

Bio 30+ Series Amino Acid Analyzer System

Results You Can Trust



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, peptides and other ingredients in foods, beverages and livestock feed, you are optimizing nutrition—and making the world a healthier and more sustainable place.

The ability to accurately qualify and quantify amino acids 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 equipment is delivering the accurate results that you need?

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 for food, beverage and feedstuff, 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.

Amino Acid Analysis...A Powerful Tool

- Evaluate food protein quality by determining the quantities of (essential) amino acids
- Assess results of experimental feeding trials and novel nutrients/formulas by analyzing the physiological fluids (such as plasma or urine) of both animals and humans
- Determine the amino acid composition of proteins and peptides and identify proteins and peptides based on amino acid profiles
- Detect odd amino acids and corroborate synthetic or recombinant proteins
- Locate 'free' amino acids in foods and feedstuffs



Healthier Food for Healthier Lives

Accurate Composition Analysis

Consumers are continuously concerned about the role of nutrition in maintaining good health. This intensifying demand has resulted in increased legislation and regulatory scrutiny of nutritional and compositional food labeling, disclosure of food and beverage origins, as well as in increased oversight of production environments to ensure a highest level of quality control.

More than ever, labs are increasingly tasked with delivery of accurate nutritional and compositional analysis of a wide range of foods, feedstuffs and beverages for both human and animals.

Accurate Nutrition Analysis

More than 20 amino acids are vital to healthy bodies, and many of these must be supplemented by diet. AAA plays an important role in accurately identifying and quantifying the true protein content of samples.

Infant formula feeds, sports nutrition products and therapeutic diets are routinely analyzed for both protein content and quality and to ensure that they contain adequate quantities of essential amino acids. AAA also facilitates determination of optimal feed and diet formulations for livestock so that supplemental amino acids can be added to feedstuff to increase animal production efficiency and help achieve least-cost formulations.

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

Ultimately, accurate AAA can provide proof of nutritional quality and data so that producers can meet stringent regulatory labeling and requirements. In fact, the United Nations (UN) recommends¹ that amino acid analysis be the only method used to analyze the protein content of:

- Foods used as the sole source of nourishment, such as infant formula and pet foods
- Cell culture based protein sources
- Foods/formulas designed specifically for special dietary conditions
- Novel foods
- Synthetic proteins



Bio 30+ Series

The Gold Standard Method

The Bio 30+ Series meets the requirements of the standard methods from the AOAC and the EU Commission Directive 98/64/EC, based on the gold standard reference method of ion exchange chromatography with post-column derivatization of samples using ninhydrin². 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 foods, beverages and feedstuffs, including the determination of a single amino acid to obtain accurate values.

Take Control—With Results You Can Trust

The Bio 30+ Series puts you in control of the major factors that commonly cause variability in amino acid analysis. Biochrom's instruments, reagents and reference materials are manufactured from high quality materials under the ISO 9001:2015 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 Certified Reference Material. The system also provides reporting and qualification tools including 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 to accommodate alternative sample types, 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
- High sample throughput due to long column life (more than 1200 samples)
- Flexible system — rapid runs and complete control
- Reproducibility of peak area and retention time <0.5%CV
- Amino and imino acid detection
- Support from application scientists and engineers dedicated to amino acid analysis
- Low interference

Dedicated Chromatographic System



Biochrom Amino Acid Analysis System

Instrument	Description	Ideal For
Bio 30+ Lithium System	Dedicated to the analysis of Physiological samples. The Biochrom 30+ Physiological system uses a combination of 5 lithium citrate buffers to achieve a full amino acid profile in less than 90 minutes using the new accelerated method.	<ul style="list-style-type: none"> • Analysis of free amino acids • Protein-bound and peptide-bound amino acid analysis for pharmaceutical applications • Food, beverages, plant extracts, fermentation products, culture media, nutraceuticals, infusion fluids, CSF, urine, plasma, tissue, fermentation products • Ideal when the separation of Asparagine and Glutamine are required
Bio 31+ Sodium Citrate Buffer System	Dedicated to the analysis of Protein Hydrolysates. Protein hydrolysates are referred to as samples containing most or all of the 20 amino acids normally found in proteins. A buffer system using 3 or 4 sodium citrate buffers is sufficient for these samples.	<ul style="list-style-type: none"> • Quality control of drug synthesis • Analysis of protein hydrolysates
Bio 32+ Sodium Oxidized Buffer System	Designed for the analysis of complex oxidized protein hydrolysates such as those obtained from food and feedstuffs.	<ul style="list-style-type: none"> • Ideal for the analysis of sulfur-containing amino acids such as Cystine and Methionine (oxidized forms) • Research of optimum diet formulation and nutritional value • Monitoring product quality throughout the production process. • Ideal for the analysis of collagen (analysis of hydroxylated amino acids such as hydroxyproline and hydroxylysine)
Bio 32+ Sodium Accelerated Buffer System	The Accelerated Buffer System enables the total analysis time to be reduced by up to 30%, which is equivalent to 7 additional runs per day. By reducing the run times, the buffer and ninhydrin consumptions are also reduced. The Accelerated Buffer System is therefore an attractive alternative to the classic oxidized system, particularly for customers for whom speed of analysis is critical.	<ul style="list-style-type: none"> • Ideal for the analysis of sulfur-containing amino acids such as Cystine and Methionine (oxidized forms). • Research of optimum diet formulation and nutritional value • Monitoring product quality throughout the production process.

