

# Innovative purification solutions for non-monoclonal biosimilars - opportunities for process improvement

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## INTRODUCTION

Non-antibody protein therapeutic development processes often require multiple steps to get to the desired purity. Being setup more than a decade ago, these production processes do not take advantage of technological innovations developed in recent times. Thermo Scientific™ CaptureSelect™ affinity chromatography resins are developed for the discovery and manufacturing of even the most demanding biotherapeutics. The affinity resins provide high target purity in a single step, independent of feedstock, offering opportunities for process improvements in biosimilar development. Here we describe the development and use of Thyroid-Stimulating Hormone (TSH) affinity chromatography resin, co-developed with our partner Genexine, Inc.

Genexine is a clinical stage biotechnology company focused on the development and commercialization of innovative immunotherapeutics and next generation novel long-acting biologics, with the goal to bring to patients medicines that will transform their lives. TSH is being developed as a diagnostic agent for the follow-up of patients with thyroid cancer.

## Development of TSH specific affinity resin

Co-development project between Genexine and Thermo Fisher Scientific to develop an affinity chromatography resin for the purification of CHO derived TSH, using CaptureSelect technology (Fig 4). The workflow for resin development is shown in figure 1.

### Required resin features:

- High selectivity for TSH with no affinity for other gonadotropins (FSH, LH & hCG)
- Efficient purification of recombinant TSH with high yield and retained activity

