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Application News

No.L433

High Performance Liquid Chromatography

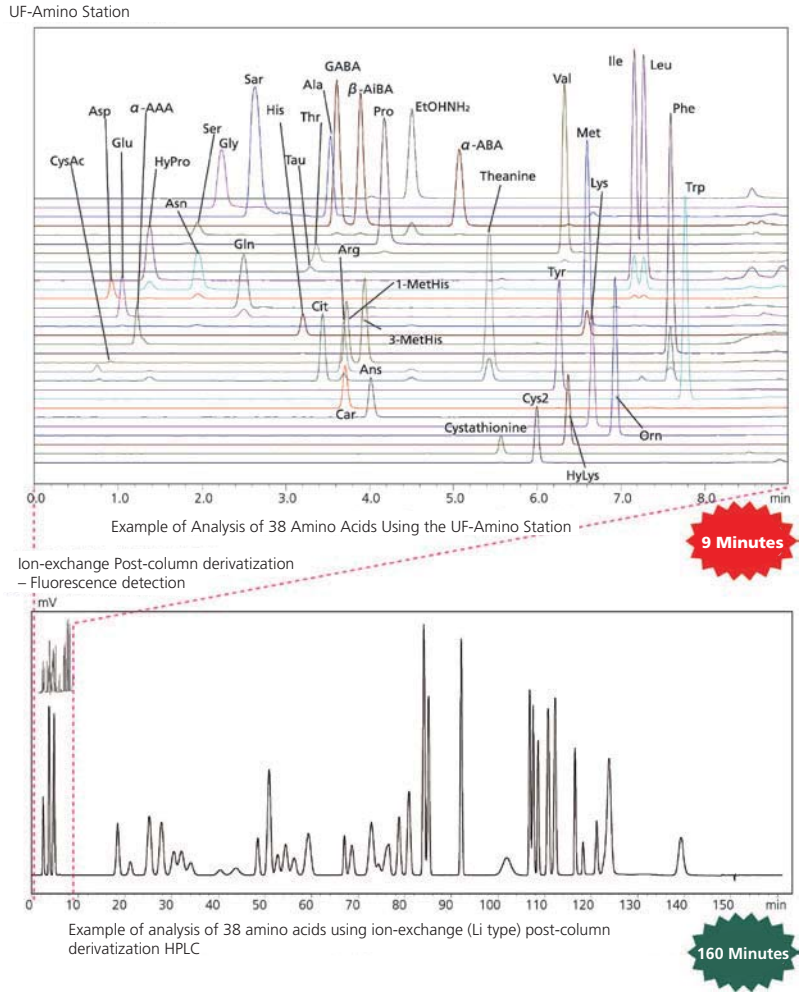
UF-Amino Station LC/MS Ultra Fast Amino Acid Analysis System (Part 1)

A Novel Approach for Ultra High Speed Analysis of Amino Acids

Ion exchange HPLC with post-column derivatization has traditionally been used for amino acid analysis, and, although effective for multiple-component amino acid analysis, this type of analysis does require considerable time. On the other hand, reversed phase pre-column derivatization with UV detection for this amino acid analysis requires much less time, but the drawback here is the limited number of possible amino acids that can be detected.
The UF-Amino Station, developed in collaboration with Ajinomoto Co., Inc., utilizes a dedicated high-speed, high-separation column together with an ultra-high-speed mass spectrometer to achieve simultaneous analysis of 38 amino acids and related substances in just 9 minutes. Furthermore, various features, including automated derivatization processing and specialized software permit simple operation and multi-sample processing.
Here, we introduce the basic principles of amino acid analysis using the UF-Amino Station which make possible ultra fast analysis of amino acids.

Simultaneous Analysis of 38 Amino Acids in 9 Minutes

Fig. 1 shows the results of simultaneous analysis of a biological standard sample consisting of 38 amino acids using the UF-Amino Station (upper), in addition to the cation-exchange post-column derivatization-fluorescence detection method (lower). The cation-exchange post-column derivatization method required more than 2 hours to complete separation of 38 components. With the UF-Amino Station, on the other hand, use of the Shim-pack UF-Amino dedicated high-speed, high-resolution column (2 µm particle size) permitted ultra-high-speed separation of the 38 amino acids. Furthermore, by using the LCMS-2020 ultra-high-speed mass spectrometer as the detector, these substances were all detected in just 9 minutes. The analytical conditions are shown in Table 1.



