Amino Acid Analysis of Foods and Feedstuffs

PARTNERS IN SCIENCE

biochrom

A balanced diet is critical for maintaining a healthy lifestyle. Consumers are becoming more and more concerned about the origins of food and the environment within which it's produced. Optimal animal nutrition is therefore critical to meet the demands of today's consumer. With the use of amino acids and vitamins, feed can be formulated which delivers exactly the nutrients required.

Precise Feed Formulation

A total of 20 amino acids have even identified of which the animal can synthesise about half; these are called non-essential amino acids. However the others cannot be synthesised and must be provided in the diet. These are called essential amino acids.

In the animal diet there are currently 4 main essential amino acids: lysine, methionine, threonine, and tryptophan. These amino acids are the most important nutritionally because limiting these will affect the growth and development of the animal.

Supplemental amino acids can be added to feedstuff to increase efficiency of animal production and achieve a least cost feed formulation.

Analysing the amino acid composition of feedstuffs ensures that nutritionists provide a more precise feed formulation.

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 protein sources
The essential amino acid found in the smallest quantity in the foodstuff.

Dedicated Amino Acid Analysis

Amino acid analysis is a technique based on ion exchange liquid chromatography used in a wide range of application areas to provide qualitative and quantitative composition analysis. The Biochrom 30 Amino Acid Analyser is a dedicated system using established post-column, ninhydrin derivatisation and photometric detection.

Analysis of both protein hydrolysates and oxidised protein hydrolysates can be performed on this system using specially formulated sodium buffers, which allows flexibility of sample type and ease of use of the instrument.

Biochrom has been a leading supplier of quality instrumentation to science and industry for more than 30 years. Continued product development drawing upon many years of experience of instrument design has resulted in reliable dedicated amino acid analysers and a large installed base of instruments.

Offering full service and support cover including user groups, training, applications and consumables for all existing and new customers world-wide, Biochrom's reputation in the field speaks for itself.

General applications for the Biochrom 30 in the food and feedstuffs industry

- Quality control of raw materials
- Identification of the source
- Identification of unwanted supplements
- Proof of quality and nutritional value of final produce
- Research into optimum feed and diet formulations
- Helping producers meet legal requirements for records of total consumption in relation to waste
- Nutritional labelling
- Determination of meat quality
- Indication of spoilage
- Indication of microbial contamination
- Monitoring product throughout the production process
- Ensuring nutritional quality

Amino Acid Analysis – the technique of choice

A number of techniques can be used to analyse amino acids, however unlike techniques such as HPLC, the Biochrom 30 Amino Acid Analyser meets the requirements of the AOAC and the EU Commission Directive 98/64/EC for the analysis of amino acids from food and feedstuffs. Described below is a case study of a quality control (QC) laboratory using HPLC to analyse amino acids.

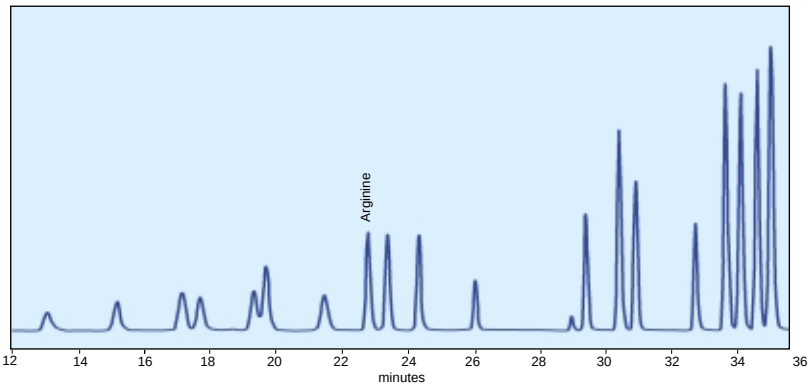


Fig 1: Amino acid standard using original HPLC method

A leading food manufacturer was using HPLC to analyse amino acids. They were encountering difficulties in obtaining accurate results because they suspected that an unknown peak was coeluting with the arginine peak in the sample, which was potentially giving rise to quantification errors (Figure 1)

