Overview

Requirements for calibration are contained in NR 149.44. This subsection specifies requirements for laboratory support equipment, such as balances, refrigerators, thermometers, and pipettes, and for laboratory analytical instruments, such as ultra-violet and visible light spectrophotometers, mass spectrophotometers, and inductively-coupled plasma emission spectrophotometers (ICP). This module of the Chapter NR 149 Implementation Guidance discusses analytical instrument calibration. Section 8 of the Guidance discusses calibration of laboratory support equipment.

The calibration requirements for laboratory analytical instruments have been expanded considerably in the revised Chapter NR 149. The new NR 149 provides specificity to the older version's directive requiring "calibration and maintenance of all test instruments and equipment as necessary to maintain accuracy." The revised Chapter NR 149 now provides calibration details that are "necessary" to maintain the accuracy of results reported by laboratories.

The requirements of the revised Chapter NR 149 are comprehensive, but may be superseded (except when otherwise noted) by stricter requirements in mandated test method or regulations. The revised Chapter NR 149 addresses instrument calibration general requirements and provisions, followed by requirements for initial instrument calibration and continuing instrument calibration verification.

This module will note the specific requirements for instrument calibration, discuss them, and describe the implications of some of the requirements for laboratories participating in the Certification and Registration Program.

General Requirements [NR 149.44(5)]

Summary

This subsection establishes two fundamental principles. Instruments must be in calibration before they are used to report results, and generally, when a method has stricter calibration requirements than Chapter NR 149, the method requirements must be followed.

Requirement: NR 149.44(5) (a)

All instruments have to be calibrated or their calibration verified before they are used to analyze samples. Instruments have to be calibrated at least once a year if they have been used.

Discussion

A laboratory that does not fully calibrate an instrument on each day of analysis and that is able to verify the instrument's calibration for an extended period of time must fully calibrate the instrument at least once in a year.

Some laboratories have been able to verify the validity of a calibration for more than a year, but now are required to perform a full calibration at least yearly.

Requirement: NR 149.44(5) (b)

If a laboratory follows a method that has more stringent requirements than those contained in Chapter NR 149, the stricter requirements must be followed, with two exceptions:

When a method calls for analyzing more than three standards for a linear calibration, a laboratory can choose to analyze three, if the calibration range is limited to two orders of magnitude.

When a method calls for verifying a linear calibration with more than one standard, a laboratory can verify the calibration with a single standard if the calibration range is limited to two orders of magnitude.

Discussion

Chapter NR 149 establishes requirements that at a minimum, must be followed by accredited laboratories, and recognizes that the minimum can be augmented or superseded by test methods or regulations. This provision restates that principle in

the context of instrument calibration, but gives two notable exceptions that apply to **linear** calibrations.

Some procedures, as for example, approved methods for analyzing phosphorus and ammonia in Standard Methods, require more than three standards (and specify their concentration) to establish a linear calibration. When a laboratory restricts the range of an analysis to two orders of magnitude, analyzing more than three standards to establish a calibration, or using more than one standard for verifying a calibration, does not improve a calibration commensurately. These provisions allow laboratories to deviate from what is a more stringent requirement and still be in compliance with Chapter NR 149.

Initial Instrument Calibration [NR 149.44(6)]

Summary

After establishing documentation requirements for calibration procedures this subsection describes the process for initial calibration following a logical progression requiring selection of:

- A calibration model.
- A number of standards appropriate for the selected calibration model.
- The concentration of the selected standards.
- A calibration function employing a reduction technique appropriate for the calibration model and number of standards selected.

Once a calibration function has been established, laboratories are required to evaluate the calibration's acceptability against set criteria. As a final check, immediately after it has been established, the calibration is checked with standards from a source different from the one used to generate the calibration.

The subsection contains specific guidance for calibrating instruments tuned to conform to a scientific law or scale, such as pH meters, ion selective electrodes, and dissolved oxygen meters, and for calibrating ICP and inductively coupled plasma mass spectrometers (ICP/MS).

Requirement: NR 149.44(6)(a)

Laboratories must include or reference in their Standard Operating Procedures (SOPs) the protocols used for calibration, including calculations, integrations, acceptance criteria, and associated statistics.

