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Application News

No.L434

High Performance Liquid Chromatography

UF-Amino Station LC/MS Ultra Fast Amino Acid Analysis System (Part 2)

Improved Sample Analysis Reliability

Analysis of amino acids is typically a time-consuming process, and although this analysis can be speeded up using HPLC or UHPLC for reversed phase pre-column derivatization of the amino acids, the reliability of the results can be adversely affected with samples containing complex matrices, such as biological or food samples. This can be due to insufficient separation of the impurities from amino acids or separation among the amino acids in the sample.
The new UF-Amino Station, a dedicated amino acid analysis system, adopts a mass spectrometer for detection. Mass spectrometry can provide excellent detection selectivity and highly reliable analysis even when the sample contains many components of interest or a complex matrix. Furthermore, the automated derivatization processing provided with the autosampler also contributes to improved reliability of quantitative values.
Here, we introduce the features of the UF-Amino Station that demonstrate its power in actual sample analysis.

Superior Selectivity with LC/MS Detection

Fig. 1 shows the results of analysis of amino acids in commercially available skim milk by the UV detection with pre-column derivatization using phenyl isothiocyanate (PITC) as a derivatization reagent (upper) and by using the UF-Amino Station (lower). If amino acids derivatized using PITC are analyzed using the same separation time as that using the UF-Amino Station, inadequate separation among amino acids or between contaminants and target amino acids may occur, adversely affecting the quantitative results. This is due mainly to the selectivity of the UV detection method, and even in the chromatogram of Fig. 1 (upper part), when the analysis time is the same as that using the UF-Amino Station, adequate separation is clearly jeopardized due to the contaminants. However, because the UF-Amino Station adopts an LC/MS as the detector, of each amino acid is conducted using a separate mass chromatogram (Fig. 1 (lower part)). This high detection selectivity minimizes the effects of amino acid interaction and the overlapping of amino acid and contaminant peaks in the quantitative results. The analytical conditions associated with the data of Fig. 1 are shown in Table 1.

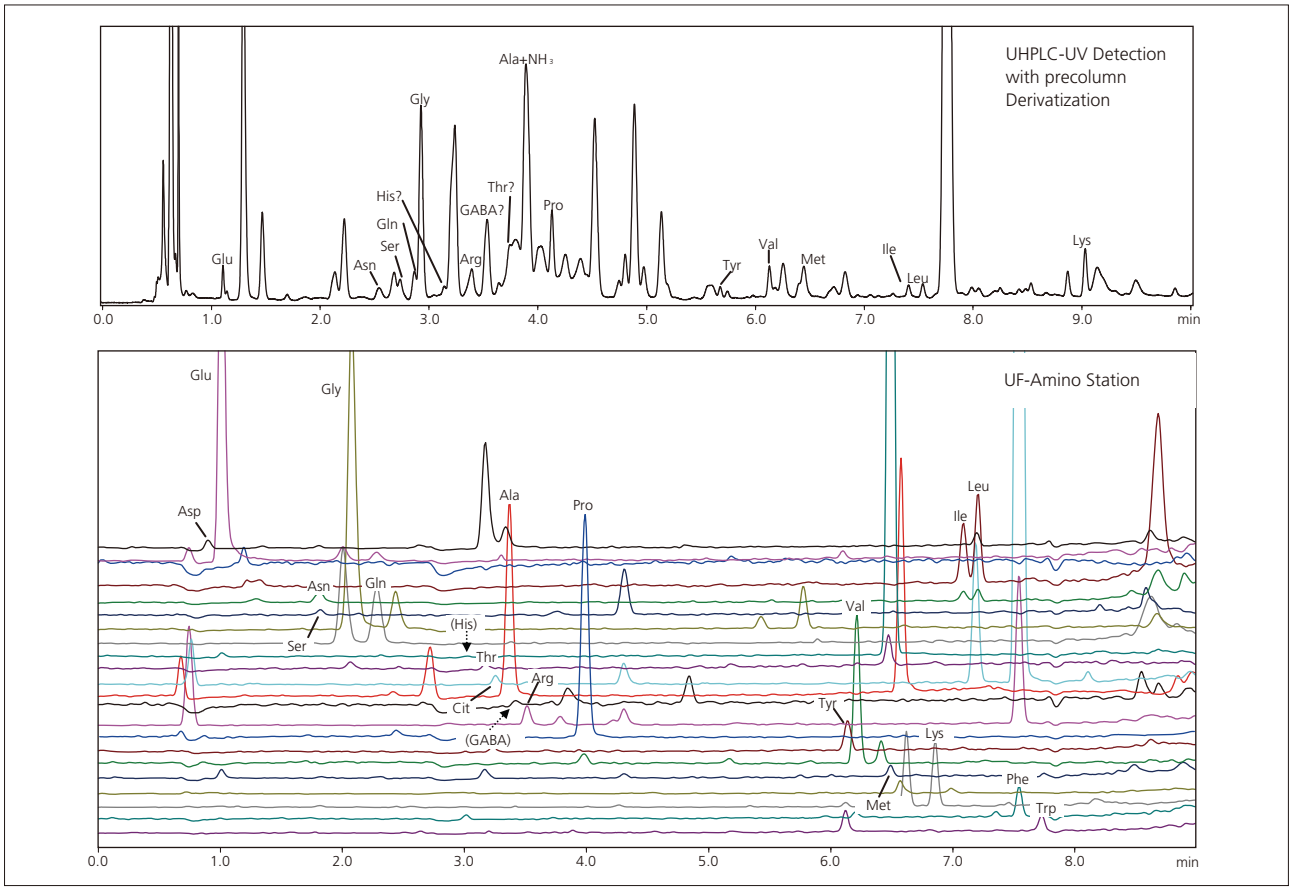

Fig. 1 Analysis of Amino Acids in Skim Milk: (Upper) UV Detection with PTC-Pre-Column Derivatization, (Lower) UF-Amino Station