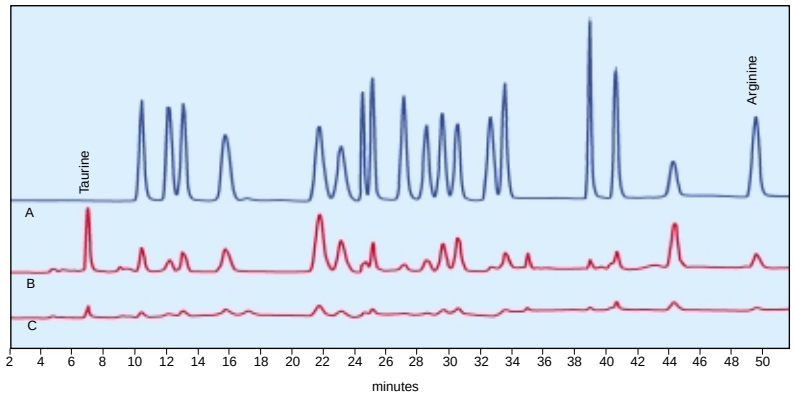


Fig 2: Biochrom 30 chromatograms A - Standard at 570nm, B - Sample 570nm, C - Sample at 440nm

The same samples were then analysed on a Biochrom 30 Amino Acid Analyser using a sodium citrate-based buffer system of increasing pH and molarity (Figure 2). By comparing the results on both systems it was confirmed that arginine was not fully resolved with the current HPLC method.

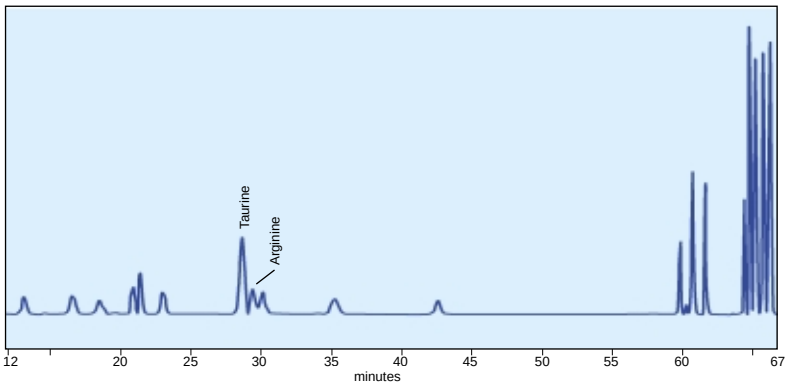


Fig 3: Standard using optimised HPLC method

Using the results obtained on the Biochrom 30, it was possible to optimise the HPLC method to resolve arginine from its coeluting peak, which was identified as taurine (Figure 3).

The method developed on the Biochrom 30 offered better separation of the amino acids and shorter analysis time than HPLC. This provides further evidence that a dedicated amino acid analyser is the technique of choice for the accurate analysis of complex mixtures found in food and feedstuff samples.

Benefits of the Biochrom 30 include:

- Better reproducibility of peak area and retention time
- Better separation: typically 90% separation between each amino acid
- High sample throughput due to long column life
- Column compatibility with sample containing high concentration of salt
- Flexibility of the stepwise elution with pH and molarity being adjusted independently
- Applications support from scientists dedicated to amino acid analysis
- Regulatory compliance

Full amino acids profiles

The Biochrom 30 Amino Acid Analyser includes a choice of buffer kits and columns specially formulated for the analysis of the most complex amino acid mixtures. Typical feedstuffs chromatograms of 24 amino acids are shown in Figure 4 and 5 using a standard mixture and an oxidised hydrolysate sample respectively.

