Instrumentation Technology
INST-1010

Calibration

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Today's meeting

- Call Attendance
- Announcements
- Collect Homework
- Give examination
  - Display time clock
- Collect examinations
- Previous examination
  - Return
  - Discussion
- Introduce topic
  - Provide Handouts
  - Socratic discussion
  - Practice – Problems
- Reminder: Administrative Rules

Themes

- Name circumstances that require calibration procedures
- Properly assemble the instruments required to perform the calibration procedure
- Explain three-step and five-step calibration procedures
- Properly assemble the instruments required to perform the calibration procedure

Themes (cont'd.)

- Recognize zero shift and span errors so that they can be properly corrected
- Describe process identification
- Use the following methods to tune a controller:
- Trial-and-error, Ziegler-Nicholas reaction curve, autotuning, and Ziegler-Nicholas continuous cycling

Themes (cont'd.)

- Define common terms associated with controller tuning
- List and describe three process models that result from a step change

MEASUREMENTS

Kinds of Signals

- Electrical
  - Analog - continuous range of values
  - Digital – discrete values, 1/0, on/off, true/false
- Pneumatic
  - Pressure (Low, High)
- Heat
  - Temperature (Low, High)
- Length
  - Level
- Acidic / Alkali
  - pH

The Purpose of Measurement

- Four main purposes:
  - Continuous input to controller
  - Monitoring of process variables or equipment
  - Recording of information for trend indication or archive
  - Spot-checking of a process variable
- Systems may be complex
  - Many processes and variables being monitored at same time

Measurement Requirements

- Accuracy and reliability of measurements depend on process requirements
  - Power plant/ Furniture kiln example
- Criticality of variables depends on process
  - Fast response for quickly changing variables
    - pressure or flow
  - Slower response for slower variables
    - Temperature or level

Errors in Measurement Systems Sources of error

- Incorrect calibration
- Noise
- Speed of response
- System degradation
- Errors of observation
- Transducer accuracy

Errors in Measurement Systems Observation Errors

- Incorrect interpretation of instrument reading
- Minimize parallax with analog pointer scales
- Verify display graduations
- Double check multi digit displays

Noise

- Noise
  - Any signal other than the desired signal
  - Many sources
  - Noise gets added to signal
  - Large SNR (Signal to Noise Ratio) desired

Response Time

- Response lag
  - Time required for measurement system to respond
  - See example pages 50, 51, 52

System Deterioration

- As system and components age, changes occur
- Minimize effect by
  - Regular routine maintenance
  - Regular recalibration
  - Replace failing components

Transmitters

- Transmitters used to condition measuring sensor output for remote display

Proportionality

- Changes in sensor output proportional to display value
  - 4-20 mA could drive a display showing level from 0 to 100%

Kinds of Displays

- Audible
  - Tones, beeps, bells, etc.
- Visual
  - Pointer
    - Edgewise, circular
    - Scale typically graduated
      - Linear, nonlinear, logarithmic
  - Digital displays
    - Direct reading of variable
    - Rapidly replacing many pointer scale displays
  - Monitors
    - Text, graphics, color used to define process, variables, …

Remote versus Local Display

- Local
  - Variable measured and displayed at source
- Remote
  - Variable measured at source displayed remotely through a separate transmitter

CALIBRATION

Calibration of Instrument System

- Measurement of Accuracy
- Establishment the relation of an instrument's accuracy to the international standard

Calibration

- Exact value of variable must be known to calibrate instrument
- All other accuracies of equipment used to perform calibration must be taken into account
  - See example page 49

Calibration

- psi is pound per square inch pressure
- psia is pound per square inch absolute (in respect to absolute vacuum)
- psig is pound per square inch gage (indicated differential pressure between ambient pressure and the measured pressure)
- all those units are outdated. Today the international units of pressure are (Pascal) Pa, hPa and Bar
- The suffix absolute or gage should never added into the dimensional unit.
- Use it as follows:
  - Absolute pressure = 5 bar
  - Differential pressure = 4 bar

Instrument Calibration

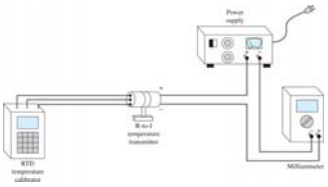
- Transmitter
  - Converts signal from a sensor to a standard analog signal used by the controller
- Calibration
  - Performed to establish zero and span settings
- Instrument
  - Adjusted using a screw, nut on a bolt, or keypad

Reasons for Performing Calibrations

- Include:
  - Wear and aging
  - Environmental conditions
  - Routine maintenance
  - Before new instrument installation
  - After extended shutdown
  - After repair
  - When products fail to meet specifications

