Amino Acid Analyzer

YL Amino Acid Analyzer is to analyze the first or second amino acids with a variety of solutions in an easy way. With a superior sensitivity, this enables to detect derivatized natural amino acids in animal tissues, broths, fruits and beverage juices and hydrolyzed amino acids in protein, collagen, peptides and processed foods.

• The need of derivatization

a. Except for tryptophane, tyrosine and phenylalanine that have an absorbance at UV/Vis wavelength range, amino acids formed in single bond have no absorbance at UV/Vis wavelength range so they are needed to be derivatized to have fluorescence by derivatization reagents.

b. Derivatization Reagents for Amino Acids

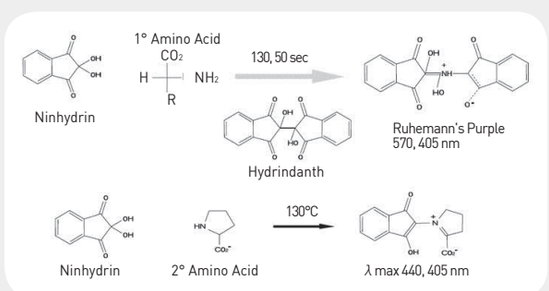
- Ninhydrin
- OPA (ortho-phthalaldehyde)
- PITC (phenylisothocyanate)
- FMOC (fluorenylmethoxycarbonyl chloride)

Post-column derivatization

This procedure is a method to detect the derivatized amino acids that are separated in a cation exchange column by adding the derivatization reagent at a reactor online in front of a detector .

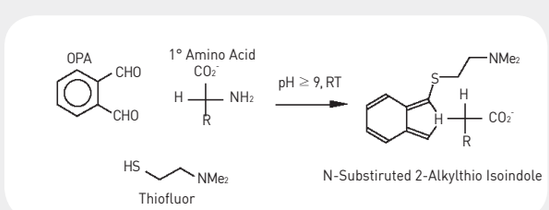
a. Ninhydrin Method (Reaction condition under temperature control)

: 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 °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 570 nm and 405 nm.



b. OPA Method

: This method utilizes the OPA reagent to react with a free amino acid that has been separated by cation exchange in the column and construct a derivative of an amino acid that is easily detected and measured by either UV/Vis detector or Fluorescence detector. The amino acid derivative is constructed at room temperature which is different from 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. (OPA method can be used for both post and pre-column derivatization.)



Sample preparation procedure

a. Decide the sample amount (Ex: 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 °C for 24 hours to hydrolyze the amino acids.
c. Dilute the hydrolyzed sample by adding 50 ml of purified water.
d. Take 1 ml in the vial and vaporize it. (Pre-column Derivatization)
e. Put 1 ml of pH 2.2 Borate buffer (or 0.02N HCl) into the vial and dissolve it.
d. Filter the sample with a 0.45 μm aqueous syringe filter
e. Mix 20 ml of HCl and 20 μl of the hydrolyzed sample

• Application

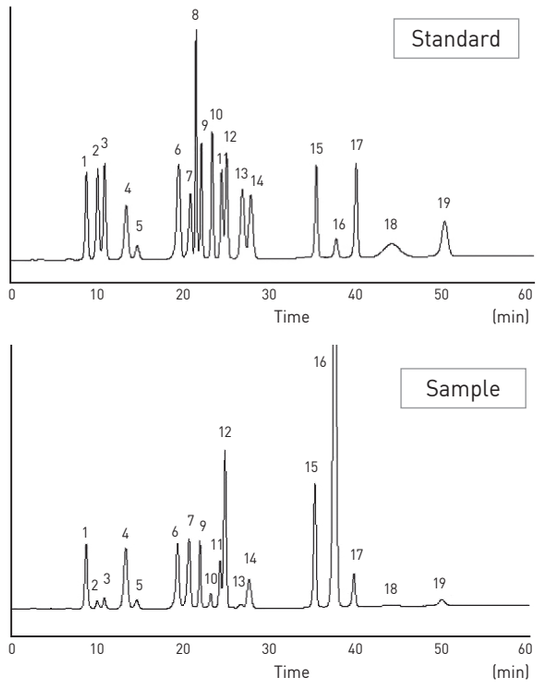
- Life Sciences: Protein structure, Synthesized peptide qualification
- Pharmaceutical: Medicine for Injection, Medicine for Nutrients
- Food and beverages: Measurement of the essential amino acids found in food
- Clinical: Diagnosis of amino acid levels and deficiencies

■ Post-Column Derivatization

- Column : High efficiency Sodium Cation-exchange (4.0 mm, 150 mm)
- Temperature : 42 °C
- Flow rate : 0.4 ml/min
- Mobile phase : 1700-0112 : Na740 : RG011 (Gradient)
- Detector : UVD 570 nm
- Injection volume : 20 µL

Post-column Conditions

- System : Pinnacle PCX with 0.5 ml reactor
- Reagent : TRIONE (Derivatizing reagent)
- Reactor condition : 130 °C, 0.3 ml/min

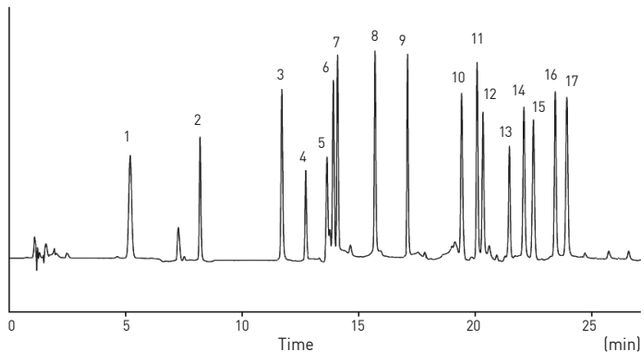


1. Aspartic acid
2. Threonine
3. Serine
4. Glutamic acid
5. Proline
6. Glycine
7. Alanine
8. Cystine
9. Valine
10. Methionine
11. Isoleucine
12. Leucine
13. Tyrosine
14. Phenylalanine
15. Lysine
16. Ammonia
17. Histidine
18. Tryptophan
19. Arginine

■ Pre-Column Derivatization

- Column : C18 (4.6 mm, 150 mm, 5 µm)
- Temperature : 40 °C
- Flow rate : 2.0 ml/min
- Mobile phase : A: 40 mM Na2HPO4 pH7.8
  B: ACN:MeOH:Water (45:45:10), Gradient
- Detector : UVD 338 nm
- Injection volume : 20 µL

	Action Type	From	To	Amount (µl)
1	Add	Sample	Destination	20
2	Add	Reagent A	Destination	30
3	Mix	3		35
4	Add	Reagent B	Destination	20
5	Mix	5	0	50



1. Aspartic acid
2. Glutamic acid
3. Serine,
4. Histidine
5. Glycine
6. Threonine
7. Arginine
8. Alanine
9. Tyrosine
10. Cystine
11. Valine
12. Methionine
13. Tryptophane
14. Phenylalanine
15. Isoleucine
16. Leucine
17. Lysine