or three times, and discard the PBS. Add a sufficient volume of trypsin solution to cover the cell layer. Cap the flask and place the flask in a humidified incubator for one or two minutes. After confirming detachment of the cell layer from the bottom of the flask by using a microscope, add an appropriate volume of the fresh culture medium and gently pipet the cells completely out of the flask by using a Pasteur pipet. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge the tube at 800 – 1000 revolutions per minute for 2 – 5 minutes. Discard the supernatant, resuspend the cells in an appropriate volume of PBS by pipetting, using a Pasteur pipet, and centrifuge the tube again. Discard the PBS, and add an appropriate volume of fresh culture medium to the flask. Resuspend the cells by pipetting and make a single cell suspension. Determine the cell concentration using a hemocytometer.

(iv) Cytotoxicity testing: Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust the cell concentration to 100 cells/mL. Place a 0.5 mL aliquot of the diluted cell suspension on each well of a sterile disposable multiple well plate. Incubate the plate in the humidified incubator for 4 – 6 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL aliquot of the test solution or fresh medium to quadruplicate wells. Place the plate immediately in the humidified incubator and incubate the plate for the appropriate period: 7 – 9 days for L929 cells; 6 – 7 days for V79 cells. After the incubation, discard the medium from the plate, add an appropriate volume of dilute formaldehyde TS to each well and allow the plate to stand for 30 minutes to fix the cells. Discard the dilute formaldehyde TS from each well and add an appropriate volume of dilute Giemsa's TS to each well. After ensuring good staining of the colonies, discard the stain solution from the wells and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the test solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the colony formation rate (%) for each extract concentration of the test solution. Plot the extract concentration (%) of the test solution on a logarithmic scale and the colony formation rate on an ordinary scale on semi-logarithmic graph paper to obtain a colony formation inhibition curve of the container. Read the % extract concentration which inhibits colony formation to 50%, IC_{50} (%), from the inhibition curve. It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable control materials or substances in the test system, if necessary.

Plastic Containers for Aqueous Injections

Plastic containers for the aqueous injections listed in Offical Monographs of JP XIII do not interact with pharmaceuticals contained therein to alter the efficacy, safety or stability, and do not permit the contamination with microorganisms. The containers meet the following requirements.

1. Polyethylene or polypropylene containers for aqueous injections

The containers are made of polyethylene or polypropylene and free from any adhesive.

(1) Transparency—The containers have a transmittance of not less than 55%, when tested as directed in Method 1 under the Transparency test. When Method 1 can not be applied, test according to the Method 2B of the Transparency test. In this case, the rate that the water-containing container is judged as "being turbid" is not more than 20%, and the rate that the reference suspension-containing container is judged as "being turbid" is not less than 80%.

(2) Appearance—The containers do not have strips, cracks, bubbles, or other faults which cause difficulties in practical use.

(3) Water vapor permeability—Proceed as directed in Method 1 of the Water vapor permeability test. The loss of mass is not more than 0.20%.

(4) Heavy metals—The turbidity of the test solution is not greater than that of the control solution when the amount of the sample taken is 1.0 g.

(5) Lead—Perform the test as directed in Method 1. The absorbance of the sample solution is not more than that of the standard solution.

(6) Cadmium—Perform the test as directed in Method 1. The absorbance of the sample solution is not more than that of the standard solution.

(7) Residue on ignition—The residue is not more than 0.10%.

(8) Extractable substances—

(i) Foaming test: the foam formed almost disappears within 3 minutes.

(ii) pH: the difference in the reading of pH between the test solution and the blank solution is not more than 1.5.

(iii) Potassium permanganate-reducing substances: The difference in the consumption of 0.002 mol/L potassium permanganate VS between the test solution and the blank solution is not more than 1.0 mL.

(iv) UV spectrum: The maximum absorbance between 220 nm and 240 nm is not more than 0.08, and that between 241 nm and 350 nm is not more than 0.05.

(v) Residue on evaporation: Not more than 1.0 mg.

(9) Cytotoxicity—IC_{50} (%) is not less than 90%. The result obtained by the other standard methods is negative.

2. Polyvinyl chloride containers for aqueous injections

The containers are composed of homopolymer of vinyl chloride, free from any adhesive, and the plasticizer added to the material should be di(2-ethylhexyl)phthalate. The containers may be covered with easily removable material to prevent the permeation of water vapor. In this case, perform the water vapor permeability test on the covered containers.