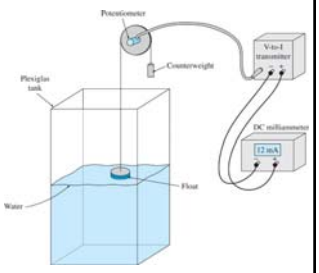
Calibration Preparation

- Five steps
- Calibration equipment connections must not introduce errors during test procedure
- Calibration circuit:



Standard Calibration Procedure

- Seven steps
- Three-point calibration check

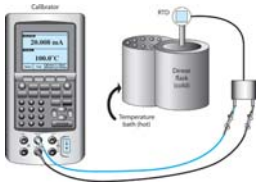


Five-Point Calibration Procedure

- Includes:
- Calibration check
- Calculating the input values
- Calculating the output values
- Analysis of calibration data
- Transmitter zero and span adjustments
- Verification of adjustments

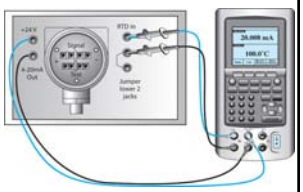
Process Calibrators

- Sensor calibration with a process calibrator



Process Calibrators

- Transmitter calibration with a process calibrator



CALIBRATION OF INSTRUMENTS AND STANDARDS LABORATORY

1. Calibration process

- The purpose of calibration is to ensure that the measuring accuracy is known over the whole measurement range under specified environmental conditions for calibration.

Calibration of Instrument

- Instrument to be calibrated
  - Whole measuring range
  - System output
- Instrument of higher standard
  - The input value with known accuracy
- Standard instrument
- Environmental conditions (modifying inputs)
  - Ensure the calibration is done under the specified environmental conditions

Calibration Process

- A proper course of action must be defined which describes the procedures to be followed when an instrument is found to be out of calibration.
- The required action depends very much upon the nature of the discrepancy and the type of instrument involved.
- For example,
  - Simple output bias can be corrected by a small adjustment
  - Alternation of scale factor may be corrected by redrawing the output scale or adjusting the amplification.

Calibration Process

- For the corrections mentioned above, the adjustment screws must be sealed to prevent tampering.
- In extreme cases, where the calibration procedure reveals signs of instrument damage, it may be necessary to send the instrument for repair or even replacement.

Calibration Process

- Calibration process must be managed and executed in a professional manner:
  - A particular place for all calibration operations to take place and keeping all instruments for calibration
  - A separate room is preferred because
    - (1) better environmental control and
    - (2) better protection against unauthorized handling or use of the calibration instruments.

Calibration Process

- The performance of all calibration operations is assigned as the clear responsibility of just one person.
- Calibration procedures, used for quality control functions, are controlled by the international standard ISO 9000.
  - It requires that all persons using calibration equipment be adequately trained.
- Instrument calibration has to be repeated at prescribed intervals because the characteristics of any instrument change over a period of time.

Factors deciding the frequency of calibration:

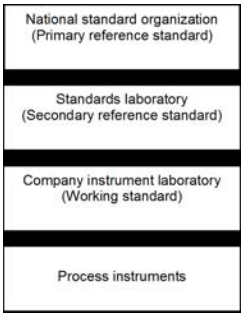
- usage rate
- conditions of use
- skill level of personnel
- degree of accuracy expected
- costs of calibration
- Maintaining proper records is an important part of fulfilling the calibration function, which is very useful in providing a feedback which shows whether the calibration frequency has been chosen correctly or not.

A typical format for instrument record

Type of Instrument:	Instrument Model:	Manufacturer Serial Number:
Company Serial Number:	Company's Part Number:	Date Introduced:
Measurement Limits:	Location:	Calibration frequency:
Person Responsible for Calibration	Name:	Signature:
Instructions for use:		
Calibration Record		
Calibration Date	Calibration Results	Calibrated by

Traceability

- As shown in Fig., calibration has a chain-like structure in which every instrument in the chain is calibrated against a more accurate instrument immediately above it in the chain.



Traceability

- The knowledge of the full chain of instruments involved in the calibration procedure is known as traceability, and is specified as a mandatory requirement in satisfying the ISO 9000 standard.
- Documentation must exist which shows that process instruments are calibrated by standard instruments which are linked by a chain of increasing accuracy back to national reference standards.

