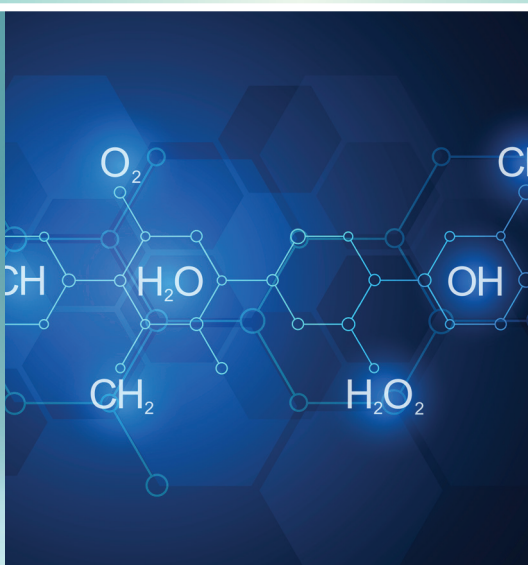


Bio 30+ Series Amino Acid Analyzer System



Results You Can Trust:

Research, Development and Manufacturing



Amino Acid Analysis

When accuracy is essential...



As a scientist involved with amino acids analysis (AAA), it's easy to get lost in your day-to-day routines and even lose sight of how important your work is to humanity. But because you are continuously monitoring and analyzing proteins and peptides in active pharmaceutical ingredients and raw materials, you are helping people around the world to optimise their health and live better lives.

The ability to accurately qualify and quantify AAs—at a molecular level, is essential to your success. Yet consistently obtaining reliable data can pose significant challenges. The risk of unexpected patterns that can skew results is real. Exceptional lab equipment and reagents are critical to your research, as is access to expert support to help you fine-tune your equipment and adjust your chemicals and formulas—to keep your work on track.

Are you truly confident that your AAA method is delivering the accurate results that you need?

The Gold Standard Method

The Bio 30+ Series meets the global pharmacopoeia guidelines for dedicated amino acid analysis by ion exchange liquid chromatography and post-column derivitisation of ninhydrin positive substances, and is a key part of the ICH Q6B guidelines for characterization of biopharmaceuticals. After optimal sample preparation using the reference methods, these instruments can accurately identify and quantify amino acids. This enables the critical estimation of the amino acid composition of proteins and peptides in pharmaceutical and therapeutics, including the determination of a single amino acid to obtain accurate values.

Amino Acid Analysis...A Powerful Tool

- Tests to identify active biopharmaceutical ingredients by compositional analysis of amino acids
- Assess results of trials and novel formulas by analyzing the physiological fluids (such as plasma or urine) of both animals and humans
- Total or single amino acid quantification in therapeutic products, including complex matrices
- Detect odd amino acids and corroborate synthetic or recombinant proteins
- Locate 'free' amino acids and identify impurities



Accurate Compositional Analysis

With an increasing understanding of the molecular mechanisms that cause disease and continual drive for new and improved medications, the demand on the pharmaceutical industry has never been so high. This intensifying demand has resulted in increased legislation and regulatory scrutiny of development practices and raw materials, as well as increased oversight of production environments to ensure the highest level of quality control.

Quantification and qualification of amino acids in the fast paced world of peptide therapeutics allows scientists to optimise formulas meaning best care for consumers combined with lowered cost of production.

Accurate Molecular Analysis

By determining the sources and quality of proteins, peptides and raw materials, AAA can also flag instances of impurity, adulteration or microbial contamination, thus fine-tuning quality control and production processes.

In a pharmaceutical setting amino acid analysis is commonly used on protein and peptide samples for the purposes of:

- identification
- quantification
- supporting structural analysis
- evaluation and mapping fragmentation
- detecting atypical amino acids



Bio 30+ Series Amino Acid Analyzer System

Count on Biochrom—for Results You Can Trust

Harvard Bioscience's division Biochrom has been delivering the industry's Gold Standard Amino Acid Analysis (AAA) instruments to life scientists throughout the world—for decades. Cited in thousands of scientific journal articles, Biochrom has been helping tens of thousands of researchers achieve their goals. When it comes to AAA pharmaceutical research and development, you can count on Biochrom for superior AAA equipment—and for unmatched expert support so you can confidently trust your results and focus on making the world a healthier place.

