

evaluated by calculating the linear regression (correlation coefficient, R), and by evaluating the back calculated concentrations of the calibration standards.

Accuracy

The accuracy of the assay was defined as the absolute value of the ratio of the back calculated mean values of the unknown samples and their nominal values expressed as a percentage. Accuracy in the range of 80 - 120% was acceptable for limit of quantitation samples (first point in the standard curve) and in the range of 85 - 115% was acceptable for other samples.

Precision

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of plasma matrix. The precision of the assay was measured by the intra-day and inter-day percent coefficient of variation over the concentration range of 50 to 8000 ng/ml for rhein during the course of the validation. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV. Precision was estimated from the standard curve and the quality control samples.

Sensitivity

The limit of quantification is 50 ng/ml for rhein calculated as 5% of the reported C_{max} for rhein.