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ANALYTICAL CHEMISTRY

Quantitation and Calibration Methods

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Chapter Description

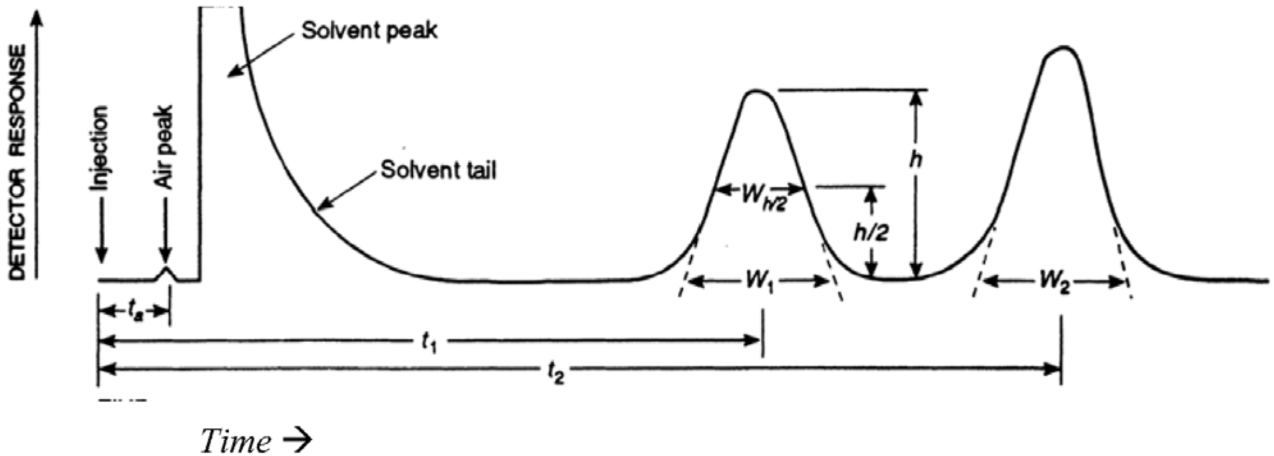
- Expected Outcomes
  - Calculate response factor, peak area and percent composition of analyte in given problem.
  - Understand and apply the quantitation methods including external standard, internal standard and standard addition method in order to calculate the unknown concentration of analyte in given sample.

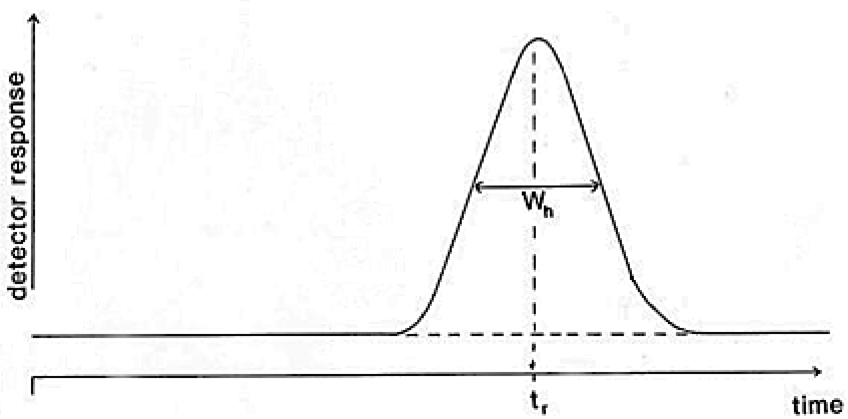
Contents

- Response Factor
- Peak Area Measurement Methods
  - Percent Composition
  - Corrected Percent Peak Area
- Quantitation
  - External Standard Calibration
  - Internal Standard Calibration
  - Standard Addition Method
- Calibration Method
- Linear Regression
- Noise

RESPONSE FACTOR

$$R_X = \frac{Peak\ area\ of\ analyte}{Concentration\ of\ analyte} = \frac{A_X}{[X]}$$





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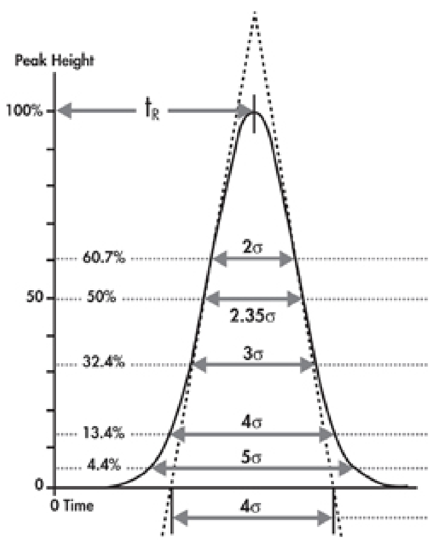
http://ocw.ump.edu.my/course/view.php?id=467

