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Analytical method Validation: ICP-OES

Purpose: The following is a summary of tests/experiments performed to validate the ICP-OES instrument and establish an analysis method for samples.

Instrument description: The instrument Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) is used in atomic spectroscopy, and during analysis the sample is decomposed by intense heat into a cloud of hot gases containing free atoms and ions of the element(s) of interest. The high temperatures cause significant amounts of collisional excitation and ionization of the sample atoms. Once the atoms or ions are in their excited state, they can decay to lower states through thermal or radiative (emission) energy transitions. During ICP-OES analysis the intensity of the light emitted at specific wavelengths is measures and used to determine the concentration of the element(s) of interest. In ICP-OES analysis the thermal excitation sources can populate a large number of different energy levels for several different elements at the same time. All of the excited atoms and ions can then emit their characteristic radiation at the same time. This results in the flexibility to choose from several different emissions concurrently and allows detection of multiple elements concurrently.

Instrument IQ/OQ/PQ

The installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) were complete by a Perkin Elmer service engineer on March 13, 2012. The instrument/system acceptance was completed on March 15, 2012 by a Perkin Elmer service engineer. The data and information associated with the studies are kept in the ICP-OES Instrument System log book which is kept by the ICP-OES.

Performance Experiments

Performance experiments are periodically conducted to check instrument performance and/or analytical method performance.

Experiments to test instrument performance are required by Perkin Elmer to obtain accurate data by the instrument during an analysis. These tests use Perkin Elmer solutions and may or may not include elements of interests to the BNL isotope production group. Some of the tests were performed at instillation, while other tests were performed after modification of the instrument by Perkin Elmer service engineer. Results of the instrument performance tests can be found in the ICP-OES Instrument System log book.

Experiments to test the analytical method performance are recommended by the FDA in the document “Guidance for Industry: Bioanalytical Method Validation.” The experiments use elements of interest to the BNL isotope production group. Results from analytical method performance experiments can be found in the ICP-OES Instrument System log book, and/or chapter 19 of the OPM’s under ICP-OES. Other performance testing such as QC sample analysis is performed with every batch of product and the results are in batch files for the product.

Experimental summary:

Instrument Performance

The following performance tests are conducted by a Perkin Elmer service engineer to check the instrument performance: Align View, Detector Calibration, UV/Vis Wavelength Calibration, Precision, Spectral Resolution, Detection Limits and Stability. The results are recorded by the Perkin Elmer service engineer and kept in the ICP-OES Instrument System log book.
Some instrument performance tests are conducted by BNL staff prior to conducting experiments to test analytical method performance or setting up selectivity (emission wavelengths). The following ICP-OES instrument performance testes were/should be performed within 1 week of the studies: Detector Calibration, Align View, UV/Vis Wavelength Calibration. For tests utilizing the plasma the tubing at the peristaltic pump should be changed if there is more than 10 hours of operating time on them. It is important that the instrument be operated with the plasma on for at least 30 minutes pumping water on both internal standard and sample lines before initiating the align view and wavelength calibration. The results for instrument performance tests should be kept in the ICP-OES Instrument System log book.

Detector Calibration

Purpose: This procedure is an electronic calibration for the instrument detectors.

Start the ICP according to OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV. In the **Tools** menu, click on **Spectrometer Control**. The Spectrometer Control window appears. Click on **Detector Calibration** in the Spectrometer Control window to display the Detector Calibration dialog. Click on **OK** to begin Detector Calibration. The system automatically closes the shutter to measure signal bias and dark current. The detector calibration routine may take up to twenty minutes. This test can be performed with or without the plasma on.

Align View
Purpose: This procedure automatically aligns the torch viewing position for the highest signal intensity.
Solutions you will need:

- A standard solution containing 1 ppm Mn for the axial view alignment.
- A standard solution containing 10 ppm Mn for the radial view alignment.

In the **Tools** menu, click on **Spectrometer Control**. The Spectrometer Control window appears. First do the alignment in the Axial viewing mode, then select the Radial viewing mode and repeat the procedure. To view spectra collected during the procedure, open the Spectra Display window. Click on **Align View** in the Spectrometer Control window. Using this dialog box: Click on the **Select Analyte** option and then select Manganese, which is typically used as the alignment wavelength for the majority of analyses. Set the Read Delay time. Aspirate a solution containing 1 ppm Mn solution for the axial and 10 ppm Mn for the radial view. The system will determine the intensity at the selected wavelength, while adjusting the viewing position in incremental steps.

