THE DEVELOPMENT OF AN ELISA ASSAY FOR THE DETERMINATION OF RITUXIMAB IN HUMAN SERUM

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Introduction

Rituximab is a chimeric monoclonal antibody used in the treatment of diseases characterized by abnormal or excessive B cells. It targets CD20, which is expressed on the surface of B cells, to induce apoptosis in B cells expressing CD20 and is used in the treatment of leukemias and lymphomas, some autoimmune disorders, and transplant rejections.

A quantitative method for rituximab has been developed and optimized for pharmacokinetic assessment of samples.

Method

Diluted serum samples were pipetted onto microplates previously coated with an appropriate capture antibody. The wells were washed to remove any unbound sample material and an enzyme-labeled antibody added. Unbound labeled antibody was removed and a chromogenic substrate added, resulting in development of the colored reaction product being directly proportional to the amount of rituximab present in the sample. The microplate was then analyzed using a colorimetric plate reader.

Results

Rituximab uses a 4-parameter logistic regression weighted $1/Y^2$ over the analytical range 1.00–50.00 µg/mL. The concentrations of rituximab standards were back-calculated using the regression equation and the coefficient of variation (C.V.) was less than or equal to 5.0 percent.

Inter-batch precision (percent CV) and inter-batch accuracy (percent Bias) of rituximab quality control samples between 1.00 and 50.0 µg/mL was less than 16.4 and -7.5, respectively.

Table 1. Rituximab Inter-Batch Precision and Accuracy

Rituximab	LLOQ QC 1.00 µg/mL	Low QC 3.00 µg/mL	Mid QC 12.0 µg/mL	High QC 37.5 µg/mL	ULOQ QC 50.0 µg/mL
Inter-Batch Mean	0.987	2.97	11.8	37.4	46.3
Inter-Batch SD	0.16	0.21	0.47	1.2	2.3
Inter-Batch % CV	16.3	7.1	4.0	3.2	5.0
Inter-Batch % Bias	-1.3	-1.0	-1.7	-0.3	-7.4
n	9	9	9	9	9

The integrity of rituximab hemolyzed samples was verified by preparing a sample at the mid QC concentration in human serum fortified with 5 percent whole blood. The hemolyzed sample quantitated 11.7 percent higher than the theoretical concentration indicating that hemolysis does not have a significant impact on the quantitation of rituximab samples.

Long-term stability of low and high QC rituximab concentrations in human serum was evaluated for samples stored at -80°C. The data met acceptance criteria indicating samples have a stability of at least 93 days.

Short-term stability of rituximab in serum was established for 20 hours at ambient temperature under white light.

Freeze and thaw stability of rituximab in serum was established for six freeze (-80°C) and thaw (ambient temperature) cycles.

Table 2. Freeze (-80°C)-Thaw and Short-Term Stability of Rituximab in Human Serum

	FT Mid QC 12.0 µg/mL	STS Mid QC 12.0 µg/mL
	11.9	11.5
	10.1	10.9
	9.90	10.1
	9.60	10.2
		9.77
		9.80
Mean	10.4	10.4
% CV	9.9	6.6
% Theoretical	86.7	86.7
n	4	6

Samples fortified at the LLOQ and high QC concentrations in 10 lots of matrix were evaluated to determine if there were any matrix effects associated with this method. Eight of ten samples prepared at the LLOQ concentration quantitated within 25 percent of their theoretical concentration and nine of nine samples prepared at the high QC concentration quantitated within 20 percent of their theoretical concentration. This data indicated there are no significant matrix effects associated with this method.

An evaluation of dilution integrity demonstrated that a dilution factor can be applied to rituximab samples to dilute them into the quantifiable range.

The absence of a hook effect (an artifact causing samples with concentrations greater than the ULOQ to back-calculate within the analytical curve range) was demonstrated for rituximab by assaying a sample with a concentration higher than the ULOQ. The sample assayed back-calculated with concentrations above the ULOQ indicated there are no hook effects associated with this method.

Conclusion

A method has been developed, that allows for a rapid, accurate, and reproducible assay of rituximab in human serum samples. This method is available for use in the comparative analysis of rituximab and a biosimilar compound.