STANDARD ADDITION AND INTERNAL ADDITION

STANDARD ADDITION:

The method of standard addition is a type of quantitative analysis approach that is used in analytical chemistry whereby the standard is added directly to the aliquots of the analyzed sample.

In the method of **Standard Additions** addition of a known amount of a standard solution of analyte is done to one portion of the sample. The responses before and after the addition are measured and are used to obtain the analyte concentration. Multiple additions are made to several portions of the sample alternatively.

MAIN BODY:

The method of standard addition is used when it is difficult or impossible to duplicate the sample matrix. Generally, the sample is **SPIKED** with a known amount of standard solution of the analyte.

In **single point standard addition** method, two portions of the sample are taken. One portion is measured as usual but addition of known amount of standard analyte solution is done in second portion. The responses for these two portions are then used to calculate the unknown concentration assuming a linear relationship between response and analyte concentration.

In **multiple addition method** known amount of standard analyte solution is added to several portions of the sample and a multiple addition calibration curve is obtained.
We can avoid the complication of matching the matrix of the standards to the matrix of the sample by conducting the standardization in the sample. This is known as the method of standard addition

SINGLE STANDARD ADDITIONS:

The simplest version of a standard addition is shown in Figure 1.1. First we add a portion of the sample, **Vo**, to a volumetric flask, dilute it to volume, **Vf** and measure its signal, **Ssamp**. Next, we add a second identical portion of sample to an equivalent volumetric flask along with a spike, **Vstd**, of an external standard whose concentration is **Cstd**. After diluting the spiked sample to the same final volume, we measure its signal, **Sspike**. The

following two equations relate **Ssamp** and **Sspike** to the concentration of analyte, **CA**, in the original sample.

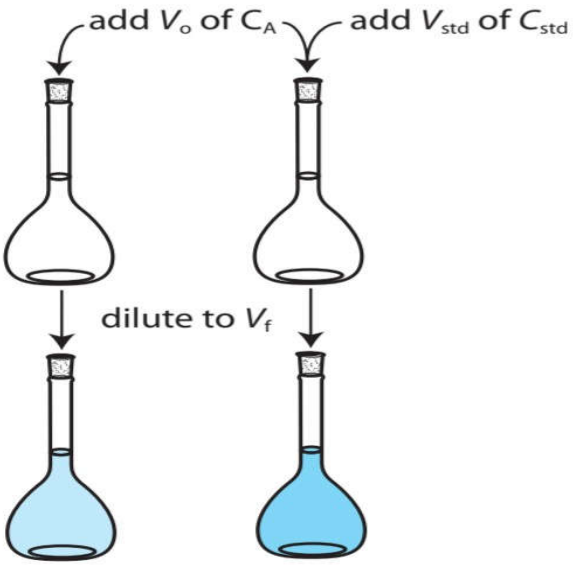


Figure 1.1 Figure showing the method of standard additions. The volumetric flask on the left contains a portion of the sample, **Vo**, and the volumetric flask on the right contains an identical portion of the sample and a spike, **Vstd**, of a standard solution of the analyte. Both flasks are diluted to the same final volume, **Vf**. The concentration of analyte in each flask is shown at the bottom of the figure where **CA** is the analyte's concentration in the original sample and **Cstd** is the concentration of analyte in the external standard.

$$C_A \times \frac{V_o}{V_f}$$

Eq.1

$$C_A \times \frac{V_o}{V_f} + C_{std} \times \frac{V_{std}}{V_f}$$

$$S_{samp} = k_A C_A \frac{V_o}{V_f}$$

$$S_{spike} = k_A \left(C_A \frac{V_o}{V_f} + C_{std} \frac{V_{std}}{V_f} \right)$$

As long as **Vstd** is small relative to **Vo,** the effect of the standard's matrix on the sample's matrix is insignificant. Under these conditions the value of the **ka** is same in both equations.

$$\frac{S_{samp}}{C_A \frac{V_o}{V_f}} = \frac{S_{spike}}{C_A \frac{V_o}{V_f} + C_{std} \frac{V_{std}}{V_f}}$$

Eq. 4

By this the concentration of analyte **CA** is calculated.

Standard addition can possibly be made directly in the sample, measuring signals both before and after the spikes. In this case the final value after the standard addition is **Vo** plus **Vstd.** Then equation 2,3 and 4 becomes

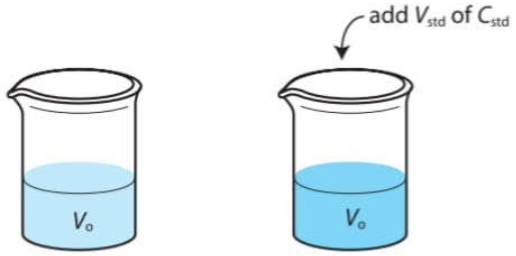


Figure 1.2 is showing the alternate method of standard addition. In this case, the spike is added to the external standard directly to the sample without any further change in volume.

