Automated harvesting and 2-step purification of eight 1-L unclarified mammalian cell-culture broths containing antibodies using a novel configuration for ÄKTATM pure

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Introduction
Therapeutic antibodies represent one of the fastest growing segments in the pharmaceutical market. The growth of the segment has necessitated development of new efficient and cost saving platforms for the preparation and analysis of early candidates for faster and better antibody selection and characterization.

A new approach on an integrated platform for unattended harvesting and 2-step purification of antibodies expressed transiently in HEK293T-cells at 1-liter scale is shown. The system consists of two bench-top chromatography instruments connected to a central unit with eight disposable filtrations devices used for loading and filtering the unclarified feeds. The configuration of the system allows to process eight samples in 24 h.

System setup

- Pressure control of the filters (ΔP) and air sensor in the inlet valves for safe and complete sample loading.
- Dual-flow valves and independent pump control for parallel loading and eluting.
- Tandem 2-step purification on 2x5 ml HiTrap™ MabSelect™ SuRe™ followed by HiLoad™ Superdex™ 200 prep grade 26/600.
- In-line dual UV (280/600 nm) watch function removes the need for intermediate peak storage.
- In-line neutralization of Protein-A eluted peak.
- Automated Cleaning in Place (CIP) and column re-equilibration (with NaOH peak contact time).

Results

Average recoveries of the automated 2-step purification were around 80% for most antibodies. The automated process showed high reproducibility, with intra- and inter-run variations around 1.1% and 2.1% respectively.

Conclusion

Our end-product QC analysis demonstrates that the quality of the material prepared by the 2-step automated purification is fully comparable to that of the material purified by standard manual 2-step purification. Average recoveries were mostly comparable and in some cases slightly higher than those obtained by our standard manual 2-step purification, indicating that this automated system allows the cost-efficient preparation of therapeutic antibodies in the 20-200 mg range, and covers the requirements for early in vitro and in vivo profiling of drug candidates.