UV/Vis Wavelength Calibration
Purpose: The process of determining the relationship between the physical settings of a spectrometer and the wavelengths at which it takes a measurement.
Solutions You will Need:

- Calmix 3 solution (Part No. N058-2152) available through PerkinElmer, which contains 100 mg/L of P, K, S; and 20 mg/L of As, La, Li, Mn, Mo, Ni, Sc, Na.
- For spectrometers that have a visible wavelength channel on the detector, you will need VIS Wavecal mix (Part No. N930-2946) available through PerkinElmer, which contains 1 mg/mL Ba, Ca; and 10 mg/L of La, Li, Mn, Na, Sr; and 50 mg/L of K.
- Rinse solution

To initiate a wavelength calibration

1. Make sure that the plasma is lit and has been on for at least one hour on Optima spectrometers.

2. In the **Tools** menu, click on **Spectrometer Control**.
The Spectrometer Control window appears.

To perform UV channel wavelength calibration:
Aspirate the Calmix 3 solution (Part No. N058-2152 Perkin Elmer). On Axial (XL) instruments, you may need to dilute the wavecal solution 1:10 to keep certain emission lines from saturating the detector. On Dual View (DV) instruments, select Radial viewing. If you have selected axial, a message appears informing you to change to radial. Select **UV**, then click on **Wavelength Calibration** in the Spectrometer Control window. In the dialog that appears, click on **OK** to perform the calibration. The UV calibration may take several minutes. You may view the spectra used for calibration by clicking on **Spectra** in the toolbar. When the UV calibration is complete, the UV coefficients and RMS (Root Means Square) value will be displayed in the Results window. These values should meet the following specifications:

UV Coefficients and RMS Specification

First Coefficient	Absolute value < 2.0
Second Coefficient	Absolute value < 2.0
Third Coefficient	Absolute value < 8.0
RMS measures variability of wavelength offsets)	< 2.0

If these specifications are not met, repeat the wavelength calibration and make sure all peaks can be seen in the Spectra Display window. If the specifications are still not met, a complete optical alignment may be required. Contact your PerkinElmer service engineer.

For spectrometers that have a visible wavelength channel on the detector, to perform VIS channel wavelength calibration:

Aspirate VIS Wavecal mix (Part No. N930-2946). On Axial (XL) instruments, you may need to dilute the wavecal solution 1:10 to keep certain emission lines from saturating the detector. On Dual View (DV) instruments, select Radial viewing. If you have selected axial, a message appears informing you to change to radial. Select **VIS**, then click on **Wavelength Calibration** in the Spectrometer Control window. The VIS calibration may take several minutes. You may view the spectra used for calibration by clicking on **Spectra** in the toolbar. When the VIS calibration is complete, the VIS coefficients and RMS (Root Means Square) value will be displayed in the Results window. These values should meet the following specifications.

VIS Coefficients and RMS	**Specification**
First Coefficient	Absolute value < 3.0
Second Coefficient	Absolute value < 3.0
Third Coefficient	Absolute value < 12.0
RMS measures variability of wavelength offsets	< 2.0

If these specifications are not met, repeat the wavelength calibration and make sure all peaks can be seen in the Spectra Display window. If the specifications are still not met, a complete optical alignment may be required. Contact your PerkinElmer service engineer.

Summarized information for analytical method establishment

The following bulleted, indented & underlined information are the information the FDA guidance documentation on bioanalytical method validation recommends be in a validation report. FDA definitions are bulleted, indented and italics. BNL responses are left justified.

- *Bioanalytical Method Validation: The fundamental parameters to ensure the acceptability of the performance of a bioanalytical method validation are selectivity, accuracy, precision, sensitivity, reproducibility, and stability.*

    - <u>An operational description of the analytical method</u>

The OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV is an operational description of the analytical method.