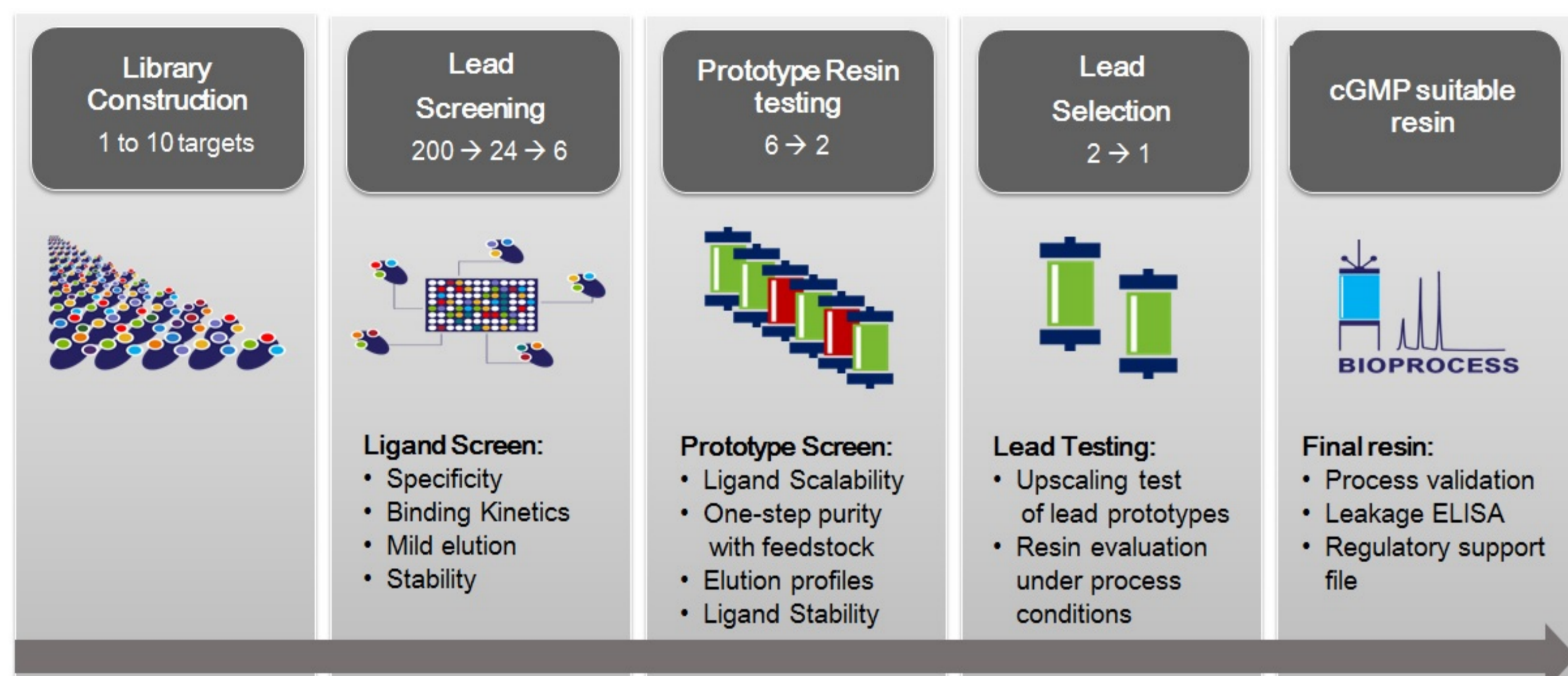


Fig.1 Workflow showing all milestones in the process of affinity resin development

## TSH prototype resin testing and lead selection process

A total of 3 ligands were tested as lead candidates for the final resin. Screening includes production scalability and chromatography performance.