Concentration of analyte **CA**

$$C_A \times \frac{V_o}{V_f} + C_{std} \times \frac{V_{std}}{V_f}$$

EQ.5

$$S_{samp} = k_A C_A$$

$$S_{spike} = k_A \left(C_A \frac{V_o}{V_o + V_{std}} + C_{std} \frac{V_{std}}{V_o + V_{std}} \right)$$

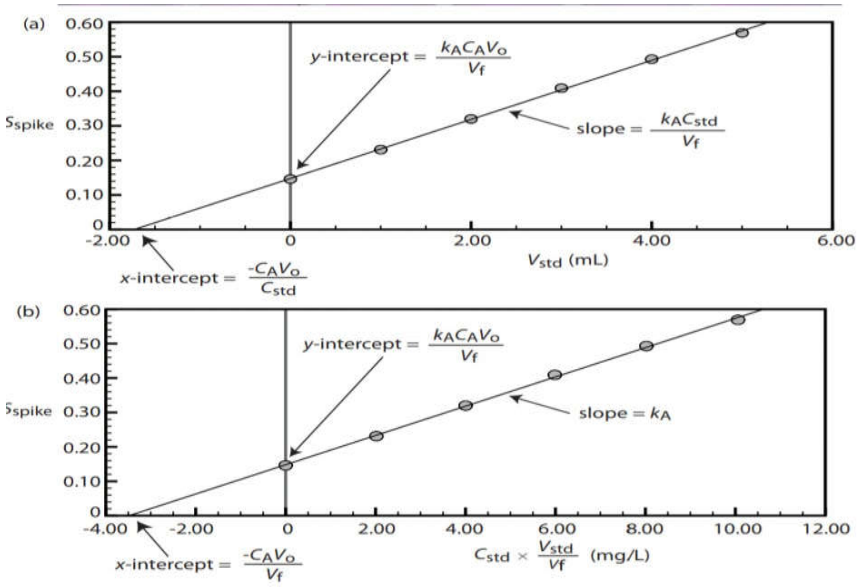
$$\frac{S_{samp}}{C_A} = \frac{S_{spike}}{C_A \frac{V_o}{V_o + V_{std}} + C_{std} \frac{V_{std}}{V_o + V_{std}}}$$

EQ. 6 & 7

MULTIPLE STANDARD ADDITIONS:

We can adapt single point standard addition into a multiple point standard addition by preparation of a series of samples containing increased amount of the external standard. we plot **Sspike** against the volume of the spikes, **Vstd**. If **kA** is constant, then the calibration curve is a straight-line. It is easy to show that the x-intercept is equivalent to **–C_A Vo/ Cstd.**

1.3 shows that at the top there is a set of six standard additions for the determination of Mn^{2+}. The flask is Figure a 25ml sample diluted to 50ml. the remaining flasks contain 25ml sample and from left to right 1,2,3,4, and 5ml of an external standard of 100.6 $\frac{mg}{l}$. Mn^{2+}.



The graphs show two ways to plot the standard addition calibration curve. The absorbance for each standard addition Sspike is shown by filled circles.

Because we know the volume of original sample, **Vo** and the concentration of external standard **Cstd**, we can calculate the analyte's concentration from the x-intercept of a multiple point standard additions.

As we construct a standard addition calibration curve in the sample, this cannot be used for the other samples. Each sample, therefore requires its own standard addition calibration curve.

FOR EXAMPLE

Suppose you have to analyze 10 samples using a three-point calibration curve. For a normal calibration curve you need to analyze only 13 samples (three standards and ten samples). If you use the method of standard addition, you have to analyze 30 solutions (each of the ten should be analyzed three times before and after the spiking.

USE OF STANDARD ADDITION TO IDENTIFY MATRIX EFFECTS

Standard additions can be used to validate the external standardization when matching of the matrix is not possible. For this purpose, first we prepare a normal calibration curve of $\mathbf{S_{std}}$ verses $\mathbf{C_{std}}$ and calculate the value $\mathbf{k_A}$ from its slope. Next, we prepare standard addition calibration curve by plotting the data. The slope of the standard addition calibration curve gives an independent determination of $\mathbf{k_A}$. If there is no noticeable difference between the two values of k_A, then we can overlook the difference between the matrix of sample and that of external standards. When there is significant

difference in the values of **kA**, then by the use of normal calibration curve introduces a proportional determinate error.

