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Application News

High Performance Liquid Chromatograph Nexera™ XR/RF-20AXS

Quantitative Analysis of Pre-Column Derivatized Amino Acids by HPLC-Fluorescence Detector Using Automatic Pretreatment Function

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User Benefits

- Quantitative analysis of amino acids can be performed with good repeatability.
- Pre-column derivatization analysis of amino acids can be easily performed by using the automatic pretreatment function of Nexera XR.
- Analysis of 20 proteinogenic amino acids can be performed in 24 minutes per cycle.

■ Introduction

Analysis of amino acids is considered necessary in a wide range of fields, such as development of food products and pharmaceuticals. Various techniques are used, including the HPLC-fluorescence detector, HPLC-UV detector, LC/MS, and GC/MS, taking advantage of the respective features of these instruments.

The Application News 01-00007-EN introduced an analysis of amino acids by the automated pre-column derivatization method using HPLC-fluorescence detector.

This article introduces an example in which an analysis method using those conditions was applied to a quantitative analysis of amino acids.

■ Analytical Condition

This analysis was performed using the pre-column derivatization method, in which amino acids are derivatized before the analysis. The derivatization reaction was carried out automatically using the automatic pretreatment function equipped in the autosampler, enabling simple and stable analysis.

The chromatograms of 21 amino acids, mainly proteinogenic amino acids, are shown in Figure 1, and an outline of the analytical conditions is shown in Table 1. For details, refer to the Application News 01-00007A-EN.

Table 1-1 Analytical Conditions

Column	: Shim-pack™ XR-ODSII*1 100 mm × 3.0 mm I.D., 2.2 µm
Mode	: Low pressure gradient
Mobile phase	: A) 20 mmol/L (Sodium) acetate buffer (pH 6) B) Water/Acetonitrile = 100:900 C) 20 mmol/L (Sodium) acetate buffer (pH 5) containing 0.5 mmol/L EDTA-2Na
Flow rate	: 1.0 mL/min
Column temp.	: 35 ˚C
Injection volume	: 1 µL
Sample cooler	: 4 ˚C
Detection	: Ch1) Ex. 350 nm, Em. 450 nm Ch2) Ex. 266 nm, Em. 305 nm (RF-20AXS, cell temperature 35 ˚C)

*1: P/N 228-41624-92

Table 1-2 Time Program

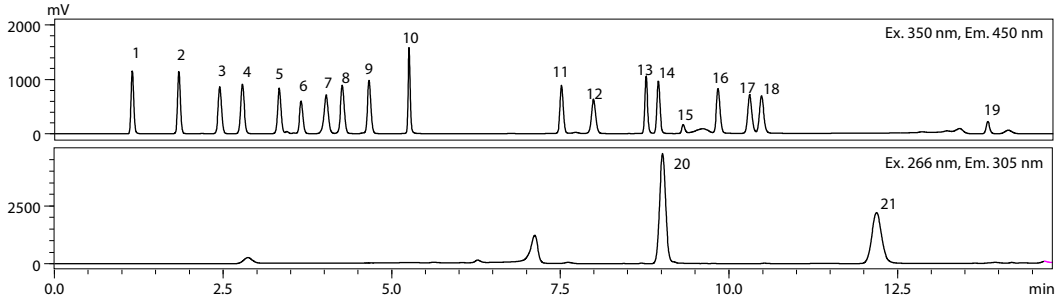
Time (min)	A.conc	B.conc	C.conc
0	95	5	0
0.2	93	7	0
1	93	7	0
4	87	13	0
5	0	15	85
7.5	0	30	70
12	0	35	65
14	0	45	55
14.01	0	95	5
16.99	0	95	5
17	95	5	0
18	95	5	0

■ Linearity and Repeatability

Linearity (r^2, coefficient of determination) of calibration curves for all amino acids were 0.999 or greater in the concentration range of 1 to 100 µmol/L. Table 2 shows the repeatability of retention time and peak area for 25 µmol/L standard solution (n=6).

Table 2 Repeatability of Retention Time and Peak Area

	Retention Time (%RSD)	Area (%RSD)		Retention Time (%RSD)	Area (%RSD)
Asp	0.46	0.55	GABA	0.07	0.73
Glu	0.14	0.57	Met	0.03	0.50
Asn	0.13	0.72	Val	0.04	0.52
Ser	0.11	0.53	Cys	0.03	0.84
Gln	0.08	0.72	Phe	0.03	0.50
His	0.07	2.49	Ile	0.04	0.44
Gly	0.08	0.75	Leu	0.05	0.43
Thr	0.08	0.68	Lys	0.03	3.85
Arg	0.10	3.07	Hy-Pro	0.03	3.13
Ala	0.05	0.77	Pro	0.04	5.09
Tyr	0.05	0.59			



1, Aspartic Acid 2, Glutamic Acid 3, Asparagine 4, Serine 5, Glutamine 6, Histidine 7, Glycine 8, Threonine 9, Arginine 10, Alanine 11, Tyrosine 12, GABA 13, Methionine 14, Valine 15, Cystine 16, Phenylalanine 17, Isoleucine 18, Leucine 19, Lysine 20, Hydroxyproline 21, Proline

Fig. 1 Simultaneous Analysis of 21 Amino Acids; Top: Ch1 (Ex. 350 nm, Em. 450 nm), Bottom: Ch2 (Ex. 266 nm, Em. 305 nm)

■ Results of Quantitative Analysis of Real Samples

The Application News M310 introduced an example of a comprehensive analysis of the components of several food samples using a GCMS-TQ™ 8040 NX and Smart Metabolites Database™. The Application News C238 introduced a similar analysis using an LCMS-8060 NX and LC/MS/MS Method Package for Primary Metabolites Ver. 2. The purpose of these analyses was to search for characteristic compounds for use in differentiation of food products. On the other hand, the method introduced in the present article provides a simpler and more stable approach for confirming the differences in quantitative values after the characteristic components have been determined .