Fig. 2 shows the results of amino acid recovery analysis of the same commercially available skim milk of Fig. 1, spiked with a standard solution of amino acids, this time using the UF-Amino Station. Preparation was conducted according to the specified method, which included deproteinization of the skim milk by dissolving it in heated water and adding acetonitrile, addition of amino acid standard solution, and addition of internal standard solution, etc. Analysis was conducted using the UF-Amino Station. The excellent recoveries shown in Fig. 2 demonstrate the high quantitative reliability that can be obtained even with a sample containing a highly complex matrix, such as that of nonfat dry milk.
Also, as introduced in Application News No. L433, the UF-Amino Station automates the derivatization of amino acids with the pretreatment feature provided in the SIL-20AC$_{PT}$ autosampler. This feature not only improves analysis efficiency, but also boosts the repeatability of the derivatization process, thus contributing to significantly higher precision.

Table 1 Analytical Conditions

[UHPLC, UV Detection with PTC-Precolumn Derivatization]	
Column	: Shim-pack XR-ODS (100 mm L. × 3.0 mm I.D., 2.6 µm)
Mobile Phase	: (Potassium) phosphate buffer (pH 7.0) and Acetonitrile, Gradient Elution
Flowrate	: 0.9 mL/min
Column Temp.	: 40 °C
Reaction Reagent	: Phenyl Isothiocyanate
Detection	: SPD-20AV at 254 nm with Semi-micro Cell
[UF-Amino Station]	
Refer to *Application News No. L433*	

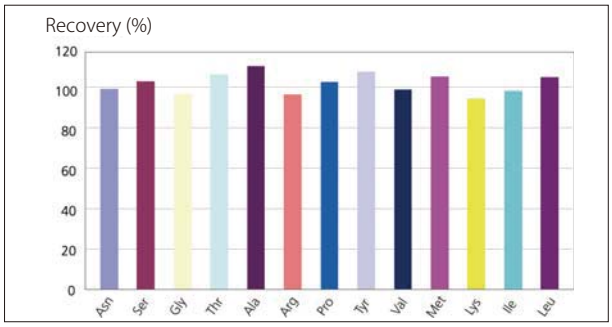

Fig. 2 Recovery of Amino Acids in Skim Milk

Biological Sample Applications

Biological samples also contain many impurities which often can adversely affect the reliability of quantitative values when using UV detection with pre-column derivatization. Fig. 3 shows the results of the analysis of amino acids in a commercially available serum-free culture medium and rat plasma using the UF-Amino Station. In both samples, all components are clearly separated without any interference effects, clearly demonstrating that the UF-Amino Station is applicable for accurate analysis of amino acids in biological samples.

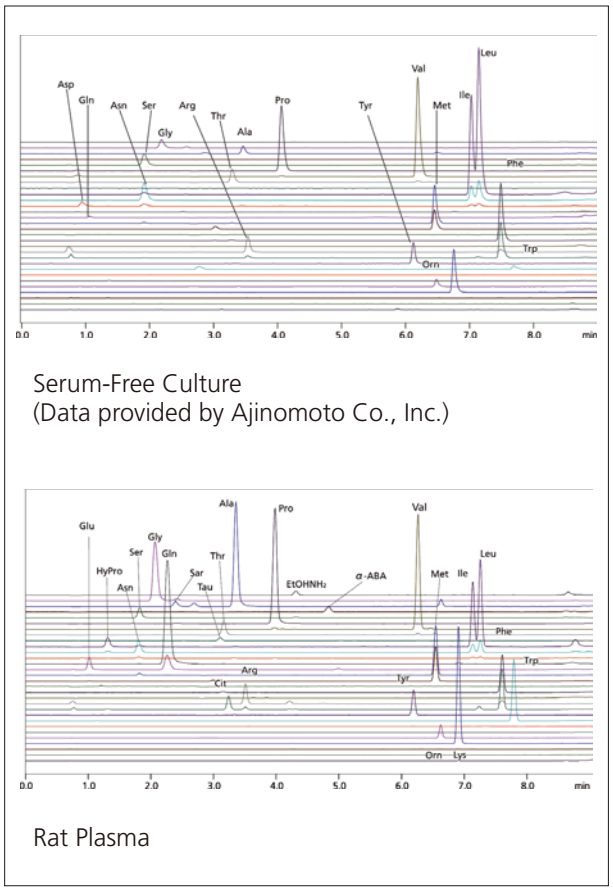

**Fig. 3 Biological Sample Applications:
(Upper) Serum-Free Culture, (Lower) Rat Plasma**

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Shimadzu Corporation
www.shimadzu.com/an/