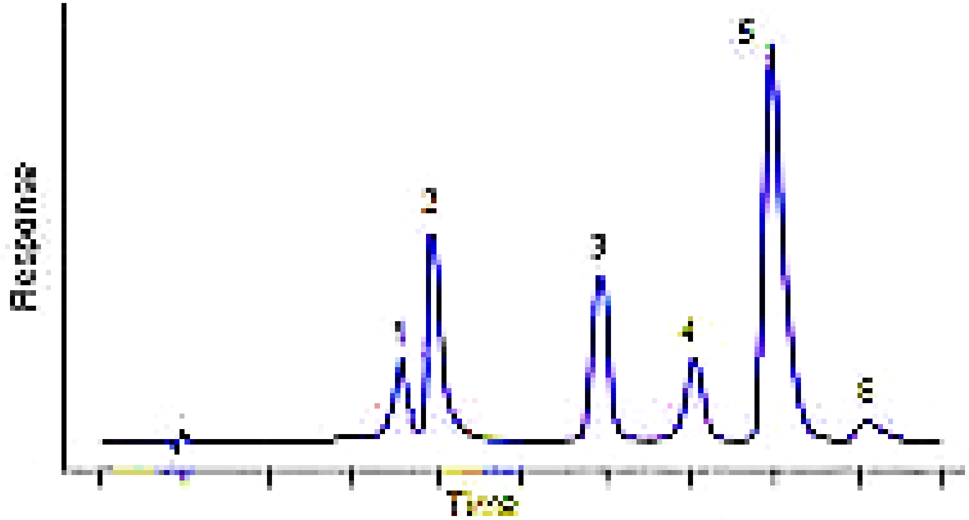
PEAK AREA MEASUREMENT METHODS

Methods

- **Cut and Weigh** — Classical
- **Area of Triangle** — $A = \dfrac{h \times w_b}{2}$
- **Integrator** — detector response is converted into an electrical response with a frequency directly proportional to the voltage
- **Computer** — *analog-to-digital converter*



PERCENT COMPOSITION



$$\%X = \frac{peak\ area\ X}{total\ peak\ area} \times 100$$

Peak	Area
1	2245
2	6488
3	5144
4	2787
5	12147
6	1023

$$Area\ (Peak\ 3) = \frac{5144}{(2245 + 6488 + 5144 + 2787 + 12147 + 1023} = 17.2\ \%$$

CORRECTED PERCENT PEAK AREA

$$A_x = A_x / R_x$$

$$A_i = A_i / R_i$$

$$\% \, x = \frac{(A_x/R_x)}{\sum(A_i/R_i)} \times 100$$

QUANTITATION

- Method
  - External Standard
  - Internal Standard
  - Standard Addition

EXTERNAL STANDARD CALIBRATION

- External std = a std solution containing a known amount of analyte, prepared separately from samples containing analyte.

- Concentration of analyte for single-point external std after standardized:

Concentration of analyte: C_A

Sample's signal: S_{sample}

Method's sensitivity: k

$$C_A = \frac{S_{sample}}{k}$$

INTERNAL STANDARD CALIBRATION

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- **Internal standard** = a reference species, chemically and physically similar to the analyte, that is added to samples, standards and blanks.

$$S_A = k_A C_A \qquad S_{IS} = k_{IS} C_{IS}$$

$$\frac{S_A}{S_{IS}} = \frac{k_A}{k_{IS}} * \frac{C_A}{C_{IS}} = K * \frac{C_A}{C_{IS}}$$

$$K = \left(\frac{C_{IS}}{C_A}\right)\left(\frac{S_A}{S_{IS}}\right)_{std} \qquad C_A = \left(\frac{C_{IS}}{K}\right)\left(\frac{S_A}{S_{IS}}\right)_{sample}$$

INTERNAL STANDARD

A spectrometric method for the quantitative determination of Pb^{2+} levels in blood uses Cu^{2+} as an internal standard. A standard containing 1.75ppb Pb^{2+} and 2.25ppb Cu^{2+} yields a ratio of S_A/S_{IS} of 2.37. A sample of blood is spiked with the same concentration of Cu^{2+}, giving a signal ratio of 1.80. Determine the concentration of Pb^{2+} in the sample.

$$\frac{S_A}{S_{IS}} = \frac{k_A}{k_{IS}} * \frac{C_A}{C_{IS}} = K * \frac{C_A}{C_{IS}}$$