Specifically for amino acids

The Bio 30+ Series 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

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 (oxidized 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:

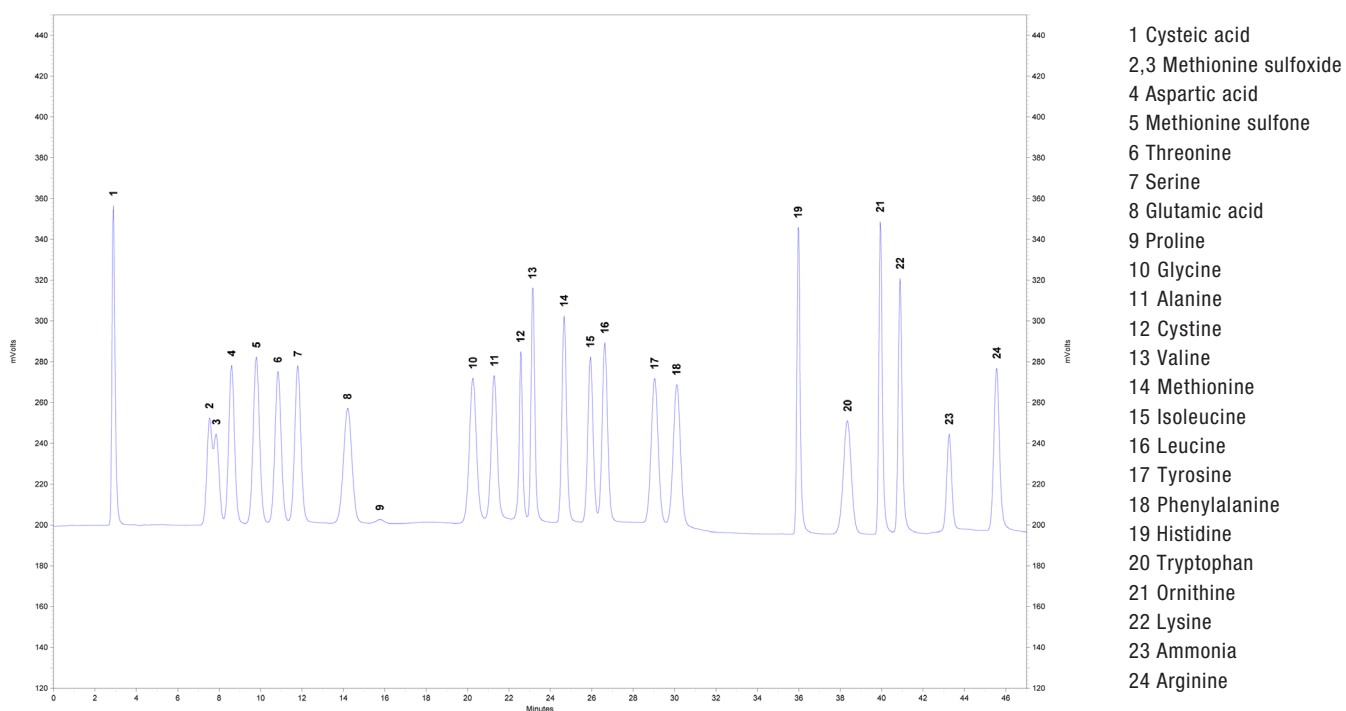
- On site installation, validation and training as part of purchase, IQ/OQ also available.
- Biochrom quality parts used for maintenance and repairs
- Access to a responsive engineering team
- Maintenance visits performed by Biochrom trained and certified field service engineers

Rapid Analysis System for Protein Hydrolysates and Oxidized Hydrolysates

Speed of analysis is critical to increase sample throughput. Biochrom has developed the Sodium Accelerated Buffer System that reduces analysis time by 33% compared to the Oxidized Protein Hydrolysate System and is suitable for both hydrolysates and oxidized hydrolysates samples.