CysAc	: Cysteic acid
Asp	: Aspartic acid
Glu	: Glutamic acid
α-AAA	: α-Aminoadipic acid
HyPro	: Hydroxyproline
Asn	: Asparagine
Ser	: Serine
Gly	: Glycine
Gln	: Glutamine
Sar	: Sarcosine
His	: Histidine
Tau	: Taurine
Thr	: Threonine
Cit	: Citrulline
Ala	: Alanine
GABA	: γ-Aminobutyric acid
Car	: Carnosine
Arg	: Arginine
1-MetHis	: 1-Methylhistidine
3-MetHis	: 3-Methylhistidine
β-AiBA	: β-Aminoisobutyric acid
Ans	: Anserine
Pro	: Proline
$EtOHNH_2$	: Ethanolamine
α-ABA	: α-Aminobutyric acid
Theanine	: Theanine
Cystathionine	: Cystathionine
Cys2	: Cystine
Tyr	: Tyrosine
Val	: Valine
HyLys	: Hydroxylysine
Met	: Methionine
Orn	: Ornithine
Lys	: Lysine
Ile	: Isoleucine
Leu	: Leucine
Phe	: Phenylalanine
Trp	: Tryptophan

Fig. 1 Simultaneous Analysis of 38 Amino Acids Standard: (Upper) UF-Amino Station, (Lower) Cation-Exchange Post-Column Derivatization - Fluorescence Detection

Table 1 Analytical Conditions

Column	: Shim-pack UF-Amino (100 mm L. × 2.1 mm I.D., 2 µm)	Reaction Reagent	: Amino Tag Wako and Amino Tag Wako Borate Buffer
Mobile Phase	: Amino Tag Wako Eluent Buffer and Acetonitrile, Gradient Elution	Detection	: LCMS-2020
		Probe	: ESI Positive
Flowrate	: 0.3 mL/min		
Column Temp.	: 40 °C		* All reagents are available from Wako Pure Chemical Industries, Ltd.

Automated Derivatization in Overlap Process Contributes to Ultra Fast Analysis

The UF-Amino Station utilizes the automatic pretreatment functions of the SIL-20AC$_{PT}$ autosampler to effectively streamline the process of amino acid derivatization. The flow line drawing of Fig. 2 shows the automated derivatization reaction. When the analysis is started after placing the standard solution, sample solution and special reagent in the autosampler rack, each of these solutions are aspirated by the autosampler, then discharged into an empty vial for mixing and dilution, and the mixture is then introduced into a heated reaction unit for derivatization. The derivatized amino acids are then injected onto the column by just switching a valve. Since these operations are conducted automatically by the autosampler, the derivatization reaction is accomplished safely and efficiently.

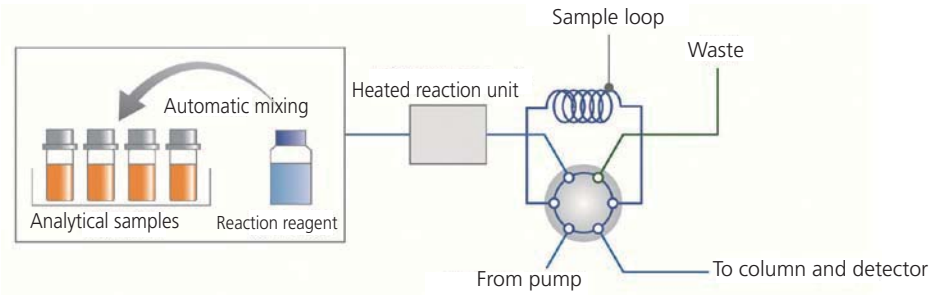


Fig. 2 Automated Derivatization by SIL-20AC$_{PT}$

Also, by conducting the automated derivatization process and analysis concurrently, this contributes to even speedier analysis. When the derivatized sample is injected onto the column, the autosampler begins pretreatment of the next sample solution. While the first injected sample is being analyzed, mixing of a new sample solution and reaction reagent, and derivatization in the heating reaction unit take place. Thus, efficient analysis is promoted by effectively utilizing one cycle of analysis time for preparation of the next analysis. Fig. 3 illustrates the pretreatment overlap process.

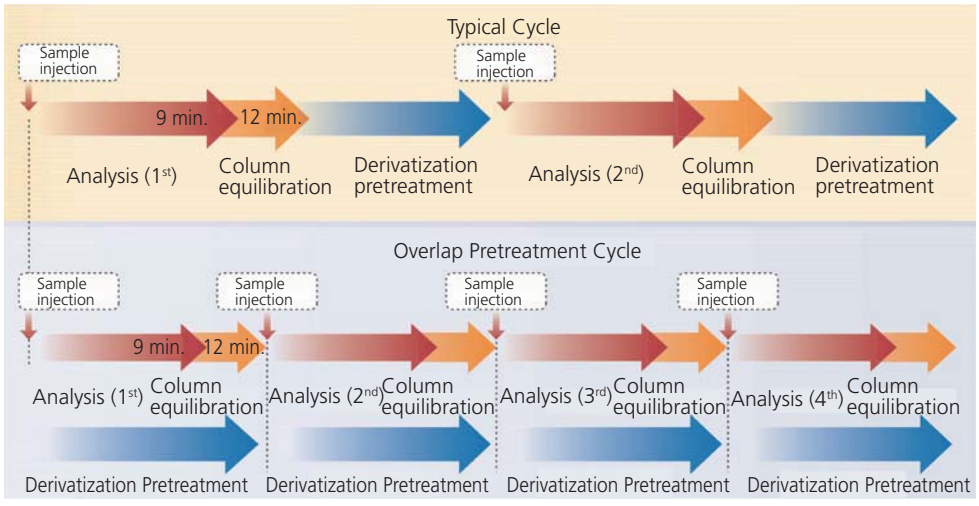


Fig. 3 Overlap Derivatization Process by UF-Amino Station

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