

filtrate and washing almost to dryness on a water bath, add 3 mL of acetone, and evaporate again to dryness on a water bath. Complete the drying between 70°C and 80°C under reduced pressure (about 2.67 kPa) for 30 minutes, allow to stand for cooling in a desiccator (reduced pressure, silica gel) for 30 minutes, and then weigh. After weighing, add 2 mL of diethyl ether and 10 mL of neutralized ethanol, and dissolve the residue by shaking well. Add a few drops of phenolphthalein TS, and titrate the remaining fatty acids in the residue with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds.

$$\text{Unsaponifiable matter (\%)} = \frac{a - (b \times 0.0282)}{\text{amount (g) of sample}} \times 100$$

a: Amount (g) of the extracts.

b: Volume (mL) of 0.1 mol/L potassium hydroxide-ethanol VS consumed for titration.

Iodine value

The iodine value, when measured under the following conditions, is the number of grams of iodine (I), representing the corresponding amount of halogen, which combines with 100 g of sample.

Procedure: Unless otherwise specified, weigh accurately the amount of sample shown in Table 2, according to the expected iodine value of the sample, in a small glass container. In a 500-mL glass-stoppered flask place the container containing the sample, add 20 mL of cyclohexane to dissolve the sample, then add exactly 25 mL of Wijs' TS, and mix well. Stopper the flask, and allow to stand, protecting against light, between 20°C and 30°C for 30 minutes (when the expected iodine value is more than 100, for 1 hour) with occasional shaking. Add 20 mL of potassium iodide solution (1 in 10) and 100 mL of water, and shake. Then, titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination.

b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed for titration of the sample.

Table 2

Iodine value	Amount (g) of sample
Less than 30	1.0
30 to 50	0.6
50 to 100	0.3
More than 100	0.2

18. Flame Coloration Test

The Flame Coloration Test is a method to detect an element, by means of the property that the element changes the colorless flame of a Bunsen burner to its characteristic color.

(1) Salt of metal—The platinum wire used for this test is about 0.8 mm in diameter, and the end part of it is straight. In the case of a solid sample, make the sample into a gruel by

adding a small quantity of hydrochloric acid, apply a little of the gruel to the 5-mm end of the platinum wire, and test by putting the end part in a colorless flame, keeping the platinum wire horizontal. In the case of a liquid sample, immerse the end of the platinum wire into the sample to about 5 mm in length, remove from the sample gently, and perform the test in the same manner as for the solid sample.

(2) Halide—Cut a copper net, 0.25 mm in opening and 0.174 mm in wire diameter, into a strip 1.5 cm in width and 5 cm in length, and wind in round one end of a copper wire. Heat the copper net strongly in the colorless flame of Bunsen burner until the flame no longer shows a green or blue color, and then cool it. Repeat this procedure several times, and coat the net completely with cupric oxide. After cooling, unless otherwise specified, apply about 1 mg of the sample to the copper net, ignite, and burn it. Repeat this procedure three times, and then test by putting the copper net in the colorless flame.

The description, "Flame coloration persists", in a monograph, indicates that the reaction persists for 4 seconds.

19. Fluorometry

The Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. The Fluorometry is also applied to the phosphorescent substances.

Fluorescence intensity *F* in a dilute solution is proportional to the concentration *c* in mol per liter of the solution and the pathlength *l* of light through the solution in centimeter.

$$F = kI_0\phi\epsilon cl$$

k: Constant

*I*₀: Intensity of exciting light

φ: Quantum yield of fluorescence or phosphorescence

$$\phi = \frac{\text{number of quanta emitted as fluorescence or phosphorescence}}{\text{number of quanta absorbed}}$$

ε: Molar extinction coefficient of the substance at the excitation wavelength

Apparatus

Spectrofluorometer is usually used. Generally, a xenon lamp, laser, an alkaline halide lamp, etc. which provide stable exciting light are used as the light source. Usually, a nonfluorescent quartz cell (1 cm × 1 cm) with four transparent sides is used as the container for sample solution.

Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of sample solution with varying excitation wavelengths at a fixed emission wavelength (in the vicinity of the fluorescence maximum) and drawing a curve showing the relationship between the excitation wavelength and the fluorescence intensity. Fluorescence spectrum is obtained by measuring fluorescence intensities of sample solution with varying emission wavelengths at a fixed excitation wavelength (in the vicinity of the excitation maximum) and drawing the same curve as described for the excitation spectrum. If necessary, the spectra are corrected with regard to the optical characteristics of the apparatus.

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-