Take Control—With Results You Can Trust

The Bio 30+ Series put you in control of the major factors that commonly cause variability in amino acid analysis. Biochrom's instruments and reagents are manufactured from high quality materials under the ISO 9001:2008 Quality System and subject to rigorous control procedures. Optimized methods and instrumentation control the analysis conditions. Biochrom's high-quality reagents allow precise control of the chemistry and each system is supplied with ISO 17034 verified reference material. The system also provides reporting and qualification tools including 21CFR part 11, IQ/OQ documentation.

Rugged

Unlike generic methods, the Bio 30+ Series can withstand both complex sample matrices and high salt concentrations—without interference. The system dramatically reduces the risk of reproducibility errors associated with studies involving high salt concentrations that can cause peak broadening and affect the resolution of methionine sulfone, aspartic acid, threonine and serine.

Flexible

The Bio 30+ Series offers ultimate flexibility. Configured to suit your particular needs based on the amino acids of interest to you at the time of purchase, the system is designed to grow with your research. The system can be easily modified, without replacing hardware. The system also has pre-defined analytical, processing and reporting methods and the flexibility to tailor analysis times to meet your laboratory's requirements.



- Better separation: typically 90% separation between each amino acid
- Reproducibility of peak area and retention time <0.5%CV
- High sample throughput due to long column life (more than 1200 samples)
- Amino and imino acid detection
- Low interference
- Flexible system — rapid runs and complete control
- Support from application scientists and engineers dedicated to amino acid analysis

Dedicated Chromatographic System



Bio 30+ Series Amino Acid Analysis System

Reagent System	Ideal For
5 Buffer Lithium Based System	• Native samples—40+ amino acids and derivatives. • Free amino acids • Milk products, cheese or formula feed e.g. baby milk • Analysis of soft drinks and beverages. • Analysis of human and animal physiological fluids • Applications that need to resolve asparagine (Asn) and glutamine (Gln) from the amino acids threonine (Thr) and serine (Ser). • Dedicated short programs of single amino acids e.g. phenylalanine and tyrosine, or the branched chain amino acids valine, leucine and isoleucine • Separation of the methylated histidines, gamma-aminobutyric acid (GABA), beta-alanine and beta-aminoisobutyric acid
4 Buffer Sodium Accelerated System	• Rapid run times • Analysis of protein or peptides hydrolysates in a variety of samples • Estimation of cysteic acid and methionine sulfone in oxidized protein or peptide hydrolysates • Tailored short programs for the analysis of single amino acids as e.g. taurine or lysine, and of natural sulfur amino acids

A Dedicated Chromatographic System

Specifically for amino acids

The Bio 30+ Series Amino Acid Analyzer is a cation exchange chromatography system coupled with a highly specific detection system using post column derivatization with ninhydrin reagent. Amino acids are separated according to their net charge determined by the pKa of their ionized groups. The mobile phase is a finely tuned set of buffers used in a stepwise elution profile of increasing pH and molarity. A temperature gradient on the column maximizes resolution. The resin bed is regenerated after each run cycle.

Highly specific detection system

The ninhydrin method is highly specific because it reacts only with amino groups giving a compound absorbing at 570nm wavelength (440nm for amino acids like proline). This response is a linear relationship between the absorbance and the amount of amino acid in the sample. The sensitivity of the ninhydrin reaction is optimized and the response is 100% linear within the expected amino acids concentration range encountered. The continuous flow of reagent ensures a reproducible derivatization giving high precision in the peak area.

Powerful System, Unmatched Support

Software

Biochrom Phoenix software controls the instrument and autosampler showing real-time operational information. To offer flexibility and efficiency, for your lab, Biochrom offers options in data handling software including OpenLab from Agilent and Clarity from DataApex. This powerful, advanced software combination is easily networked for secure data storage and includes special tools for compliance with regulatory demands e.g. FDA 21 CFR part 11. It offers a wide variety of functions to set up methods and sample sequences, process and visualise data and compile custom reports. Data can be exported easily to other programs or other Windows applications.

