Evaluation of the Data obtained by the different instrumental methods

- **Analytical Chemistry** deals with methods for determining the chemical composition of samples.
  - Qualitative Analysis (identification) provides information about the identity of species or functional groups in the sample (an analyte can be identified).
  - Quantitative Analysis provides numerical information of analyte (quantitate the exact amount or concentration).

Analytical Methods

- **Classical Methods**: Wet chemical methods such as precipitation, extraction, distillation, boiling or melting points, gravimetric and titrimetric measurements.

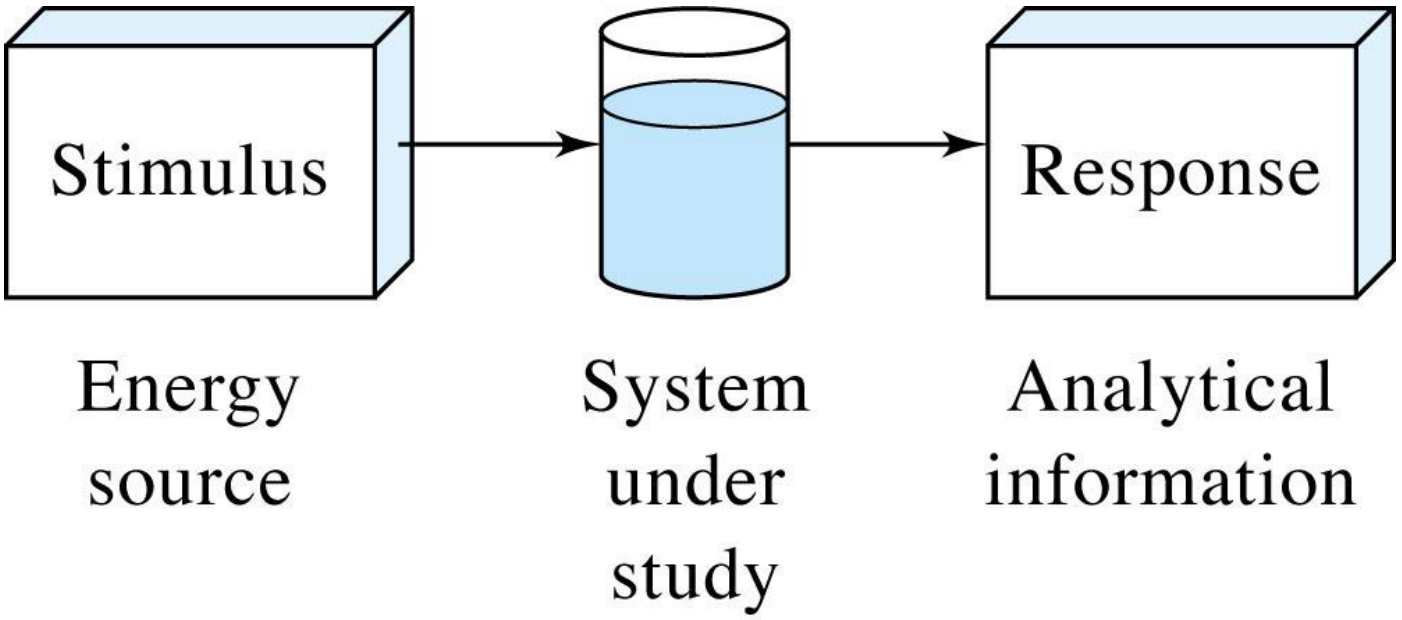
- **Instrumental Methods**: Analytical measurements (conductivity, electrode potential, light absorption or emission, mass-to-charge ratio, fluorescence etc.) are made using instrumentation.