Here, quantitative analyses were carried out using the same two types of tomato juices (with/without labeling showing the tomato variety) and a wine and nonalcoholic wine as in the above-mentioned Application News. Table 3 and Fig. 2 show the results for the tomato juices, and Table 4 and Fig. 3 show the results for the two wines. The supernatant of the tomato juices after centrifugation was diluted with water, and the wine and nonalcoholic wine were simply diluted with water.

Table 3 Quantitative Values of 2 Types of Tomato Juices (mmol/L)

	Tomato variety indicated	Tomato variety not indicated		Tomato variety indicated	Tomato variety not indicated
Asp	11.06	11.74	GABA	12.93	9.97
Glu	17.77	16.66	Met	0.30	0.31
Asn	6.53	6.80	Val	0.64	0.56
Ser	2.55	2.04	Cys	-	-
Gln	0.06	0.13	Phe	1.80	1.90
His	0.95	0.90	Ile	0.71	0.61
Gly	0.89	0.85	Leu	0.75	0.67
Thr	1.97	1.80	Lys	0.90	0.77
Arg	0.83	0.68	Hy-Pro	-	-
Ala	7.01	6.91	Pro	0.73	0.47
Tyr	0.46	0.44			

Table 4 Quantitative Values of Wine and Nonalcoholic Wine (mmol/L)

	Wine	Nonalcoholic wine		Wine	Nonalcoholic wine
Asp	0.67	0.56	GABA	1.12	2.69
Glu	0.64	0.86	Met	0.15	0.12
Asn	0.25	0.22	Val	0.43	0.40
Ser	0.43	0.74	Cys	-	-
Gln	0.00	0.00	Phe	0.34	0.36
His	0.27	0.44	Ile	0.19	0.18
Gly	0.83	0.20	Leu	0.51	0.37
Thr	0.42	0.69	Lys	0.56	0.23
Arg	0.35	6.74	Hy-Pro	0.25	0.05
Ala	1.74	2.20	Pro	41.06	6.77
Tyr	0.18	0.22			

The quantitative values of the amino acids which were regarded as characteristic components in the above-mentioned Application News were also greatly different from those of the other amino acids.

■ Conclusion

In this article, an example in which a pre-column amino acid analysis using an automatic pretreatment function with an HPLC-fluorescence detector was introduced. This method is suitable for the quantitative analysis of amino acids.

This system provides stable quantitation performance, with good repeatability of both the retention time and area values. In addition, the automatic pretreatment function enables the automatic derivatization of amino acids, making it easy to use. Based on these advantages, we propose quantitative analysis using the HPLC-fluorescence detector system in cases where quantitative analysis is necessary after a comprehensive component search using another instrument.

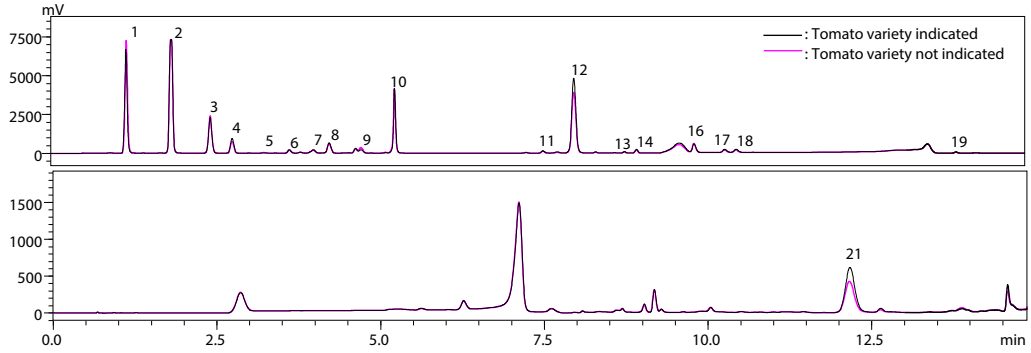


Fig. 2 Chromatograms of 2 Types of Tomato Juices
(Top: Results for Samples Diluted 100 times Detected by Ch1, Bottom: Results for Samples Diluted 100 times Detected by Ch2)

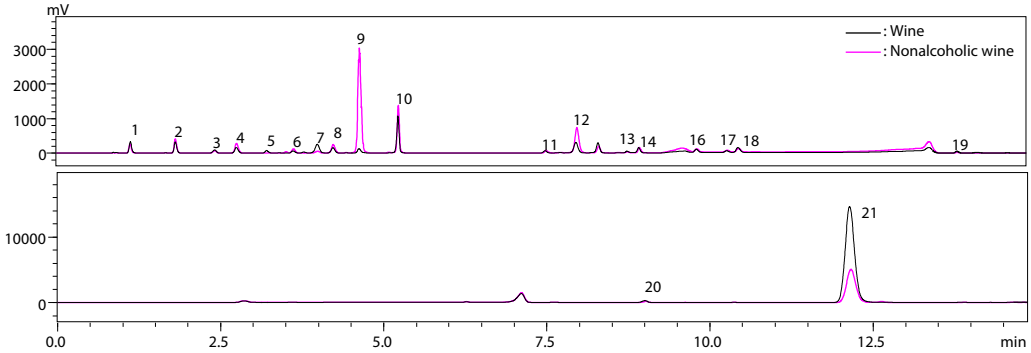


Fig. 3 Chromatograms of Wine and Nonalcoholic Wine
(Top: Results for Samples Diluted 100 times Detected by Ch1, Bottom: Results for Samples Diluted 100 times and 200 times Detected by Ch2)

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