Best fit calibration lines of chromatographic response versus concentration were determined by weighted least squares regression analysis with a weighing factor equal to 1/X. The coefficient of determination was consistently greater than 0.99 during the course of the analysis.

The following conditions should be met in developing a calibration curve:

- A standard curve should be defined by at least 5 concentrations.
- 15% deviation from nominal concentration for each concentration except the first concentration 20%.
- At least 75% out of the non-zero standards should meet the above criteria.
- Intercept not more than 20% of the value of the ratio of the lowest point in the standard curve.
- Correlation coefficient should be more than 0.98
- Rejected points must not be any two successive points.
- Rejected points must not be the first or last point in the standard curve.
- Best fit calibration lines of chromatographic response versus concentration were determined by weighted least square regression analysis with the Suitable weighing factor.

3. Linearity and linear working range

Six calibration curves each consisting of a blank, zero (blank and internal standard) and seven non-zero standards prepared in human plasma were chromatogramed. The concentrations of calibration standards cover the range from lower limit of quantitation 50 ng rhein /ml to the highest expected concentration of rhein about 8000 ng/ml. The calibration standards were prepared as above and the linearity was