STANDARD OPERATING PROCEDURE	
Department: Quality Control	**SOP No.:**
Title: Operation and Calibration of UV-Visible Spectrophotometer	**Effective Date:**
Supersedes: Nil	**Review Date:**
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1.0 OBJECTIVE:

To lay down a procedure for Operation & Calibration of UV-Visible spectrophotometer.

2.0 SCOPE:

The scope of this SOP covers the operation and calibration of UV-Visible spectrophotometer as per the current pharmacopoeias (IP/BP) and In-House requirements in Quality control department Make: Shimadzu, Model: UV-1800 with software UV probe 2.50.

3.0 RESPONSIBILITY:

Executive, Officer – Quality Control.

Head – Quality control

4.0 PROCEDURE :

4.1 Operation:

4.1.1 Ensure that instrument is clean and free from dust and instrument is calibrated.

4.1.2 Switch ‘ON’ the mains.

4.1.3 Switch ‘ON’ the power button on the instrument; wait for pre initialization of the instrument.

4.1.4 The instrument will do automatically self-diagnosis for following initialization.

4.1.5 Select and double click on UV Probe 2.50 icon available on the desktop of monitor.

4.1.6 UV Probe window display (Photometric mode or Spectrum mode or Kinetic) on the screen.

4.1.7 Click on connect button and connect the software with instrument.

4.1.8 Select and click on the photometric mode or spectrum mode or kinetic available Icon as per requirement.

4.1.9 For Photometric mode analysis:

4.1..1 For preparation of new method, click on the method icon (M), method wizard will be display on the screen.

4.1.10.2 Add required wavelength and click on next button, Select type Raw data and then click next button, again click on next button and then click Finish button.

4.1.10.3 Select file menu and click on save as, add file name and save the file with selecting in a UV probe method folder.

4.1.10.4 For opening method, click on file menu, select open and select respective method available in a UV Probe method folder.

4.1.10.5 Fill the blank solution in the cuvette and put the cuvettes in the sample compartment, then click on the Autozero button.

4.1.10.6 After the Auto zero, process over fill the cuvettes with standard and except reference cuvettes put the cuvettes on the sample compartment, give the sample ID and comments as per requirement and then click on Read Unk button.

4.1.10.7 Fill the cuvetts with sample and put the cuvettes on the sample compartment and click on Read Unk button.

4.1.10.8 After completion save the data file with respective folder. For printing click on report icon, report window display on the screen. Click on file menu and select open, select the report template and click on preview and click on print button.

4.1.11 For Spectrum mode analysis:

4.1.11.1 For preparation of new method, click on the method icon (M), method wizard will be display on the screen.

4.1.11.2 Add required range and click on OK button.

4.1.11.3 Select file menu and click on save as, add file name and save the file with selecting in a UV Probe method folder.

4.1.11.4 For opening method, click on file menu, select open and select respective method available in a UV Probe method folder.

4.1.11.5 Fill the blank in the cuvette and put the cuvette on the sample compartment, then click on the Baseline button.

4.1.11.6 After the baseline correction, fill the cuvette with standard and put the cuvette on the sample compartment, give the sample ID and comments as per requirement and then click on Read Unk button.

4.1.11.7 Fill the cuvette with sample and put the cuvette on the sample compartment and click on Read Unk button.

4.1.11.8 After completion save the data file with respective folder. For printing click on report icon, report window display on the screen. Click on file menu and select on open, select the report template and click on preview and click on print button.

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4.2 Calibration:

4.2.1 Control of absorbance:

4.2.1.1 **Preparation of 0.005 M Sulphuric acid: -** 0.6ml (2 to 3 drops) of sulphuric acid in 1000ml of purified water.

Weigh accurately about 57-63 mg of Potassium Dichromate, previously dried at 130°C for two hours for constant mass (Dry until two consecutive weighings do not differ by more than 0.5 mg), in a 1000 ml dried and clean volumetric flask, dissolve and makeup to volume with 0.005 M sulphuric acid. Check the absorbance of solution against 0.005 M sulphuric acid at the 235nm, 257nm, 313nm and 350nm.

