COURSE ON INTRODUCTION AND APPLICATION OF ENVIRONMENTAL FORENSICS

Environmental Forensics

Lecture 2: Environmental Forensics Sampling

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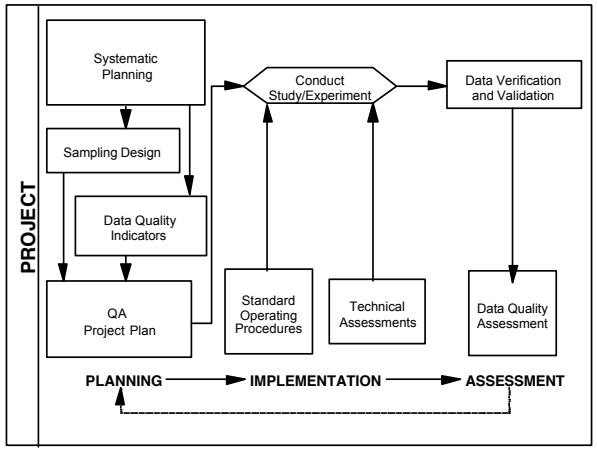
Life-cycle of Environmental Data



GIGO

Garbage In, Garbage Out

"Fancy statistical methods will not rescue garbage data"

What is Quality?

- Quality Assurance
  - Defined as everything done with respect to producing reliable data.
- Quality Control
  - Defined as a single step or procedure to evaluate a process or test

Why Quality?

- Recognize, quantitate, and minimize errors.
- Describe the accuracy and precision of the data.
- Assess the legality of the reported results, i.e., defensible in the US court of law.

Quality starts with field sampling

- A majority of all errors in environmental analysis result from incorrect sampling.
- Factors
  - Safety
  - Obtaining a representative sample
  - Preventing contamination
  - Legal documentation
  - Protecting the sample from changing prior to analysis (preservation)
- Detailed standard operating procedures
  - Typically not covered by audits or PE programs.
- Detailed record keeping
  - Field records
  - Chain of Custody

Field Quality Control

- Field blanks
- Trip blanks
- Equipment blanks
- Preservatives
  - Ice
  - Bottles
  - Chemicals

Custody

- Important to document where and who has the sample at all times.
- Chain of Custody - Legal document
  - Description of the sample
  - Who collected, what container, where and when.
  - Requested tests
  - Signature and dates/times when the sample was passed from person to person
  - Included with the final report.
- Internal Custody
  - Often provided by internal quality system
  - Custody of each bottle – standard method

Laboratory Quality Control

- Quality Control designed to check four general areas.
  - Calibration of instrument
  - Process is contamination free
  - Process is efficient and accurate
  - Matrix effects

Level I Report

- Sample information
- Parameter details
- Custody documents

Example Report

Analytical Laboratory Report

EXAMPLE REPORT

Lab Sample ID: S00001.01
Sample Tag: Sample Name
Collected Date/Time: 04/18/2007 12:00
Matrix: Groundwater
COC Reference: 000000

Sample Containers

#	Type	Preservative(s)	Refrigerated?	Arrival Temp. (C)	Thermometer #
1	4 oz. glass	None	Yes	4.5	IR

Sample Information

Analysis	Results	Units	RDL	Method	Run Date/Time	Analyst	CAS #	Flags
Extraction / Prep.								
Extraction, PCB	Completed			3510C	04/20/07 10:10	ARCSV		
Oil & Grease n-Hexane Extract	13	mg/L	1	1664A	04/18/07 15:10	TS		
Organics - PCBs/Pesticides								
PCB								
PCB-1016	Not detected	ug/L	0.1	8082	04/22/07 12:30	ARCSV	12674-11-2	
PCB-1221	Not detected	ug/L	0.1	8082	04/22/07 12:30	ARCSV	11104-28-2	
PCB-1232	Not detected	ug/L	0.1	8082	04/22/07 12:30	ARCSV	11141-16-5	
PCB-1248	Not detected	ug/L	0.1	8082	04/22/07 12:30	ARCSV	12672-29-6	
PCB-1254	5,000	ug/L	0.1	8082	04/22/07 12:30	ARCSV	11097-69-1	
PCB-1260	Not detected	ug/L	0.1	8082	04/22/07 12:30	ARCSV	11096-82-5	
PCB-1242	Not detected	ug/L	0.1	8082	04/22/07 12:30	ARCSV	53469-21-9	
PCB, Total	5,000	ug/L	0.1	8082	04/22/07 12:30	ARCSV	1336-36-3	