This system of 4 buffers reduces analysis time without compromising peak resolution and offers the possibility of creating short programs when only certain amino acids are required. Standard feedstuffs chromatograms of 24 amino acids are shown below. Figures 1 and 2 demonstrate the baseline free of artifact peaks.

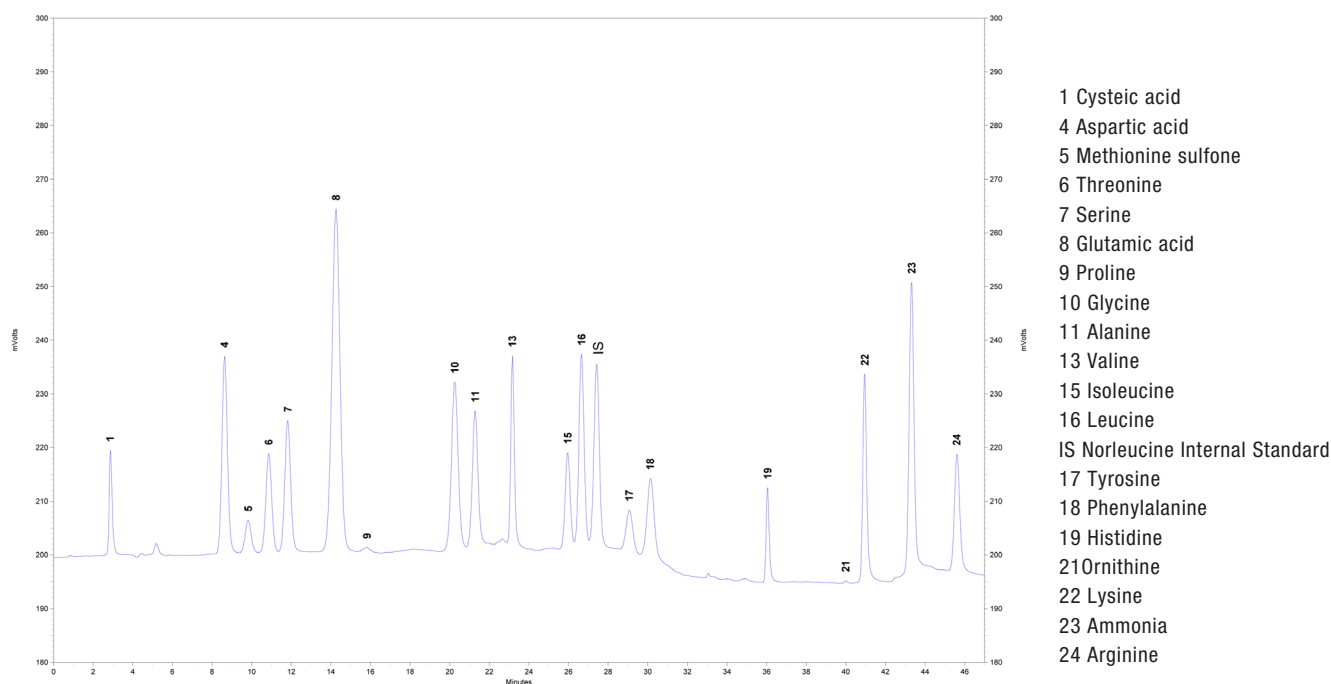
Figure 1: Standard mixture containing the amino acids typically found in feedstuffs – Sodium Accelerated Buffer System



The Bio 30+ Series

- Bio 30+ Series with 84 position air cooled autosampler
- Choice of column (with top-up resin)
- Starter pack of ready-to-use reagents
- 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

Figure 2: Oxidized hydrolysate sample prepared according to the EC directive – Sodium Accelerated Buffer System



