

 <p><b>BLDEA'S Shri Sanganabasava Mahaswamiji College of Pharmacy &amp; Research Centre Vijayapur 586103</b></p>	<b>DEPT:</b> PHARMACEUTICAL CHEMISTRY	<b>SOP NO.:</b> <b>BCP/PC/SOP/013</b>
	<b>INSTRUMENT:</b> UV-VISIBLE SPECTROPHOTOMETER	<b>PAGE NO.:</b> 01-02
	<b>MAKE:</b> SHIMADZU <b>MODEL:</b> UV-1900i <b>PROCURED ON:</b> 10-07-2020	<b>EFFECTIVE DATE:</b> <b>01/01/2022</b>
	<b>SUBJECT:</b> <b>SOP FOR UV- VISIBLE SPECTROPHOTOMETER</b>	<b>REVIEW PERIOD:</b> <b>31/12/2022</b>

**Objective:**

The following document describes the standard operating procedure for UV-Visible spectrophotometer..

**Scope:**

UV-Visible Spectrophotometer is used to detect maximum absorbance for given sample, unknown concentration of sample, carry out calibration studies, etc.

**Procedure:**

1. Plug in to ensure the power supply.
2. Switch **ON** the main power supply and instrument mains.
3. The instrument will auto ensure that the program is functioning properly by displaying **OK** on the screen. This will take approximately 3-4 min.
4. From the Configure drop-down menu, select Parameters.
  - i. You may use the default parameters
  - ii. **OR** Adjust Wavelength Range before starting the test. Wavelength range is between 200-800 nm.
  - iii. Recording Range can be changed at any time. It is recommended to set Scan Speed to fast and Sample Interval to Auto.
5. For determining maximum wavelength for the given sample, go to **SPECTRUM** mode.
6. Select the wavelength scanning range between 800-200nm.

7. Fill the two cuvettes with blank solution and place both of them in two separate cuvette holder.
8. Click on **BASE** Correction to detect and nullify any background interference.
9. After the base correction is done the instrument will beep.
10. Replace one of the cuvettes with the sample under study.
11. Press the **START** button and wait till the scanning is completed.
12. A graph will be displayed on the screen with the highest peak as the maximum absorption for the given sample.
13. For obtaining linear curve/ calibration curve, go to **PHOPTOMETRIC** mode.
14. Click on **GO TO WAVELENGTH** and set the maximum absorbed wavelength.
15. Fill the two cuvettes with blank solution and place both of them in two separate cuvette holder.
16. If some amount of the light will be absorbed by the blank, the reading will be displayed on the screen, then click on **Auto Zero** and wait until it reads 0.000A
17. Replace one of the cuvettes with the different serially diluted samples under study.
18. Note the absorbance values for each diluted sample and plot a graph of **ABSORBANCE** v/s **CONCENTRATION**.

**Precautions:**

- Before start of the instrument, ensure to remove silica bag from the cuvette holder chamber.
- Ensure that cuvettes are cleaned thoroughly after every using.
- The concentrations of the solutions under study by dilute so as to obtain accurate results.

	<b>PREPARED BY</b>	<b>CHECKED BY</b>	<b>APPROVED BY</b>
<b>NAME and DESIGNATION</b>	Ms.Hasti.Kenia Assistant professor	Dr. S.M.Metri Associate Professor	Dr.B.Shivakumar Professor & Head
<b>SIGNATURE &amp; DATE</b>			