A. **PURPOSE**:

The acceptability of quantitative standard solutions, prepared in-house, will be determined using a UV spectrophotometer to determine the true concentration of the solution. This will be performed when the solution is prepared; prior to use with case materials. The specific wavelength and absorptivity of the solution will be determined utilizing a reference such as *Clark's Isolation and Identification of Drugs.* Absorptivity (A^1_1) is defined as "the absorbance of a 1%w/v solution in a cell of 1cm path-length" This laboratory utilizes a Shimadzu UV-2401PC, UV-VIS Recording Spectrophotometer with UV Probe version 2.1 software.

B. **SAFETY**:

The UV spectrophotometer utilizes a light source which should not be viewed. As with any electrical device there is a chance of electrical shock if not handled properly, do not perform maintenance on this instrument unless trained to do so.

C. **RESPONSIBILITY**:

All analysts (however titled) assigned to the CS section are responsible to follow the guidance of this SOP when utilizing the UV spectrometer.

Reagents

a. Aqueous acid solution:
   1% Hydrochloric acid: 1 ml of Hydrochloric acid into 100 mls of deionized water
b. Aqueous alkaline solution:
   0.05M Borate buffer: dissolve 19.97 grams of Borax in deionized water, bring to 1000 mls

D. **PROCEDURE**:

The UV is used in the CS laboratory as a mechanism of verifying the concentration of standards prepared in-house. Standards are prepared by weighing a portion of a standard and diluting the solution in the appropriate solvent (this will be analyte specific). To correct for any "human error" in this process the solution is validated by UV. Substances were UV data is not available the concentration will be based on the weighed values. Certified reference standards used to make controls or calibration

standards do not need to be validated by UV; concentration will be based on certificate of analysis provided by the manufacturer.

After validating the solution if there is a variation greater than 20% the analyst should consult with the section Supervisor. Analysts must take into account the form of the standard they are using, if a salt form of the drug is used the salt needs to be accounted for in determining the theoretical concentration.

1. Sample Preparation:
   a. Based on the reference data for absorptivity, dilutions will be prepared from the stock standard solutions which will give an UV absorbance in a range of 0.5Au to 1.5 Au. The solutions are to be made in aqueous acid or aqueous alkaline, according to the reference data.
2. Controls:
   a. A matrix blank is run with the standards to correct for interference that may occur.
   b. Daily clean the sample compartment if spills occur
   c. Daily clean sampling cells to verify there are no finger prints on the glass cuvettes
   d. Semi-annually check wave-length accuracy (see user's manual)
3. Instrument Set-UP
   a. Turn on the UV spectrophotometer and open the UV probe software.
   b. Click the connect icon to initiate the communication between the instrument and computer.
   c. Run a Baseline Scan
   d. Set up the method under the Spectrum program.
   e. In general the instrument is set to scan from 350-200Au
      i. Log that the daily check was performed in the maintenance book. (See CS-9.1
   f. Under the method in put the standard information
      i. Weight, volume, dilution, path length and additional information.
   g. Fill a quartz cuvette with blank (sample matrix) and place this in the back cell holder. Put the standard in a quartz cuvette and place this in the front cell holder.
   h. Run the method

i. Click the peak point icon to label the peaks

ii. Go to the Reporting software open the stndval.rpt report format and print the report.

4. Calculation:

a. Concentration (mg/ml) = (absorbance (Au) x 10 mg/ml)/Absorptivity (Au)

i. The absorbance is the reading at the specified peak

ii. The 10 mg/ml factor is based on the fact that the (A^{1}_{1}) information is based on a 1%w/v solution.

iii. The absorptivity is from the reference

5. Reporting:

a. The ctroprt2 format in the reporting software is set up to provide spaces for all the needed data.

b. Fill in the needed information and calculate the concentration.

c. File the report as per the Preparation and Validation of Standards Method.

E. **SOURCES OF ERROR**:

1. Placing the sample in the wrong position in the sample chamber
2. Not using a matrix blank or using the wrong matrix
3. Putting the sample in the wrong matrix (aqueous alkaline instead of aqueous acid)
4. Applying the calculation incorrectly

F. **REFERENCES**:

1. Shimadzu UV-2450/2550 Instruction Manual
2. Shimadzu Instruction Manual UV-2401PC/2501PC User's System Guide
3. Clark's Isolation and Identification of Drugs in pharmaceuticals, body fluids, and post-mortem materials, The Pharmaceutical Society of Great Britain.