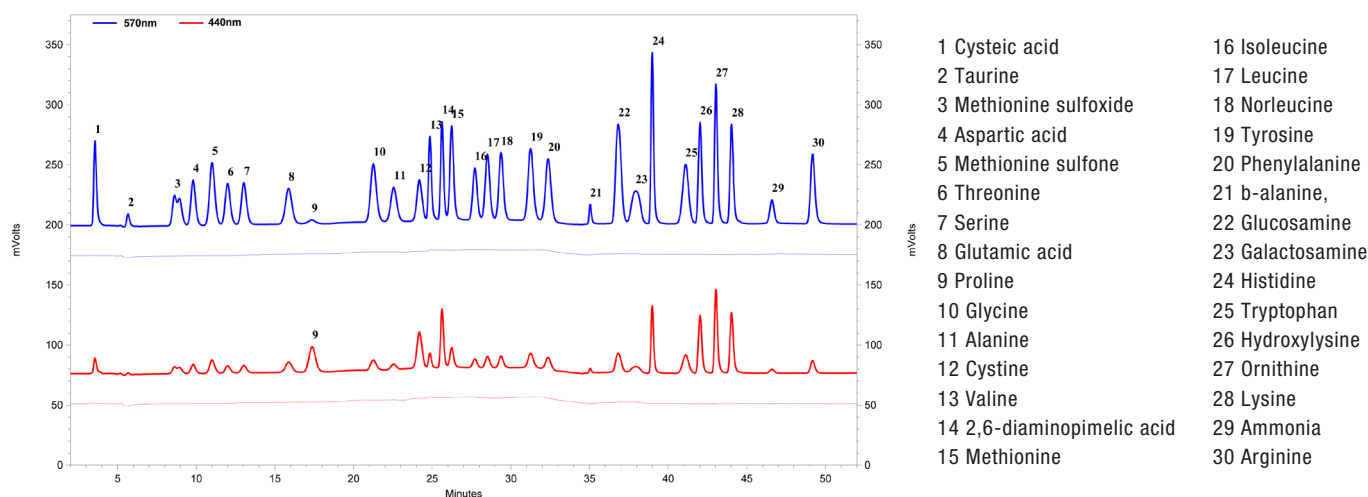
Additional amino acids such as taurine, 2,6-diaminopimelic acid, beta-alanine, glucosamine and galactosamine can also be analyzed using the Accelerated Sodium Buffer System (Figure 3).

The 30 amino acids can be separated in less than 50 minutes (64 minutes injection to injection). A smooth baseline is observed at both wavelengths, particularly under cystine, allowing small amounts of cystine to be accurately quantified.

The chromatograms also show accurate quantitation of ornithine and hydroxylysine which in many existing ion-exchange buffer systems co-elute with lysine and histidine respectively and hence give rise to considerable quantitation errors.

The system can also be used for analyzing hydrolysates prepared from organic acids or alkaline hydrolysis where tryptophan may be preserved.

Figure 3: Chromatogram of a standard mixture containing 30 amino acid and blank (sodium citrate loading buffer), detection at 570 nm and 440 nm.



Case Study 1

A Feedstuffs Manufacturer Requiring Rapid Analysis of Lysine in Feedstuff Samples

Lysine is a limiting amino acid in cereal grains and in some vegetable protein sources, therefore often requiring synthetic supplementing to meet the needs of the animal diet. The optimal lysine content in the diet prevents lysine deficiency and improves the animal's performance through better amino acid balance. Rapid analysis of lysine enable adjustments to the formulation of feedstuff to be made.

The samples tested were supplied by an external laboratory. They were prepared according to the EC official method and analyzed using the full program. These were compared to the Bio 32+ Sodium Accelerated Buffer System, using the short lysine method. This accurately quantified lysine in less than 10 minutes (Figure 4). Three replicates were tested for each sample.

Dietary Protein Source	Limiting Amino Acid
Wheat	Lysine
Rice	Lysine and Threonine
Maize	Tryptophan and Lysine
Pulses	Methionine
Beef	Methionine and Cysteine

Table 1: Limiting amino acids in some protein sources: the essential amino acid found in the smallest quantity in the foodstuff.

Samples (hydrolysates)	Lysine (% of raw material)	
	Biochrom Short Method	External Laboratory Full Program
Feedstuff 60 989 H	1.14	1.13
Pig feed 60 354 H	0.83	0.86
Feedstuff 60 287 H	1.06	1.05
Piglet feed control 29/11 H	1.29	1.28

Table 2: Results obtained with the short program on hydrolysate samples.

