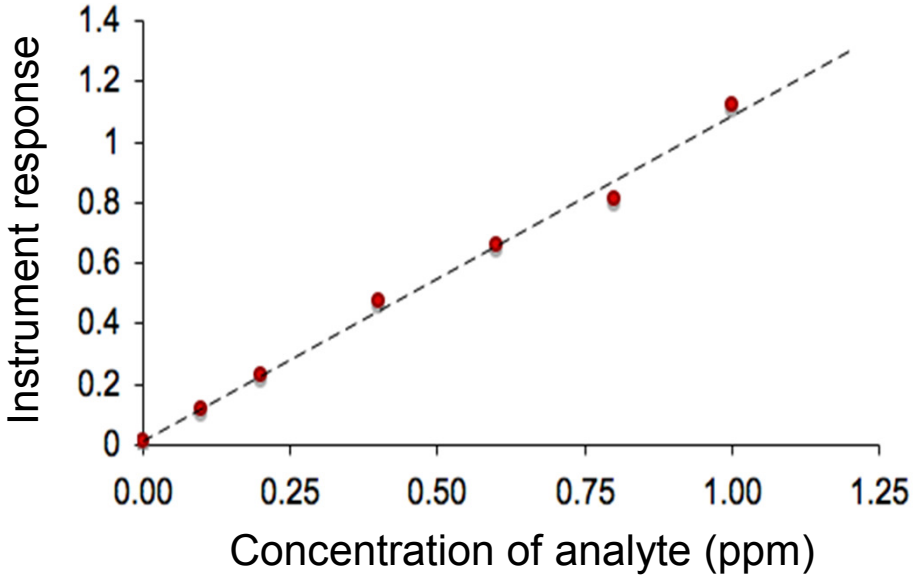
Calibration methods

- How do we relate the signal from an instrument to the concentration of a sample?

Response α concentration

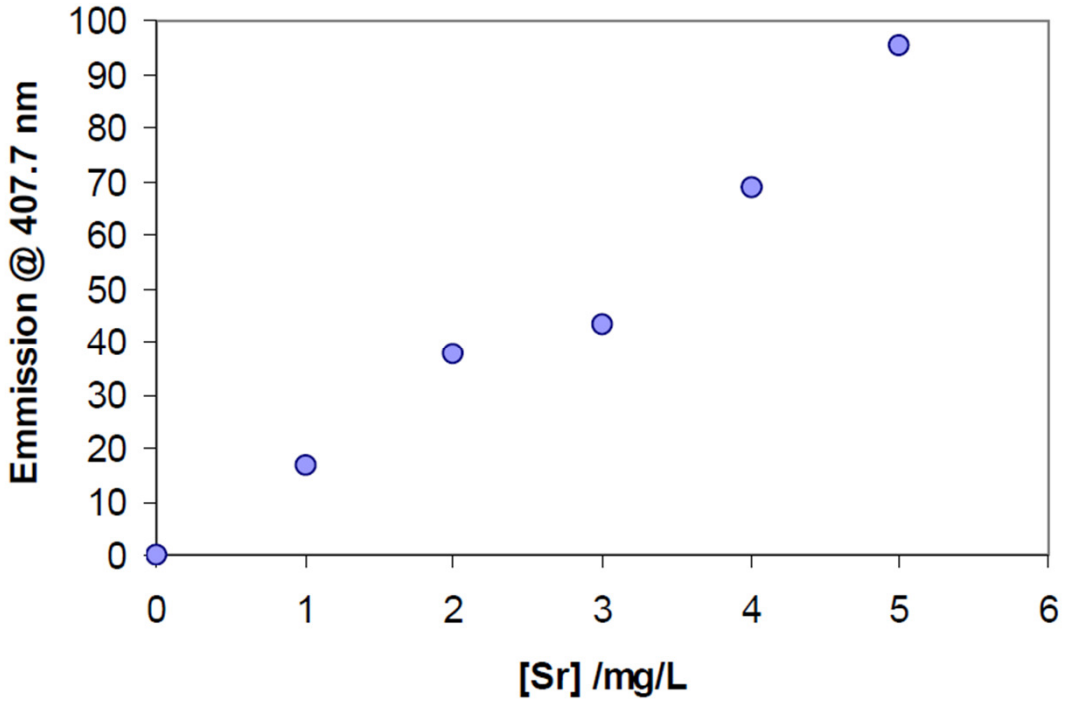
- The instrument response is measured against a series of standard solutions with a known concentration of analyte.

Calibration curve:
shows a response of an analytical method to known quantities of analyte.

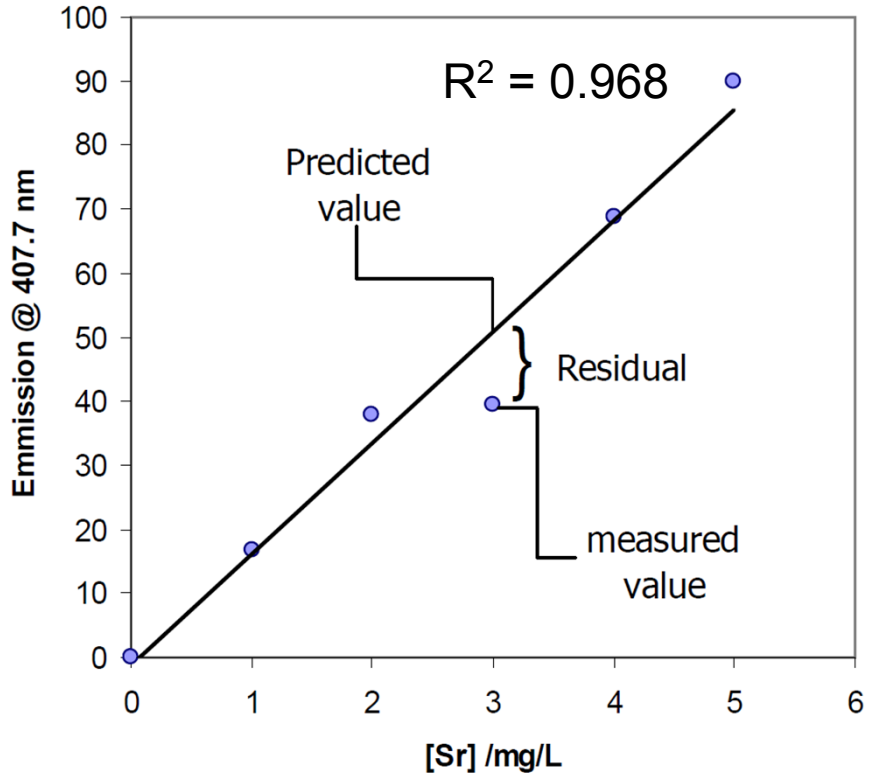


Fitting the best line

[Sr] (mg/L)	Emission Intensity @407.7 nm
0.00	0.0
1.00	16.6
2.00	37.8
3.00	43.2
4.00	68.7
5.00	95.2