Parameter Details

Sample Information

- Project ID
- Sample ID
- Field Personnel
- Date & Time collected
- Date & time Received
- Laboratory ID

Correct Methods

EXAMPLES

- Correct method depends on regulatory program
  - NPDES/IPP - 40 CFR 136
  - Waste/RCRA - SW-846
  - Monitoring/Landfill - SW-846 or 40 CFR 136
  - Drinking Water
- Alternate Test Procedures
  - NPDES/IPP
  - Parameters not listed in 40 CFR 136
  - In house method

Detection limits

- MDL - Method Detection Limit
  - The minimum level of an analyte that can be qualitatively identified with 99% confidence.
- Reporting Limits
  - The minimum level of an analyte that can be reasonably reported by the laboratory taking into consideration the sample matrix.
  - PQL, LOQ

Qc Organization - Batches

- Sample and QC data are grouped into *batches*
  - A batch may be defined by *sample count* (e.g. 20)
  - A batch may be defined by *time elapsed* (e.g. 12 hr.)
- Each batch has QC validating its samples
  - Example: LCS, Blank, MS, MSD, Duplicate
- Types of batches:
  - *Preparation* (e.g. digestion, extraction)
  - *Analytical* (e.g. GC/MS, ICP/MS)
  - Samples may belong to both types of batches

Evaluating Qc - Level II Data

- Evaluate *Preparation QC Items* (as analyzed):
  - Method Blank
  - Laboratory Control Sample (LCS)
  - Matrix Spike (MS)
  - Duplicate and/or Matrix Spike Duplicate (Dup, MSD)
  - Surrogates (for organic analyses)
- *Instrument QC Items* are **not** included

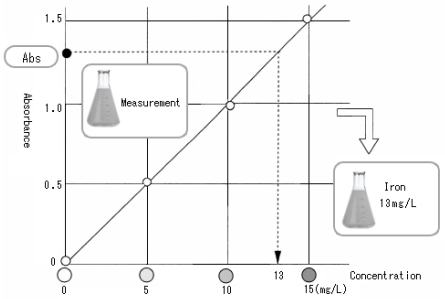
Standardisation and calibration

Standardisation

- In titrimetric analysis, a standard solution is added to the sample and the concentration of analyte is determined from the volume and the standard solution is used up
- The exact conc of the standard solution is not known at the onset but it can be found by titrating the standard solution with another solution called a primary standard

Standardisation and calibration

Standardisation is the name given to this process of accurately determining the concentration of a standard solution



Concentration

The amount of solute in a solution

A. mass % = $\frac{\text{mass of solute}}{\text{mass of sol'n}}$

% by mass – medicated creams
% by volume – rubbing alcohol

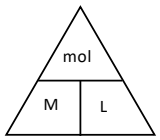
B. parts per million (ppm) → also, ppb and ppt

- commonly used for minerals or contaminants in water supplies

C. molarity (M) = $\frac{\text{moles of solute}}{\text{L of sol'n}}$

- used most often in "Chemistry class"

$$M = \frac{mol}{L}$$



D. molality (m) = $\frac{\text{moles of solute}}{\text{kg of solvent}}$

Glassware – Precision and Cost

beaker vs. **volumetric flask**

When filled to 1000 mL line, how much liquid is present?

beaker
5% of 1000 mL = 50 mL

Range: 950 mL – 1050 mL

imprecise; cheap

volumetric flask
1000 mL ± 0.30 mL

Range: 999.70 mL– 1000.30 mL

precise; expensive

Markings on glassware

Beaker	500 mL ± 5%	Range = 500 mL ± 25 mL 475 – 525 mL
Graduated Cylinder	1000 mL ± 5 mL	Range = 1000 mL ± 5 mL 475 – 525 mL
Volumetric Flask	500 mL ± 0.2 mL	Range = 499.8 – 500.2 mL

TC 20°C "to contain at a temperature of 20 °C"

TD "to deliver" T_s^{22} "time in seconds"

