# PHARMA DEVILS

## QUALITY CONTROL DEPARTMENT

| STANDARD OPERATING PROCEDURE | |
|---|---|
| **Department:** Quality Control | **SOP No.:** |
| **Title:** Operation, cleaning and calibration of UV Spectrophotometer | **Effective Date:** |
| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

**1.0 OBJECTIVE:**

To lay down procedure for operation, cleaning and calibration of UV Spectrophotometer .

**2.0 SCOPE:**

This SOP is applicable for operation, cleaning and calibration of UV Spectrophotometer (Make: Perkin elmer, Model : LAMBDA 25).

**3.0 RESPONSIBILITY –** Execution- Executive QC
Checking -Assistant Manager QC

**4.0 ACCOUNTABILITY -** Manager Quality Control

**5.0 PROCEDURE:**

**5.1 OPERATING PROCEDURE:**

5.1.1 Switch on the instrument. Switch on the computer. Enter the login name and password to run the windows.

5.1.2 Click on "Perkin elmer UV win lab".

5.1.3 Enter the login name and password.

5.1.4 Go to METHOD, select method. Then go to task,data collection,Instrument. Enter wavelengths, scan speed, data interval, ordinate mode, Slit width,lamp change as per the requirement.

5.1.5 Select SAMPLE INFORMATION,enter sample ID description.

5.1.6 Go to processing.

5.1.7 Go to ACTION press START. Make it auto zero to blank put blank solution click "OK" then put the sample solution click "OK".Instrument will start automatically.

5.1.8 After completing the process, go to reporting Press PRINT to print the result.

5.1.9 In case of any problem / clarification contact LAB INCHARGE.

**5.2 CALIBRATION PROCEDURE**

(Calibration Frequency: After every 6 months)

**5.2.1 CONTROL OF ABSORBANCE**

**5.2.1.1 PREPARE THE FOLLOWING REAGENTS:**

5.2.1.1.1 0.005M Sulphuric Acid : Add carefully 7.0 ml of conc. sulphuric acid in 100 ml of cold water and make up the volume to 250 ml. Further dilute 10 ml of this solution to 1000 ml with DI water in a 1 lt.volumetric flask.

5.2.1.1.2 Potassium dichromate Solution : Dissolve 57 to 63 mg of Potassium dichromate AR previously dried at 130 deg.C to constant weight, in 100 ml of 0.005 M sulphuric acid and dissolve by keeping in an ultrasonic bath. Make up the volume to 1000 ml with 0.005 M sulphuric acid.

5.2.1.1.3 Clean the cell chamber of the instrument. Soak the cuvettes in 2% v/v solution of detergent in water and wash with water.

5.2.1.1.4 Switch on the power supply of the instrument

5.2.1.1.5 Switch on the instrument. Switch on the computer. Enter the login name and password to run the windows.

5.2.1.1.6 Click on Perkin elmer UV win lab.

5.2.1.1.7 Enter the login name and password.

5.2.1.1.8 Go to METHOD select method. Then go to task,data collection,instrument. Enter START and END wavelengths, lambda max. first and then lambda min. scan speed, data interval, ordinate mode, Slit width, lamp change.

**5.2.1.2 INSTRUMENT SETUP**

5.2.1.2.1

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| | |
|---|---|
| METHOD TYPE | SCAN |
| START WL | 400 nm |
| STOP WL | 200 nm |
| ORDINATE MODE | A |
| SLIT WIDTH | 1.00 nm |
| SCAN SPEED | 120 nm/min |
| DATA INTERVAL | 1 nm |
| NUMBER OF CYCLE | 1 |
| CYCLE TIME | 1 SEC. |
| UV LAMP | ON |
| VISIBLE LAMP | ON |
| LAMP CHANGE | 326 nm |

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5.2.1.2.2 Then go to SAMPLE INFORMATION, enter sample ID description.

5.2.1.2.3 Go to processing, add peak table.

**5.2.1.3 PROCEDURE**

5.2.1.3.1 Fill the quartz cells with 0.005 M sulphuric acid and start the baseline scan by go to ACTION then START. After the completion of the scanning the instrument will display 0.000 against the absorbance.

5.2.1.3.2 Fill the sample quartz cell with potassium dichromate solution after thoroughly rinsing it with potassium dichromate solution. Press 'OK'.

5.2.3.3 Determine absorption at 235, 257, 313 and 350 nm. The permitted tolerance for Ultraviolet range is +/- 1 nm.

**5.2.1.4 CALCULATIONS**

5.2.1.4.1 Calculate the A(1%, 1 cm) as given below:

$$A\ (1\%, 1\ cm) = \frac{\text{Absorbance}}{\text{Weight per 100 ml.}}$$

5.2.1.4.2 Check the absorbance using solution of potassium dichromate at the wavelengths indicated in the below table.

5.2.1.4.3 The values should conform to the values given in the following table.

| WAVELENGTH(nm) | SPECIFIC ABSORBANCE A (1%, 1cm) | MAXIMUM 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 |

**5.2.2 CONTROL OF WAVELENGTHS**

**5.2.2.1 PREPARATION OF REAGENTS**

5.2.2.1.2 Holmium Perchlorate solution:

Prepare 4% w/v solution of Holmium oxide in 1.4 M Perchloric acid.