4.2.1.2 For control of absorbance at 430nm use solution prepared by dissolving an accurately weighed quantity of about 57-63mg of potassium Dichromate, previously dried at 130°C to constant mass (Dry until two consecutive weighings do not differ by more than 0.5 mg), in a 100 ml dried and clean volumetric flask, dissolve and makeup to volume with 0.005 M sulphuric acid.

4.2.1.3 **Acceptance Table**

Wavelength (nm)	E (1%, 1 cm)	Maximum tolerance limits
235	124.5	122.9 to 126.2
257	144.5	142.8 to 146.2
313	48.6	47.0 to 50.3
350	107.3	105.6 to 109.0
430	15.9	15.7 to 16.1

Calculation for Wavelengths (235, 257, 313 & 350 nm)

$$= \frac{\text{Obtained Absorbance} \times 1000}{\text{Weight of } K_2Cr_2O_7 \text{ in g} \times 100}$$

Calculation for Wavelength (430 nm)

$$= \frac{\text{Obtained Absorbance} \times 100}{\text{Weight of } K_2Cr_2O_7 \text{ in g} \times 100}$$

4.2.2 Limit of stray light:

4.2.2.1 Weigh accurately about 1.2 g of potassium chloride in 100 ml dried and clean volumetric flask and make up the volume with water.

4.2.2.2 Scan the sample in the range 190 to 230 nm in a 1 cm cell path length against water and the record the absorbance at 198 nm.

4.2.2.3 The absorbance of the above solution in a 1 cm cell path length increases steeply between 220 nm and 200 nm and the absorbance at 198 nm is greater than 2.0.

4.2.3 Resolution :

4.2.3.1 Prepare a 0.02%v/v solution of toluene in hexane as follows :

Transfer 2.0 ml of toluene into a 100 ml clean and dry volumetric flask. Dilute to volume with hexane. Dilute 1.0 ml of this solution to 100 ml with hexane.

4.2.3.2 Take the spectrum of the solution in the range 225 nm to 290 nm.

4.2.3.3 The ratio of the absorbance at the maximum at about 269 nm to that the minimum at about 266 nm is not less than 1.5

4.2.4 Control of wavelength:

4.2.4.1 **Preparation of Holmium Percholate solution:** Weigh accurately about 400 mg of Holmium oxide in 10 ml of 1.4 M Perchloric acid

Preparation of 1.4 M Percholric Acid: 11.4ml of Perchloric in100 ml of purified water.

Verify the wavelength scale using the absorption maxima of Holmium perchlorate solution & record the absorbance in the range 200 nm to 400 nm & 400 nm to 600 nm.

4.2.4.2 The permitted tolerance is + 1 nm for the range 400 nm to 200 nm.

The permitted tolerance is + 3 nm for the range 600 nm to 400 nm.

Wavelengths	**Limit**
241.15 nm	240.15 to 242.15
287.15 nm	286.15 to 288.15
361.5 nm	360.50 to 362.50
536.3 nm	533.30 to 539.30

4.2.5 Resolution power using 0.02% v/v Toluene in Methanol:

4.2.5.1 Record the second order-derivative spectrum of a 0.02 per cent V/V toluene in methanol ,using methanol as the compensation liquid

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4.2.5.2 Take the spectrum of the above solution in the range 255 nm to 275 nm.

4.2.5.3 **Acceptance criteria:**

4.2.5.3.1 The spectrum shows a small negative extremum located between 2 large negative extrema at 261 nm and 268 nm, respectively.