Traceability

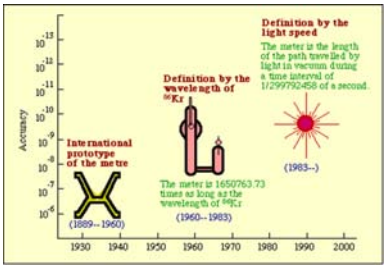
- In engineering measurement and calibration, the 'ten-to-one' rule is usually applied to the choice of instrument, which states:
  - For a particular measuring application, choose an instrument the discrimination of which splits the permissible tolerance on the dimension to be measured into approximately 10 parts.
- For example:
  - If the tolerance on a dimension is $\pm$ 0.5 mm, choose an instrument which has a resolution of 0.1 mm.
  - If tolerance is 0.02 mm on a part, then choose an Instrument with a resolution 0.002 mm

Example

- Iodine stabilized Helium-Neon laser (uncertainty 1 to 10^9)
- Spectral lamp(uncertainty 1 to 10^7)
- Reference gage range blocks (uncertainty 1 to 10^6)
- Standard gage range blocks (uncertainty 1 to 10^5)
- Micrometer (uncertainty 1 to 10^4)

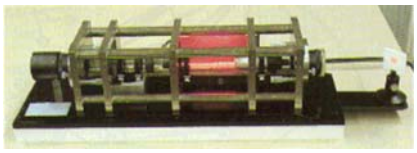
- The National physical laboratory
- The National physical laboratory
- A specialized service calibration company
- Standard laboratory of the company
- Shop floor of the company

Evolution of the length standard.



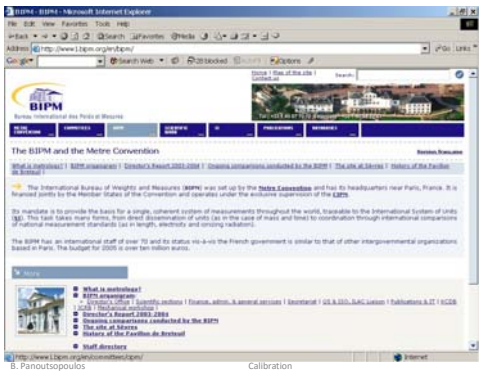
Evolution of the length standard.

- The length standard: 10^{-10} to a greater accuracy.

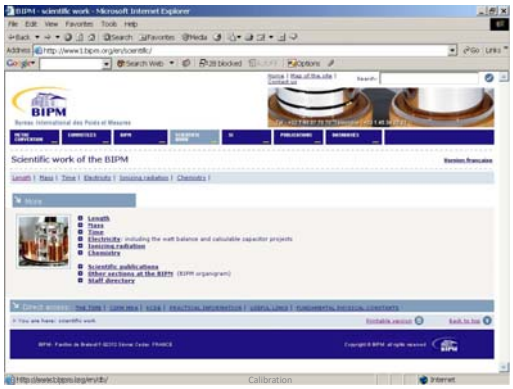


- Iodine-stabilized He-Ne laser at 633 nm.

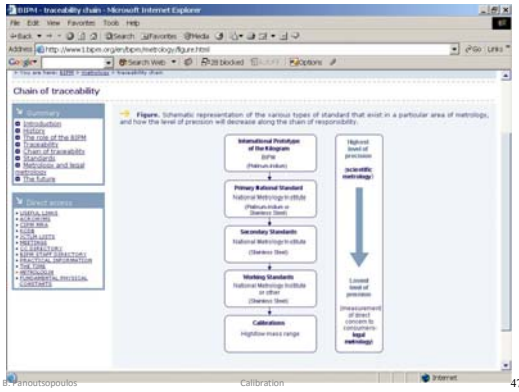
The International Bureau of Weights and Measures (**BIPM**)
http://www1.bipm.org/en/bipm/



http://www1.bipm.org/en/scientific/



http://www1.bipm.org/en/bipm/metrology/figure.html



calibration certificate

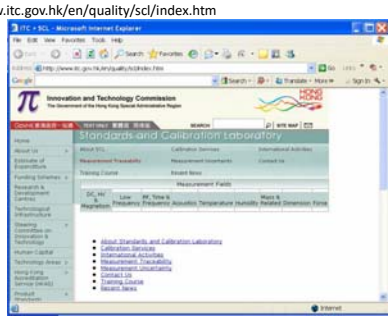
- The instrument used for calibrating working standard instruments is known as a secondary reference standard.
- When the working standard instrument has been calibrated by an authorized standards laboratory, a calibration certificate will be issued.
- This will contain at least the following information:
  - The identification of the equipment calibrated.
  - The calibration results obtained.
  - The measurement uncertainty.
  - Any use limitations on the equipment calibrated.
  - The date of calibration.
  - The authority under which the certificate is issued.

Standard Laboratories

- The establishment of a company standards laboratory to provide a calibration facility of the required quality is economically viable only in the case of very large companies where large numbers of instruments need to be calibrated.
- In the case of small to medium size companies, the cost of equipping such instruments is not justified.
- Therefore, they would normally use the calibration service provided by various companies which specialize in offering a standards laboratory.
- Such standards laboratories are closely monitored by national standards organizations (ISO/IEC Guide 25, General Requirements for the Technical Competence of Testing Laboratories).

