Quantitative Analysis in HPLC:

Quantitative column chromatography is based upon a comparison of either:

1) the height of the analyte peak with that of one or more standards.

2) the area of the analyte peak with that of one or more standards.

If conditions are properly controlled, these parameters vary linearly with concentration.

Areas are a more satisfactory analytical parameter than peak heights. On the other hand, peak heights are more easily measured and for narrow peaks, more accurately determined. (accurate results results are obtained with peak heights only if variables in column conditions do not alter the peak widths during the period required to obtain chromatograms for sample and standards because the peak heights are inversely related to peak widths.

"**Calibration**" is the process of establishing a calibration curve of the specified analyte from a set of injected calibration standard solutions.

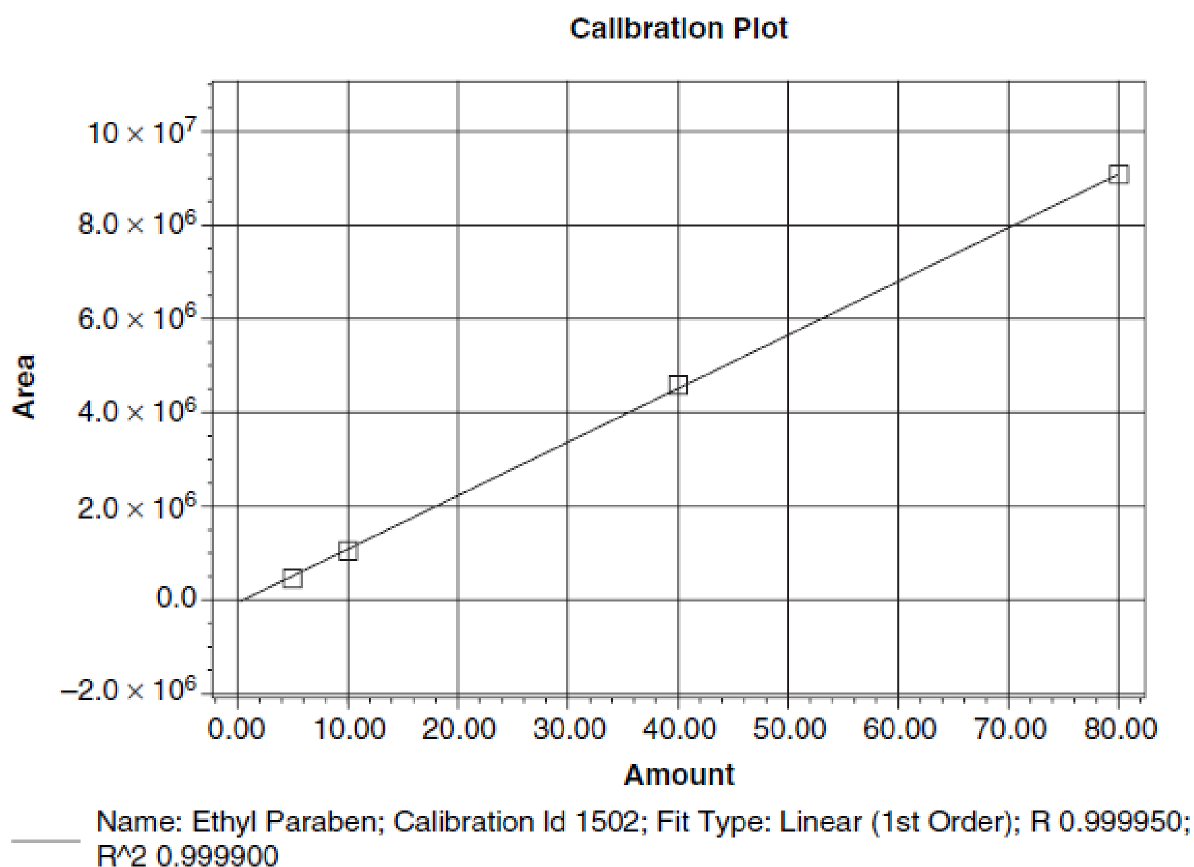

Figure 5.10. Typical calibration curve using external standardization (linear calibration through origin). Note that the slope of the curve is equal to the response factor of the analyte.

This Figure shows a calibration curve plotting the peak area of the analyte against the amount injected. The response factor (R_f) can be calculated from the slope of the curve or by dividing the peak area with the amount according to the equation below. In "quantitation," peaks in the unknown samples are identified by comparing them with retention times of the component list in the processing method. The amount of the sample can then by

calculated by dividing the peak area by its respective response factor.

$$Response\ Factor\ (R_f) = \frac{Area_{Std}}{Amount_{std}} = \frac{Area_{sample}}{Amount_{sample}}$$

The Internal Standard Method:

To avoid the uncertainties introduced by sample injection, a carefully measured quantity of an internal standard substance is introduced into each standard and sample, and the ratio of analyte to internal standard peak areas (or heights) serves as the analytical parameter.The internal standard peak should be separated well from the peaks of all other components of the sample ($R_s > 1.25$).

The Area Normalization Method:

Another method to avoid the uncertainties associated with sample injection is the area normalization method where the areas of all eluted peaks are computed, the concentration of the analyte is found from the ratio of its area to the total area of all peaks.

Example:

The following area data were obtained from a chromatogram of a mixture of butyl alcohols:

Alcohol	Peak area, cm^2	Detector response factor	Reduced areas, cm^2
n-butyl	2.74	0.603	4.54
i-butyl	7.61	0.530	14.36
s-butyl	3.19	0.667	4.78
t-butyl	1.66	0.681	2.44
			26.12

Each entry in column 4 is the quotient of the data in columns 2 and 3. To normalize,

% *n*-butyl = (4.54/26.12) x 100 = 17.4

% *i*-butyl = (14.36/26.12) x 100 = 55.0

% *s*-butyl = (4.78/26.12) x 100 = 18.3

% *t*-butyl = (2.44/26.12) x 100 = 9.3

100.0%