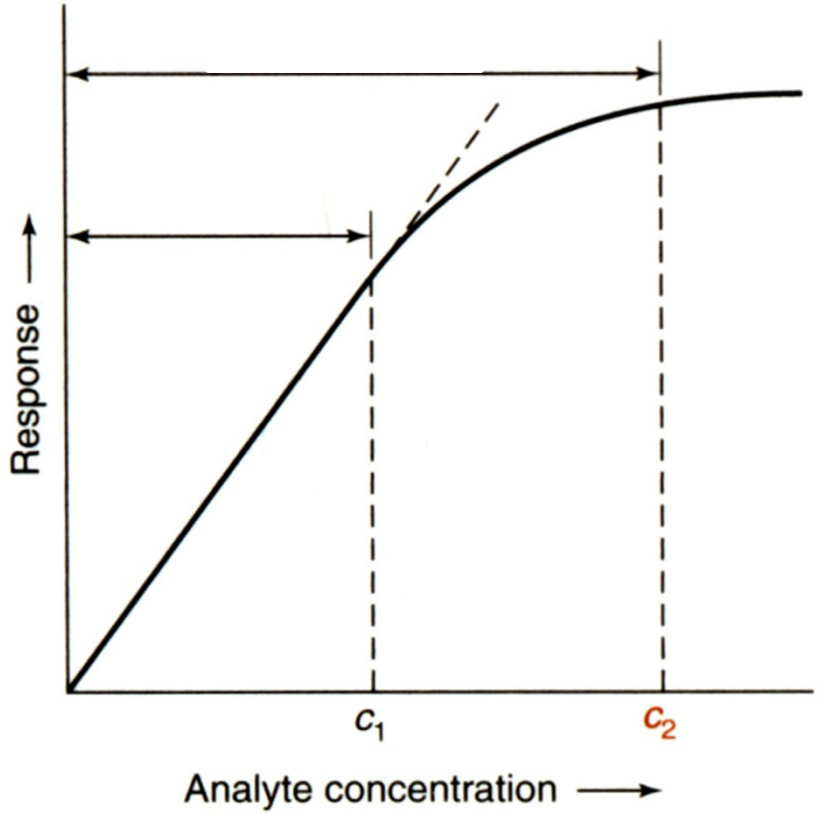


Method of least squares: minimises the sum of the squares of all the residuals

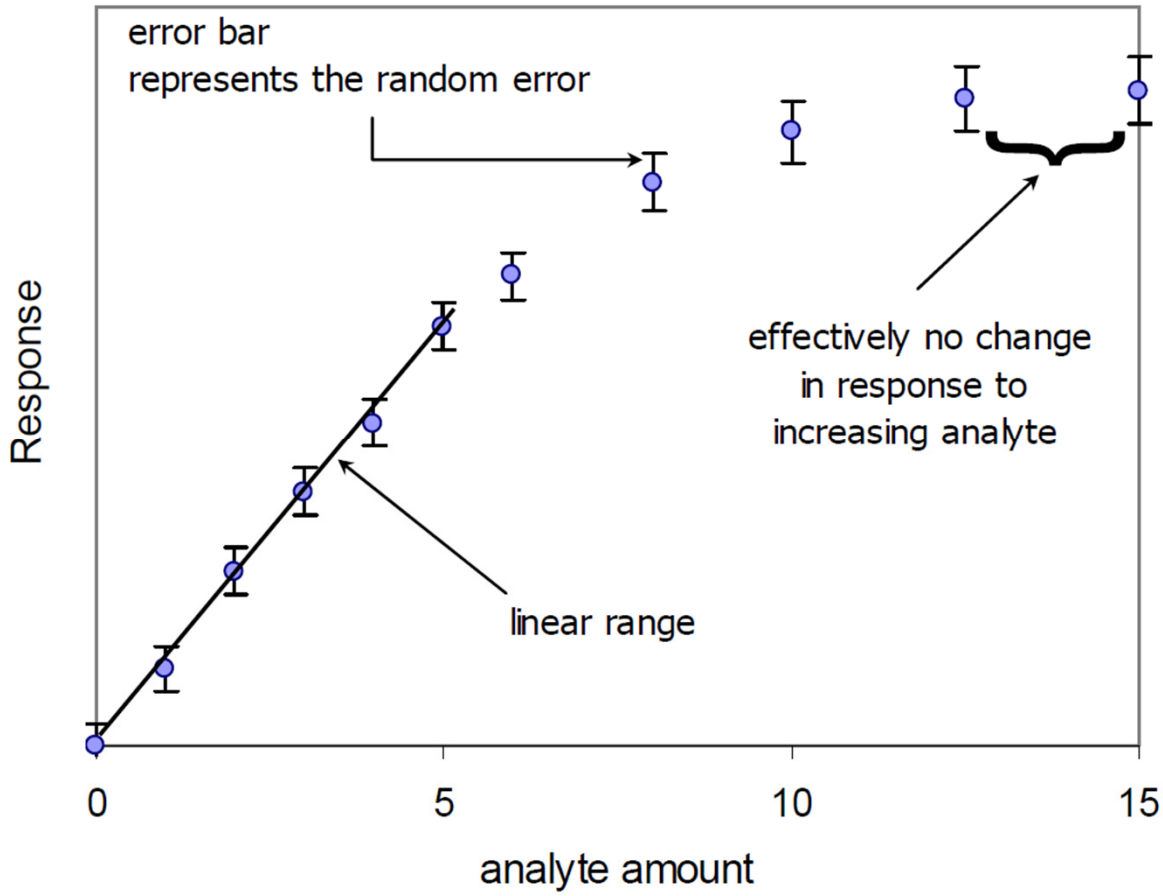


- Find the concentration of an unknown solution using the equation of the line (y = mx +c)
- Correlation coefficient (R^2 value): Tells you how well the data fit the line. We typically aim for >0.95

Using a Calibration Curve



- Calibrations tend to deviate from linearity at high concentrations due to non-ideal detector response or chemical effects.
- The linear range varies depending on the instrumental technique used.

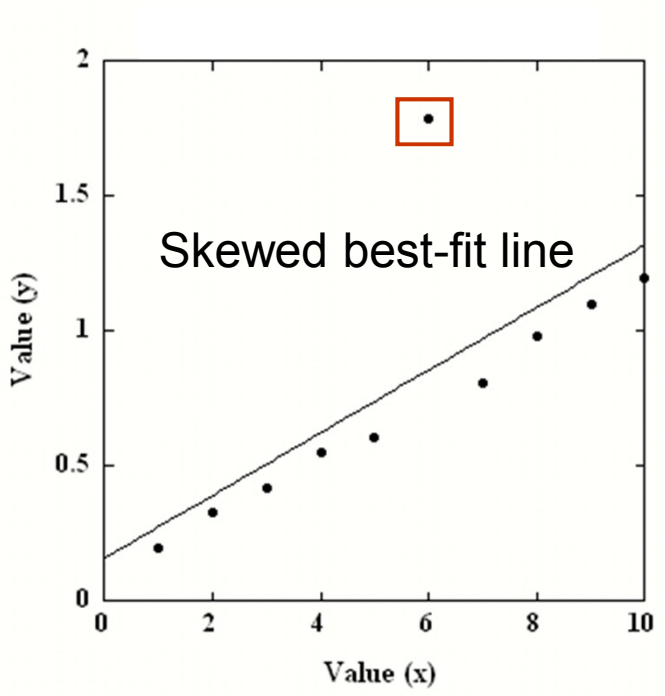


Impact of “Bad” Data Points:

Identification of erroneous data point.