Discussion

Most laboratories will include calibration details in a designated section of their SOPs. NR 149.40(2) (d) requires including or referencing calibration and standardization in the analytical methods manual. When this information is not included directly in an SOP, the referenced document containing the information must include sufficient information to allow reproducing calibrations performed at the laboratory.

Requirement: NR 149.44(6)(b)

A laboratory must select a calibration model appropriate to the expected behavior of the analytical instrument to be calibrated.

Discussion

The majority of the instruments used in environmental chemistry analyses have detectors that behave linearly or have their responses transformed to operate linearly. Spectrophotometers within their operating range obey Beer's Law, which is a linear function. ICPs and ICP/MS have extensive linear ranges. The output of ion selective electrodes is transformed logarithmically to yield linear responses. Almost all instruments that exhibit deviations from linearity will remain linear within a defined range.

There are times when an analyte's response in a detector that usually behaves linearly will not follow a linear model. This is more common for methods that in a single run, as for example many gas chromatography/mass spectrometers (GC/MS) procedures, detect many analytes. This not only results from the nature of the interaction between analyte and detector, but also from the fact that optimizing response for some analytes compromises the response of others.

Detectors that behave according to a cubic (third order) response are rare. Having to resort to a cubic model usually indicates that an instrument is being used beyond its recommended concentration range.

Chapter NR 149 allows the use of any calibration model that can be chosen to be indicative of a detectors or analyte response, as long as the choice is not used to compensate for saturation of signal, lack of sensitivity or malfunction. The Chapter minimizes the risks of using higher order models inappropriately by requiring more stringent calibration verification for non-linear models.

Requirement: NR 149.44(6) (c), (d)

A laboratory must select a number of non-zero standards appropriate to the calibration model selected and the expected range of concentrations. The minimum number of standards is three, except as noted below, and increases with the complexity of the calibration model chosen. For quadratic and cubic calibration models, the minimum number of concentrations is five and seven, respectively.

Some instruments can be accurately calibrated with fewer than three standards: ion selective electrodes and pH meters can be calibrated with a minimum of two, and ICP and ICP/MS can be calibrated with a minimum of one non-zero standard.

Dissolved oxygen meters are calibrated against an aliquot of water-saturated air, air-saturated water, or by Winkler (iodometric) titration

Discussion

The minimum number of standards needed to characterize a calibration is, except as noted, one more than twice the order of the function describing the calibration model. This is known as the "2n + 1" rule, where "n" is the order of the function. Therefore for a cubic, third order function, the minimum number of standards is seven.

The minimum number can be and should be increased to define a calibration range accurately, particularly for calibrations that span large concentration ranges. A good calibration, when plotted should look like a well-labelled highway, with posts marking distances at set intervals.

Requirement: NR 149.44(6)(e)

The concentration of the standards chosen to establish a calibration must be within the expected concentration range of the samples to be quantitated. When a laboratory needs to report results at or near the limit of detection of an analysis, the initial calibration must include a standard with a concentration near the limit of quantitation of the analysis.

Discussion

Laboratories that analyze the same type of samples, usually those laboratories that are associated with a treatment plant or an industry, have the luxury of knowing the expected concentrations of the samples they analyze and can tailor their calibration ranges to fit their samples. More accurate quantitations result when the concentration of the samples quantitated closely match the concentration of the calibration standards chosen.

Commercial laboratories receiving samples from many sources tend to calibrate at wider ranges to minimize dilutions. Nevertheless, it is a good practice to keep the scale of the calibration within the scope of the concentration of samples to be analyzed. A yardstick cannot measure the length of a plasma cell anymore accurately than an electron microscope could give the dimensions of a picnic table quickly. Scaling is everything.

