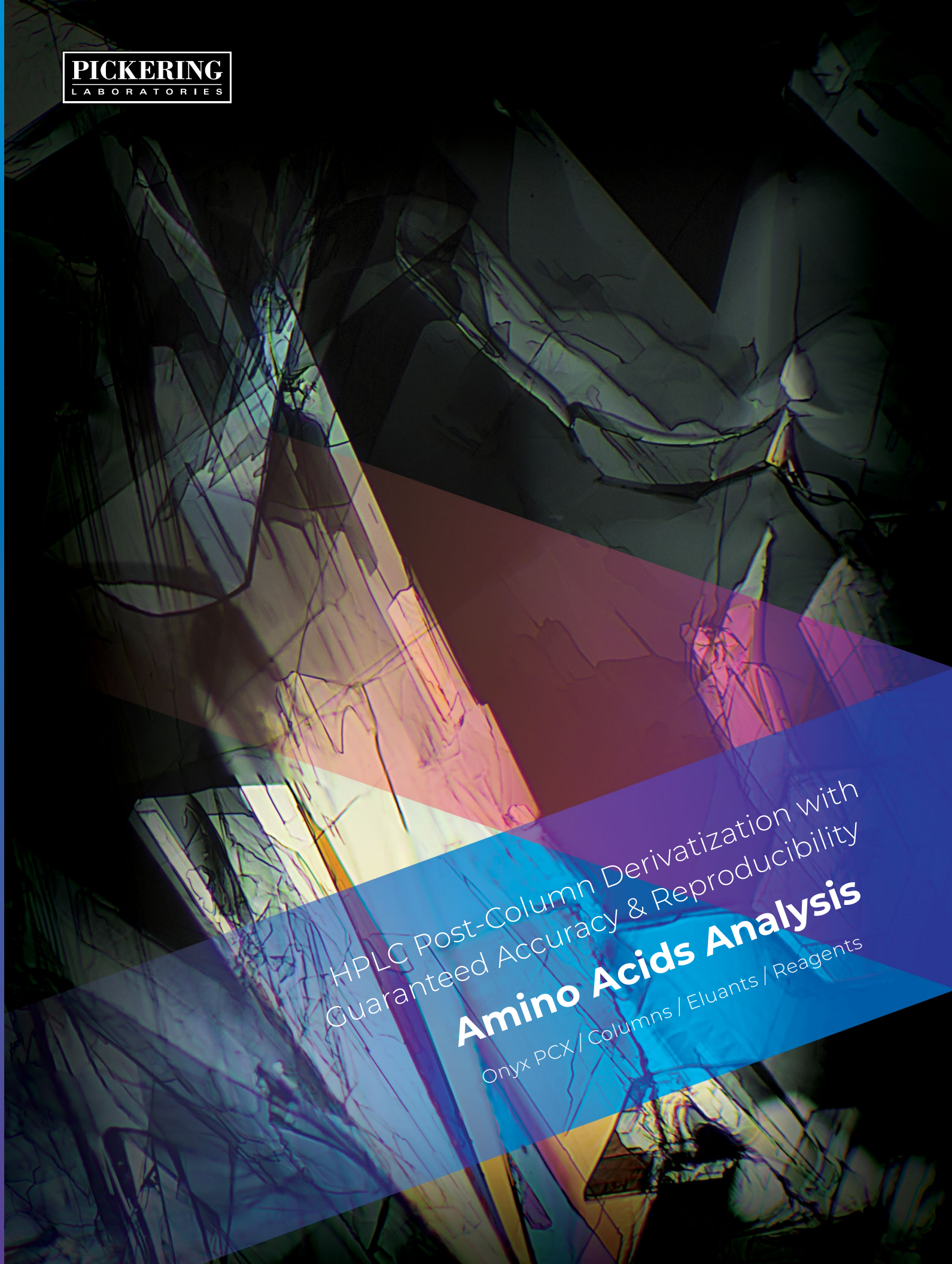
PICKERING
LABORATORIES



HPLC Post-Column Derivatization with Guaranteed Accuracy & Reproducibility

Amino Acids Analysis

Onyx PCX / Columns / Eluants / Reagents

Looking for something?

It doesn't need to be difficult. HPLC analysis with post-column derivatization has been and continues to be the method of choice for detecting amino acids in biological matrices, food products & pharmaceuticals. It offers sensitive and reliable results that have been trusted by chemists for over 50 years.

Amino Acids Analysis

Unequaled Accuracy & Reproducibility

No other technique, including pre-column derivatization followed by reversed-phase chromatography, has been shown to match post-column ion-exchange methods in accuracy and reproducibility. This is because the retention mechanism in ion exchange provides for chromatography that is almost completely matrix-insensitive. Simple sample preparation for native samples is an added benefit of the ion-exchange method.