Calibration

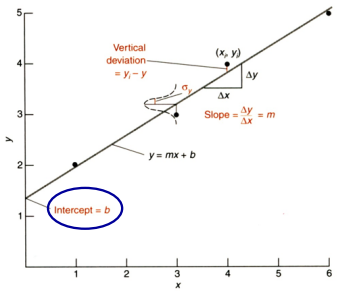
- Quantifying the results of an instrumental analysis generally involves the construction of a calibration graph
- The direct response of the instrument, or the peak height obtained on a chart recorder, is plotted as a function of the analyte concentration for a series of standard solutions containing differing concentrations of the analyte substance
- The sample is then analysed in the same way and the concentration in the sample is then determined by interpolation

Calibration Methods

Finding the "Best" Straight Line

Many analytical methods generate calibration curves that are linear or near linear in nature

(i) Equation of Line:



$$y = mx + b$$

x = independent variable
y = dependent variable
m = slope
b = y-intercept

$$slope = \frac{\Delta y}{\Delta x} = m$$

Calibration Methods

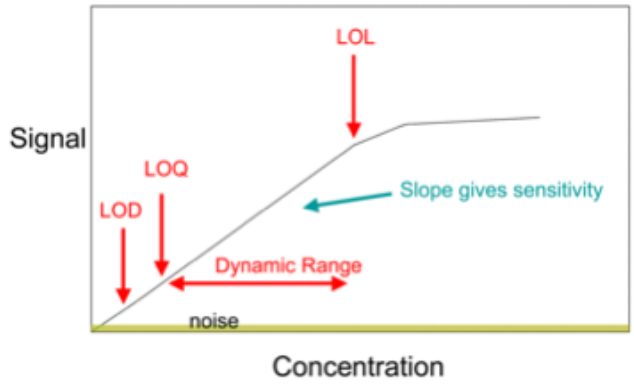
The data - the concentrations of the analyte and the instrument response for each standard - can be fit to a straight line, using linear regression analysis.

This yields a model described by the equation y = mx + y0, where

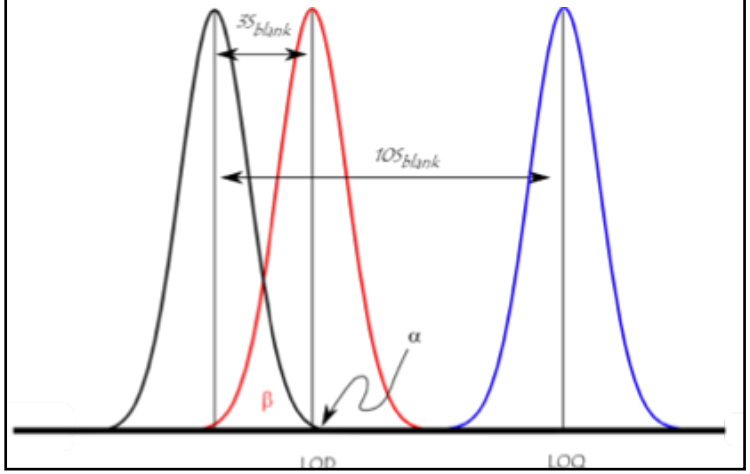
- ***y is the instrument response,***
- ***m represents the sensitivity, and***
- ***y0 is a constant that describes the background.***

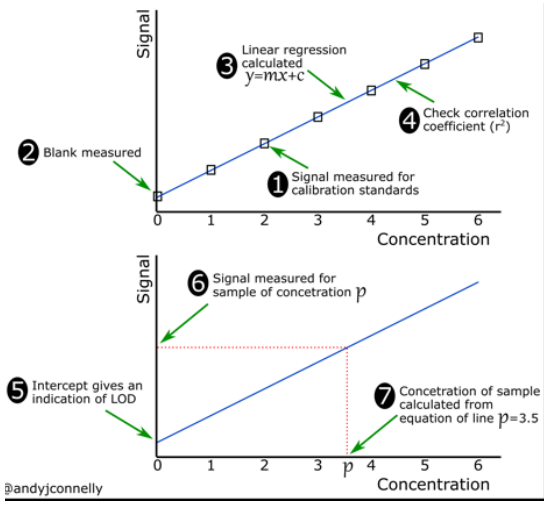
The analyte concentration (x) of unknown samples may be calculated from this equation.