There is not a misprint when Chapter NR 149 requires that a standard near the **limit of quantitation** of an analysis be included in a calibration when a laboratory has to report results down to the **limit of detection**. By definition, numerical results between the limit of detection and the limit of quantitation are unreliable because

in this region, the presence of an analyte can be affirmed, but its quantity cannot be confirmed. It would be inappropriate to allow this lack of quantitative certainty to influence the calibration function. Therefore the lowest standard, in cases where reporting to the detection limit is required, should be set as close as possible to the detection limit, but within the region where quantitative results can be expected. This is of course, the limit of quantitation. To allow laboratories to set a manageable single concentration for multi-analyte methods, Chapter NR 149 allows setting the concentration of the standard uniformly by allowing its concentration to be "near" the limit of quantitation and not insisting it be exactly at that limit.

Requirement: NR 149.44(6) (f)

To generate a calibration function, a laboratory must select a reduction technique or algorithm that fits the calibration model and the number of standards chosen.

A laboratory must provide a mathematical description of the reduction technique or algorithm selected and any parameters needed to identify the function uniquely. For dissolved oxygen meters and ion selective electrodes mathematical characterization is not necessary.

When options to use more complex calibration functions are available, a laboratory must choose a linear function, unless it can demonstrate that a non-linear function defines the calibration range better. A laboratory may use weighted algorithms or reduction techniques. However, using non-linear functions or weighted algorithms to compensate for instrument saturation, insensitivity, or malfunction is not allowed. Reduction techniques or algorithms that force calibration functions through zero are not allowed.

Discussion

A function is a rule that relates values to a variable according to a discernible pattern. Functions assign values uniquely. A calibration function relates a detector or instrument response to a given concentration of analyte; a response is assigned a corresponding concentration and the same response cannot be associated with more than one concentration. The assignment can be made following a universal rule or agreed upon scale, as is the case with ion selective electrodes, which obey the Nernst Equation, pH electrodes, which are tuned to conform to the pH scale, and dissolved oxygen meters, which are most often tuned to conform to the known relationship between oxygen gas in a fluid at a given temperature and pressure. More often, the assignment is made anew at each calibration event, establishing a relationship experimentally, or empirically, by analyzing a set of standards at a known concentration and relating their individual responses mathematically. That mathematical relationship becomes the calibration function.

The function chosen to describe the calibration must fit the calibration model and the number of standards analyzed to establish calibration. When the calibration function must be determined empirically because there is not an applicable universal rule to establish a relationship between response and concentration, the number of standards analyzed and the model selected limit the calibration function that can be chosen. Analyzing three standards with a detector that behaves linearly would allow a laboratory to choose either average response factors or linear regression to obtain a unique calibration. Quadratic regression could not be used under those circumstances.

When the calibration function is determined empirically, a mathematical description of the relationship between concentration and response is necessary to describe the relationship uniquely and to be able to reproduce the laboratory's results. When the calibration function follows a universal law, mathematical characterization is not required because in essence, that relationship is known and available. In these cases, a given response will only yield a specific concentration. In other words, the calibration function is the same for all users of associated instrumentation.

For calibration functions, it is best to keep choices simple. Nevertheless, with the advent of computers and quantitation software, laboratories that analyze many calibration standards, can, at the touch of a button, reduce calibration data by several functions and compare acceptance criteria readily. A laboratory analyzing eight standard concentrations can compare acceptability criteria for response factors, and linear, quadratic, and cubic regression. It is tempting to go with the highest order that meets acceptance criteria on the grounds that more is better.

Chapter NR 149 requires that to make the switch to a non-linear function, laboratories demonstrate that the calibration range is better defined using a non-linear function. The Chapter is silent on how to do this, but plotting the resulting curves and evaluating acceptability criteria could help make the case. In any event, since choosing non-linear functions requires stricter and more involved calibration verification, most laboratories will tend to choose a non-linear function only when a calibration model is not linear or when it Is otherwise necessary. Choosing non-linear functions to correct for lack of sensitivity, detector saturation, or instrument malfunction is not allowed.

Chapter NR 149 now explicitly allows the use of weighted algorithms or reduction techniques. In the past, laboratories were allowed to use weighted calibration if they could demonstrate that the variance of responses of standards along the calibration range was not constant. Determining what degree of variation constituted a lack of constant variance was subject to interpretation and virtually every laboratory that attempted the demonstration could show a lack of constant

variance. Quantitation software has made this option virtually universal. It has been demonstrated that for regression techniques, which tend to favor better fits for higher concentrations, using weighted regression reverses the trend and may improve quantitations at the lower end of a calibration. As long as a laboratory can provide the mathematical characterization of the weighted function and as long as the resulting weighted technique's order is considered in choosing the number of standards for creating and verifying the calibration, using weighted calibration techniques is allowed. However, choosing weighted algorithms to correct for lack of sensitivity, detector saturation, or instrument malfunction is not allowed.