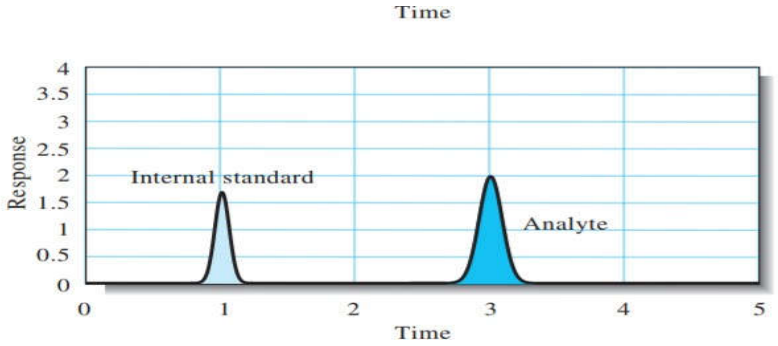
INTERNAL STANDARD METHOD

- The internal standard is used to improve the precision of quantitative analysis.
- An internal standard is a substance of known concentration that is present in every sample that is analyzed.
- The internal standard is a reference species, physically and chemically similar to the analyte, that is added to standards, samples and blanks. The ratio of the analyte response to that of the internal standard is plotted against the concentration of the analyte.

EXPLANATION

In the internal standard method, addition of a known volume of the reference species to all the standards, samples and blanks take place. The signal of the response is not the signal of the analyte but the ratio of the signal of the analyte to the signal of reference species. A calibration curve is prepared where the Y-axis is the ratio of the responses and the X-axis is the ratio of the concentration of the analyte in the standards.

The use of internal standard method for peak-shaped responses is illustrated in the figure 1.4.



If the analyte and the reference species influence to the same proportional extent, the internal standard method can compensate for certain types of errors. For example, if the temperature affects both the reference species and the analyte to the same extant, taking ratio can compensate for variations in temperature. For the compensation to occur the chosen reference species have very similar chemical and physical properties to the analyte.

For internal standard method to compensate for the errors, a suitable reference species must be available. The reference species must not have unique interferences different from the analyte. For the preparation of the internal standard, there must be no analyte contamination in the material used. Concentration of both the species must be in linear potion of their calibration curves. Because of the difficulty in finding an appropriate

internal standard species, the internal standard method is not as commonly as some other error compensating method.

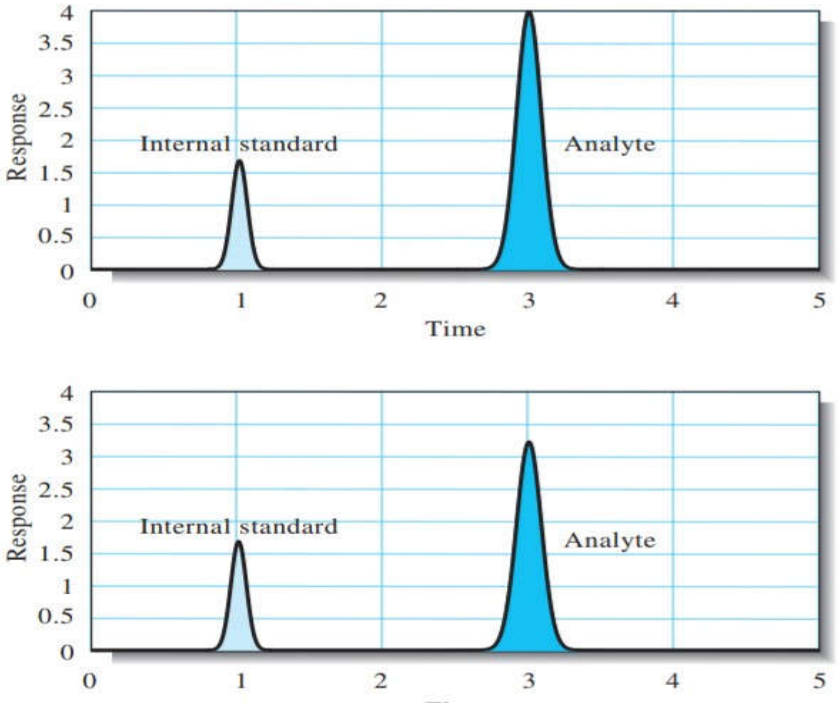


Figure 1.4shows the internal standard method. To all the samples, blanks and standards a fixed amount of internal standard species is added. The calibration curve gives the ratio of the signal of the analyte to the signal of the internal standard against the concentration of the analyte.

