

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on either side of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

(2) Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

### Terminology

The definition of terms described under the Liquid Chromatography shall apply in the Gas Chromatography.

Note: Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the column packing material, concentration of the stationary phase, column temperature, and flow rate of carrier gas may be modified within limits which allow the required elution order, resolution, symmetry factor and relative standard deviation to be obtained. The sample injection port and the operating conditions for headspace may be also modified within limits which allow the accuracy and precision more than those of a prescribed method to be obtained.

## 22. Heavy Metals Limit Test

The Heavy Metals Limit Test is a limit test of the quantity of heavy metals contained as impurities in drugs. The heavy metals are the metallic inclusions that are darkened with sodium sulfide TS in acidic solution, as their quantity is expressed in terms of the quantity of lead (Pb).

In each monograph, the permissible limit for heavy metals (as Pb) is described in terms of ppm in parentheses.

### Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as directed in the following:

(1) Method 1

Place an amount of the sample, directed in the monograph, in a Nessler tube. Dissolve in water to make 40 mL. Add 2 mL of dilute acetic acid and water to make 50 mL, and designate it as the test solution.

The control solution is prepared by placing the volume of Standard Lead Solution directed in the monograph in a Nessler tube, and adding 2 mL of dilute acetic acid and water to make 50 mL.

(2) Method 2

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500°C and 600°C. Cool, add 2 mL of hydrochloric acid, evaporate to dryness on a water bath,

moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Then add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid on a water bath, further evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

(3) Method 3

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, heat cautiously, gently at first, and then increase the heat until incineration is completed. After cooling, add 1 mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate 1 mL of aqua regia to dryness on a water bath. Hereinafter, proceed as directed for the test solution, and add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

(4) Method 4

Place an amount of the sample, directed in the monograph, in a platinum or porcelain crucible, mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and carbonize by gradual heating. Cool, add 1 mL of sulfuric acid, heat carefully, and incinerate by ignition between 500°C and 600°C. If a carbonized substance remains, moisten with a small amount of sulfuric acid, and incinerate by ignition. Cool, dissolve the residue in 3 mL of hydrochloric acid, evaporate on a water bath to dryness, wet the residue with 3 drops of hydrochloric acid, add 10 mL of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a pale red color develops, then add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and the washing to a Nessler tube, add water to make 50 mL, and use this solution as the test solution.

The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. Cool, add 1 mL of sulfuric acid, heat carefully, and ignite between 500°C and 600°C. Cool, and add 3 mL of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

### Procedure

Add 1 drop of sodium sulfide TS to each of the test solution and the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colors of both solu-

tions by viewing the tubes downward or transversely against a white background. The test solution has no more color than the control solution.

## 23. Infrared Spectrophotometry

Infrared Spectrophotometry is a method of measurement of the extent, at various wave numbers, of absorption of infrared radiation when it passes through a layer of a substance. In the graphic representation of infrared spectra, the plot usually shows units of wave numbers as the abscissa and units of transmittance or absorbance as the ordinate. Wave number and transmittance or absorbance at each absorption maximum may be read graphically on an absorption spectrum and/or obtained by a data-processor. Since the wave number and the respective intensity of an absorption maximum depend on the chemical structure of a substance, this measurement can be used to identify or determine a substance.

### Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about  $2851\text{ cm}^{-1}$  to the minimum at about  $2870\text{ cm}^{-1}$  should be not less than 18% transmittance and that from the maximum at about  $1583\text{ cm}^{-1}$  to the minimum at about  $1589\text{ cm}^{-1}$  should be not less than 12% transmittance.

The wave number ( $\text{cm}^{-1}$ ) scale is usually calibrated by the use of several absorption bands of a polystyrene film, shown below. The numbers in parentheses indicate the precision with which these values have been established.

3027.1 ( $\pm 0.3$ )	1801.6 ( $\pm 0.3$ )	1069.1 ( $\pm 0.3$ )
2924 ( $\pm 2$ )	1601.4 ( $\pm 0.3$ )	1028.0 ( $\pm 0.3$ )
2850.7 ( $\pm 0.3$ )	1583.1 ( $\pm 0.3$ )	906.7 ( $\pm 0.3$ )
1944 ( $\pm 1$ )	1181.4 ( $\pm 0.3$ )	698.9 ( $\pm 0.5$ )
1871.0 ( $\pm 0.3$ )	1154.3 ( $\pm 0.3$ )	

The difference of transmittance should be within 0.5% when the spectrum of a polystyrene film is measured twice at several wave numbers from  $3000$  to  $1000\text{ cm}^{-1}$ , and the difference of wave number should be within  $5\text{ cm}^{-1}$  at about  $3000\text{ cm}^{-1}$  and within  $1\text{ cm}^{-1}$  at about  $1000\text{ cm}^{-1}$ .

### Preparation of samples and measurement

Unless otherwise specified, when it is directed to perform the test "after drying the sample", use a sample dried under the conditions specified in the monograph. Prepare the specimen for the measurement according to one of the following procedures so that the transmittance of most of the absorption bands is in the range of 5% to 80%. Single crystals of sodium chloride, potassium bromide, etc. are available for the optical plate. Generally, the reference cell or material is placed in the reference beam for double-beam instruments, while for single-beam instruments, it is placed in the same optical path in place of the specimen and measured separately

under the same operating conditions. The composition and preparation of the reference depend on the sample preparation methods, and sometimes the background absorption of the atmosphere can be utilized.

Unless otherwise specified in the monograph, the spectrum is usually recorded between  $4000\text{ cm}^{-1}$  and  $400\text{ cm}^{-1}$ . The spectrum should be scanned using the same instrumental conditions as were used to ensure compliance with the requirements for the resolving power and for the precision of wave number scale and of wave numbers.

(1) *Potassium bromide disk or potassium chloride disk method*—Powder 1 to 2 mg of a solid sample in an agate mortar, triturate rapidly with 0.10 to 0.20 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption, and compress the mixture with a press in a suitable die (disk-forming container) to make the sample disk. If necessary to obtain a transparent disk, press the mixture under vacuum in a die with pressure applied to the die of 50 to  $100\text{ kN per cm}^2$  for 5 to 8 minutes. Prepare a potassium bromide reference disk or a potassium chloride reference disk in the same manner as the sample disk.

(2) *Solution method*—Place the sample solution prepared by the method directed in each monograph in a fixed cell for liquid, and usually measure the spectrum against the reference solvent used for preparing the sample solution. The solvent used in this method should not show any interaction or chemical reaction with the specimen to be examined and should not damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

(3) *Paste method*—Powder 5 to 10 mg of a solid specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 to 2 drops of liquid paraffin to give a homogeneous paste. After spreading the paste to make a thin film in the center of an optical plate, place the plate upon another optical plate with precautions against intrusion of air, bubbles in the film, and examine its absorption spectrum.

(4) *Liquid film method*—Examine 1 to 2 drops of a liquid specimen as a thin film held between two optical plates. When the absorption intensity is not sufficient, place spacers of aluminum foil, etc., between the two optical plates to make a thicker liquid film.

(5) *Film method*—Examine a thin film just as it is or a prepared thin film as directed in each monograph.

(6) *Gas sampling method*—Put a sample gas in a gas cell previously evacuated under the pressure directed in the monograph, and examine its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm, but, if necessary, may exceed 1 m.

(7) *ATR method*—Place a specimen in close contact with an attenuated total reflectance (ATR) prism, and examine its reflectance spectrum.

(8) *Diffuse reflectance method*—Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about  $50\text{ }\mu\text{m}$  particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

### Identification

When the spectrum of a specimen and the Reference Spec-