- compare points to the best-fit line
- compare value to duplicate measures

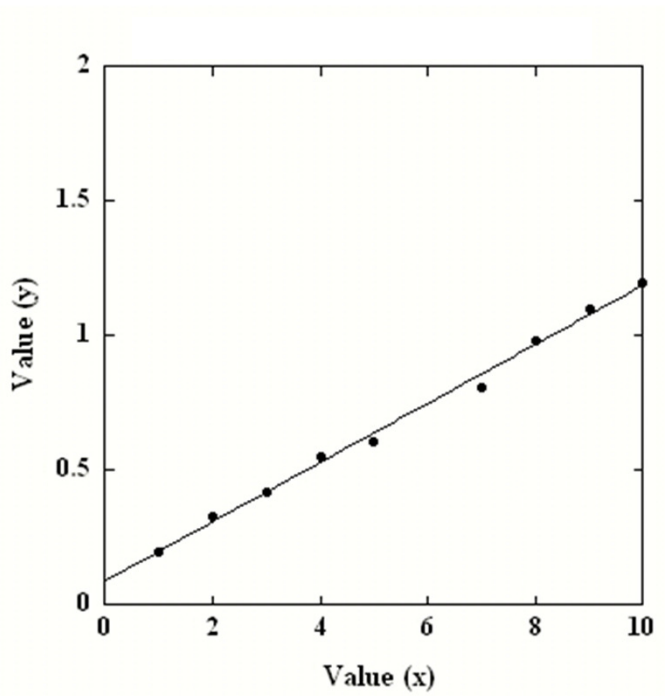
$R^2 = 0.53261$



Remove “bad” point

Improve fit and accuracy

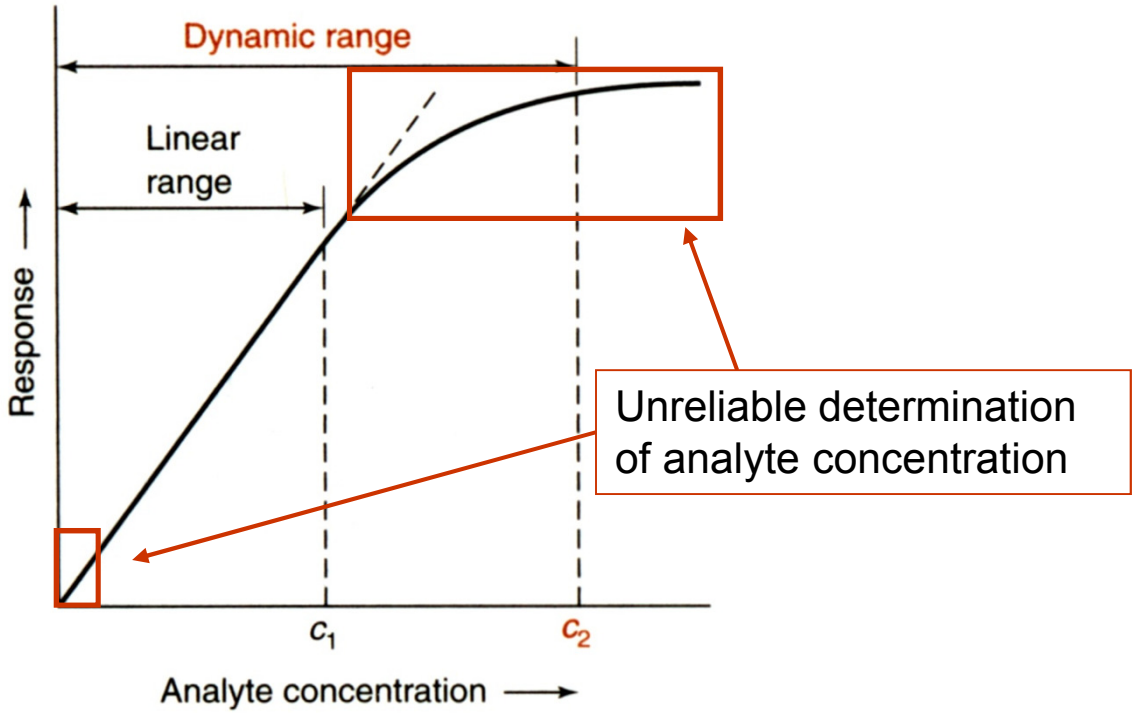
$R^2 = 0.99518$

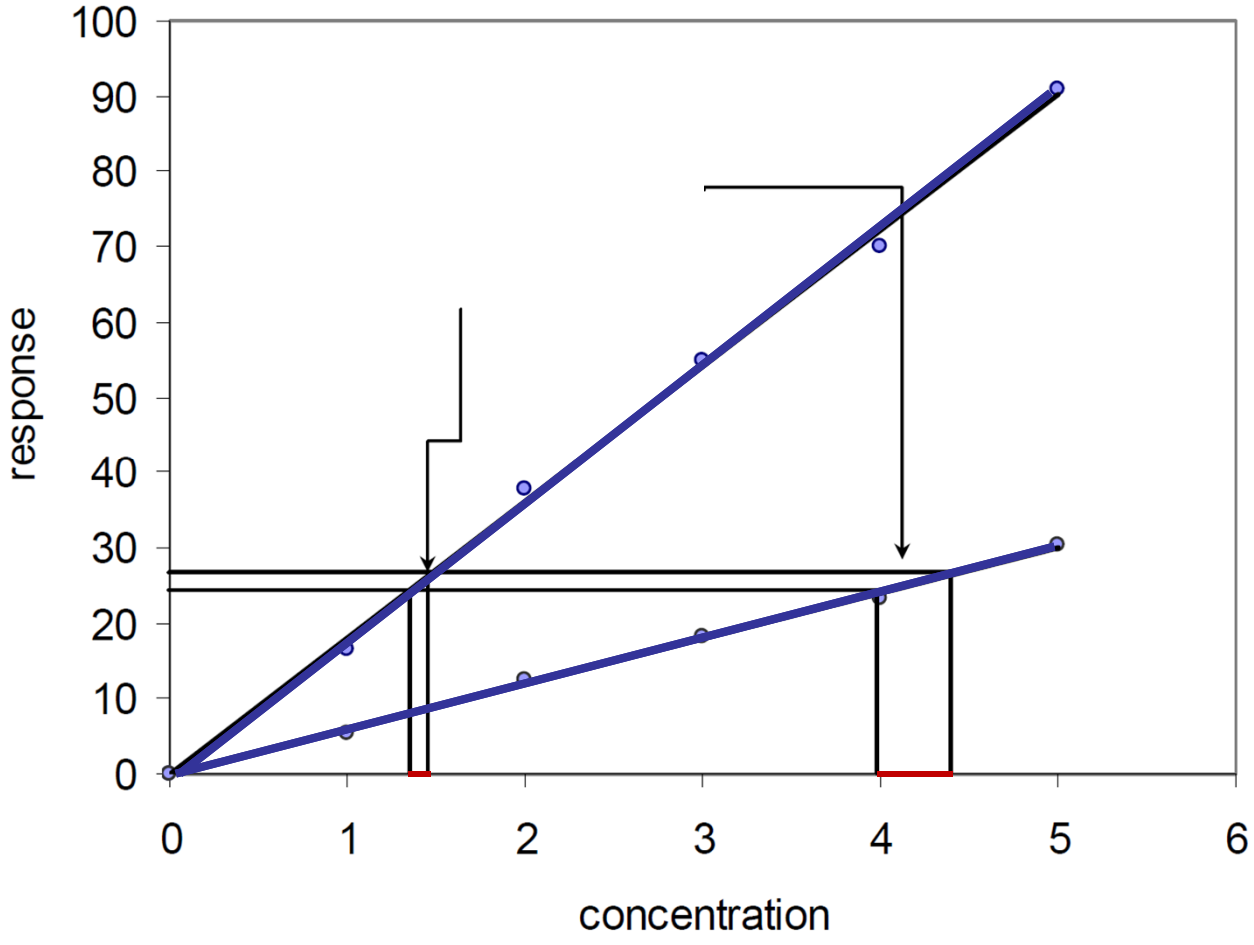


Limitations in a Calibration Curve

Limited application of calibration curve to determine an unknown.

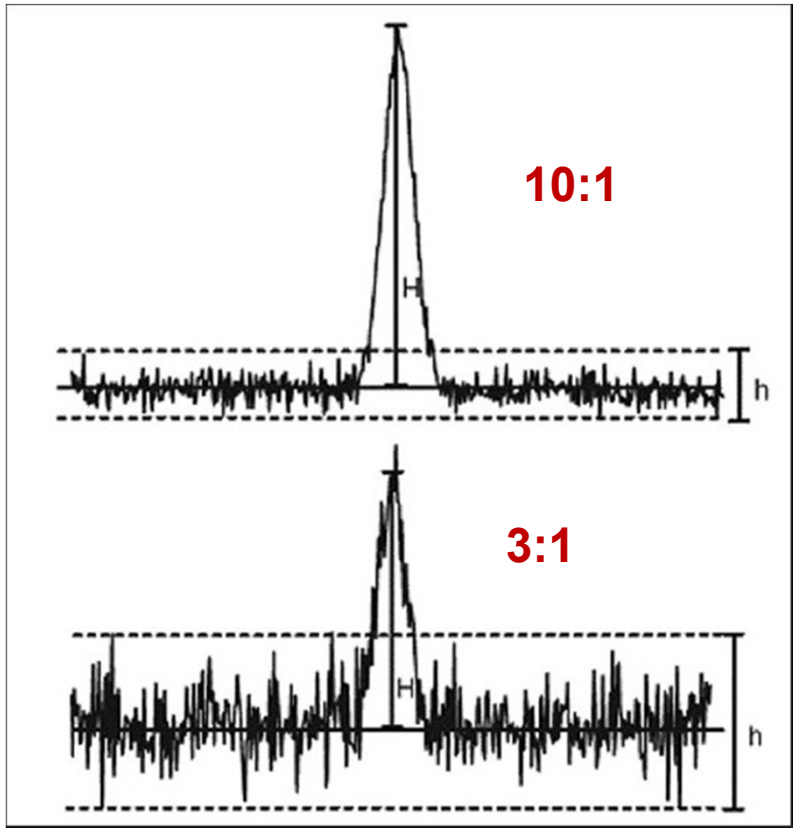
- Limited to linear range of curve
- Limited to range of experimentally determined response for known analyte concentrations
- Detection limit




100
90
80
70
60
50
40
30
20
10
0
response
0
1
2
3
4
5
6
concentration

Limit of detection

- There is no such thing as a zero concentration!
- You simply reach a point where you can no longer detect the presence of an analyte.
- You should report your detection limit.

