

Abstract

The aim of this project is to solve the problems of time consuming, yield/ quality/ quantity various when using 2-stage ammonium sulfate precipitation on purification of horse IgG F(ab)₂. Base on the present manufacture protocol, we reformed and improved some of the protocols, which include (1) removed the precipitate from 14% ammonium sulfate reacted mixture, (2) collected the precipitate from 36% ammonium sulfate reacted mixture, (3) shorten the dialysis period, (4) filtrated the drug substance, (5) clarified the manufactory parameter, and(6) established the check point. So as to standardize the batch manufacture.

In this project, we established the manufacture process as the scale in 1.0kg and 3.0kg and inferred to the scale in 20L. To improve the filtrate efficiency of precipitate removed from 14% ammonium sulfate reacted mixture, we change the material from TOYO 131 paper (3um) to medical grade filtrate bag (10um and 1um) followed by disposable capsule filter (1.2um). Flow rate greater than 2.0 L/min could be achieved in new process. Precipitate collecting from 36% ammonium sulfate reacted mixture was performed by medical grade filter bag (1um). After re-suspend the precipitate in DI water, sterilize the drug substance by filtrated with 0.45 / 0.2um disposable capsule filter. For dialysis process, we conjugated Tangential flow filtration (TFF) with diafiltration system to replace the traditional dialysis, to shorten the processing period from 7 days to 2-3 hours. Linear scale-up on manufacture can be easily achieved by parallel connection with several apparatus. To ensure the sterility of the drug substance, 0.45um filter in original process was replaced with 0.45/0.22um sterilized disposable capsule filter.

Quality control check points, including activity, quantity, yield and sterility, were built in the manufacture process as the index of process change. To be aimed at intermediate and final product evaluation, several quality control methods were established, including protein quantitation, horse IgG activity quantity by ELISA, F(ab)₂ activity quantity by ELISA, purity by SDS-PAGE, identification by Western blot and sterility test.

Keywords : diafiltration ; cGMP ; trangular flow filtration