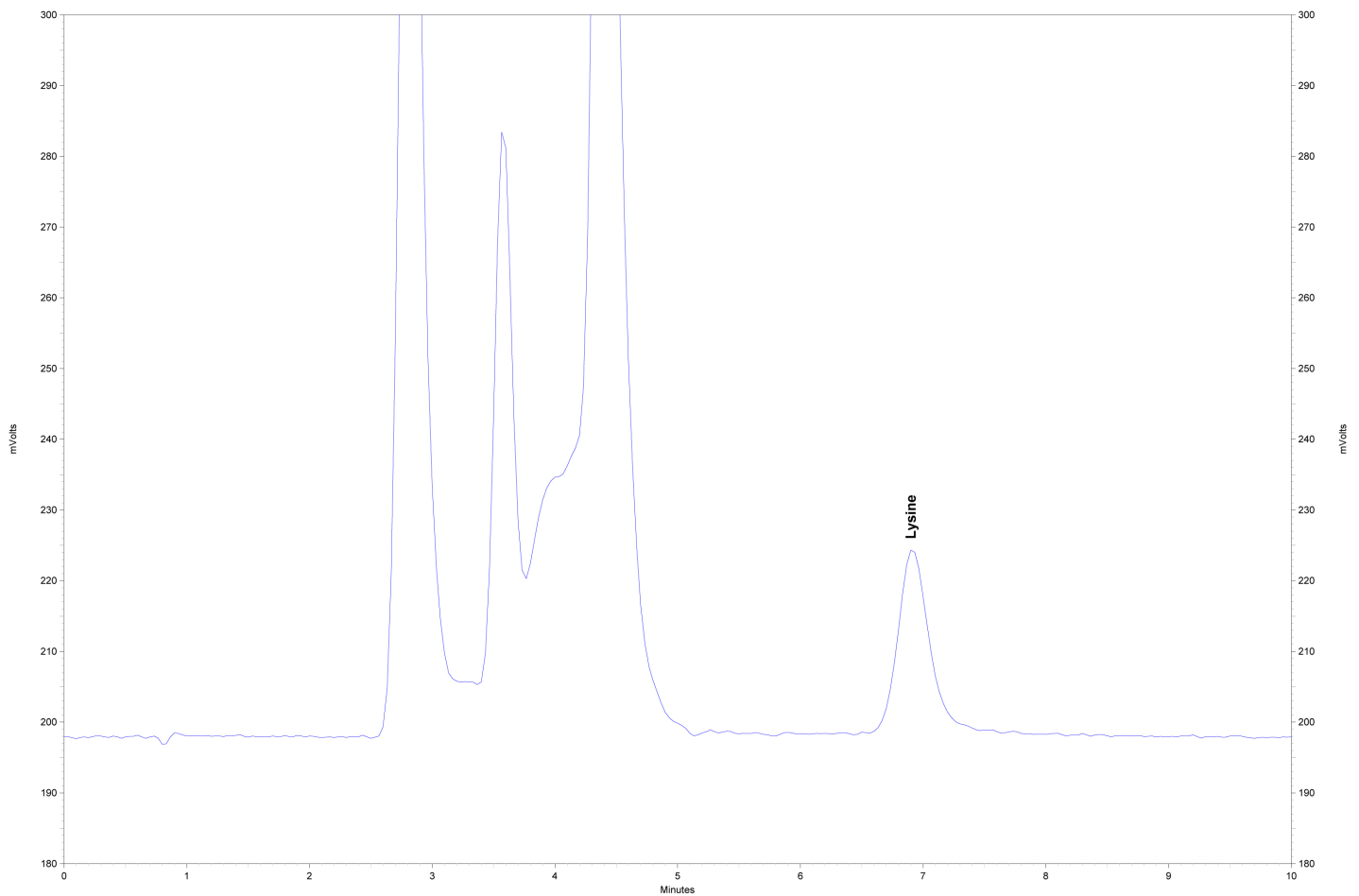
Run Time (injection to injection)	20 Min
Intermediate precision	0.9% (5 nmol / 20 µL injection)
Detection limit	14 pM / 20 µL
Quantification limit	48 pM / 20 µL

Table 3: Analytical performance short method

The results obtained on the feedstuff samples with the short program showed a good correlation with the results obtained with the full program as well as with the results obtained when tested by an external laboratory. The program also gave very good analytical performance with good repeatability and low detection and quantification limits.

The short program for the analysis of lysine allows more than 70 analyzes to be performed per day, making it a critical tool for busy quality control labs.

Figure 4: Hydrolysate sample (pig feed) obtained with the short program (Lysine retention time: 6.9 min compared to 41.6 min using the full program)

