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

Pet food is mainly made from grains, livestock meat, fish, etc., and it is necessary to always secure safe and excellent quality raw materials [1]. 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.
The results show that each pet food contains well-balanced amino acids, and PH-Nle High-speed analysis method is very useful when quick analysis is required.

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



LA8080 HIGH SPEED AMINO ACID ANALYZER (AminoSAAYA)

Analysis of Amino Acid Standard Solution

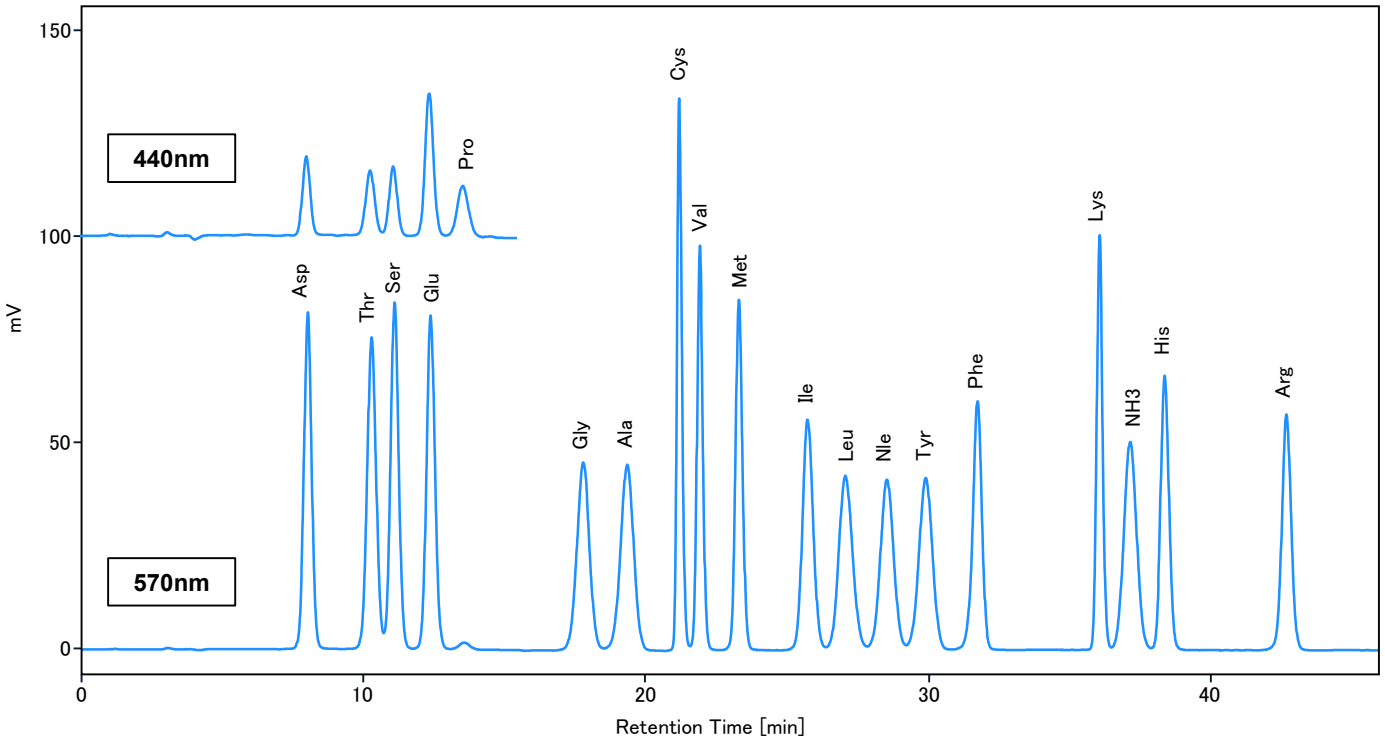

Fig.1 Analysis of Amino Acid Standard Solution

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

Column	#2620M 4.6 mm I.D. × 60 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:modifyed	Reaction temperature	135 ºC
Flow rate	0.22 mL/min	Detection wavelength	VIS 440 nm、570 nm
Column temperature	50~80 ºC	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

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Analysis of hydrolyzed pet food samples

- ✓ Each pet food contains well-balanced amino acids.
- ✓ Hydrolyzed samples can be analyzed within 45 minutes using the PH-Nle High-speed analysis method.

