ELITE

Amino Acid Analyzer

ELITE HPLC

Determination of Amino Acids by HPLC Post-column Derivatization Method

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HPLC-Post Column Derivatization is a widely used analytical technique for amino acid determination, particularly suitable for detecting amino acids that lack inherent UV absorbance or fluorescence properties. The principle involves separating amino acids on the column, then introducing a derivatization reagent into the eluates to convert the amino acids into derivatives with strong detection signals, such as fluorescence or UV absorbance, thereby enhancing both the detection sensitivity and selectivity.

Chromatographic Separation

The amino acid mixtures are separated using a high-performance liquid chromatography column such as C18 column, based on differences in the partition coefficients of each amino acid in the stationary phase and the mobile phase.

Post-column Derivatization

After separation, the amino acids are eluted from the column and mixed online with derivatization reagents such as ninhydrin or o-phthalaldehyde (OPA). These reagents react with the amino acids to form derivatives with UV absorption or fluorescence properties, making them detectable by UV or fluorescence detectors.

Detection and Quantification

Qualification is based on the retention time of the derivatives and quantification is based on peak area or peak height. External or internal standard method is used to calculate the content of amino acids in the sample.

Applications

- Biomedicine: Determination of free amino acids or hydrolyzed amino acids in blood, urine, and tissues for diagnosis of metabolic diseases, nutritional assessment, etc.;
- Food and Agriculture: Analyzing the amino acid composition and content in food and crops to evaluate the nutritional value of protein;
- Pharmaceutical R&D: Determination of the amino acid sequence of peptide drugs, as well as the amino acid impurities in drug substances, etc.;
- Environmental monitoring: Analyzing the amino acid content in water and soil to evaluate the nitrogen cycle or pollution of the ecosystem.

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The amino acid analyzer is established based on the EClassical 3200 HPLC and the HP-HS20 post-column derivatization system, featuring excellent separation, high sensitivity, and a high degree of automation.

The analyzer complies with the requirements of GB 5009.124-2016 "National Food Safety Standard - Determination of Amino Acid in Foods" and GB/T 30987-2020 "Determination of Free Amino Acids in Plants".

High-Performance Liquid Chromatography System - EClassical 3200 HPLC

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Liquid Chromatography Pump

- Patented adjustable pulse damper design ensures high-precision and high-stability mobile phase delivery;
- Equipped with an 800 μL high-efficiency mixer, enabling uniform mixing of the mobile phase even with low organic content;
- The highly integrated solvent switching system enables dual high-pressure binary operation of the high-pressure binary assembly.

Autosampler

- The S3200 autosampler robotic arm is capable of performing 100,000 successive injections without operational deviation, as verified by reliability test;
- The injector delivers consistent sample volumes, with less than 0.5% RSD in peak area over 50,000 consecutive injections;
- Supports online derivatization and dilution, enabling unattended sample dilution and derivatization.

Column Oven

- Frequency-conversion temperature control technology keeps temperature fluctuation within ±0.1°C, minimizing retention time drift and selectivity changes caused by temperature variations;
- Pre-heating design of the tubing optimizes separation efficiency and reproducibility.

Detector

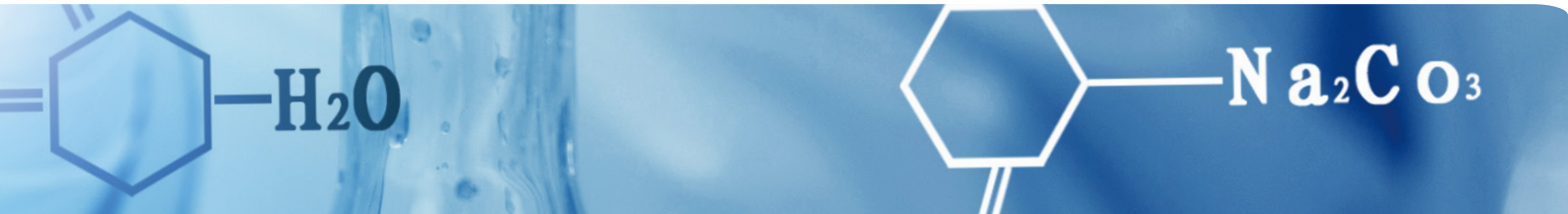
- The light path adopts dark array technology to achieve automatic calibration of dark current;
- Thermostatic flow cell design minimizes signal interference caused by changes in solvent refractive index.

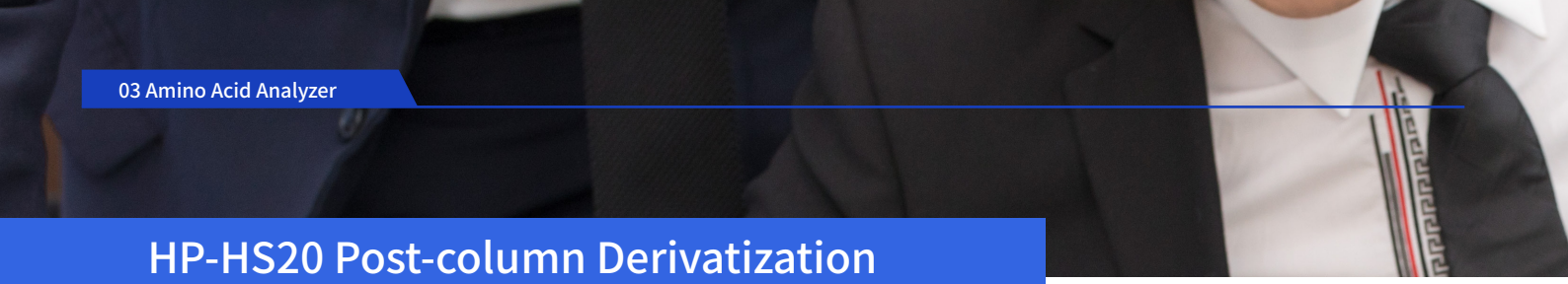
Workstation

- Kromstation liquid chromatography workstation, compliant with 21CFR Part11 requirements.