    - <u>Evidence of purity and identity of drug standards, metabolite standards, and internal standards used in validation experiments</u>

Certificate of Analysis was provided by Spex CertiPred for standards and internal standards and were approved by Quality Control. The standards are NIST traceable. Identity is confirmed by ICP-OES analysis of the emission wavelengths of the elements in the solution.

- A description of stability studies and supporting data

Standards, QC samples, and internal standards are certified for stability for 1 year by Spex CertiPrep. Solution prepared from Spex CertiPrep are used during one run season, and prepared from Spex CertiPrep solutions that are in specification during the time of analysis.

- A description of experiments conducted to determine selectivity, calibration curve, accuracy, precision, recovery, limits of quantification and relevant data obtained from these studies.

Selectivity:

- *Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample.*

After the wavelength calibration the following emission wavelengths (nm) were setup and are used by the ICP-OES for analysis of each of the elements: Cu 324.752, Pb 220.353, Zn 213.857, Co 228.616, Cr 205.560, Cd 214.440, Ni 232.003, Fe 238.204, Mn 257.610, Al 396.153, Ga 294.364, Ge 209.426, Sr 460.733, Sr 407.771, Sr 421.552, Be 313.107, Mg 279.077, Rb 780.023, Ca 317.933, Ba 233.527, Nb 309.418 and the Internal standard Y 371.029.

Calibration curve:

- *A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte. The standard curve should cover the entire range of expected concentrations.*

Performing a calibration curve and analyzing samples and quality control checks are described in OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV. A blank, and a five point calibration curve were/are generated using the following concentrations: 0.05, 0.1, 0.2, 0.5, 1.0 ppm of the 19 element standard. A linear fit of the curve were/are chosen in the software. The calibration, QC samples and productions samples were analyzed in 2% nitric acid. The QC and desired production samples contain elements with concentrations on the range of the calibration curve.

- *Calibration curve acceptance or rejection criteria*

From OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV: After analysis of the last standard the R^2 for the calibration curve should be 0.99 to 0.9999 depending on the element. Quality Control (QC) samples are analyzed to check the accuracy and precision of the calibration curve for each analysis and are used to accept or reject the calibration curve and the batch run. As described in OPM 19.2.24 a low QC sample and a high QC sample are performed before the analysis of an isotope product. After the analysis of an isotope product the middle QC sample is analyzed for precision and accuracy. The acceptance of the precision and accuracy of the QC samples demonstrates the calibration curve can be used to accurately and precisely determine concentrations.

Accuracy, Precision:

The accuracy and precision of an analytical method is determined by analysis of Quality control samples.

- *The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte.*
- *The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV.*

Perkin Elmer definition: During ICP-OES analysis the precision is expressed as the % RSD or the %CV (coefficient of variation).

- *FDA Acceptance criteria of QC samples: At least 67% (4 out of 6) of QC samples should be within 15% of their respective nominal value, 33% of the QC samples (not all replicates at the same concentration) may be outside 15% of nominal value*.

  - *Quality control sample and assay run acceptance or rejection criteria*

From OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV: The determined concentration should be within 20% of the true value and the % RSD should be less than 10% for the 0.075ppm QC check, unless it is below the LLOQ for the element. For the 0.25 and 0.75 ppm QC checks the samples should be within 10% of the true value and the %RSD below 6% for the 0.25 and 0.75 ppm QC check. If QC values are outside the accepted range then the analysis will be repeated and/or a service call to Perkin Elmer will be made and the instrument serviced, and the day to day variation of QC standards will be determined again.

Reproducibility:

- *Reproducibility represents precision of the method under the same operating conditions over a short period of time.*

Experiment:

OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV was followed to generate the calibration curve. Three quality control samples: one high concentration QC sample (0.750 ppm), one low concentration QC sample (0.075 ppm), and one mid-range concentration QC sample (0.225 ppm) were prepared. Each QC sample was analyzed on the calibration curve. This was repeated over a four day period such that analysis was performed on three days with at least 1 day in between where analysis was not performed. After the initial IQ/OQ/PQ and subsequent instrument performance testing by the Perkin Elmer service engineer the reproducibility study for the 19 elements associated with the analytical method was determined.

