

Amino acid analysis in Pet food (PH-Nle High-resolution analysis method)

Pets are kept by more than half of the world's people ¹⁾, and dedicated pet food is widely distributed. Pet food is mainly made from grains, livestock meat, fish, etc., and it is necessary to always secure safe and excellent quality raw materials ²⁾. Furthermore, it is important that amino acids are contained in a well-balanced manner as nutrients necessary for growth and health maintenance.

This report introduces measurement examples of samples of cat, dog, bird, and goldfish pet food hydrolyzed with hydrochloric acid. The PH-Nle High-resolution analysis method (refer to AS / AAA-029E) was used to analyze the 18 components of protein hydrolyzed amino acids and norleucine (Nle) added as an internal standard at once. Norleucine (Nle) was added as an internal standard. Nle can be analyzed in any sample, and each amino acid component can be analyzed with high resolution. The PH-Nle High-resolution analysis method is useful for amino acid analysis in pet food.



**LA8080 HIGH SPEED
AMINO ACID ANALYZER
(AminoSAAYA)**

1) "Man's best friend: global pet ownership and feeding trends" Growth from Knowledge (GfK)

2) "Ingredients for pet food" ? Japan Pet Food Association (JPFA)

Analysis of Amino Acid Standard Solution

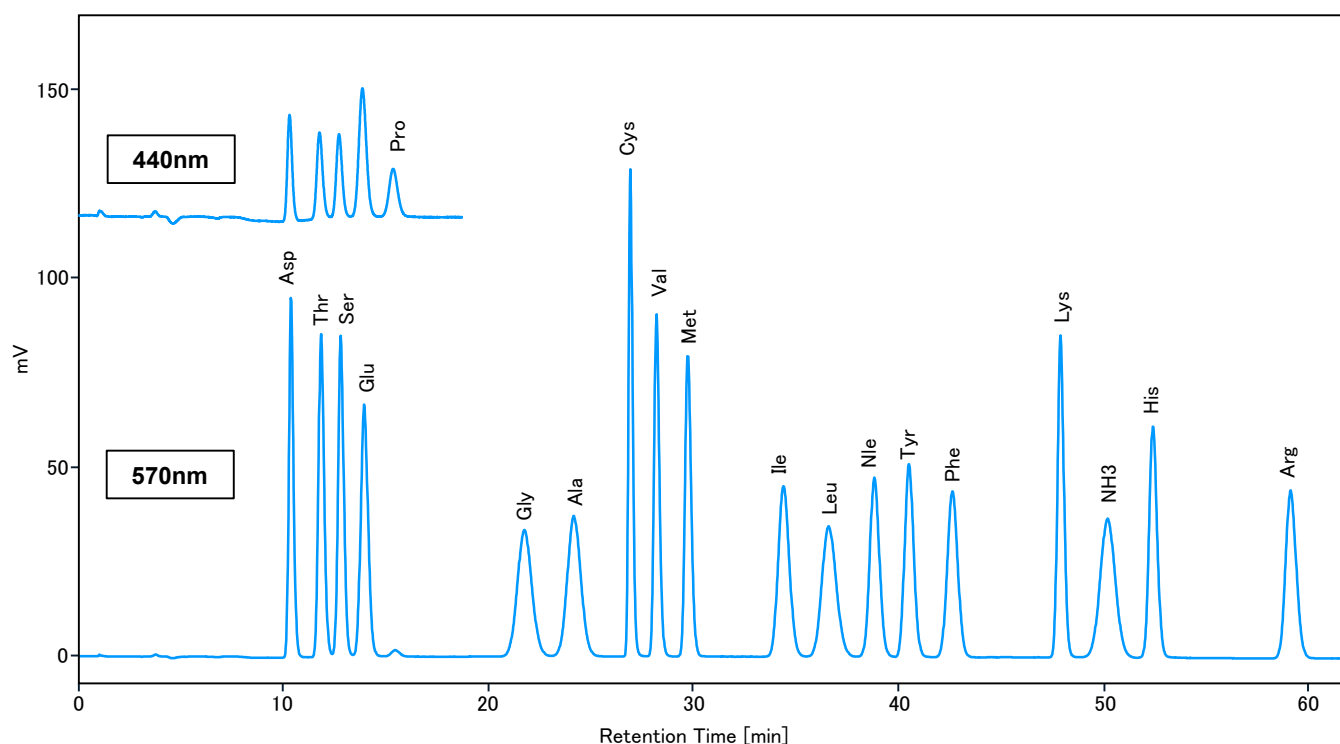


Fig.1 Analysis of Amino Acid Standard Solution

Table 1. Analytical Conditions for PH-Nle High-resolution method

Column	#2620M 4.6 mm I.D. × 80 mm	Reaction reagent	Ninhydrin Reagent Wako Amino Acid Automated Analyzer Kit (ID code: For Hitachi) (*)
Ammonia filter column	#2650L 4.6 mm I.D. × 60 mm	Reaction reagent flow rate	0.30 mL/min
Eluent	MCI buffer L-8500 PH-Kit (*) B1:modified	Reaction temperature	135 °C
Flow rate	0.22 mL/min	Detection wavelength	VIS 440 nm、570 nm
Column temperature	55~90 °C	Injection volume	20 µL

The standard sample is Amino Acids Mixture Standard Solution, Type H(*) with Nle added.

This analysis method is only available on the LA8080.

(*) FUJIFILM Wako Pure Chemical Corporation

Analysis of hydrolyzed pet food samples

- ✓ Hydrolyzed samples could be analyzed with high resolution within 60 minutes.
- ✓ Each pet food contains well-balanced amino acids.

