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

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Introduction

Pegfilgrastim is a PEGylated form of filgrastim, a recombinant human granulocyte colony-stimulating factor (GCSF). The PEGylation of the molecule (with the n-terminus addition of 20 kDa polyethylene glycol) extends the half-life of the protein from 3 – 4 hours to 15 – 80 hours. The use of pegfilgrastim treatment serves to stimulate the bone marrow to produce more white blood cells (neutrophils) to help fight infection in patients undergoing chemotherapy.

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

Method

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 pegfilgrastim present in the sample. The microplate was then analyzed using a colorimetric plate reader.

Results

Pegfilgrastim uses a 4-parameter logistic regression weighted $1/Y^2$ over the analytical range 0.200 – 10.0 ng/mL. The concentrations of pegfilgrastim standards were back-calculated using the regression equation and the coefficient of variation (C.V.) was less than or equal to 5.5%.

Inter-batch precision (%CV) of pegfilgrastim quality control samples between 0.200 and 10.0 ng/mL was less than 10.2. Inter-batch accuracy (% Bias) of the same quality controls samples was between -11.6 and +5.0.

Table 1. Pegfilgrastim Inter-Batch Precision and Accuracy

Pegfilgrastim	LLOQ QC 0.200 ng/mL	Low QC 0.600 ng/mL	Mid QC 2.00 ng/mL	High QC 7.50 ng/mL	ULOQ QC 10.0 ng/mL
Inter-Batch Mean	0.199	0.630	2.08	6.63	9.07
Inter-Batch SD	0.0148	0.0219	0.0644	0.446	0.926
Inter-Batch % CV	7.5	3.5	3.1	6.7	10.2
Inter-Batch % Bias	99.5	105.0	104.0	88.4	90.7
n	15	15	15	14	15

Combined short-term and freeze-thaw stability was established at the low and dilution (200 ng/mL) QC concentrations for three freeze (-20°C) and thaw (ambient temperature) cycles with a longest thaw period of 25 hours and a total of 31 hours at ambient temperature under white light.

Table 2. Freeze (-20°C)-Thaw and Short-Term Stability of Pegfilgrastim in Human Serum

Pegfilgrastim	Low QC 0.600 ng/mL	Dilution QC 200 ng/mL
	0.587	177
	0.532	182
	0.613	180
	0.630	183
	0.577	142
	0.565	158
Mean	0.584	170
% CV	6.0	9.8
% Theoretical	97.3	85.0
n	6	6

The integrity of pegfilgrastim hemolyzed samples was verified by preparing a sample at the low and high QC concentrations in five different human serum lots fortified with 5% whole blood. Four of the five lots quantitated within ±20.0% of the theroretical concentration for both concentrations indicating that hemolysis does not have a significant impact on the quantitation of pegfilgrastim samples.

Samples fortified at the LLOQ and high QC concentrations in 8 lots of matrix were evaluated to determine if there are any matrix effects associated with this method. Seven of eight samples prepared at the LLOQ concentration quantitated within 25.0% of their theoretical concentration and seven of eight samples prepared at the high QC concentration quantitated within 20.0% of their theoretical concentration. This data indicates there are no significant matrix effects associated with this method.

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

Table 3. Dilution Integrity of Pegfilgrastim in Human Serum

Pegfilgrastim	DF = 50 200 ng/mL	DF = 40 200 ng/mL	DF = 100 200 ng/mL
	189	168	172
	177	170	164
	171	163	168
	208	167	174
	183	184	170
	204	225	176
Mean	189	180	171
% CV	7.8	13.0	2.5
% Theoretical	94.5	90.0	85.5
n	6	6	6

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 pegfilgrastim by assaying samples with three different concentrations higher than the ULOQ. The samples assayed back-calculated with concentrations above the ULOQ indicating 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 pegfilgrastim in human serum samples. This method is available for use in the comparative analysis of pegfilgrastim and a biosimilar compound.