Name of Procedure:

Ultraviolet Spectroscopy

Suggested Uses:

1. Preliminary qualitative analysis of unknown substances (liquid or solid).

2. Quantitative analysis of known compounds.

Apparatus Used to Perform Procedure:

Ultraviolet Spectrophotometer
Fume hood
Gloves
Eye protection
Laboratory coat
Graduated cylinder
Storage container
Dispensing bottle
Hydrochloric acid (concentrated)
Distilled - deionized water
Funnel
Quartz UV cuvette
Volumetric flasks
Pipettes
Spatula

Formula for Preparing Reagent:

1. Concentrated hydrochloric acid is 12 N. Use a ratio of 1 mL $HCl_{(conc.)}$ to 250 mL distilled water to obtain a 0.05 N HCl solution.
2. Add the water to a storage container before adding HCl. Mix well.
3. Label the container with "0.05 N HCl", the date, preparer's initials, & "no expiration".

Expiration Date of Chemical Reagent:

The 0.05 N HCl solution has an indefinite shelf life if stored in an airtight reagent bottle.

Operational Procedures for Using the UV/Vis Spectrophotometer:

1. General Start-up of Instrument:

a. With the compartment door closed, switch instrument on. Allow the instrument to initialize and warm-up for a minimum of 20 minutes prior to use.
b. Check that the exterior of the cuvette is free of water and fingerprints. Use a Kimwipe® with methanol to clean the surface. Handle only the frosted sides.
c. Fill a clean cuvette with the 0.05 N HCl solution (or desired solvent).
d. Place the cuvette in the cell holder nearest the operator, with the non-frosted sides of the cuvette in the light path.
e. Close the door completely and perform a scan of the blank UV cell.
f. If there is no significant absorption in the scan, then the clean cuvette is now ready for qualitative or quantitative analysis. If a significant absorption is observed clean the cuvette and repeat.

2. Calibration Verification:

a. Set the following parameters (may store as a file):
   1. Scan range: 350 - 210 nm
   2. Slit width: 2.0nm (if adjustable)
   3. Absorbance range: 0 – 1.0 (or adjust as needed)
   4. Scan speed: medium
   5. Sampling interval: 0.2 nm (if adjustable)
b. While the cell holder is empty, perform a baseline correction.
c. Place the standard (Holmium Oxide Solution) into the cell holder.
d. Perform a scan of the standard.
e. Print the scan of the standard; include the peak list, the scan parameters, and instrument identification information.
f. Compare the peaks identified to the following table corresponding to the instrument model number.
   •Peaks shall be identified at the wavelengths listed in column A within the range specified in column B.
   •If a discrepancy is noted the instrument shall be removed from service and the instrument coordinator shall be notified.
   •The instrument coordinator shall correct any problems with the instrument or request service.
   •The Calibration verification shall be successfully completed prior to placing the instrument back in service.

Shimadzu UV-1601

Peak (nm)	Acceptable Range (nm)
241.12	240.1 – 242.2
250.03	249.0 – 251.0
278.10	277.1 – 279.1
287.52	286.5 – 288.5
333.47	332.5 – 334.5
345.42	344.4 – 346.4

Shimadzu UV-1650 and UV-2401

Peak (nm)	Acceptable Range (nm)
241.12	240.3 – 242.0
250.03	249.2 – 250.8
278.10	277.3 – 279.0
287.52	286.7 – 288.3
333.47	332.7 – 334.3
345.42	344.6 – 346.2

3. Qualitative Analysis:

a. Set the following parameters (may store as a file):
   1. Scan range: 350 - 210 nm
   2. Slit width: 2.0nm (if adjustable)
   3. Absorbance range: 0 – 1.0 (or adjust as needed)
   4. Scan speed: medium
   5. Sampling interval: 0.2 nm (if adjustable)

b. Rinse and fill a cuvette with the desired solvent (typically 0.05 N HCl). Ensure that the cuvette exterior is clean and dry. Ensure that no air bubbles are trapped on the inside of the cuvette. Place the cuvette in the instrument with the non-frosted sides in the light path. Close the instrument door. Perform a background correction.