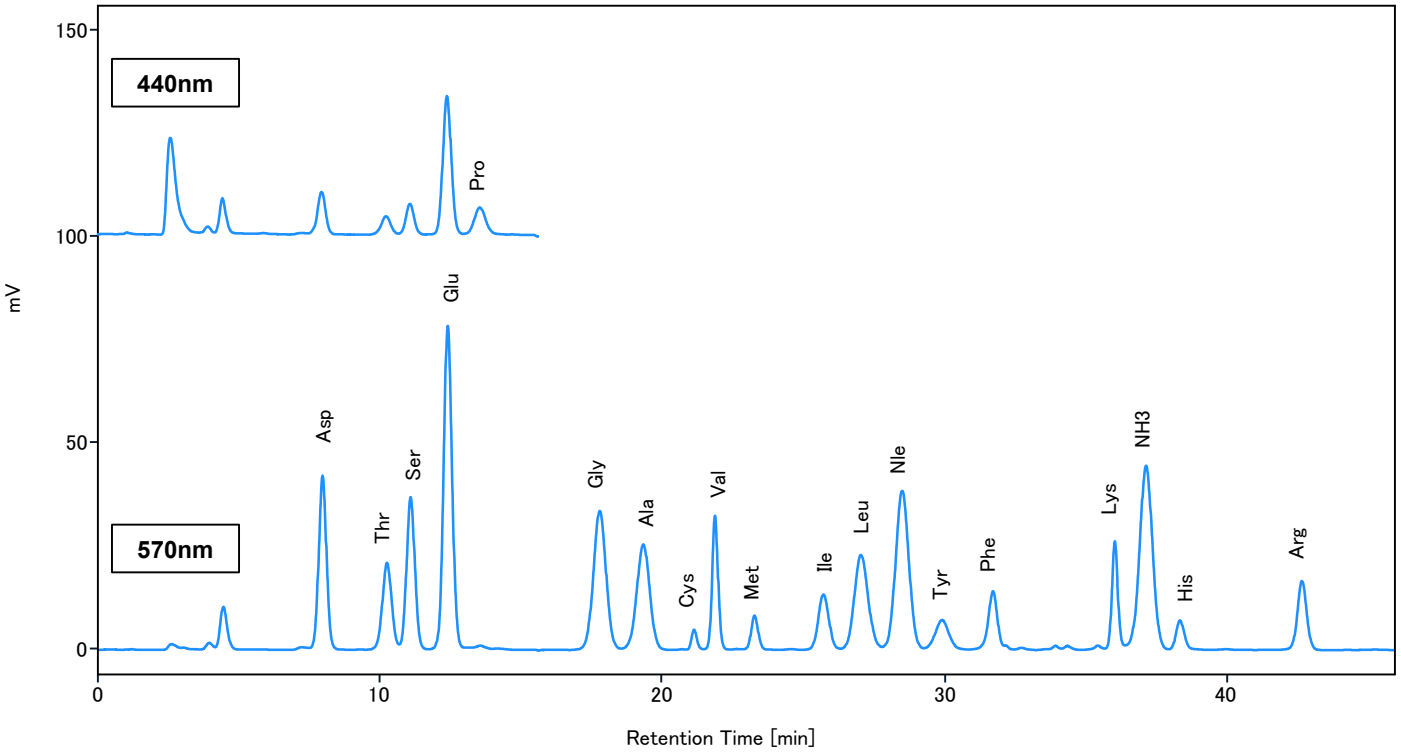

Fig.2 Analysis of Pet food for Cat

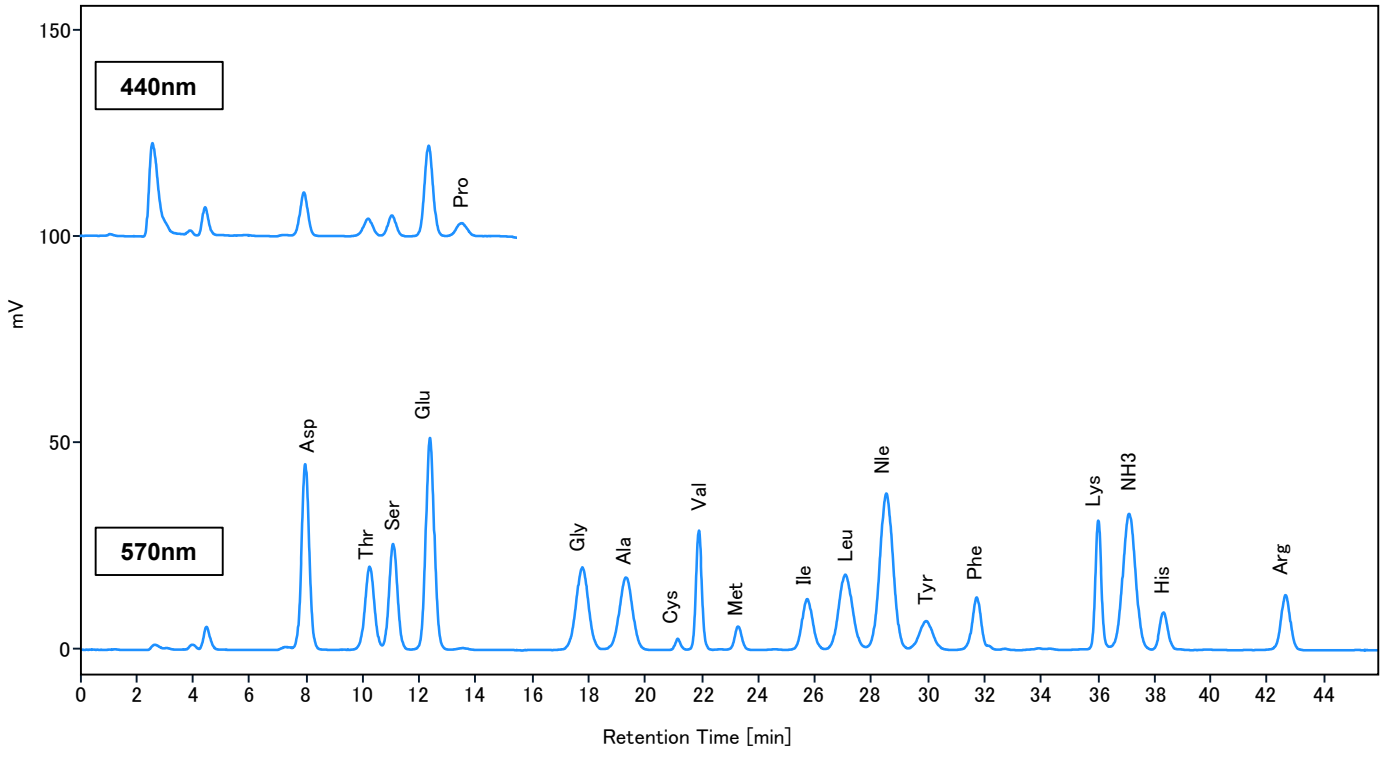

Fig.3 Analysis of Pet food for Dog

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Analysis of hydrolyzed pet food samples