EXAMPLE

To use the method of standard additions or an external standardization, we should treat all the samples and standards identically. When it is not possible, the accuracy and precision of our standardization may suffer. For example, if the analyte is a volatile solvent, then its concentration increases on its exposure to evaporation. Suppose we have standard and sample with the identical concentration of the analyte an identical signal. If both of them experience same loss of solvent, then there are corresponding concentrations of analyte and signals continue to be identical. In effect if the samples and standards experience equivalent loss of solvent we can ignore evaporation. If they lose different amount of solvents, then their respective concentrations and signals will no longer be equal. In this case a simple external standardization or standard addition is not possible.

We can complete the standardization if we reference the signal of the analyte to a signal from another species that is added to all samples and standards. The species is called **internal standard**, must be different from the analyte.

In any sample or standard, the analyte and the internal standard receive the same treatment, any lack of reproducibility in their procedure would unaffected the ratio of their signals. if a solution contains an analyte of concentration C_A and internal standard of concentration C_{IS}, then the signals due to analyte S_A and internal standard S_{IS} are:

$$S_A = k_A C_A$$

$$S_{IS} = k_{IS} C_{IS}$$

Where k_A and k_{IS} are the sensitivities of the analyte and internal standard. Taking the ratio of two signals gives the fundamental equation for internal standardization.

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

Because **K** is a ratio of the sensitivity of the analyte and the internal standard, it is not necessary to independently determine values of k_A and k_{IS}.

SINGLE INTERNAL STANDARD

In a single point internal standardization, containing the analyte and internal standard we prepare the single standard and use it to determine the value of **K**.

MULTIPLE INTERNAL STANDARDS

For the construction of an internal standard calibration curve we prepare a series of standards, each containing different concentrations of the analyte and same concentration of internal standard. A calibration curve of $(S_A \; S_{IS})_{std}$ verses C_A is linear with a slope of $K\, C_{IS}$.

In some cases, it is not possible to prepare standards so that each contains the same concentrations of internal standards. This is the same case, for example when the preparation of the samples is done in mass instead of volume.

APPLICATIONS

standard addition method gor the determination of pharmaceutical residues in rinking water by lc-ms/m.

The study of occurrence and the outcome of pharmaceutical products in drinking or waste water systems has become very popular in recent years. In the determination of pharmaceutical residues at trace level in water, LC- MS/MS is a powerful analytical tool used. Many stages may disturb the analytical procedure and the results. A list of 27 environmentally related molecules including therapeutic classes were selected. In the determination of the concentration of the 27 targeted pharmaceutical products at the nano gram per liter level, a method is developed using Ultra Performance Liquid Chromatography coupled to tandum Mass spectrometry (UPLC-MS/MS) and solid phase extraction (SPE) at different treatment stages, the matrix effect is evaluated from water samples. Traditional methods with external calibration and Internal standard correction were compared to the **standard addition method.** By the standard addition method associated with UPLC-MS/MS, an accurate determination of pharmaceutical compounds on drinking water were obtained. The developed method was used to calculate the occurrence and outcome of pharmaceutical compounds in drinking water treatment plants (DWTPs) in the west of France.

RESULT

In 16 samples, matrix effects were calculated for 29 pharmaceuticals. Even in the internal standard correction, the matrix effects were severe, for the standard addition method was necessary for an accurate determination. The developed analytical method was then used to calculate the occurrence and outcome of drug residues in water treatment systems.

Evaluation and application of the internal standard technique for the direct determination of copper in fruit juices employing fast sequencial flame atomic absorbtion spectrometry

By employing Fast Sequential Flame Atomic Absorption Spectrometry (FS-FAAS), the evaluation and application of internal standard for the determination of copper in fruit juices was done. Using the correlation graphs, the internal standards were tested indium, cobalt and nickel. By considering the composition of the samples, the indium was used. The copper was evaluated in fruit juices using indium as internal standard, after the stage. The selected samples of fruit juice for the analysis were of grapes, orange, pineapple, peach, cashew and strawberry. With a limit of quantification of 0.011 mg/L, this method allows the determination of copper. The contents of copper in these samples varied from

the 0.02 to 0.42mg/L. After complete mineralization using acid digestion and employing FS-FAAS, the analytical results were compared with the results obtained by the analysis of these samples.

RESULT

The statistical comparison by a t-test showed no noticeable difference between the results. With and without the use of internal standard the relative standard deviations (RSD) for copper solution containing 0.4mg/L were of 0.62 and 1.94% respectively. The use of indium as internal standard provided more accurate analytical results, as well as better analytical performance for the evaluation of copper in juice samples.