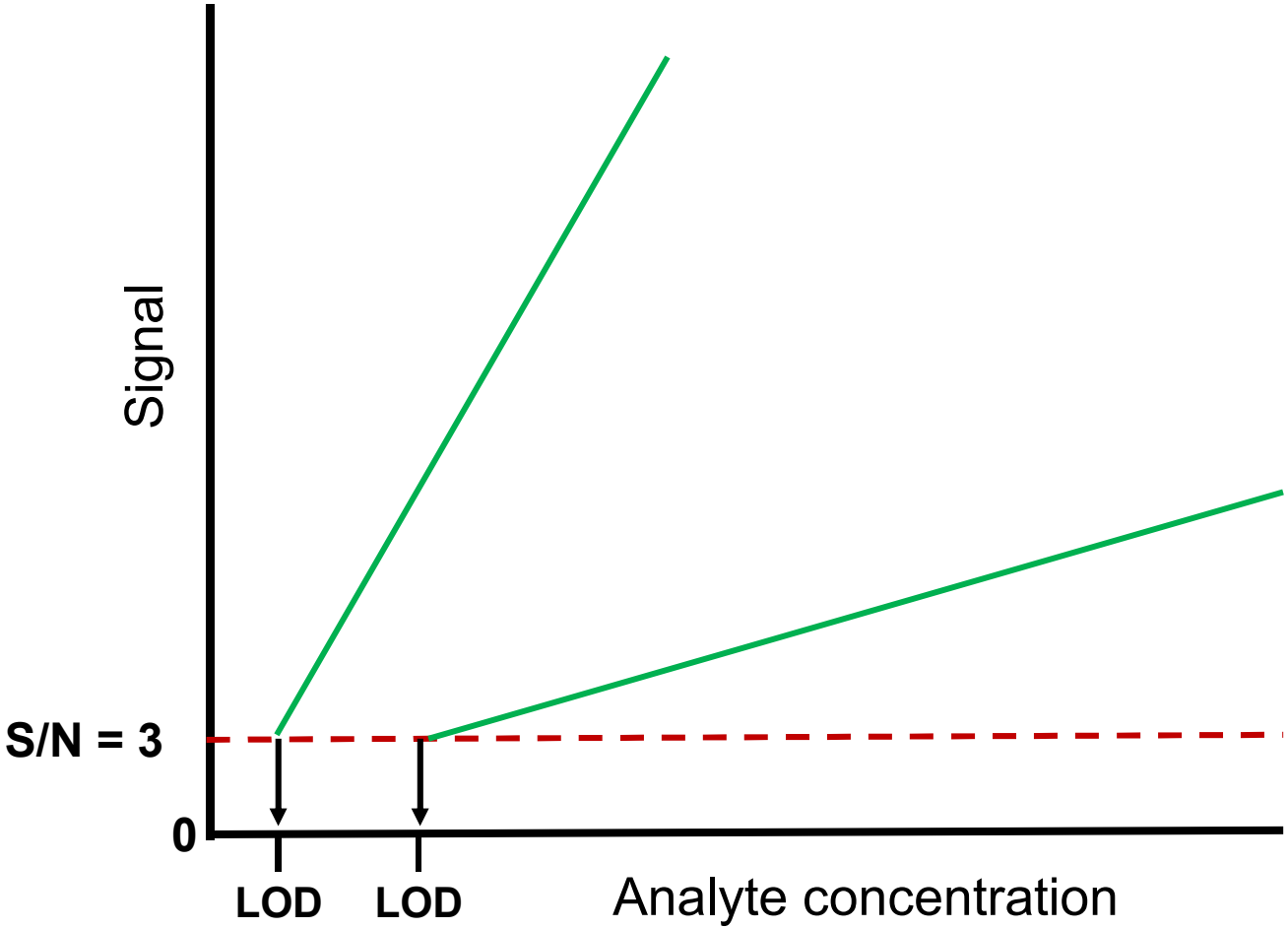


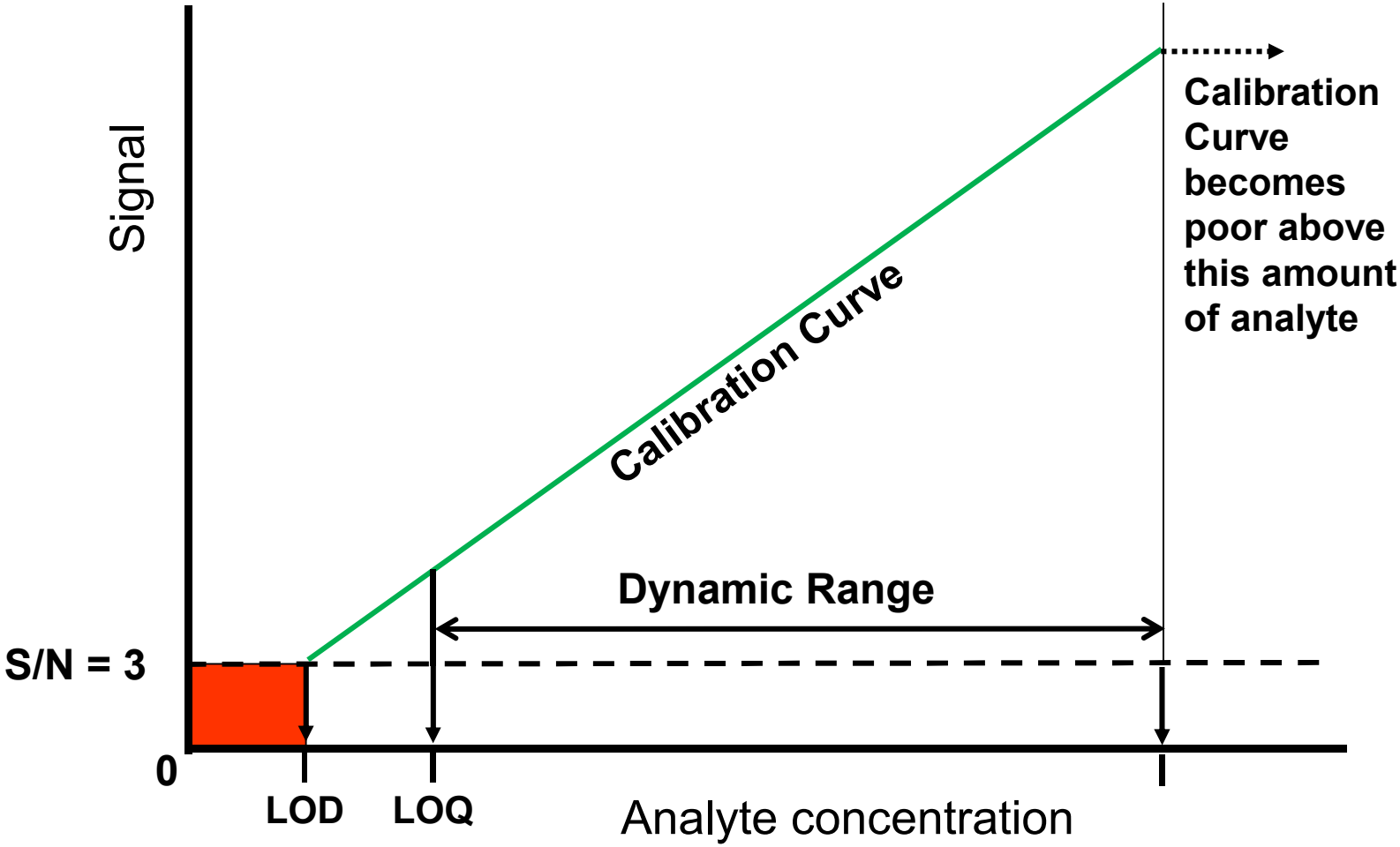
Baseline noise is the unwanted random change in the output signal

Limit of Quantification (LOQ):
signal > 10 x σ noise

Limit of Detection (LOD):
signal > 3 x σ noise

Sensitivity vs. Limit of Detection




Signal
Calibration Curve
Calibration Curve becomes poor above this amount of analyte
Dynamic Range
S/N = 3
0
LOD
LOQ
Analyte concentration

