Standard Operating Procedure of UV-Vis spectrometry

1- Purpose

This instruction describes the use of a UV-Vis model Shimadzu UV 1800 made in Japan with an identification number of 0208 Located in the Chemometrics Laboratory of the Pharmaceutical Analysis Research Center.

2- Scope

This method is used to measure the pharmaceutical and non-pharmaceutical compounds that have detectable peaks in the UV-Vis region.

3- Responsibility

The laboratory assistance is responsible for the accuracy of the apparatus.

4- Materials and equipment

4-1) equipment

Name: UV-Vis spectrophotometer

4-2) Materials

Potassium dichromate powder and hemoglobin drabkin for quality control

5- Guidelines for use

- *Plug the apparatus into a power outlet.*
- *Make sure that the cuvette is not inside the cell holder and its lid is closed.*
- *Turn on the device with a key on the right hand of the device. After the power button is pressed, the model of the device will be displayed on the screen.*
- *After that, the apparatus automatically performs some operations called Initialization and waits until PC Control appears on the monitor.*
- *Give the devise 5 minutes to warm up.*
- *Run the device software called UV Probe 2.61.*
- *In the opened window, press the "Connect" key to connect the device to the software.*
- *Then according to the below path in the "Spectrum" window, enter the wavelength range that you want to scan.*

  Edit → Spectrum method → Measurement → Wavelength range
- *Fill the cuvettes with the solvent used for the analyte and place them in the cell holders.*
- *Press the "Baseline" key to set the background absorbance to zero.*
- *Empty the first cuvette and fill it with the analyte solution and place it back in the cell holder.*
- *Press the "Start" key to draw the spectrum of the analyte.*

- *From the "Save as" option in the "File" section on the "Spectrum" window, save the spectrum in Spc. or TXT format.*
- *To turn off the device, exit from the software, press the power button and unplug the device.*

Note: To read the absorbance in a single beam mode, select the "Go To" at the bottom of the "Spectrum" window, enter the desired wavelength and press the Enter key to move to the desired wavelength. Fill both cuvettes with sample solvent and place in the cell holders. Click on the "Auto Zero" button to set the background absorbance to zero. Then pour the analyte solution into the cuvette and the absorbance will be displayed at the opened window.

6- Warning and safety precautions

- *Connect the device to the power stabilizer to prevent serious damage to the device due to power fluctuations.*
- *Avoid handing over the device to beginners and unfamiliar with spectrophotometric principles.*
- *For measurement, use a sample free of foreign materials and particles.*
- *When operating with the cuvette, ensure that the inside and outer walls of the cuvette are clean.*
- *Avoid placing the cuvette inside the cell holder after the work is completed.*
- *Avoid pouring any solution into the cell holder.*

- *After work is done, it is recommended that the device is unplugged and covered.*
- *Since the spectrophotometer is a precise and sensitive instrument, do not replace the device parts without the approval of the relevant agency.*

7- Maintenance

The spectrophotometer should be kept dry and away from direct sunlight. After work is done, turn off the device and cover it.

8- Quality control

The spectrophotometer should be qualitatively controlled for 6-month periods in terms of linearity control, photometric accuracy, wavelength accuracy, stability test or photometric drift. Solution No#1. Dry the potassium dichromate powder in an oven at 110 °C for one hour and diluted 100 mg of it with 0.01 N sulfuric acid to 1 L. Store this solution in the dark flask. This solution is used to linearity control.

In 10 tubes, pour 2 mL of 0.01 N sulfuric acid, respectively, except for tube number 1 and in tube 1 add 2 mL of stock solution and transfer 1 mL of it to tube number and stir well. 1 mL is then transferred from tube 2 to tube 3, and then goes to tube 10 (serial dilution is prepared).

Solution No#2.

Drabkin hemoglobin solution (used to control the accuracy of wavelength or maximum wavelength) Solution No#3.

Dilute one second of solution number 1 (be sure to prepare it in the volumetric flask Class A).

This solution is used to control the photometric accuracy. Solution No#4.

Hemoglobin Drabkin Solution is used to control photometry drift. The spectrophotometer quality control form is then filled.

9- References

Catalog and Instructions given by Manufacturer and Instructions of Iranian Reference Laboratory

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