Types of Instrumental Methods

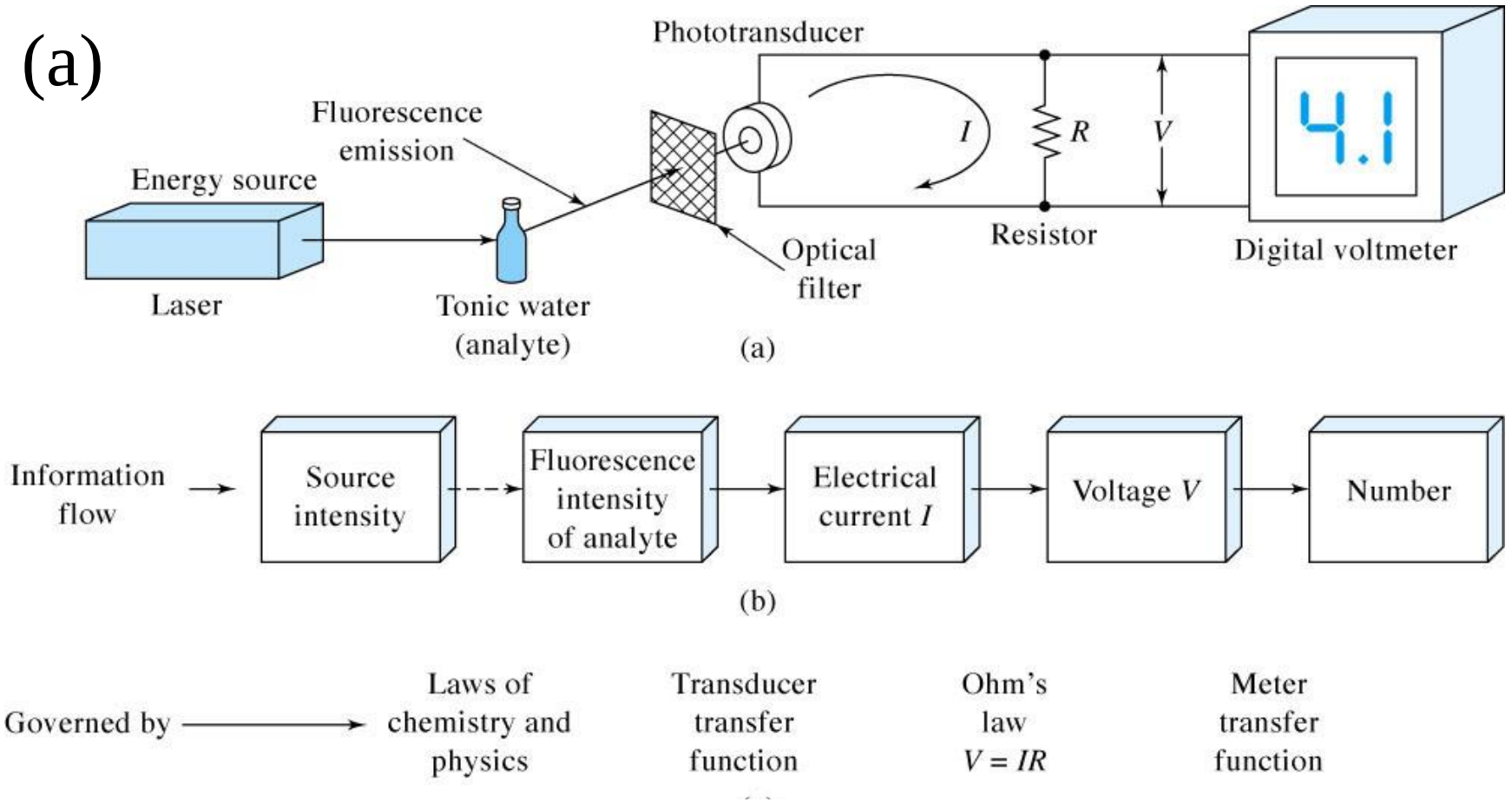
1. Spectroscopic methods:
    a. Atomic spectroscopy
    b. Molecular spectroscopy

2. Chromatographic methods (separations):

3. Electrochemistry:



Block diagram of an instrumental measurement



Block diagram of a fluorometer

Applications of Instrumental Methods

1. Bioanalytical: biological molecules and/or biological matrices (e.g., proteins, amino acids, blood, urine)
2. Environmental: pesticides, pollution, air, water, soil
3. Material science: polymers, characterization of new materials
4. Forensic science (application of science to the law): body fluids, DNA, gun shot residue, hair, fibers, elemental analysis, drugs, alcohols, poisoning, fingerprints, etc.

