

The fluorescence intensity is usually measured at the excitation and the emission wavelengths in the vicinity of excitation and emission maxima of the fluorescent substance. The fluorescence intensity is expressed as a value relative to that of a standard solution, because it is readily affected even by a slight change in the condition for the measurement.

Unless otherwise specified, the instrument is operated as follows with standard, sample, and reference solutions prepared as directed in the monograph: Fix the excitation and fluorescence wavelength scales at the designated positions, adjust the dark current to zero, put the quartz cell containing the standard solution in the light path, and adjust the instrument so that the standard solution shows the fluorescence intensity of 60% to 80% of full scale. Then perform the measurements with the cells containing the sample solution and the control solution, and read the fluorescence intensity as % under the same condition. Set the width of the wavelength properly unless otherwise specified.

Note: The fluorescence intensity is readily affected by the concentration, temperature and pH of the solution, and nature and purity of solvents or reagents used.

20. Foreign Insoluble Matter Test for Injections

The Foreign Insoluble Matter Test for Injections is a test method to examine foreign insoluble matters in injections.

Method 1. This method is applied to injections either in solutions, or in solution constituted from sterile drug solids.

Clean the exterior of containers, and inspect with the unaided eyes at a position of light intensity of approximately 1000 luxes under an incandescent lamp: Injections must be clear and free from readily detectable foreign insoluble matters. As to Injections in plastic containers for aqueous injections, the inspection should be performed with the unaided eyes at a position of light intensity of approximately 8000 to 10,000 luxes, with an incandescent lamp at appropriate distances above and below the container.

Method 2. This method is applied to injections with constituted solution.

Clean the exterior of containers, and dissolve the contents with constituted solution or with water for injection carefully, avoiding any contamination with extraneous foreign substances. The solution thus constituted must be clear and free from foreign insoluble matters that is clearly detectable when inspected with the unaided eyes at a position of light intensity of approximately 1000 luxes, right under an incandescent lamp.

21. Gas Chromatography

Gas Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or

vaporizable sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio (k) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

Since the relation given below exists among the ratio (k), the time for which the mobile phase is passed through the column (t_0 : time measured from the time of injection of a compound with $k = 0$ to the time of elution at the peak maximum), and the retention time (t_R : time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1 + k) t_0$$

Apparatus

Basically, the apparatus required for the gas chromatographic procedure consists of a carrier gas-introducing port and flow regulator, a sample injection port, a column, a column oven, a detector and a recorder. Gas introducing port and flow regulator for a combustion gas, a burning supporting gas and an accessory gas and sample injection port for headspace are also used, if necessary. The carrier gas-introducing port and flow regulator serves to deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure regulation valve, a flow rate regulation valve and a pressure gauge. The sample injection port is used to deliver a quantity of the sample to the flow line of carrier gas with high reproducibility. There are sample injection ports for packed column and for capillary column. There are both divided injection mode and non-divided injection mode to sample injection port for capillary column. The columns are usually classified as packed column or capillary column. The packed column is a tube made of inert metal, glass or synthetic resin, in which a packing material for gas chromatography is uniformly packed. The packed column with not more than 1 mm in inside diameter is also called a packed capillary column (micro packed column). A capillary column is a tube made of inert metal, glass, quartz or synthetic resin, whose inside wall is bound chemically with stationary phase for gas chromatography. The column oven has the setting capacity for a column with required length and the temperature regulation system for keeping the constant column temperature. The detector is used to detect a component separated on the column, and may be an alkaline thermal ionization detector, a flame photometry detector, mass spectrophotometer, hydrogen flame-ionization detector, an electron capture detector, a thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

Procedure

Unless otherwise specified, proceed by the following method. Fix the detector, column and carrier gas to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column system through the sample injection port. The sepa-

rated components are detected by the detector, and recorded by the recorder as a chromatogram.

Identification and purity test

Identification of a component of a sample is performed by confirming agreement of the retention time of the sample with that of an authentic specimen, or by confirming that the peak shape of the sample is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the sample solution with a standard solution which is prepared by diluting the sample solution to a concentration corresponding to the specified limit amount of the impurity, or by the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its relative sensitivity to the principal component.

Assay

In general, perform the assay by using the internal standard method. The absolute calibration curve method is used when a suitable internal standard is not available. Perform the assay by using the standard addition method when the effect of the component other than the compound to be assayed on the quantitative determination is not negligible against a result of the determination.

(1) Internal standard method—In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the gas chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under

fixed conditions to determine the amount of the objective compound. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the ratios of the peak area or peak height of the objective compound to those of the internal standard, which are obtained by repeating the injection of a fixed volume of the standard solution.

(2) Absolute calibration curve method—Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the liquid chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the peak areas or peak heights of the objective compound, which are obtained by repeating the injection of a fixed volume of the standard solution.

(3) Standard addition method—Pipet a fixed volume of more than 4 sample solutions, add exactly the standard solution so that stepwise increasing amounts of the object compound are contained in the solutions except 1 sample solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the sample solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual sample solutions, measure the peak area of individual sample solutions. Calculate the concentration of standard objective compound added into each sample solution, plot the amounts (concentration) of added standard object compound on the abscissa and the peak area on the ordinate on the graph, extend the calibration curve obtained by linking the plots, and determine the amount of object compound to be assayed from the distance between the origin and the intersecting point of the calibration curve with the abscissa. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the peak areas of the objective compound, which are obtained by repeating the injection of a fixed volume of the standard solution. This method is available only in the case that the calibration curve is a straight line, and passes through the origin when the absolute calibration curve method is employed. In this method, all procedures must be carried out under a strictly constant condition.

Method for peak measuring

Generally, the following methods are used.

(1) Peak height measuring method

(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-