$$2.37 = K* \frac{1.75 \text{ ppb}}{2.25 \text{ ppb}}$$

$$2.37 = K* 0.778$$

$$K = 3.05$$

$$1.8 = 3.05* \frac{C_A}{2.25 \text{ ppb}}$$

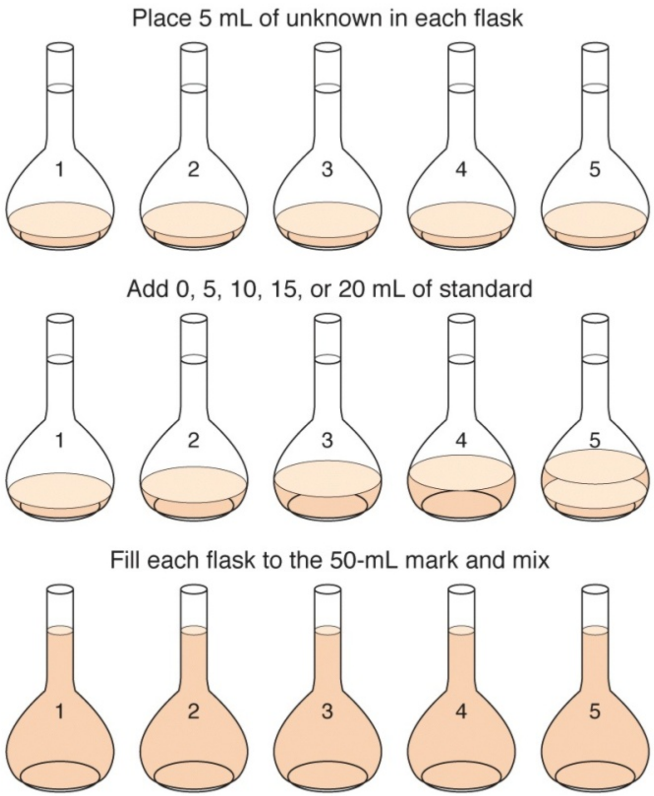
Ans: C_A = 1.33ppb Pb^{2+}

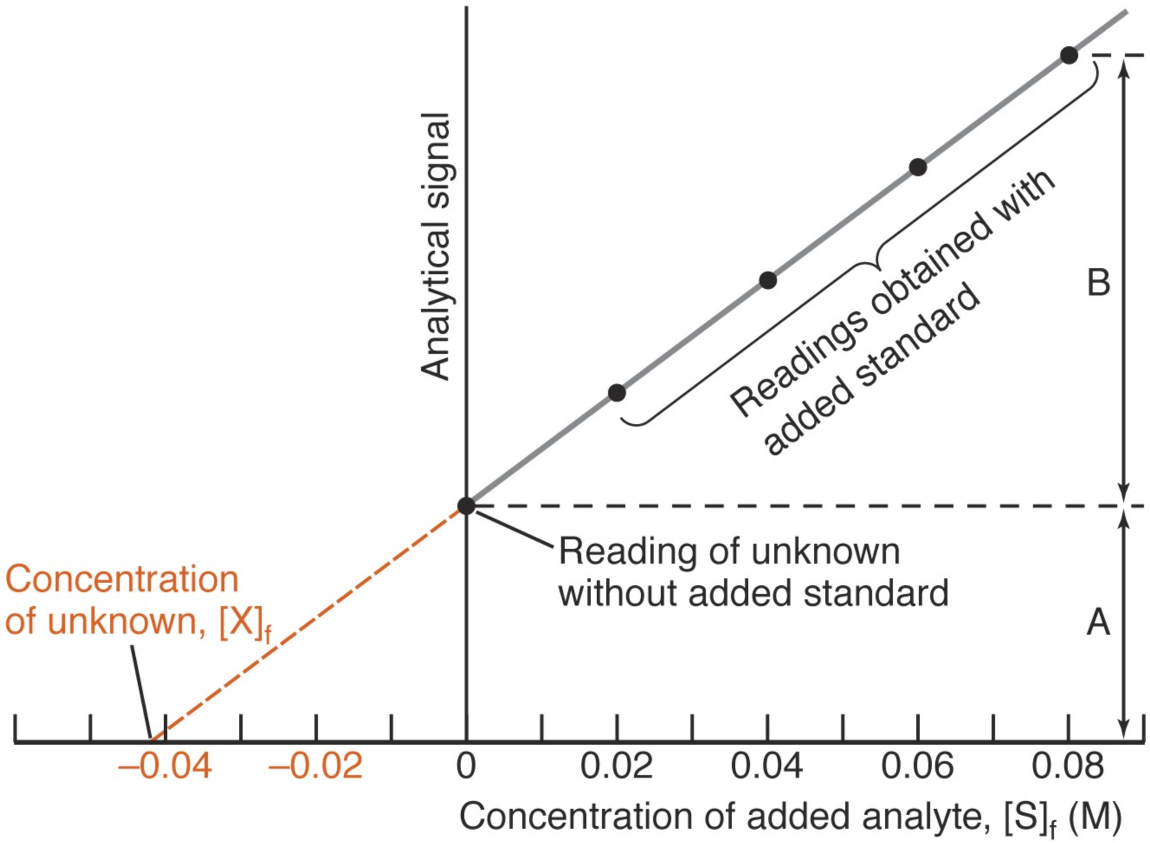
STANDARD ADDITION METHOD

- A method to determine the amount of analyte in an unknown where known quantities of analyte (standard) are added to an unknown (sample).

