

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