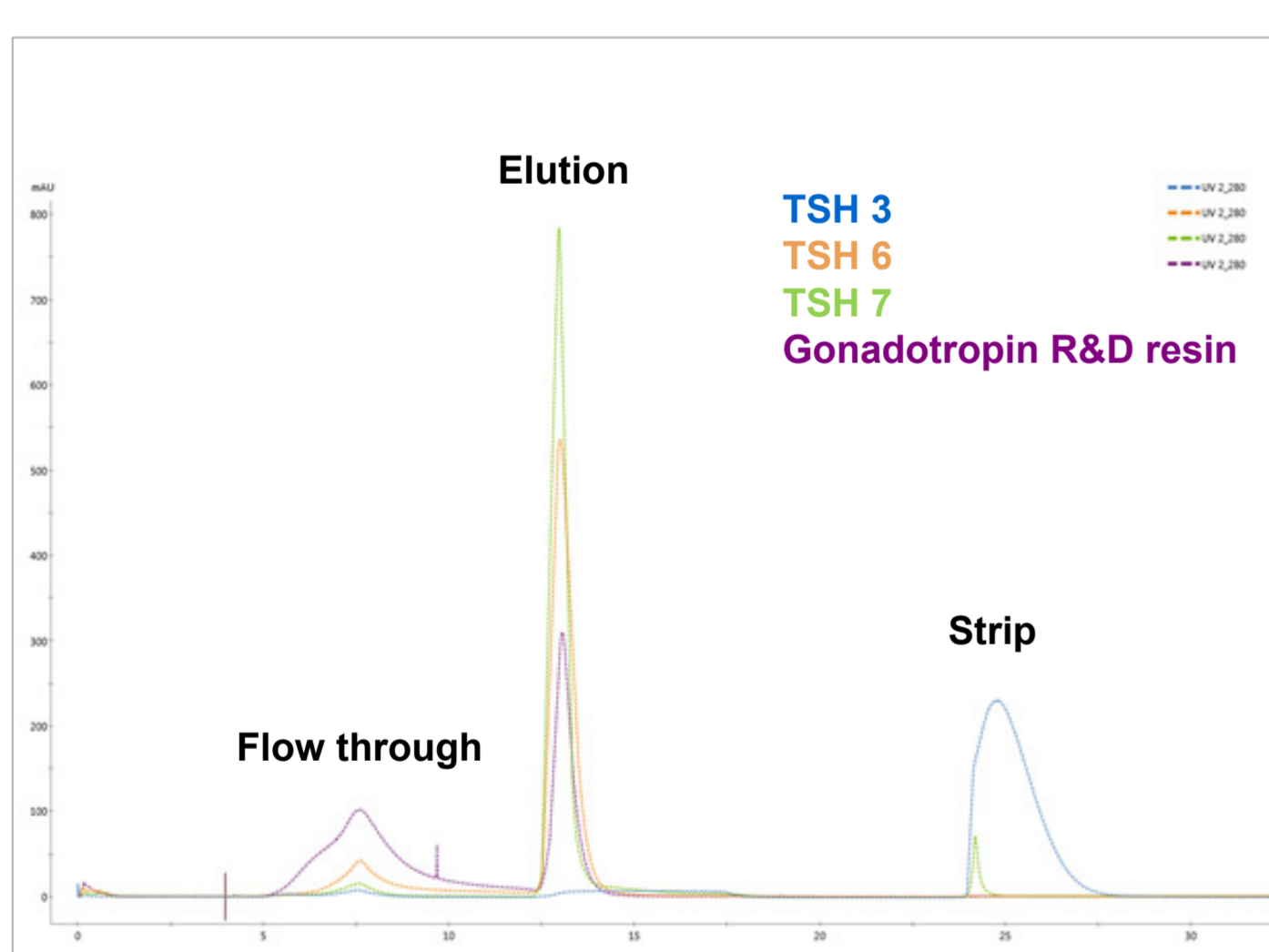


Fig. 2 The lead ligands TSH-6 and TSH-7 have a good binding capacity and show efficient elution with 0.1M Citric acid pH 3.0, TSH-3 does not elute.

Next step is check TSH 6 and 7 with feed stock (figure 3)