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

STANDARD ADDITION GRAPHICALLY





Add V_o of C_A

Add V_o of C_A

Add V_S of C_S

Dilute to V_f

Dilute to V_f

$$S_{sample} = kC_A \frac{V_o}{V_f}$$

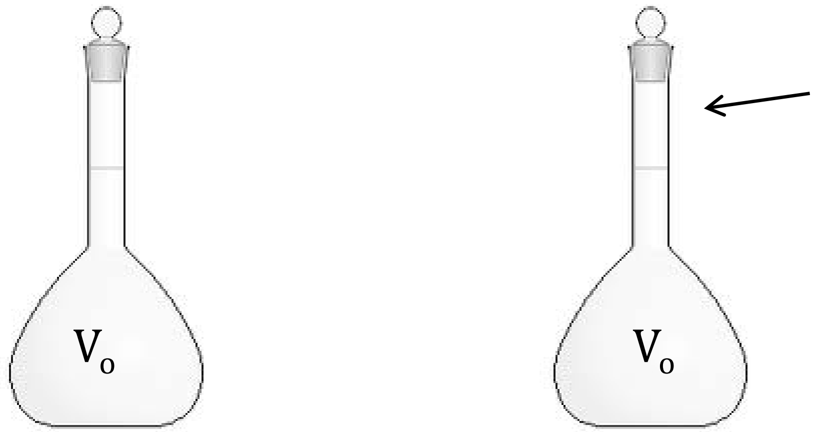
$$S_{spiked} = k\left(C_A \frac{V_o}{V_f} + C_s \frac{V_s}{V_f}\right)$$

Conc = C_A*V_o/V_f

Conc = C_A*V_o/V_f + C_S*V_S/V_f

$$\frac{S_{sample}}{C_A \frac{V_o}{V_f}} = \frac{S_{spiked}}{\left(C_A \frac{V_o}{V_f} + C_s \frac{V_s}{V_f}\right)}$$

SINGLE POINT STANDARD ADDITION



Add V_s of C_s

Conc = C_A

Conc = $C_A*V_o/(V_o+V_s) + C_S*V_s/(V_o+V_s)$

$$\frac{S_{sample}}{C_A} = \frac{S_{spiked}}{\left(C_A \frac{V_o}{V_o + V_s} + C_s \frac{V_s}{V_o + V_s}\right)}$$

STANDARD ADDITION METHOD

A spectrometric method for the quantitative determination of the concentration of Pb^{2+} in blood yields an S_{sample} of 0.712 for a 5.00mL sample of blood.

After spiking the blood sample with 5.00µL of a 1560ppb Pb^{2+} standard, a S_{spike} of 1.546 is obtained.

Determine the concentration of Pb^{2+} in the original sample.

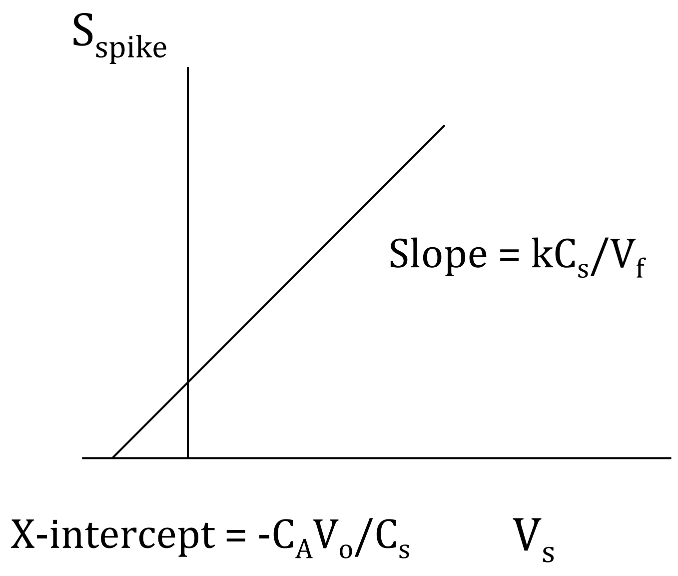
$$\frac{S_{sample}}{C_A} = \frac{S_{spiked}}{\left(C_A \frac{V_o}{V_o + V_s} + C_s \frac{V_s}{V_o + V_s}\right)}$$

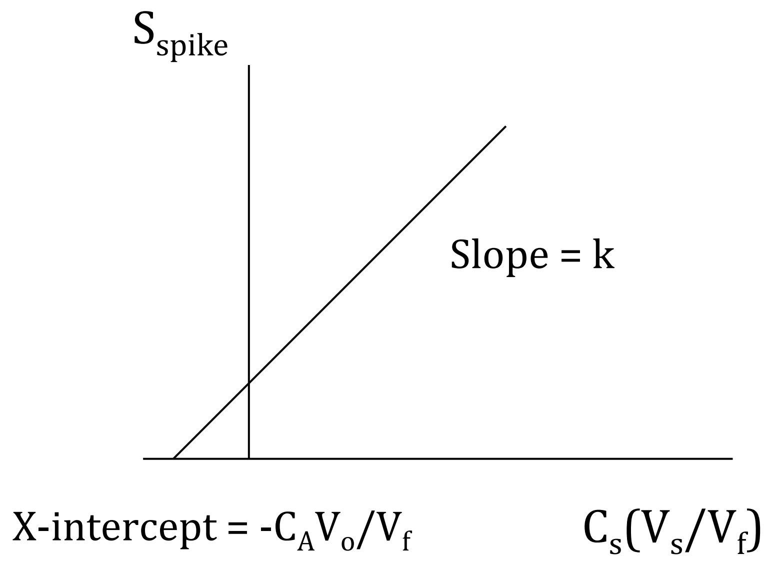
$$\frac{0.712}{X} = \frac{1.546}{X^* \frac{5\ \text{mL}}{5.005\ \text{mL}} + 1560^* \frac{0.005\ \text{mL}}{5.005\ \text{mL}}}$$

