Analysis of the Influence of Five Variables on an Established Immunoaffinity Chromatography Procedure to Purify a Picchia pastoris Yeast Derived-HBsAg for Pharmaceutical Use

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SUMMARY. Immunoaffinity chromatography (IAC) has a wide application in protein purification. The aim of the study was to investigate the influence of five operational factors on an IAC procedure to purify a yeast derived-Hepatitis B surface antigen (rHBsAg) for pharmaceutical use. The immunosorbert adsorption capacity of the rHBsAg is affected at 4 °C. The applied antigen concentration, 100-1000 μg mL–1, does not have influence on this IAC efficiency. The residence time, applied antigen amount and column geometry have a significant influence on the adsorption and elution capacity, and recovery of the rHBsAg. There is a marked retention of the rHBsAg into the matrix, which is reduced at residence times higher than 2 h. A high height/diameter ratio of the column, 3.75, reduces the antigen adsorption to the matrix but increases the recovery and the productivity for this high molecular weight (multiple chemical forms) antigen and a high affinity constant ligand. The solution of the rHBsAg retention into the matrix could increase by 30% the active pharmaceutical ingredient purification and the Hepatitis B vaccine production.

KEY WORDS: Hepatitis B surface antigen, Immunoaffinity chromatography, Purification of rHBsAg, Protein purification.

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