Honk Kong Laboratory Accreditation Scheme (HOKLAS)

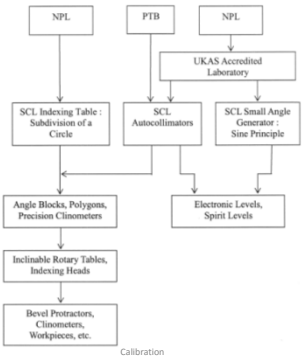
http://www.itc.gov.hk/en/quality/scl/index.htm



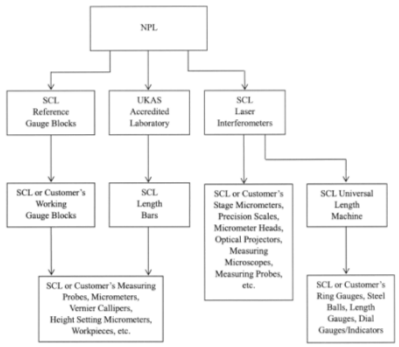
Honk Kong Laboratory Accreditation Scheme (HOKLAS)

http://www.itc.gov.hk/en/quality/scl/trace.htm

Traceability of Angle Measurement



Traceability of Length Measurement



Accreditation of Standards Laboratories

- The Accreditation Scheme
  - It is a voluntary scheme
  - It is open to any laboratory that performs objective testing within the scope of the Scheme and meets criteria of competence.
  - May also accept applications for accreditation from laboratories located outside thw country of origin.

Accreditation of Standards Laboratories

- The aims are:
  - to upgrade the standard of testing and management of laboratories,
  - to identify and officially recognize competent testing laboratories,
  - to promote the acceptance of test data from accredited laboratories, both locally and internationally.

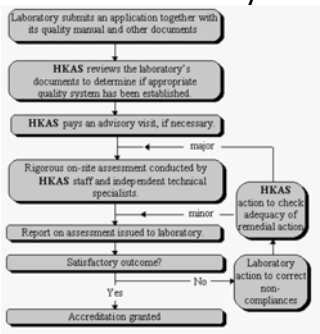
Accreditation of Standards Laboratories

- For non-medical testing and calibration laboratories, accreditation criteria are in accordance with ISO/IEC 17025:2005 "General Requirements for the competence of testing and calibration laboratories".
- For medical testing laboratories, accreditation criteria are in accordance with ISO 15189:2007(E) "Medical laboratories – Particular requirements for quality and competence".
- For proficiency testing providers, the accreditation requirements are in accordance with those stated in ISO/IEC 17043:2010 "Conformity assessment - General requirements for proficiency testing".
- For reference material producers, the accreditation criteria is given in the Accreditation of Reference Material Producers. The main text of this document is a verbatim reproduction of ISO Guide 34:2009.
  - IEC - International Electrotechnical Commission

Accreditation of Standards Laboratories

- Accreditation is recognition of a laboratory's capability to perform specific tests and does not guarantee individual test results or equate with product certification.
- Laboratories accredited or seeking accreditation are required to have their testing and measuring equipment regularly calibrated by a competent calibration organization which can offer calibrations traceable to international standards of measurement.
- The Standards and Calibration Laboratory - has been designated as one of the competent calibration organizations able to provide the necessary measurement traceability.

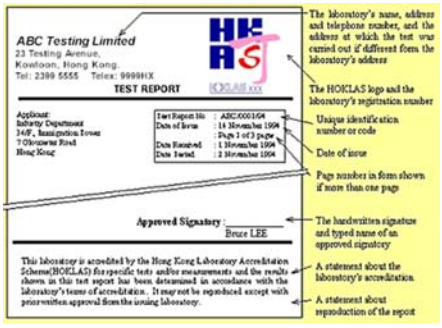
The accreditation process of a standard laboratory.



A Sample of HOKLAS Endorsed Test

- HOKLAS accreditation is also granted for specific tests.
- Laboratories are allowed to issue HOKLAS Endorsed Test Certificates (Fig.) only for tests which are accredited.

A Sample of HOKLAS Endorsed Test Certificate



national standards organization

- In the United Kingdom, the appropriate national standards organization for validating standards laboratories is the National Physical Laboratory,
- In the United States of America, the equivalent body is the National Bureau of Standards.
- To achieve confidence in the goods and services which move across national boundaries, international agreements have established the equivalence of the different accreditation schemes in existence.
- HKAS is a member of the
  - International Accreditation Forum (IAF),
  - International Laboratory Accreditation Cooperation (ILAC),
  - Pacific Accreditation Cooperation (PAC) and
  - Asia Pacific Laboratory Accreditation Cooperation (APLAC).

