TELEDYNE
CETAC TECHNOLOGIES
Everywhereyoulook™

14306 Industrial Road
Omaha, NE 68144
USA
+1.402.733.2829
cetac.com

Application Note

High Throughput Automation of the ÄKTA™ pure 25 via the ASX-560

Florian Madura[1], Penny Hamlyn[2], Niel Williams [3] and Nick Westaway[3]

(1) Kymab, The Bennet Building (B930), Babraham Research Campus, Cambridge CB22 3AT, UK, florian.madura@kymab.com
(2) GE Healthcare, Amersham Place, Little Chalfont, Bucks, HP7 9NA, UK, penny.hamlyn@ge.com
(3) Teledyne CETAC Technologies, 14306 Industrial Road, Omaha, NE 68144, USA, niel.williams@teledyne.com

INTRODUCTION

The ÄKTA pure (GE Healthcare) is a widely used system for single-step (e.g. protein A, IMAC, IEX) or multiple-step (e.g. protein A followed by desalting or gel filtration) protein purification processes. The ÄKTA pure is however currently limited to a maximum of 14 (2 sample inlet valves, no limit on volume), 24 (10mL samples with Alias autosampler) or 192 (2mL samples with Alias autosampler) samples being analysed per run. Following on the work from Walker et al. (J. Chromatogr. A (2014); 1344:23-30) on the ÄKTA AS, GE Healthcare and Teledyne CETAC have developed an off-the-shelf solution for a fully automated high-throughput medium scale purification system: the ÄKTA pure 25 – ASX-560 (**Figure 1**). This system can easily manage up to 240 samples of 14mL, up to 84 samples of 50mL or even larger sample volumes (e.g. 21 samples of 200mL).

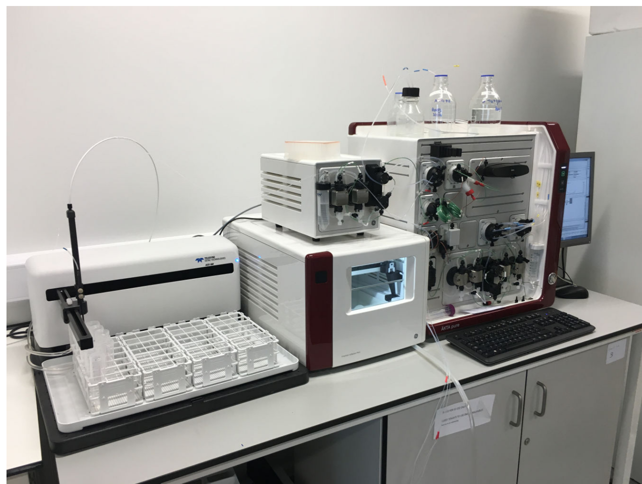

Figure 1: ÄKTA pure 25 with ASX-560 autosampler. ASX-560 (left), sample pump (middle top), fraction collector (middle bottom) and ÄKTA pure 25.

This "next generation ÄKTA AS" system was optimized and tested at Kymab in Cambridge (UK) by analysing and comparing a two-step purification process on different antibodies.

MATERIAL & METHODS

Hardware and software configuration

This study utilised the ÄKTA pure 25 with UNICORN™ 7 software. The ÄKTA pure had the following configuration – inlet valve IA, inlet valve IB, injection valve, 5 position column valve (V9-C), 10 port outlet valve (V9-0), sample pump, sample inlet valve (V9-IS) and Frac 9-C. The ASX-560 was connected via an internal I/O board to the ÄKTA pure's external I/O board via a custom cable developed specifically for this application. The cable was created so that the output pins of the ASX-560 I/O board connected to the input pins of the ÄKTA pure I/O box while the input pins of the ASX-560 were connected to the output pins on ÄKTA pure I/O box. The ASX-560 was connected to the host computer via USB, which runs both the UNICORN 7 software for the ÄKTA pure and the AScript software for the ASX-560. The digital output signals within the ÄKTA pure's I/O box settings were set to 0 for all 4 of the outputs. The method within UNICORN 7 was altered to send digital output signals via the I/O box at key points within the sequence. A customised method script for the ASX-560 was developed to run in parallel with the UNICORN script so that when digital output signals were sent from UNICORN the AScript software would trigger a sequence of actions for the ASX-560.

