Appendix XIV: Ultraviolet Spectrophotometry and Colourimetry

Calibration and performance test of the spectrometer

(1) **Wavelength** – The performance of the spectrometer can be affected by environmental factors, leading to a drift of wavelength scale. Therefore, it is necessary to perform the regular calibration for the spectrometer and the calibration of wavelength should be done prior to the experiment. Mercury lamp is considered as the common choice of light source for calibration. The spectral lines of 237.8 nm, 253.7 nm, 275.3 nm, 296.7 nm, 313.2 nm, 334.2 nm, 365.0 nm, 404.7 nm, 435.8 nm, 546.1 nm and 577.0 nm can be used. The wavelength scale can also be calibrated by using deuterium discharge lamp with spectral lines of 486.0 nm and 656.1 nm. In addition, Holmium glass filter exhibits sharp absorption peaks at 279.4 nm, 287.5 nm, 333.7 nm, 360.9 nm, 418.5 nm, 460.0 nm, 484.5 nm, 536.2 nm and 637.5 nm, which can also be used for calibration of wavelength. However, the exact peak position may differ slightly depending on the commercial source and the age of filter.

(2) **Accuracy of absorbance** – The absorbance scale may be checked with a solution of dichromate in sulphuric acid. Potassium dichromate certified reference material is dried to a constant weight at 120°C. Then, accurately weigh about 60 mg of potassium dichromate certified reference material and dissolve in 1000 mL of 0.005 mol/L sulphuric acid. Determine the absorption coefficient at wavelengths shown in the following table and the absorption coefficient of the potassium dichromate measured should be within the acceptable range.

Wavelength/ nm	235 (min)	257 (max)	313 (min)	350 (max)
Absorption coefficient ($E^{1cm}_{1\%}$)	124.5	144.0	48.6	106.6
Acceptable range of Absorption coefficient ($E^{1cm}_{1\%}$)	123.0-126.0	142.8-146.2	47.0-50.3	105.5-108.5

(3) Presence of stray light – The solutions of sodium iodide and sodium nitrite used for checking stray light are prepared according to the following table. The transmittance of these solutions measured in a 1-cm quartz cell against water at the specific wavelength should not exceed 0.8.

Reagent	Concentration / % (g/ml)	Wavelength / nm	Transmittance / %
Sodium iodide	1.00	220	< 0.8
Sodium nitrite	5.00	340	< 0.8

Requirement of the solvent

The cut-off wavelength of methanol and ethanol is 205 nm. The presence of impurity or interfering substances in solvent such as highly conjugated compounds may affect the absorbance measured. Thereby, in this regard, it is necessary to check for any absorption peak of the solvent around the wavelengths selected for the test solution being examined. This can be done by determining the absorbance of the solvent in a 1-cm quartz cell with air as blank. The absorbance of a solvent in 1-cm quartz cell against air should not exceed 0.40 in the range of 220 - 240 nm, 0.20 in the range of 241 - 250 nm, 0.10 in the range of 251 - 300 nm, and 0.05 at the wavelengths above 300 nm.

Procedure

Solvent served as blank and the solvent used for preparing the test solutions should be from the same batch, unless otherwise specified. The absorbance of the test solution at the absorption maximum near the wavelength specified in the monograph is determined. The difference of the absorption maximum wavelength of the test solution and wavelength specified in the monograph should not exceed ±2nm, unless otherwise specified. The absorbance reading is usually more accurate in the range of 0.3 to 0.7. The width of the spectral slit must be smaller than one tenth of half-width of the absorption band, otherwise, may result in low absorbance. The slit width is appropriate if further reduction does not result in an increase of the absorbance. The absorbance due to the quartz cell and the solvent used must be subtracted from the absorbance due to the substance being examined or automatically subtracted by the instrument.

If the absorbance of the testing solution can be affected by pH, the pH of the blank and testing solution should be the same.

(1) **Identification and quality tests** – refer to the method described in the corresponding monograph.

(2) **Determination of the content** – refer to the method described in the following:

(a) **Comparison with Standard solution method** – Prepare the standard solutions from the CRS according to the method described in the corresponding monograph. The concentration of content of CRS standard solution used should be 100% ±10% of the test solution being examined. Measure the absorbances of test and standard solutions at the specified wavelengths and determine the concentration of the test solution according to the following equation:

$$C_X = (A_X/A_R)\, C_R$$

Where C_X is the concentration of the test solution; A_X is the absorbance of the test solution; C_R is the concentration of the standard solution; A_R is the absorbance of standard solution.

(b) **Absorption coefficient method** – Prepare the test solution according to the method described in corresponding monograph. Then measure the absorbance at the specified wavelength. The concentration of test solution is determined by calculating the absorption coefficient. This method can be used when the absorption coefficient is larger than 100 and the instrument should be regularly checked and calibrated.

(c) **Colourimetry** – The content of test solution being examined is determined by measuring the absorbance of the test solution treated with a suitable colour-developing agent.

Colourimetry determination can be carried out with a series of standard solution. A blank is prepared in the same manner as the test and standard solution, containing equal volume of solvent added with the same amount of colour-developing agent. Prepare a 5-point calibration curve by plotting the absorbances of the standard solutions at the specified wavelength against the corresponding concentrations (in milligram per litre) of the standard solutions. Obtain the slope, y-intercept, the regression equation and the r^2 value from the calibration curve. The concentration of the analyte in the test solution is calculated by using the following equation –

$$\text{Concentration of the analyte} = \frac{A - I}{m}$$

Where A = the absorbance of the analyte in the test solution,
I = the y-intercept of the 5-point calibration curve,
m = the slope of the 5-point calibration curve.

Calculate the percentage content of the analyte in the sample by using the following equation –

$$\text{Content (\%) of the analyte} = \frac{C \times V \times D}{10000\ W}$$

Where C = the concentration, in mg/L, of the analyte in the test solution,
D = dilution factor, if any,
V = the final make-up volume, in mL, of the test solution,
W = the weight, in g, of the sample used for the preparation of the test solution.

The concentration of the test solution can also be determined by measuring the absorbance of the test and standard solution, after adding colour-developing agent and using the equation stated in the method (a).