



## Stability of Glutamine and Tryptophan

In the commercially available mixed standard sample solution used for the amino acid analysis, glutamine (GluNH<sub>2</sub>) and tryptophan (Trp) that are considered unstable are not included. For the quantitative analysis of those, an analyst adds the separately prepared GluNH<sub>2</sub> or Trp standard solution to the commercially available mixed standard sample. Then, to adjust the pH close to that of the mobile phase, the dilution with dilute hydrochloric acid is performed for the analysis.

This time, the solution for dissolving GluNH<sub>2</sub> and Trp to be added and the storage method were studied. As a result, the stability was found to be different depending on the components, and the study results are introduced here.



L-8900 High-speed Amino Acid Analyzer

### Verification Method for Stability

- ✓ The standard samples prepared by the method described in Figure 1 were analyzed and the stability was verified by comparing the peak areas.

- (1) Weigh 7.3 mg of GluNH<sub>2</sub> and 10.2 mg of Trp
- (2) Make up the volume to 20 mL with dilute hydrochloric acid (20 mmol/L HCl) or ultrapure water to prepare the 2.5 μmol/mL solution
- (3) Store at 4°C or -20°C
- (4) Perform the step (1) to (3) every 2 weeks
- (5) After 12 weeks, dilute the solutions that had been prepared with dilute hydrochloric acid to 0.1 μmol/mL
- (6) Analyze

Figure 1 Verification Method

Table 1 Analytical Conditions

Column	#2622PH 4.6 mm I.D. × 60 mm
Guard column	#2619 4.0 mm I.D. × 5 mm
Ammonia filter column	#2650L 4.6 mm I.D. × 40 mm
Mobile phase	MCI buffer L-8500 PH-Kit*
Flow rate	0.40 mL/min
Column temperature	57°C
Reaction reagent	Ninhydrin coloring solution kit for Hitachi*
Reaction reagent flow rate	0.35 mL/min
Reaction temperature	135°C
Detection wavelength	VIS 440 nm, 570 nm
Injection vol.	20 μL

\* Supplier: Wako Pure Chemical Industries, Ltd.