Analytical Methodology

1. Plan: Qualitative or quantitative or both; what kind of information have; which technique is suitable etc.
2. Sampling: Accuracy depends on proper sampling, characteristic of sample is very important, required good representative sample (from top, middle and bottom and mix up and take average sample).
3. Sample preparation: depends on analytical techniques.
4. Analytical measurement:
5. Data Analysis: Whether the data make sense or not.

Selecting an Analytical Method

- In order to select an analytical method intelligently, it is essential to define clearly the nature of the analytical problem. In general, the following points should be considered when choosing an instrument for any measurement.

1. Accuracy and precision required
2. Available sample amount
3. Concentration range of the analyte
4. Interference in sample
5. Physical and chemical properties of the sample matrix
6. Number of sample to be analyzed
7. Speed, ease, skill and cost of analysis

Figures of Merit

- Precision
- Bias
- Sensitivity
- Detection limit
- Concentration range (Dynamic range)
- Selectivity

- **Precision:** How close the same measurements are to one another. The degree of mutual agreement among data that have been obtained in the same way. Precision provides a measure of the random or indeterminate error of an analysis
- **Accuracy:** How close the measurement approaches the real value.
- **Bias:** Bias provides a measure of the systematic, or determinate error of an analytical method.

  bias = $\mu - x_t$, where, μ is the population mean and

  x_t is the true value

TABLE 1-5 Figures of Merit for Precision of Analytical Methods

Terms	Definition*
Absolute standard deviation, s	$s = \sqrt{\dfrac{\sum_{i=1}^{N} (x_i - \bar{x})^2}{N - 1}}$
Relative standard deviation (RSD)	$\text{RSD} = \dfrac{s}{\bar{x}}$
Standard error of the mean, s_m	$s_m = s/\sqrt{N}$
Coefficient of variation (CV)	$\text{CV} = \dfrac{s}{x} \times 100\%$
Variance	s^2

- **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.

  $S = mc + S_{bl}$

  S = measured signal; c = analyte concentration;

  S_{bl} = blank signal; m = sensitivity (Slope of line)

  Analytical sensitivity (γ): $\gamma = m/s_s$

  m = slope of the calibration curve

  s_s = standard deviation of the measurement

- **Detection Limit (Limit of detection, LOD):** The minimum concentration of analyte that can be detected with a specific method at a known confidence level.

  LOD is determined by S/N, where, S/N = Signal-to-noise ratio

  = (magnitude of the signal)/(magnitude of the noise)

- Noise: Unwanted baseline fluctuations in the absence of analyte signal (standard deviation of the background)
- The detection limit is given by,

  $C_m = (S_m - S_{bl})/m$, where, C_m = minimum concentration i.e., LOD, S_m = minimum distinguishable analytical signal (i.e., S/N = 2 or S/N = 3), S_{bl} = mean blank signal

