2370

ALTA BIOSCIENCE LTD

Amino Acid Analysis

Technical Document

AltaBioscience offers amino acid analysis accredited to ISO 17025:2017 (2370) standard as defined by the United Kingdom Accreditation Service (UKAS).

Amino acid analysis is an important tool in research and product development in the food, animal feed, drug and supplement industries. It is the most accurate way to determine the composition and quantity of protein in a sample.

Uses for amino acid analysis:

- Accurate protein quantification for a variety of sample types
- Quantification of free amino acids in serum and physiological samples
- Calibration of Extinction Coefficient (protein assays)
- Exploring protein structure and function in basic biological research and in pharma R&D
- Monitoring fermentation and cell culture processes
- Quantifying the amounts of protein and amino acids and the relative amounts of bound and free amino acids in protein chemistry applications, synthetic peptides, serum, growth media and food, drink and animal feed
- Characterising recombinant proteins and registering protein and peptide drugs
- Nutritional analysis of food and animal feed, including specialist dietary products such as low protein foods or foods containing phenylalanine
- Detection of non-standard amino acids, such as taurine in animal foods and sports drinks, intermediates in biosynthesis and non-proteogenic amino acids in synthetic biology
- Quantitation of non-amino acid compounds e.g. amino sugars
- Quantification of net peptide content of synthesised peptides

Accredited to ISO/IEC17025 (2370)

Fast sample turnaround with option for Express Service

Ideal for food, animal feed, supplement and drink analysis

Method development to suit your specific requirements

Professional technical assistance throughout the whole project

Testing to European Pharmacopoeia 2.2.56 (Ph. Eur.) monographs avaialble

Advancing science through analytical chemistry and synthesis services in the life and food science sectors worldwide

Accredited by UKAS to ISO/IEC 17025:2017 (2370) for amino acid analysis, our laboratories use state of the art technology and optimised processes to carry out fast, accurate, quantitative amino acid analysis using the most robust analytical method, post-column detection.

The advantage of post column detection means a wide range of sample types, including samples of food, drink, serum and animal feed can be analysed not just pure proteins required by other methods.

Turnaround is fast, with multiple instruments to avoid unnecessary delays and an optional Express Service when results are required urgently.

AltaBioscience offers two types of analysis, 'total' and 'free' amino acid analysis

• Total Amino Acid Analysis

Where the amino acid composition of a protein or peptide is of interest, or if the total protein concentration of a sample is required, then this is the method of choice. The proteins and/or peptides within the sample are broken down by acid hydrolysis into their individual amino acids, the final extract is therefore a sum of amino acids which were free in solution and those which were previously incorporated into proteins. The amino acids within the hydrosylate are then separated using a sodium citrate buffer system prior to detection. This method measures 18 of the 20 standard amino acids (see figure1). If required, we can also quantify hydroxyproline however this requires additional analysis steps.
Tryptophan and cysteine/cystine are usually lost during acid hydrolysis, however, estimates can be given for these amino acids. For specific quantification of tryptophan or cysteine/cystine, separate additional testing methods are available.