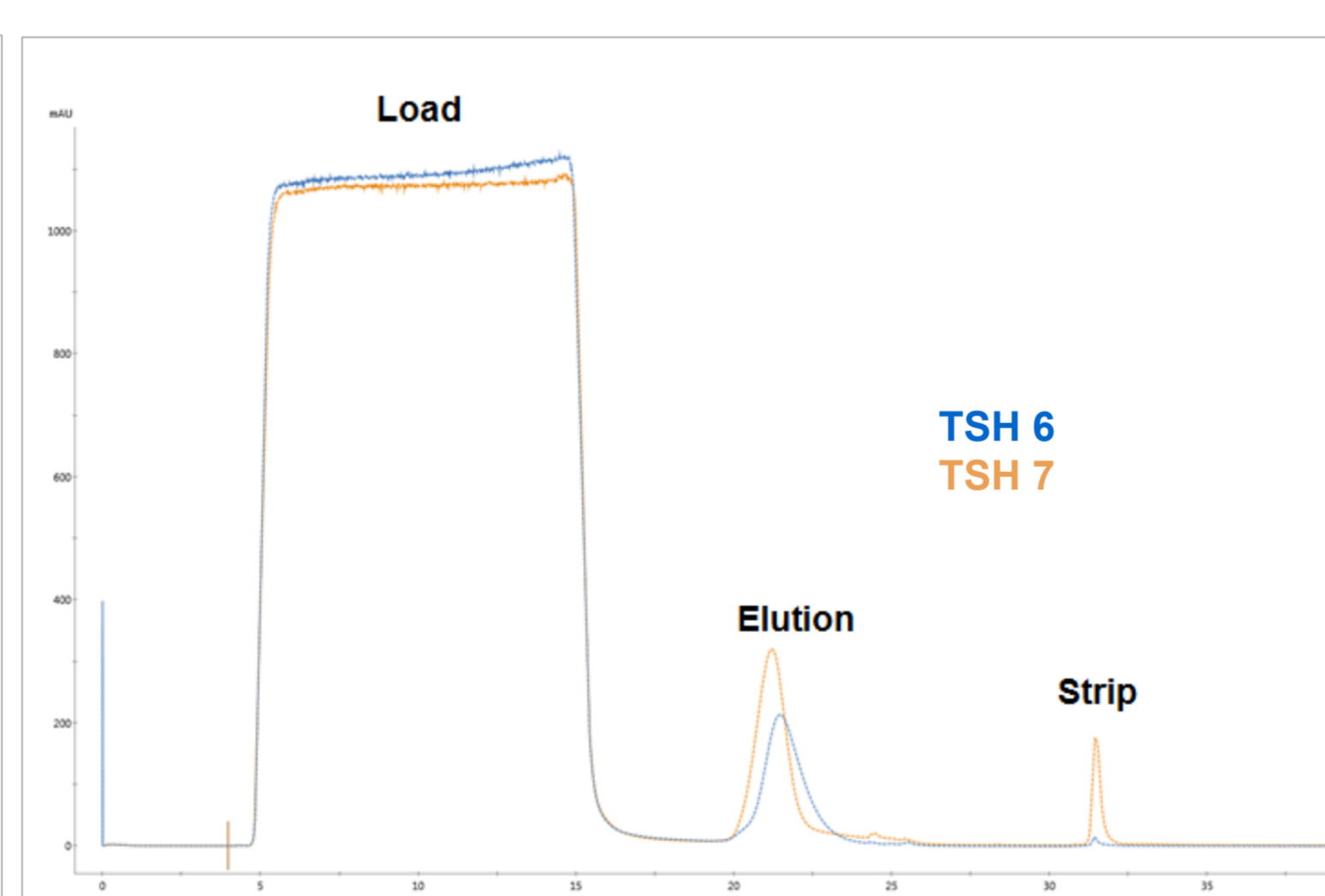


Fig. 3 TSH 6 and 7 tested with Genexine feed stock. Based on performance, ligand 6 was selected for final cGMP resin development.

0.4 ml column (5\*20 mm), 0.5 ml/min (150 cm/hr)  
10.3 ml feed loaded (0.29 mg/ml TSH; 3 mg TSH in total)  
Equilibration buffer PBS pH 7.4  
Elution: 0.1 M Citric acid pH 3.0  
Strip: 0.1 M Phosphoric acid

## CaptureSelect Technology – unique affinity purification solution

- Affinity through antibody selectivity: technology based on Camelid-derived single domain [ $V_{H1}$ ] antibody fragments
- Unique screening technology for target specificity, mild elution & stability
- Animal origin free production process (*Saccharomyces cerevisiae*)
- Technology used in commercial purification processes

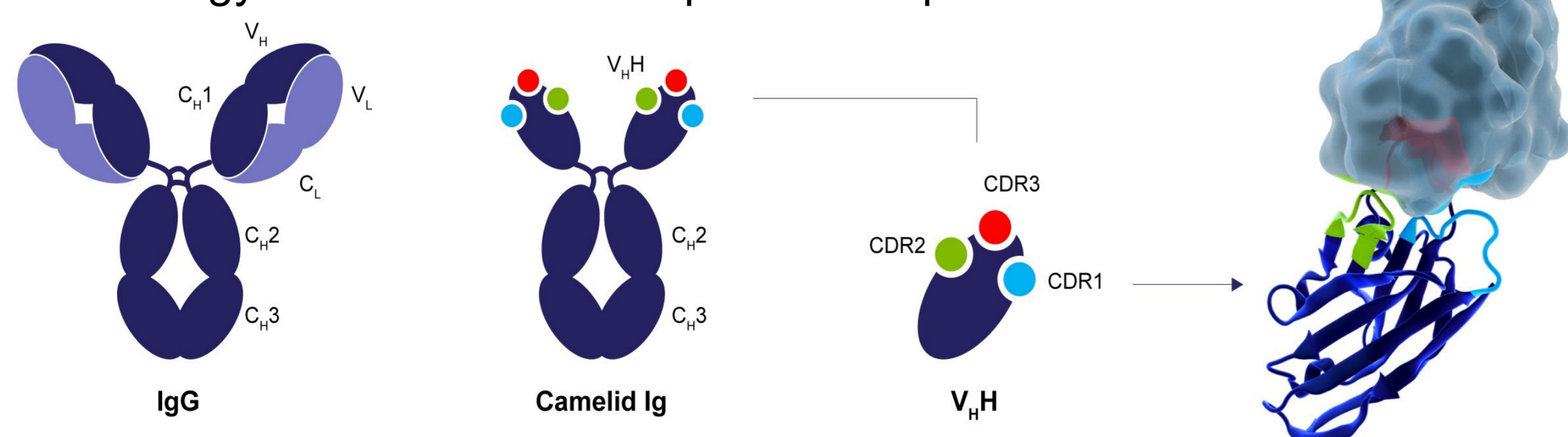


Fig. 4 Regular IgG antibody compared to a Camelid heavy-chain antibody. The  $V_{H1}$  antibody fragments offer high specificity, affinity and stability.