Chapter NR 149 now explicitly disallows the use of calibrations forced through zero. The program has always prohibited using these calibrations on several grounds, one of them implied by the technique's name. Using this type of algorithm alters the natural tendency of a set of responses below the lowest calibration standard to conform to the theoretical notion that a blank should not register a discernible response when analyzed. In reality we know that appreciable amounts of analyte yield no responses, which is one of the reasons laboratories determine limits of detection. An analytical zero is not necessarily the same as a theoretical zero. The forcing technique disregards the effect that detector noise has on discerning a true signal and eliminates an indicator of instrumental sensitivity, namely, the "y" intercept. The information on the note to this subparagraph indicating that forcing through zero results in a null response for a zero standard that has a non-zero response, or yields a theoretical null response without the analysis of a calibration blank summarizes why the technique is objectionable.

The Certification Program is aware that forcing through zero is allowed by some EPA programs in some specific methods. We consider the prohibition of the technique's use to be a stricter requirement than a method's allowance.

Requirement: NR 149.44(6) (g)

A laboratory must evaluate a calibration for acceptability against set criteria appropriate for the type of analytes to be quantitated, and the calibration model and reduction technique or algorithm selected. The table below summarizes the criteria.

Acceptability Criteria for Initial Calibration

Reduction Technique	Evaluation Parameter	Inorganic Analytes and Metals	Organic Analytes
Average Response Factors	Relative Standard	≤ 20%*	≤ 20%*

(RF)	Deviation (RSD)		
Linear Regression	Correlation Coefficient	≥ 0.995	≥ 0.99
Quadratic Regression	Coefficient of Determination	≥ 0.995	≥ 0.99

*Unless an approved method of analysis allows a larger percentage.

Discussion

The table summarizes the acceptability criteria for initial calibrations. At the moment, Chapter NR 149 does not explicitly state acceptability criteria for reduction techniques other than average response factors and linear and quadratic regression. Laboratories using other acceptable reduction techniques must choose criteria appropriate to the model and technique chosen.

Some laboratories use software that provides residuals, that is, the difference between the true concentration of a standard and what is obtained when the response of the analyzed standard is entered into the derived calibration function. Residuals can be very useful in evaluating the acceptability of a calibration, but at the moment, there is no uniform agreement on when they should trigger recalibration, elimination of a standard from a calibration, or in general, acceptability of a calibration. This is partly because each standard is associated with a residual and so there is not a single indicator for the overall "goodness" of a calibration. The Certification Program may provide guidance on the use of residuals in evaluating calibration in the future.

Note also how in this section Chapter NR 149, in discussing the RSD of average response factors, explicitly permits broader criteria when a method calls for it.

Requirement: NR 149.44(6) (h)

A laboratory must establish procedures for zeroing instruments and treating calibration blanks when a method requires a calibration blank to be part of a calibration function.

Discussion

This is not an easy matter to tackle. There are several valid ways, depending on the calibration function and nature of the instrument, to establish or include a calibration blank in a calibration function. The Certification Program knows that there are several ways in which laboratories account for blank responses, from zeroing with a calibration blank, to zeroing with a method blank for procedural standards, those submitted to the same preparation steps as samples. And the

manner of zeroing has some effect on the treatment of a blank in a calibration function.

Logically, if an instrument's response can be adjusted so that a calibration blank can have a response of zero, then it is indicated to include an entry of zero concentration and zero response in a regression array. But what is the proper way to proceed when a calibration blank's response cannot be adjusted to zero? Does a laboratory include the actual response of the blank in a regression array, or does it ignore it and let regression alone predict the calibration's zero? Because the limits of detectability continue to be pushed down, treatment of calibration blanks is not a trivial matter.

