Calibration/standard curve

A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte. A calibration curve, constructed with blank sample and zero-sample (blank with internal standard) and 7 non-zero standard samples, including lower limit of quantification (LLOQ) sample was used in the validation and the analysis of the study. Concentrations of standards were 50 ng/ml, 100 ng/ml, 500 ng/ml, 1000 ng/ml, 2500 ng/ml, 5000 ng/ml and 8000 ng/ml. They were chosen on the basis of the concentration range expected in the study.

1. Lower limit of quantification (LLOQ)

The lowest standard point on the calibration curve (CC) was accepted as the lower limit of quantification (LLOQ) if the following conditions were met:

a. No interference presented in blanks at the retention time or the analytes
b. Analyte peak identifiable, discrete and with an accuracy between 80% - 120% in relation to the nominal standard concentration.
c. Precise on (CV %) and accuracy of 20%, calculated among duplicate standards when both are approved if a sample has an estimated concentration that falls below the lower limit of quantification (LLOQ) its value was not extrapolated and, therefore, reported as zero or BLQ (Below the Limit of Quantification).

2. Calibration curve /concentration-response

The linearity of the method was illustrated by weighted least squares regression analysis of standard plots associated with a seven point validation and graphically by plotting peak area ratios of standards versus concentration for each sample set.