4.2.5.3.2 The absorbance ratio A/B should be not less than 0.2.

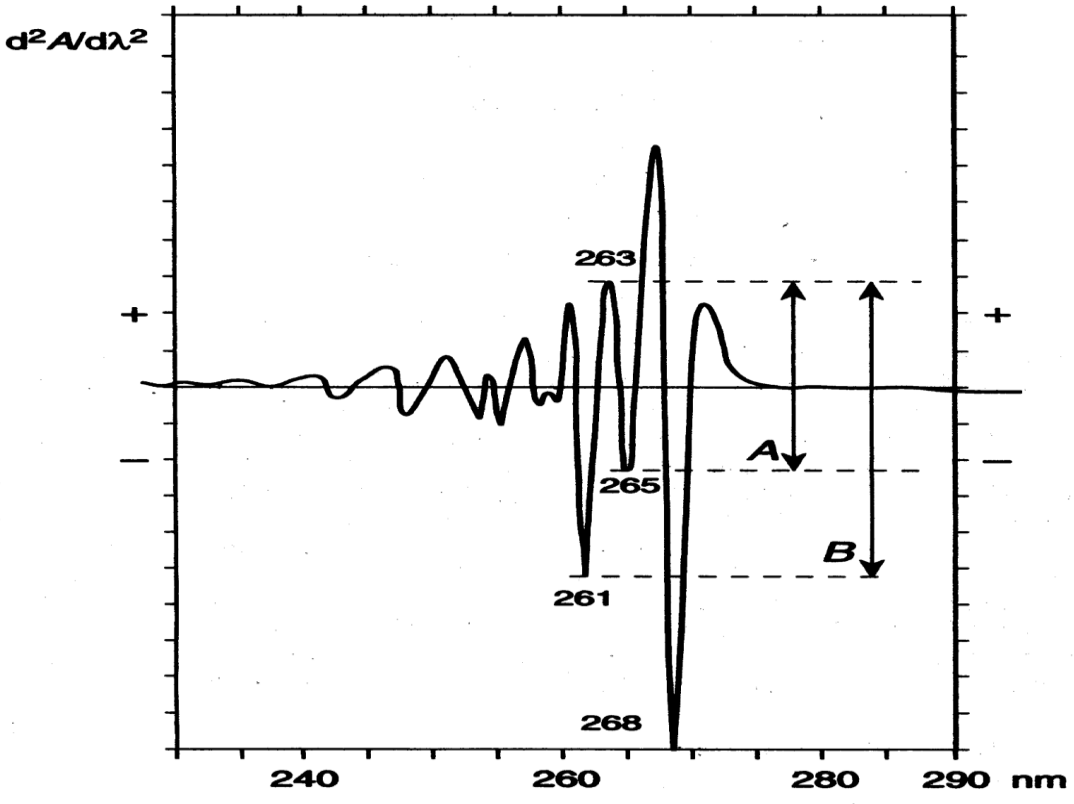

Figure 2.2.25.-1

4.2.6 Frequency:

Quarterly: All Calibration Parameter

Monthly: Control of absorbance and Control of wavelength

4.2.7 If the instrument is out of calibration, Affix an "OUT OF CALIBRATION" tag, and proceed as per SOP No..

4.2.8 Record the calibration as per Annexure – I.

5.0 ANNEXURE (S):

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Annexure – I : Calibration Record of UV-Visible spectrophotometer.

6.0 REFERENCE (S):

SOP: Preparation, Approval, Distribution control, revision and destruction of Standard operating Procedure (SOP).

7.0 ABBREVIATION (S) /DEFINITION (S):

nm - nanometer

λ - wavelength

$K_2Cr_2O_7$ - potassium Dichromate

REVISION CARD

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REASON (S) FOR REVISION	REFERENCE CHANGE CONTROL No.
1	00	----	------	New SOP	-

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ANNEXURE – I
Calibration Record of UV–Visible Spectrophotometer

Location		**Page No.:**	1 of 5
Manufactured By	Shimadzu	**Model No.**	
Frequency		**Identification No.**	
Date of Calibration		**Next Calibration Due on**	

Balance ID:	**Oven ID:**
Batch No. of Potassium Dichromate : _____ Grade : _____	
Drying procedure of Potassium dichromate (Dry at 130°C to constant mass) : Weight of LOD bottle + standard : Weight of LOD bottle + standard (after drying) : (I)_____ g, (ii.) _____ g	
1.1 For control of absorbance at 235 nm, 257 nm, 313 nm and 350 nm	

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preparation of standard solution:

Weight of potassium dichromate (previously dried to constant mass at 130°C) _____ g (0.057-0.063g) diluted to _____ ml (1000 ml) with 0.005 M sulphuric acid.

