PICKERING LABORATORIES
CATALYST FOR SUCCESS

AMINO ACIDS ANALYSIS OF CELL CULTURE MEDIA

Cell cultures are widely used to produce biopharmaceuticals and other biologically active compounds. The composition of the cell culture media affects the yield and structure of the desired products and must be carefully optimized. Cell culture media is typically composed of mixtures of amino acids, vitamins, carbohydrates, inorganic salts as well as different peptides, proteins and other compounds. As the cells grow, they consume nutrients and release target biopharmaceuticals as well as waste products.

Amino Acids serve as the building blocks of proteins, as well as intermediates in many metabolic pathways. Amino Acids are typically added to cell culture media to provide nutritional requirements for the cells. Monitoring and adjusting Amino Acid composition is an essential part of optimizing the manufacturing process to ensure high quality and optimum yield of the final product.

Amino Acids Analysis using cation-exchange chromatography with post-column Ninhydrin derivatization allows for easy determination of Amino Acid concentrations in many complex matrices, including cell culture media. The post-column method is very sensitive, reproducible and rugged. It has been and continues to be a method of choice for laboratories running biological samples, protein, peptides and foods analysis. Most chemical compounds present in the media do not interfere with analysis, so the majority of samples only need diluting with citric buffer and filtering before analysis. If serum is added to the media, then the proteins need to be precipitated using either Seraprep™ solution or ultrafiltration.

Pickering Laboratories, Inc. offers the complete solution for Amino Acids Analysis, including post-column derivatization instruments, columns, eluants, reagents and standards. The Pinnacle PCX derivatization system has a programmable column oven to allow for shorter run times and easy method optimization.

METHOD

Analytical conditions

Column: High-efficiency Lithium cation-exchange column, 4.6 x 75 mm, Catalog Number 0354675T

Flow Rate: 0.55 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	1700-1125, %	LI365, %	LI375, %	RG003, %
0	100	0	0	0
10	100	0	0	0
19.0	40	60	0	0
32.0	0	100	0	0
43.0	0	100	0	0
43.1	0	0	100	0
57.0	0	0	100	0
57.1	0	0	70	30
72.0	0	0	70	30
72.1	100	0	0	0
84.0	100	0	0	0

TABLE 2. COLUMN OVEN PROGRAM

TIME	Temp, °C
0	34
6	34
17	65
25	70
70	70
71	34

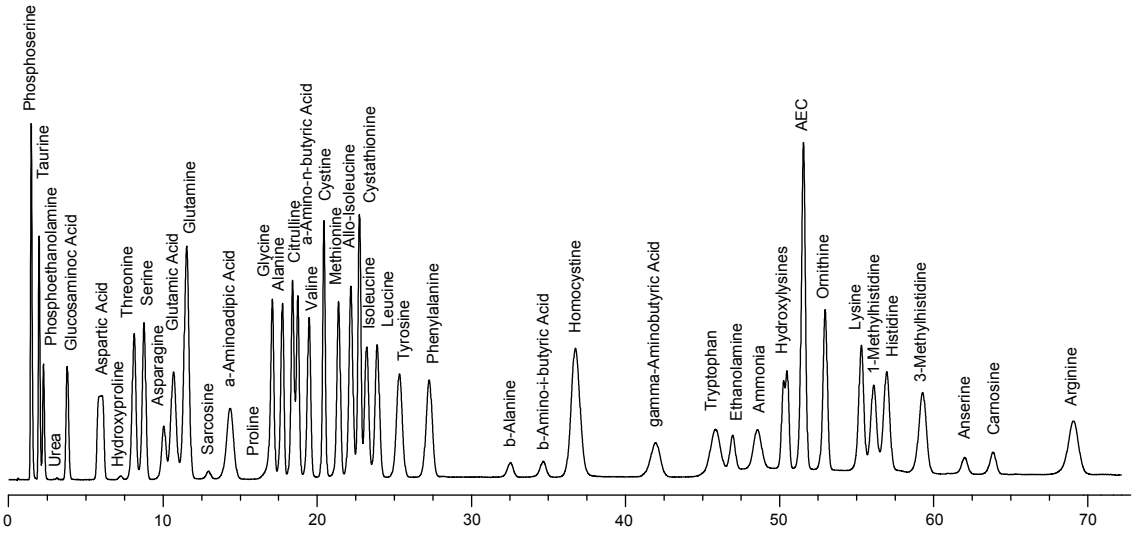

Fig. 1. Chromatogram of Amino Acids standard

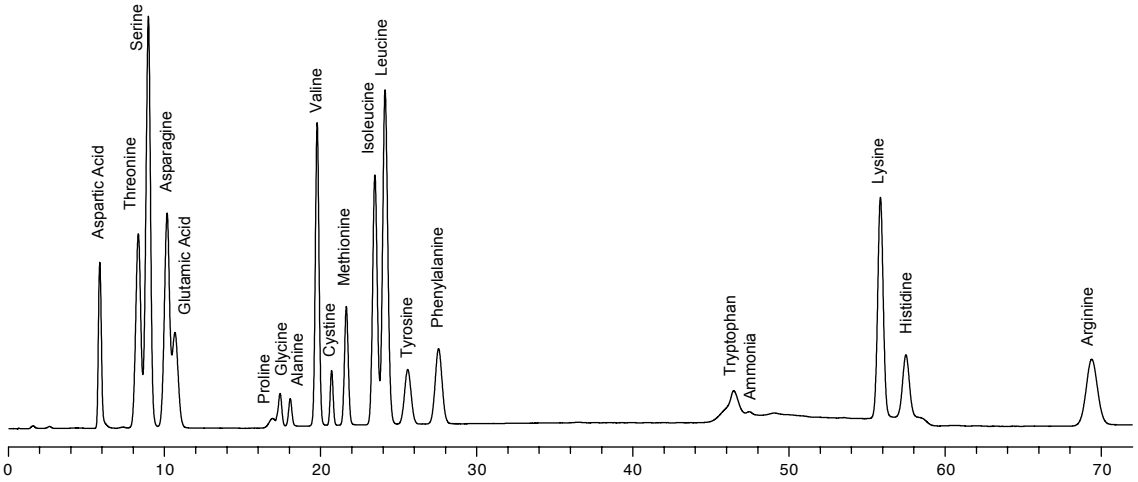

Fig. 2. Chromatogram of cell culture media sample

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