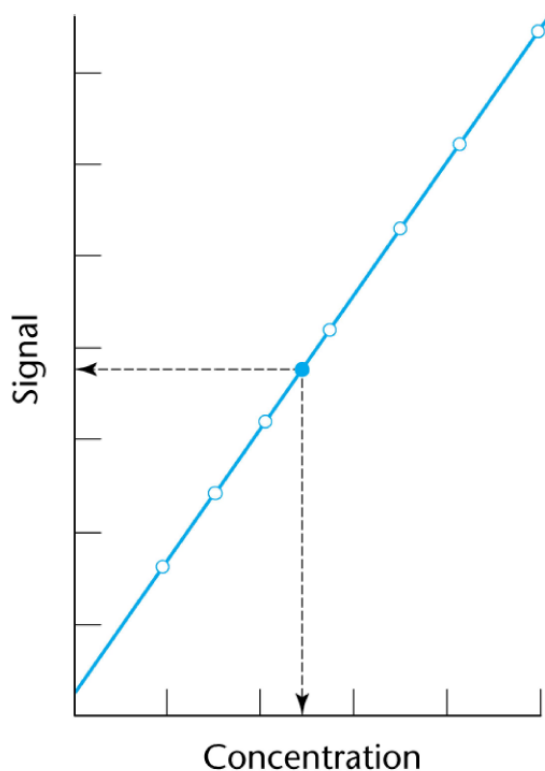## Calibration

- Take a calibaration sample with known but different concentrations.
- Based on measurements plot response curve.
- Is it linear?
- Make prediction for concentration between calibrated points.

(Remark: In analytical chemistry, the response variable is often call the *signal*)

Determining chemical concentration with standard addition: An application of linear regression

One of the most common tasks in chemistry is to determine the concentration of a chemical in an aqueous solution (i.e., the chemical is dissolved in water, with other chemicals possibly in the solution).

A common way to accomplish this task is to create a calibration curve by measuring the signals of *known* quantities of the chemical of interest - often called the **analyte** - in response to some analytical method (commonly involving absorption spectroscopy, emission spectroscopy or electrochemistry); the calibration curve is then used to interpolate or extrapolate the signal of the solution of interest to obtain the analyte's concentration.

Distortion due to the Matrix Effect

However, what if other components in the solution distort the analyte's signal? This distortion is called a **matrix interference** or **matrix effect**, and a solution with a matrix effect would give a different signal compared to a solution containing purely the analyte.

Consequently, a calibration curve based on solutions containing only the analyte cannot be used to accurately determine the analyte's concentration.

Overcoming Matrix Interferences with Standard Addition

An effective and commonly used technique to overcome matrix interferences is **standard addition**.

This involves adding known quantities of the analyte (the *standard*) to the solution of interest and measuring the solution's analytical signals in response to each *addition.* (Adding the standard to the sample is commonly called "*spiking* the sample.")

Assuming that the analytical signal still changes proportionally to the concentration of the analyte in the presence of matrix effects, a calibration curve can be obtained based on **simple linear regression**.

The analyte's concentration in the solution before any additions of the standard can then be extrapolated from the regression line.

Procedurally, here are the steps for preparing the samples for analysis in standard addition:

1) Obtain several samples of the solution containing the analyte in equal volumes.
2) Add increasing and known quantities of the analyte to all but one of the solutions.
3) Dilute the mixture with water so that all solutions have equal volumes.

These three steps are shown in the diagram below. Notice that no standard was added to the first volumetric flask.
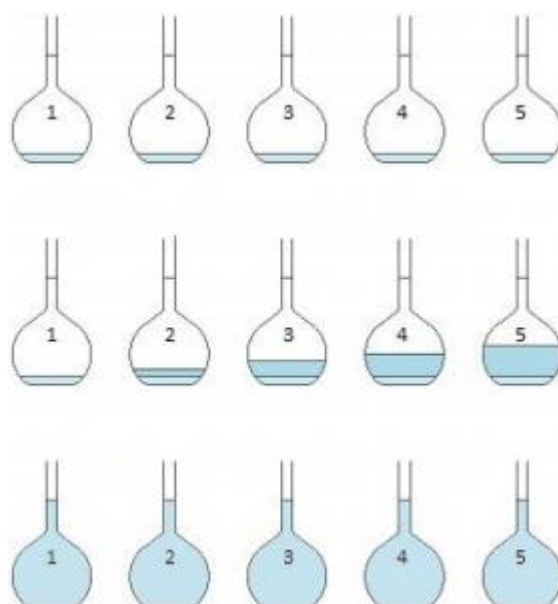
At this point, the five solutions are now ready for analysis by some analytical method.