Calibration Methods



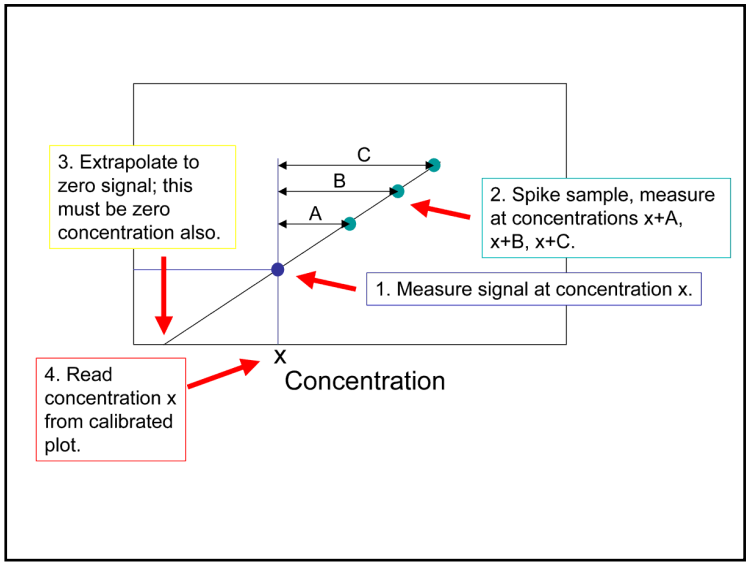
LOD and LOQ





Standard addition

- An alternative way to quantify the analyte conc is by means of the standard additions method
- In this case, a series of solutions containing both the sample and varying concentrations of the substance to be determined are prepared by adding aliquots of a standard solution to the sample
- The solutions are analysed and the response of the instrument is plotted against the conc due to the added standard
- The –ve intercept on the x-axis gives the conc in the sample



Standard Addition

Standard addition is a method to determine the amount of analyte in an unknown.

- In standard addition, known quantities of analyte are added to an unknown.
- We determine the analyte concentration from the increase in signal.

Standard addition is often used when the sample is unknown or complex and when species other than the analyte affect the signal.

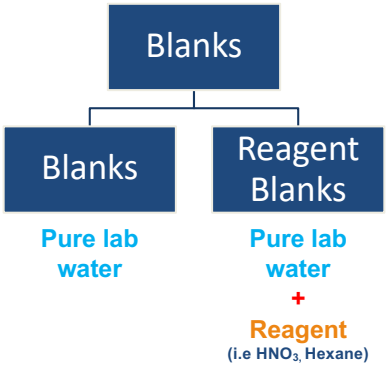
- The matrix is everything in the sample other than the analyte and its affect on the response is called the matrix effect

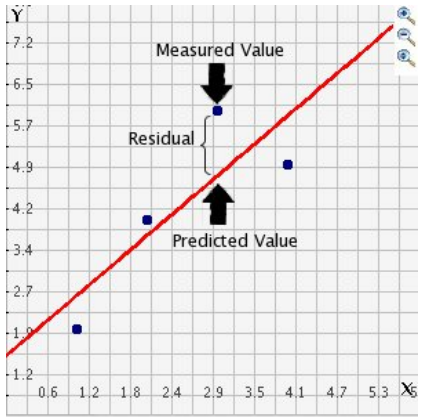
Why ?

- This method is used to eliminate matrix effects, i.e interferences by other components that may be present in the sample
- Graph obtained from the calibration method and the standard additions method can be treated using the method of least squares to obtained the best fit line through the data points

Blanks

- The water for preparing various reagents and standard solutions should be of the highest purity
- Doubly distilled, deionised, or various waters obtained from laboratory purification systems (e.g. Milli-Q) can be used
- With many types of analyses, it is also necessary to analyse blanks
- Blanks consist of pure laboratory water, while reagent blanks contain pure lab water and the various reagents used in the analysis
- Blank should be analysed frequently as they can reveal sources of contamination



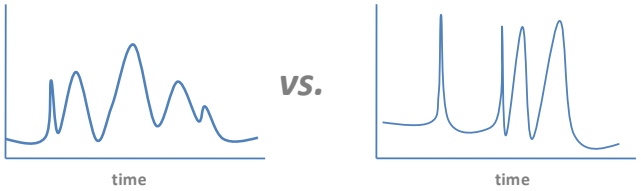


Method Validation

- Specificity
- Linearity
- Accuracy
- Precision
- Range
- Limits of Detection and Quantitation