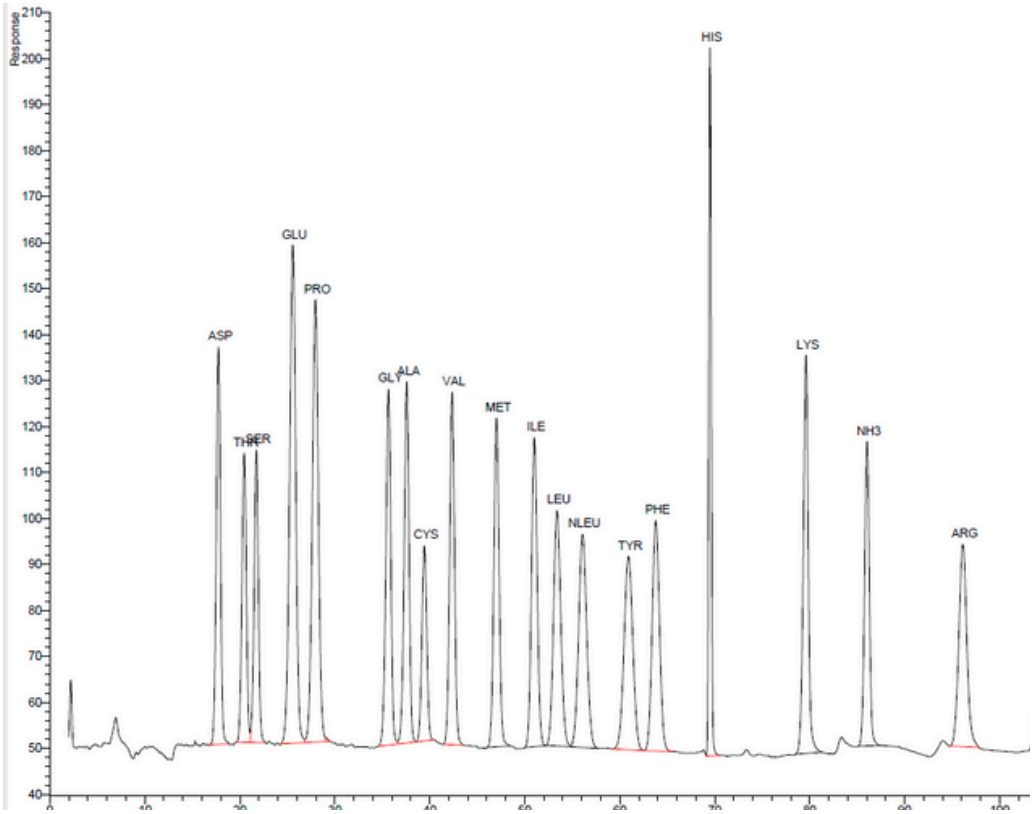

Figure 1. Example chromatogram of an amino acid standard separated on the protein hydrolysate (sodium) system.

• Free Amino Acid Analysis

For the analysis of samples such as physiological fluids including serum or cell growth media, the amino acids incorporated into proteins or peptides are not of interest, and it is the concentration of the individual unbound amino acids which is required. Therefore the protein and interfering compounds are removed, leaving the amino acids free in solution. The free amino acids are then separated using a lithium citrate buffer system prior to detection. This high resolution method quantifies up to 40 components, including many non-standard amino acids.