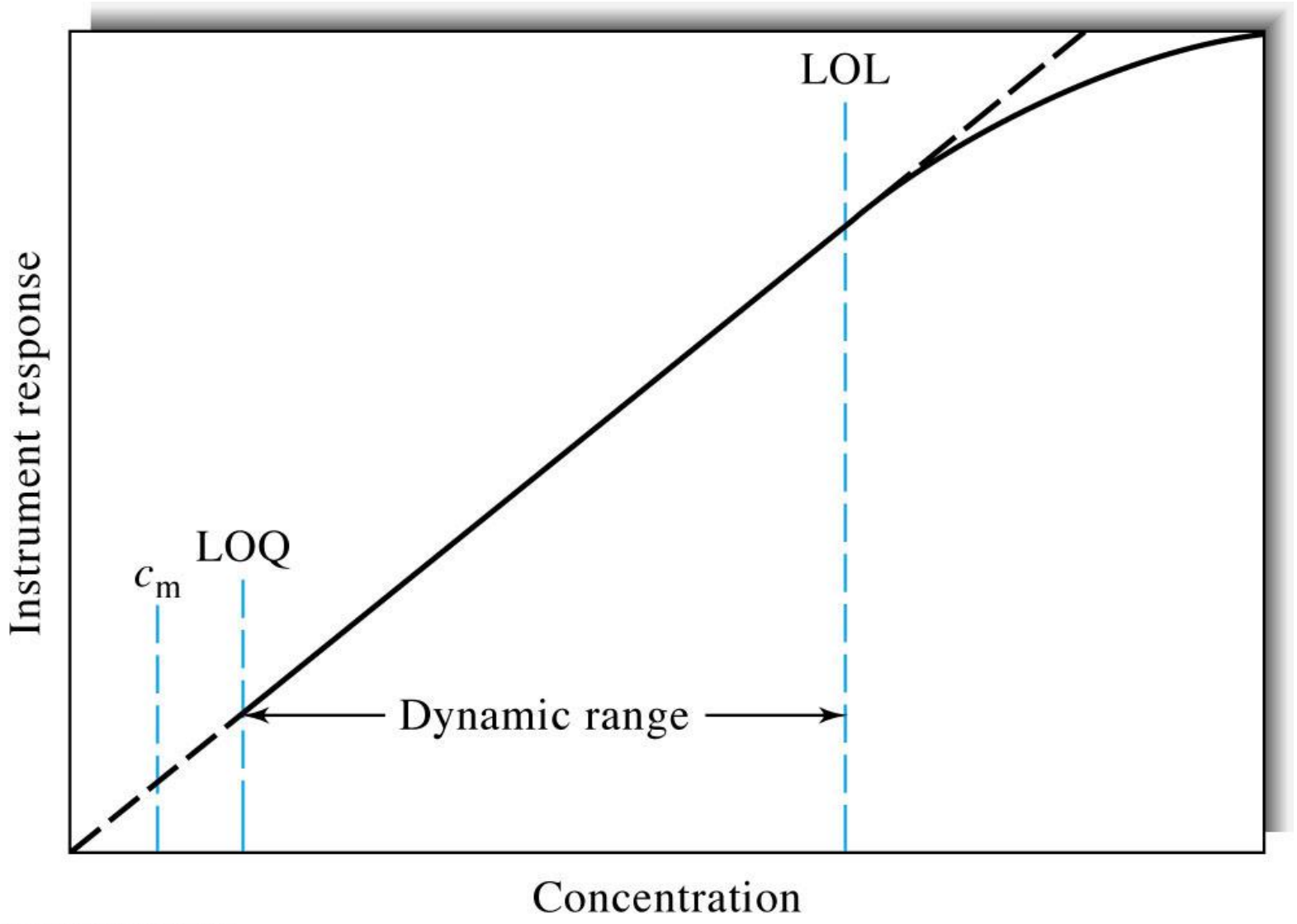
  m = sensitivity (i.e., slope of calibration curve)

- **Dynamic Range:** The lowest concentration at which quantitative measurements can be made (limit of quantitation, or LOQ) to the concentration at which the calibration curve departs from linearity (limit of linearity, or LOL).

  The lower limit of quantitative measurements is generally taken to be equal to ten times the standard deviation of repetitive measurements on a blank or 10 S_{bl}.

  Dynamic range is the range over which detector still responds to changing concentration (at high concentrations – usually saturates – quits responding)

  An analytical method should have a dynamic range of at least two orders of magnitude, usually 2-6 orders of magnitude.



- **Selectivity:** Selectivity of an analytical method refers to the degree to which the method is free from interference by other species contained in the sample matrix. No analytical method is totally free from interference from other species, and steps need to be taken to minimize the effects of these interferences. Selectivity coefficient gives the relative response of the method to interfering species as compared with analyte. Selectivity coefficient can range from zero (no interference) to values greater than unity. A coefficient is negative when the interference caused a reduction in the intensity of the output signal of the analyte.

