Amino Acid Analyzer

YOUNG LIN INSTRUMENT

1. Product

The Young Lin Amino Acid Analysis System

2. Purpose

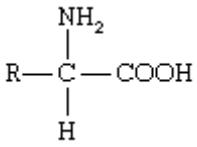
The Young Lin Amino Acid Analysis System is a special analyzer for detecting the presence of amino acids in a variety of solutions, such as extracellular and intracellular fluids, plant and animal tissues, broths, and fruits and beverage juices.

It also is capable of detecting the presence of hydrolyzed amino acids, such as is found in protein, collagen, peptides, and processes foods.

3. Principle of Analysis

1) Definition of Amino acid

Amino acids are organic molecules that possess both an amino group (NH_2) and a carboxyl group (COOH), and a variable group symbolized by R. The R-group may be as simple as a hydrogen atom (glycine), or the R-group may be a carbon skeleton with various functional groups attached. The basic molecular structure of an amino acid is:



The basic amino acid formula is written as: R-CH(NH_2)-COOH

Characteristic profile of amino acids are established according to three functional groups of an amino group; a carboxylic acid group and side groups. Especially, 22 essential amino acids that have the basic formula $NH_2CHRCOOH$ are composed of 17 the first amino acids and 5 the second amino acids.

Amino acids are the basic molecular units that make up proteins. They first form short polymer chains called peptides or polypeptides, which in turn are assembled into proteins. Twenty amino acids are encoded by the standard genetic code, and are often referred to as the standard amino acids. More complicated amino acids are produced by the human body and are referred to as the nonstandard amino acids.

Twenty-two amino acids have been identified as important to biological systems - glysine, alanine, valine, leucine, isoleucine, threonine, serine, cysteine , cyctine, methionine, aspartic

acid, asparagines, glutamic acid, di-iode-tyrosine, lysine, arginine, hystidie, phenylalanine, tyrosine, tryptopan, proline and oxyproline. The essential amino acids are those that cannot be synthesized by the human body from other compounds via chemical reactions. In humans, the essential amino acids include lysine, leucine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine, and, for children, histidine and arginine.

Detection of Amino Acids

(1) Proteins are easily quantified by ultraviolet (UV) spectrophotometry due the presence of three aromatic amino acids (tryptophan, tyrosine, and phenylalanine), which often account for a small portion of the amino acids in proteins. The other 19 amino acids, which are non-aromatic, cannot be detected by UV spectrophotometry. This necessitates constructing derivatives of those 19 amino acids, which can be detected and quantified by UV/VIS spectrophotometry. Such a procedure can indirectly detect and quantify these 19 amino acids.

(2) Reagents for Constructing Derivatives of Amino Acids

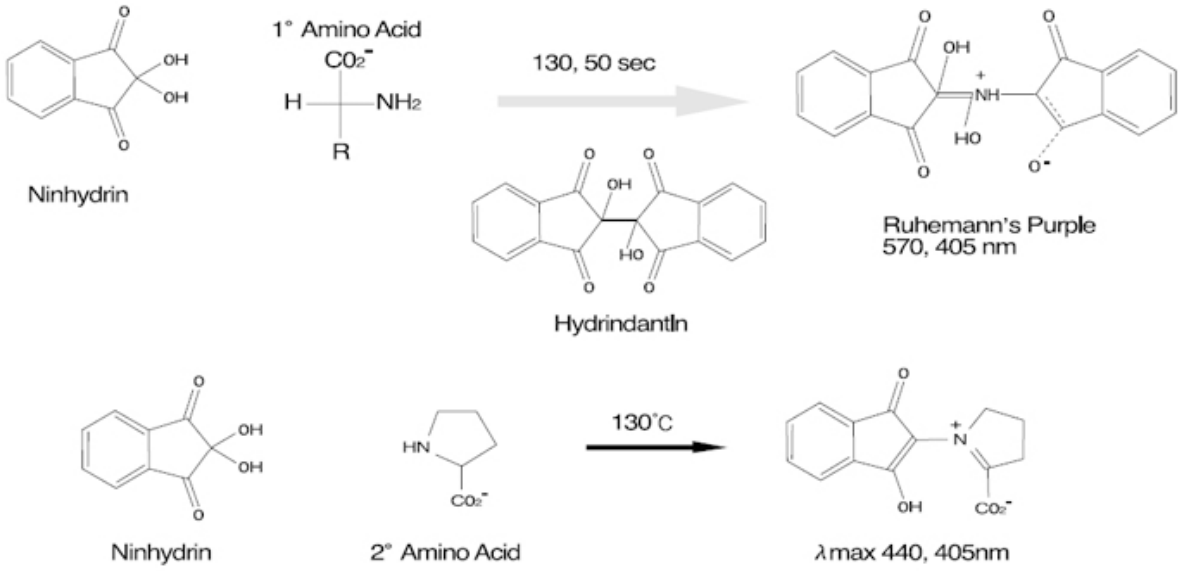
- Ninhydrin
- OPA (ortho-phthalaldehyde)
- PITC (phenylisothocyanate)
- FMOC (fluorenylmethoxycarbonyl chloride)