Maintenance of Accreditation of a Laboratory

- Mandatory reassessments are conducted one year after the granting of accreditation and at two-year intervals thereafter.
- Surveillance visits, announced or unannounced, are also conducted.
- Accredited laboratories are visited at least once a year and are required to participate in proficiency testing activity at least once every four years for each major sub-area of major disciplines.

Documentation of Calibration

- An essential element in the operation of calibration is the provision of full documentation that consists of the following:
  - Measurement requirements (such as environmental conditions)
  - Instruments used
  - Calibration system and procedures operated
  - Calibration record
  - Traceability of the calibration system back to national reference standards must be defined and supported by calibration certificates.
  - Training programmes
  - The above-mentioned are also important to the maintenance of measurement system and form a necessary part of the quality manual.

REFERENCES

References

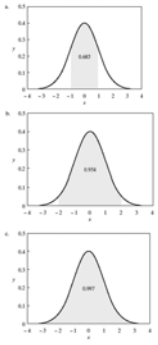
APPENDIX:

THE MATHEMATICS OF CALIBRATION

arithmetic mean

- The is the "standard" average, often simply called the "mean"

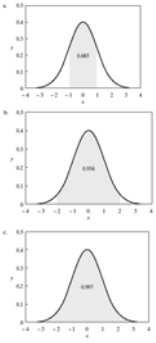
$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$



standard deviation

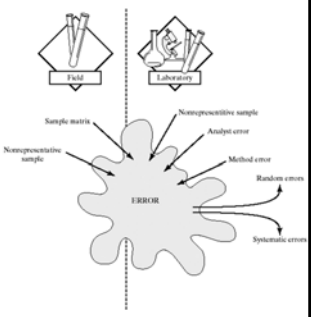
- The standard deviation (SD) quantifies variability.
- If the data follow a bell-shaped Gaussian distribution, then 68% of the values lie within one SD of the mean (on either side) and 95% of the values lie within two SD of the mean.
- The SD is expressed in the same units as your data.

$$s = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{n-1}}$$



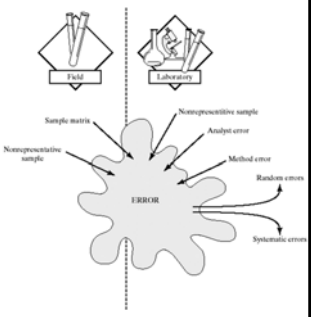
errors

- Errors separate into two broad categories: those originating from the analyst and those originating with the method.
- The former is an error that is due to poor execution,
- the latter an error due to an inherent problem with the method.
- Method validation is designed to minimize and characterize method error.
- Minimization of analyst error involves education and training.

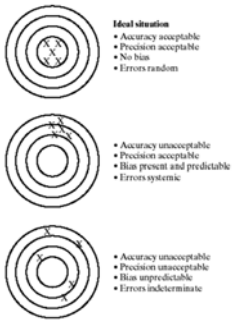


errors

- A second way to categorize errors is by whether they are systematic.
- Systematic errors are predictable and impart a bias to reported results.
- These errors are usually easy to detect by using blanks, calibration checks, and controls. In a validated method, bias is minimal and well characterized.
- Random errors are equally positive and negative and are generally small.



Accuracy - Precition



Calibration of equipment

- The accuracy and precision of analytical devices must be known and monitored.
- Devices requiring calibration include refrigerators, balances, pipets, syringes, pH meters, microscopes, and so on.
- In short, if the equipment provides a measurement that is related to generating data, it must be calibrated.

Initial performance	
Stable water temperature	26.0°C
Date	2/2/2006
Analyst	S.H.
Pipette serial number	1098CX-3
Received	2/1/2006
Density H_2O	
CRC 2004	0.9967867

Trial	Weight	Volume (calc, uL)
1	0.2003	200.9
2	0.1995	200.1
3	0.2004	201.1
4	0.1993	199.9
5	0.2004	201.0
6	0.2007	201.3
7	0.1998	200.4
8	0.2003	200.9
9	0.2010	201.6
10	0.1999	200.5

Volume (calc, uL)	
Mean	200.8
Standard error	0.168
Standard deviation	0.531
Sample variance	0.282
%RSD	0.265
Range	1.7
Minimum	199.9
Maximum	201.6
Count N	10
Confidence level (95%)	0.38

UWL: 201.1
LWL: 200.5
UAL: 201.3
LAL: 200.3

$$\bar{x} \pm \frac{2s}{\sqrt{N}}(95\%) \qquad \bar{x} \pm \frac{3s}{\sqrt{N}}(99.7\%)$$

Calibration of equipment

- Suppose an Eppendorf pipet arrives at a lab.
- The analyst immediately validates this performance by repeatedly pipetting what the device records as aliquots into dried, tared containers on a calibrated analytical balance.
- By recording the water temperature and using a chart that relates density to temperature, the analyst converts the weight of water, in milligrams, to a volume delivered by the pipet.

