1.0 Purpose and Scope

This procedure prescribes the Sandia National Laboratories (SNL) Waste Isolation Pilot Plant (WIPP) process for the calibration, operation, and maintenance of the Cary 300 Conc UV-Visible spectrophotometer as part of the laboratory research activities in the support of the WIPP Project.

This procedure is applicable only for the Cary 300 Conc UV-Visible spectrophotometer for the following applications: inorganic anions and metals. This document is concise, and is not meant to substitute for the manufacturers’ instruction manuals. The user is responsible for reading and understanding the appropriate manuals (see references).

Acronyms and definitions for terms used in this procedure may be found in the Glossary located at the Sandia National Laboratories (SNL) WIPP Online Documents website.

2.0 Implementation Actions

2.1 Safety

The activities described in this SP shall conform to the SNL Environmental Safety and Health programs (ES&H) as described in the current laboratory standard operating procedure (SOP SNL/CPG Building NPHB).

2.2 Responsibility

The Principal Investigator (PI), or designee, whose activities warrant the use of this procedure, is responsible for implementing the requirements of this procedure.

The PI or designee is responsible for performing the calibrations and measurements following the requirements of this procedure, documenting calibrations, and assuring that the latest revision of this document is followed.
If the procedure cannot be implemented as written, the user has the responsibility to stop work and resolve all concerns with the PI/designee or safety, as appropriate, prior to proceeding with the work. The user has to seek other analytical techniques if any concern persists.

2.3 Standards

The standards used for calibration and calibration checks will be prepared from primary standards. The standards will be prepared in the matrix as close to that of the samples as possible, based on the colorimetric method being utilized. The primary standards will be commercially obtained and traceable to NIST (National Institute of Standards and Technology) or other nationally recognized standards, or alternatively may be prepared from ACS (American Chemical Society) grade salts. The certificates of analysis for the primary standards provided by the manufacturer will be submitted to the WIPP Records Center. The preparation of the standards shall be documented in the Scientific Notebook in accordance with NP 13-1, Control of Samples and Standards, and NP 20-2, Scientific Notebooks (see Appendix A). The standards will not be used past the expiration date listed on the container by the manufacturer.

2.4 Quality Control

If the results of the calibration and calibration check samples are not within the acceptance/tolerance limits (described in subsequent sections), a corrective action will be documented and the affected samples will be flagged in the data report use-as-is if that is the determination from the corrective action.

2.5 Corrective Action

If during calibration or calibration check, the tolerance limit cannot be met, a Corrective Action Request (CAR) will be issued to document the results and impact relative to data collection. Corrective action would include an evaluation to any data collected. Results of all activities related to the out-of-tolerance will be summarized in the CAR per the requirements in NP 16-1, Corrective Action.

2.6 Calibration

Calibration will be performed using at least three standards which bracket the range of expected measurements. A calibration curve will be obtained prior to the sample measurements. A new calibration curve will be obtained when major instrument changes have occurred (i.e. change in cuvette lightpath, use of different coloring reagents, or cuvettes of different specifications), and when the calibration check standard fails. The linearity of the calibration curve should be better than 0.98 in terms of squares of linear correlation coefficients ($R^2$), i.e., $R^2$ value of the calibration needs to be higher than 0.98. If any sample concentration exceeds the highest concentration of the calibration standards, it must be diluted and re-analyzed. Alternatively in the case that a sample is already used up, a new calibration standard to bracket the concentration of the sample will be prepared and analyzed. This standard will then be processed in the calibration method and the sample result re-processed.

Pull up one of the programs for the spectrophotometer from “Start” menu to collect absorbance readings (e.g., “Simple Reads”). Put the cuvettes filled with standards into the holder beneath the cover of the spectrophotometer. Close the cover. It is recommended to start with background. Typically, background consists of the same matrix as other standards but with no analyte in it, and it can be considered equivalent to the 0 concentration standard. Record reading in the appropriate scientific notebook or supplemental binder. Zeroing the background reading prior to proceeding to
remaining standards helps find deterioration of calibration standards and/or optics of the spectrophotometer. Copy the collected absorbance readings into spreadsheet (e.g., Microsoft Excel) to calculate the slope, intercept, and $R^2$ values from the plot of absorbance versus standard concentration. Note that one of the programs for the spectrophotometer is capable of defining the calibration curve as the readings are obtained from standards. The calculated slope, intercept, and $R^2$ values, along with the data inputs will be printed out and included in the appropriate scientific notebook or supplemental binder. Using the readings from standards, define a calibration curve in the following form;

$$\text{Absorbance} = \text{slope} \times \text{standard concentration} + \text{intercept}, R^2$$

### 2.7 QC Sample Criteria

A calibration check standard will be analyzed as a sample immediately after the calibration and prior to each day of analysis if a previous calibration curve is being utilized. This calibration check standard is one of the standards used for establishing the calibration curve. The check standard will be analyzed after every twenty samples, and at the end of the analytical run. The results of these standards should agree with their respective expected values within 15%. If not, the check standard may be immediately re-analyzed. If this check standard fails, the analysis will be terminated (or see sect 2.4). After the problem is corrected, re-analyze the calibration check standard. If the tolerance limit is still not met, the instrument will be re-calibrated and samples will be re-analyzed.

### 2.8 Instrument Start-up

1. Turn on the power switch located at the lower-left corner on the front panel of the spectrophotometer. Allow one hour for warming-up.
2. Power up the computer connected to the spectrophotometer.
3. Open the program.
4. Change the wavelength specific to your analysis.

### 2.9 Sample Analysis

Collect absorbance readings for samples in the same way as used for standards (See section 2.6). Typical procedure is described below. Determine the concentration of analyte in the sample using the values of slope and intercept of the calibration curve above.

1. Open the cover.
2. Place sample into the holder, and close the cover.
3. Record reading in the appropriate scientific notebook or supplemental binder.
4. Repeat above for remaining samples. You can save the readings on the hard disk of the computer for future reference.
5. Readings from the samples will be used to determine the concentrations based on the calibration curve.

### 2.10 Instrument Shut-down

After the analysis is completed, do the following to shut-down the instrument:

1. Turn off the spectrophotometer.
2. Turn off the computer.

### 2.11 Maintenance

Please refer to the Cary 300 Conc UV-Visible spectrophotometer users’ manual.

- Use cuvette caps to prevent any spill out of the cuvette during the measurement.
- It is recommended to calibrate before every use; this will help to find out instrumental shift due to aging of the optics.

2.12 References

- Cary 300 Conc UV-Visible spectrophotometer users’ manual.

3.0 Records

The following records, generated through implementation of this procedure, shall be prepared and submitted to the WIPP Records Center in accordance with NP 17-1 (Records):

QA Record

- Scientific Notebook
- Scientific Notebook Supplement

4.0 Appendices

There are no appendices associated with this document.