1. **PURPOSE:**

To describe the procedure for Operation and calibration of UV Visible spectrophotometer.

1. **SCOPE:**

The procedure applies to Operation and calibration of UV Visible spectrophotometer.

Make : Agilent Technologies

Model : Carry G6860A

ID No. : DIPL/QC/INS/UV/001

1. **RESPONSIBILITY:**
   1. Analyst-QC is responsible to follow this SOP.
   2. Head-QC/Designee is responsible for ensuring implementation of this SOP.
   3. Head-QA/Designee is responsible for monitoring overall compliance of this SOP.
2. **DEFINITIONS:**

Nil.

1. **PROCEDURE:**
   1. **OPERATIONS:**
      1. **ADVANCED READ** (to measure OD at a particular Wavelength)
         1. Switch on the Instrument wait for green color blinking
         2. Switch ON PC.
         3. Open ‘Cary Win UV’ folder from desktop.
         4. Open ‘Advanced Read’.
         5. Click ‘Setup’🡪put required wavelength’🡪Click OK.
         6. Put Blank on cell holder.
         7. Click ‘Zero’🡪OK.
         8. Put 1st Sample on cell holder.
         9. Click ‘Start’🡪OK🡪Write a file name🡪Save.
         10. Click ‘OK’🡪Put 2nd Sample & click ‘OK’ & CONTINUE.
         11. After completion 🡪Click ‘Cancel’🡪’OK’🡪close the window.
      2. **SCAN** (to measure λ max of a sample)
         1. Switch ON PC.
         2. Open ‘Cary Win UV’ folder from desktop.
         3. Open ‘Scan’.
         4. Click ‘Setup’🡪Put WL range at Start & Stop
         5. On Baseline Tab select ‘Baseline correction’. Click ‘OK’.
         6. Put Blank on Cell holder & Click ‘Baseline’🡪OK.
         7. Click ‘Start’🡪Write any File name & click ‘Save’.
         8. Put Sample on cell holder. Click ‘OK’.
      3. **CONCENTRATION** (to measure the concentration of unknown samples)
         1. Switch ON PC.
         2. Open ‘Cary Win UV’ folder from desktop.
         3. Open ‘Concentration’.
         4. Click ‘Setup’
         5. Put required Wavelength🡪On Standard tab write the concentration of prepared standards.
         6. Put Blank on cell holder. Click ‘Zero’🡪OK
         7. Click ‘Start’🡪OK🡪Write any file name🡪Click ‘Save’
         8. Put 1st Standard on cell holder🡪Click OK.
         9. Follow Screen Instruction.
         10. After completion close the window.
      4. **VALIDATION** (to check weather the instrument is running OK)
         1. Switch ON PC
         2. Open ‘Cary Win UV’ folder from desktop.
         3. Open ‘Validate’
         4. Select ‘Tests’
         5. Select the radio button ‘Instrument Performance Tests’
         6. Click OK.
         7. Click ‘Zero’.
         8. Click ‘Start’🡪Click ‘OK’.
         9. Wait until the completion of the test (approximately 6~7 minutes).
      5. Check all the 4 tests result & report it to Varian Tele Support No (033-64570930).
      6. To take any print out of report click print.
   2. **CALIBRATION:**
      1. **Control of Wave length:**
         1. **Preparation of 1.4 M Perchloric acid solution:**

* Take 11.5 mL of perchloric acid (60% w/w strength) into a clean and dried 100 mL volumetric flask.
* Dissolve and dilute to 100 mL with water.

**Note:** Dilute 8.5 mL to 100 mL with water in case of 70% w/w strength perchloric acid.

* + - 1. **Preparation of Holmium per chlorate:**
* Weigh accurately about 400 mg of Holmium oxide into a 10 mL volumetric flask containing about 5 mL of 1.4M perchloric acid solution. And kept aside the solution for 24 hours.
* Shake the solution well to dissolve Holmium oxide completely.
* Dilute to 10 mL with 1.4M perchloric acid solution.
  + 1. **Procedure:**
       1. Take a pair of matched quartz cells having a path length of 1cm.
       2. Perform the baseline correction with 1.4M perchloric acid solution from 200 nm to 600 nm
       3. Remove the cell from sample compartment.
       4. Rinse the sample cell with Holmium per chlorate solution fill the cell with the same, clean the smooth surface of the cell with tissue paper and place within the sample compartment.
       5. Scan from 200 nm to 600 nm.
       6. The spectrum should show the maximum at the following wave lengths.