Ans: C_A = 1.33ppb

MULTIPLE POINT STANDARD ADDITION

- Plot S_{spike} vs an appropriate measure of the amount of added standard





CALIBRATION METHOD

Least square method: y = mx + c

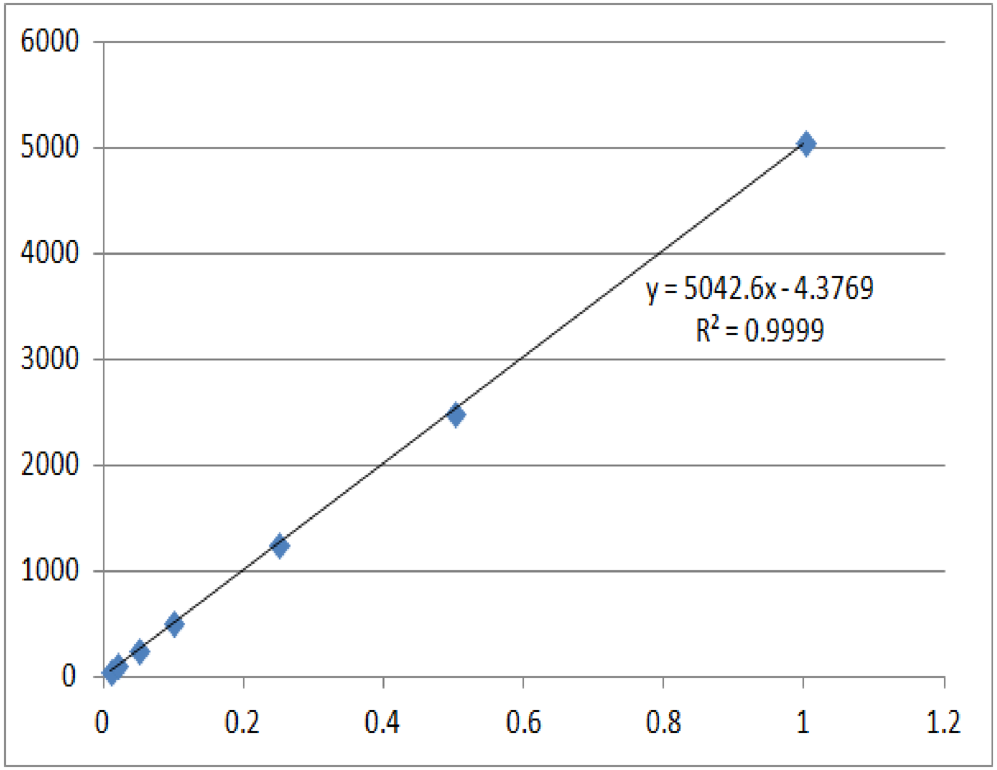
Correlation coefficient, R: $R = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}$

Calibration curve

EXAMPLE

Calculate the molar concentration of NaCl in sea water. Sea water was diluted 1000 times that detection sensitivity is 3265 mV.

NaCl	
Concentration / mM	Detection sensitivity / mV
0.01	51.51
0.02	106.3
0.05	249.8
0.1	500.9
0.25	1251
0.5	2489
1	5053