Preparation of standards

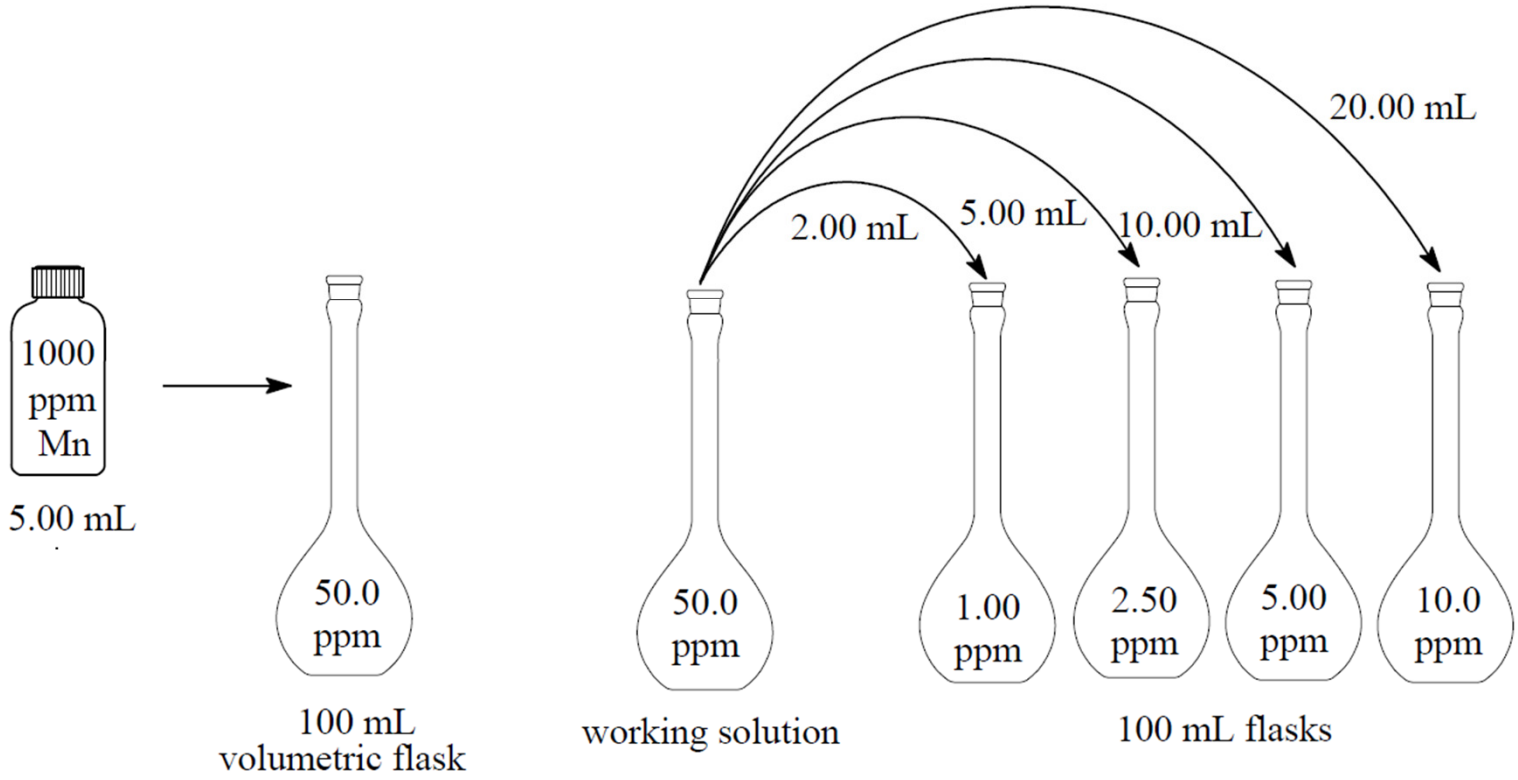
- Analytical chemists need to know how to prepare solutions with *accurately known concentrations*
- The accuracy and precision of your results can be no better than the accuracy with which you make your standards!

Working with solutions

Moles or mass of analyte taken from concentrated solution.

$C_1 \times V_1 = C_2 \times V_2$

Moles or mass of analyte placed in dilute solution.

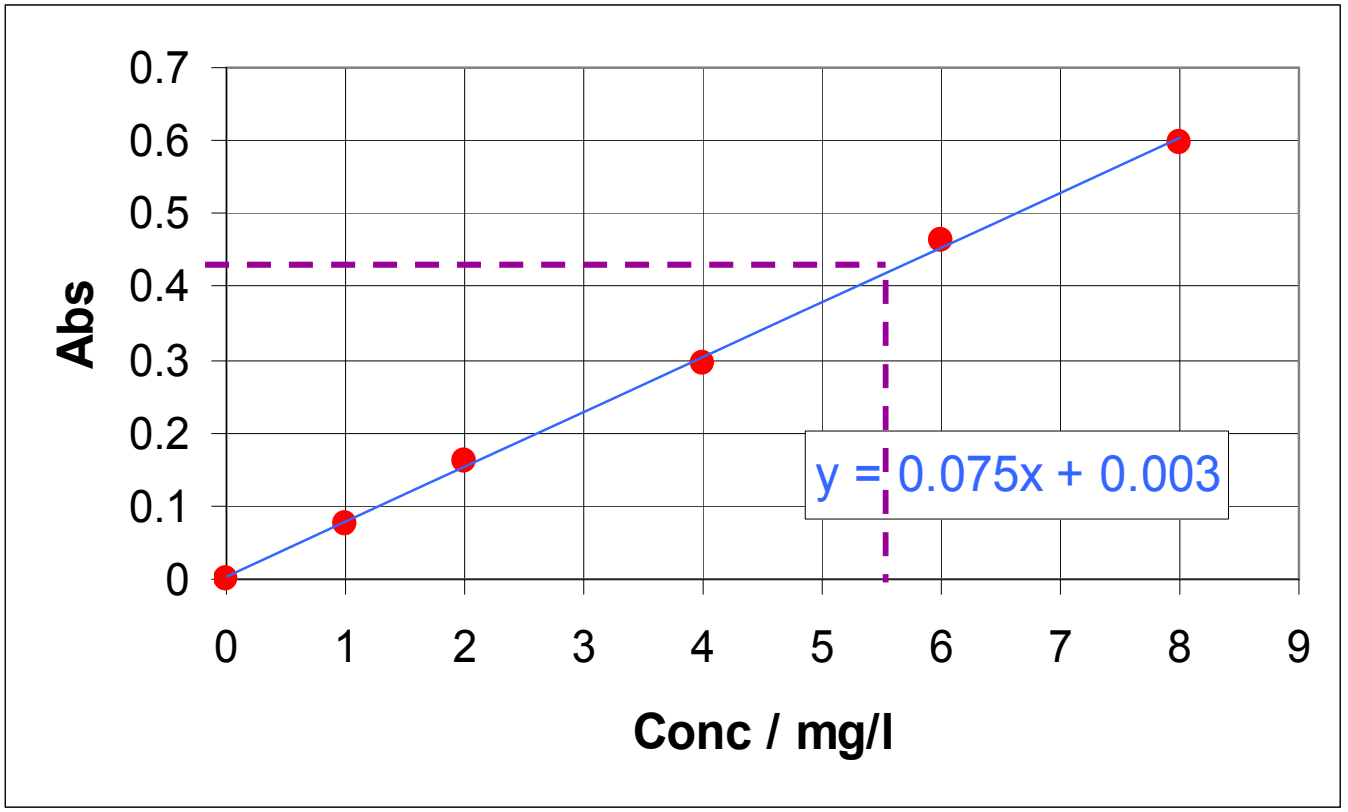


Example:

I prepared 6 solutions with a known concentration of Cr^{6+} and added the necessary colouring agents. I then used a UV-Vis spectrophotometer and measured the absorbance for each solution at a particular wavelength. The results are given in the table below.

Concentration mg L^{-1}	**Absorbance**	**Corrected absorbance**
0 (blank)	0.002	0.000
1	0.078	0.076
2	0.163	0.161
4	0.297	0.295
6	0.464	0.462
8	0.600	0.598

Best fit straight line:



Calibration techniques

1. External standard (e.g. lab 5)
2. Standard addition (e.g. lab 7)
3. Internal standard

Things to note:

- A calibration should be carried out using standards that are similar to the sample, i.e. the same **matrix**
- Components within the matrix may contribute or interfere with the analyte signal.
- The calibration concentration range should cover the sample range. If not - either change your standards or dilute your samples.

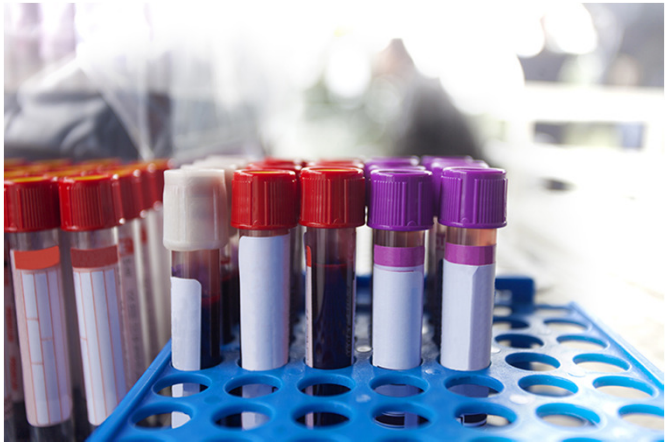
Recall:
A matrix is all other components of the sample except the analyte.

Real-life calibration

Overcoming matrix effects:

- Complex samples such as biological fluids, geological materials (salts, oils, contaminants, particulates).

- Several clever schemes are employed to solve real-world calibration problems:
  - Standard Addition
  - Internal Standard



You have a bag that contains smarties. The bag has no mass and you cannot see into it. The only thing you can do is find the mass and add smarties. Can you figure out a way to determine the number of smarties?