With the alternative pre-column derivatization method, the prepared sample is subject to a chemical reaction that takes place in a very complex medium: the residual matrix. Matrix complexity produces both competition and inhibition in chemical synthesis, resulting in decreased reproducibility in peak areas and retention times. Very often, the pre-column method must be optimized for each different sample matrix. In contrast, ion-exchange chromatography followed by post-column derivatization is intrinsically more rugged and repeatable, since the matrix components do not retain on the cation-exchange column and have no influence on separation or derivatization of amino acids. The same method can be used for variety of samples, from plant extracts to serums or spinal fluids.

Pickering Laboratories, Inc. provides a complete solution to Amino Acid Analysis. We supply columns, eluants, reagents and post-column derivatization instruments that work seamlessly with virtually any modern HPLC, so we can guarantee the accuracy and reproducibility of the analysis. Our chemists will help you choose the method and instrumentation best suited to your requirements, work with you on any custom method optimization and offer continuous support in day-to-day operation.

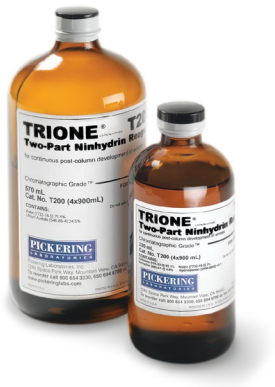
Onyx PCX

The Better Way for Amino Acids Analysis

Onyx PCX is the next generation of HPLC post-column derivatization instruments, resulting from Pickering Laboratories' 40 years of experience manufacturing post-column instruments. This instrument combines unique features that are essential for successful Amino Acids Analysis and reflects the ease of use, reliability and ruggedness customers have come to expect from Pickering Laboratories.

System design advancements result in optimized analysis:

- All Onyx PCX components are specifically designed for post-column derivatization to ensure optimum performance and simplified serviceability.
- Onyx PCX can be connected to any HPLC system, allowing for flexibility in the equipment set-up.
- The electronic syringe pump delivers true pulse-free flow for superior sensitivity and consistency without additional pulse-dampening components. The pump cylinder is made from a single piece of inert ceramic for durability and non-reactivity.
- Fully inert, oxygen-impermeable flow path protects reagent and amino acid derivatives from oxidation. It also saves instrument from the effects of corrosion, thereby reducing maintenance and extending system life.
- Color LCD display provides for continuous system monitoring and critical message alerts.
- The column oven utilizes circulating air for consistency of heating and quick cooling between runs.
- Column oven temperature gradient programming improves separation and lowers analysis times.
- Electronic valves eliminate troublesome check valves and allow for automated pump flushing.
- The quick-change reactor cartridge makes replacements quick and inexpensive.
- The PCX Control software, compatible with Windows 7 or newer Windows operating systems, allows for precise control of the reagent flow rate and conservation of reagent during the necessary column re-equilibration.
- Software stores methods and sequences, allowing for flexible application setup and switching.
- Log files collect continuous data, from system status to error messages, for traceability and in-depth troubleshooting.
- Field calibration now available by trained Pickering Laboratories service engineers to support recertification.



Post-Column Reagents for Amino Acids Analysis

Two Options for Flexibility in the Lab

Pickering Laboratories offers two post-column reagents for Amino Acid Analysis: our patented Ninhydrin reagent, called Trione®, and *o*-Phthalaldehyde (OPA) reagent. The choice of reagent is based on compounds of interest, sensitivity requirements and available instrumentation (Table 1). Both reagents can be used with any amino acid cation-exchange column and set of eluants.

Table 1. Post-Column Reagents for Amino Acid Analysis

Category	Trione® Reagent	OPA Reagent
Products	**T100** - Ready to use reagent; 4-month shelf life from the date of manufacture; Each (950 mL/bottle) **T100C** - Ready to use reagent; 4-month shelf life from the date of manufacture; Case of 4 (950 mL/bottle) **T200** - 2-part reagent, combine and use, 12-month shelf life from the date of manufacture; To prepare case of 4 (900mL/bottle)	**OD104** - *o*-Phthalaldehyde diluent for Amino Acid analysis; Case of 4 (950 mL/bottle) **O120** - *o*-Phthalaldehyde (OPA) crystals; Each (5g/bottle, makes 16x950 mL of reagent) **3700-2000** - Thiofluor™; Each (10g/bottle, makes 5x950 mL of reagent) All three products are necessary to prepare OPA reagent for amino acid analysis
Analytes	Primary and secondary amino acids	Primary amino acids. To detect secondary amino acids oxidation step is required prior to reaction with OPA. Sensitivity of detection of primary amino acids decreases when oxidation step is used.*
Detector	UV/VIS	Fluorescence
Sensitivity	10 pmole on column	2 pmole on column
Columns & Eluants	Works with any cation-exchange column for amino acid analysis	Works with any cation-exchange column for amino acid analysis
Onyx PCX Configuration	Single-pump Onyx PCX with 0.5 mL reactor	Single-pump Onyx PCX with 0.15 mL reactor. * Two-pump Onyx PCX with 0.5 mL and 0.1 mL reactors is required to detect secondary amino acids. The sensitivity for primary AA is decreased in this mode.

