UPSTREAM PROCESS DEVELOPMENT APPROACH TO SELECTIVELY REDUCE ONE OR MORE UNPAIRED CYSTEINES OF A MONOCLONAL ANTIBODY

Coralie Borrossi, Fresenius Kabi SwissBioSim
coralie.borrossi@fresenius-kabi.com
Simon Fradin, Fresenius Kabi SwissBioSim
Periklis Selevos, Fresenius Kabi SwissBioSim
Dean Houston, Fresenius Kabi SwissBioSim

Key Words: Upstream process development (USP), Quality by design (QbD), critical quality attributes (CQA) tuning, unpaired cysteines reduction

Over the last couple of decades, biosimilar medicines have been successfully incorporated in the healthcare market and have experienced an ever-accelerating growth since. Developing a biosimilar represents unique technical challenges, as their development requires strict quality, safety and efficacy targets.

During the early stages of biosimilar process development, one of the main focuses is to achieve biosimilarity for quality target via precise critical quality attributes (CQAs) tuning. As resorting to additive supplementation can involve additional regulatory and quality challenges, the preferred approach is usually to tune CQA levels by changing process parameters setpoints, when possible.

During early development of the presently investigated antibody, produced by a CHO cell line, one of the main challenges encountered was a high level of paired cysteine measured in the molecule (and more specifically in the Complementarity-Determining Regions) at the end of the cell culture which needed to be removed, with significant impact on the process yield. Indeed, for this molecule to retain a biological activity, the free status of unpaired cysteines (or unblocked e.g. uncysteinylated) is a requirement.

As a consequence, and as part of the upstream process development (USP), the impact of seeding density, culture duration, and the presence and timing of temperature and pH shifts on the cysteinylation levels (measured by HIC-HPLC or Cysteinylation by Intact Mass -CysIM- methods) were studied following a Quality by Design approach and Design of Experiment methodology. More specifically, a full factorial design approach was derived to be able to estimate the impact of the parameters' potential interactions.
All fed batch cell cultures were performed in bioreactors and the main output, i.e. cysteinylation levels, was fed back into the design for modeling.

During several rounds of experiments, conclusions on the importance of decreasing the temperature and keeping a low pH at the end of the process could be drawn. Moreover, a longer fed-batch culture duration led to a decrease in the cysteinylation levels.

A decrease of 4°C in temperature (on either day 10 or day 12 of culture) in combination with a longer culture duration and a constant lower pH setpoint and tighter deadband throughout culture (pH 6.95 ±0.15) led to a decrease in cysteinylation levels compared to control (≈26% vs. 2%; with the CysIM method). The effects of these temperature and pH conditions on cysteinylation levels was shown across a range of cell seeding densities (0.2, 0.4 and 0.6 10^6 cells/mL).

In summary, upstream control on the key culture parameters of temperature, seeding density, culture duration and pH, identified through Quality by Design approach, allowed selective reduction of unpaired cysteines of a monoclonal antibody whilst keeping conserved antibody disulfide bonds intact. This led to a robust level of uncysteinylated species >90% in the final version of the process.

This study forms the basis of international patent application WO2022117773A1.