water suspension of powdered solid substance for the stationary phase, directed in the monograph, on one side of the glass plate to make a uniform layer of 0.2 to 0.3 mm in thickness. After air-drying, dry further by heating at a fixed temperature between 105°C and 120°C for 30 to 60 minutes. A suitable plastic plate may be used instead of the glass plate. Preserve the dried plate with protection from moisture.

Procedure

Unless otherwise specified, proceed by the following method.

Designate a line about 20 mm distant from the bottom of the thin-layer plate as the starting line, spot 2 to 6 mm in diameter the directed volumes of the sample solution or the standard solution in the monograph using micropipets at points on this line, separated by more than 10 mm, and air-dry. Unless otherwise specified, attach the filter paper along with the inside wall of the container, and wet the filter paper with the developingt solvent. In the container, the developing solvent is placed up to about 10 mm in height from the bottom beforehand, seal the container closely, and allow it to stand for 1 hour at ordinary temperature. Place the plate in the container, avoiding contact with the inside wall, and seal the container. Develop it at ordinary temperature.

When the solvent front has ascended from the starting line to the distance directed in the monograph, remove the plate from the container. Immediately put a mark at the solvent front. After air-drying, observe the location, color, etc., of each spot by the method specified in the monograph. Calculate the *R*f value by using the following equation:

$$Rf = \frac{\text{distance from the starting line to the center of the spot}}{\text{distance from the starting line to the solvent front}}$$

64. Ultraviolet-visible Spectrophotometry

The Ultraviolet-visible Spectrophotometry is a method to measure the degree of absorption of light between the wavelengths of 200 nm and 800 nm by substances for the tests of their identity and purity, and for assay. When an atomic absorption spectrophotometer is used for these purposes, proceed as directed under the Atomic Absorption Spectrophotometry. When monochromatic light passes through a substance in the solution, the ratio of transmitted light intensity I to incident light intensity I_0 is called transmittance t; transmittance expressed in the percentage is called percent transmission T, and common logarithm of the reciprocal of transmittance is called absorbance A.

$$t = \frac{I}{I_0} \quad T = \frac{I}{I_0} \times 100 = 100t \quad A = \log\frac{I_0}{I}$$

The absorbance A is proportional to the concentration c of a substance in the solution and the length l of the layer of the solution through which light passes.

$$A = kcl \quad (k: \text{constant})$$

The absorbance, calculated on the basis that l is 1 cm and c is 1 mol/L, is called molar absorption coefficient ε. The molar absorption coefficient at the wavelength of maximum absorption is expressed as ε_{max}.

When a light beam passes through a substance in the solution, the absorbance by the sample differs depending on the wavelengh of the light. So, an absorption spectrum is obtained by determining the absorbances of a light beam at various wavelengths and by graphically plotting the relation between absorbance and wavelength. From the absorption spectrum, it is possible to determine the wavelength of maximum absorption λ_{max} and that of minimum absorption λ_{min}.

The absorption spectrum of a substance in the solution is characteristic, depending on its chemical structure. Therefore, it is possible to identify a substance by comparing the spectrum of a sample within the specified wavelength range with the Reference Spectrum or the spectrum of Reference Standard, by determing the wavelengths of maximum absorption, or by measuring the ratio of absorbances at two specified wavelengths. For the purpose of assay, the absorbance by a sample solution with a certain concentration is measured at the wavelength of the maximum absorption λ_{max} and compared it with the absorbance of a standard solution with a certain concentration.

Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of absorbance.

After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it should be confirmed that the wavelength and the transmission rate meet the specifications of the tests described below.

The calibration of wavelength should be carried out as follows. Using an optical filter for wavelength calibration, measure the transmission rate in the vicinity of the standard wavelength value shown in the test results form, under the test conditions given in the test results form attached to each of the filters. When performing a test to determine the wavelength which shows minimal transmission rate, the difference between the measured wavelength and the standard wavelength value should be within ± 0.5 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm. It is also possible to carry out the test using a low-pressure mercury lamp at bright line wavelengths of 253.65 nm, 365.02 nm, 435.84 nm and 546.07 nm, or a deuterium discharge lamp at bright line wavelengths of 486.00 nm and 656.10 nm. In the case of these tests, the difference between the measured wavelength and the wavelength of the bright line should be within ± 0.3 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm.

The calibration of transmission rate or absorbance should be carried out as follows. Using an optical filter for transmission rate calibration, determine the transmission rate at the standard wavelength value under the test conditions given in the test results form attached to each of the filters. The difference between the measured transmission rate and the standard transmission rate value should be within the range of from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the test results form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmission rate) should be within the mean ± 0.002 when the absorbance is not more than 0.500, and within the mean ± 0.004 when the absorbance is more than 0.500. In addition, it will be desirable to confirm the linearity of transmission rate at

the same wavelength using several optical filters for calibration of transmission rate with different transmission rates.