Chapter NR 149, at the moment, requires laboratories to establish procedures for zeroing instruments and treating calibration blanks in relation to calibration functions. In the future, the Certification Program may develop guidance on how to address these issues. In the meantime, requiring laboratories to establish procedure to address zeroing and calibration blanks will get laboratories to discuss these issues and to start evaluating the validity of a contemplated approach.

Expect laboratory evaluators to ask questions about how a laboratory addresses these concerns.

Requirement: NR 149.44(6) (i), (j)

A laboratory must verify all initial calibrations with a second source standard unless:

- An instrument is tuned to conform to a universal scale or law, as pH meters, ion selective electrodes, and dissolved oxygen meters.

- The laboratory analyzes quality control standards for the analyte or analyte groups.

Unless otherwise specified in a method or regulation, the acceptance criteria for the second source standard are identical to the corresponding criteria for continuing instrument calibration verification.

Discussion:

Second source standards are like second opinions for medical procedures. When a second opinion agrees with that of the first physician, the course of action seems clear. But what happens when the two opinions do not agree? Regardless, it seems that in matters of importance it is always wise to check with a different set of ears, eyes, relatives, doctors, experts, or psychics. This is the perspective that using a second source standard can contribute.

A second source standard is the proverbial outside check; it verifies that another laboratory, using a different set of standards, could obtain comparable results to the ones a laboratory would obtain using the calibration being verified. Second source standards are good indicators of inter-laboratory agreement, your laboratory against others, but do not strictly verify intra-laboratory agreement, or how your calibration function deviates from its initial state overtime. A second source standard can also spot systematic errors in the preparation of a set of calibration standards.

Second source standards are commonly referred to as initial calibration verification (ICV) standards and when employed, are required to be analyzed immediately after generating an initial calibration and before samples are analyzed. ICVs do not have to be analyzed on each day when a laboratory does not generate a full calibration to quantitate samples.

There are two valid exceptions for requiring checking a calibration with a second source standard. First, when an instrument is tuned to conform to a universally accepted scale or law, tuning to that function is in itself a second source check because that function is known and it applies to all laboratories, not just the one performing the calibration.

Secondly, when a laboratory analyzes quality control standards (QCS), which are samples of known concentration obtained from a source outside the laboratory and different from the one used to prepare the calibration standards, and the QCS are analyzed and evaluated three times per year at evenly spaced intervals, the laboratory has an established system for checking its calibrations against those of others. This is not as immediate a check as verifying each calibration with a second source standard, but for those laboratories that do not calibrate daily or at a high frequency, this is a valid alternative.

Generally, the costs of using a second source standard are less than those of purchasing quality control standards three times per year. Most laboratories will institute second source checks readily, as is the industry norm, instead of risking producing inaccurate results for four months before analysis of a QCS unveils a hidden problem.

It makes sense to set the criteria for acceptance of a second source standard to be equal to that of the calibration verification standard, if the second source standard is to perform its duties properly. It also could make sense to set the acceptance criteria of the second source standard to be stricter than that of the calibration verification standard. It is not sensible to set the acceptance criteria of the second source standard to be broader than that of the calibration verification standard. This is one more reason why using second source standards may be

more protective than analyzing QCS three times per year: the acceptance limits of QCS are derived statistically and are generally broader than the acceptance criteria of calibration verification standards.

Requirement: NR 149.44(6) (k)

A laboratory must quantitate sample results from an established full initial calibration, unless a regulation, method, or program instructs otherwise.

Discussion

Years ago, laboratories participating in EPA's Superfund Contract Laboratory Program (CLP) had to be continuously admonished against quantitating samples for other programs against the response of the continuing calibration verification standard. The Statement of Work (SOW) for organic analyses required this mode of quantitation and because it was convenient, this rule became the norm for any organic samples analyzed in those CLP laboratories.

Nowadays, laboratories do not have to be reminded as much to quantitate samples against full initial calibration curves (otherwise, why bother establishing a full initial calibration); most laboratories do, and when they do not, there are mandated reasons for it. Chapter NR 149 allows an exception when methods or programs allow otherwise. That provision accommodates laboratories quantitating some polychlorinated biphenyls (PCBs) congeners by high resolution mass spectrometry, and those that are not Aroclors 1016 and 1260 by method 8082. This provision can also help those few laboratories that are part of the CLP and that have to analyze samples by antiquated SOWs.