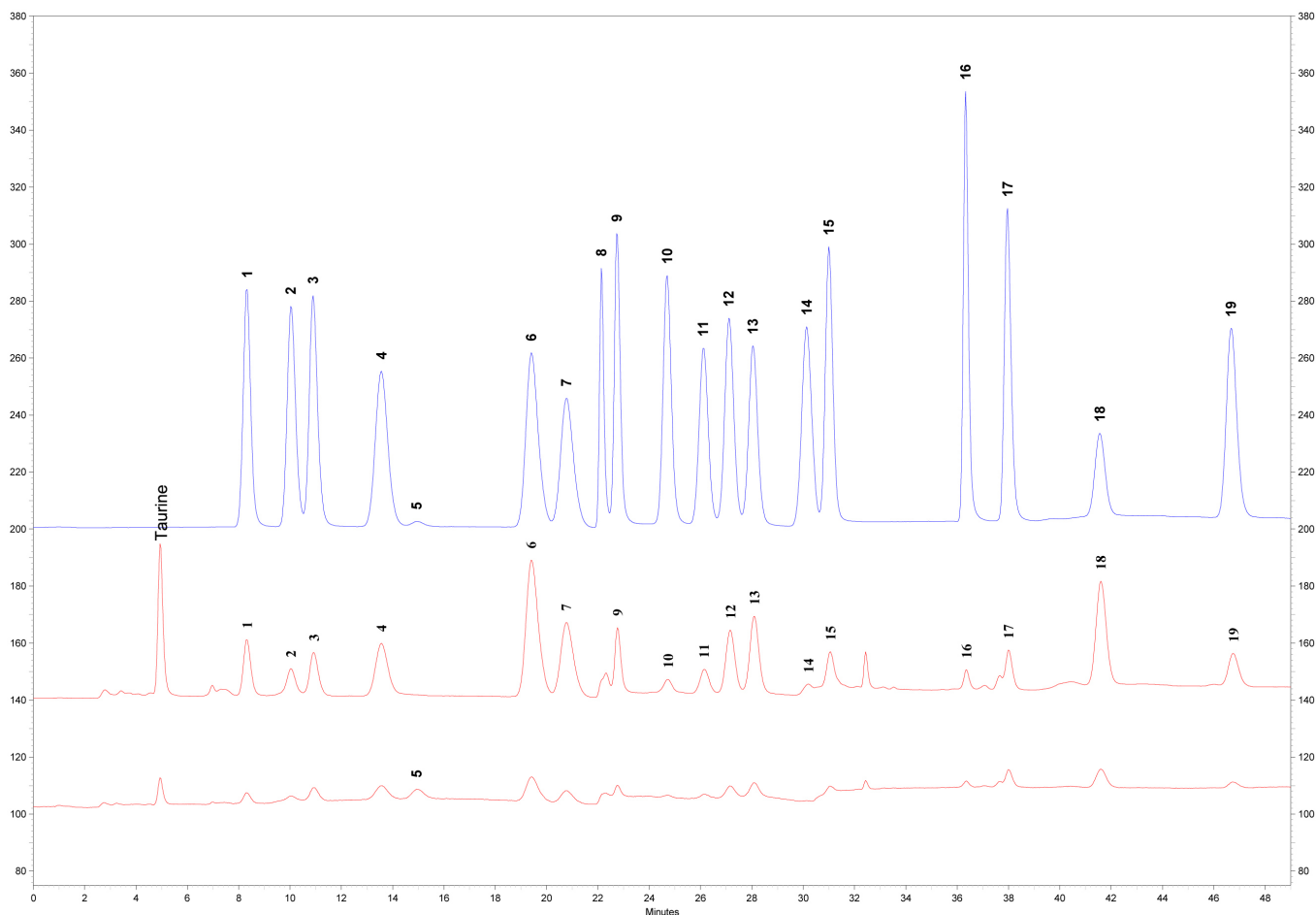


Case Study 2

A Food Manufacturer Using HPLC

A leading food manufacturer using HPLC to analyze amino acids suspected that an unknown peak was co-eluting with arginine in their samples giving inaccurate results. Even optimizing the HPLC method by extending the analysis time could not fully resolve the peaks. The samples were analyzed on a Biochrom amino acid analyzer using a sodium citrate based buffer system. This resolved arginine from the co-eluting peak and identified the co-eluting compound as taurine (Figure 5). The method developed on the Biochrom AAA offered both more accurate separation of the amino acids and a shorter analysis time than the optimized HPLC method. This shows that dedicated amino acid analysis is the technique of choice for the analysis of complex mixtures found in food and feedstuffs. Amino Acid Analysis gives better separation and shorter analysis time than the HPLC method.

Figure 5: Chromatograms from Bio 31+ System



Shown with standard (blue) at 570 nm,
sample at 570 nm (top red) and
sample at 440 nm (bottom red)

- 1 Aspartic acid
- 2 Threonine
- 3 Serine
- 4 Glutamic acid

- 5 Proline
- 6 Glycine
- 7 Alanine
- 8 Cystine
- 9 Valine
- 10 Methionine
- 11 Isoleucine

- 12 Leucine
- 13 Internal Standard Norleucine
- 14 Tyrosine
- 15 Phenylalanine
- 16 Histidine
- 17 Lysine
- 18 Ammonia

Case Study 3

Rapid Analysis of Sulfur Amino Acids in Food & Feedstuffs

Sulfur containing amino acids (i.e. methionine and cystine) are critical limiting components of the feed proteins. Although methionine can meet the total need for sulfur amino acids in the absence of cystine, it cannot be synthesized from cystine, and therefore it is classified as essential.

The hydrolysis procedure to determine the amino acids partially oxidizes methionine into methionine sulfoxide and methionine sulfone. Cysteine is also partially oxidized to cystine and cysteic acid. These reactions are non-reproducible and can result in quantitation errors.

