Technical Procedure for Ultraviolet Spectroscopy

1.0 Purpose - This procedure specifies the required elements for the performance check and use of the ultraviolet spectrophotometers.

2.0 Scope - This procedure applies to all ultraviolet spectrophotometers used in the Drug Chemistry Section at the Raleigh location of the State Crime Laboratory.

3.0 Definitions

- **Performance verification –** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) check –** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Reference material –** Material sufficiently homogenous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Ultraviolet Spectrophotometer

4.2 Materials and Reagents

- Holmium Oxide Reference Material
- Fume Hood
- Gloves
- Eye protection
- Laboratory coat
- Graduated cylinder
- Storage container
- Dispensing bottle
- Funnel
- Quartz UV cuvette
- Volumetric flasks
- Pipettes with bulb
- Spatula
- Water (deionized)

4.3 Commercial Reagents

- Hydrochloric acid (concentrated)
- Other solvents, depending on sample needs

4.4 Formula for Preparing Reagents - The reagent may be prepared in any amount provided that the component ratios are kept constant.

4.4.1 Concentrated hydrochloric acid is 12 N. Use a ratio of 1mL concentrated hydrochloric acid to 250 mL water to obtain a 0.05 N HCl solution.

4.4.2 Add water to the storage container before carefully adding the acid.

4.4.3 Mix well.

4.4.4 Lot number: Eight digit year format year/month/day/0.05NHCl/initials of preparer. Example: 201012310.05NHClXXX

4.4.5 Expiration date - There is no expiration date for this reagent.

4.4.6 Quality control check: Quality control re-checks shall be performed every six months by ensuring the solution is acidic with litmus paper.

5.0 Procedure

5.1 General Start-up/Shutdown of Instrument

5.1.1 With the compartment door closed, switch instrument on. Allow the instrument to initialize and warm-up according to manufacturer's instructions.

5.1.2 Check that the exterior of the cuvette is free of liquid and fingerprints. Clean if any are present. Handle only the frosted sides.

5.1.3 Fill a clean cuvette with the 0.05 N HCl solution (or other suitable solvent).

- 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.

5.1.4 Place the cuvette in the cell holder nearest the operator, with the non-frosted sides of the cuvette in the light path.

5.1.5 Close the door completely and perform a scan of the blank UV cell.

5.1.6 If there is no significant absorption in the scan, the clean cuvette is ready for qualitative or quantitative analysis.

5.1.7 If a significant absorption is observed in the scan, clean the cuvette and repeat.

5.1.8 The UV instrument and computer (if applicable) may be turned off at the end of business each day.

5.1.9 When the UV has been placed out of service (e.g., maintenance, malfunction, leaving direct control of the Laboratory), correct operation shall be demonstrated by a performance verification.

- The performance verification shall follow the requirements for a monthly QC check outlined below.
- Laboratory personnel shall examine the effect(s), if any, of a malfunction on analysis results and implement the Laboratory Procedure for Corrective Action as required.

5.2 Standards and Controls

5.2.1 Maintenance

- UV instruments shall be serviced yearly by an outside approved vendor.
- Any maintenance or repairs performed on the instrument shall be recorded in the log book along with the date and person performing the maintenance or report. The log book shall be kept near each instrument. (See UV Maintenance Log in Section Files.) If converted to electronic format, maintenance/repair records shall be maintained by the UV Key Operator and stored in section files.

5.2.2 Monthly QC Check

- A monthly QC Check shall be performed by the Section UV Key Operator or his/her designee, using a standard Holmium Oxide Solution. If the data meets the criteria set out below in **5.2.2**, the Section UV Key Operator or his/her designee shall store hard copies in a notebook with each instrument, or electronically with other instrument data.
- After general start-up of instrument is complete (see below), set the following parameters (may store these as a file on the instrument):
- Scan range: 700-210 nm
- Spectral bandwidth: 1.0 nm (if adjustable)
- Absorbance range: 0-2.2 (or adjust as needed)
- Scan speed: medium
- Sampling interval: 0.2 nm (if adjustable)
- While the cell holder is empty, perform a baseline correction.
- Place the standard (Holmium Oxide Solution) into the cell holder.
- Perform a scan of the standard using the parameters listed above.
- Print the scan of the standard; include the peak list, the scan parameters, and the instrument identification information.
- 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 key operator shall be notified.
- The instrument key operator shall correct any problems with the instrument or request service.
- The QC check shall be successfully completed prior to placing the instrument back in service.

Shimadzu UV-1650PC **Accuracy(σ) = ± 0.3 nm** **Spectral Bandwidth = 2.0 nm (fixed)** **(Range set to ±0.9 nm = 3 σ at 2 nm Spectral Bandwidth)**	
***Peak(nm)**	**Acceptable Range (nm)**
241.12	**240.22 - 242.02**
250.03	**249.13 - 250.93**
278.10	**277.20 - 279.00**
287.52	**286.62 - 288.42**
333.47	**332.57 – 334.37**

345.42	**344.52 – 346.32**

*Peak wavelengths taken from current National Institute of Standards and Technology Certificate for Holmium Oxide Solution Standard Reference Material at noted Spectral Bandwidth. (Referenced to Air)

5.2.3 Performance Verification for New Instrument Set Up

5.2.3.1 Follow the same procedure as a monthly QC check using standard Holmium Oxide.

5.2.3.1.1 If the criteria are acceptable according to specifications listed in the monthly QC Check, file the initial Holmium Oxide solution scan with the instrument files as performance verification.

5.2.3.2 Select three compounds and obtain UV spectra for each.

5.2.3.2.1 If the data obtained matches the known standards for each compound, file the data collected and a copy of the standards with the instrument files as performance verification.