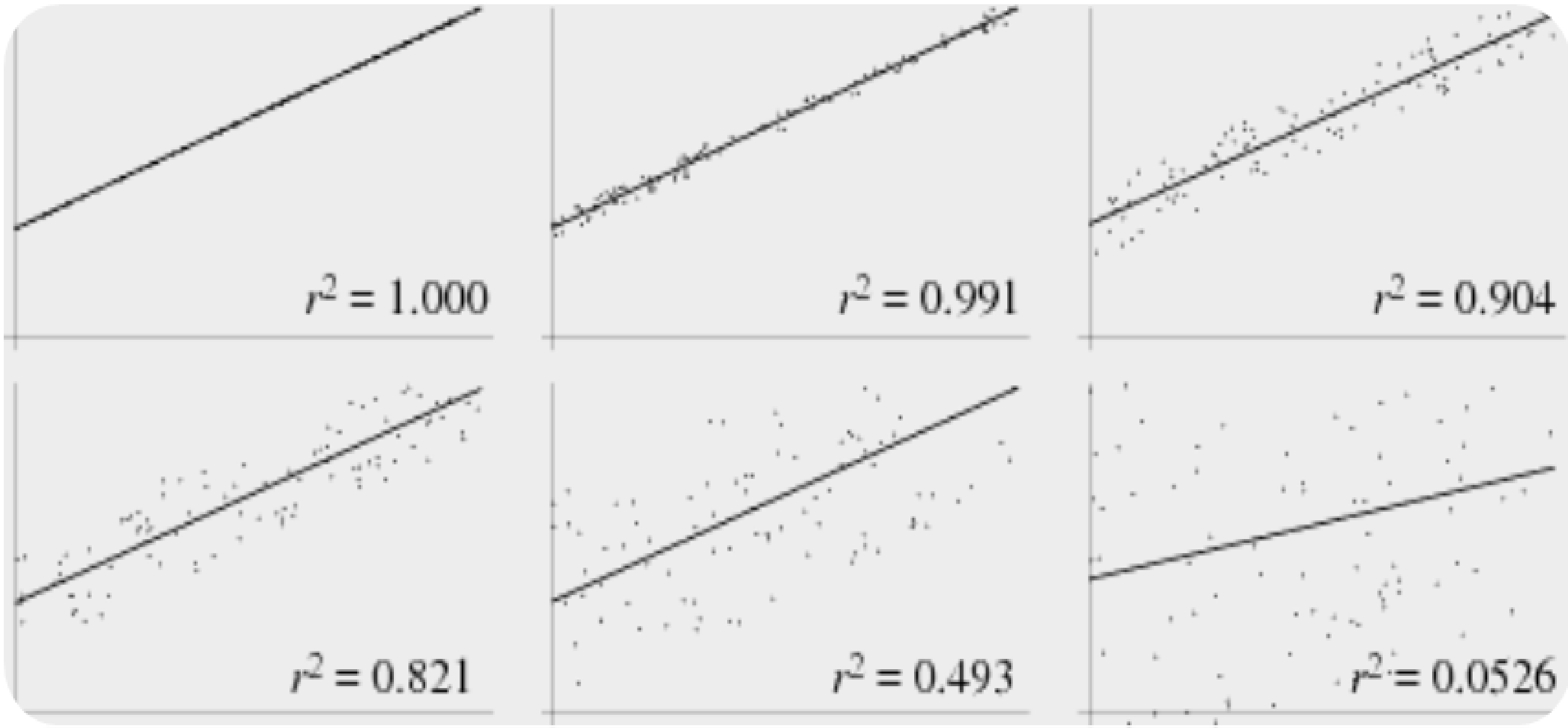


Concentration of NaCl in diluted sea water = (3265+4.3769)/5042.6
= 0.6484 mM

Concentration of NaCl in sea water =
0.6484*1000 = 648.4 mM

LINEAR REGRESSION

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

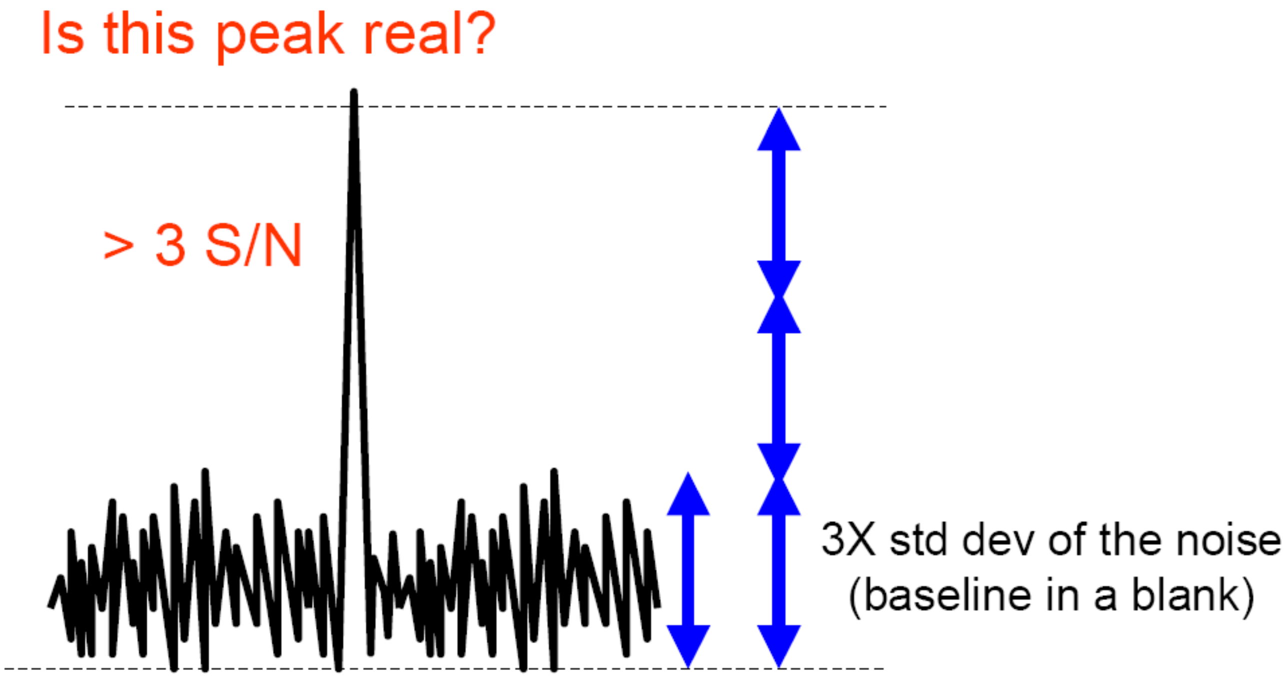


LOD AND LOQ

- 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."

