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 and calibration of UV-Visible Spectrophotometer.

2.0 SCOPE:

This procedure is applicable for operation and calibration of UV-Visible Spectrophotometer in the Quality Control Department.

3.0 RESPONSIBILITY:

Officer, Executive – Quality Control Department

Head – Quality Control Department

4.0 DEFINITION(S):

NA

5.0 PROCEDURE:

Make : Shimadzu, Model : UV-1700

5.1 Operation:

5.1.1 Ensure that instrument is clean and free from dust.

5.1.2 Switch ‘ON’ the mains.

5.1.3 Switch ‘ON’ the power button of the instrument, wait for pre initialization of the instrument.

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

LSI initialize

ROM check

RAM check

Filter origin

Light Source Org.

λ org. (coarse)

W Lamp Energy

λ org. (Fine)

D_2 Lamp Energy

λ checks

5.1.5 After the initialization, display will show following modes.

1. Photometric
2. Spectrum
3. Quantitation
4. Kinetics
5. Time Scan
6. Multi Component
7. Photometric (Multi λ)
8. Optional Program Pack
9. Utilities

5.1.6 Select the mode by pressing their respective serial number, as per requirement.

5.1.7 For Photometric mode analysis:

5.1.7.1 Press 1 and Photometric mode will display.

5.1.7.2 Press GO TO WL and put λ value as per requirement, then press ENTER.

5.1.7.3 Display will show the λ value as entered.

5.1.7.4 Clean both the cuvettes with methanol properly and set blank in both cuvettes, then place the transparent face of cuvettes in the path of light.

(Before putting cuvette inside the compartment wipe the cuvette properly with tissue paper from outside)

5.1.7.5 Check the Absorbance, if it is not showing the 0.000 ABS, then press AUTOZERO and wait for 0.000 ABS.

5.1.7.6 Take out first one cuvette and put the standard & sample as required and take the print of the result.

5.1.7.7 If samples are more than one, press START/STOP and display will show result into tabulation form.

5.1.7.8 Put samples one by one and press every time START/STOP. Maximum 8 samples can display on the screen and can take out print. If samples are more than 8, then we can take print out with the help of F3 and F4.

5.1.7.9 After analysis press RETURN and MODE to get original mode.

5.1.8 For Spectrum mode analysis:

5.1.8.1 Press 2, then instrument will go in spectrum mode automatically.

5.1.8.2 Display will show spectrum mode.

1. Meas. Mode
2. Scanning Range
3. Rec. range
4. Scan speed
5. No. of scans
6. Display mode

5.1.8.3 After setting the above parameters as per our requirement put the blank into both cuvette and press F1 for base line correction.

5.1.8.4 After base line correction remove first one cuvette, rinse and then put standard & samples as required and press START/STOP for scanning. Display will show a graph on the screen of the instrument.

5.1.8.5 Press F2 for the data proc as display on the screen of instrument.

5.1.8.6 Press 3 to check the peak.

5.1.8.7 Press 4 to Area calc, Press 5 to point pick, display will ask λ value and interval (put the value as per our requirement)

5.1.8.8 Press RETURN and MODE to get original mode.

5.1.8.9 Record the operation detail in instrument log book of UV – Visible Spectrophotometer.

5.2 Calibration:

5.2.1 Control of absorbance:

5.2.1.1 Weigh accurately about 60 mg of previously dried at 130°C Potassium Dichromate in 1000 ml dried and clean volumetric flask, dissolve and makeup to volume with 0.005 M sulphuric acid.