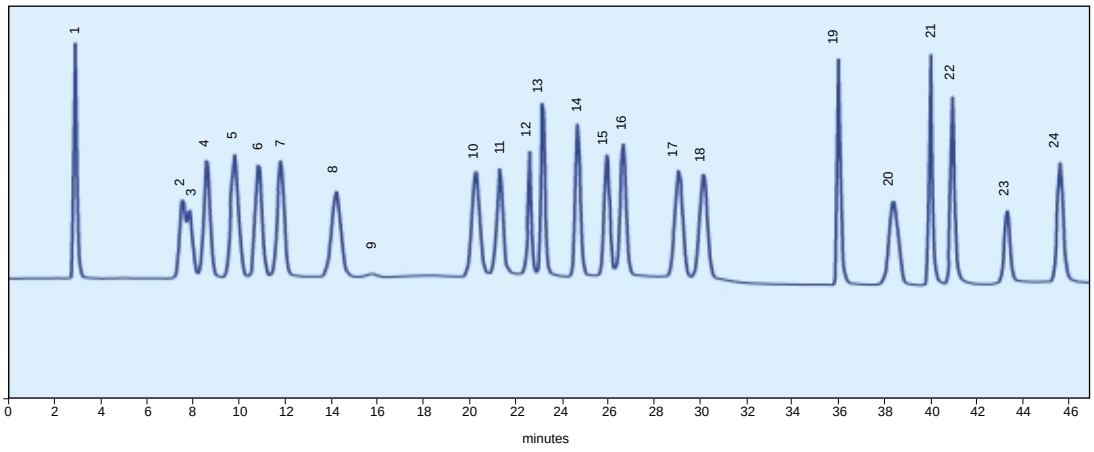


Fig 4: Standard mixture containing the amino acids typically found in feedstuffs

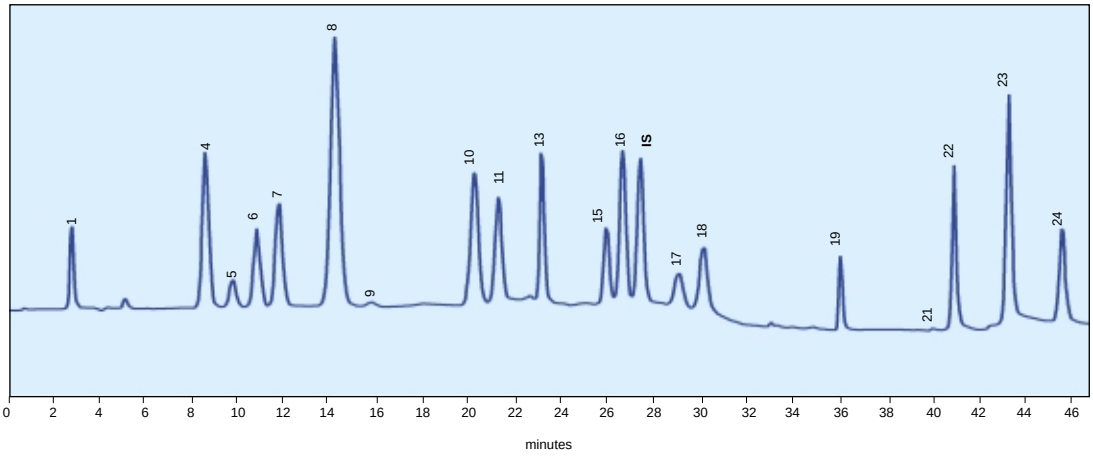


Fig 5: Oxidised hydrolysate sample prepared according to the EC directive (IS - Internal standard)

1-Cysteic acid
2,3- Methionine sulphoxide
4-Aspartic acid
5-Methionine sulphone
6-Threonine
7-Serine
8-Glutamic acid
9-Proline
10-Glycine
11-Alanine
12-Cystine
13-Valine
14-Methionine
15-Isoleucine
16-Leucine
17-Tyrosine
18-Phenylalanine
19-Histidine
20-Tryptophan
21-Ornithine
22-Lysine
23-Ammonia
24-Arginine