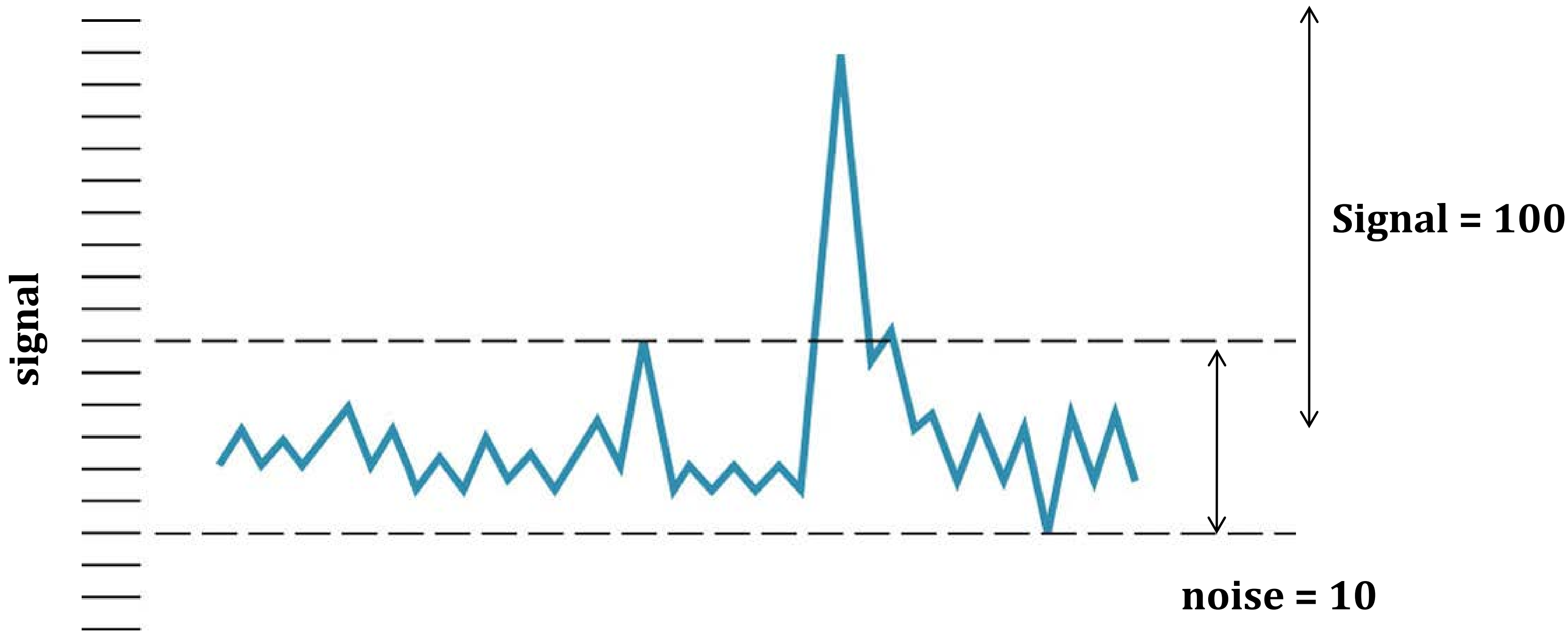
LOD

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

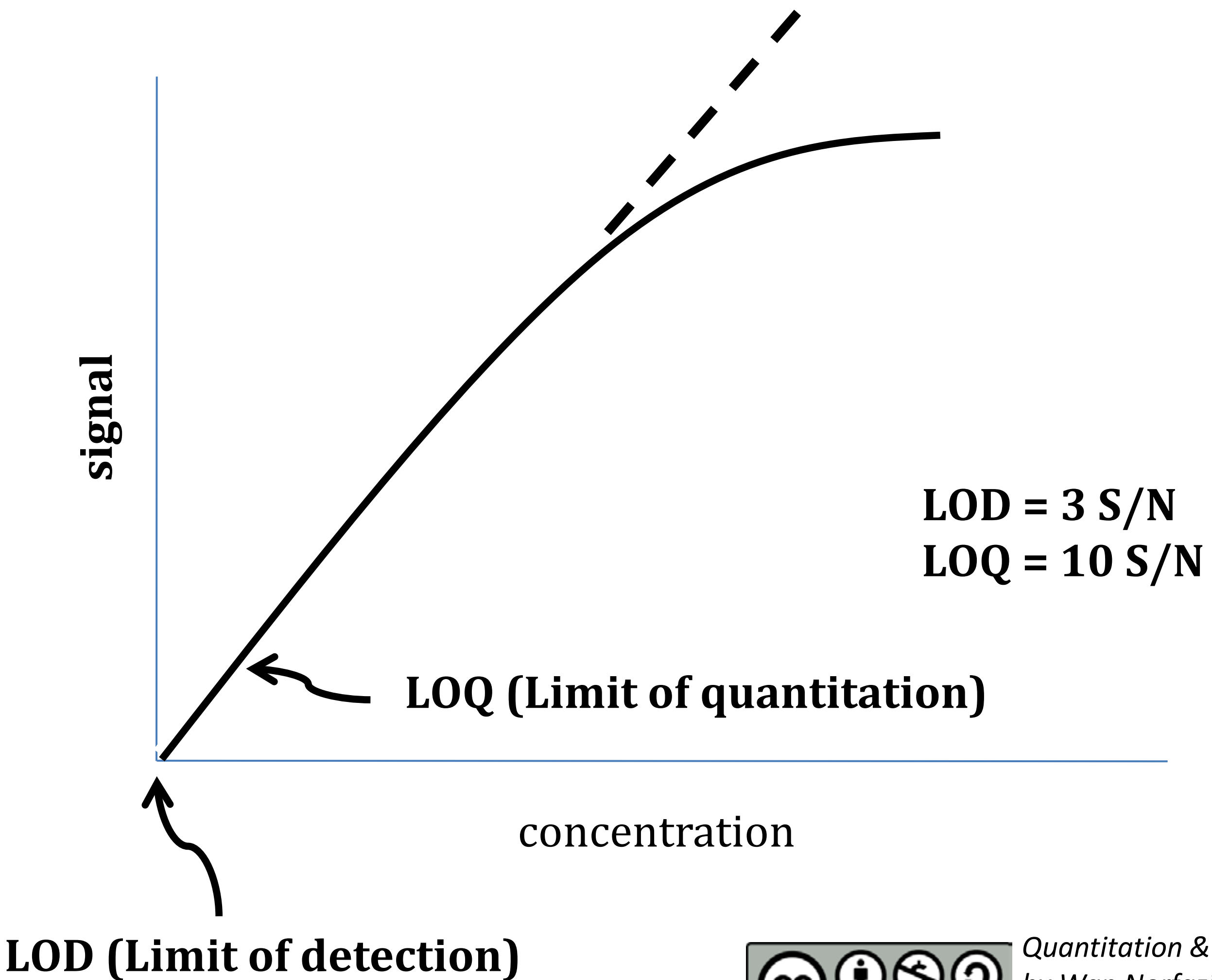


LOQ

Typically 10 times the signal-to-noise (based on standard deviation of the noise)



LOD AND LOQ

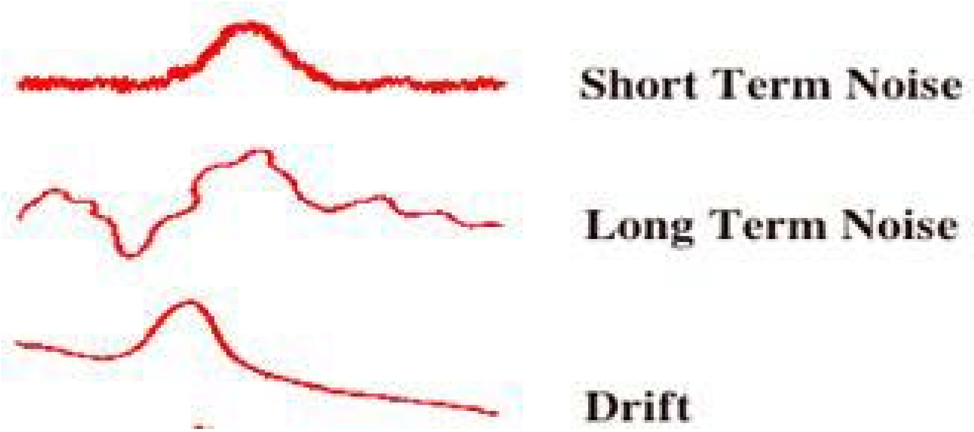


signal
LOD = 3 S/N
LOQ = 10 S/N
LOQ (Limit of quantitation)
concentration
LOD (Limit of detection)

NOISE

- Noise = uncertainty in the value of baseline in the absence of analyte

- 3 basic categories:
  - Short-term noise
  - Long-term noise
  - Baseline drift



NOISE

- Short-term noise can be due to a number of factors
  - Detector noise
  - Pulsations of pumping system
  - Electronic noise in the integration system

- Long-term noise
  - Temperature variations
  - Bleed of stationary phase from the column

- Baseline drift occurred when the composition of the solvent is deliberately changed during elution.

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