Validation for the quality control samples, low, medium and high, were carried out before the start of the study for three days.

2. Calibration curve in plasma for three days before the start of the study.
3. Quality control samples in plasma during the analysis of the subjects' plasma.

A (low, medium and high), quality control samples were conducted during the analysis of the plasma samples.

4. Standard curve in plasma during the analysis of the subjects' plasma samples

Recovery

The percent recovery was determined by measuring the absolute peak area of rhein and the internal standard from a plasma sample prepared according to the method of analysis. Plasma samples containing concentrations of {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml} were prepared in triplicate. The absolute peak areas obtained from the injections of the prepared plasma standards were compared to the absolute peak areas of an equivalent aqueous standard, which was prepared to contain a concentration of rhein or internal standard assuring 100% recovery. The extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible.

The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV). The recovery of rhein was acceptable at the concentrations studied.