B. 95% confidence interval was calculated for each specificity cut point to ensure a rate of 5% false positives (t0.05 = 1.645). After outlier determination using the boxplot method, data for the % inhibition was divided into runs and the corresponding % inhibition was performed on the individual run data using a parametric and non-parametric approach.

C. The % inhibition is calculated using the following formula:

\[
\% \text{inhibition} = 100 \times \left(1 - \frac{\text{drug inhibited sample}}{\text{uninhibited sample}}\right)
\]

D. The applied statistical procedure allowed an objective setting of the cut points. More differences in the specificity cut point calculated by the non-parametric and parametric approach were found, resulting in a change in the cut point values using the parametric method. Moreover, in both cases, the cut point values were lower than the desired cut point values which were set to 0.10. Thus, it might be necessary to re-evaluate the % inhibition using a higher threshold for % inhibition to be acceptable. This will be evaluated in future studies.

E. The Levene’s test showed that run variances are statistically different (p-value 0.031). According to the recommendations given in [1], an instrument or analyst specific floating cut point or a dynamic cut point should be applied then. However, the applied statistical method of outlier identification could possibly be more feasible for the determination of the specificity cut point.

F. The observed ratio of falsely confirmed positives exceeded the target range by more than a factor 10 because of the matrix effect. The applied statistical procedure allowed an objective setting of the cut points.

G. The CONCLUSION section states that the assay was validated according to the recommendations given in [1]. The assay sensitivity was calculated at a spike concentration of 91.7 ng/mL. The matrix effect on the recovery of low spike concentrations was evaluated, resulting in a matrix effect of 85.0% - 107.1% at the 91.7 ng/mL spike concentration. The recovery of low spike concentrations in human serum samples and assay buffer was evaluated, resulting in a matrix effect of 91.9% - 105.3% at the 91.7 ng/mL spike concentration.

H. The assay sensitivity for 9230 human serum samples was determined at a spike concentration of 91.7 ng/mL. The assay sensitivity was calculated at a spike concentration of 91.7 ng/mL. The matrix effect on the recovery of low spike concentrations was evaluated, resulting in a matrix effect of 85.0% - 107.1% at the 91.7 ng/mL spike concentration. The recovery of low spike concentrations in human serum samples and assay buffer was evaluated, resulting in a matrix effect of 91.9% - 105.3% at the 91.7 ng/mL spike concentration.