THEORETICAL AND EXPERIMENTAL STUDIES FOR DEVELOPMENT AND OPTIMIZATION OF LIQUID CHROMATOGRAPHY STEPS FOR PURIFICATION OF A BIOTHERAPEUTIC PROTEIN

by

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to the

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.....Dedicated

to my

Parents and sister

CERTIFICATE

This is to certify that the thesis entitled **" THEORETICAL AND EXPERIMENTAL STUDIES FOR DEVELOPMENT AND OPTIMIZATION OF LIQUID CHROMATOGRAPHY STEPS FOR PURIFICATION OF A BIOTHERAPEUTIC PROTEIN"** being submitted by RAHUL S. BHAMBURE to the Indian Institute of Technology, Delhi, for the award of the degree of Doctor of Philosophy, is a record of bonafide research work carried out by him. RAHUL S. BHAMBURE has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

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…….RAHUL

ABSTRACT

In today's competitive market, besides being first to the market, quality and cost-effectiveness of the product are also key drivers for process development. Protein biopharmaceutical products are typically produced along with a variety of impurities, some of which are product-related and have very similar physicochemical properties as the product itself. Methionine oxidized, reduced and fMet forms of native recombinant protein are often some of the critical quality attributes (product related impurities) associated with proteins expressed as bacterial inclusion bodies. Such oxidized and reduced impurities differ from native protein in their structural and functional aspect, causing loss of biological activity. In bacterial expression systems, such as *E. coli.* all synthesized proteins retain an amino-terminal formyl-methionine or methionine residue. This particular variant may lead to immunogenic response in patient. Lack of selective removal of these product related variants using conventional ion exchange or hydrophobic interaction chromatography is a critical bottleneck of existing manufacturing processes.

The key objective of the thesis is creation of efficient approaches for downstream processing of biotech therapeutics for removal of various product as well as host-cell related impurities. Granulocyte colony stimulating factor (GCSF) is selected as a model protein for the study. QbD based process development approach was adopted for evaluation of the various chromatography (Ion exchange and multimodal chromatography) and non chromatography (Aqueous two phase separation) based purification techniques for isolation of product variants and host-cell impurities. The experimental investigation reported in this thesis has led to several significant findings. First, a high throughput process development platform has been created to facilitate thorough but time and resource efficient examination of process chromatography steps. Second, highly efficient novel multimodal chromatography based platform has been proposed for removal of the various product as well as host cell related

impurities. And finally an aqueous two phase assisted precipitation platform has been proposed for the removal of various host related impurities. We believe that together these proposed tools can be used to create highly efficient and productive biopharmaceutical processes.

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