Robust and Stable Chemistry

Biochrom's patented EZ Nin solution is a unique form of Ninhydrin which needs no preparation or special conditions to remain stable long-term, meaning less time to set up, and no more wasting the last few mL in the bottle. Biochrom's chemical kits contain everything you need for routine analysis of up to 320 runs (protein hydrolysate method). Chemicals and consumables are available either as complete kits or as individual buffers to enable continuity of analysis. All reagents are stable at room temperature and guaranteed to give accurate and reproducible results with a 3-year shelf life. On the instrument, buffers and reagents are stored under an inert gas to ensure stability.



Re-usable Columns

Manufactured from PEEK material, the columns are free from corrosion and metal contamination and packed with optimally sized cation exchange resin. Columns are installed with finger-tight fittings so no special spanners are required to ensure a leak free seal. All columns are fully tested and optimized under strict QC criteria. To minimize waste and reduce costs, our columns are fully recyclable at the end of their life thanks to our unique repacking and cleaning service.

Peace-of-Mind

Biochrom's dedicated Technical Support team offers training and installation services to get your lab up and running quickly. Biochrom's Applications Team is available to assist with full screening methods or specific short methods derived from an extensive application database. A partnership with Biochrom gives you peace of mind and includes:

- Maintenance visits performed by trained and certified field service engineers.
- Biochrom quality parts used for maintenance and repairs.
- Access to a responsive engineering team



The Biochrom AAA System

- Bio 30+ Series Analyzer with 84 position air cooled autosampler
- Choice of column (with top-up resin)
- Starter pack of ready-to-use reagents including ISO 34 verified reference standard
- Spare parts and consumables kit
- HP computer and monitor, Windows® operating system and all cables
- Easy to use control software compatible with popular data handling and analysis platforms
- Manuals and Qualification & Performance Verification Logbook
- On-site customer training

Case Study 1

Infusion Solutions

Experimental data

The Bio 30+ Series are available in various configurations based on either sodium or lithium buffer chemistry depending on the type of product to be analysed, and the amino acids of interest.

In the case of infusion solutions, lithium systems (also called physiological systems) are often preferred as they enable the separation of amino acids such as cysteine/proline and asparagine/glutamine, which are difficult to separate using the sodium system.

As infusion fluids are used intravenously, manufacturers must abide by very strict legislation in manufacture and quality control. A dedicated amino acid analyser can rapidly deal with complex samples, requiring virtually no sample preparation, making it an essential quality control technique for infusion solutions containing amino acids.

Several infusion samples from various global manufacturers were run on the Biochrom 30+ Physiological Lithium System in the Biochrom Applications Laboratory. The experimental data focuses on two key points for method validation: linearity and repeatability.

Accelerated program and repeatability studies

Since infusion liquids usually contain a limited number of amino acids the analysis time can be greatly reduced. The short program used below enables the analysis time to be reduced by up to 44% while still retaining an excellent separation.