2) Post-column derivatization

This procedure is an on-line method for detecting derivatives of amino acids. Amino acids are first separated by a cation exchange column to which one or more of the reagents for constructing amino acid derivatives is added.

Ninhydrin Method

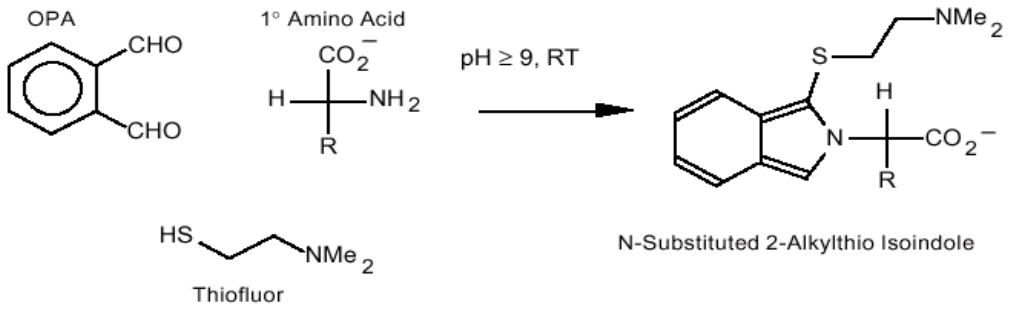
This procedure uses Ninhydrin to indirectly detect amino acids with UV/VIS spectrophotometry after the amino acids have been separated by the cation exchange column. The Ninhydrin reagent, which constructs derivatives of both the first and second amino acid groups. The amino acids of the first group would have reacted with the Ninhydrin reagent at 130^{o}C as it passed through Hydrindatin in the column. The derivatives of the first group produce purple products, with a UV/VIS absorbance values at 570nm and 405nm.



[Procedure for Using the Ninhydrin Method]

Derivatization using the OPA reagent

This method utilizes the OPA reagent to construct a derivative of an amino acid that is easily detected and measured by UV/VIS spectrophotometry. The OPA reagent reacts with a free amino acid that has been separated by cation exchange in the column. The amino acid derivative is constructed at room temperature in a different way the Ninhydrin method. Isoindole, which fluoresces under UV light, is produced as the first amino acid reacts with the OPA reagent and Thioflour, allowing for the detection of amino acids by UV/VIS fluorescent methods.



[Procedure for Using the OPA Reagent Method]

(1) Sample preparation procedure

a. Decide the sample amount (Example: Place 200 mg of soy sauce in a vial)
b. Add 30 ml of 6 *N* HCl to the vial. Incubate the sample at 130^{o}C for 24 hours to hydrolyze the amino acids.
c. Dilute the hydrolyzed sample by adding 50 ml of purified water.
d. Filter the sample with a 0.45μl aqueous syringe filter
e. Mix 20ml of HCl and 20ul of the hydrolyzed sample

(2) Analytical Condition

UV/Vis detector (Ninhydrin reagent) - The first amino acid and second amino acid groups at 405nm Fluorescence detector (OPA reagent) – Ex 330nm, Em 465nm Column: Cation column

