

Gas Chromatography is a useful tool that allows us to identify and quantitate individual components in a mixture. Using individual standards and reproducible conditions enables peak identification by retention time. In most cases this is absolute, that is unless there are two peaks with exactly the same retention time under the analysis conditions. This same "absolute" property cannot be applied to quantitation which is affected by numerous variables.

Quantitation uses chromatographic data to determine the amount of a given component in a mixture. This data can be in the form of either peak height or peak area which is obtained from an integrated chromatogram. It is very important that this data is gathered accurately. It is best if the peak is totally resolved from any neighboring peaks. A co-elution or other anomalies such as tailing or fronting will distort or obscure the beginning and ending points of the peak making it difficult to accurately determine the size of the peak.

Quantitation Methods

There are several types of quantitation methods commonly used. The five most common are area percent, single point external standard, multiple point external standard, single point internal standard, and multiple point internal standard.

Area Percent Method

Area percent is the simplest quantitation method. This method assumes that the detector responds identically to all compounds. This assumption, however, is not valid. This method provides a rough estimate of the amounts of analytes present.

To calculate area percent take the area of an analyte and divide it by the sum of areas for all peaks. This value represents the percentage of an analyte in the sample.

Single Point External Standard

Unlike the area percent method, the Single Point External Standard method requires the analysis of more than just the sample of interest. Analyze a sample containing a known amount of analyte or analytes and record the peak area. Then calculate a response factor using **Equation 1**.

EQUATION 1

$$\text{response factor} = \frac{\text{peak area}}{\text{sample amount}}$$

Inject a sample with the unknown analyte concentration and record the peak area. Then calculate the amount of analyte using **Equation 2**.

EQUATION 2

$$\text{amount of analyte} = \frac{\text{peak area}}{\text{response factor}}$$

Calculate an individual response factor for each compound of interest.

SINGLE PT. EXT. STD. EXAMPLE

An injection containing benzene at a concentration of 2,000 $\mu\text{g}/\text{ml}$ is made and results in a peak area of 100,000. Calculate the response factor for benzene using **Equation 1**.

$$\text{response factor} = \frac{100,000}{2,000} = 50$$

An injection of the sample with the unknown concentration of benzene has a peak area of 57,000. Calculate the amount of benzene present using **Equation 2**.

$$\text{amount of benzene} = \frac{57,000}{50} = 1,140 \mu\text{g}$$

Multiple Point External Standard

The Single Point External Standard method assumes analyte response to be linear over a range of concentrations. (**Figure 1**). Use the Multiple Point External Standard method when the concentration range is large or if the single point external standard method is not linear (**Figure 2**). The samples used in this method cover the expected analyte concentration range. Use a line fitting algorithm such as point to point, linear least squares, or quadratic least squares to produce a calibration curve. See **Figure 2**. Most modern data systems include one or all of these algorithms.

FIGURE 1

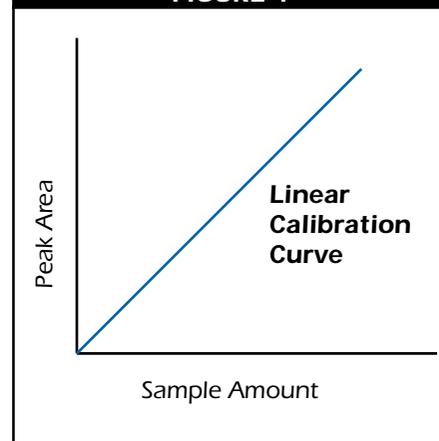
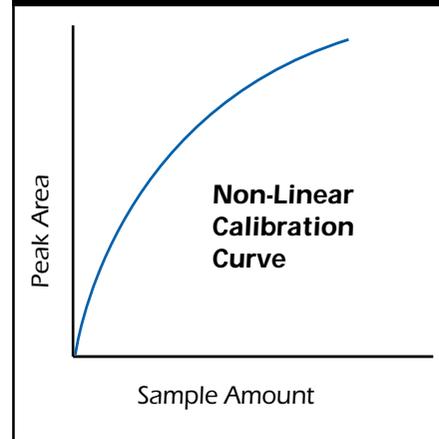


FIGURE 2



Single Point Internal Standard

Unlike external standard methods, the internal standard method accounts for any variances in gas chromatograph performance. The analyte chosen for the internal standard has a predictable retention time and area, allowing it to be used to determine if abnormalities have occurred.

The Single Point Internal Standard method requires at least two analyses. The first analysis contains a known amount of internal standard and the compounds of interest. Calculate the response factor using **Equation 3**.

EQUATION 3

Internal Response Factor =

$$\frac{\text{area}_{\text{IS}} \times \text{amount}_{\text{SC}}}{\text{amount}_{\text{IS}} \times \text{area}_{\text{SC}}}$$

IS = Internal Standard
SC = Specific Compound of Interest

Then add a known amount of the internal standard to the sample containing analytes of unknown concentrations. Calculate the amount of the unknown analyte using **Equation 4**.

EQUATION 4

amount of specific compound =

$$\frac{\text{amount}_{\text{IS}} \times \text{area}_{\text{SC}} \times \text{IRF}_{\text{SC}}}{\text{area}_{\text{IS}}}$$

IS = Internal Standard
SC = Specific Compound of Interest
IRF = Internal Response Factor

Multiple Point Internal Standard

This method, like the Multiple Point External Standard, uses several analyses. Each analysis contains the internal standard whose concentration is kept constant and the analyte of interest whose concentration covers the range of concentrations expected. Plot the results with the ratio of the area of the analytes to the area of the internal standard on the y-axis and the ratio of the concentration of the analytes to the concentration of internal standard on the x-axis. Fit this data to a curve using methods previously described.

Analyze the samples with unknown analyte concentrations. Determine the ratio of the analyte area to internal standard area from the data. The corresponding ratio of analyte concentration to internal standard concentration is determined from the graph. Multiply the concentration of internal standard in the sample by this ratio. This yields the concentration of the analyte in the unknown sample.

Quantitation can be as easy as the area percent method or as in depth as the multiple point internal standard method. Each method varies with the degree of accuracy and ease of performance. You should pick the one which is right for you.

SINGLE POINT INTERNAL STANDARD EXAMPLE

Prepare a sample containing 2,000 µg/mL of toluene (the internal standard) and 1,000 µg/mL benzene (the analyte). Then inject the sample. The resulting peak areas are 120,000 for toluene and 67,000 for benzene. Using **Equation 3** the response factor for benzene is:

$$\begin{aligned} \text{Internal Response Factor} &= \\ & \frac{120,000 \times 1,000}{2,000 \times 67,000} \\ &= 0.8955 \end{aligned}$$

Inject the sample containing 2,000 µg/mL of toluene and an unknown amount of benzene using the same chromatography conditions. The resulting areas are 122,000 for toluene and 43,000 for benzene. Calculate the amount of benzene present using **Equation 4**.

$$\begin{aligned} \text{Amount of Benzene} &= \\ & \frac{2,000 \times 43,000 \times 0.8955}{122,000} \\ &= 631 \mu\text{g} \end{aligned}$$