Important: The signals are quantified and plotted against the concentrations of ***the standards that were added to the solutions***, including one sample that had no standard added to it.

A simple linear regression curve can then be fitted to the data and used to extrapolate the chemical concentration.
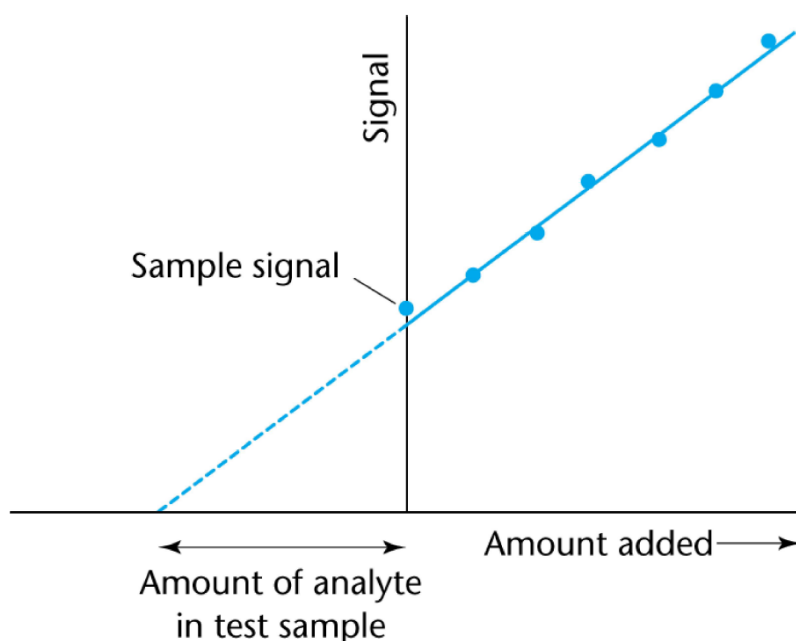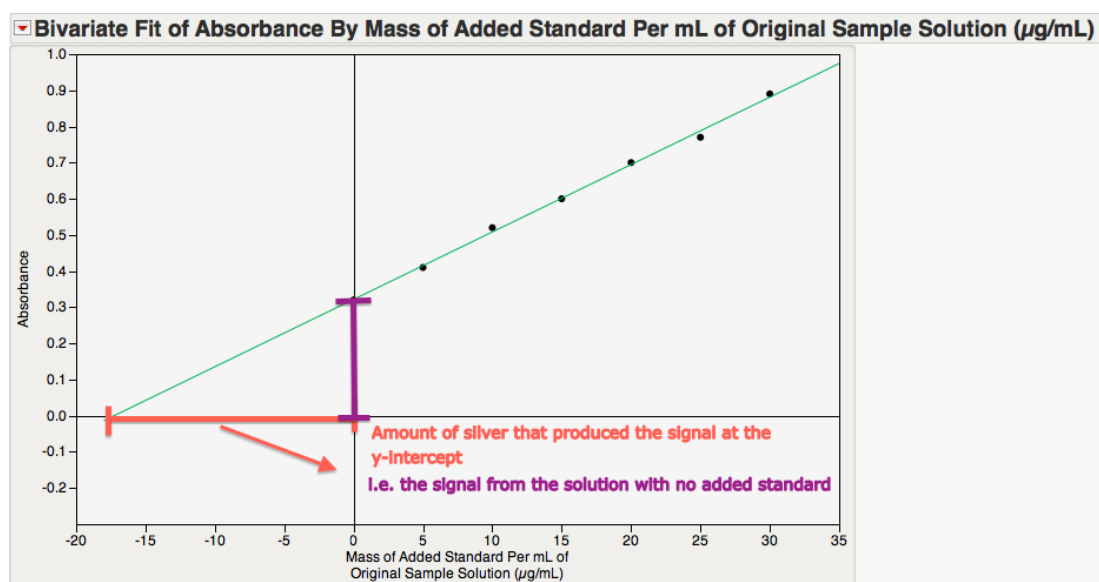
Example:

The following example is from pages 117-120 in "*Statistics for Analytical Chemistry*" by J.C. Miller and J.N. Miller (2nd edition, 1988). The light-sensitive chemicals on photographic film are silver halides (i.e., ionic compounds made of silver and one of the halogens: fluorine, bromine, chlorine and iodine). Thus, silver is often extracted from photographic waste for commercial reclamation.