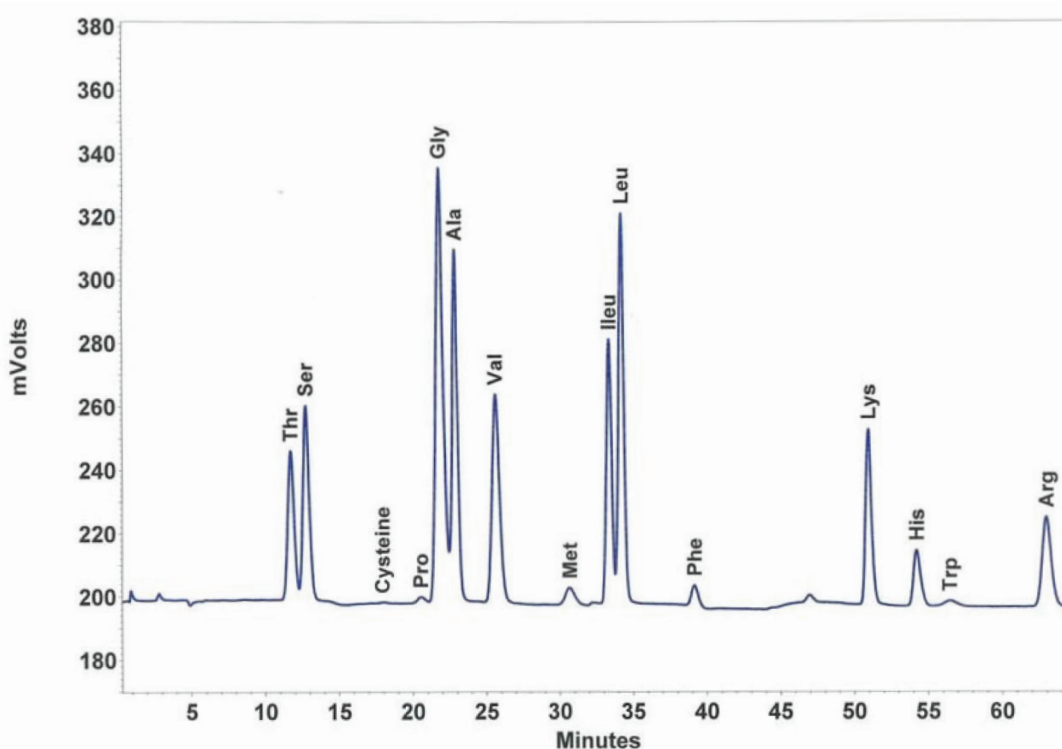


Figure 1: Infusion fluid (x 200 dilution)

Repeatability

The sample was analysed 6 times consecutively using the accelerated program. An overlay of the chromatograms is displayed below (figure 2).

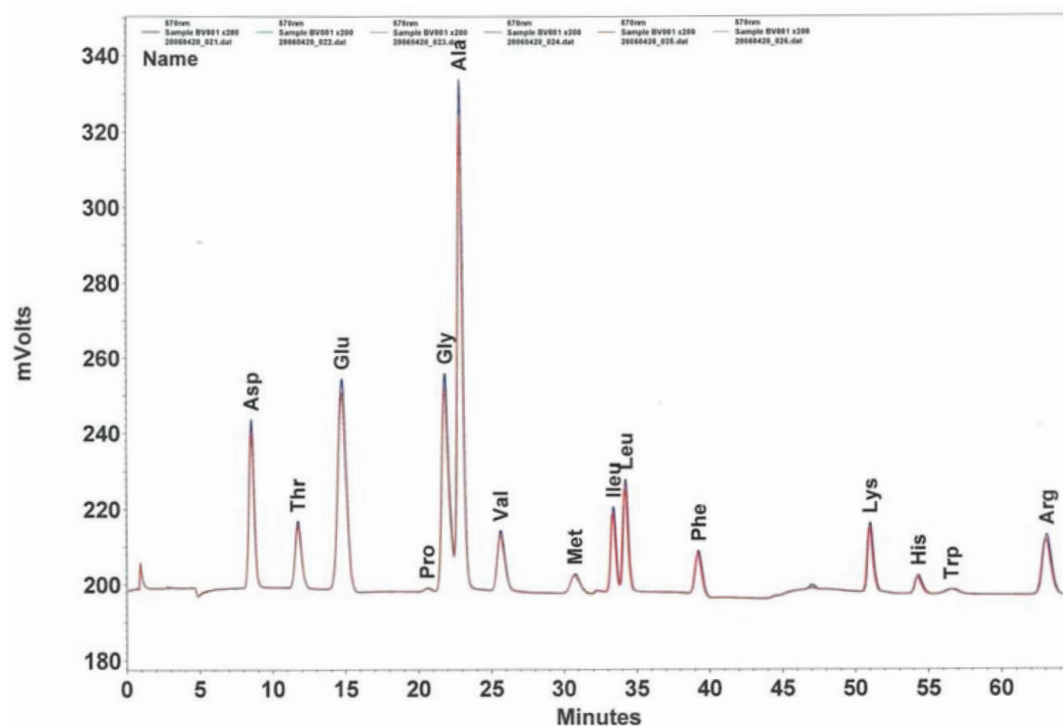


Figure 2: Overlay of 6 replicates

Compound	Concentration (g/L)	RSD (%)
L-Alanine	8.4	0.03
L-Arginine base	3.1	0.02
L-Aspartic acid	3.7	0.02
L-Glutamic acid	8.6	0.02
Glycine	3.4	0.04
L-Histidine base	0.6	0.02
L-Isoleucine	2.2	0.03
L-Leucine	2.6	0.02
L-Lysine base	1.9	0.02
L-Methionine	0.8	0.02
L-Phenylalanine	1.6	0.02
L-Proline	3.7	0.02
L-Threonine	1.6	0.02
L-Tryptophan	0.6	0.02
L-Valine	2.0	0.03