## Improving downstream processing for TSH

After development of the TSH affinity resin, performance tests were conducted by Genexine and final parameters for their TSH purification process were defined.

1. Determination of dynamic binding capacity (DBC) of CaptureSelect TSH resin
2. Design of experiments (DoE) to determine optimal parameters for recovery and purity
3. Evaluation of purified product
4. Upscaling to 200L purification

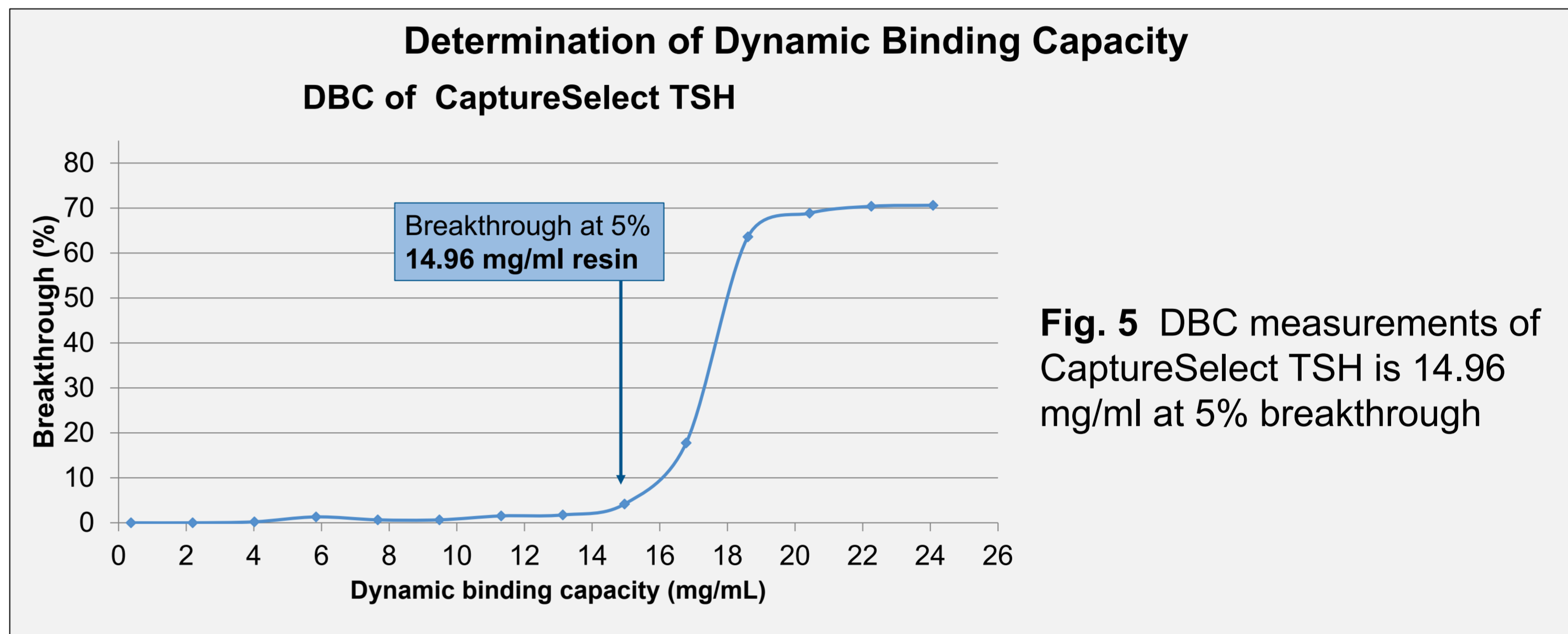


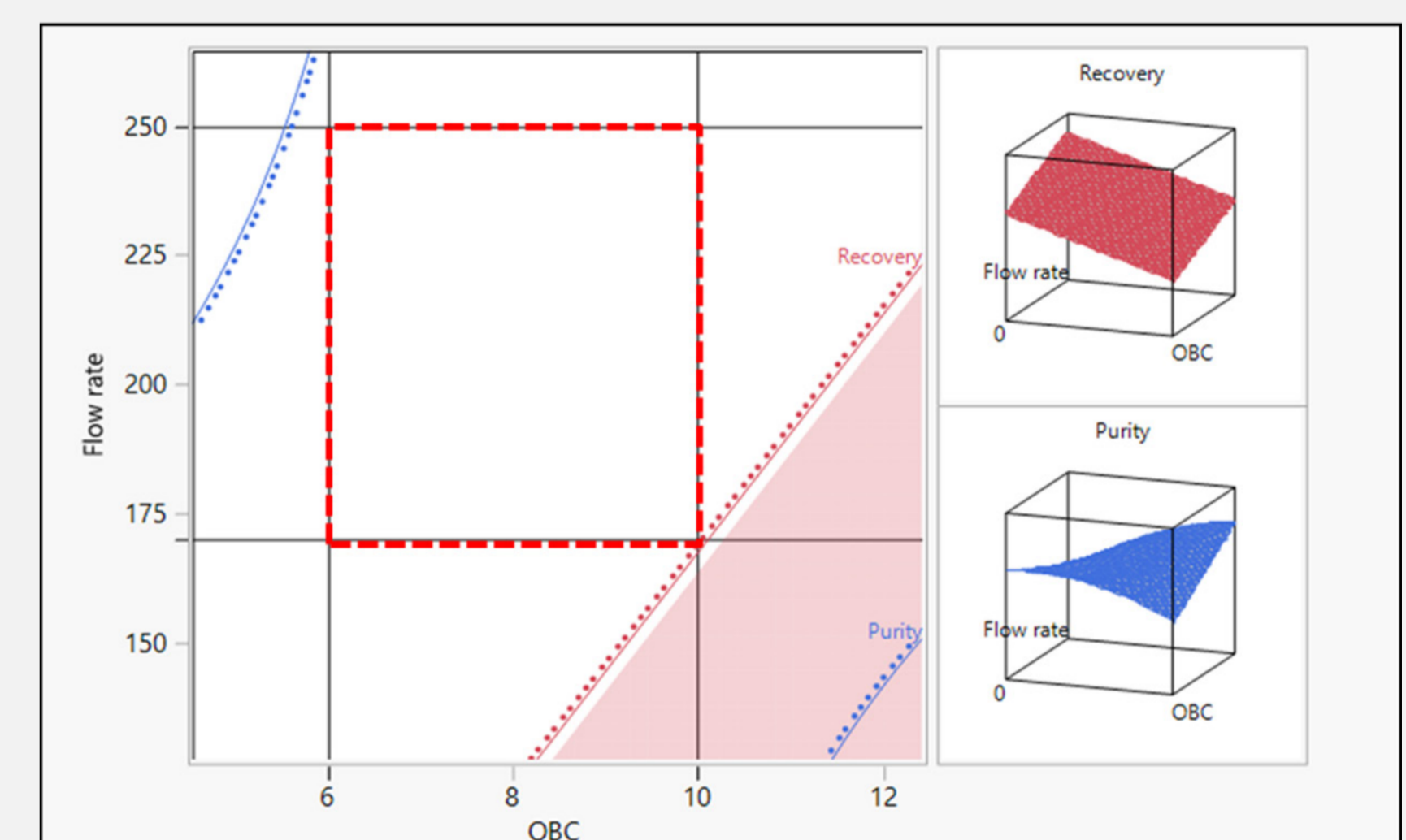
Fig. 5 DBC measurements of CaptureSelect TSH is 14.96 mg/ml at 5% breakthrough

## Design of Experiments

Fig.6 Optimization of process parameters: flow rate and operating binding capacity (OBC)

