b. Limit of Quantization= 50 ng/ml.
c. The standard curve was chosen to be from 50 to 8000 ng/ml and three quality control samples, {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml} were prepared in triplicate.

The lower limit of quantification of rhein 50 ng/ml, where this value is considered to be sufficient in order to achieve the aim of this study.

The method validation (US FDA guideline, 2001; Shah et al., 2000) include a pre-study validation with determination of stability of the stock solutions and of the rhein in the biological matrix under processing conditions and during the entire period of storage, selectivity, recovery, accuracy, precision, limit of quantification (sensitivity), dilution integrity and system suitability, as well as online validation during analysis with control samples at three concentration levels.

Method Validation

Validation of the analytical method was performed in order to evaluate the method in terms of recovery, linearity of response, accuracy, precision, sensitivity, stability and specificity. The validation was performed on three separate days with a seven point standard line (not including zero) on each day. Each day of validation included plasma samples representing ten Q.C.s of each level {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml}.

The validation included:

1. Quality control (Q.C.) samples in plasma for three days before the start of the study.