Begin by finding the mass of the smarties in the bag.

Our bag weighs 40 g

Next you add 5 smarties to the bag and find the new mass.

We add 5 smarties, the new mass of the bag is 50 g

You can now determine the mass of the 5 smarties that you added.

Since you know how many smarties you added, you can determine the mass of each.

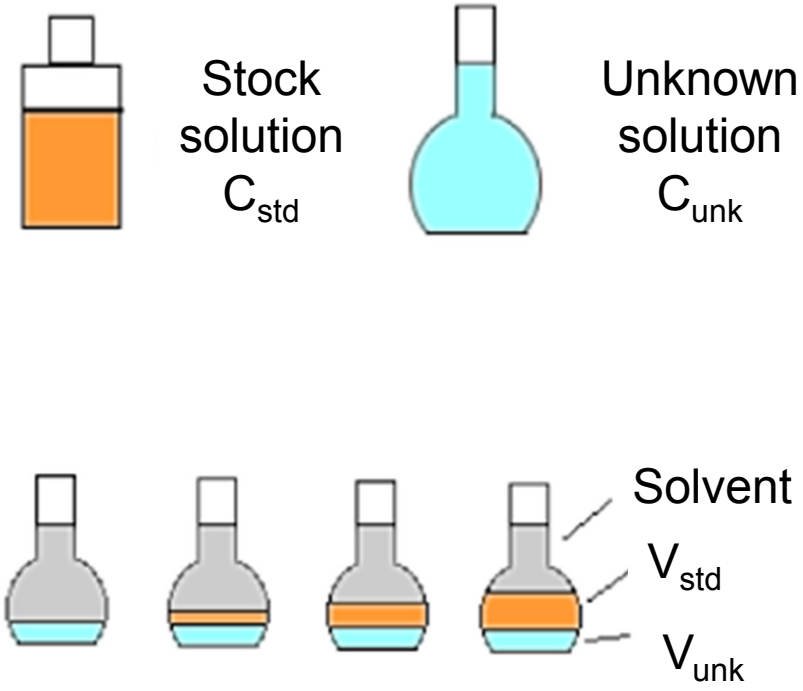
Now you know the mass of a single smartie, so you can determine the number of smarties initially in the bag from the initial mass.

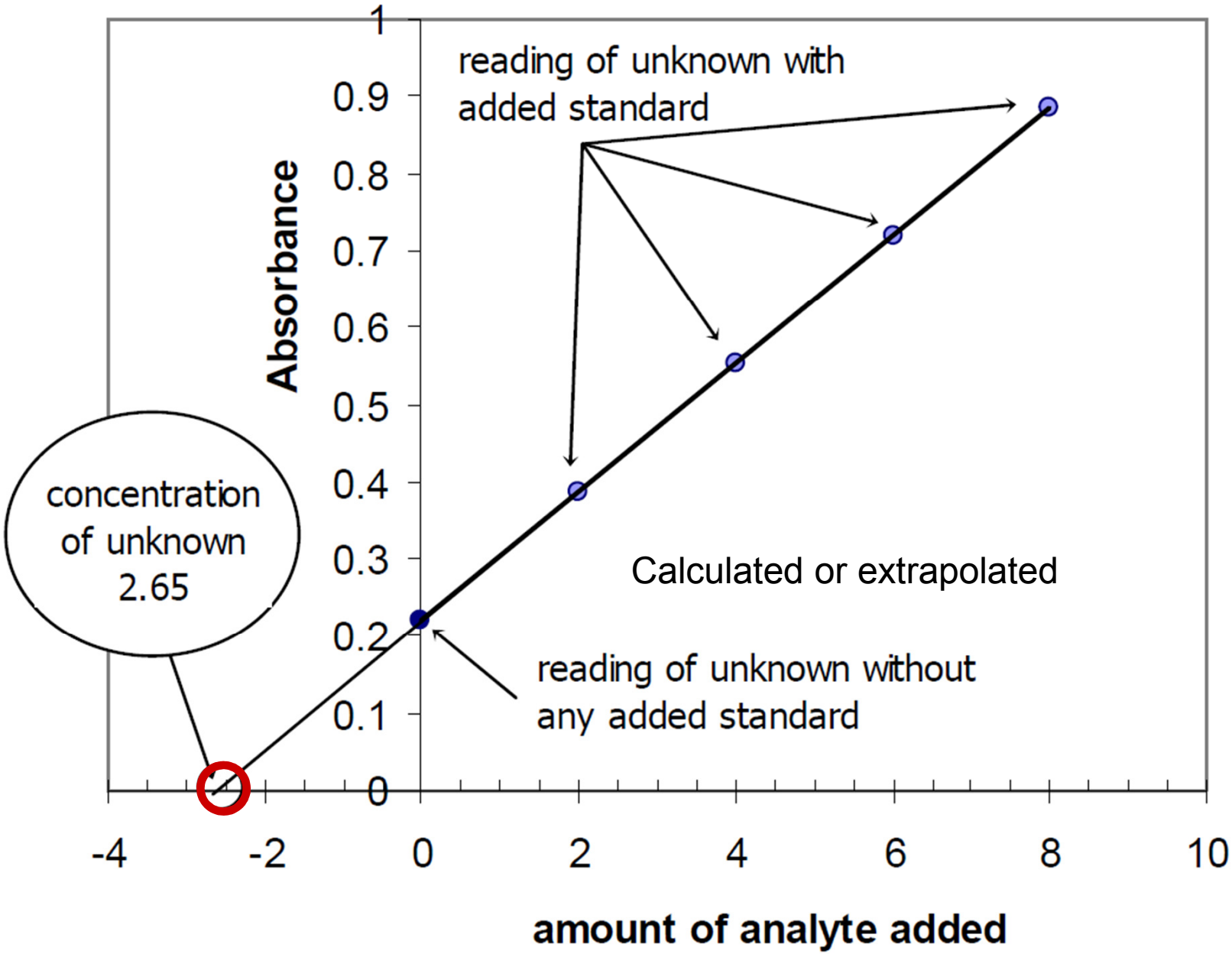
Method of standard addition

We can correct for matrix effects through the method of standard addition

Increasing quantities of a standard solution are added to aliquots (fixed volume) of the unknown sample.

The increase in signal allows one to deduce how much analyte was originally present.




reading of unknown with
added standard
Absorbance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
concentration
of unknown
2.65
Calculated or extrapolated
reading of unknown without
any added standard
-4
-2
0
2
4
6
8
10
amount of analyte added

Example:

Gold was determined in a waste stream using voltammetry. The peak height of the current signal is proportional to concentration.

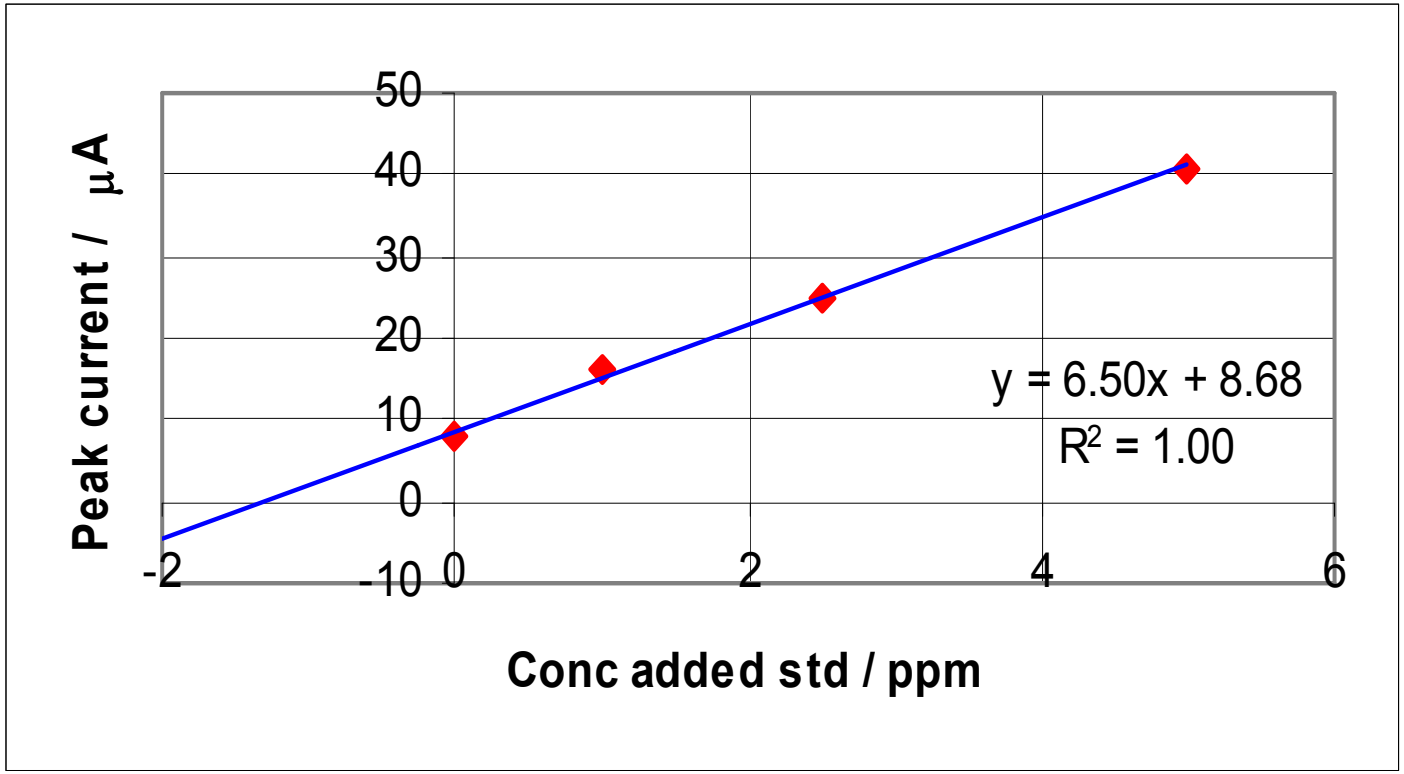
A standard addition analysis was done by adding 10 ppm Au solution to the sample as shown in the table below. All solutions were made up to a final volume of 20 ml. The peak currents obtained from the analyses are tabulated below.

