avantor™

Chromatography Solutions

Technical note #022

High Resolution Separation of BSA Tryptic Digest using Coupled Avantor® ACE® Columns

INTRODUCTION

Protein therapeutics are a fast-growing area of the pharmaceutical industry, with thousands of biopharmaceutical drugs currently in clinical development. It is crucial that these biologic drugs are thoroughly characterised during production due to the heterogenous nature of these compounds. The International Council for Harmonisation (ICH) has issued guidelines for testing of these biopharmaceutical products. Peptide mapping is one of the tests included in the ICH Q6B recommendations.

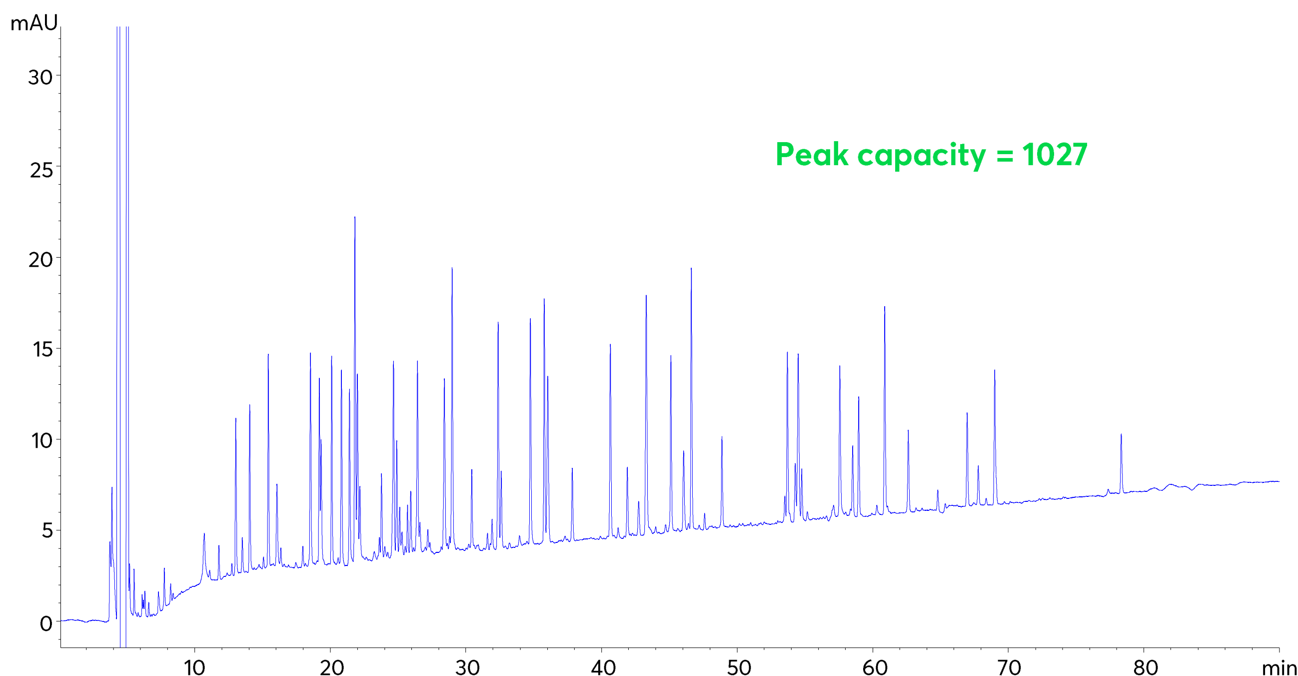
PEPTIDE MAPPING

Peptide mapping is described as a Critical Quality Attribute test, which is used to confirm the primary amino acid sequence of the protein therapeutic. Changes to a protein's primary amino acid sequence can occur through post-translational modifications, such as deamidation and oxidation, during the production process. Small changes to the basic amino acid structure of a protein can affect the safety and efficacy of a drug, so it is imperative that any changes be closely monitored.

For peptide mapping to be performed, the protein must first be broken down into its constituent peptides. This is achieved via enzymatic digestion with an enzyme such as trypsin. This process then allows the reversed-phase separation of peptides based on their hydrophobicity. HPLC analysis at the peptide level can give information on post-translational modifications and can also be used in the comparison of biosimilars with originator drugs.

