



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Forensic Chemistry Standard Operating Procedure Manual

Ultraviolet-Visible Spectrophotometry

19.0 ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY

19.1 Application

Ultraviolet-visible spectrophotometry (UV-Vis) is only used for presumptive testing in the TBI FCU. Compounds of interest typically absorb between 200-400 nm. Different types of compounds will produce different bands and intensities of absorption based on the functional groups present in the substance.

19.2 Equipment

The TBI FCU currently utilizes two models of UV-Vis spectrophotometers (HP 8453 and Cary 60) supplied by Agilent Technologies (formerly Hewlett Packard). The HP 8453 is equipped with a diode array, while the Cary 60 uses two silicone diodes for detectors. The hardware systems are controlled by a proprietary Agilent software package. Quartz cuvettes are used for sampling.

19.3 Solvents and Standards

The following reagents and solvents are typically used in analysis.

- Methanol
- Reagent alcohol (ethanol)
- 0.1 N or 0.5 N hydrochloric acid
- 0.5 N sodium hydroxide
- 0.5 N sulfuric acid

This list is not all inclusive since other reagents or solvents may be required depending on the nature of the analyte.

Holmium oxide QC kits will be used to verify that the UV-Vis is reading accurate wavelengths for the HP 8453 **only**.

19.4 Method

An aliquot of the sample is added to a cuvette and dissolved or diluted with the appropriate solvent. The class of compound may also determine the amount of sample used. The sample concentration can be adjusted as necessary by dilution or adding additional sample.

19.5 Quality Assurance

Running daily primary standards for making spectral comparisons to unknown samples is not required since all primary standards and samples are run using the same spectral parameters. Consult the instrument logbook for these parameters.

The end solvent used for sample analysis must be the same as the solvent used to obtain the primary standard spectrum for a valid comparison.

A procedural blank will be performed prior to each sample in which the UV-Vis is used. This blank is accomplished by running the solvent/reagent blank in the same manner as the sample. This spectrum will be retained in the appropriate case record(s). For shifts requiring multiple



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steps, the final mixture will be treated as a single solvent/reagent for the blank.

Care should be taken to prevent spilling of samples or solvents into the sampling chamber. Spills or splashes must be cleaned up immediately.

Cuvettes must be cleaned prior to blank and sample collection.

19.6 Performance Verification and Acceptance Criteria

19.6.1 HP 8453

Performance Verification

The HP 8453 instrument and its lamps will be checked monthly using the instrument's internal diagnostic software. This software evaluates light intensity, noise, baseline flatness, and wavelength placement. The results of the monthly checks will be kept in the appropriate instrument manual. The unit may also run checks using a known standard and comparing the result with the standard UV-Vis data if desired.

Source lamps can vary in intensity from time to time, but they will usually produce an adequate response to the detector. Lamps will be replaced if they do not produce the required wavelengths for analysis.

Acceptance Criteria

The spectrophotometer's proprietary software will generate a pass/fail result for the following parameters:

- Self-test
- Maximum and minimum dark current
- Current lamp intensity
- Stability
- Wavelength accuracy and repeatability

Self-test failure will most likely occur when the lamp needs replacement. The tests should be repeated after the lamp has been replaced. The instrument will be removed from service if any of these criteria fail the repeated tests, and the unit supervisor will be notified.

Some older instruments do not have the capability to generate pass/fail results for wavelength accuracy. Comparison of the current holmium oxide spectrum against the previously generated instrument performance check's spectra will be performed to determine wavelength accuracy acceptability criterion.



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19.6.2 Cary 60

Performance Verification

The Cary 60 will be checked monthly using the instrument's internal diagnostic software. This software evaluates wavelength accuracy (xenon line), noise, and baseline flatness. The xenon lamp creates intense pulses of light **only** during scanning allowing for extended bulb life (approximately 10 years.)

Holmium oxide tests are not necessary on this instrument since the high intensity light flashes from the xenon lamp do not cause excess photometric noise. Therefore, wavelength shift errors associated with traditional scanning methods are eliminated.

Acceptance Criteria

The spectrophotometer's proprietary software will generate a pass/fail result for the following parameters:

- Wavelength accuracy (xenon line scan)
- Photometric noise
- Baseline flatness

Instrument performance test failure will most likely occur when the instrument has been powered on for an extended period of time. Restarting the instrument will allow it to internally recalibrate. The tests should be repeated after this internal recalibration is complete. The instrument will be removed from service if any of these criteria fail the repeated tests, and the unit supervisor will be notified.

Refer to Appendix E for maintenance schedules.

19.7 Interpretation

Direct comparison between the primary standard and the sample UV-Vis spectra must be made for the analyst to determine consistency with the primary standard. The sample wavelength(s) must be within ± 3 nm of the standard for the analyst to conclude it is consistent with the compound.

A slight shift in absorption bands between the primary standard and sample spectra may be due to sample concentration or adulterating substances present in the sample. These effects should be considered when making consistency determinations.

General peak shape should also be a consideration. Peaks that are above 2.0 absorbance units are off-scale and unusable for comparison.