Requirement: NR 149.44(6) (L)

A laboratory must quantitate sample results from responses that are within the range of the standards in the initial calibration. If dilution is required to obtain a sample result within the calibration range, the dilution should be the lowest one required to bring a sample response within the initial calibration range, except as noted below:

- For samples analyzed by ICP and ICP/MS that have responses below 90% of the upper limit of the respective instrument's linear dynamic range, but above the response of the highest concentration standard, a laboratory may report the corresponding result without having to dilute and re-quantitate the samples.

- For samples analyzed by ICP and ICP/MS, a laboratory must dilute and reanalyze all samples with responses at or above 90% of the upper limit of the

respective instrument's linear dynamic range. When this is not possible, sample results must be reported with qualifiers or narratives.

- For all other samples that have responses above that of the highest initial calibration standard when a calibration function that requires at least three different standard concentrations is used, the samples must be diluted and reanalyzed. When this is not possible, sample results must be reported with appropriate qualifiers.

Discussion

This requirement seems self-evident but for the exceptions offered explicitly to accommodate ICP and ICP/MS and those tests for which calibration does not require at least three different standards.

Outside of the range of the calibration standards, it is "no analyst" land. The regions below the lowest calibration standard and above the highest calibration standard are alien planets, quantitation wise. This part of Chapter NR149 deals only with responses that are above those of the highest calibration standard.

Unless a laboratory is analyzing samples for metals by ICP, ICP/MS, ion selective electrodes, dissolved oxygen electrodes, or pH meters, samples having responses above that of the highest calibration standard must be diluted to fit within the calibration range. The code requires the dilution to be the lowest one to bring a sample's response within the calibration range. This specific requirement is not meant to have laboratories analyze a series of decreasing dilutions until one is the lowest that is still within calibration range. The intent of this requirement is to prevent dilutions required for analytes to be within the calibration range from making other analytes fall below the calibration range. Naturally, this may require analyzing more than one dilution for tests that detect multiple analytes in a single run.

For samples analyzed by ICP or ICP/MS, a laboratory must dilute all samples having responses at or above 90% of the upper limit of an instrument's linear dynamic range. This 10% buffer zone allows fluctuation in the linear range (it is after all, dynamic) to have little effect on the accuracy of quantitations of high concentration samples. Samples having responses at or below 90% of the linear dynamic range of an ICP or ICP/MS can be reported as such, without dilution.

Samples with responses that exceed that of the highest calibration standard using any instrument allowed to be calibrated with one or two standards need not be diluted and reanalyzed. For pH meters and dissolved oxygen meters, dilution is not required. For ion selective electrodes, dilution may be required if an approved method requires three or more standards for calibration, and if the range of

calibration exceeds more than two orders of magnitude. In practical terms, this means that for most analyses performed by ion selective electrode, dilution will be needed for samples exceeding the response of the highest calibration standard.

Whenever a required dilution is not performed, associated data must be reported with appropriate qualifiers, as in for example: "Reported results are above the concentration of the highest calibration standard. The laboratory consumed the entire sample on analysis and it was not possible to analyze a diluted aliquot.""

Requirement: NR 149.44(6) (m)

After a calibration is finalized, its model and function cannot be changed after samples have been analyzed without performing another initial calibration.

Discussion

Once an initial calibration is finalized, it is "locked" and a laboratory cannot go back and change its conditions or parameters after samples have been analyzed. It is not permissible to change a calibration model or function to alter a quantitated response for a sample. In the future, the program may provide guidance on removing responses of calibration standards from an established or to be established calibration function.

Requirement: NR 149.44(6)(n)

A laboratory must perform an initial calibration after an instrument's initial calibration cannot be verified, or after an instrument undergoes non-routine maintenance, or when the instrument's performance is contrary to its expected behavior.

Discussion

When in doubt, recalibrate. After a calibration fails verification criteria, and the failure is confirmed, recalibration is required. However, even if calibration verification is acceptable, after non-routine maintenance calibration is in order because operating conditions have changed. In an ideal world where initial calibration would not be so time consuming, all quantitations would be based on calibrations performed on the same day that samples are analyzed.