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Calibration of Instruments Concentration and Response

- This type of calibration, typically for instruments like spectrometers, requires the use of linear regression. earlier in the chapter.
- A good regression line is required for a valid calibration.
- A calibration curve has a lifetime that is linked to the stability of the instrument and the calibration standards.

Calibration of Instruments Concentration and Response

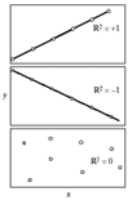
- Another aspect of curve validation is the use of calibration checks.
  - The ideal calibration check (CC) is obtained from a traceable standard that is independent of the solutions used to prepare the calibration standards.
  - This is the only method that facilitates the detection of a problem in the stock solution.
- Finally, blanks must be analyzed regularly to ensure that equipment and instrumentation have not been contaminated
- Thus, four factors contribute to the validation of a calibration curve:
  - correlation coefficient (R^2),
  - the absence of a response to a blank
  - the time elapsed since the initial calibration or update,
  - and performance on an independent calibration-check sample.

Calibration of Instruments Concentration and Response

- The goodness of fit of the line is measured by the correlation coefficient or more frequently as its squared value R^2, and is a measure of linearity of the points.
- If the line is perfectly correlated and has a positive slope whereas describes a perfectly correlated line with a negative slope.
- If there is no correlation, It is important to remember that r is but one measure of the goodness of a calibration curve, and all curves should be inspected visually as a second level of control.

Calibration of Instruments Concentration and Response

$$R^2 = \frac{\left[\sum (x_i - \overline{x})(y_i - \overline{y})\right]^2}{\sum (x_i - \overline{x})^2 \sum (y_i - \overline{y})^2}$$



▲ **Figure 3.4** *Relationship of correlation coefficient (R^2) to linear fit. Typical calibration curves are at least "two nines," or 0.99. The value of (R^2) is an important criterion, but not the only one, for describing the goodness of fit of the calibration curve.*

Predictive modeling and calibration

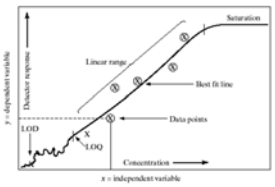
- Involves multivariate statistics (the application of statistics to data sets with more than one variable)
- Regression lines take the form (y = mx + b)
  - where m is the slope and
  - b is the y-intercept, or simply intercept.
- The variable y is called the dependent variable, since its value is dictated by x, the independent variable.
- All linear calibration curves share certain generic features.

Predictive modeling and calibration

- The range in which the relationship between concentration and response is linear is called the linear range and is typically described by "orders of magnitude."
- A calibration curve that is linear from 1 ppb to 1 ppm, a factor of 1000, has a linear dynamic range (LDR) of three orders of magnitude.
- At higher concentrations, most detectors become saturated and the response flattens out; the calibration curve is not valid in this range of higher concentrations, and samples with concentrations above the last linear point on the curve must be diluted before quantitation.
- The concentration corresponding to the lowest concentration in the linear range is called the limit of quantitation (LOQ).
- Instruments may detect a response below this concentration, but it is not predictable and the line cannot be extrapolated to concentrations smaller than the LOQ.
- The concentration at which no significant response can be detected is the limit of detection, or LOD.

fitting empirical data to a line

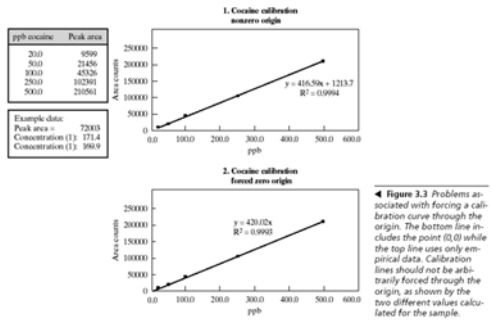
- The line generated by a least-squares regression results from fitting empirical data to a line that has the minimum total deviation from all of the points.
- The method is called least squares because distances from the line are squared to prevent points that are displaced above the line (signified by a plus sign,) from canceling those displaced below the line (signified by a minus sign,).
- Most linear regression implementations have an option to "force the line through the origin," which means forcing the intercept of the line through the point (0,0).
- This might seem reasonable, since a sample with no detectable cocaine should produce no response in a detector, but must be used with care.



plot

- Forcing the plot through (0,0) is not always recommended, since most curves are run well above the instrumental limit of detection (LOD).
- Arbitrarily adding a point (0,0) can skew the curve because the instrument's response near the LOD is not predictable and is rarely linear, as show below.
- As illustrated forcing a curve through the origin can, under some circumstances, bias results.

