

ULTRA-VIOLET VISIBLE SPECTRO PHOTOMETER

Ultra-Violet Visible Spectrophotometer (UV-Vis)

Principle:

Measures absorbance of UV (190–400 nm) and Visible light (400–800 nm) based on **Beer-Lambert's Law**.

Wavelength Range: 190–800 nm

Light Source: Deuterium (UV), Tungsten (Visible)

Detector: Photodiode / PMT

Cuvette: Quartz (UV), Glass (Visible)

Standard Operating Procedure (SOP)

1. Instrument Start-Up

- Switch ON the UV-Vis spectrophotometer
- Allow warm-up for **15–20 minutes**
- Select required mode (UV / Visible / Scan)

2. Sample Preparation

- Prepare blank using solvent/reagent
- Prepare test solution of known concentration
- Filter sample if turbid

3. Cuvette Handling

- Use **quartz cuvette for UV**, glass for visible
- Rinse cuvette with sample before filling
- Fill cuvette up to 2/3 level
- Wipe cuvette with lint-free tissue

4. Baseline / Blank Correction

- Insert blank cuvette
- Set absorbance to **zero (AUTO ZERO)**
- Remove blank

5. Sample Analysis

- Insert sample cuvette
- Set wavelength (λ_{\max})
- Record absorbance / spectrum
- Repeat for replicates if required

6. Data Recording

- Note wavelength, absorbance values
- Plot calibration curve if quantitative analysis
- Save or print results

7. Instrument Shutdown

- Remove cuvettes
- Switch OFF instrument
- Clean cuvettes and workspace

Applications

- Drug estimation
- Phytochemical analysis
- Protein & nucleic acid analysis
- Quality control
- Research & teaching labs

Precautions

- Do not touch optical surfaces
- Avoid air bubbles in cuvette
- Always match cuvette orientation
- Use freshly prepared solutions