

Rapid Analysis of Amino Acids Using the Hitachi LaChromUltra® High Speed Liquid Chromatography System

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Amino acid analysis is an important technique involved in a wide range of bioanalytical applications including protein and peptide analysis, monitoring of amino acid uptake in cell culture media, quality assessment of foodstuffs and clinical diagnosis of metabolic disorders. Historically, this technique has been performed on highly specialized chromatographic systems dedicated for comprehensive (up to 50-plus components) and quantitative amino acid analysis. While the data from these platforms are extremely reproducible and informative, analysis time can be lengthy (up to 150 minutes) and some sensitivity may be lost. Here we describe an alternative methodology for the rapid analysis of amino acids suitable for the analysis of proteins and peptides, cell culture media, and foodstuffs using the highly flexible Hitachi LaChromUltra® U-HPLC system. Following pre-column derivatization using 4-fluoro-7-nitrobenzo-furazan (NBD-F), amino acids are analyzed via reversed phase separation and ultraviolet or fluorescence detection. The data here specifically describe the analysis of a commercial sports drink via fluorescence detection in under eight minutes¹.

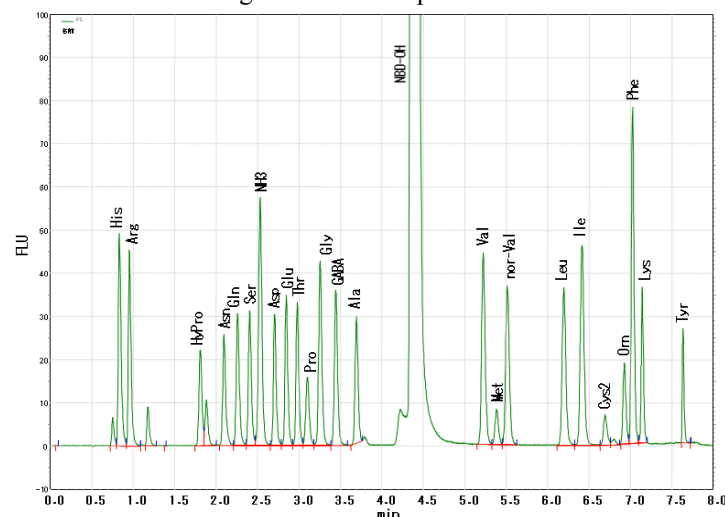
Experimental Conditions

Module	Conditions
Pump (L-2160U)	Mobile Phase A: 120mM Ammonium Formate + 1g/L Tetrabutylammonium Bromide/H3PO4 concentration (v/v): 0.15% Mobile Phase B: Acetonitrile/Tetrahydrofuran (95:5) + 1g/L Tetrabutylammonium Bromide Flow Rate: 0.55mL/min
Autosampler (L-2200U)	Injection Volume: 10µL
Oven (L-2300)	Temperature: 37°C
Detector (L-2485U)	Ex: 470nm; Em: 540nm
Standard	23 amino acid mixture

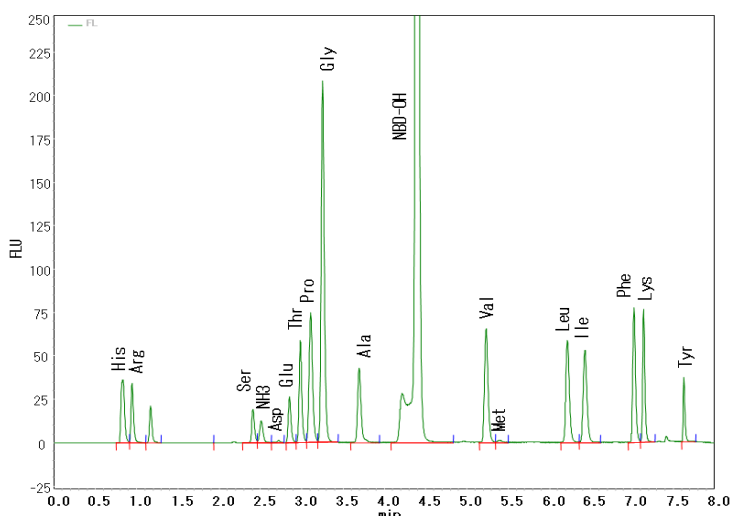
Results – Performance

Peak Area Reproducibility	0.38 – 0.95% RSD
Linearity (0.2 – 20.0µmol)	0.9991 – 0.9999% RSD
Detection Limit (S/N=3)	<40fmol/10uL (except Pro=60, Orn=70, Cys2=110 and Met=120)
Quantitative Limit (S/N=10)	<100fmol/10uL (except Pro=200, Orn=230, Cys2=375 and Met=400)

Results – Chromatogram of 23-Component Mixture



Results – Chromatogram of Commercial Sports Drink



Discussion

Hitachi's LaChromUltra® liquid chromatography system is extremely effective at rapid analysis of amino acids in a variety of matrices. The system exhibits a linear response over a broad concentration range, excellent reproducibility and very low detection and quantitation limits.

Reference:

1 – Technical Data, Hitachi High Technologies Corporation.

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