plot



◀ **Figure 3.3** *Problems associated with forcing a calibration curve through the origin. The bottom line includes the point (0,0) while the top line uses only empirical data. Calibration lines should not be arbitrarily forced through the origin, as shown by the two different values calculated for the sample.*

Calibration of instruments Concentration and response

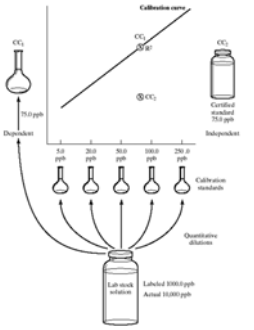
- External Standard:
- This type of curve is familiar to students as a simple concentration-versus-response plot fit to a linear equation.
- Standards are prepared in a generic solvent, such as methanol for organics or 1% acid for elemental analyses.
- Such curves are easy to generate, use, and maintain.
- For example, if an analysis is to be performed on cocaine, some sample preparation and cleanup is done and the matrix removed or diluted away.
- In such cases, most interference from the sample matrix is inconsequential, and an external standard is appropriate.
- External standard curves are also used when internal standard calibration is not feasible, as in the case of atomic absorption spectrometry.

Calibration of instruments Concentration and response

- Internal Standard:
- External standard calibrations can be compromised bycomplex or variable matrices.
- In toxicology, blood is one of the more difficult matrices to work with, because it is a thick, viscous liquid containing large and small molecular components, proteins, fats, and many materials subject to degradation.
- A calibration curve generated in an organic solvent is dissimilar from that generated in the sample matrix, a phenomenon called matrix mismatch. Internal standards provide a reference to which concentrations and responses can be ratioed.
- The use of an internal standard requires that the instrument system respond to more than one analyte at a time.
- Furthermore, the internal standard must be carefully selected to mimic the chemical behavior or the analytes.

Calibration

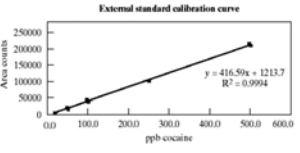
- Example:
- Calibration standards are prepared from a certified stock solution of cocaine in methanol, which is diluted to make five calibration standards.
- The laboratory prepares a calibration check in a diluted blood solution using certified standards, and the concentration of the resulting mixture is 125.0 ppb cocaine.
- When this solution is analyzed with the external- standard curve, the calculated concentration is found to be about half of the known true value.
- The reason for the discrepancy is related to the matrix, in which unknown interactions mask nearly half of the cocaine present.



Calibration

ppb cocaine	Peak area
20.0	9599
50.0	21456
100.0	45326
250.0	102391
500.0	210561

Calibration check:	
True ppb =	125
Area counts:	65.8
Calculated:	28632
% error:	-47.3



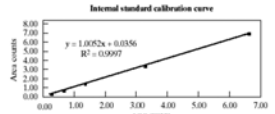
◀ **Figure 3.22**
Demonstration of a matrix effect. The matrix reduces the response of the analyte cocaine by half, and as a result, the percent error is nearly 50%.

Calibration

- Now consider an internal-standard approach
- An internal standard of xylocaine is chosen because it is chemically similar to cocaine, but unlikely to be found in typical samples.
- To prepare the calibration curve, cocaine standards are prepared as before, but 75.0 ppb of xylocaine is included in all calibration solutions.

Calibration

ppb cocaine	Peak area	ppb xylocaine	Peak area	[] ratio	Area ratio
20.0	9599	75.0	31063	0.27	0.31
50.0	21456	75.0	30099	0.67	0.71
100.0	45326	75.0	32051	1.33	1.41
250.0	102391	75.0	31004	3.33	3.30
500.0	210561	75.0	31100	6.67	6.77



True =	125
Area counts cocaine =	28832
Area counts IS =	16723
Area ratio (x)	1.712
Calculated [] ratio	1.668
Calculated cocaine:	125.1
% error:	0.1

▶ **Figure 3.23** *An internal standard corrects for the matrix effect as long as the internal standard is affected similarly to the way the analyte is affected.*

National Institute of Standards and Technology

Certificate of Analysis

Standard Reference Material 1511

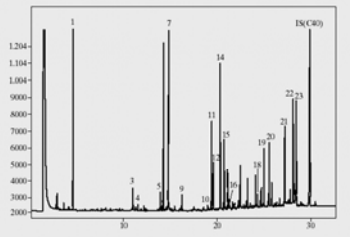
Multi-Drugs of Abuse in Freeze Dried Urine

The Standard Reference Material (SRM) is intended primarily for verifying the accuracy used for the determination of morphine, codeine, cocaine metabolite (benzoylecgonine), marijuana metabolite (THC-9-COOH), and phencyclidine in human urine. SRM 1511 consists of three (3) bottles of freeze-dried urine with all of the analytes in each bottle. (See reconstitution procedure for reconstitution to 25 mL). There is no blank urine with this SRM.