Purification method

Two-step human antibody purifications were performed on a 1mL HiTrap™ MabSelect SuRe™ column (GE

Healthcare) followed by a 5mL HiTrap Desalting (Desalt) column (GE Healthcare). Columns were equilibrated and washed with 3 column volumes (CVs) of D-PBS without divalent cations (Life Technologies) at 4mL/min and 15mL/min on HiTrap MabSelect SuRe and Desalt respectively. Antibodies were eluted at 2mL/min on HiTrap MabSelect SuRe and desalted at 10mL/min. HiTrap MabSelect SuRe peak elution was collected in a 4mL loop when eluates had an absorbance above 20mAU at 280nm and injected at 10mL/min onto the Desalt. Desalt peak elution was collected in a 24-well plate, 1 sample per well, when eluates had an absorbance above 20mAU at 280nm. Cleaning-in-place (CIP) was performed at 2mL/min and 10mL/min on HiTrap MabSelect SuRe and Desalt, respectively, with a 3CV Water/3CV 0.1M NaOH/3CV PBS sequence for each sample (**Figure 2**).

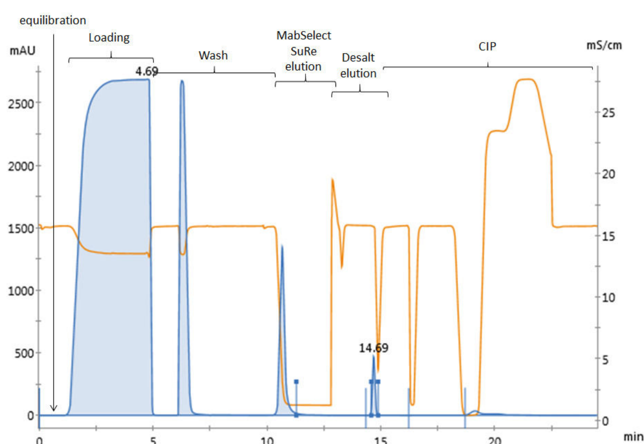

Figure 2: Representative chromatogram on ÄKTA pure 25 – ASX-560. Absorbance in mAU and conductivity in mS/cm throughout the run are represented in blue and orange, respectively.

RESULTS

A capacity study was carried out by loading (at 2mL/min) in triplicate 250µg, 500µg, 1mg, 2.5mg, 5mg, 10mg, 20mg and 30mg of purified human antibody diluted in 10mL of D-PBS and antibody recovery was analysed (**Figure 3**). As expected, the recoveries were lower but nonetheless respectable for loads ≤1mg (45% to 65%). Recoveries reached their peak (75%) between loads of 2.5mg and 20mg making this system optimal for high throughput medium scale purifications. A load of 2.5mg per run was therefore chosen for a loading flow-rate study.

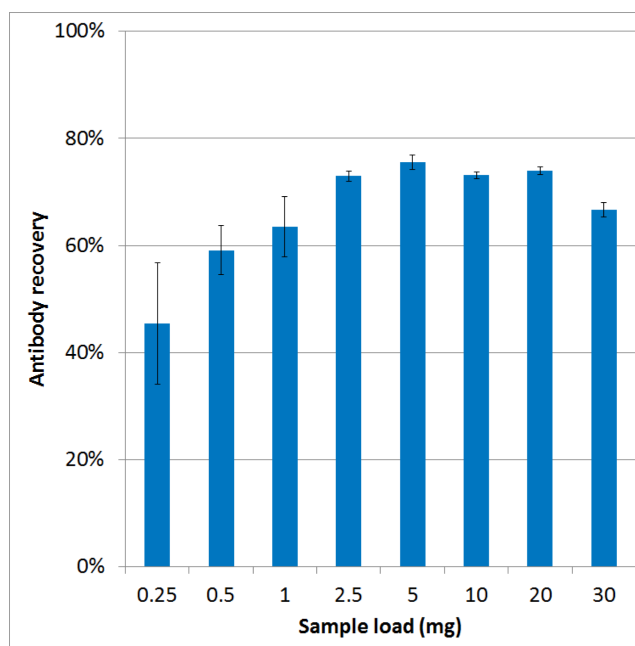

Figure 3: Capacity study of the ÄKTA pure 25 – ASX-560 with 1mL HiTrap MabSelect SuRe and 5mL HiTrap Desalting. Antibody recovery reaches its maximum between 2.5mg and 20mg (75%), ideal for medium scale purifications.

A loading flow-rate study was carried out by loading 2.5mg in triplicate of either already purified antibody diluted in 10mL of D-PBS (Ab1, Ab2 and Ab3) or 10mL of clarified supernatant from CHO cell expression containing 2.5mg of Ab2. Loading flow-rates of 1mL/min, 2mL/min, 3mL/min and 4mL/min were tested with a HiTrap MabSelect SuRe wash flow-rate of 4mL/min (**Figure 4**). A similar experiment with a HiTrap MabSelect SuRe wash flow-rate of 2mL/min was carried out in order to determine if antibodies were eluted during a wash at 4mL/min but comparable results were obtained (data not shown).

