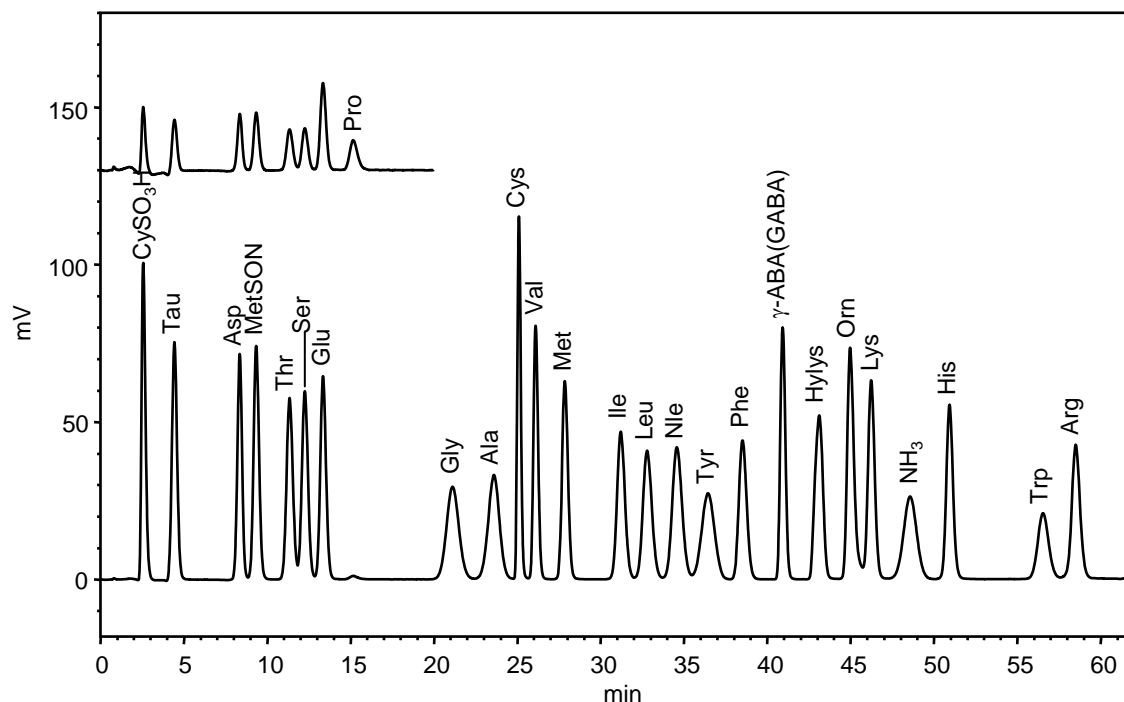


Analysis of Amino Acid Standards
(L-8900, Special Amino Acid Analysis Method)

In pet foods, it is important that well-balanced amino acids are contained as the nutrients essential for the growth. An example of the analysis in which the amino acids in a commercially available rabbit fodder are analyzed is introduced here.

For the preparation, the hydrochloric acid hydrolysis method is generally used. However, this method cannot be used for the analysis as cysteine forms a different derivative. In addition, the recovery of methionine may be reduced depending on the decomposition conditions.

To analyze these components, cystine/cysteine and methionine should be oxidized with performic acid and assayed as cysteic acid (CysO₃H) and methionine sulfone (MetSON), respectively. As tryptophan is destroyed in hydrochloric acid hydrolysis, it should be hydrolyzed by alkali and analyzed.



SAMPLE	20 μL of Std. Soln. (2 nmol/ 20 μL)	PRESSURE	
PACKING MATERIAL	#2620M [HITACHI]	TEMPERATURE	35 – 80°C
COLUMN SIZE	for separation 4.6 mm I.D. × 60 mm [#2620M]	SEPARATION METHOD	Ion Exchange
	for ammonia trap 4.6 mm I.D. × 60 mm [#2650L]	DETECTOR	VIS 570 nm, 440 nm
ELUENT	L-8500 PH-Kit	INSTRUMENTS	L-8900 (Amino Acid Analyzer)
FLOW RATE	0.2 mL/min		

NOTE

Reaction reagent = Ninhydrin coloring solution set for L-8900 R1/R2 – 50/50, Reaction reagent flow rate = 0.3 mL/min, Reaction unit temperature = 135°C, L-8900 special amino acid analysis method was used for this analysis.

For the information on the eluent composition and method, please contact our sales department or the contact site of our HP by quoting the instrument model used currently.