Calibration of Instrumental Methods

- All types of analytical methods require calibration for quantitation. Calibration is a process that relates the measured analytical signal to the concentration of analyte. We can't just run a sample and know the relationship between signal and concentration without calibrating the response.

  The **three** most common calibration methods are:

- Calibration curve
- Standard addition method
- Internal standard method

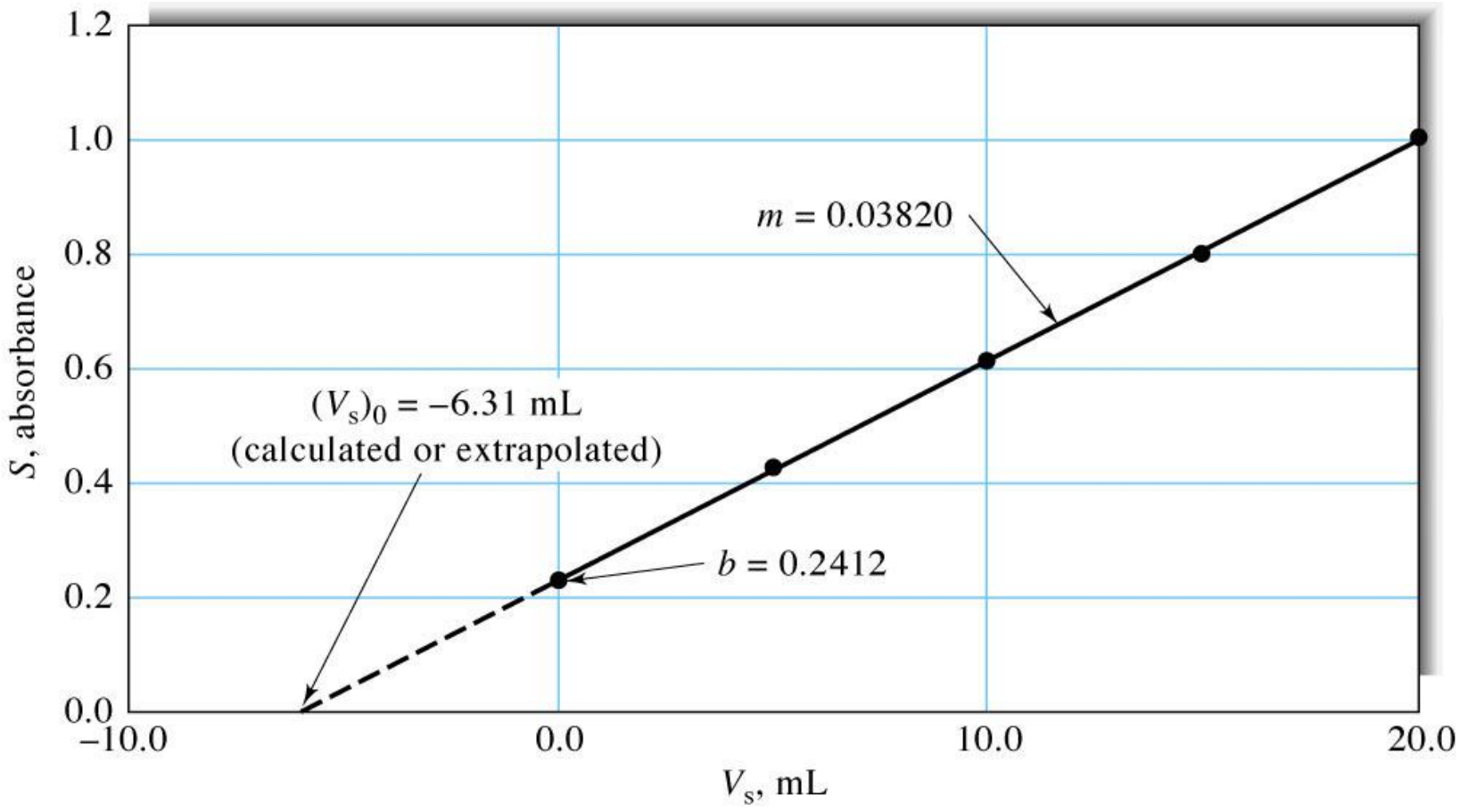
Calibration Curves

- Several standards (with different concentrations) containing exactly known concentrations of the analyte are measured and the responses recorded.
- A plot is constructed to give a graph of instrument signal versus analyte concentration.
- Sample (containing unknown analyte concentration) is run, if response is within the LDR of the calibration curve then concentration can be quantitated.
- Calibration curve relies on accuracy of standard concentrations.

- It depends on how closely the matrix of the standards resemble that of the sample to be analyzed.
- If matrix interferences are low, calibration curve methods are OK.
- If matrices for sample and standards are not same calibration curve methods are not good.
- Need to consider the linear part of the curves.

Standard Addition Method

- Better method to use when matrix effects can be substantial
- Standards are added directly to aliquots of the sample, therefore matrix components are the same.
- Procedure:
  - Obtain several aliquots of sample (all with the same volume).
  - Spike the sample aliquots ==> add different volumes of standards with the same concentration to the aliquots of sample.
  - Dilute each solution (sample + standard) to a fixed volume.
  - Measure the analyte concentration.



Standard Addition Method

- Instrumental measurements are made on each solutions to get instrument response (S). If the instrument response is proportional to concentration, we may write,

  $S = (kV_sC_s)/V_t + (kV_xC_x)/V_t$