(1) Thickness—Measure the thickness of a container at five different locations. The difference between the maximum and minimum values of thickness is 0.05 mm or less.

(2) Transparency—Proceed as directed in (1) under Polyethylene or polypropylene containers for aqueous injections.

(3) Appearance—Proceed as directed in (2) under Polyethylene or polypropylene containers for aqueous injections.

(4) Leakage—Proceed with the test according to Leakage test. The solution contained does not leak.

(5) Flexibility—Insert the spike needle for infusion through a rubber closure of the container used in (4) Leakage. The contained solution is almost completely discharged without displacement by air.

(6) Water vapor permeability—Proceed as directed in (3) under Polyethylene or polypropylene containers for aqueous injections.

(7) Heavy metals—The turbidity of the test solution is not greater than that of the control solution when the amount of the sample taken is 1.0 g.

(8) Lead—Perform the test as directed in Method 2. The absorbance of the sample solution is not more than that of the standard solution.

(9) Cadmium—Perform the test as directed in Method 2. The absorbance of the sample solution is not more than that of the standard solution.

(10) Tin—The absorbance of the sample solution is not more than that of the standard solution.

(11) Vinyl chloride—Wash a cut piece of a container with water, wipe thoroughly with a filter paper, subdivide into pieces smaller than 5-mm square, and place 1.0 g of them in a 20-mL volumetric flask. Add about 10 mL of tetrahydrofuran for gas chromatography, dissolve by occasional shaking in a cold place, add tetrahydrofuran for gas chromatography, previously cooled in a methanol-dry ice bath, to make 20 mL while cooling in a methanol-dry ice bath, and use this solution as the sample solution. Perform the tests as directed under Gas Chromatography according to the operating conditions 1 and 2, using 10 μL each of the sample solution and Standard Vinyl Chloride Solution. Under either operating condition, the peak height of vinyl chloride from the sample solution is not more than that from the Standard Vinyl Chloride Solution.

***Operating conditions* 1—**

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and 2 to 3 m in length, packed with 150 to 180 μm siliceous earth for gas chromatography coated with 15% to 20% polyalkylene glycol monoether for gas chromatography.

Column temperature: A constant temperature of between 60°C and 70°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 1.5 minutes.

Selection of column: Proceed with 10 μL of Standard Vinyl Chloride Solution under the above operating conditions. Use a column from which vinyl chloride and ethanol are eluted in that order, with a good resolution between their peaks.

Detection sensitivity: Adjust the detection sensitivity so that the peak height from 10 μL of the Standard Vinyl Chloride Solution is 5 to 7 mm.

***Operating conditions* 2—**

Detector: A hydrogen flame-ionization detector. Column: A column about 3 mm in inside diameter and about 1.5 m in length, packed with 150 to 180 μm porous acrylonitrile-divinylbenzene copolymer for gas chromatography (pore size: 0.06 - 0.08 μm; 100 - 200 m^2/g).

Column temperature: A constant temperature of about 120°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 3 minutes.

Selection of column: Proceed with 10 μL of Standard Vinyl Chloride Solution under the above operating conditions. Use a column from which vinyl chloride and ethanol are eluted in that order, with a good resolution between their peaks.

Detection sensitivity: Adjust the detection sensitivity so that the peak height from 10 μL of the Standard Vinyl Chloride Solution is 5 to 7 mm.

(12) Fine particles—The number of fine particles in 1.0 mL of the test solution is counted as not more than 100 of 5 to 10 μm, not more than 10 of 10 to 25 μm and not more than 1 of 25 μm or more.

(13) Residue on ignition—The residue is not more than 0.10%.

(14) Extractable substances—Proceed as directed in (8) under Polyethylene or polypropylene containers for aqueous injections.

(15) Cytotoxicity—Proceed as directed in (9) under Polyethylene or polypropylene containers for aqueous injections.

3. Plastic containers for aqueous injections being not described above

The containers meet the following specifications and other necessary specifications for their materials with regard to heavy metals, residue on ignition and extractable substances, etc.

(1) Transparency—Proceed as directed in (1) under Polyethylene or polypropylene containers for aqueous injections.