**5.2.2.2 INSTRUMENT SETUP**

5.2.2.2.1Set the following values against the menu displayed as done above :

| | |
|---|---|
| METHOD TYPE | SCAN |
| START WL | 600 nm |
| STOP WL | 200 nm |
| ORDINATE MODE | A |
| SLIT WIDTH | 1.00 nm |
| SCAN SPEED | 120 nm/min |
| DATA INTERVAL | 1 nm |
| NUMBER OF CYCLE | 1 |
| CYCLE TIME | 1 SEC. |
| UV LAMP | ON |
| VISIBLE LAMP | ON |
| LAMP CHANGE | 326 nm |

**5.2.2.3 PROCEDURE**

5.2.2.3.1 Fill the quartz cells with 1.4 M Perchloric acid and start the baseline scan by go to ACTION then START.After the completion of the scanning the instrument will display 0.000 against the absorbance.

5.2.2.3.2 Fill the sample quartz cell with Holmium perchlorate solution after thoroughly rinsing it with Holmium perchlorate solution. press "OK".

5.2.2.3.3 Determine maxima.

5.2.2.3.4 The maxima shall be at the following wavelengths with permitted tolerance of ± 1nm for ultraviolet range and ± 3 nm for visible range.

| WAVELENGTH | TOLERANCE |
|---|---|
| 241.15 nm | ± 1 nm |
| 287.15 nm | ± 1 nm |

| | |
|---|---|
| 361.5 nm | ± 1 nm |
| 536.3 nm | ± 3 nm |

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5.2.2.3.5 Go to reporting,select templet save method and task ,print the result.

**5.2.3. LIMIT OF STRAY LIGHT**

**5.2.3.1 PREPARATION OF REAGENTS**

5.2.3.1.1 Potassium Chloride solution:

Prepare 12 gm/l solution of Potassium chloride in water.

**5.2.3.2 INSTRUMENT SETUP**

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| | |
|---|---|
| METHOD TYPE | WAVELENGTH PROGRAM |
| WAVELENGTH | 200 nm |
| ORDINATE MODE | A |
| SLIT WIDTH | 1.00 nm |
| RESPONSE | 0 S |
| NUMBER OF CYCLE | 1 |
| CYCLE TIME | 1 SEC. |
| UV LAMP | ON |
| VISIBLE LAMP | ON |
| LAMP CHANGE | 326 nm |

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**5.2.3.3 PROCEDURE**

5.2.3.3.1 The absorbance of above Potassium Chloride solution in water. Check the absorbance of Potassium chloride solution at 200 nm in 1 cm cell using water as blank.

**5.2.4 RESOLUTION**

5.2.4.1 Record the spectrum of a 0.2 ml of Toluene AR in 1000 ml of Hexane AR from 260 nm to 275 nm.

5.2.4.2 Calculate ratio of minimum peak height to maximum peak height using Hexane as blank.

5.2.4.3 The minimum ratio of the absorbance at the maximum at 269nm to that at the minimum at 266nm shall be more than 1.5

5.2.4.4 INSTRUMENT SETUP

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| | |
|---|---|
| METHOD TYPE | SCAN |
| START WL | 275 nm |
| STOP WL | 260 nm |
| ORDINATE MODE | A |
| SCAN SPEED | 120 nm/min |
| DATA INTERVAL | 1 nm |
| SLIT WIDTH | 1.00 nm |
| NUMBER OF CYCLE | 1 |
| CYCLE TIME | 1 Sec. |
| UV LAMP | ON |
| VISIBLE LAMP | ON |
| LAMP CHANGE | 326 nm |

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**5.2.5. RESOLUTION POWER**

5.2.5.1 Record the second derivative spectrum of 0.2 gm solution of Toluene AR in 1000 ml of methanol AR using methanol as the compensation liquid.

5.2.5.2 The spectrum shows the negative extreme located between two large negative extreme at 261 nm and 268 nm respectively.

5.2.5.3 The ratio of absorbance difference at 263 and 265 nm to the absorbance difference at 263 and 261 nm shall not be less than 0.2.