Method Validation - Specificity

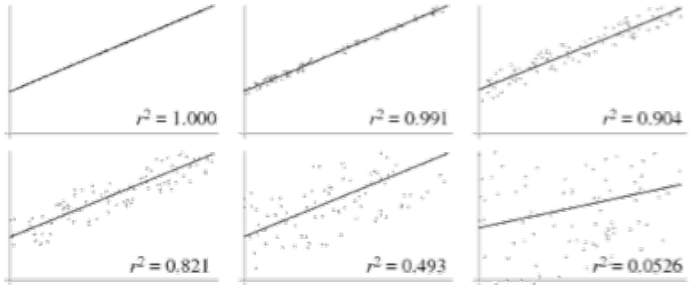
- How well an analytical method distinguishes the analyte from everything else in the sample.
- Baseline separation



Method Validation- Linearity

- How well a calibration curve follows a straight line.
- R^2 (Square of the correlation coefficient)

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

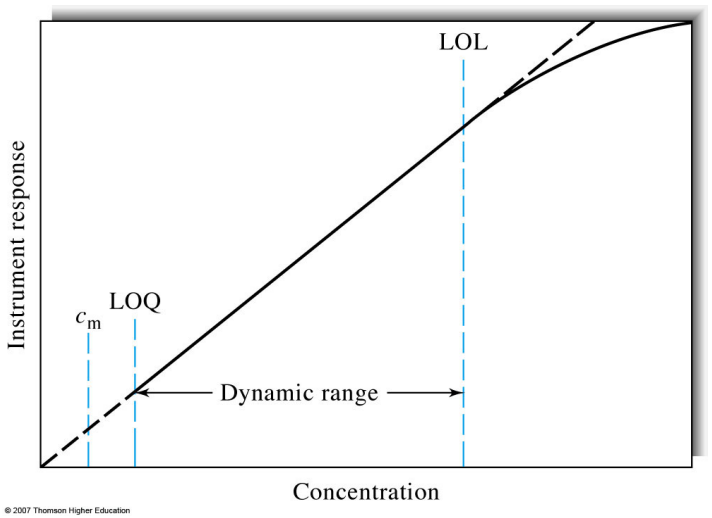


Method Validation- LOD and LOQ

Sensitivity

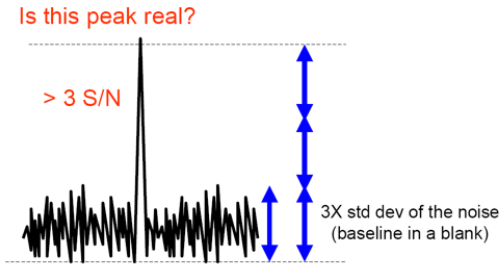
Sensitivity of an instrument is a measure of its ability to discriminate between small differences in analyte concentration. The change in signal per unit change in analyte concentration. The slope of the calibration curve at the concentration of interest is known as calibration sensitivity.

- Limit of detection (LOD) – "the lowest content that can be measured with reasonable statistical certainty."
- Limit of quantitative measurement (LOQ) – "the lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test."



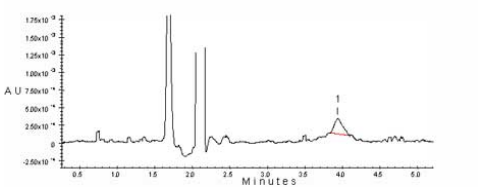
Limit of Detection (LOD)

- Typically 3 times the signal-to-noise (based on standard deviation of the noise)

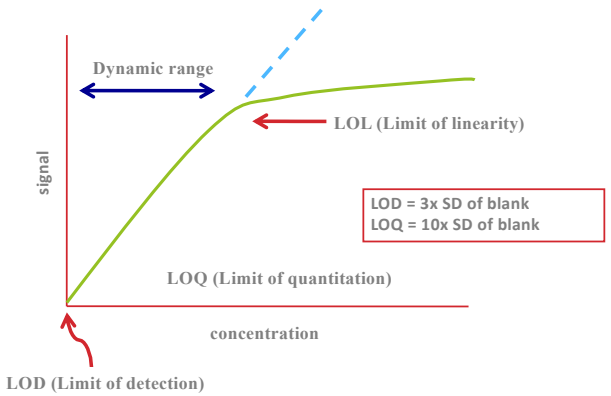


Limit of Linear Response (LOL)