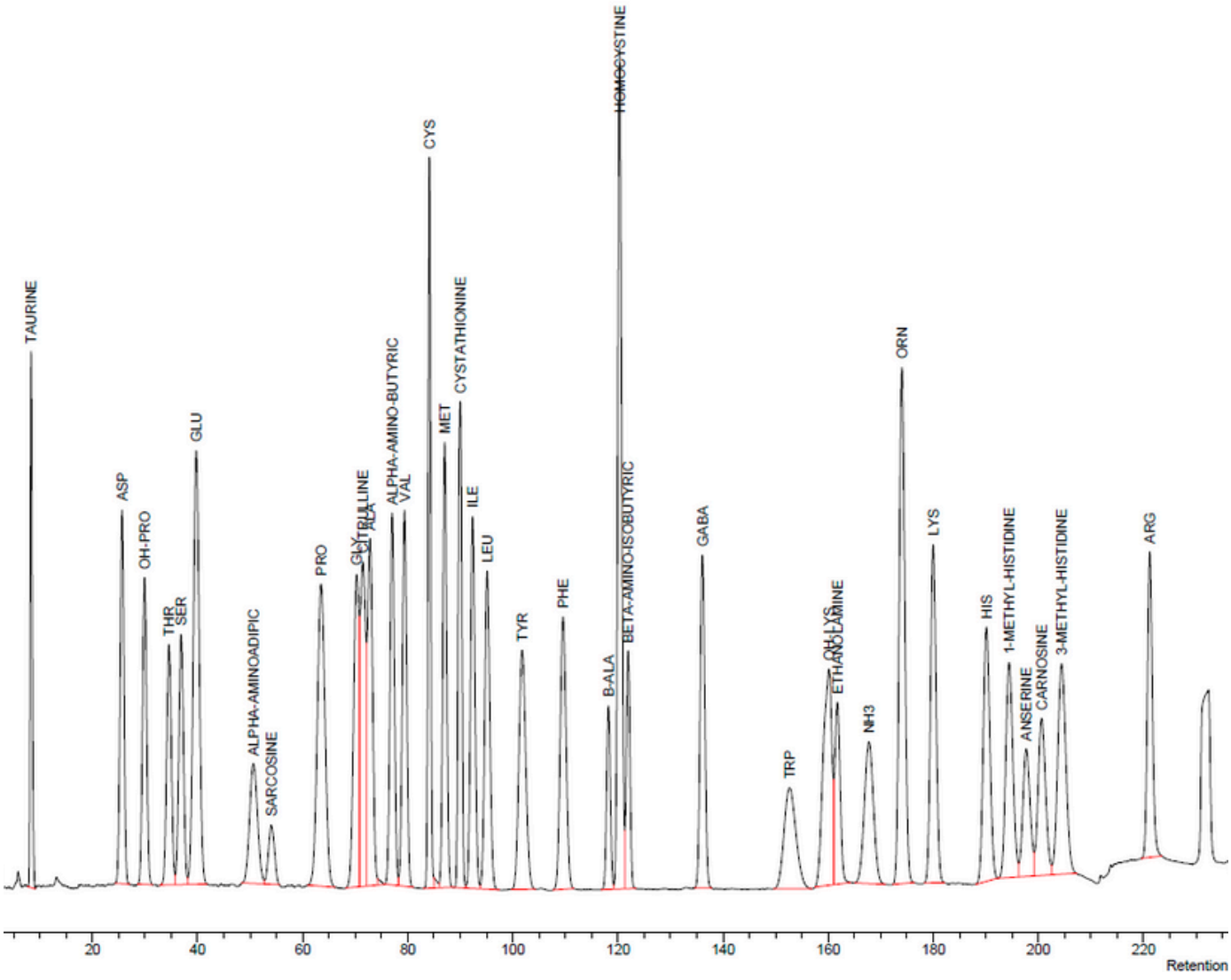

Figure 2. Example of chromatogram of an amino acid standard spearated on the physiological fluid (lithium) system.

For both types of analyses, in addition to our standard service, AltaBioscience offers an extended choice of service levels dependent on your specific requirements. Whichever service options you choose, you can be assured of the highest level of quality in-line with our ISO 17025:2017 (2370) accredited status.

Service options:

- **Standard service:** Ideal for routine testing, each sample is hydrolysed or precipitated once, followed by a single analytical separation and quantification. There are options to add duplicate sampling if required.

- **Premium service:** Ideal for samples from GMP processes or where rigorous QA monitoring and additional testing is required. Sample weighings and hydrolysis/extraction are carried out in duplicate, followed by duplicate analysis of each hydrolysate/precipitation, a total of 4 separations in all. This data is calibrated against the average of 2 standards specific to your sample batch. Rigorous QA checks are applied throughout the process. A complete validation study with application report can be performed on request

- **Method specific testing:** Ideal for analyses following EP methods: samples are analysed in duplicate using a test and reference sample and includes comparison of ammonium content as required.

Method Considerations

Some amino acids are sensitive to the acid hydrolysis process and therefore accurate quantitation can be achieved using special hydrolysis methods:

- **Cysteine and Cystine must be converted to cysteic acid before the acid hydrolysis step.**
- **Tryptophan** requires a alkaline hydrolysis prior to analysis. For this method, we require at least 5mg of sample.
- **Glucosamine and Galactosamine** require a specific hydrolysis step and a change to the separation method. Other amino sugars can be analysed, but would require method development, please enquire.
- **Asparagine and Glutamine** are converted to Aspartic acid and Glutamic acid during acid hydrolysis when analysed using the 'Total' amino acid method

Method Limitations

- Creatine and Creatinine cannot be measured using this methodology.
- D and L isomers are not resolved by ion exchange chromatography.
- Compounds containing carotene or acrylamide cannot be hydrolysed due to their explosive potential when heated.

Sample Requirements

Amino acid analysis can be performed on virtually any type of sample matrix and can be submitted in solid, liquid or lyophilised form.

Please see below for minimum quantities for analysis. If you are unable to supply these weights/volumes, please contact us to discuss your needs. Analysis of cysteine and tryptophan will require a separate hydrolysis and analysis operation and additional quantities of sample, please contact us for more information.

Total amino acid analysis

Dry samples

For peptides at least 5mg of material is required if the sample is to be weighed in house, although we can process pre weighed peptides if required. Please inform us if you are aware of any of the following:

- Any known solubility issues.
- Any Ile-Ile, Ile-Val or Val-Val bonds as these are abnormally stable to the hydrolysis process and we will need to perform additional validation.