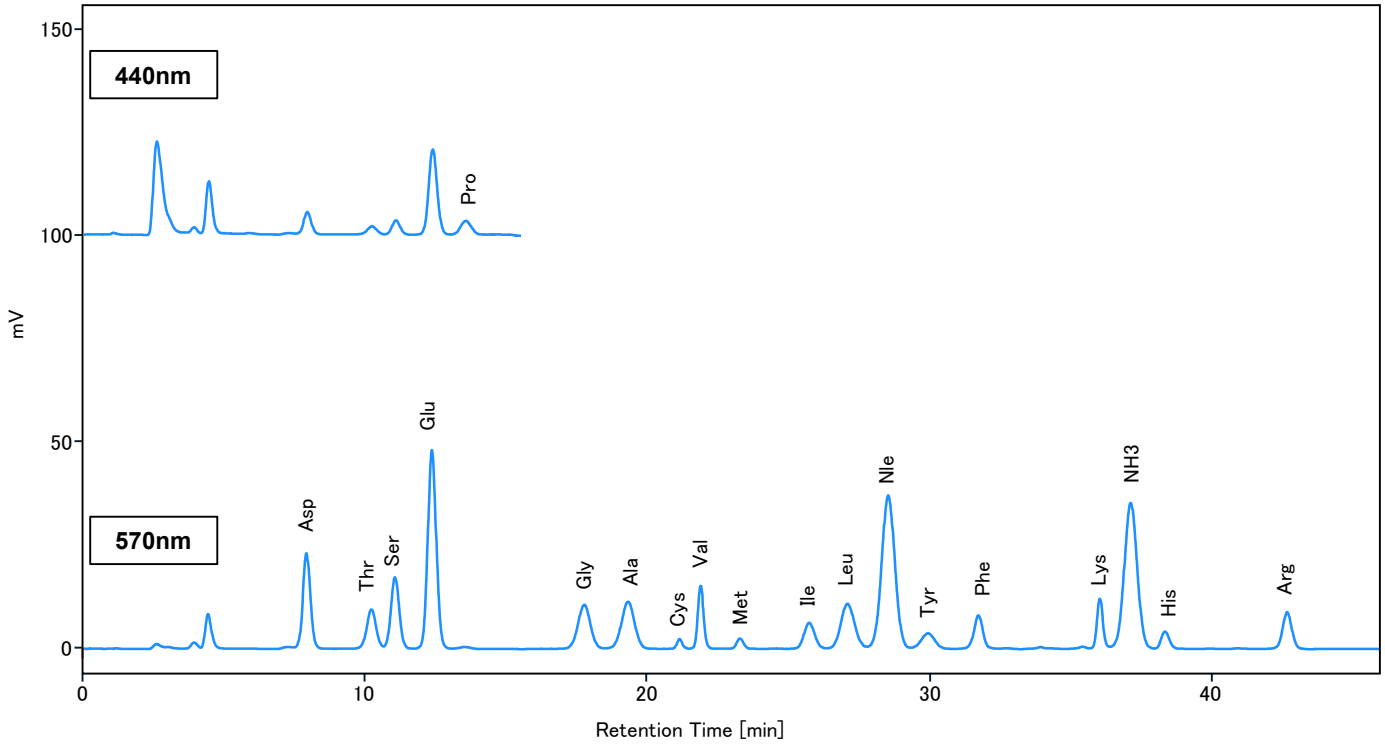

Fig.4 Analysis of Pet food for Bird

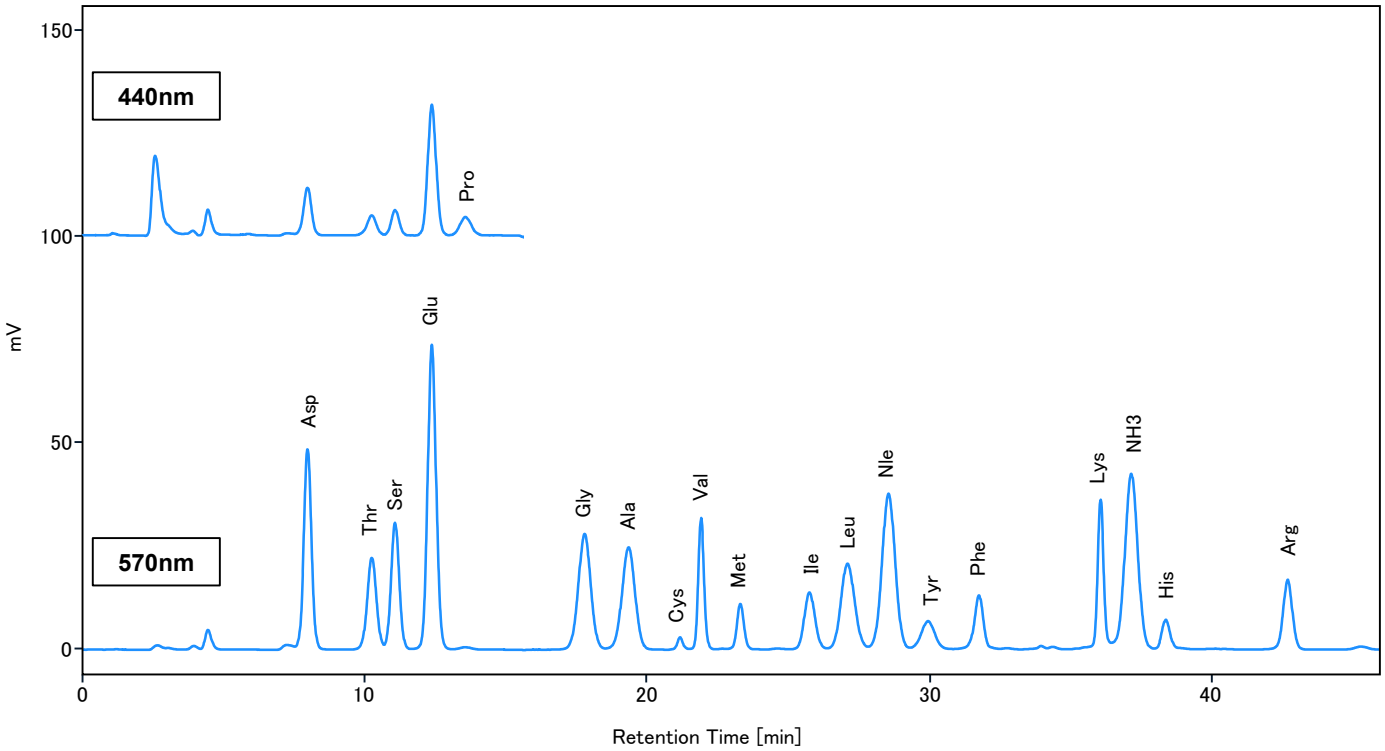

Fig.5 Analysis of Pet food for goldfish

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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℃ 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

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