Table 1: Results obtained on 6 replicates. The Bio 30+ Physiological Lithium System gave an excellent repeatability of areas and retention times over 6 runs. The precision on concentrations was typically better than 0.05%.

Case Study 2

Quantitative Analysis of Desmopressin Acetate to European Pharmacopeia Standards

3 samples of Desmopressin Acetate in powder form were submitted by a pharmaceutical manufacturer for amino acid analysis in order to demonstrate the performance of the Bio 31+ Sodium Hydrolysate System.

The analytical method used is based on Cation Exchange Chromatography coupled with post column ninhydrin derivatization for the detection of amino acids. This method ensures high reproducibility and accuracy in the results thanks to high tolerance of complex sample matrices.

The Instrument used was the Bio 31+ Sodium Hydrolysate System running a set of Sodium Hydrolysate Buffers, coupled with ninhydrin detection using EZ Nin Reagent. This system delivers a maximum runtime of 60min injection to injection and allows the separation of 18 protein amino acids.

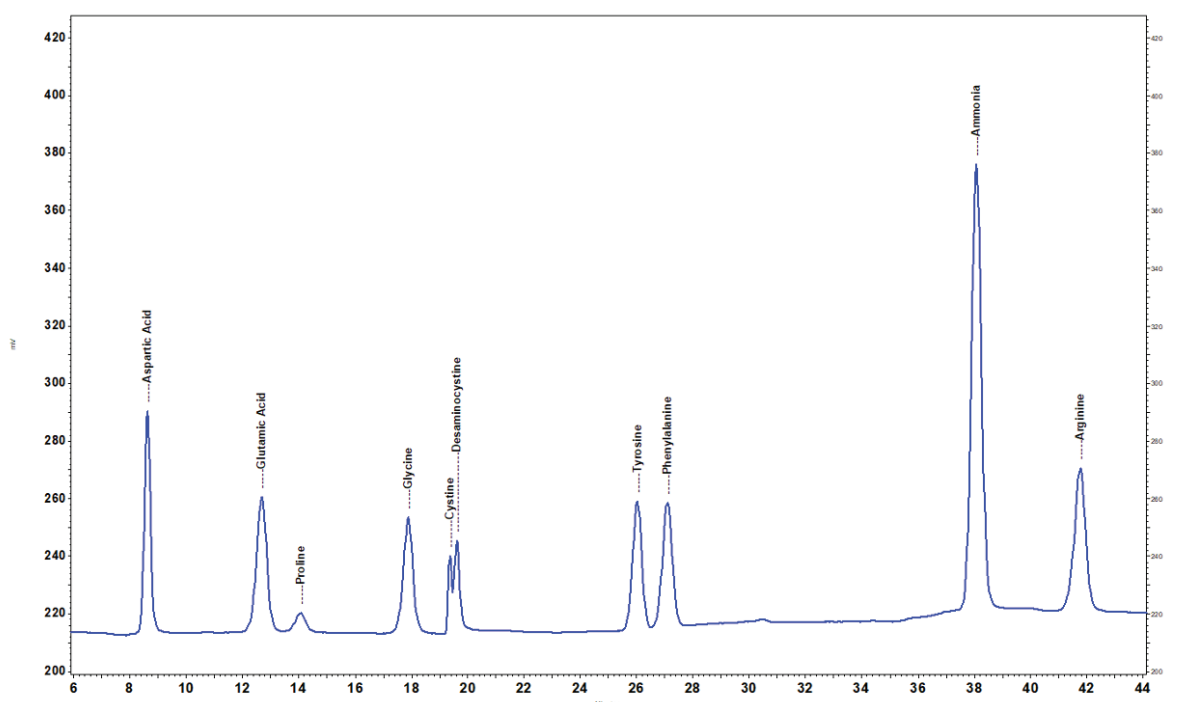
All amino acids were measured at 570nm except Proline at 440nm.