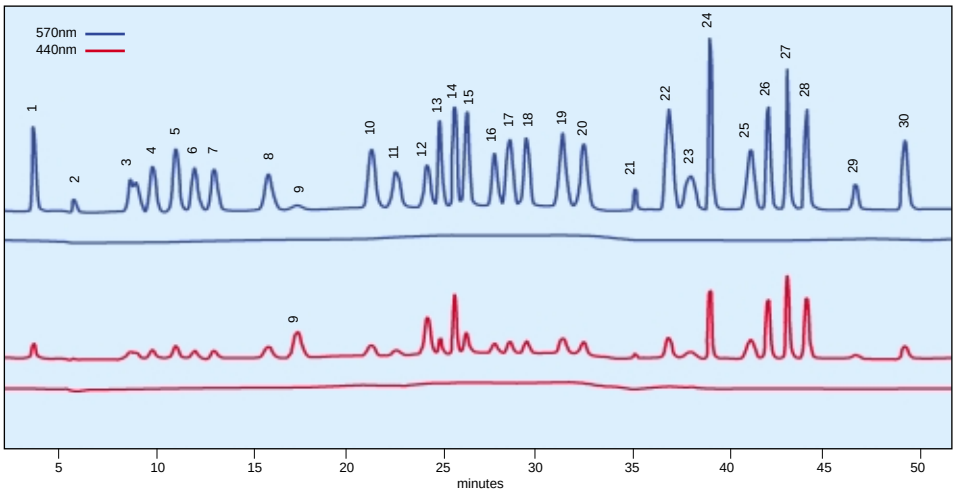


Fig 6: Chromatogram of a standard mixture containing 30 amino acids and blank (sodium citrate loading buffer). Detection at 570 nm and 440 nm. 1-Cysteic acid, 2-Taurine, 3-Methionine sulphoxide, 4-Aspartic acid, 5-Methionine sulphone, 6-Threonine, 7-Serine, 8-Glutamic acid, 9-Proline, 10-Glycine, 11-Alanine, 12-Cystine, 13-Valine, 14-2,6 -Diaminopimelic acid, 15-Methionine, 16-Isoleucine, 17-Leucine, 18-Norleucine, 19-Tyrosine, 20-Phenylalanine, 21-β-alanine, 22-Glucosamine, 23-Galactosamine, 24-Histidine, 25-Tryptophan, 26-Hydroxylysine, 27-Ornithine, 28-Lysine, 29-Ammonia, 30-Arginine

Additional amino acids such as taurine, 2,6-diaminopimelic acid, ß-alanine, glucosamine, galactosamine, and hydroxylysine can also be analysed using the sodium buffer system (Figure 6).The 30 amino acids are separated in less than 50 minutes (64 minutes injection to injection), which represents significant decrease in individual run times, thus increasing throughput. A smooth baseline is observed at both wavelengths, avoiding baseline artefacts incurred by the buffer changes.

Benefits of the Biochrom 30 include:

- A baseline free of artefact peaks which could otherwise interfere with an amino acid peak.
- Smooth baseline under cystine, allowing small amounts of cystine in the sample to be accurately quantified.
- Ability to withstand high salt concentrations, which can cause peak broadening and affect the resolution of methionine sulphone, aspartic acid, threonine, and serine hence giving rise to reproducibility errors.
- The accurate quantification of ornithine and hydroxylysine which in many existing ion-exchange buffer systems coelute with lysine and histidine respectively.
- Ability to analyse hydrolysates prepared from organic acids or alkaline hydrolysis where tryptophan may be preserved.

Rapid quantification 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.

Improvement of animal performance through better amino acid balance, better carcass quality and prevention of lysine deficiency are some of the benefits associated with the right lysine content in the animal diet. For example for piglets and pigs the use of synthetic lysine allows an increase in feed consumption and in body weight gain; and an improvement in feed conversion and in nitrogen retention. Particularly with pigs, its use allows an improvement of the carcass quality.

Using the Biochrom 30 sodium buffer system, lysine can be accurately quantified in less than 10 minutes to enable adjustments to the formulation of feedstuff.

The samples were supplied by an external laboratory and prepared according to the EC official method. (See references.)