c. Add the sample to the cuvette. Cover the cuvette and invert it several times to mix. Ensure that the cuvette exterior is clean and dry and that there are no air bubbles trapped on the inside of the cuvette. Place the cuvette in the instrument with the non-frosted sides in the light path. Close the instrument door.

d. Perform a scan and observe the sample absorption.

e. Readjust parameters (absorbance range) or sample concentration as necessary to obtain a scan with a peak maxima ~1 A (desired range: 0.5 to 1.0 A).

f. Print the scan, the peak list (if peaks were identified) and scan parameters.

g. Remove the cuvette and rinse well with the solvent used, followed by purified water, and allow to air dry.

4. General Quantitation of Known Single Drug (No Interfering Analytes):

a. Prepare a calibration curve of the standard to obtain the absorptivity (a or ε).
   1. Prepare a minimum of three solutions of the standard at concentrations ranging from 0.1 - 1.0 mg/ml (or other concentrations as needed).
   2. For each solution: accurately weigh an amount of standard (10 - 100 mg) into a 100 ml volumetric flask (or equivalent ratio) and bring to volume with the desired solvent (typically 0.05 N HCl). Alternatively, prepare a stock solution by accurately weighing an amount of standard into a volumetric flask and preparing serial dilutions to obtain the desired concentrations.
   3. Scan each solution according to the procedure above for Qualitative Analysis, rinsing the cuvette with the solution prior to filling, and record the absorbance at the desired wavelength.
   4. Construct a calibration curve to determine the molar absorptivity (ε) (i.e., the slope of the line produced is the molar absorptivity).

b. Prepare a solution of the sample and quantitate.
   1. Accurately weigh an amount of sample into a volumetric flask [approximately 50 mg (or other amount as needed) into a 100-mL flask (or equivalent ratio)].
   2. Bring to volume with the desired solvent (typically 0.05 N HCl).
   3. Scan the sample according to the procedure above for Qualitative Analysis, rinsing the cuvette with the sample solution prior to filling, and record the absorbance at the same wavelength used for the standard solutions.
   4. Use the following equation to determine the concentration of the analyte in the prepared sample solution.

      $A/\varepsilon = c_{analyte}$ A = absorbance (measured)

      ε = molar absorptivity (liter / mol / cm)

      (calculated from standard measurements)

      $c_{analyte}$ = concentration of analyte (mol / liter)

      Use the following equation to determine the percentage of analyte in the sample.

      $c_{analyte}/c_{sample} \times 100$ = % analyte in the sample

      $c_{analyte}$ = concentration of analyte (calculated above)

      c_{sample} = concentration of prepared sample solution

Safety Concerns:

Eye protection, appropriate gloves, and a laboratory coat should be worn when making the reagent, as concentrated hydrochloric acid is highly corrosive.

Other:

1. Normal parameter settings include:
   - absorbance mode
   - sampling interval: 0.2 nm (if adjustable)
   - medium scan speed
   - 0 to 1, 2, or 3 Abs. range
   - wavelength range: 350 to 210 nm
   - 2.0 nm slit width (if adjustable)

2. Solvents other than 0.05 N HCl may be used (although most literature references are in 0.05 N HCl) due to pH shifts of absorbance maxima and peak absorbance in non-aqueous media.

3. Molar absorptivities (ε) are available in literature sources and can be used only for approximating quantitation value. This is useful when calibration standards (e.g.: LSD) are not available. Skoog recommends against using literature values or using a single standard value in quantitation because Beer's law cannot be assumed to be met (see references).

4. Calculations may be done using other units as long as consistency is maintained between standards and sample.

Literature References:

Denney, R.; Sinclair, R. **Visible and Ultraviolet Spectroscopy (Analytical Chemistry by Open Learning)**; John Wiley and Sons;1987.

Skoog, D. **Principles of Instrumental Analysis**; Saunders College,1985; pp 160-224.

Silverstein, Brassler, Clayton, Terance, Morrill, **Spectrometric Identification of Organic Compounds**, New York, Wiley, 1991.

Instrument manuals.