ACE 300Å Wide Pore HPLC Columns for the Separation and Purification of Proteins

ACE Technical Note #003

The analysis and characterisation of peptides and proteins in biotechnology is an important activity to ensure the quality and safety of bioproducts. It is estimated that there are currently approximately 400 biopharmaceuticals in the clinical trial phase of development. Due to the chemical diversity and complexity of proteins, chromatography plays a crucial role in providing a comprehensive analytical characterisation, confirming product identity, structure and detection of related impurities.

A combination of chromatographic techniques is typically used to characterise proteins, both in their native and fragmented states (e.g. enzymatic digestions of therapeutic proteins). With respect to intact proteins, a number of analytical challenges exist, including their highly diverse and complex structures, the need to separate closely related variants and their existence in highly complex matrices. These challenges require robust and highly efficient separation techniques that can also utilise complimentary detection methods, such as mass spectrometry, to provide maximum structural information for the analytes of interest.

Reversed phase liquid chromatography (RPLC) is a powerful and widely utilised tool for the separation and characterisation of both intact and fragmented proteins and peptides which successfully meets these challenges. In addition, RPLC is highly compatible with mass spectrometry. High resolution separations of complex protein digests can be readily achieved using columns packed with high purity, high performance silica based particles with a pore size of approximately 100 Å (e.g. ACE Excel C18). However, the analysis of intact proteins requires a wider pore size to fully accommodate the physically larger analytes. The ACE range of 300 Å wide pore columns has been specifically engineered to produce highly reproducible separations for a wide range of peptides, proteins and other high molecular weight biomolecules. Based on ultra high purity silica, they provide exceptional chemical stability, peak shape, sensitivity and column lifetime, allowing highly efficient separations of proteins to be achieved.

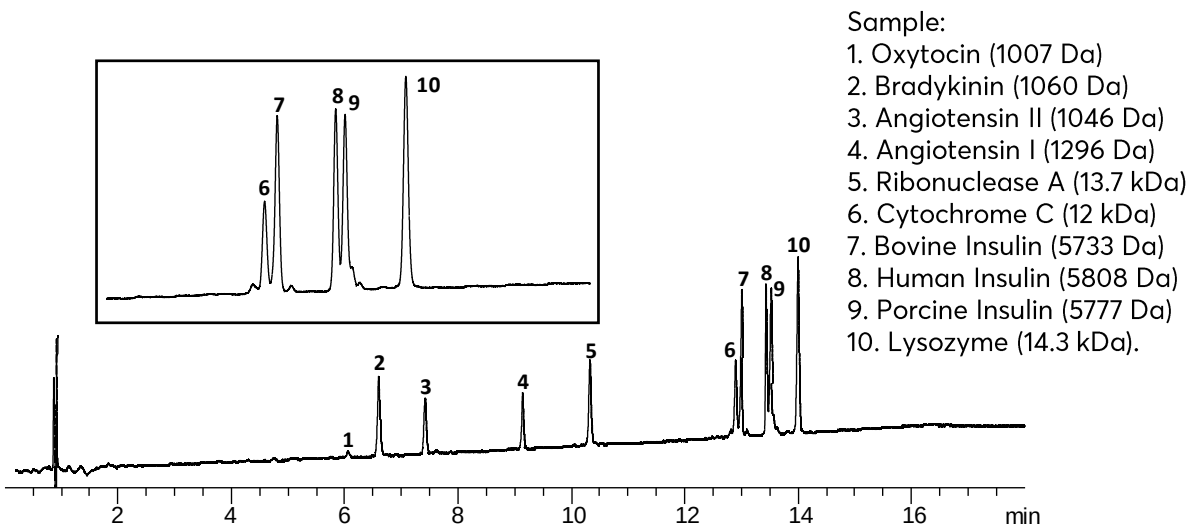
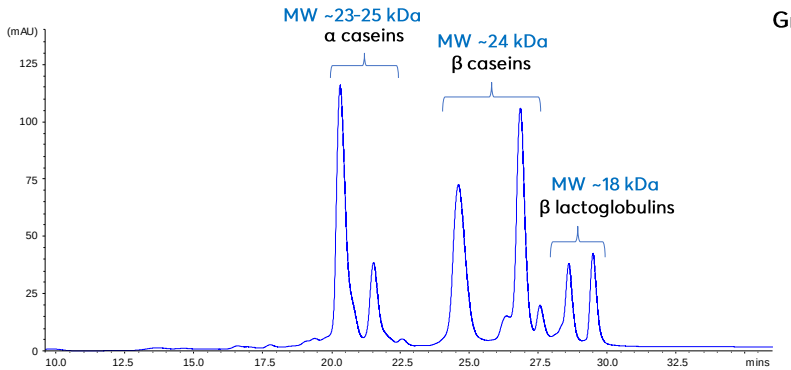