We also require the following information for peptides:

- The salt form of the peptide, e.g. TFA or HCl salt.
- The theoretical ratios of the amino acids.
- Information regarding any modifications e.g. acetylated or modified amino acids.

For other sample types including food at least 1g is typically required, unless the sample needs to be homogenised prior to analysis (please contact us for further information).

Solutions

For peptides and proteins, we can calculate accurate minimum sample volumes if the sequence is known.
For other sample types including drink products 2mL is typically required.

Free amino acid analysis

Dry samples

For most sample types we require at least 1g of material, but can work with less depending on the sample type. If the sample needs to be homogenised prior to analysis more than 1g may be required, please contact us for further information.

Solutions

For most sample types including drink products 2mL is typically required.
For physiological samples including cell culture media and plasma, 200uL is typically required but please contact us if you are unable to supply this.

Sample Considerations

- If samples are hygroscopic, it may not be possible to obtain a steady weighing and hence an accurate value for amino acid content.
- Samples containing Glycerol, Acrylamide and Mannitol cannot be processed due to risk of explosion when hydrolysed.
- PEG can react with some amino acids during hydrolysis, affecting recovery, and should therefore be avoided. Samples with a high salt content can affect the ion exchange column. Ammonium salts should also be avoided in the last stage of purification as large amounts of ammonia will cause the analysis reagent to precipitate out in the reaction coil.
- Glycine buffers should never be used during the work up of proteins that have to be amino acid analysed.
- Glycine is very difficult to remove afterwards, with obvious effect on the accuracy of the measurement of glycine. Proteins adsorbed on nitrocellulose membranes cannot be analysed (use PVDF filters instead).

Table 1 lists the amino acids which are offered routinely as part of the Total and Free analysis methods. However these lists are not exhaustive as we can perform custom analysis and method development and would be happy to discuss your individual requirements.

Amino Acid	Total AA Standard resolution separation	Free AA High resolution separation
Hydroxyproline	Specific analysis	✓
Aspartic Acid	✓	✓
Threonine	✓	✓
Serine	✓	✓
Glutamic Acid	✓	✓
Proline	✓	✓
Cysteine	Specific hydrolysis	✓
Glycine	✓	✓
Alanine	✓	✓
Cystine	Specific hydrolysis	✓
Valine	✓	✓
Methionine	✓	✓
Isoleucine	✓	✓
Leucine	✓	✓
Norleucine	✓	✓
Tyrosine	✓	✓
Glucosamine	Specific hydrolysis	✓
Phenylalanine	✓	✓
Histidine	✓	✓
Tryptophan	Specific hydrolysis	✓
Lysine	✓	✓
Ammonia	✓	✓
Arginine	✓	✓

Amino Acid	Total AA Standard resolution separation	Free AA High resolution separation
Taurine	×	✓
Phosphoserine	×	✓
Asparagine	Converted to Aspartic Acid	✓
Glutamine	Converted to Glutamic Acid	✓
α-Aminoadipic acid	×	✓
Sarcosine	×	✓
Citrulline	×	✓
α-Amino-butyric acid	×	✓
Cystathionine	×	✓
β-Alanine	×	✓
β Amino-butyric acid	×	✓
γ-Amino-butyric acid	×	✓
δ-Hydroxylysine	×	✓
Ornithine	✓	✓
1-Methylhistidine	×	✓
Anserine	×	✓
Carnosine	×	✓
3-Methylhistidine	×	✓
Unusual Amino Acids		
Theanine	×	✓
Norvaline	×	✓
Homocystine	×	✓

Table 1: List of amino acids available routinely

About AltaBioscience

AltaBioscience, was founded in 1973, originally within The University of Birmingham. The ISO 9001 certified company provides services for academic research and for the pharmaceutical, healthcare and food, drink and animal feed industries.

CONTACT US

For more details on sending samples including our sample submission form, visit **www.altabioscience.com**

To discuss any aspect of this service please contact us by phone on **+44 (0)1527 584495**
or email us at **info@altabioscience.com**

Advancing science through analytical chemistry and synthesis services in the life and food science sectors worldwide