Abstract

The goal of this project is using chromatographic process to substitute the conventional ammonium sulfate salting –out procedure in the purification from anti-venom horse plasma. This project is to establish a stable and fast process to meet the cGMP compliance.

In this project, we employed 100 ml and 1L horse sera as the study model and then to apply the experience in a 20L purification process in ratiocination. In the first step, pepsin was added for 3 hours at 37℃and the pH was stayed at 3.2. Horse IgG was then digested into F(ab)2 and Fc regions. The F(ab)2 was then extracted by 14% ammonium sulfate. The intermediate product was then filtered with a 10μm and a 1μm pharma-grade filter bags. It was then filtered with a 0.2 m filter for aseptic filtration to eliminate the contamination. After the above steps, tangential flow filtration and diafiltration were employed. In the process, a 30 Kd 6 ft^2 column was conducted for de-salting, concentration and 10 fold quantity of the formulation buffer exchange. The intermediated product was then purified with a SP cation exchange chromatography. Most of the product was collected in the elution peak. The elution peak was then conducted with the tangential flow for formulation buffer exchange, concentration and diafiltration. The product was then filtered with a 0.2 m filter for aseptic filtration. The product from the new process was compared with the one from CDC traditional process and it showed equal effectiveness and purity. The whole process can be shortened into 2 days in a 1L horse plasma origin.

Some problems were in the process development, such as the incompleteness and storage too long in the raw material. Once the problems were solved, the newly developed process which used 14% ammonium sulfate precipitation and followed by a SP cation exchange chromatography is worthy for application in the anti-venom product for the reasons of less contamination, more controllable, a shorter process time, higher purity and yield.

Keywords：diafiltration；cGMP；tangential flow filtration and ion exchange