To determine the sulfur amino acids accurately, samples must be first oxidized with performic acid to quantitatively convert methionine and cyst(e)ine to methionine sulfone and cysteic acid, respectively. If methionine sulfoxide was present in the sample protein prior to performate oxidation and hydrolysis it would also be converted to methionine sulfone. Performic oxidation prior to hydrolysis is a widely used method and it is also the method recommended by the EU Commission Directive 98/64/EC.

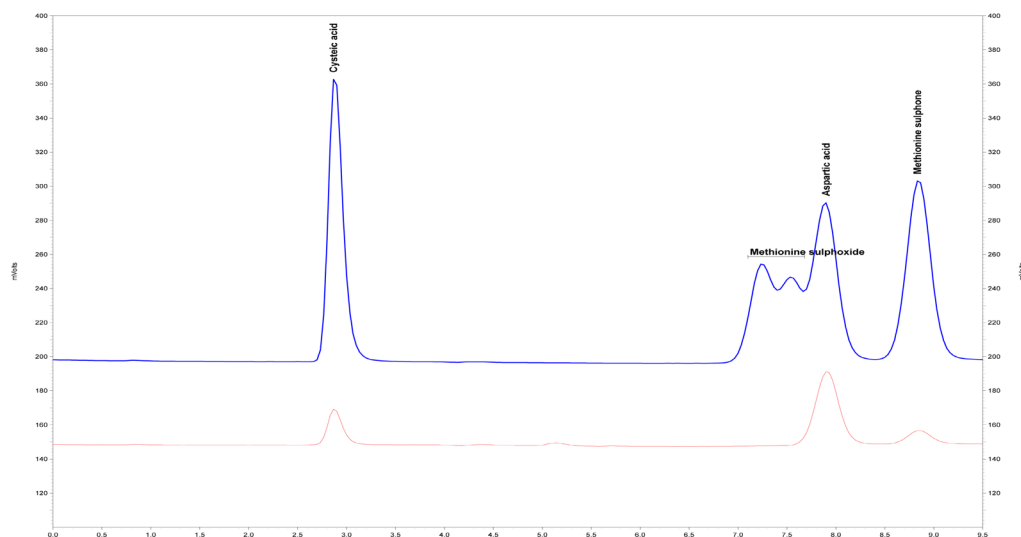
Biochrom has developed a short program to quantify sulfur containing amino acids in oxidized protein hydrolysates. Using this program with the accelerated buffers, methionine sulfone is well resolved from aspartic acid and any methionine sulfoxide that could remain from incomplete oxidation.

Cysteic acid and methionine sulfoxide elute in less than 10 minutes with a total cycle time of less than 20 minutes injection to injection (Figure 6).

The results showed very good reproducibility of areas and retention times as well as good general analytical performance. This program can be modified to accommodate the use of an internal standard if required (Table 4).

Not all amino acids can be quantified using oxidized material. Tyrosine must be determined on unoxidized samples. Thanks to the flexibility of the Bio 32+ Accelerated System, this program can be easily included within a sequence of hydrolyzed samples for the determination of the amino acid profile. As both methods are using the same column and buffers, the analysis of sulfur containing amino acids becomes even more straightforward.

Figure 6: Oxidized protein standard spiked with methionine sulphoxide compared with feed sample hydrolysate after performic acid oxidation



Run Time (injection to injection)	19 Min
Typical retention time	Cysteic acid: 2.9 min Methionine sulphone: 8.9 min
Intermediate precision	<0.5% (5 nmol / 20 µL injection)
Detection limit	10 pmol / 20 µL
Quantification limit	30 pmol / 20 µL
Linearity range	10 pmol to 5 nmol per 20 µL injection