First, no significant differences in recoveries between already purified antibody in D-PBS and antibody purified from tissue culture supernatant (Ab2) were observed. Recoveries were similar (75%) to the recovery observed in the previous experiment for a 2.5mg load highlighting the robustness of the system. Then, as expected, some slight differences between Ab1, Ab2 and Ab3 were reported due to the difference in dynamic binding capacity (DBC) on HiTrap MabSelect SuRe of each antibody.

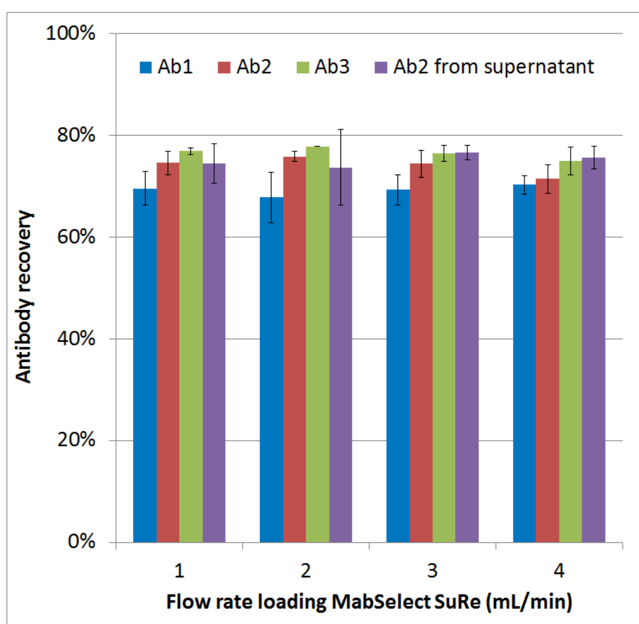

Figure 4: Loading flow-rate study on ÄKTA pure 25 – ASX-560. Already purified antibodies diluted in D-PBS (Ab1, Ab2 and Ab3 in blue, red and green, respectively) and Ab2 from CHO expression in tissue culture supernatant (purple) were purified with a 2-step process. No significant differences between flow-rates have been observed.

However, no significant differences have been observed for a given antibody between the different flow-rates. A loading flow-rate and wash flow-rate of 4mL/min was therefore chosen for further experiments in order to decrease the run time per sample. Furthermore, this is a highly reproducible system highlighted by relatively narrow error bars in both the capacity study for loads between 2.5mg and 30mg and the flow-rate study.

DISCUSSION & CONCLUSION

The ÄKTA pure 25 – ASX-560 has been investigated at Kymab for a 2-step purification process. The results of the different studies show that this is a highly reproducible fully automated high-throughput medium scale purification system. The average antibody recovery for loads between 2.5mg and 20mg is 75% which is as good as or better than the recoveries from the innovative initial ÄKTA AS from Walker et al. (J. Chromatogr. A (2014); 1344:23-30).

Despite the original ÄKTA AS revolutionizing the high-throughput medium scale purification process, this "next generation ÄKTA AS" displays brand new features and capabilities. First, there is no need to modify the ÄKTA pure 25 in any way (e.g. new valves, filters, etc.). For example, the "up-flow elution" feature is integrated to the new UNICORN 7 software. Because of the additional pressure monitors, this new system can run methods at higher flow-rates without generating alarm pressures. Additionally, while the original ÄKTA AS was only dedicated to high-throughput purifications, the ÄKTA pure 25 – ASX-560 displays more flexibility by having up to 14 sample inlets. Therefore, sample inlet 1 would be dedicated to the autosampler but the other inlets can be used for other type/scale of purifications. Moreover, the ÄKTA AS is using discontinued equipment (ÄKTA purifier and ASX-520). The run time of a 10mL sample (20-25min) is similar to the ÄKTA AS but can be further decreased by optimizing the CIP procedure (i.e. by increasing flow-rates, decreasing CVs, etc.). Finally, such system could also be useful for other type of purifications (i.e. 1-step process for dynamic binding capacity analysis or other 2-step processes like IMAC followed by desalting) and the ASX-560 and methods are easily transferable on the ÄKTA avant as the latter is also running on UNICORN 7.

In conclusion, the ÄKTA pure 25 – ASX-560 allies the robustness and the reliability of the new ASX-560 autosampler with the flexibility and the efficiency of the ÄKTA pure 25.