5.2.5.4 INSTRUMENT SETUP

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| | |
|---|---|
| METHOD TYPE | SCAN |
| START WL | 290 nm |
| STOP WL | 220 nm |
| ORDINATE MODE | A |
| SCAN SPEED | 30 nm/min |
| DATA INTERVAL | 0.1 nm |
| SLIT WIDTH | 0.5 nm |
| NUMBER OF CYCLE | 1 |

CYCLE TIME 1 Sec.
UV LAMP ON
VISIBLE LAMP ON
LAMP CHANGE 326 nm

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**5.3 CLEANING PROCEDURE**

**Frequency:** Daily or after each use.

5.3.1 Open the front part of the instrument .

5.3.2 Wipe out any material in the quartz cell holder assembly by means of tissue paper.

5.3.3 Clean all the sampling accessories with tissue paper after analysis and keep them in proper place.

5.3.4 Clean the outer surface of the instrument with dry cotton cloth.

5.3.5 Record the details of cleaning in log card.

**6.0 SAFETY & PRECAUTIONS:**
Not Applicable

**7.0 REVISION HISTORY:**

| Revision No. | Reason for Revision | Superseded from & Date |
|---|---|---|
| | | |

**8.0 DISTRIBUTION:**

| Copy No. | Issuance Record | | | | Withdrawal Record | | Destruction Record | |
|---|---|---|---|---|---|---|---|---|
| | Date | Dept. issued | Name / Signature of receiver | Issued By Name / Signature | By | Sign/ Date | By | Sign/ Date |
| | | | | | | | | |

**9.0 REFERENCES:**

Not Applicable

**10.0 ABBREVIATIONS & ANNEXURES:**

SOP : Standard Operating Procedure

No. : Number

QC : Quality Control

**Annexure I :** Calibration Record of UV Spectrophotometer

# PHARMA DEVILS

## QUALITY CONTROL DEPARTMENT

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|---|---|
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| **Supersedes:** Nil | **Review Date:** |
| **Issue Date:** | **Page No.:** |

### ANNEXURE-I
### CALIBRATION RECORD OF UV SPECTROPHOTOMETER

| PERFORMANCE DATE | DATE OF LAST PERFORMANCE DONE | NEXT DUE FOR PERFORMANCE |
|---|---|---|
| | | |

| INSTRUMENT DETAILS | | |
|---|---|---|
| INSTRUMENT NAME | INSTRUMENT MAKE | **INSTRUMENT IDENTIFICATION NO.** |
| | | |

### 1. Control of Absorbance:

Calculated Specific Absorbance A (1%, 1cm) = $\frac{\text{Absorbance x 1000}}{\text{W in gms}}$

W= weight of $K_2Cr_2O_7$ in mg =_____

| S.No. | Wavelength (nm) | Observed Absorbance | Calculated Specific Absorbance |
|---|---|---|---|
| 1 | 235 | | |
| 2 | 257 | | |
| 3 | 313 | | |
| 4 | 350 | | |

| S.No. | Wavelength (nm) | Calculated Specific Absorbance A (1%, 1cm) | Maximum Tolerance |
|---|---|---|---|
| 1 | 235 | | 122.9 – 126.2 |
| 2 | 257 | | 142.8 – 146.2 |
| 3 | 313 | | 47.0 – 50.3 |
| 4 | 350 | | 105.6 – 109.0 |

**Remarks:** Satisfactory/Not Satisfactory

### 2. Control of Wavelength:

| Wavelength (nm) | Measured maxima at (nm) | Tolerance |
|---|---|---|
| 241.15 | | ± 1 nm |
| 287.15 | | ± 1 nm |
| 361.5 | | ± 1 nm |
| 536.3 | | ± 3 nm |

**Remarks:** Satisfactory / Not Satisfactory

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### 3. Limit of stray light:

Absorbance of KCl (12 gm/lit) solution in water (at 200nm) _____

**Remarks:** Satisfactory/Not Satisfactory

### 4. Resolution:

Ratio of absorbance at 269nm / Absorbance at 266nm =

| Wavelength (nm) | Measured Absorbance |
|---|---|
| 269 | |
| 266 | |

**Remarks:** Satisfactory/Not Satisfactory

### 5. Resolution Power:

Wavelength scan (290 – 220 nm)

Absorbance difference at 263nm -265nm (A) =_____

Absorbance difference at 263nm -261nm (B) =_____

| **Wavelength (nm)** | **Measured Absorbance** |
|---|---|
| 261 | |
| 263 | |
| 265 | |

Ratio of (A/B) =_____

**Remarks** : Satisfactory/Not Satisfactory

Calibration done by : Date :

Checked by : Date :

| CONCLUSION | |
|---|---|
| **INSTRUMENT WORKING SATISFACTORY** | **INSTRUMENT NOT WORKING SATISFACTORY** |

| PERFORMED BY | | | CHECKED BY | | |
|---|---|---|---|---|---|
| **NAME** | **SIGN** | **DATE** | **NAME** | **SIGN** | **DATE** |
| | | | | | |