Normal operating range:  
Flow rate: 210 ± 40 cm/hr  
OBC: 8 ± 2 mg/ml

Recovery: 70%  
Purity: >99%



## Evaluation of purified product

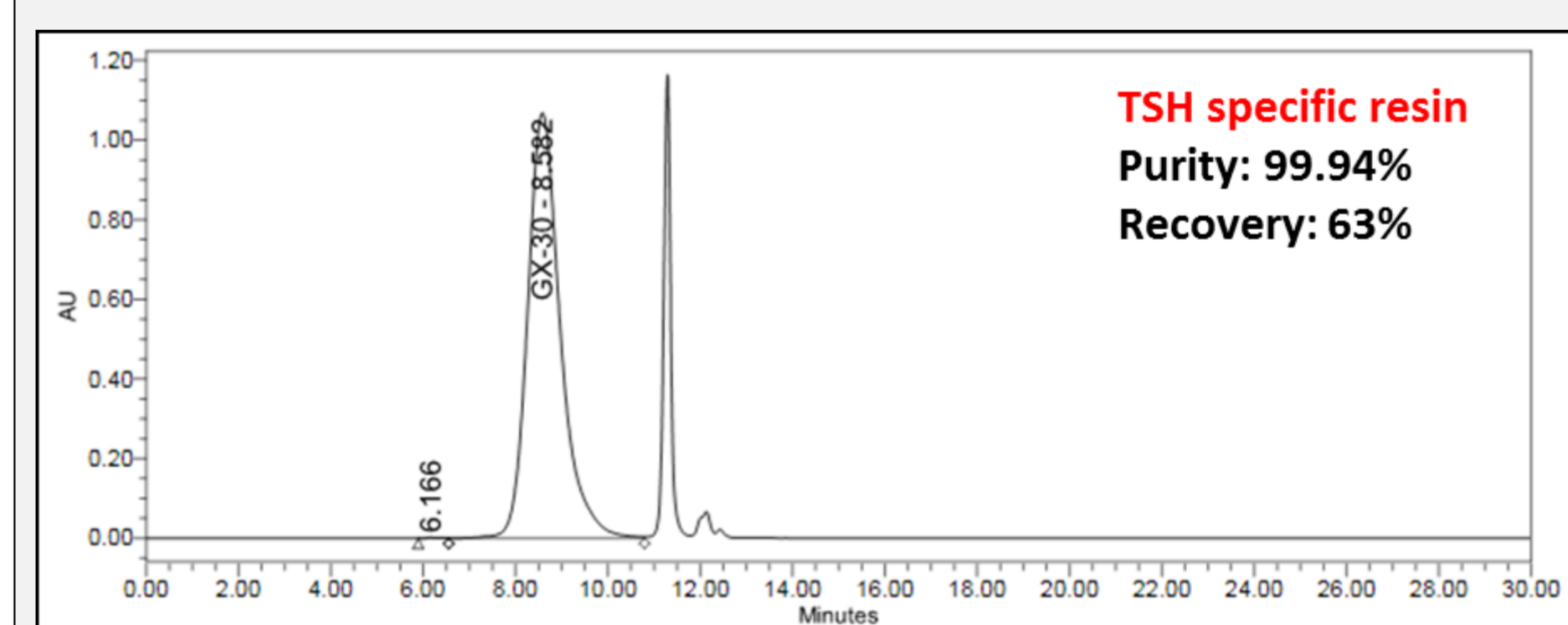


Fig. 7 SEC-HPLC analysis revealed a purity of 99.94% with a recovery of 63%

## Conclusions

1. Successful co-development between Genexine and Thermo Fisher Scientific of a cGMP suitable affinity resin for commercial production of TSH
2. High DBC of ~ 15 mg/ml with high purity (>99%) in a single capture step
3. Only 2,5L resin required for purification of a 200L feed stock (resin cost saving of 30% compared to old process)

## CaptureSelect products and development pipeline for therapeutic proteins

Product Stage	Therapeutic proteins
Bioprocess resin	FSH, HSA, hCG, tPA, C-tagXL, hGH, TSH
Research scale resin	Antithrombin III, Fibrinogen, Transferrin, ApoH, C1-inhibitor, GM-CSF
Lead Selection	Prothrombin,
Prototype resin testing	DNase, Uni-tag, FX
Lead screening	IFN $\alpha/\beta$ , hIL2, FV, FXI, FXII, FXIII, FH, EPO, insulin