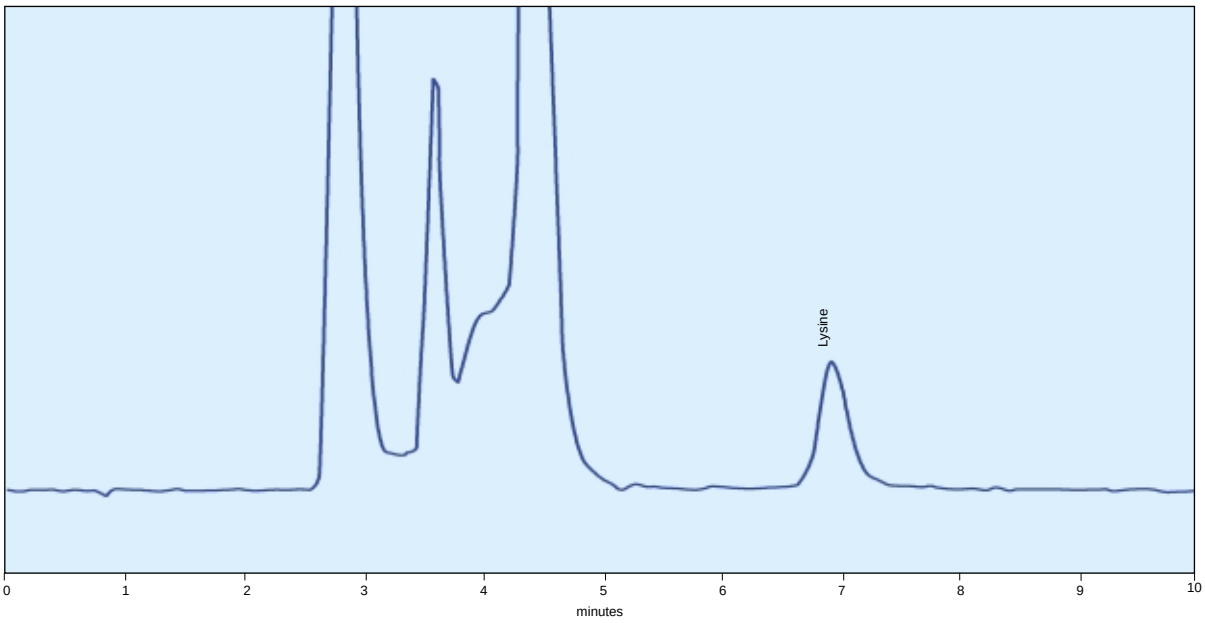


Fig 7: Hydrolysate sample (pig feed) obtained with the short program
(Lysine retention time: 6.9 min compared to 41.6 min using the full program)

Samples (hydrolysates)	Lysine concentration (nmol/20µL) Short program	Lysine (% of raw material)	
		Biochrom	External laboratory
Feedstuff Sample A	1.877	1.14	1.13
Feedstuff Sample B	1.745	1.06	1.05
Pig feed Sample	1.368	0.83	0.86
Piglet feed Control	2.120	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/20µl injection)
Detection limit	14 pmol/20µl
Quantification limit	48 pmol/20µl

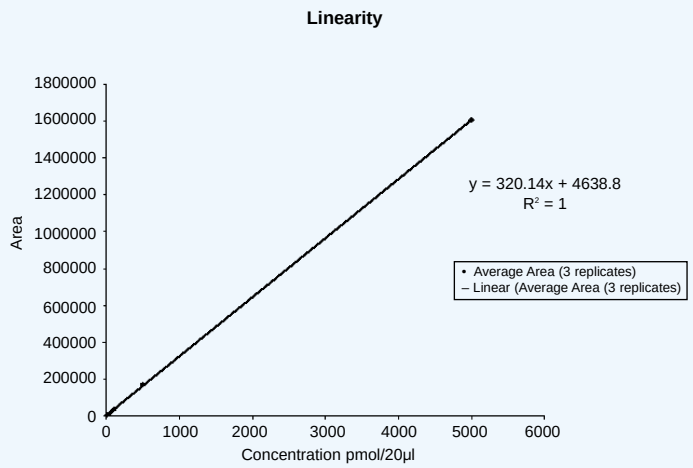


Table 3: Analytical performance

Conclusion

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 analyses to be performed per day, making it a critical tool for busy QC labs.

Rapid analysis of Sulphur Amino Acids

Sulphur-containing amino acids (e.g. methionine and cystine) are considered the most critical limiting components of the feed proteins. Although methionine can meet the total need for sulphur amino acids in the absence of cystine, it cannot be synthesised from cystine, and therefore it is classified as essential.

Hydrolysis is required to determine the amino acid composition of feed proteins. During this process methionine is partially oxidised to methionine sulphoxide and methionine sulphone. This reaction occurs in a non reproducible way thus giving rise to quantitation errors. Cysteine is also partially oxidised to Cystine and Cysteic acid. In order to determine the sulphur amino acids accurately, the sample must be primarily oxidised with performic acid to quantitatively convert methionine and cyst(e)ine to methionine sulphone and cysteic acid, respectively.