|  |  |
| --- | --- |
| **Maxima (nm)** | **Tolerance (nm)** |
| 241.15 nm | + 1 |
| 287.15 nm | + 1 |
| 361.5 nm | + 1 |
| 536.3 nm | + 3 |

* + 1. **Control of Absorbance:**
       1. **Preparation of 0.005M Sulphuric acid:**
* Add cautiously 2.8 mL conc. Sulphuric acid into 50 mL water and dilute to 100 mL with water.
* Take 1 mL of above prepared solution into a 100 mL volumetric flask.
* Dilute to 100 mL with water.
  + - 1. **Preparation of K2Cr2O7 UV solution:**
* Weigh accurately about 200 mg of AR grade K2Cr2O7 into a weighing bottle and heat at 200oC for 2 hours.
  + 1. **Preparation of Stock solution:**
       1. Cooled it in a desiccator and weigh about 57.00 to 63.00 mg into a 100 mL volumetric flask containing 50 mL of 0.005M Sulphuric acid.
       2. Shake the flask well to dissolve completely.
       3. Dilute to 100 mL with 0.005M Sulphuric acid.
    2. **Preparation of UV solution:** 
       1. Take 1mL of above prepared stock solution into a 10 mL volumetric flask.
       2. Dilute to 10 mL with 0.005M Sulphuric acid solution.
       3. Examine this UV solution for the Control of Absorbance at 235nm, 257nm, 313nm and 350nm
       4. For the Control of Absorbance at 430nm examine with stock solution.
    3. **Procedure:**
       1. Take a pair of matched quartz cells having a path length of 1cm.
       2. Perform base line correction with 0.005M Sulphuric acid solution from 200 nm to 450 nm.
       3. Remove the cell from sample compartment. Rinse the sample cell in K2Cr2O7 UV solution, fill the cell with same solution, clean the smooth surface with tissue paper.
       4. Place the cell in sample compartment, scan from 200 nm to 450 nm and measure the absorbance at 235nm, 257nm, 313nm and 350nm.
       5. Remove the cell from sample compartment. Rinse the sample cell in K2Cr2O7 stock solution, fill the cell with same solution, and clean the smooth surface with tissue paper.
       6. Place the cell in sample compartment, scan at 430 and measure the absorbance at 430nm.
       7. Calculate the Specific absorbance using the following formula:

|  |  |
| --- | --- |
| Concentration of K2Cr2O7  (For stock solution at 430 nm) |  |
|  | |

* + - 1. The specific absorbance against each wavelength should be as follows:

|  |  |  |
| --- | --- | --- |
| **Wave Length**  **(nm)** | **Specific Absorbance**  **(1% 1cm)** | **Max Tolerance**  **(nm)** |
| 235 | 124.5 | 122.9 – 126.2 |
| 257 | 144.5 | 142.8 – 146.2 |
| 313 | 48.6 | 47.0 – 50.3 |
| 350 | 107.3 | 105.6 – 109.0 |
| 430 | 15.9 | 15.7 – 16.1 |

* + 1. **Limit of Stray light:**
       1. **Preparation of 1.2% w/v Potassium Chloride solutions:**
* Weigh accurately about 2 g of AR grade KCl into a weighing bottle and heat at 120oC for 2 hours.
* Weigh accurately about 1.2 g of KCl into a 100 mL volumetric flask.
* Dissolve and dilute to 100 mL with water.
  + - 1. **Procedure:**
* Take a pair of matched quartz cells having a path length of 1cm.
* Perform base line correction with water.
* Remove the cell from sample compartment. Rinse the sample cell KCl solution, fill the cell with same solution, and clean the smooth surface with tissue paper.
* Measure the absorbance between 220 nm to 190 nm.
* Acceptance **criteria:** The Absorbance should be more than 2.00 at 198 nm Calibration shall be done for every six month **(±7** Days)

1. **FORMATS / ANNEXURE(S):**
   1. Instrument Usage log : QC048-FM086
   2. Visible spectrometer Calibration Record : QC034-FM070
2. **CHANGE HISTORY:**

| **Revision No.** | **Effective Date** | **Details of Revision** | **Ref CCF No.** |
| --- | --- | --- | --- |
| 00 | 11.09.2013 | New SOP “Operation and calibration of UV Visible spectrophotometer.” is introduced across all the API manufacturing facilities of Discovery. | -- |
| 01 | 01.06.2014 | Formats are the part of SOP. So prepared separately. | -- |
| 02 | 01.01.2017 | SOP format changed make to in line with SOP-QA-001-04 | QC-CRF-025-16 |