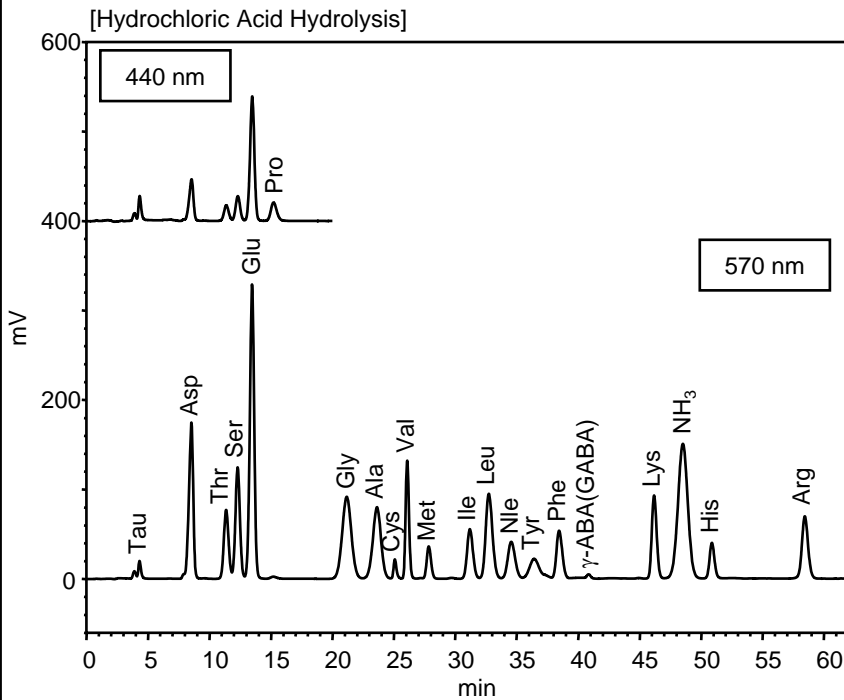
KEY WORDS

Bio-Medical Science-Food-Pharmaceutical, Fertilizer-Fodder, Biochemistry, Agriculture, Fodder Component, Fodder, Hydrolysis, Amino Acid, Norleucine, Rabbit, Special Amino Acid Analysis Method, Ninhydrin Method, UV-VIS Spectrometry, Environment, L-8900, Ion Exchange

High Performance Liquid Chromatograph (HPLC)

Sheet No. LC090034-01A

Analysis of Amino Acids in Hydrolyzed Fodders for Rabbit
(L-8900, Differences in Preprocessing Method)



[Preparation Method]

Weigh out 6 mg of the pulverized sample and place in a hydrolysis tube

- ← Add 1 mL of 6 mol/L HCl
- ← Add 0.5 mL of 0.4 μmol/mL Nle (as the internal standard)

Degas by an aspirator for 15 min

110°C, 22 hrs

Dry under vacuum

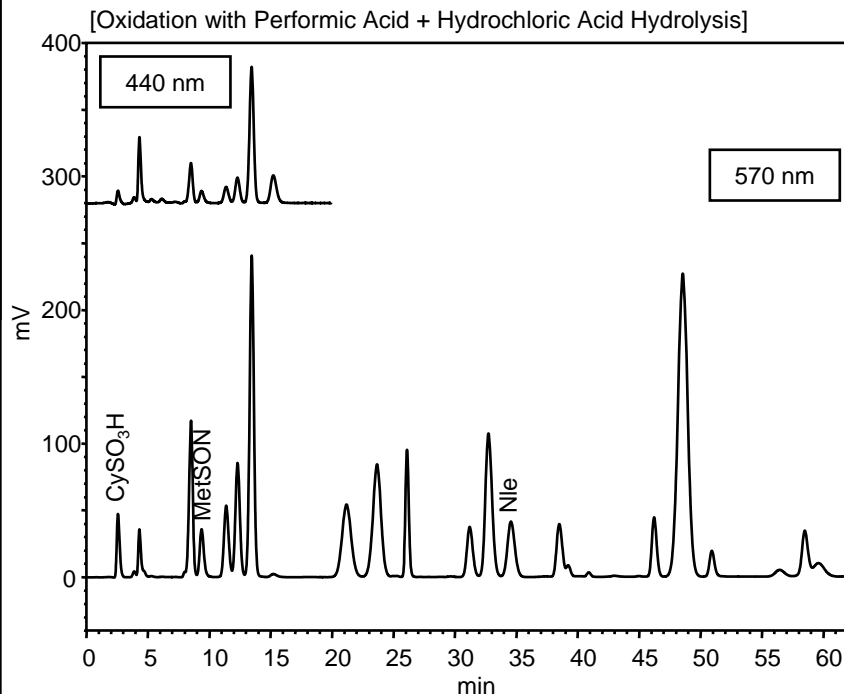
- ← Add 2 mL of 0.02 mol/L HCl

Dissolve

- Centrifuge at 10,000 rpm for 10 min

Filter the supernatant through a 0.2 μm filter

Sample for injection



[Preparation Method]