### Change When Stored at -20°C After Dissolving with Dilute Hydrochloric Acid

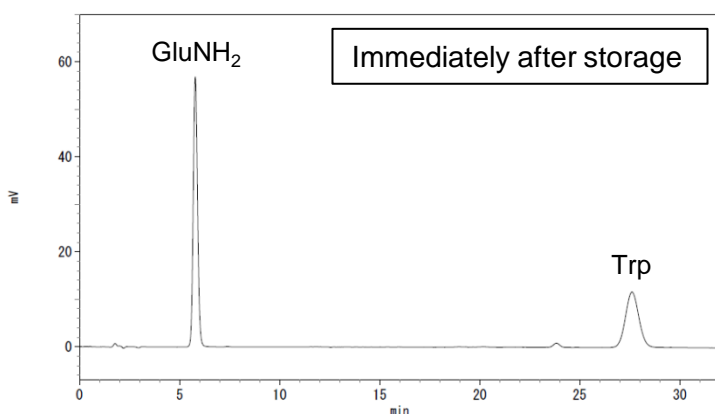


Figure 2 Chromatogram of Standard Sample Immediately After Storage

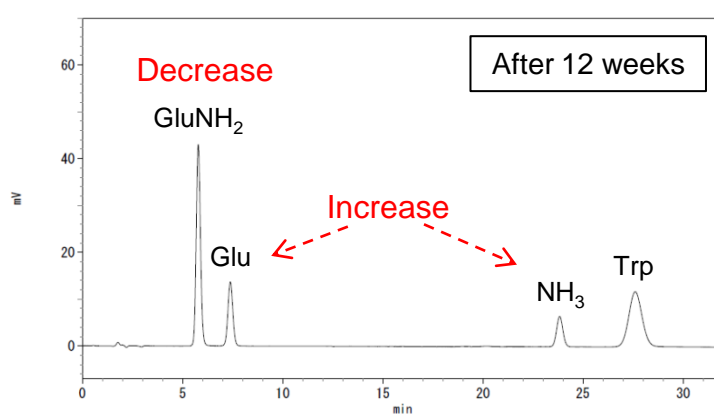


Figure 3 Chromatogram of Standard Sample After 12 Weeks



## Change When Stored at 4°C After Dissolving in Ultrapure Water

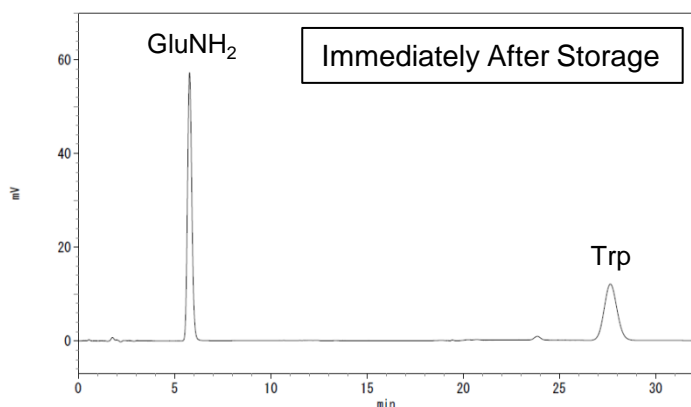


Figure 4 Chromatogram of Standard Sample Immediately After Storage

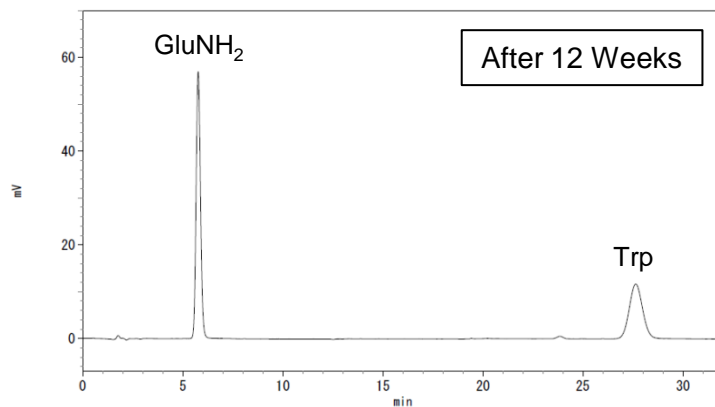


Figure 5 Chromatogram of Standard Sample After 12 Weeks

## Comparison of Changes Over Time for Different Solutions and Storage Temperatures

- ✓ Based on the peak area obtained immediately after dissolving in ultrapure water, the area ratios (%) were determined.
- ✓ The concentration of glutamine dissolved in dilute hydrochloric acid decreased gradually. However, the change was suppressed when ultrapure water was used for the dilution (Figure 6).
- ✓ For tryptophan, regardless of the solutions and storage temperatures, no significant change was observed (Figure 7).

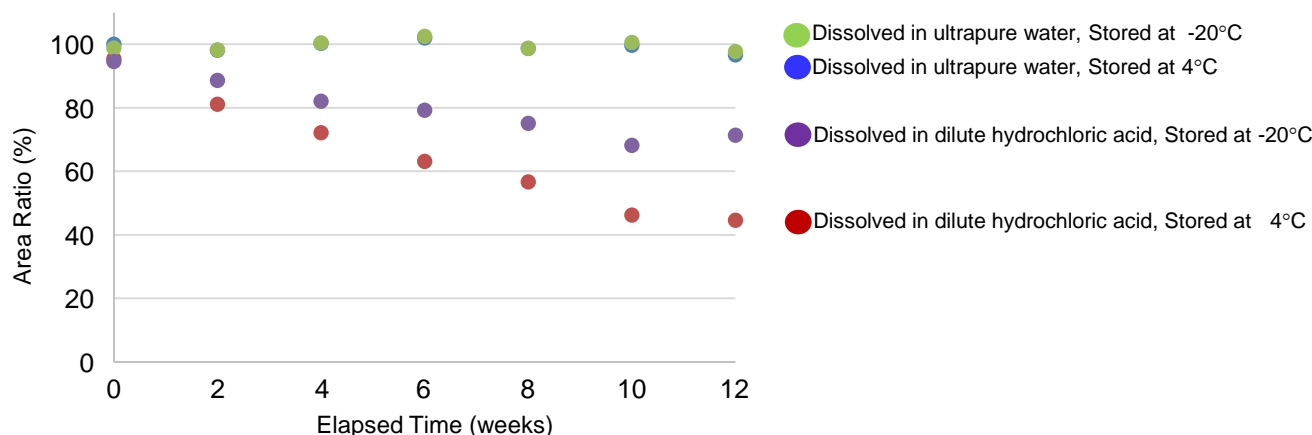


Figure 6 Variation with Time for Glutamine

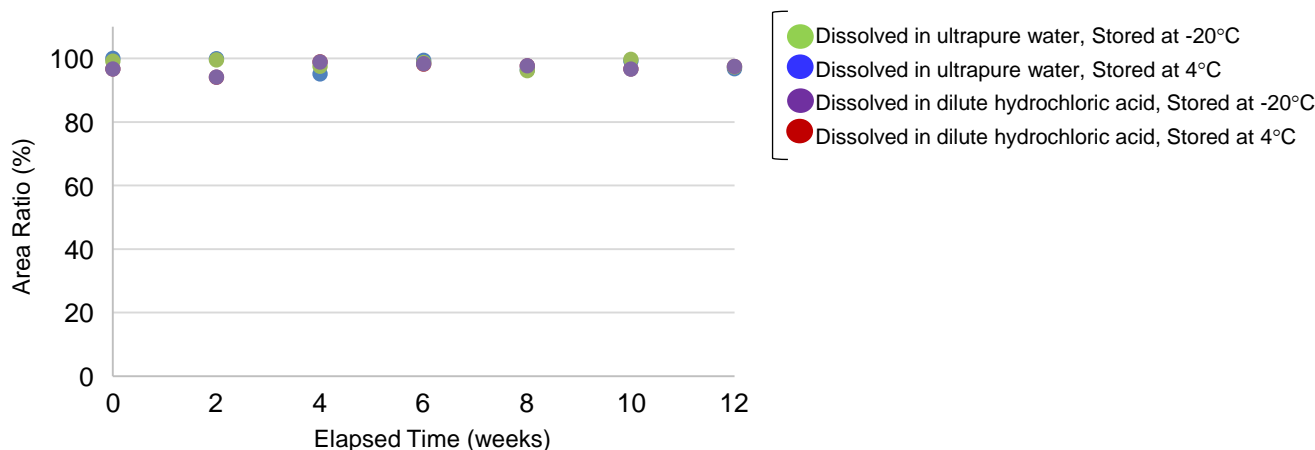


Figure 7 Variation with Time for Tryptophan

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.