Specific method conditions must be implemented to achieve optimum separation of peptides, due to the large number of peaks which are produced from trypsin digestion. The use of trifluoroacetic acid (TFA) as an ion-pair reagent is commonplace in peptide mapping due to the mostly positively-charged nature of the analytes. Elevated temperatures and gradient methods are also typically employed to improve peak shape and resolution.

Figure 1 shows the reversed-phase analysis of the component peptides of bovine serum albumin (BSA) after digestion with trypsin enzyme. BSA is a 66.5 kDa protein which is made up of 583 amino acids.[1] The use of coupled columns, totalling 45 cm in length, packed with solid-core particles, has resulted in a highly efficient separation of BSA tryptic peptides with a peak capacity of over 1000. Trypsin acts via cleavage of the peptide bond at the C-terminus of arginine and lysine amino acid residues (see figure 2), unless they are followed by a proline residue, resulting in approximately 80 peptides during BSA digestion.[2]



Column	ACE UltraCore 2.5 SuperC18
Dimensions	(3x) 150 x 2.1 mm
Mobile phases	A: 0.05% TFA in H_2O B: 0.05% TFA in MeCN
Gradient	10 to 40% B in 90 mins
Flow Rate	0.21 mL/min
Injection	20 µL
Temperature	60 °C
Detection	UV, 214 nm

Figure 1: Peptide map from tryptic digest of BSA on three ACE UltraCore 2.5 SuperC18 columns coupled in series. (Avantor® ACE® Application Note #6910)

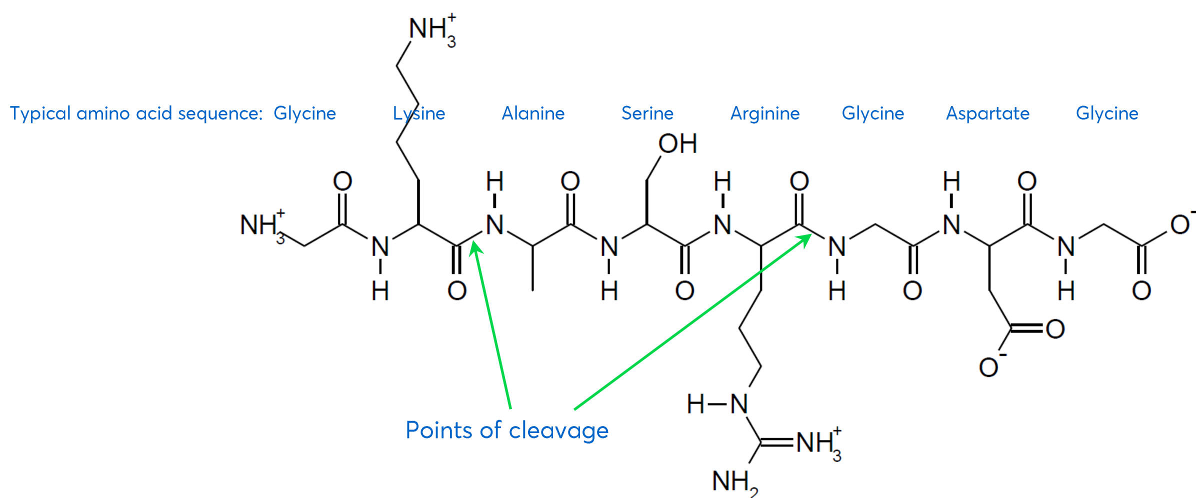


Figure 2: Trypsin catalyses hydrolysis only at the points shown (carboxyl side of lysine and arginine)

ORDERING TABLE

Product	Details	Size	Part number
ACE UltraCore 2.5 SuperC18	HPLC column	150 x 2.1 mm	CORE-25A-1502U

REFERENCES

[1] Majorek KA, Porebski PJ, Dayal A, Zimmerman MD, Jablonska K, Stewart AJ, Chruszcz M, Minor W. Structural and immunologic characterization of bovine, horse, and rabbit serum albumins. *Molecular Immunology*. 2012; 52 (3–4): 174–82

[2] Sandra K, Joseph M, Sandra P, Strategies for the Characterisation of Biopharmaceuticals, *Chromatography Today*. Aug/Sept 2015