Certified concentration: The certified values and uncertainties for the analytes, as free bases, are calculated and given in the table below. For benzoylecgonine, morphine, codeine, and phencyclidine, GC/MS and LC/MS data were used and the uncertainty is a 95% confidence interval for the mean. For the THC-9-COOH, the mean concentration was computed from GC/MS and GC/MS/MS measurements taken at NIST and the uncertainty is also a 95% confidence interval for the mean. However, this confidence interval also includes variability observed between NIST and five military labs which had been used to demonstrate the suitability of the material. It is assumed that systematic errors are very small compared to random errors.

	Concentration	
Analyte	**nmole/L**	**ng/mL**
Morphine	$1.08 \pm 0.07 \times 10^4$	309 ±20
Codeine	$9.62 \pm 0.37 \times 10^4$	288 ±11
Benzoylecgonine	$3.60 \pm 0.28 \times 10^4$	162 ±8
THC-9-COOH	$4.09 \pm 0.23 \times 10^4$	14.1 ±0.8
Phencyclidine	$9.74 \pm 0.33 \times 10^4$	23.8 ±0.8

The certified concentration apply only to urine reconstituted as specified under "Reconstruction Procedure" and are based upon the concordant results from two different analytical methods for each analyte. Brief descriptions of the methods are given under "Analytical Methods." *Note:* This material also contains amphetamine and methamphetamine, but these analytes were not certified as analytical results that indicated probable degradation of these constituents with time.



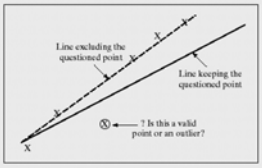
▶ *The positions of the 16 selected reference peaks and the internal standard (IS) in the profile obtained with the 'IRA-standard' sample. Figures reproduced with permission from the reference cited below. Copyright 2000, Elsevier Science.*

sample extracts, and 16 analytes were selected as shown in the chromatogram. Internal standard calibration was used, a topic to be addressed shortly. The compounds selected were minor constituents of a typical heroin exhibit, but the group of 16 did not include heroin. Using similar analytical protocols, each laboratory performed various repetitive analyses with a standard, and the reproducibility of the results was a measure of the %RSD of the 16 components. The details of the analyses were compared and refined, and the experiment was repeated with the results shown in the figure above. Significant improvements in precision between the laboratories is clearly shown in the decrease in %RSD values.

Source: Stromberg, L., et al. "Heroin Impurity Profiling: A Harmonization Study for Retrospective Comparisons." *Forensic Science International* 114 (2000), 67–88.

Exhibit A: Just Do It (Again)

There are a number of statistical tests and analyses that can be used to evaluate a calibration curve and to weigh the "goodness" of each calibration data point. As shown in the accompanying figure, one of the calibration points appears to be an outlier and including it would skew the calibration curve significantly. What is the best course of action? Clearly the point "looks funny," a phrase invoked in the previous chapter as part of the discussion on outliers. When a forensic chemist examines these data, what should the next action be?



There is no justification for throwing the point away on a hunch. Of course it looks suspicious, but the question is, Why? Is it a symptom of an underlying problem, or is it just a fluke? There is only one way to resolve the issue, and it does not involve a calculation or a hypothesis test. The next step involves laboratory work. The suspicious solution would be rerun to see if the results are replicated. If so, then a fresh solution should be prepared and retested. Depending on the results, the entire calibration series may have to be prepared fresh and reanalyzed. Even then, though, the answer to this question has little to do with calculations, identifying outliers, or weighting the questionable point less than that of the other calibration points. Once again, it's a matter of *chemistry before statistics.*

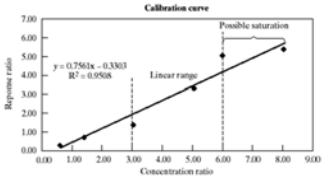
Construction and evaluation of a calibration curve

- Given the following data, construct an internal standard calibration curve and evaluate the results.

ppb codeine	Peak area	ppb internal standard	Peak area	[] ratio	Area ratio
15.0	9599	25.0	29,933	0.60	0.32
35.0	21,456	25.0	30,099	1.40	0.71
75.0	45,326	25.0	32,051	3.00	1.41
125.0	102,391	25.0	31,004	5.00	3.30
150.0	157,342	25.0	31,100	6.00	5.06
200.0	162,309	25	30,303	8.00	5.36

Construction and evaluation of a calibration curve

- The calibration has problems that become clear when the curve is plotted.
- The response at the upper concentration is flattering out, indicating detector saturation.
- At lower concentrations, there is what appears to be a linear relationship, but it differ from that in the middle of the curve.
- The LDR is approximately 35-150 ppb codeine; the calibration should be redone in this range.



Calibration - References