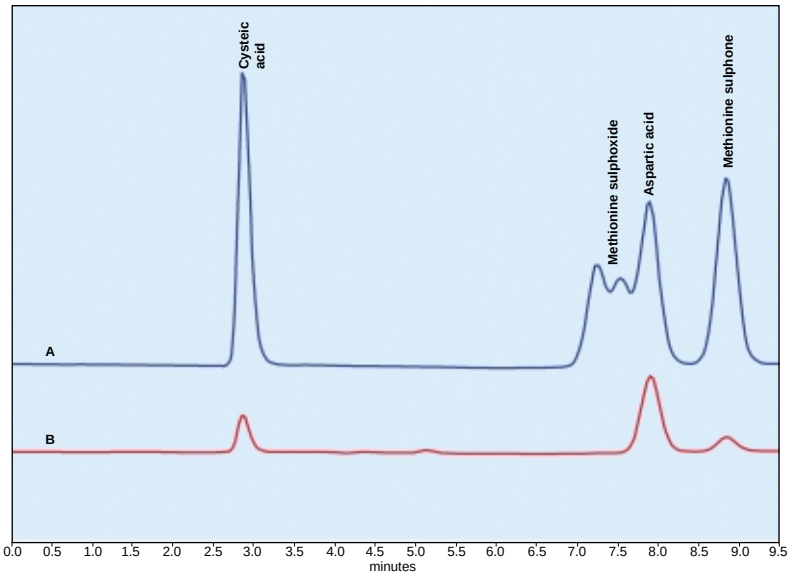


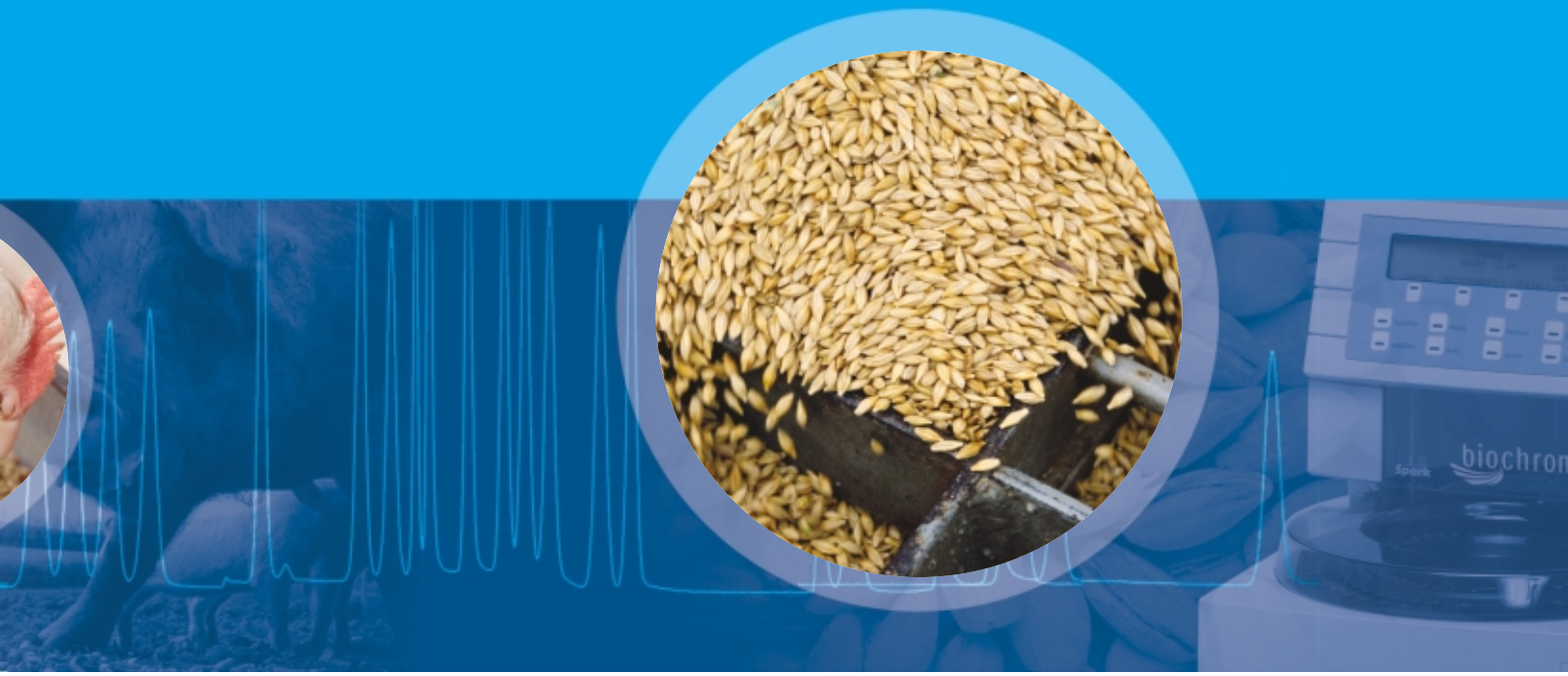
Fig 8: A - Oxidised protein standard, B - Feed hydrolysate after performic acid oxidation

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

Conclusion

A short program was developed for the quantification of sulphur containing amino acids in oxidised protein hydrolysates. Using this program methionine sulphone is well resolved from aspartic acid and any methionine sulphoxide that could remain from incomplete oxidation. Cysteic acid and methionine sulphoxide are eluted in less than 10 minutes with a total cycle time of less than 20 minutes injection to injection (Figure 8). The results showed very good reproducibility of areas and retention times as well as good general analytical performances (Table 4). This program could also be modified to accommodate the use of an internal standard if required.



