Midwest Laboratories®

LAB BRIEFS

AMINO ACID PROFILE

AOAC 988.12, AOAC 988.15 & AOAC 985.28

The analysis of Amino Acids is based on the traditional method of sample hydrolysis and the analysis of the individual amino acids using HPLC (High Performance Liquid Chromatography) with Post Column Derivatization. The method is based off of AOAC 994.12. To determine the total amino acids, three (3) methods are required, including the 994.12 (acid hydrolysis), but in addition, analyses of tryptophan requires base hydrolysis (AOAC 988.15), and the sulfur-containing amino acids (methionine and cystine) require a pre-oxidation step (Modified AOAC 985.28).

The process of sample preparation requires use of a relatively small amount of sample, generally less than 0.50 grams, thus the sample used for analyses must be well ground and representative of the submitted sample. A small amount of a non-homogenous sample will alter the final results. To hydrolyze the sample, the sample is placed in a container with acid and then the acid heated for twenty-four (24) hours to break down the protein into the constituent amino acids. A second sample is required for analysis of tryptophan, but instead of using an acid to break down (hydrolyze) the protein, a base (alkalai) is used. The preparation of methionine and cystine requires a pre-oxidation step that needs a twenty-four (24) hour process and then the acid hydrolysis step, so the minimum time of sample preparation for methionine and cystine is forty-eight (48) hours.

After the samples are hydrolyzed, the extracts are filtered to remove any remaining particulates, and then analyzed by HPLC. The HPLC columns obtain separation of the various individual amino acids. When the amino acids leave the HPLC column, they react with a special chemical (Ninhydrin) that allows the detector to see the amino acids. To quantitate the amount of amino acid present, a known amount of amino acid (a standard) is analyzed at the same time. By calculating the instrument response to the amount found with the sample, it is possible to quantitate the individual amino acids.

In doing Quality Control, it is important to compare the amount of amino acid to the total protein because the amino acid (natural) comprises the protein. If nitrates or non-protein nitrogen is present, the amount of protein (if using a nitrogen analyses) will be higher than the calculated amount from the amino acid profile.

EXAMPLE REPORT

Analysis	Level Found	Units	Detection Limit	Method	Analyst-Date	Verified-Date
Alanine	2.33	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Arginine	1.41	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Aspartic acid	1.85	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Cystine	0.72	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Glutamic acid	4.96	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Glycine	1.03	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Histidine	0.79	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Isoleucine	1.09	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Leucine	3.30	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Total lysine	0.76	%	0.01	HPLC/PCD	tjp-11/13	tjp-11/15
Methionine	0.62	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Phenylalanine	1.36	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Proline	2.48	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Serine	1.41	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Threonine	1.04	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Tyrosine	1.20	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15
Tryptophan	0.28	%	0.01	HPLC-PCD	tjp-11/15	tjp-11/15
Valine	1.21	%	0.01	HPLC-PCD	tjp-11/13	tjp-11/15

REFERENCE ID: F421