Qualitative Analysis:

The following amino acids were detected: Tyr, Phe, Glu, Asp, Cys, Pro, Arg and Gly. An extra ninhydrin positive compound was also detected eluting close to Cystine. This is believed to be a residue of S-(Carboxyethylthio)-L-Cysteine formed during the hydrolysis of desmopressin.

The qualitative results correlate with the known formula of desmopressin (Mercaptopropionyl-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH₂).

Note: Asn and Gln are converted into their respective acids during hydrolysis and are detected as being Asp and Glu. The trace of Cystine is created by fission of the S-(Carboxyethylthio)-L-Cysteine during acid hydrolysis. The combination of cysteine monomers then forms Cystine.



Bio 30+ Series Technical Specifications

Reproducibility	Area: Better than 0.5% RSD at 10 nanomoles. Retention time: Better than 0.1%RSD
Detection Limit	9 - 15 pmoles Ninhydrin (depending on individual response of amino acid)
Analysis Time	Biochrom 30+: Lithium System 115 minutes injection to injection Biochrom 31+: Sodium citrate buffer system for hydrolysed proteins Biochrom 32+: Sodium Accelerated Buffer System 60 minutes injection to injection
Analytical Column	High pressure PEEK column packed with Ultropac 8 cation exchange resin. Peltier heating/cooling system.
Eluent System	Up to 6 buffers (5+1 regeneration solution) stored on the instrument at room temperature in graduated 1L glass bottles under nitrogen pressure. Ninhydrin reagent: Stored on the instrument at room temperature under nitrogen pressure in a 2L plastic coated glass bottle
Temperature	Column temperature variable between 20°C and 99°C. Reaction coil temperature adjustable between 40°C and 145°C (138°C is optimum when using EZ Nin reagent).
Photometric Detection	Single flow cell with optical beam splitter. Dual channel detection at 440 nm and 570 nm
Sample Injection	3 injection modes (full loop, partial loop and micro), 84 position autosampler (cooling optional) Sample volumes from 1 µL to 5000 µL. 200 µL loop supplied as standard.
Software	Phoenix software for system and autosampler control software Choice of Data Handling software package — Clarity from Data Apex or Openlab EZ Chrom Edition from Agilent (21 CFR part 11 compliant)
Dimensions and Weights	Bench top fluidics cabinet: 48 x 59 x 57 cm, 19 x 23 x 22 inches (w x d x h) - Weight: 50 kg, 110 lbs - Autosampler: 30 x 57.5 x 36 cm, 12 x 23 x 14 inches (w x d x h) - Weight: 21 kg, 46 lbs
Operating Conditions	Operating temperature: 15 °C to 25 °C Maximum humidity: 80% at 25 °C
Required Services	Oxygen free nitrogen gas (99.99%) or Argon regulated to 73.5 psi (5bar). Drainage facility. 240V/100V, 50Hz/60Hz, 300 VA mains supply.
Safety System	Automatic shut-down and reaction coil flushing in the event of: • photometer lamp failure • incorrect ninhydrin / buffer / coil / nitrogen pressures • incorrect coil and column temperatures power failure

Biochrom, a division of Harvard Bioscience, is a world leader in amino acid analysis. The Biochrom 30+ Series is recognized as the gold standard dedicated amino acid analyzer used by hospitals, pharmaceutical and industrial labs worldwide. Applications are available both for clinical analysis metabolic disorders, for drug synthesis, infusion fluids and for industrial applications in food, beverage and feedstuffs.

Biochrom is a leading manufacturer of scientific instruments with over 50 year's experience. The Biochrom Group manufactures a range of instruments covering amino acid analysis, UV/Vis spectroscopy, and microplate instrumentation. Laboratories worldwide trust our products and we are a valued OEM partner of many of the world's finest scientific instrumentation companies.

All our instruments are available through a growing global network of independent distributors, backed by our commitment to customer support.



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