Calculate the concentration of Au in the original sample.

Volume of sample (ml)	**Volume of std (ml)**	**Peak current (μA)**
10	0	8
10	2	16
10	5	25
10	10	41

Calculate the concentration of Au added to each sample:

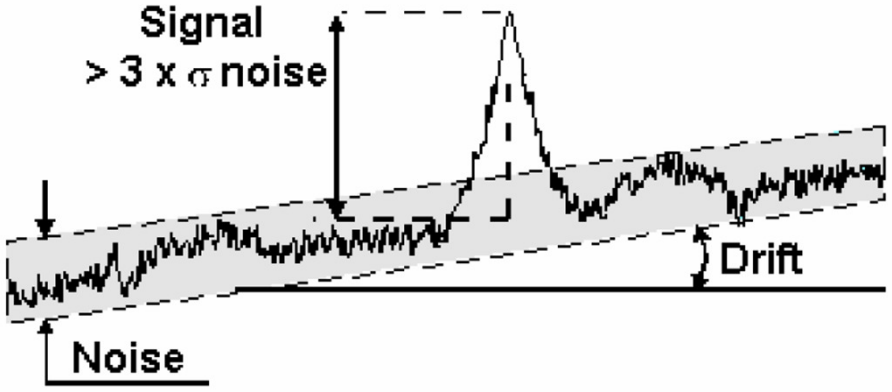
Volume of std (ml)	Conc. of added std (ppm)	Peak current (μA)


Peak current / µA
50
40
30
20
10
0
-10
-2
0
2
4
6
y = 6.50x + 8.68
R2 = 1.00
Conc added std / ppm

Internal standards

- When working with small samples or trace concentrations → error may be large.
- An *Internal Standard* is a substance that is added in a constant amount to all samples, blanks and calibration standards.
- Used effectively when a *small variation* in signal leads to a *large change* in concentration.

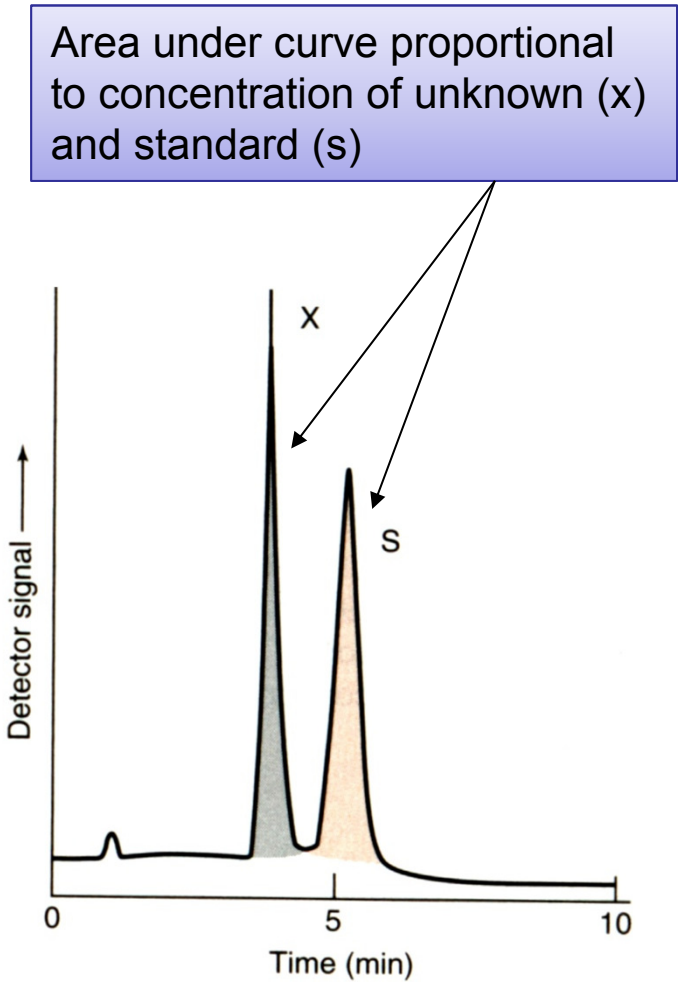
What kind of variations are we likely to encounter in the lab?



Calibration involves plotting the *ratio* of the analyte signal to the internal standard signal as a function of analyte concentration of the standards.

Properties of an IS

- Must not be present in the sample
- Must be compatible with the sample
- Must be miscible with the sample
- Signal must be fully resolved
- Of similar concentration to the analyte
- Structurally similar to analyte



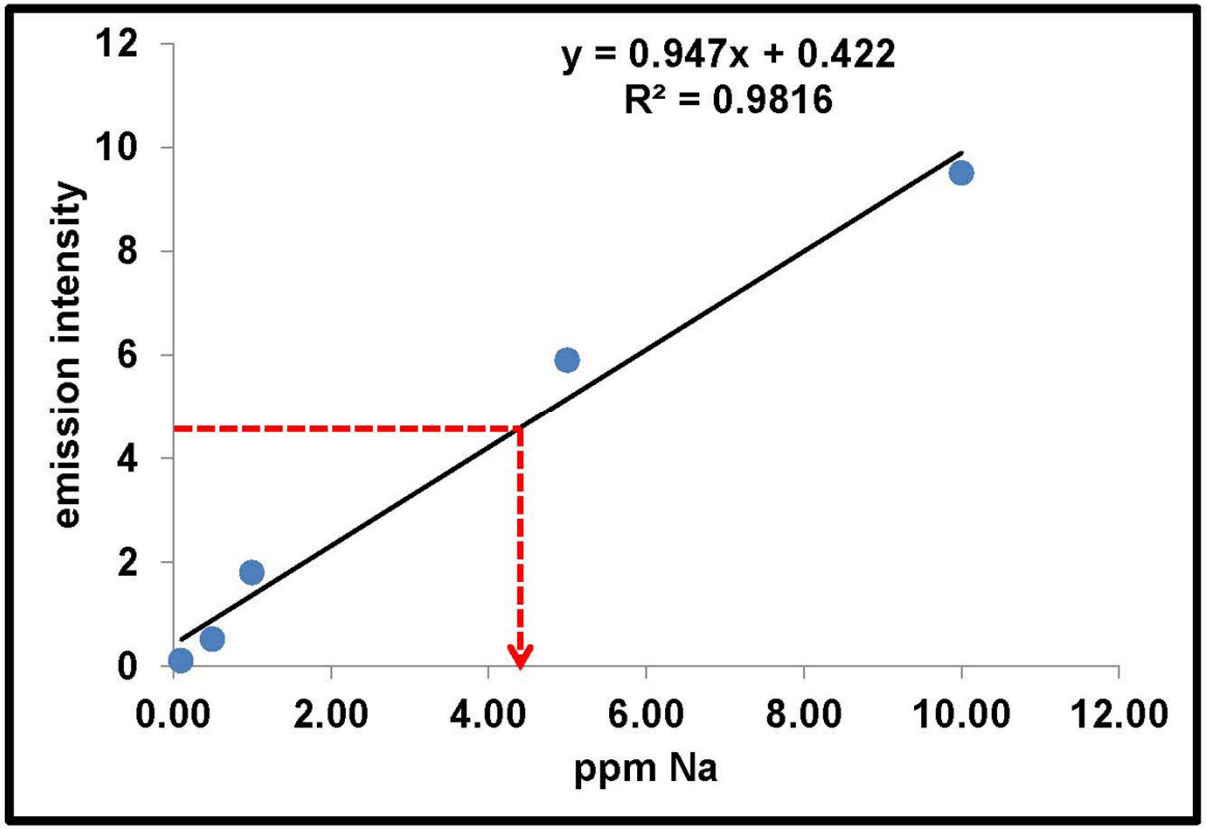
Example

- Li is used an internal standard for the spectroscopic analysis of Na in glass.
- A 1000 ppm Li solution was used as an internal standard in differing concentrations of sodium.
- 1000 ppm Li was also added to the sample of glass.