(2) Appearance—Proceed as directed in (2) under Polyethylene or polypropylene containers for aqueous injections.

(3) Vapor permeability—Proceed as directed in (3) under Polyethylene or polypropylene containers for aqueous injections.

(4) Cytotoxicity—Proceed as directed in (9) under Polyethylene or polypropylene containers for aqueous injections.

62. Thermal Analysis

"Thermal Analysis" is a generic term for a variety of techniques to measure the physical properties of a substance as a function of temperature and/or time.

Among the physical properties, phase transitions such as solid/liquid phase transition (melting, freezing) and crystal polymorphism or thermal behavior such as heat evolution or absorption accompanying thermal degradation or chemical reaction can be detected by the techniques of differential thermal analysis (DTA) or differential scanning calorimetry (DSC). DTA is a method for detecting the thermal behavior of a specimen in terms of the temperature change, while DSC employs the heat quantity (enthalpy) change. There is also a method, thermogravimetry (TG), in which the mass change of a specimen caused by dehydration, adsorption, elimination or oxidation etc., is detected as a function of temperature and/or time.

Among the above three different methods, TG can be used as an alternative method for "Loss on Drying" or "Water Determination". However, it must be confirmed beforehand that no volatile component except for water is included in the test specimen when TG is used as an alternative method for "Water Determination".

Method 1 Differential Thermal Analysis (DTA) or Differential Scanning Calorimetry (DSC)

Apparatus Apparatus for DTA or DSC is usually composed of a heating furnace, a temperature-controller, a detector, a device for controlling the atmosphere, and an indicator/recorder.

Differential Thermal Analysis (DTA) In a DTA apparatus, a sample specimen and an inert reference material placed in the heating furnace are heated or cooled at a constant rate, and the temperature difference evolved between the sample and reference material is detected continuously by a device such as a thermocouple and recorded as a function of time and/or temperature. As an inert reference material, α-Alumina for thermal analysis is usually adopted.

Differential Scanning Calorimetry (DSC) Two kinds of DSC apparatus, based upon different principles are available as shown below.

1. Input compensation-type differential scanning calorimetry (Input compensation DSC)

A sample specimen and the reference material in twin furnaces are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference, which is detected by a device such as a platinum resistance thermometer, is kept at null by controlling the heating unit with a compensation feed-back circuit. The instrument is designed to measure and record continuously the balance of thermal energy applied to each furnace as a function of temperature and/or time.

2. Heat flux-type differential scanning calorimetry (Heat flux DSC)

A sample specimen and the reference material in twin furnaces are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference is detected as a difference of heat flux and recorded as a function of temperature and/or time. In heat flux DSC, thermal conductors are adopted so that the heat flux between the sample and the heat reservoir is proportional to the temperature difference between them.

In usual DSC analysis, α-Alumina is used as a reference material, both in Input compensation DSC and in Heat flux DSC. But in some cases, an empty sample container can also be used without any reference material.

Procedure

A sample specimen and the reference material are put in sample pans, and the furnace is heated or cooled under a controlled temperature program. As the temperature changes, the temperature difference (DTA) or heat quantity change (DSC) that develops between the specimen and the reference is detected and recorded continuously. Apparatus equipped with a data-processor is operated according to the instruction manual provided with the instrument.

A preliminary experiment is needed to determine the appropriate temperature range of measurement, within which a predicted physical change such as melting or polymorphic phase transition will occur, and to confirm that unpredicted thermal changes are not induced in a specimen in that temperature range. In this preliminary test, a wide temperature range (room temperature-the temperature at which degradation begins) can be scanned at a rapid heating rate (10 – 20°C/min). Thereafter, tests by DSC or DTA should be performed at a low heating rate, usually 2°C/min, in the chosen temperature range. However, when a clear heat change cannot be observed, such as in a case of glass-transition, the heating rate may be changed to a higher or a lower rate, as appropriate for the kind of physical change being observed. By analyzing the measured DTA-curve or DSC-curve, a quantity of heat change and/or a specific temperature (ignition, peak or end temperature) that accompanies a physical change, such as melting or polymorphic phase transition, can be obtained.

Calibration of the apparatus

1. Temperature calibration for DTA and DSC

Temperature calibration for DTA and/or DSC apparatus can be performed by using reference substances having an intrinsic thermal property, such as melting point of pure