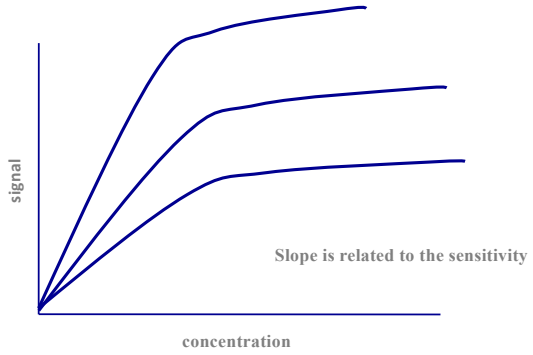
- Point of saturation for an instrument detector so that higher amounts of analyte do not produce a linear response in signal.



Useful Range of an Analytical Method



Method Validation- Linearity



Method Validation- Accuracy and Precision

Precision - reproducibility

- Accuracy – nearness to the truth
- Compare results from more than one analytical technique
- Analyze a blank spiked with known amounts of analyte.

Method Validation- LOD and LOQ

- Detection limit (lower limit of detection – smallest quantity of analyte that is "statistically" different from the blank.

- HOW TO:
- Measure signal from n replicate samples (n > 7)
- Compute the standard deviation of the measurments
- Signal detection limit: $y_{dl} = y_{blank} + 3s$
- $y_{sample} - y_{blank} = m \cdot$ sample concentration

- Detection limit: 3s/m
- Lower limit of quantitation (LOQ) : 10s/m

Example: sample concentrations: 5.0, 5.0, 5.2, 4.2, 4.6, 6.0, 4.9 nA
Blanks: 1.4, 2.2, 1.7, 0.9, 0.4, 1.5, 0.7 nA
The slope of the calibration curve for high conc. m= 0.229 nA/mM
What is the signal detection limit and the minimum detectable concentration?
What is the lower limit of quantitation?

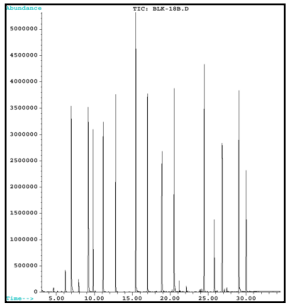
The Matrix Effect

- The matrix effect problem occurs when the unknown sample contains many impurities.

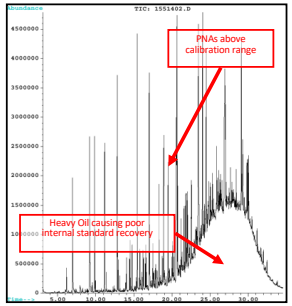
- If impurities present in the unknown interact with the analyte to change the instrumental response or themselves produce an instrumental response, then a calibration curve based on pure analyte samples will give an incorrect determination

Example - Matrix Interference

Actual sample matrices may contain target and non-target interferences that preclude the analysis of a sample in an undiluted state and result in **elevated reporting limits**.

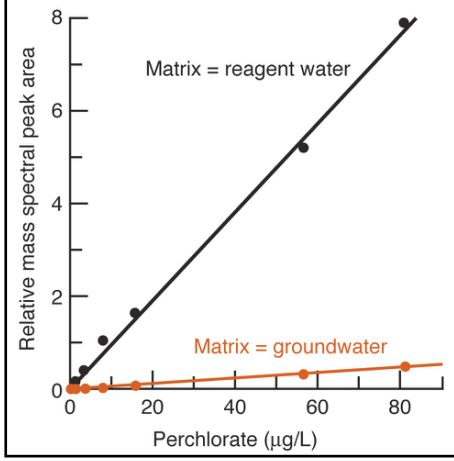


Method Blank



Sample Exhibiting Interference

Calibration Curve for Perchlorate with Different Matrices



Perchlorate (ClO_4^-) in drinking water affects production of thyroid hormone. ClO_4^- is usually detected by mass spectrometry, but the response of the analyte is affected by other species, so you can see the response of calibration standards is very different from real samples.