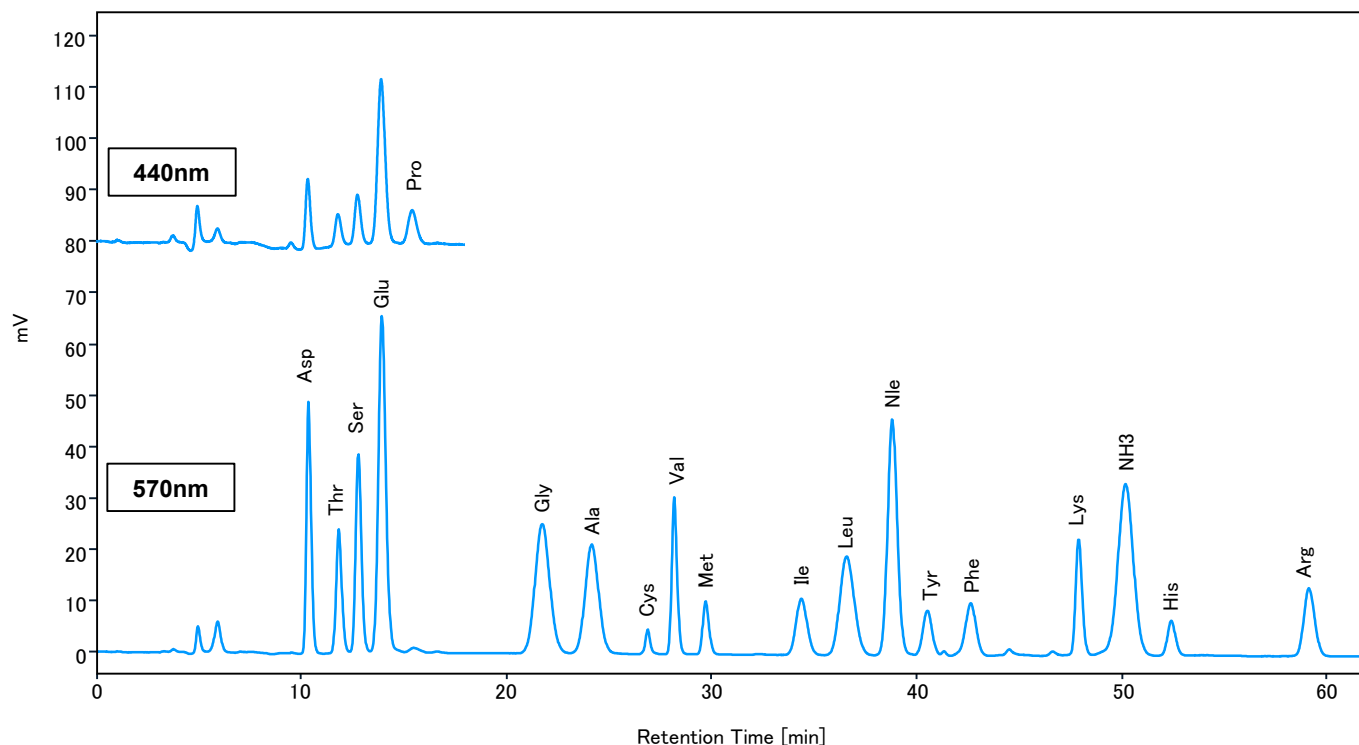


Fig.2 Analysis of Pet food for Cat

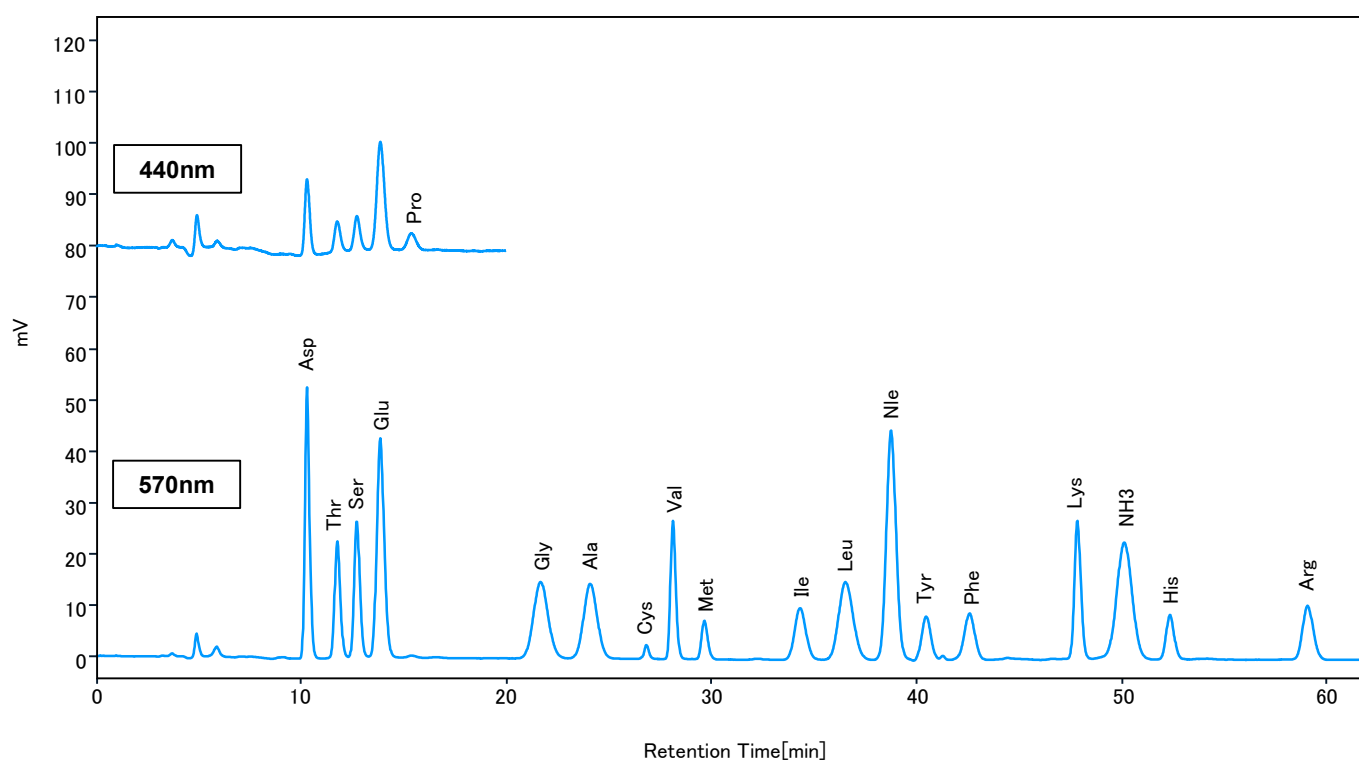


Fig.3 Analysis of Pet food for Dog

Analysis of hydrolyzed pet food samples

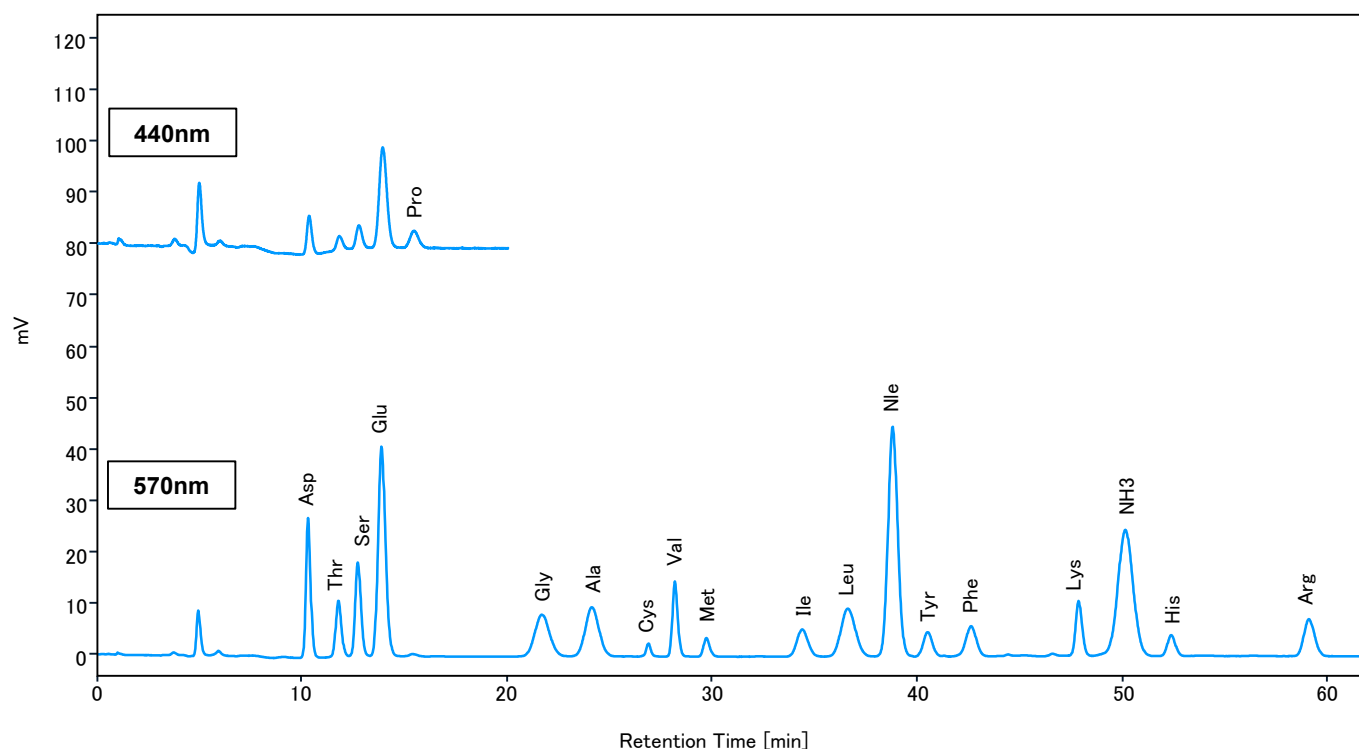


Fig.4 Analysis of Pet food for Bird

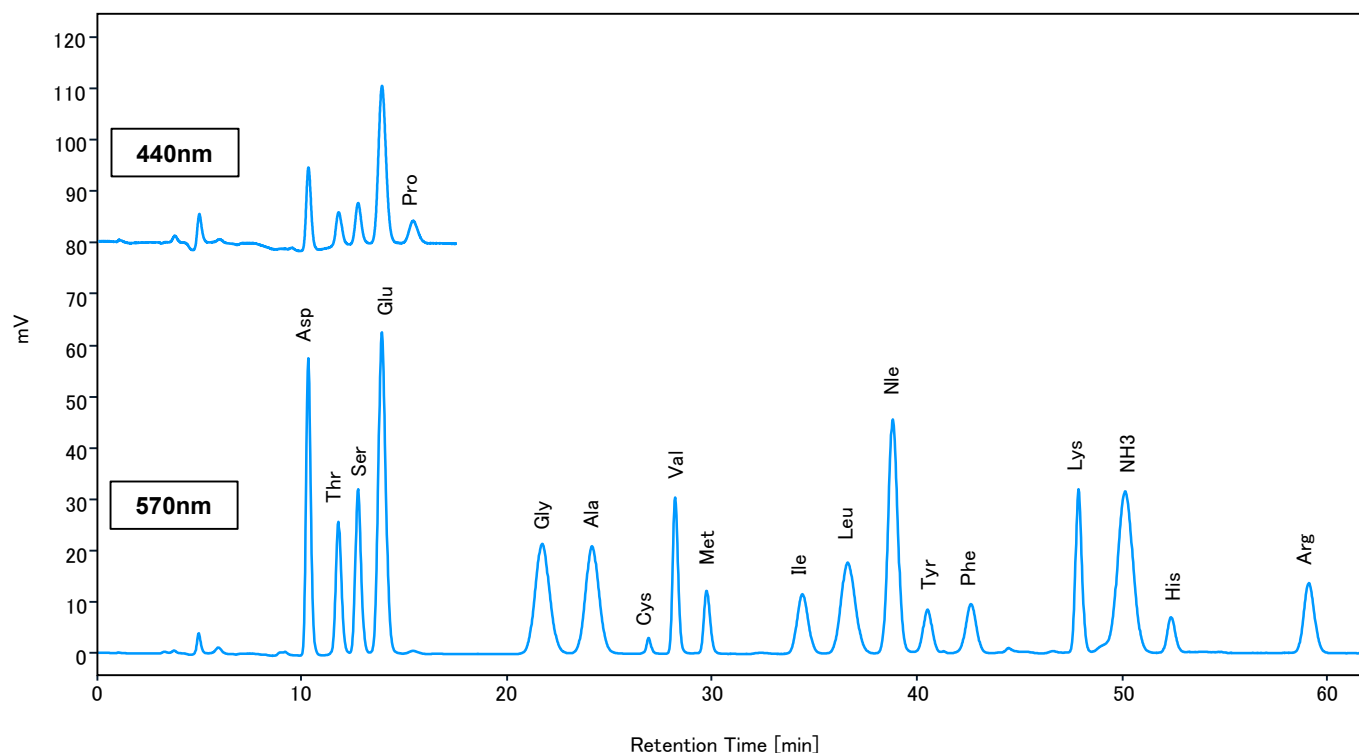