5.2.1.2 Check the absorbance of solution against 0.005 M sulphuric acid at the following wavelength.

Wavelength (nm)	E (1%, 1 cm)	Maximum tolerance limits
235	124.5	122.9 to 126.2
257	144.0	142.4 to 145.7
313	48.6	47.0 to 50.3
350	106.6	104.9 to 108.2

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

5.2.2 Limit of stray light:

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

5.2.2.2 Measure the absorbance of solution at 200 nm against water.

5.2.2.3 Absorbance of the above solution at a path length of cell 10 mm at about 200 nm should be greater than 2.0.

5.2.3 Resolution power:

5.2.3.1 Prepare a 0.02 % v/v solution of toluene in hexane.

5.2.3.2 Take the spectrum of the solution in the range 225 nm to 275 nm.

5.2.3.3 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

5.2.4 Control of wavelength:

5.2.4.1 Verify the wavelength scale using the absorption maxima of Holmium per chlorate solution(4 % solution of Holmium oxide in 1.4 M Perchloric acid) & record the absorbance in the range 200 nm to 600 nm.

5.2.4.2 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.

Wavelengths

241.15 nm

287.15 nm

361.5 nm

536.3 nm

5.2.5 Resolution:

5.2.5.1 Record second derivative spectrum in the range 255 to 275 nm of a 0.02 % v/v solution of toluene in Methanol using Methanol in the reference cell.

5.2.5.2 For second derivative spectrum, feed following parameters after pressing 2 for derivative.

Order : 2

$\Delta \lambda$ (N), Δ T (N) : 2

5.2.5.3 A small negative extremum located between two large negative extrema at about 261 nm and 268 nm should be clearly visible.

5.2.6 Frequency - Quarterly

5.2.7 If instrument is out of calibration, affix “UNDER MAINTENANCE” label and call for service engineer.

5.2.8 Fill the calibration status on metallic calibration label of the instrument, record the calibration results in annexure - I

5.3.0 Cleaning:

5.3.1 Clean the Instrument properly with cotton cloth.

5.3.2 Wash the cuvette with water after analysis.

6.0 ABBREVIATION(S):

QCD – Quality Control Department

SOP – Standard Operating Procedure

NA – Not Applicable

nm - nanometer

$K_2Cr_2O_7$ - Potassium Dichromate

IP – Indian Pharmacopoeia

7.0 REFERENCE(S):

IP -1996

8.0 ANNEXURE(S):

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

PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

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REVISION CARD:

S.No.	REVISION No.	REVISION DATE	DETAILS OF REVISION	REVISION FOR REVISION

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ANNEXURE I

1.0 Control of absorbance:

Batch No. of Potassium Dichromate:

Weight of potassium dichromate _____ g diluted to 1000 ml with 0.005 M Sulphuric acid.

Wavelength in nm	A (1%, 1 cm)	Absorbance	Result	Tolerance
235	124.5			122.9 to 126.2
257	144.0			142.4 to 145.7
313	48.6			47.0 to 50.3
350	106.6			104.9 to 108.2

Calculation: Observed Absorbance x 1000 = _____ x _1000 =

Weight of $K_2Cr_2O_7$ in g x 100 100

2.0 Limit of stray light:

Batch No. of Potassium chloride:

Weight of potassium chloride _____ g diluted to 100 ml with distilled water.

Absorbance: _____ (Limit NLT 2.0)

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3.0 Resolution power:

Batch No. of Toluene:

Batch No. of Hexane:

Prepare a 0.02% v/v solution of toluene in hexane.

Absorbance at 269 nm: _____

Absorbance at 266 nm: _____

Ratio of absorbance of 269 nm/266 nm : _____ / _____ = _____ (Limit NLT 1.5)

4.0 Control of wavelength:

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

Wavelengths	Observed Wavelength(nm)	Remarks
241.15 nm		
287.15 nm		
361.50 nm		
536.30 nm		

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5.0 Resolution:

Batch No. of Toluene:

Batch No. of Methanol:

Prepare a 0.02% w/v solution of toluene in methanol. Record second derivative spectrum in the range 255 to 275 nm

Small negative extremum located between two large negative extrema at about 261 nm and 268 nm is clearly visible / not visible.

Acceptance Criteria: Small negative extremum located between two large negative extrema at about 261 nm and 268 nm should be clearly visible.

Remarks: The Instrument Calibration **Complies/Does not Comply.**	
Calibrated By: **Date**	**Checked By:** **Date**