5.3 Application of Procedure on Evidence

5.3.1 Qualitative Analysis

5.3.2 Follow general start-up procedures.

5.3.3 Set the following parameters (may store as a file):

- Scan range: 350-210 nm
- Spectral Bandwidth: 2.0 nm (if adjustable)
- Scan speed: medium
- Sampling interval: 0.2 nm (if adjustable)

5.3.4 Rinse and fill a cuvette with the desired solvent (typically 0.05 N HCl).

5.3.5 Ensure that the cuvette exterior is clean and dry and no air bubbles are trapped on the inside of the cuvette.

5.3.6 Place the cuvette in the instrument with the non-frosted sides in the light path.

5.3.7 Close the instrument door and perform a background correction.

5.3.8 Add the sample to the cuvette.

5.3.9 Cover the cuvette and invert it several times to mix, if needed.

5.3.10 When cuvette is clean and dry with no air bubbles, place it in the instrument with the non-frosted sides in the light path.

5.3.11 Close the instrument door and perform a scan to observe the sample absorption.

5.3.12 Adjust parameters (absorbance range) or sample concentration to obtain a scan with a peak maxima approximately 1 A. (Desired range: 0.5 to 1.0 A)

5.3.13 Print the scan, the peak list (when applicable) and scan parameters.

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

5.3.15 UV 1650-PC Software Instructions:

5.3.15.1 Turn on computer and open *UV Probe* software.

5.3.15.2 Choose "Connect" at the bottom of screen, so the UV unit will communicate with the computer.

5.3.15.3 Holmium Oxide Standard Run:

5.3.15.3.1 Make sure there is no cuvette in the sample compartment.

5.3.15.3.2 Choose "Baseline" hotkey located at the bottom of the screen, and wait for baseline data collection.

5.3.15.3.3 Place the Holmium Oxide cuvette in the sample compartment.

5.3.15.3.4 Choose "File" (Located at the top of the toolbar).

5.3.15.3.4.1 Choose "Open."

5.3.15.3.5 Choose AHolmium Oxide Standard@ file and make sure that type of file at the bottom of screen is set for A*.smd@ before proceeding.

5.3.15.3.6 Choose "OK."

5.3.15.3.7 Choose "Start" hotkey at the bottom of the screen, and wait for data collection.

5.3.15.3.8 Choose AOK@ to save the data so you can print it.

5.3.15.3.9 Choose "Operations" (Located at the top of the toolbar).

5.3.15.3.9.1 Choose "Peak Pick" (or the hot key for this).

5.3.15.3.10 Choose "Window" (Located at the top of the toolbar).

5.3.15.3.10.1 Choose "Report generator" (or the hot key for this).

5.3.15.3.10.2 Choose "Print" (or "File Print Preview").

5.3.15.4 Casework Sample Run:

5.3.15.4.1 Make sure there is no cuvette in the sample compartment.

5.3.15.4.2 Choose "Baseline" hotkey located at the bottom of the screen, and wait for baseline data collection.

5.3.15.4.3 Place sample cuvette in sample compartment.

5.3.15.4.4 Choose "File" (Located at the top of the toolbar).

5.3.15.4.5 Choose "Open."

5.3.15.4.6 Choose ASample@ file and make sure that type of file at the bottom of the screen is set for A*.smd@ before proceeding.

5.3.15.4.7 Choose "OK."

5.3.15.4.8 Choose "Start" hotkey located at the bottom of the screen, and wait for data collection.

5.3.15.4.9 Choose AOK@ to save the data so you can print it.

5.3.15.4.10 Choose "Operations" (Located at the top of the toolbar).

5.3.15.4.11 Choose "Peak Pick" (or the hot key for this).

5.3.15.4.12 If you need to remove old data from the screen, go to peak pick and remove the file from the list above the new one.

5.3.15.4.13 Choose "Window" (Located at the top of the toolbar).

5.3.15.4.14 Choose "Report generator" (or the hot key for this).

5.3.15.4.15 Choose "Print" (or "File Print Preview").

5.4 General Quantitation of Known Single Drug (No Interfering Analytes)

5.4.1 Prepare a calibration curve of the standard to obtain the absorptivity (a or ε).

5.4.1.1 Prepare a minimum of three solutions of the standard at concentrations ranging from 0.1 - 1.0 mg/mL (or other concentrations as needed).

5.4.1.2 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. Using serial dilutions, prepare solutions at desired concentrations using the stock solution.

5.4.1.3 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. (Use medium speed for quantitation work.)

5.4.1.4 Construct a calibration curve to determine the molar absorptivity (ε) (i.e., the slope of the line produced is the molar absorptivity).

5.4.2 Prepare a solution of the sample and quantitate.

5.4.2.1 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)].

5.4.2.2 Bring to volume with the desired solvent (typically 0.05 N HCl).

5.4.2.3 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.

5.5 Sampling - See Drug Chemistry Section Technical Procedure for Sampling.

5.6 Calculations

5.6.1 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)

5.6.2 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

5.6.3 Molar absorptivities (ε) are available in literature sources and may be used only for approximating quantitation value.

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

7.0 Safety - See State Crime Laboratory Safety Manual

8.0 References

Denney, R.C. and R. Sinclair. *Visible and Ultraviolet Spectroscopy (Analytical Chemistry by Open Learning)*. New York: Wiley, 1987.

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

Silverstein, Robert M., et al. *Spectrometric Identification of Organic Compounds.* 5th Edition. New York: Wiley, 1991.

Instrument Manuals.

Current National Institute of Standards and Technology Certificate for Holmium Oxide Solution Standard Reference Material.

9.0 Records

- FA Case files.
- UV Maintenance Log (see FORMS for printable version)

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Technical Procedure E-01 converted to ISO Standards.