4. Configuration

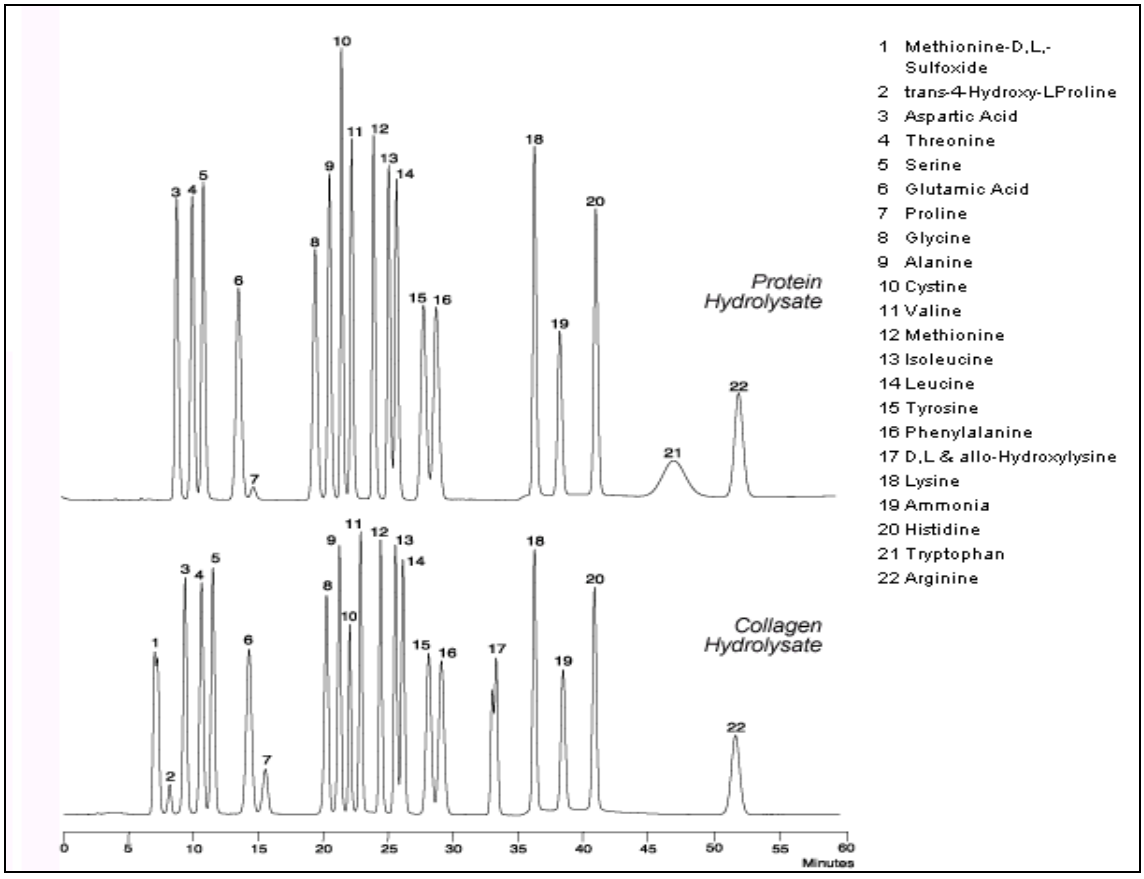
Equipment

(1) Post-column derivatization system
(2) Solvent delivery module
(3) 4 channel vacuum degasser & mixer
(4) Manual Sample Injector with switch
(5) UV/Vis detector(for Ninhydrin) or Fluorescence detector(for OPA)
(6) Data system
(7) Application kit for Post-column derivatization
(8) Performance kit
(9) Solvent Clarification kit
(10) 100ul syringe
(11) Autosampler (Optional)

5. The Advantages of Post-column

	Pre-column	Post-column
Resolution	Good	Excellent (No interrupted substances)
Run time	6~45 min	45~120min
Easy to use	Good	Fair
Cost	Moderate	High
Reproducibility	Excellent	Excellent
Sensitivity	Pico-femto mole	Nano-pico mole
Status	State-of-the art	Traditional

6. Reference



TIME	1700-0112 %	Na740 %	RG011 %
0	100	0	0
12	100	0	0
34	0	100	0
53	0	100	0
53.1	0	0	100
55	0	0	100
55.1	100	0	0
67	100	0	0

[Experimental Condition and Chromatogram]

7. Application

- Life Sciences: Protein structure, Synthesized peptide qualification
- Pharmaceutical: Medicine for Injection , Medicine for Nutrients
- Food and beverages, measurement of the essential amino acids found in food
- Clinical: Diagnosis of amino acid levels and deficiencies

8. System Configurations

The Young Lin Amino acid Analyzer

OPA	Ninhydrin
Post-column derivatization system	Post-column derivatization system
Solvent delivery module	Solvent delivery module
4 channel vacuum degasser	4 channel vacuum degasser
Fluorescence detector	UV/Vis detector
Data system	Data system
Manual Sample Injector with switch	Manual Sample Injector with switch
Performance kit	Performance kit
Solvent Clarification kit	Solvent Clarification kit
100ul syringe	100ul syringe
Application kit for Post-column derivatizaion	Application kit for Post-column derivatizaion