  Where, V_x =Volume of sample = 25 mL (suppose)

  V_s = Volume of standard = variable (5, 10, 15, 20 mL)

  V_t = Total volume of the flask = 50 mL

  C_s = Concentration of standard

  C_x = concentration of analyte in aliquot

  k = proportionality constant

- A plot of S as a function of V_s is a straight line of the form,

  $S = mV_s+b$

  Where, slope, $m = (kC_s)/V_t$ and intercept, $b = (kV_xC_x)/V_t$

  Now, $b/m = (kV_xC_x)/V_t \times V_t/(kC_s)$

  $C_x = bC_s /mV_x$

Standard Addition Method

Another approach to determine C_x

- Extrapolate line on plot to x-intercept
- Recall: At $V_s = 0$ ➔ instrument response (relating to concentration of x in sample)
- At x-intercept, you know the volume of analyte added to (i.e., inherent in) the sample.
- Another way: This value S = 0 (no instrument response) ➔ no analyte present in sample.

In any case, Since $S = 0$,

Therefore, $S = (kV_sC_s)/V_t + (kV_xC_x)/V_t = 0$

Solve for C_x, $C_x = - (V_s)_oC_s / V_x$

Standard Addition Method

- In the interest of saving time or sample, it is possible to perform standard addition analysis by using only two increments of sample. A single addition of V_s mL of standard would be added to one of the two samples and we can write, $S_1 = (kV_xC_x)/V_t$ and $S_2 = (kV_xC_x)/V_t + (kV_sC_s)/V_t$

$$\frac{S_2}{S_1} = \frac{k(V_xC_x + V_sC_s)}{V_t} X \frac{V_t}{kV_xC_x}$$

$$= 1 + \frac{V_sC_s}{V_xC_x}$$

$$\frac{V_sC_s}{V_xC_x} = \frac{S_2 - S_1}{S_1}$$

$$C_x = \frac{S_1VC_s}{V_x(S_2 - S_1)}$$

Internal standard Method

- An Internal Standard is a substance that is added in a constant amount to all samples, blanks and calibration standards in an analysis.
- Calibration involves plotting the ratio of the analyte signal to the internal standard signal as a function of analyte concentration of the standards.
- This ratio for the samples is then used to obtain their analyte concentrations from a calibration curve.
- Internal standard can compensate for several types of both random and systematic errors.