PICKERING LABORATORIES
CATALYST FOR SUCCESS

AMINO ACID ANALYSIS OF MONOCLONAL ANTIBODIES

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

Post-Column Conditions

Post-column System: 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

Injection Volume: 10-50 uL

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

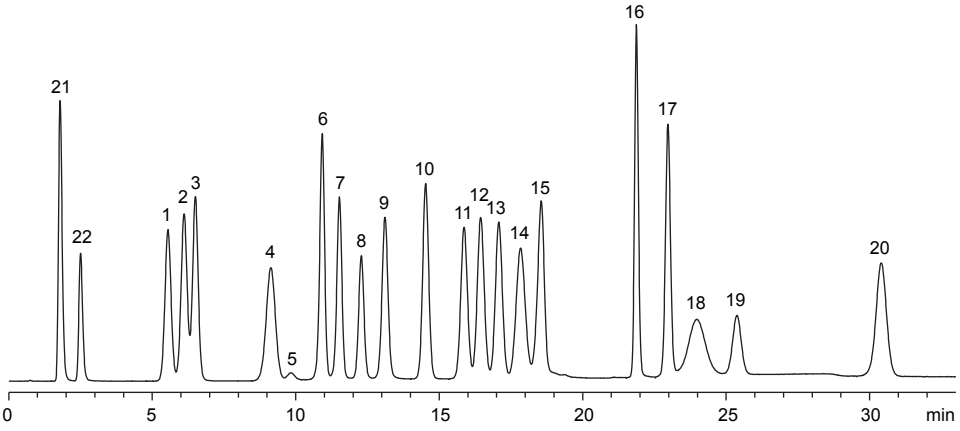

Fig. 1. Chromatogram of Amino Acids standard

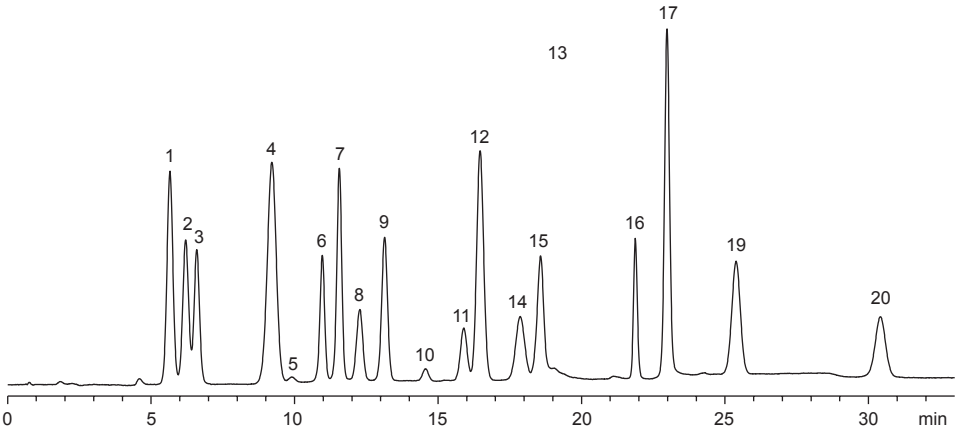

Fig. 2. Chromatogram of hydrolyzed sample of IL-17F monoclonal antibody

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

ACKNOWLEDGEMENTS

We would like to thank Frank N. Konstantinides and AminoAcids.com for their contribution to this project.

PICKERING LABORATORIES
1280 Space Park Way / Mountain View, CA 94043
sales@pickeringlabs.com / support@pickeringlabs.com
800-654-3330 / 650-694-6700 / Fax: 650-968-0749