Internal Standards

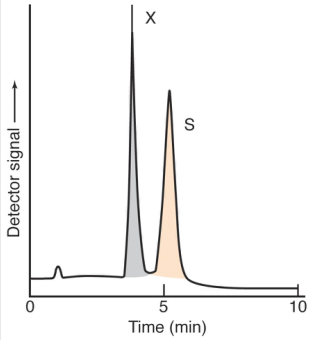
An internal standard is a known amount of a compound, different from the analyte, added to the unknown sample.

Internal standards are used when the detector response varies slightly from run to run because of hard to control parameters.

- e.g. Flow rate in a chromatograph

But even if absolute response varies, as long as the relative response of analyte and standard is the same, we can find the analyte concentration.

Response Factors



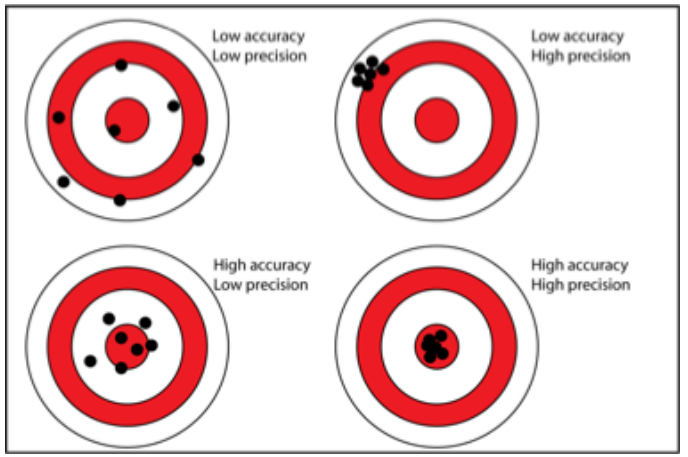
For an internal standard, we prepare a mixture with a known amount of analyte and standard. The detector usually has a different response for each species, so we determine a **response factor** for the analyte:

$$\frac{A_X}{[X]} = F\left(\frac{A_S}{[S]}\right)$$

[X] and [S] are the concentrations of analyte and standard after they have been mixed together.

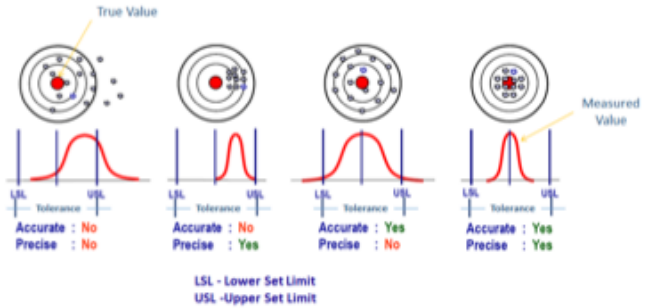
$$\frac{\text{Area of analyte signal}}{\text{Concentration of analyte}} = F\left(\frac{\text{area of standard signal}}{\text{Concentration of standard}}\right)$$

Obtained data (standard solutions)

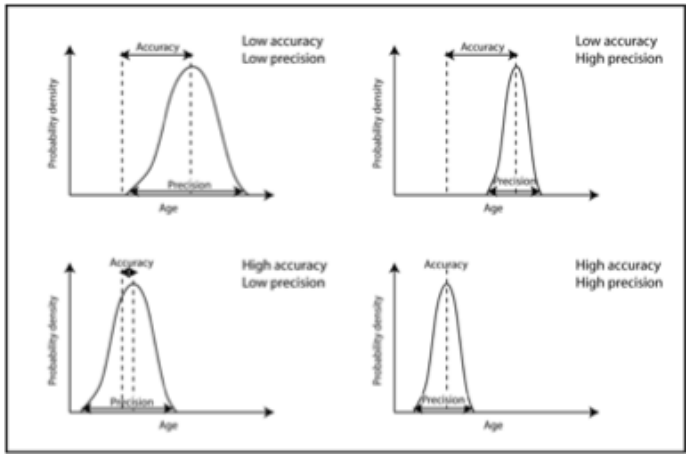


Concepts Accuracy and precision

Preferably we would like analytical methods to be both precise and accurate but in practice this is not possible. There will always be some inaccuracy and imprecision in the method as a result of errors and the aim of the analytical chemist is to minimize the sources of these errors.



