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 Sections 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

- Standard Reference Material Holmium Oxide Solution
- 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.

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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).

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.

5.1.9.1 The performance verification shall follow the requirements for a monthly QC check.

5.1.9.2 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.

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5.2 Standards and Controls

5.2.1 Maintenance

5.2.1.1 UV instruments shall be serviced yearly by an outside approved vendor.

5.2.1.2 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

5.2.2.1 A monthly QC Check shall be performed by the Section UV Key Operator or his/her designee, using Standard Reference Material Holmium Oxide Solution. The specific operating instructions at each laboratory shall be followed. If the data meets the criteria set out below in Appendix A, 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.

5.2.2.1.1 Acceptable variance for the Monthly QC check is calculated by adding the following factors:

5.2.2.1.1.1 Uncertainty of the instrument, or wavelength accuracy (Denoted by "A" in **Appendix A**) and

5.2.2.1.1.2 Uncertainty of Standard Reference Material Holmium Oxide Solution (Denoted by "B" in **Appendix A.**)

5.2.2.2 If a discrepancy is noted, the instrument shall be removed from service and the Section UV Key Operator shall be notified.

5.2.2.3 The UV Key Operator shall correct any problems with the instrument or request service.

5.2.2.4 The QC check shall be successfully completed prior to placing the instrument back in service.

5.2.3 Performance Verification for New Instrument Set Up

5.2.3.1.1 Follow the same procedure as a monthly QC check using Standard Reference Material Holmium Oxide Solution.

5.2.3.1.1.1 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.

5.2.3.1.2 Select three compounds and obtain UV spectra for each from primary reference standards.

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5.2.3.1.2.1 If the data obtained matches the primary 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: 1.0 or 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 If using the UV 1650-PC Software or the OMNIC Software, follow the operating instructions.

5.4 Determination of Dilution/Diversion of a Single Drug – Liquid Samples

5.4.1 This procedure is used for multi-injection vials and syringes of pharmaceutically manufactured/labeled controlled substances.

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5.4.2 A known standard of the same concentration as the labeled unknown is required for comparison purposes.

5.4.3 Before proceeding with the analysis of dilution/diversion unknowns, the first set of data obtained from the standard solution shall be compared to literature references for peak shape and proper absorbances.

5.4.3.1 If the matrix interferes, a base extract of the standard shall be performed prior to UV analysis.

5.4.3.2 The unknown solution(s) are then extracted in the same manner. Worksheet notes shall contain a detailed description of any extractions performed.

5.4.4 Analysis of the standard solution:

5.4.4.1 Run a blank before each analysis. print the results and import into the case record.

5.4.4.2 Partially fill a clean cuvette with 0.05N HCl.

5.4.4.3 Add a few drops of the standard solution and then continue to fill the cuvette to the 1.0 ml indicator line of the cuvette.

5.4.4.3.1 Glass pipettes with a dropper bulb are acceptable for use in this analysis as long as the pipette size and the dropper used stays consistent throughout the procedure.

5.4.4.4 Read the absorbance of the standard solution. If the absorbance is above 3 or below 1, wash out the cuvette and repeat the steps above until an absorbance of between 1 and 3 is obtained.

5.4.4.4.1 The desired absorbance level will depend on the strength and amount of the standard present. A larger absorbance is preferred, so there is ample room to detect dilution/diversion of an unknown.

5.4.4.4.2 There is no set value for maximum absorbance in this step, as long as the sample is not overloaded. Amounts of unknown solution(s) available for analysis should also be taken into account.

5.4.4.4.3 The same number of drops of standard and unknown shall be required in the analysis.

5.4.4.5 Clean the cuvette and rinse it with UV solution between each run.

5.4.4.6 Analysis shall be done in triplicate once the acceptable number of drops has been determined.

5.4.4.7 All data shall indicate how many drops of the standard were used.

5.4.5 Analysis of the unknown(s):

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5.4.5.1 Run a blank before each analysis, print the results and import into the case record.