Procedure

After adjusting the apparatus as directed in the Apparatus and adjustment, select and set the light source, detector, mode of measurement, measuring wavelength or wavelength range, spectrum width and scanning speed.

Subsequently, allow the apparatus to stand for a certain time to confirm its stability. Then, usually adjust the apparatus so that the transmittance is 0% at measuring wavelength or over measuring wavelength range after shutting the sample side of light path. Then open the shutter and adjust the transmittance to 100% (the absorbance is zero). Adjusting the transmittance to 100% is usually done by putting cells containing the control solution in both light paths. For the control solution, unless otherwise specified, blank solvent is used.

Then perform the measurement with the cell containing the sample solution, and read the absorbance at measuring wavelength, or measure the spectrum over measuring wavelength range. Unless otherwise specified, a cell with a path length of 1 cm, made of quartz for ultraviolet range and of quartz or glass for visible range, is used. Special consideration is needed with the absorption of solvents in the ultraviolet range; use a solvent which does not disturb accurate measurement.

Specific absorbance

In the Japanese Pharmacopoeia, the absorbance, calculated on the basis that l is 1 cm and c (concentration of a medicament) is 1 w/v%, is called specific absorbance, and is expressed as $E^{1\%}_{1cm}$.

$$E^{1\%}_{1cm} = \frac{A}{c \times l}$$

l: Length of the layer of the solution (cm)
A: Absorbance value
c: Concentration of the sample in the solution (w/v%)

The description of, for example, "$E^{1\%}_{1cm}$ (241 nm): 500 – 530 (after drying, 2 mg, methanol, 200 mL)" in the monograph, indicates that observed $E^{1\%}_{1cm}$ value is between 500 and 530, when the test is performed in the following manner: The sample is dried under the conditions specified in the Test for Loss on Drying, and about 2 mg of the sample is weighed accurately with a microbalance, and dissolved in methanol to make exactly 200 mL, then the absorbance of the solution is measured as directed in the Procedure at a wavelength of 241 nm using a cell with a path length of 1 cm.

Identification

Prepare the sample solution as directed in the monograph, and test as directed in the Procedure. Usually, the test is performed by a single method or in a combination of a few methods in the following methods using the absorbance or absorption spectrum obtained from the sample solution. Subtle differences in the absorption spectrum arising from differences in the apparatus used may be neglected.

(1)   Identification using Reference Spectrum

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the Reference Spectrum, the identity of the sample and the reference may be confirmed.

In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

Reference spectrum: Reference spectra are specified under the Ultraviolet-visual Reference Spectra, which are used as the reference for the test of identification specified in the monograph.

(2)   Identification using Reference Standard

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the spectrum obtained from the Reference Standard, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum. When the relevant Reference Spectrum is not available, the range is that specified in the monograph.

(3)   Identification using absorption wavelength

When maximum absorption wavelengths of the spectrum obtained from the sample solution match the wavelengths specified in the monograph, the identity of the substance may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

(4)   Identification using the ratio of the absorbances obtained at two or more wavelengths

When the ratios of absorbances at the specified wavelengths in the spectrum obtained from the sample solution meet the specifications in the monograph, the identity of the substance may be confirmed.

Assay

Prepare the control solution, the sample solution and the standard solution as directed in the monograph, measure the absorbances of the sample solution and the standard solution according to the method described in the Procedure, and determine the amount of the substance to be assayed in the sample by comparing the absorbances.

65.   Viscosity Determination

Viscosity determination is a method to determine the viscosity of liquid samples using a viscometer.

When a liquid moves in a definite direction, and the liquid velocity has a gradient with respect to the direction rectangular to that of flow, a force of internal friction is generated along both sides of a hypothetical plane parallel to the movement. This flow property of a liquid is expressed in terms of viscosity. The internal friction per unit area on the parallel plane is called slip stress or shear stress, and the velocity gradient with respect to the direction rectangular to that of flow is called slip velocity or shear velocity. A liquid of which the slip velocity is proportional to its slip stress is called a Newtonian liquid. The proportionality constant, η, is a characteristic of a liquid at a certain temperature and is called viscosity. The viscosity is expressed in the unit of Pascal second (Pa·s), and usually milli-Pascal second (mPa·s).

A liquid whose slip velocity is not proportional to its slip stress is called a non-Newtonian liquid. Since the viscosity for a sample of a non-Newtonian liquid changes with its slip velocity, the viscosity measured at a certain slip velocity is called an apparent viscosity. In that case, the value of slip stress divided by the corresponding slip velocity is called an