

The peptide and protein based pharmaceuticals are a rapidly expanding class of therapeutical agents that are used to treat a wide variety of health conditions, including cancer, metabolic and auto-immune diseases, HIV and more. Biologic drugs, such as monoclonal antibodies, are derived from living organisms and are usually very expensive. As many biologics are coming off of patents, the market is ready for cost-saving biogenerics. But all proteins, including monoclonal antibodies, have complex structures that determine their function. Differences in structure would alter biological activity leading to changes in safety and efficacy of the drug.

ICH Q6B is a guidance document that provides a set of internationally accepted specifications for biotechnological and biological products to support new marketing applications. It establishes the set of criteria to which a drug substance, drug product or material should conform to be considered acceptable for intended use.

Determining Amino Acid composition following hydrolysis is listed in ICH Q6B as a way to characterize the protein and to confirm its identity by comparing with Amino Acid composition deduced from the gene sequence of the desired product. Amino Acids Analysis data is also used to accurately determine the protein content.

The Amino Acids Analysis with post-column derivatization is a very sensitive, reproducible and rugged method and it has been a preferred approach for laboratories running biological samples, protein, peptides and foods analysis. Pickering Laboratories Inc. offers many Amino Acids Analysis products including post-column derivatization instruments, columns, eluants, reagents and standards. All products are designed to work together to deliver optimum results for any chosen sample.

Method

Analytical Conditions

Column: High-efficiency Sodium cation-exchange column, 4.6 x 110 mm, Catalog Number 1154110T

Flow Rate: 0.6 mL/min

Mobile Phase: See method in Table 1

Injection Volume: 10-50 uL

Post-Column Conditions

Post-column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Table 1. HPLC Program

Time	Na315, %	Na425, %	Na640, %	RG011, %
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40.0	100	0	0	0

Table 2. Column Oven Program

Time	Temp, °C
0	46
4	46
9	70
32	70
33	46

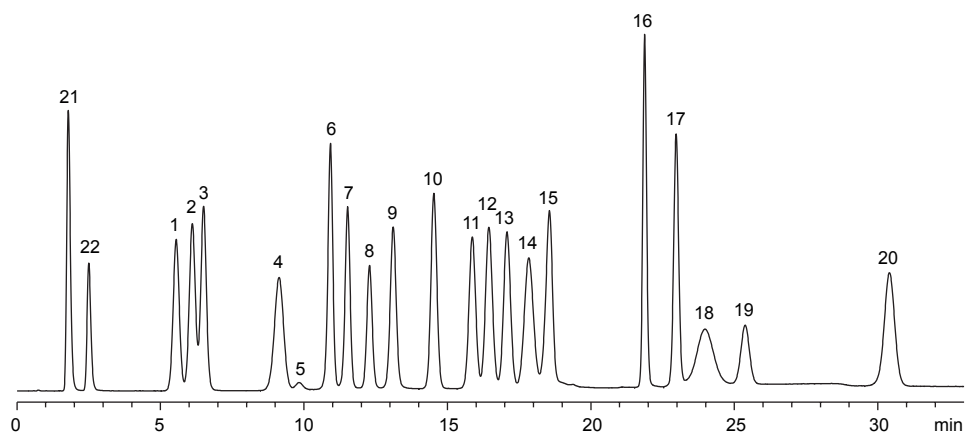


Fig 1. Chromatogram of Amino Acids standard

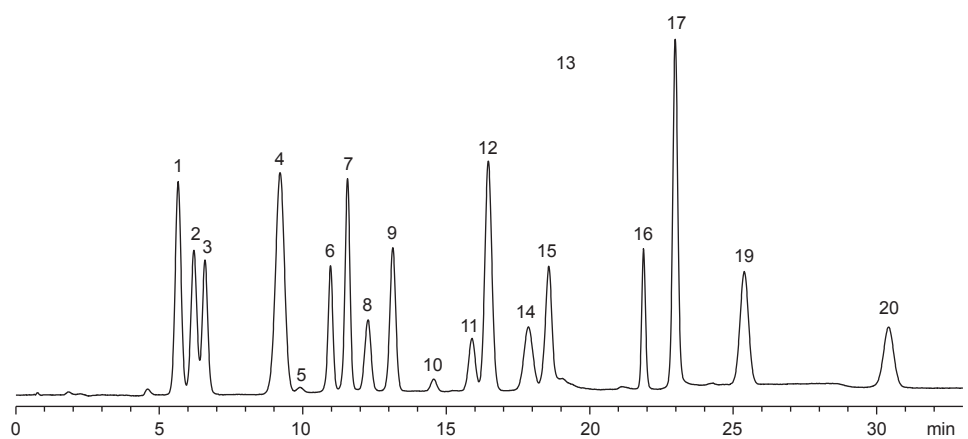


Fig 2. Chromatogram of hydrolyzed sample of IL-17F monoclonal antibody

1 Aspartic Acid	9 Valine	17 Lysine
2 Threonine	10 Methionine	18 Tryptophan
3 Serine	11 Isoleucine Alanine	19 Ammonia
4 Glutamic Acid	12 Leucine	20 Arginine
5 Proline	13 Norleucine	21 Cysteic Acid
6 Glycine	14 Tyrosine	22 Taurine
7 Alanine	15 Phenylalanine	
8 Cystine	16 Histidine	

Acknowledgements

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