5.4.5.2 Repeat the steps above in triplicate using the same number of drops that were used with the standard in 5.4.4.4.

5.4.5.3 All data shall indicate how many drops of the unknown were used.

5.4.5.4 Compare the absorbance values and peak shapes of the standard and the unknown(s) to determine if dilution/diversion has occurred.

5.4.6 Specific percentages of concentration shall not be determined or reported in casework when this method is utilized. Below are examples of results statements:

5.4.6.1 Standards are reported as: This item was used as a reference standard.

5.4.6.2 Unknown(s) are reported as: Analysis of this item revealed it has been diluted (or has not been diluted) from the original concentration.

5.4.7 If sample size allows, color tests may also be used to give indications of dilution/non-dilution. For consistent sample sizes, the strength of a Marquis color test of a full strength standard will be stronger than the Marquis of a diluted unknown for compounds that react with the Marquis color test. (See the Technical Procedure for Preliminary Color Tests.)

5.4.8 Dilution/diversion cases do not address the qualitative analysis of the controlled substance present. The laboratory report will reflect "Examine for dilution/diversion" under "Type Examination Requested." Full chemical qualitative analysis is not performed nor reported for this type of analysis.

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

5.6 Calculations – See **5.2.2.1**.

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.

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9.0 Records

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

10.0 Attachments

- Appendix A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Technical Procedure E-01 converted to ISO Standards.
02/15/2013	2	**2.0** – Changed Scope to cover all three laboratories. **5.1.9** – Removed reference to "outlined below." **5.2.2** – Removed specific instructions for Raleigh lab and added reference to "The specific working instructions at each laboratory shall be followed." Removed Peak table for Raleigh Shimadzu UV. **(Original 5.3.15)** – Removed work instructions for the Raleigh UV1650-PC Software. Added "If using the" UV-1650-PC Software, "follow the work instructions provided at the laboratory." **Attachments** – Added Appendix A for Holmium Oxide parameters for each laboratory.
05/10/2013	3	**4.2 - Revised name of Holmium Oxide Standard.** **5.1.3 -** Removed bullet point. **5.2.2.1, 5.2.3.1.1** –Added reference to Standard Reference Material. **5.2.2.1.1 -** Added explanation of how acceptance criteria for QC check is calculated. **5.2.2.2, 5.2.2.3** – Revised name of Section UV Key Operator. **5.2.3.1.1, 5.2.3.1.2** – Added reference to primary standard. **5.3.3** – Revised Spectral Bandwidth to account for multiple laboratories. **5.4** – Removed original quantitation procedure and replaced with Determination of Dilution/Diversion procedure. **5.5 -** Revised name of Sampling Plan from Technical to

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		Administrative Procedure. **5.6** – Added reference to line **5.2.2.1.** **Appendix A** – Reformatted/revised data to accommodate all three laboratories.
07/31/2013	4	**Appendix A –** Adjust slit width for Western Regional Laboratory
11/15/2013	5	Added issuing authority to header.

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Appendix A

Holmium Oxide Standard Parameters
for QC Checks

Raleigh Laboratory- Shimadzu UV-1650PC **Band width = 2.0nm** **Wavelength accuracy +/- 0.3nm** **Triad Regional Laboratory - Shimadzu UV-1800** **Bandwidth = 1.0nm** **Wavelength accuracy +/-0.3nm** **Western Regional Laboratory - Nicolet Evolution 300** **Bandwidth = 1.0nm** **Wavelength accuracy +/-0.3nm**	
Wavelength accuracy (A) ***Uncertainty of Holmium Oxide Standard (B)** **A + B = Total Variance for QC Check**	
***Peak(nm)**	**Acceptable Range (nm)**
241.XX	**+/- (0.3 nm + B*)**
250.XX	**+/- (0.3 nm + B*)**
287.XX	**+/- (0.3 nm + B*)**
333.XX	**+/- (0.3 nm + B*)**
345.XX	**+/- (0.3 nm + B*)**

*For each laboratory, see current certificate for Holmium Oxide Solution Standard Reference Material at noted Spectral Bandwidth for full wavelength numbers and Uncertainty **(B)**. (Referenced to Air)

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