ppm Na	Na emission intensity	Li emission intensity
0.10	0.11	86
0.50	0.52	80
1.00	1.8	128
5.00	5.9	91
10.00	9.5	73
unknown	4.4	95

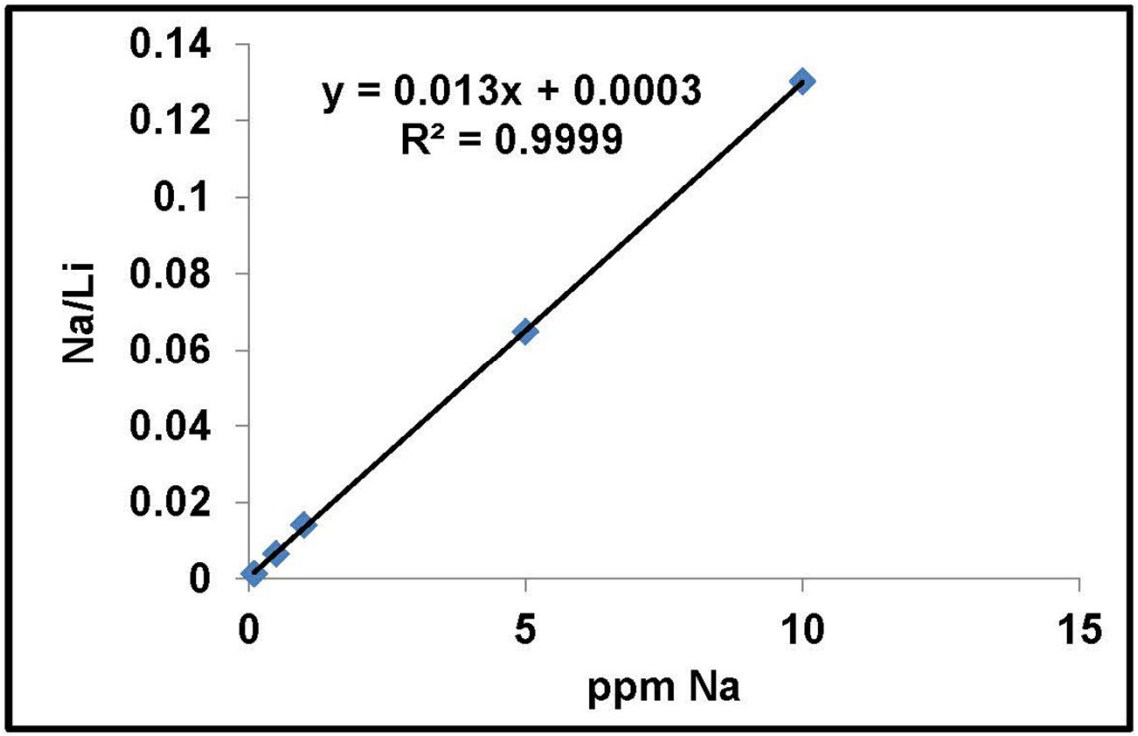
Traditional approach

ppm Na	Na emission intensity
0.10	0.11
0.50	0.52
1.00	1.8
5.00	5.9
10.00	9.5
unknown	4.4



With an internal standard

ppm Na	Na / Li emission intensity
0.10	0.001279
0.50	0.0065
1.00	0.014063
5.00	0.064835
10.00	0.130137
unknown	0.046316



Internal standard compensates for flame temperature, flow rate, efficiency in uptake rate and droplet size.

Remember: Dilutions don't matter since it is the ratio of analyte to IS that is important.

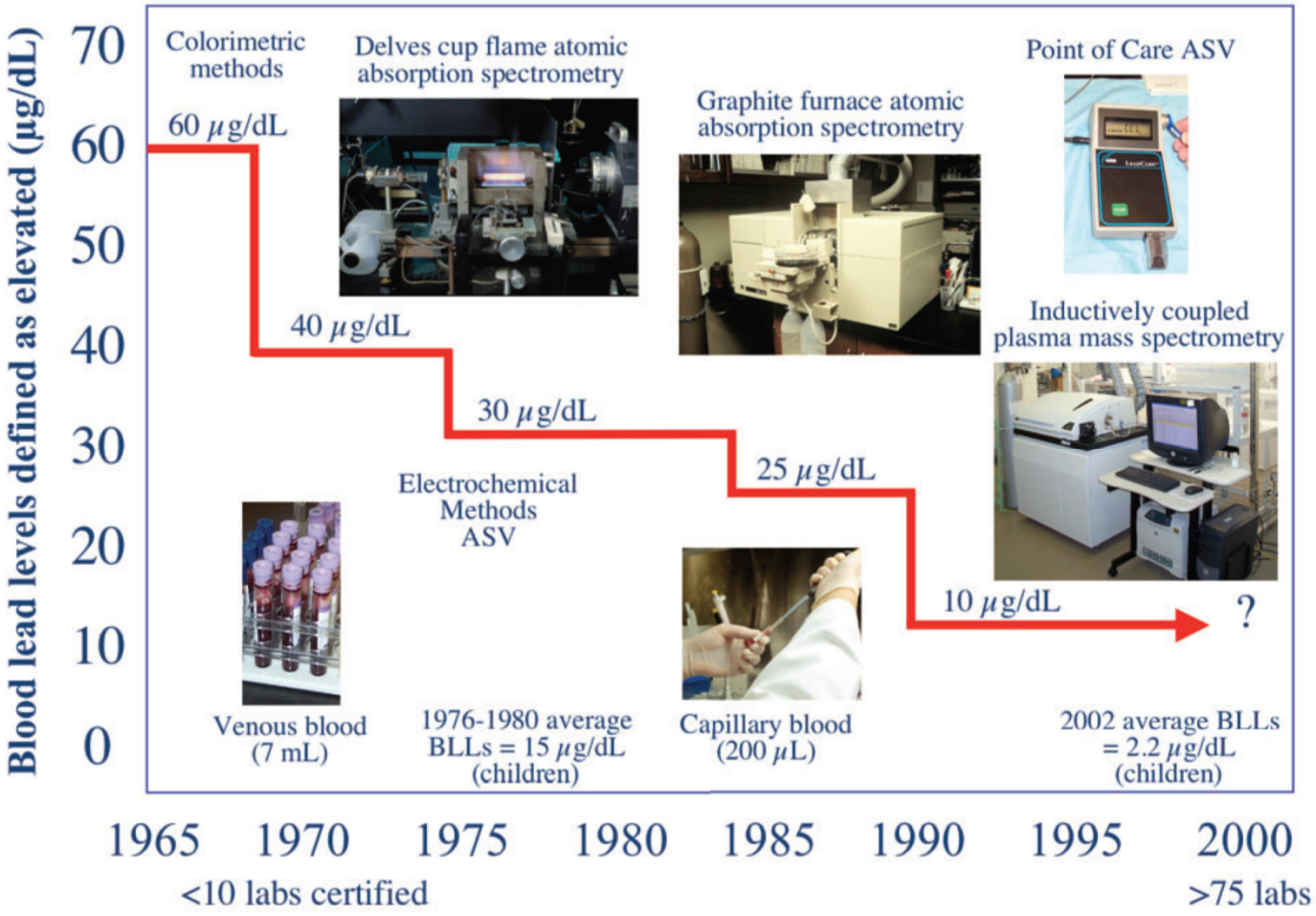
Choice of calibration method

Dependent upon several factors:

1. potential matrix effects
2. number of samples
3. consistency of matrix across samples

Can you think of advantages and disadvantages of the methods discussed?

Analytical advancements: Human exposure to lead



Lead content regulations for children's products