Fig.5 Analysis of Pet food for goldfish

Sample Preparation

Weigh out 3 mg of the pulverized sample and place in a hydrolysis tube

← Add 1 mL of 6 mol/L HCl

← Add 0.5 mL of 2 μmol/mL Nle (as the internal standard)

Degas by an aspirator for 15 min

At 110°C for 22 hrs

Dry under vacuum

← Volume 10 mL with 0.02 mol/L HCl

Filtration Pore Size 0.2 μm

Sample for injection

List of Amino Acids

Abbrev.	Amino acid	Molecular weight	Std. concentration (nmol/ 20 μL)
Asp	Aspartic acid	133.1	2
Thr	Threonine	119.1	2
Ser	Serine	105.1	2
Glu	Glutamic acid	147.1	2
Pro	Proline	115.1	2
Gly	Glycine	75.1	2
Ala	Alanine	89.1	2
Cys	Cystine	240.3	2
Val	Valine	117.1	2
Met	Methionine	149.2	2
Ile	Isoleucine	131.2	2
Leu	Leucine	131.2	2
Nle	Norleucine	131.2	2
Tyr	Tyrosine	181.2	2
Phe	Phenylalanine	165.2	2
Lys	Lysine	146.2	2
NH3	Ammonia	17.0	2
His	Histidine	155.2	2
Arg	Arginine	174.2	2

NOTE: All data on this report are examples of measurement; the individual values are NOT guaranteed. Page.4