Figure 1: Reversed-phase separation of a range of peptides and proteins with varying molecular weights on the ACE 3 C4-300 (150 x 2.1 mm).Mobile phase A: 0.1% TFA (aq), B: 0.1% TFA in MeCN:water 80:20 v/v. Gradient: 10 to 50%B in 15 minutes, Flow rate: 0.5 ml/min, Temperature: 60 °C, Detection: UV (220 nm)

Intact protein separations are typically achieved using a water:acetonitrile gradient, with the addition of TFA as an ion pairing agent. Figure 1 demonstrates the high efficiency separation of a set of peptides and proteins with varying molecular weights using a broad scouting gradient run on an ACE 3 C4-300. Of particular note is the ability to separate the three insulin variants of human, bovine and porcine origin. These insulins have molecular weights of approximately 5,800 Da with only slight variation in their amino acid sequences. Despite this, the ACE 3 C4-300 provides sufficient chromatographic performance to separate these sample components.

The separation of closely related protein variants is a common theme in protein characterisation. In this situation, shallower gradient profiles are often employed to provide enhanced resolution of closely related proteins. Figure 2 shows how this approach can be used for the analysis of milk proteins. A much shallower acetonitrile:water gradient was employed on the ACE 5 C18-300 to elute a series of casein and lactoglobulin proteins between 20 and 30 minutes. The high efficiency and excellent peak shape of the ACE column allows for the successful separation of casein variants which differ in their structure by a little as one amino acid substitution. In this example, the concentration of TFA in the mobile phase has been reduced by a factor of 10 to just 0.01%, which would dramatically improve sensitivity if MS detection was employed. This is possible due to the high inertness of the ACE silica surface, which allows excellent peak shape to be obtained for proteins, even at low concentrations of TFA.



Gradient Profile:

Time (mins)	%B
0.0	33
5.0	33
9.0	35
18.0	37
22.0	40
27.5	41
28.0	41
43.0	43

Figure 2: Separation of milk proteins using the ACE 5 C18-300. Column dimension: 150 x 2.1 mm, Mobile phase A: 0.01% TFA (aq),B: 0.01% TFA in MeCN. Gradient: as shown above, Flow rate: 0.2 ml/min., Temperature: 45 °C, Detection: UV (214 nm). Reproduced with permission of The Chemical Analysis Facility, University of Reading, UK.

ACE wide pore phases are based on 300 Å ultra inert silica. As for all ACE phases, their extremely low silanol activity results in excellent peak shape, sensitivity and reproducibility when separating compounds that contain polar functional groups making ACE 300 Å phases an ideal choice for the separation of peptides, proteins and other high molecular weight biomolecules. Five column chemistries are available (C4, C8, C18, CN and Phenyl) to fully explore column selectivity during method development. ACE 300 Å columns are available in an extensive range of dimensions and particle sizes for use in micro-scale separations, LC-MS analyses and high speed preparative analyses up to process scale.

ACE Bioanalytical 300 Å Method Development kits: 3 columns for the price of 1!
ACE® Method Development Kits (MDK) are designed to maximise selectivity, offering a powerful and reliable approach to method development. A complete range of kits for all requirements is available, including porous, solid-core and bioanalytical 300 Å column kits. These kits group together the essential column chemistries for method development and are available in a wide range of column formats including 0.5 mm and 1.0 mm microbore IDs.

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