

PH-Nle High-resolution analysis method (compliant with European Pharmacopoeia)

The European Pharmacopoeia (EP) is an international regulation used in the European region. It is recommended to use Norleucine (Nle) as an internal standard for the amino acid analysis listed in the EP. Moreover, the Resolution of Isoleucine (Ile) and Leucine (Leu) is required 1.5 or higher for system suitability.

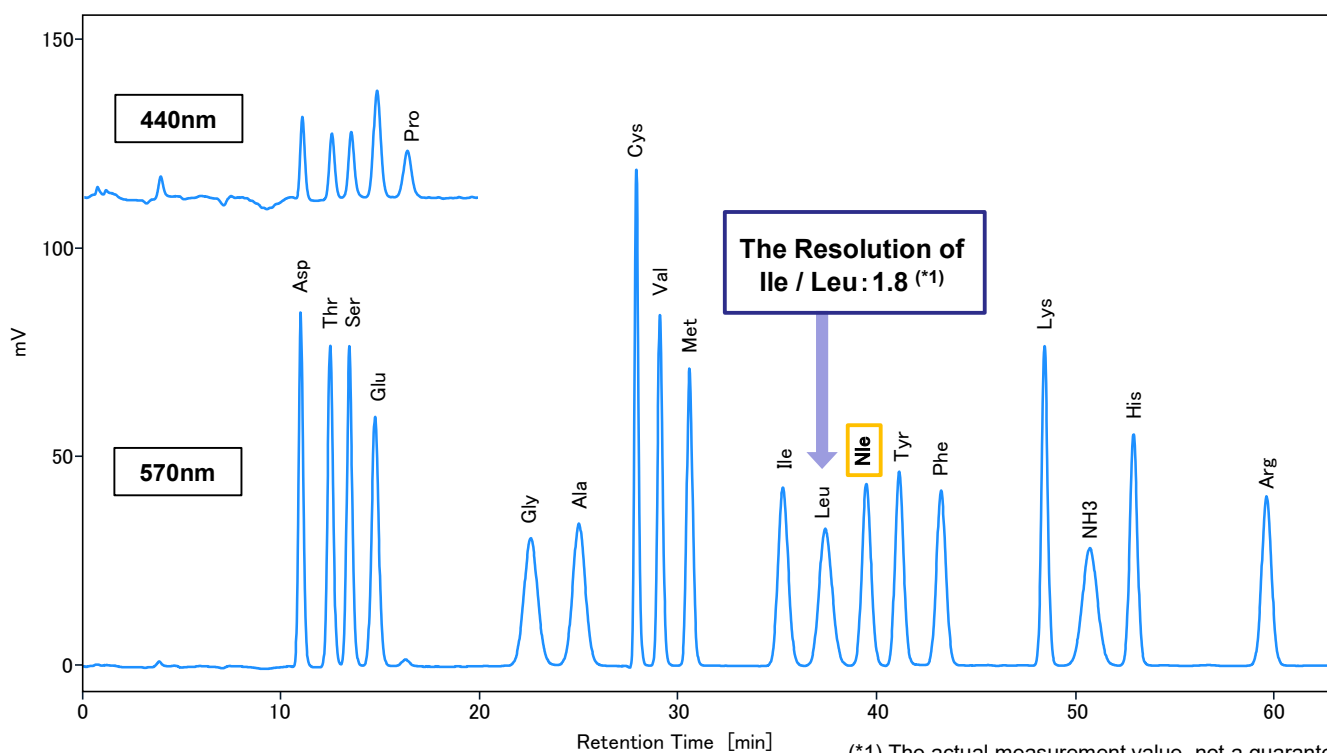
As an EP-compliant analytical method, we have developed a PH-Nle high-resolution method in which Nle can be used as an internal standard and the Resolution of Ile and Leu is 1.5 or higher.

The PH-Nle High-resolution analysis method is suitable for precise quantification, which enables amino acid analysis with high-resolution even for components other than Ile / Leu. In addition to EP, this analysis method also complies with COMMISSION REGULATION (EC) No 152/2009 and ISO 13903-2005 Animal feeding stuffs, which stipulate analysis methods for feed (including pet food).



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

Analysis of Amino Acid Standard Solution



(*1) The actual measurement value, not a guaranteed.

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) ^{(*)2}
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 ^{(*)2} 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^{(*)2} with Nle added.

This analysis method is only available on the LA8080.

(*2) FUJIFILM Wako Pure Chemical Corporation

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.2