A sample of photographic waste containing an unknown amount of silver was determined by standard addition with atomic absorption spectroscopy.

Mass of Added Standard Per mL of Original Sample Solution (µg/mL)	Absorbance
0	0.32
5	0.41
10	0.52
15	0.60
20	0.70
25	0.77
30	0.89

Bivariate Fit of Absorbance By Mass of Added Standard Per mL of Original Sample Solution (µg/mL)

Amount of silver that produced the signal at the y-intercept

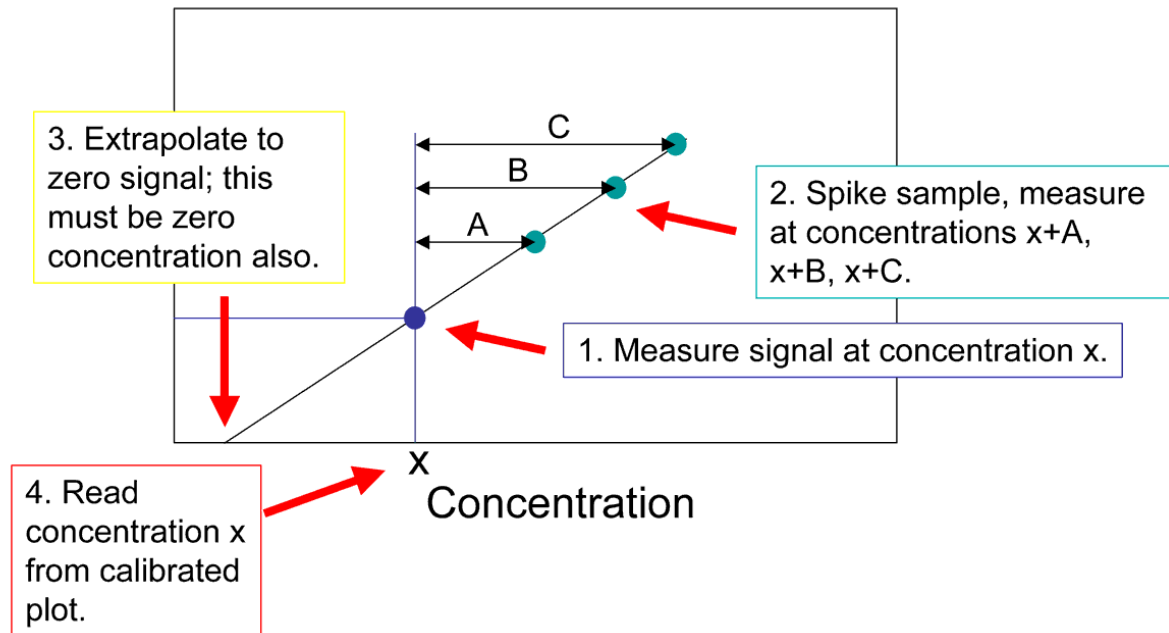i.e. the signal from the solution with no added standard

This plot illustrates the key idea behind using this calibration curve. The magnitude of the x-intercept is the concentration of the silver in the original solution.

To understand why this is so, consider the absorbance at the following two values:

- at x = 0, the value of y is the absorbance of the solution with no added standard (i.e., it corresponds to the concentration of silver that we ultimately want).
- at the x-intercept, there is no absorbance.

Thus, the magnitude of the difference between x=0 and the x-intercept is the concentration of silver that is needed to produce the signal for the original solution of interest.

Steps in computation

3. Extrapolate to zero signal; this must be zero concentration also.

2. Spike sample, measure at concentrations x+A, x+B, x+C.

1. Measure signal at concentration x.

4. Read concentration x from calibrated plot.

Concentration

X

(Source: Wikipedia)

1) Suppose the signal for unknown concentration is 25.
2) Carryout procedure as stated previous.

Addition	Signal
0	25
2	30
4	35
6	40
8	45

Intercept $b_0 = 25$ (i.e. where there was no addition).

Slope can be determined using basic co-ordinate geometry to be $b_1 = 2.5$

(For every unit increase in addition, the signal increases by 2.5)

3) *Zero Signal : $0 = 25 + 2.5 X$*

solving for X we get X = -10.

There are 10 units in the "no addition" sample : we have to remove 10 units to get a zero signal.