Table 4: Analytical performances using the short program

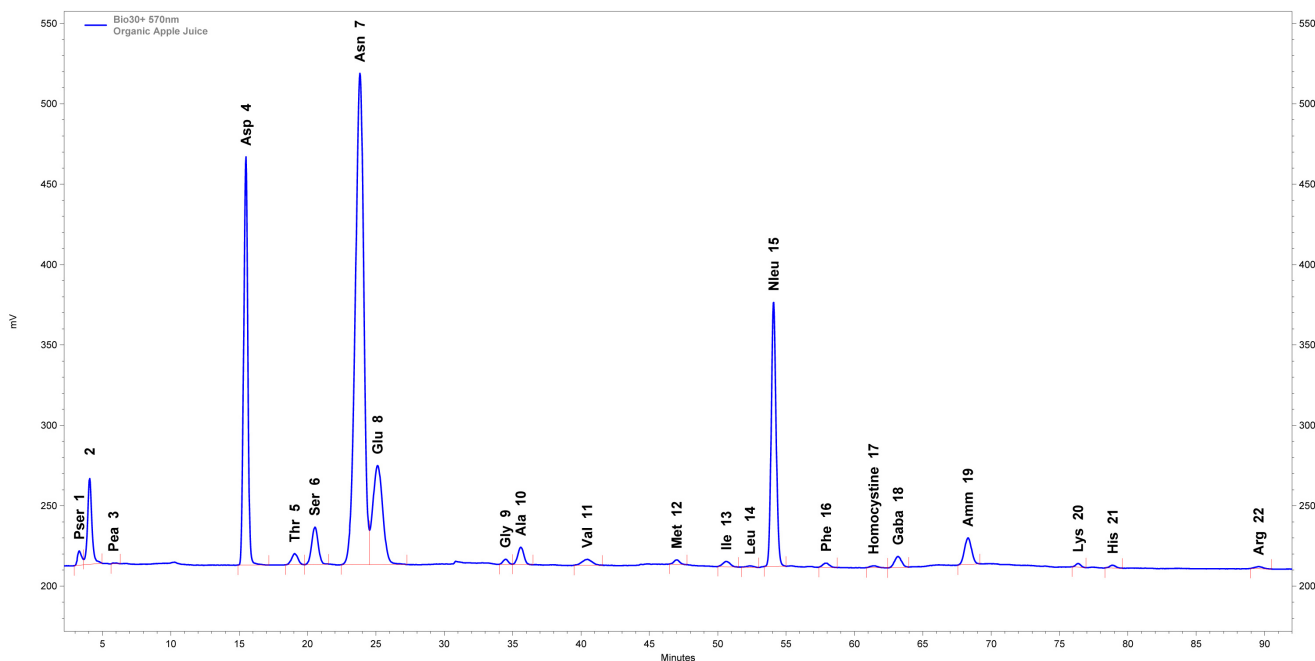
Case Study 4

Chromatography Using Lithium Citrate Buffers to Determine Free Amino Acid Content of Organic Apple Juice

Amino acid analysis of free amino acid by ion exchange chromatography is specified as one of the standard tests for commercial production by the International Federation of Fruit Juice Producers³. Fruit and fruit juices have their own characteristic profiles depending predominately on genetic factors, enabling an unknown sample to be compared with a standard of known compositional analysis to establish authenticity and

as an indicator of quality. Figure 7 shows analysis of free amino acids in organic cold-pressed pasteurized apple juice from mixed species on the Bio 30+ System using lithium buffers. Sample preparation was a simple centrifugation step at 10,000 g.

Figure 7: Analysis of organic cold-pressed pasteurized apple juice on the Bio 30+ System using the lithium buffers. Norleucine (Peak 15) is used as an internal standard.



Peak 2 comprises of carbohydrates but is clearly separated from all amino acids in the sample. No matrix interferences are seen and the amide asparagine is separated from glutamic acid. This analysis shows that the Bio 30+ can be used for detailed quantification of amino acids enabling decisions on quality and purity.

References

1. Food energy — methods of analysis and conversion factors. FAO food and nutrition paper 77. Food and Agriculture Organization of the United Nations Rome, 2003
2. Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, andolaquinoxin in feeding stuffs and amending Directive 71/393 (OJ L257, 19.9.1998, p.14)
3. Determination of free amino acids. International Federation of Fruit Juice Producers. IFU analysis No. 57 (Revised. 2005). Download from <http://www.ifu-fruitjuice.com/ifu-methods>

Bio 30+ Series Technical Specifications

Reproducibility	Area: Better than 1.5% RSD at 10 nanomoles. Retention time: Better than 0.5% RSD
Detection Limit	9 - 15 pmoles (primary amino acids detection at 570nm)
Analysis Time	Biochrom 30+: Lithium System 115 minutes injection to injection Biochrom 31+: Sodium citrate buffer system for hydrolysed proteins, 60 minutes injection to injection Biochrom 32+: Sodium Accelerated Buffer System, 60 minutes injection to injection/Sodium Oxidised System, 90 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	Software for system and autosampler control software Data Handling software package – Openlab EZ Chrom Edition from Agilent
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: <ul style="list-style-type: none"> • photometer lamp failure • incorrect ninhydrin / buffer / coil / nitrogen pressures • incorrect coil and column temperatures power failure

****Please Note: Use of non-Biochrom approved columns, reagents, buffers, etc. may invalidate the warranty of the Biochrom Amino Acid Analyzer.**

Biochrom, a division of Harvard Bioscience, is a world leader in amino acid analysis. The Bio 30+ System is recognized as the gold standard dedicated amino acid analyzer used by pharmaceutical and industrial labs worldwide. Applications are available both 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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