Ordering Information

PART NUMBER	DESCRIPTION
80-2111-01-SYS	Biochrom 31 Protein Hydrolysate and Chemical Kit
80-2111-02-SYS	Biochrom 32 Oxidised Hydrolysate and Chemical kit

The Instrument package consists of the operating unit, which includes electronics unit, pumps, detection system, and cooled autosampler. Also included in the package are the items listed below.

- Analytical column and top-up resin
- Prewash column
- Operating software and interface cables
- Chemical kit
- Calibration Standard
- Power cables
- Qualification and Performance Verification log book
- Ninhydrin and buffer bottles
- Instruction manual
- Tool kit
- Spare parts and consumables kit
- Signal lead
- Biosys control software
- Compaq desktop PC
- Compaq TFT monitor
- Deskjet printer
- EZChrom Elite Software

Due to the flexible setup of the instrument and Biochrom's commitment to customer service, all customers may have their instrument tailored to their specific application. Biochrom offers 2 applications free of charge when ordered with a new instrument. A charge will be applied if more applications are required.

References:

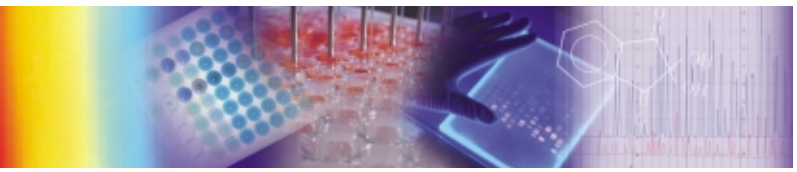
Andrews R.P., Baldar N. A., Science Tools, Vol 32, No. 2, 1985, pp. 44-48
Davies M., The Biochrom Handbook of Amino Acids
Commission Directive 98/64/EC

UV/Visible spectrophotometry

UV/Visible Spectrophotometry is a fundamental analytical technique and, together with suitable sample handling accessories, is used in laboratories for absorbance and transmission measurements of samples in all application areas. Biochrom, using its Novaspec, Ultrospec, GeneQuant, Libra and WPA brand names, manufactures an extensive range of attractive UV/Visible products and accessories, with performance and reliability guaranteed by over 20 years experience in the field. Amongst other technological advances, these instruments feature PTR (Press To Read) capability, which dramatically extends the lifetime of the source lamps.

Microtitre plate readers, washers and dispensers

In the food testing, clinical, biotech and pharmaceutical industries, the demand is for ever increasing sample throughput and smaller and smaller volumes. This is where the microtitre plate comes into its own and Biochrom offer an excellent range of fast, versatile and reliable plate readers with robot friendly designs, via its Asys Hitech subsidiary company. In addition, a range of washers is available, with a unique manifold design for minimised residual volumes and digitally controlled aspiration and dispensing pumps for high accuracy and low noise performance. To minimize human intervention and possible error, there is a growing requirement to dispense low volumes of liquids rapidly, accurately and reproducibly. Biochrom's liquid dispensers meet these needs exactly, with units for two or six, any well format, microtitre plates and the ability to deliver volumes of liquid down to two microlitres using a non-contact delivery technique, thereby eliminating cross contamination.



Gel electrophoresis

Gel Electrophoresis remains one of the most important techniques in the life sciences. Biochrom, via its Hoefer and Scie-Plas sister companies, offers a full range of electrophoresis products for analytical and preparative nucleic acid studies and manual DNA sequencing, including both horizontal and vertical units together with all appropriate buffers, sampling and blotting accessories.

Amino Acid Analysis

Biochrom has been in the field of dedicated Amino Acid Analysis for over 30 years using established ion exchange chromatography to provide rapid, specific amino acid analysis for clinical, pharmaceutical, proteomics, food and feedstuff industries. These state-of-the-art bench top products feature proven ninhydrin detection technology fully integrated into a complete package utilising the latest graphical software, active components in ceramic and PEEK for long life and elimination of contamination and a range of robust ion exchange columns for customised applications.

If you want to know more about us, or our products, please get in touch . . . .

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