Weigh out 6 mg of the pulverized sample and place in a round bottom flask

- ← Add 2 mL of performic acid reagent
- ← Add 0.5 mL of 0.4 μmol/mL Nle (as the internal standard)

Let it stand in a refrigerator for about 20 hrs

- ← Add 0.3 mL of 48% HBr

Dry under vacuum

- ← Add 1 mL of 6 mol/L HCl

Transfer into a hydrolysis tube

Degas by an aspirator for 15 min

110°C, 18 hrs

Dry under vacuum

- ← Add 2 mL of 0.02 mol/L HCl

Dissolve

- Centrifuge at 10,000rpm for 10 min

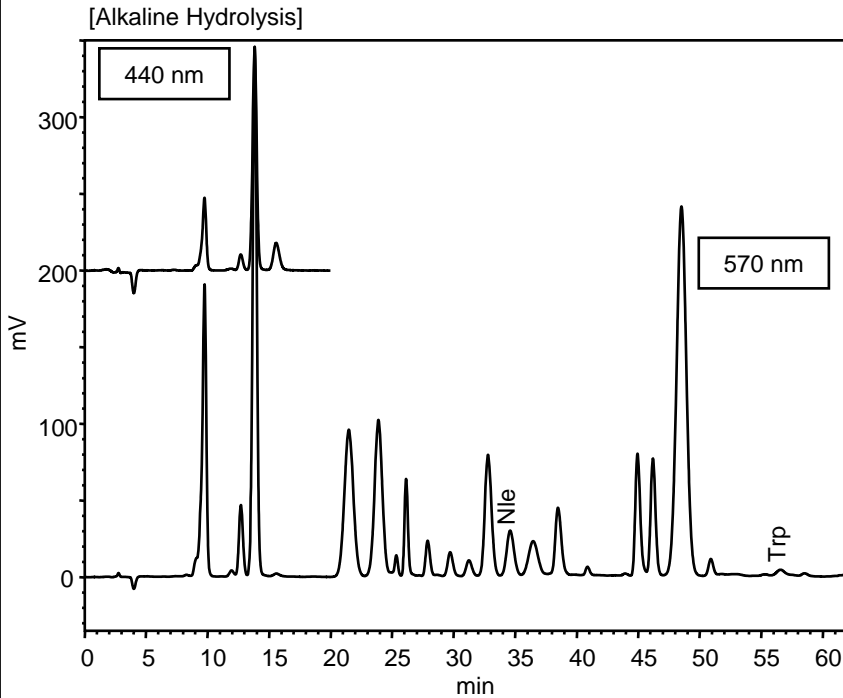
Filter the supernatant through a 0.2 μm filter

Sample for injection

High Performance Liquid Chromatograph (HPLC)

Sheet No. LC090034-02A

Analysis of Amino Acids in Hydrolyzed Fodders for Rabbit
(L-8900, Differences in Preprocessing Method)



[Preparation Method]

Weigh out 60 mg of pulverized sample and place in a hydrolysis tube

- ← Add 1.5 mL of 5 mol/L NaOH
- ← Add 0.5 mL of 4 μ mol/mL Nle (as the internal standard)

Degas for 15 min by an aspirator

110°C, 16 hrs

- Centrifuge at 3,000 rpm for 10 min

Collect 0.5 mL of supernatant

- ← Add 0.5 mL of 6 mol/L HCl
- ← Add 4 mL of purified water

Mix

- Centrifuge at 10,000rpm for 10 min

Filter the supernatant through a 0.2 μ m filter

↓

Sample for injection

[Abbreviations of Amino Acids]

Abbrev.	Amino Acid	Abbrev.	Amino Acid
CySO ₃ H	Cysteic acid	Ile	Isoleucine
Tau	Taurine	Leu	Leucine
Asp	Aspartic acid	Nle	Norleucine
MetSON	Methionine sulfone	Tyr	Tyrosine
Thr	Threonine	Phe	Phenylalanine
Ser	Serine	γ -ABA	γ -Amino-n-butyric acid
Glu	Glutamic acid	Hylys	Hydroxylysine
Pro	Proline	Orn	Ornithine
Gly	Glycine	Lys	Lysine
Ala	Alanine	NH ₃	Ammonia
Cys	Cystine	His	Histidine
Val	Valine	Trp	Tryptophan
Met	Methionine	Arg	Arginine

High Performance Liquid
Chromatograph (HPLC)

Sheet No. LC090034-03A