Results:

The data for the QC samples was collected on 3/26/2012, 3/27/2012, and 4/10/2012. The data is summarized in OPM 19.2.24.a ICP–OES Operation Model Optima 7300 DV Attachments.

Lower Limit of Quantification (LLOQ):

- *LLOQ is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision. The LLOQ should be established using at least five samples independent of standards and determining the coefficient of variation and/or appropriate confidence interval. The LLOQ should serve as the lowest concentration on the standard curve and should not be confused with the limit of detection and/or the low QC sample. The highest standard will define the upper limit of quantification (ULOQ) of an analytical method.*
- *For validation of the bioanalytical method, accuracy and precision should be determined using a minimum of five determinations per concentration level (excluding blank samples).*
- *The mean value should be within 15% of the theoretical value, except at LLOQ, where it should not deviate by more than 20%. The precision around the mean value should not exceed 15% of the CV, except for LLOQ, where it should not exceed 20% of the CV.*

Experiment:

OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV was followed to generate the calibration curve and QC checks were performed. A sequence of progressively lower concentrations of known samples were run as unknowns to determine the lower limit of quantification for the 19 elements. The concentrations used were 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 ppm. The %RSD and the measured value for each element at each concentration is added to the first page of the ICP results Excel report page. The % error of the measured sample compared to the true value is determined by the following equation: % error = Absolute value [(true value-measured value)/true value]*100. Acceptance criteria is a %RSD and a % Error less than 10%.

Results:

The lower limit of quantification was determined on 4/13/2012 and is summarized in the first page of the ICP-OES report. The data can be found in the ICP-OES validation summary located in OPM 19.2.24.a ICP–OES Operation Model Optima 7300 DV Attachments. The lower limit of quantification was re-analyzed and reported on 11/13/2012. The new LLOQ was added to the ICP-OES reported on 11/13/2012 and 19.2.24.b ICP–OES Results Form which was posted 11/14/2012. An excel spread sheet has been prepared that summarizes both sets of LLOQ data and will be updated periodically with any re-analysis of the LLOQ. The Excel spread sheet file with both the LLOQ and the detection limit will be updated periodically and posted as a report on OPM Chapter 19.

Detection limit:

A detection limit is the concentration of an analyte that results in signal intensity that is three times the standard deviation of the background intensity at the measurement wavelength.

> *"Detection limits by themselves, however, are not very diagnostic indicators. If the measured detection limit are not within their expected ranges, there could be several different causes. Detection limits are best used for diagnostic purposes when combined with a series of other more specific tests." Boss & Fredeen*

Detection limit testing for the instrument performance was established by the Perkin Elmer service engineer, and the data is kept in the ICP-OES Instrument System log book. The method detection limits for the analytical method are performed by BNL staff. The data should be recorded/kept in the

Analytical method detection limits: OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV should be followed to generate the calibration curve and the low and High QC checks should be analyzed to establish an acceptable calibration curve (defined below). The Yttrium internal standard should be aspirated, and 2% HNO_3 should be aspirated on the sample line. The name of the sample should be either "2% nitric acid" or "detection limit" and click on Analyze Sample. The reported standard deviation numbers should be multiplied by 3 to obtain the detection limits. The data will be added to the LLOQ & Detection Limit Excel spread sheet and maintained on chapter 19 OPM. Results from re-analysis of the detection limits are updated periodically in the Excel spread sheet and reposted onto chapter 19.

Application to Routine Drug Analysis

OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV is followed for routine analysis and the document outlines a report that is prepared, included in the batch record and disseminated to QA, TPL manager and the Production Manager.

Other Information

Other information applicable to both method development and establishment and/or to routine sample analysis could include:

- *Lists of abbreviations and any additional codes used, including sample condition codes,*

integration codes, and reporting codes

ND: below the lower limit of quantification

- *SOPs or protocols covering the following areas:*

OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV.

- *Sample code designations, and bioassay sample code*

Analysis of isotope sample are given the Batch number designation as the sample name, and the raw data is given the Batch number.

- *Reference lists and legible copies of any references*

OPM 19.2.24 ICP-OES Operation Model Optima 7300 DV

Perkin Elmer Optima 7100, 7200, and 7300 series hardware guide.

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