Overall System

- The entire system features a low-dispersion flow path design, including narrow-bore tubing and zero dead-volume connectors, ensuring sharp peak shapes and consistent retention times;
- Leak alarm is highly sensitive, with a minimum trigger volume of 0.1 μL and a response time of ⩽ 2 seconds; The leakage guide groove diverts the leakage directly to the waste tubing, preventing contamination of the system;
- The system is built for high-pressure operation, with patented pump head seals, connector seals, and vent valve seals, plus a high-pressure switching valve. Maximum pressure tolerance is up to 600 bar (~8700 psi), supporting sub-2 μm columns for improved separation efficiency.





HP-HS20 Post-column Derivatization System

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Precision

- High-pressure isocratic pump with a extended flow range and intelligent flow control;
- The built-in flow regulator effectively regulates the flow rate, ensuring precise delivery and minimum pulsation;
- Dedicated temperature control system maintains stable temperature uniformityin derivatization cells.

Intelligence

- A centralized intelligent control systemserves as the operation hub, with a 7-inch HMI touchscreen enabling intuitive data visualization;
- User-programmable methods with storage/recall function, ensuring operational flexibility and user accessibility;
- Optional photochemical derivatization modulesupporting switchable derivatization modes(photo/chemical).

Safety Features

- Reverse-flow protection system with multiple check valves and auto-triggeredpressure relief;
- Comprehensive alert mechanisms monitoring pressure, temperature, and leakage;
- Optional nitrogen protection and online degassing modules for enhanced reagent stability.

Worry-free Operation

- Optimized PEEK tubing offers excellent acid and alkali resistance for long lifetime;
- The pump, made of stainless steel and sapphire, offers high-pressure and corrosion resistance;
- Automated post-column and system self-cleaning functions enable smart, hassle-free maintenance;
- We provide comprehensive solutions for derivatization assays and can help develop fit-for-purpose applications and protocols based on your specific needs - saving you time, effort, and costs while boosting efficiency.

System Configuration

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· EClassical 3200 HPLC System

Part	Name	Quantity
Delivery pump	P3230 quaternary constant-flow pump	1
Sampler	S3210 autosampler	1
Column oven	O3230L oven	1
Detector	D3210 Multi-UV-Vis detector	1
Liquid chromatography workstation	Kromstation liquid chromatography workstation	1

· HP-HS20 Post-column Derivatization System Consumables

Part	Specification & Quantity
Delivery pump	Dual pump
Derivatization cell	Dual cell
Derivatization heating module	Dual heating mode
Valve switching device	2
Power controller cable	1
Nitrogen protection device	Optional
Online degassing device	Optional
Relief valve	Optional

Part	Specification & Quantity
Amino acid standard	2.5 μmol/mL of 18 amino acids, 1 mL/vial
Mobile phase (A, B, C, and D, one bottle each)	4 × 500 mL
Dedicated column for amino acid analysis	4.6 × 150 mm, 5 μm
Amino acid derivatization reagent (R & W solutions, one bottle each)	2 × 500 mL
Maintenance and supplementary reagent (A & D solutions, one bottle each)	2 × 500 mL

Determination of 17 Amino Acids by HPLC Post-column Derivatization Method

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Liquid Chromatography Pump

- Mobile phase: A, B, C, D
- Injection volume: 20 µL
- Column temperature: 60°C
- Detection wavelength: 570 nm and 440 nm
- Gradient and flow rate

Time, min	A%	B%	C%	D%	Flow rate, mL/min
0	100	0	0	0	0.3
14	100	0	0	0	0.3
14.1	75	25	0	0	0.3
24	75	25	0	0	0.3
24.1	0	100	0	0	0.3
40	0	100	0	0	0.3
40.1	0	0	100	0	0.3
58	0	0	100	0	0.3
58.1	0	0	0	100	0.3
65	0	0	0	100	0.3

Derivatization Conditions

- Derivatization reagent: R, W
- Derivatization principle: Primary amino acids react with ninhydrin to produce a blue-violet derivative known as Ruhemann's purple, which exhibits UV absorption at 570 nm. Secondary amino acids react with ninhydrin to produce a yellow derivative exhibiting UV absorption at 440 nm.

Time, min	R	W	Flow rate, mL/min	Derivatization temperature, °C
58	on	off	0.13	130
7	off	on	0.13	130
15	on	off	0.13	130

Fast and Comprehensive After-Sales Service

ELITE has established over 20 service centers nationwide, offering 24/7 support and rapid response. We provide our customers with regular theoretical and hands-on training, on-site maintenance, as well as online and offline method development and guidance. Our comprehensive services ensure that your instruments consistently meet the quality system requirements of various industries.

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