Requirement: NR 149.44(6) (o)

A laboratory must retain all raw data necessary to reconstruct, independently of analytical instruments, all calibration functions associated with initial calibrations. Older models of ICPs are exempted from this requirement.

Discussion

Retaining raw data to reconstruct initial calibrations is not just desirable for evaluators assessing a laboratory. Given the speed of changes to quantitation software and the incompatibility of subsequent versions, retaining this information is critical for all laboratories.

The exception to this requirement for older models of ICPs is a historical precedent, acknowledged in Chapter NR 149 and here without any claim to its validity.

Continuing Instrument Calibration Verification [NR 149.44(7)]

Summary

The process for continuing instrument calibration verification is followed when a full calibration is not generated on an analysis day, after analyzing a specified number of consecutive samples, and after a specified time period elapses.

Chapter NR 149 requires laboratories to select a number of verification standards that is appropriate for the calibration model and reduction technique chosen. The calibration is verified when the verification standards meet established acceptance criteria. The Chapter defines procedures to follow when the continuing calibration verification fails acceptance criteria.

Requirement: NR 149.44(7)(a)

Whenever a full initial calibration is not performed on a day when samples are to be analyzed, the last valid initial calibration must be verified before samples are analyzed and after the analysis of each group of 20 samples in an analytical run.

Continuing calibration verification is not required for analyses not amenable to fortification and for titrimetric assays.

Discussion

Calibration verification is performed before any samples are analyzed on any day when an initial calibration is not performed to demonstrate that an instrument can produce accurate sample results. After the analysis of 20 samples in a run, not counting quality control samples, the instrument is checked once again.

Many laboratories conclude their runs with an analysis of a continuing calibration verification standard (CCV), therefore bracketing all quantitated results with valid calibration checks. Traditionally, laboratories that do not use the "internal standard" mode of quantitation analyze a CCV at the end of a run.

Laboratories do not have to perform continuing calibration verification for biochemical oxygen demand (BOD), carbonaceous oxygen demand (cBOD), total suspended solids (TSS), all titrimetric analyses, and any tests for which spiking is not possible.

Requirement: NR 149.44(7) (b)

The continuing calibration verification standard may be obtained from the same source used to generate an initial calibration.

Discussion

Ideally, CCVs should be prepared from the same source as that used to generate the calibration standards. This is the most defensible practice and maintains the distinction made in Chapter NR 149 between ICVs and CCVs. Because a CCV's function is to verify the integrity of the calibration function that is used to produce sample results, it is correct to prepare the CCV from the same source used to prepare the calibration standards. In a sense, the argument about sourcing for CCVs and ICVs relates to parallel comparisons, or comparing "apples to apples". A passing CCV from the same source as the calibration standards tells a laboratory that everything is in order in the laboratory's own domain, while a passing ICV tells the laboratory that it is in harmony with the rest of the world.

Many methods require CCVs to be prepared from the same source as that used to prepare the calibration standards. This practice has now become the norm in the environmental community. The best CCVs are prepared at concentrations different from the concentrations used for the calibration standards, but of course, within the calibration range.

Requirement: NR 149.44(7) (c)

The number and concentration of calibration standards required to verify an initial calibration function, by calibration function type, are summarized below.

Calibration Verification Requirements

Calibration Function Type	Minimum Number of Verification Standards	Concentration of Verification Standards
Tuning to conform to a universally accepted law or scale*	One	Within the range of the initial calibration
Average response factor, linear regression, or other linear models	One	Within the calibration range
Quadratic regression, second order polynomial, or other quadratic models	Two	One must be at a concentration with a response near the inflection point of the function.
Cubic regression, third order polynomial, or	Three	Two must be at concentrations with

other cubic models		responses near the inflection points of the function.
Discrete or non-smooth segments	One per segment	Different from the ones used to establish each segment

- Applicable to ion-selective electrodes. Continuing calibration verification is not required for BOD, cBOD, and pH.

Discussion

The table summarizes the number and concentration of calibration standards required to demonstrate continuing calibration.

