

### Selectivity and matrix interferences

The absence of matrix interferences was demonstrated by the analysis of 6 independent sources of control matrix. Plasma samples from different volunteers were taken, and treated as per the analytical method adopted for the analysis of rhein. Each independent control matrix after being assessed and proved no interference was used to produce standard curves and quality control samples throughout the whole study.

### Dilution Integrity

The ability to dilute samples originally above the upper limit of the standard curve is demonstrated by accuracy and precision parameters.

### Stability

For the determination of rhein stability under the analytical conditions of the assay method plasma samples containing rhein concentration of {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml} were prepared in triplicate and the stability was studied as indicated. The analytical results for the stored samples were compared with those for the freshly prepared samples.

The stability of rhein was determined as follows:

#### a. Short Term Stability at Room Temperature

Three samples with concentrations of {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml} were thawed at room temperature and kept at this temperature and analyzed at 0.0 and 6.0 hours.