Calculation : $\frac{\text{Obtained Absorbance X 1000}}{\text{Weight of } K_2Cr_2O_7 \text{ in g X 100}}$

1) At 235 nm = ———————— X $\frac{1000}{100}$ =

2) At 257 nm = ———————— X $\frac{1000}{100}$ =

3) At 313 nm = ———————— X $\frac{1000}{100}$ =

4) At 350 nm = ———————— X $\frac{1000}{100}$ =

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Observation:

Wavelength in nm	E 1% 1 cm	Absorbance	Result	Tolerance
235	124.5			122.9 to 126.2
257	144.5			142.8 to 146.2
313	48.6			47.0 to 50.3
350	107.3			105.6 to 109.0

Complies /Does not comply

1.2 For control of absorbance at 430 nm

Preparation of standard solution :

Primary standard solution make / batch No. : _____

Weight of potassium dichromate (previously dried at 130°C to constant mass) _____ g (0.057 - 0.063g) diluted to _____ ml (100 ml) with 0.005 M sulphuric acid.

Calculation : $\frac{\text{Obtained Absorbance X 100}}{\text{Weight of } K_2Cr_2O_7 \text{ in g X 100}}$ At 430 nm = _____ X $\frac{100}{100}$ =

Observation :

Wavelength in nm	E 1% 1 cm	Absorbance	Result	Tolerance
430	15.9			15.7 to 16.1

Complies /does not comply

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2. Limit of stray light:

Balance ID:	

Batch No. of Potassium chloride : _____ Grade : _____

Mfr : _____

Preparation of Standard solution :

Weigh accurately _____ g (about 1.2 g) of potassium chloride in _____ ml (100 ml) dried and clean volumetric flask and make up the volume with water.

Scan the sample in the range 190 to 230 nm in a 1 cm cell path length against water and the record the absorbance at 198 nm.

Observation :

1) The absorbance of the standard solution in a 1 cm cell path length increases steeply between 220 nm and 200 nm: Comply / Does not comply.

2) The absorbance of standard solution at 198 nm is _____ (greater than 2.0).

Complies /Does not comply

3. Resolution:

Batch No. of Toluene : _____ Grade : _____ Mfr : _____

Batch No. of Hexane : _____ Grade : _____ Mfr : _____

Standard solution :

Transferred _____ ml (2.0 ml) of toluene into _____ ml (100 ml) clean and dry volumetric flask. Diluted to volume with hexane. Diluted _____ ml (1.0 ml) of this solution to _____ ml (100 ml) with hexane.

Calculation :

Absorbance at 269 nm : _____ Absorbance at 266 nm : _____

Ratio of absorbance of 269 nm/266 nm = _____ =

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4. Control of wavelength:

Lot No. of Holmium per chlorate standard solution	

PREPARATION OF HOLMIUM PERCHOLATE SOLUTION-400 mg of Holmium Oxide in 10 ml of 1.4 M percholic acid (11.4 ml percholic acid in 100ml of purified water.)

Verify the wavelength scale using the absorption maxima of Holmium per chlorate solution & record the absorbance in the range 200 nm to 600 nm & 400 nm to 600 nm.

The permitted tolerance is ± 1 nm for the range 200 nm to 400 nm.

The permitted tolerance is ± 3 nm for the range 400 nm to 600 nm.

Observation :

Wavelengths (nm)	**Observed Wavelength (nm)**	**Limits**
241.15		240.15 to 242.15
287.15		286.15 to 288.15
361.50		360.50 to 362.50
536.30		533.30 to 539.30

Complies /does not comply

5. Resolution power:

Batch No. of Toluene : _____ Grade : _____ Mfr : _____

Batch No. of Methanol : _____ Grade : _____ Mfr : _____

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Standard solution :

Transferred _____ ml (2.0 ml) of toluene into _____ ml (100 ml) clean and dry volumetric flask. Diluted to volume with methanol. Diluted _____ ml (1.0 ml) of this solution to ____ ml (100 ml) with methanol.

Observation - I:

The spectrum shows a small negative extremum located between 2 large negative Extrema at _____ nm (261 nm) and _____ nm (268 nm), respectively.

Observation - II:

The absorbance ratio between two minima and one maximum at 261 nm to 268 nm should be not less than 0.2.

A: _____ B: _____ The ratio A/B is _____ (Not less than 0.2).

Complies /Does not comply

Remarks: The Instrument Calibration is **OK/ Not OK** as per **IP/BP/In-House** requirements.

Calibrated By :	**Checked By :**	**Approved By :**
Date :	**Date :**	**Date :**