Concepts Accuracy and precision



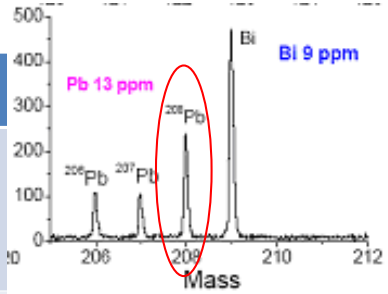
Selection & preparation of standard solution

- The **external standard** prepare by diluting the ICP Multi Element Standard Solution V CertiPUR® (Merck®, Darmstadt, Germany) with the **same acid mixture used for sample dissolution (high-purity nitric acid)**. (avoid chloric acid to prevent molecular ion interference.
- Why nitric acid?
- Gravimetrically preparation approach? Why?

- Stock standard solution = 1-10mg/L last for 1 year if properly preserved and keep in PTFE bottle, place in dark at room temperature.
- Dilution of stock standard at level µg/L have shelf life less than a month,
- Dilution at level ng/L will last in a day to a week depend on the element.
  - Important to label the bottle.
    - Name :100 dilution
      MERCK
      ICP std solution IV
    - Date : 21.1.2008
    - Prepared by : Zaharin

Selection of elements in sample

Element & Atomic Mass	Abundance	Sharing elements
Zn		
64	48.63	Ni (0.93)
66	27.90	
67	4.10	
68	18.75	
70	0.62	Ge (20.84)
Pb		
204	1.4	Hg (6.87)
206	24.1	
207	22.1	
208	52.4	



Selection of the concentration for SS

The best accuracy for complex material (acid digest, fusion and high con.), obtain by preparing a matrix-matching technique (concentration above, middle, almost similar and below the unknown concentration in the sample).

How to get those concentration estimation?

- Literature
- Preliminary test

Typical calibration curve – linear with r = 0.999

Scanning techniques – measuring analyte ion at the appropriate *m/z* values.

Selection & preparation of internal standard

- To obtain the highest quality concentration data, require IS in the direct calibration curve.
- Internal standard is recommended to correct any matrix effects and instrumental drift in the ICP-MS using mixture of scandium (Sc), indium (In) and bismuth (Bi).
  - Elements that is closely located to the analyte in the mass spectrum.

Criteria for IS

1. The element must be absent or at significantly low concentration in the samples so no indigenous levels mitigate the process.
2. The elements must be available in a high-purity form so no contamination of analyte elements.
3. At least one un-interfered isotope available for measurement.
4. Must not interact with indigenous matrix or analyte elements from the sample.

Elements that have been successfully used for internal standards

Element	*m/z*
Germanium	72
Indium	113
Lithium	6
Rhodium	103
Scandium	45
Terbium	159
Thallium	169
Thorium	232
Yttrium	89

- Maximum accuracy of the method is obtain by recoveries of the element (Trace metals & Methylmercury) in the **Standard Reference Materials** ®
- SRM is prepared and certified under National Institute of Standards and Technology, USA, or by National Research Council, Canada.
- Recoveries of the selected trace metals in SRM must ranged between 90% - 110%, (example in Table 5.3).

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1944

New York/New Jersey Waterway Sediment

Example of SRM

National Research Council of Canada	
CASS-4	Near shore seawater
NASS-5	Open ocean seawater
SLEW-3	Estuarine water
DORM-2	Dogfish muscle
U.S. National Institute Standards and Technology	
11648	Urban air particulate matter (air particulate)
1400	Bone ash (Biological and clinical relevance)
1413	Glass sand (Glass)
137	Tin ore (Minerals and Ore)
1630	Mercury in coal (coal)
1083	Wear metals in lubricating oil (oil)
1641	Mercury in water (Water and Precipitation)

Table 1 Percentage recoveries of trace metals by ICP-MS

Metals	Recovery (%)	Detection limit ranges (ppt)
Al	100.28	1–10
Ba	99.01	<0.1–1
Cd	102.05	1–10
Cu	101.87	1–10
Fe	98.46	1–10
Pb	102.12	<0.1–1
Mn	97.57	1–10
Zn	101.97	1–10
Co	100.53	1–10
Be	103.08	1–10
As	102.29	1–10
Cr	101.53	1–10
Ni	101.72	1–10
Se	103.43	10–100

THANK YOU

"We have made clear to you the signs;
perhaps you will understand."

(57:17)

H_2O

Water Research

http://research.upm.edu.my/hydro