The minimum number of standards for each calibration type is obtained by examining the slope (the first derivative in calculus) of a function. The more complex the function, the more standards needed to demonstrate continued calibration.

The concentrations chosen for preparing CCVs tend to be towards the middle of a calibration range. Selecting the concentration of standards correctly for quadratic and cubic models requires plotting the calibration function to determine inflection points. In any event, using the highest or the lowest calibration standard concentration for a CCV is not a good practice. Should the response of a CCV at the highest concentration of the calibration exceed the response of the same concentration in the initial calibration, the CCV would have to be diluted to be quantitated and dilution introduces additional error. Similarly if a the concentration of a CCV is chosen to be that of the lowest calibration standard and the CCV's response is below that of the same concentration of standard in the initial calibration, the CCV cannot be defensibly quantitated.

For ICP and ICP/MS, the concentration of the CCV can be any value as long as it does not exceed 90% of the linear dynamic range (LDR) of the instrument. Varying the concentration of the CCV within this region of the LDR may be useful, particularly if the concentration of the CCV is at times below the highest calibration standard and at others above it but within 90% of the LDR.

Some regulatory programs and methods require verification of continued calibration at concentrations at or near regulatory limits. Typically, the acceptance criteria for those types of verifications are broader than the criteria for the more conventional verifications.

Note that here Chapter NR 149 mentions discrete or non-smooth segments as a calibration function type and how verifying this calibration is perhaps the most

involved of the options. Very few laboratories use this type of function for quantitation.

Requirement: NR 149.44(7) (d)

A laboratory must follow the acceptance criteria for continuing calibration verification standards specified in methods of analysis. When a method does not contain criteria, verifying the concentration of an inorganic standard within 10% of its true value or verifying the concentration of an organic standard within 15% of its true value is required.

Discussion

A laboratory must default to method-specified criteria for determining the acceptability of a CCV. The values given here when a method does not contain criteria were included in the former version of Chapter NR 149 and are reasonable for most inorganic analyses, and for organic analyses by gas chromatography (GC) (not mass spectrometry) and high performance liquid chromatography (HPLC).

Requirement: NR 149.44(7) (e)

When a continuing calibration verification standard fails acceptance criteria, a laboratory must analyze another verification standard. If the second verification standard fails, a laboratory must take corrective action. After taking corrective action, a laboratory must analyze two consecutive standards that meet acceptance criteria or must perform a complete initial calibration.

Discussion

The process for addressing a failed CCV is progressive and logical: confirm the failure, take corrective action, demonstrate definitively that the action was effective, and if not, recalibrate.

If a second CCV does not confirm the failure of the first, that is, the second CCV passes, the laboratory may continue using the initial calibration for quantitation. If the second CCV fails, the laboratory takes corrective action, and the first of the subsequent CCV fails, recalibration is required.

Requirement: NR 149.44(7) (f)

When continuing calibration cannot be verified, a laboratory must reanalyze the associated samples or report their results with qualifiers.

Discussion

If after attempting the scheme in NR 149.44(7) (e) the laboratory cannot verify calibration, the associated samples need to be reanalyzed. If reanalysis is not possible for whatever reason, all sample results must be accompanied with qualifiers.

There are a few cases where even after a calibration verification fails, reanalysis may not be required and results may be reported with a qualified certainty. For example, if a CCV consistently exceeds the upper limit of its acceptance criteria and all analyzed samples have results that are below the reporting limit of the analysis, results may be reported (with a qualifier). When a CCV consistently exceeds the lower limit of its acceptance criteria, and all analyzed samples have results that exceed a regulatory limit, results may also be reported (with a qualifier).

Requirement: NR 149.44(7) (g)

Laboratories must include or reference in their SOPs the details of their continuing instrument calibration processes, including calculations and associated statistics.

Discussion

Most laboratories will include calibration details, including continuing calibration verification specifics, in a designated section of their SOPs. NR 149.40(2) (d) requires including or referencing calibration and standardization in the analytical methods manual. When this information is not included directly in an SOP, the referenced document containing the information must include sufficient information to allow reproducing calibration verifications performed at the laboratory.