Columns and Eluants

For Speed or Higher Resolutions

We manufacture a wide range of cation-exchange columns and eluants to suit your analytical needs. Sodium cation-exchange chromatography is used for the fast analysis of amino acids commonly found in hydrolyzed protein samples or simple formulations. Lithium cation-exchange is a slower technique with higher resolution, used to separate up to 50 amino acids found in complex matrixes such as biological fluids, plant extracts, foods or beverages.

Tables 2 and 3 summarize Pickering Laboratories' columns and eluants systems. The columns that use analytical methods with temperature gradient allow for the fastest analysis. If temperature gradient capabilities are not available, you have a choice of standard high-efficiency columns offering high selectivity and reasonable run times.

Long columns (3.0x250 mm), despite having the longest run times, can be useful due to their unique selectivity, which allows separating drug metabolites and other compounds not commonly found in native samples. If you don't find your compound of interest on the chromatograms shown, please contact Pickering Laboratories for advice on which column is best suited for your analysis.

It is highly recommended to use a guard column or cartridge in order to capture any remaining strongly retained matrix components prior to them fouling the analytical column. Pickering Laboratories offers a novel GARD™ column protection system that can be used with any cation-exchange column for amino acid analysis.

Our unique GARD™ uses proprietary material to prevent irreversibly bound matrix compounds from fouling the column while allowing compounds of interest to pass unimpeded into the analytical column. The replaceable GARD™ significantly prolongs column life without added band spreading or backpressure.

Table 2. Sodium Amino Acid Analysis			
Analytical Column	**Guard Column**	**Samples/Eluants**	**Run Time**
1154110T (4.0x110 mm)	GARD™ column protection system	For protein and collagen hydrolysates with temperature gradient: Na315, Na425, Na640, RG011 For oxidized feed hydrolysates with temperature gradient: Na270, Na425, Na640, RG011 Internal Standard: Norleucine	30 min
1154150T (4.0x150 mm)	GARD™ column protection system	For protein and collagen hydrolysates with constant column temperature: Na315, Na740, RG011 For oxidized feeds samples with constant column temperature or temperature gradient: Na270, NA740, RG011 Internal Standard: Norleucine	55 min 60 min
1154150 (4.0x150 mm)	GARD™ column protection system	For protein and collagen hydrolysates with constant column temperature: 1700-0112, Na740, RG011 Internal Standard: Norleucine	55 min
1193250 (3.0x250 mm)	GARD™ column protection system	For protein hydrolysates with constant column temperature: Na328, Na740, RG011 Internal Standard: Norleucine	60 min

Table 3. Lithium Amino Acid Analysis			
Analytical Column	**Guard Column**	**Samples/Eluants**	**Run Time**
0354675T (4.6x75 mm)	GARD™ column protection system	For physiological samples with temperature gradient: 1700-1125, Li365, Li375, RG003 Internal Standards: Glucosaminic Acid, 2-Aminoethyl-cysteine	70 min
0354100T (4.0x100 mm)	GARD™ column protection system	For physiological samples with constant column temperature: Li275, Li750, RG003 Internal Standards: Norleucine and α-Amino-β-guanidinopropionic acid For physiological samples with temperature gradient: 1700-1125, Li365, Li375, RG003 Internal Standards: Glucosaminic Acid, 2-Aminoethyl-cysteine	95 min
0393250 (3.0x250 mm)	GARD™ column protection system	For physiological samples with constant column temperature: Li275, Li750, RG003 Internal Standards: Norleucine and α-Amino-β-guanidinopropionic acid	185 min

Table 4. GARD™ Column Protection System	
Catalog Number	**Product Description**
1700-3102	Cation-Exchange GARD™ assembly: Includes holder and 2 replaceable GARD™s
1700-3101	Replacement cation-exchange GARD™s (2/pack)
1700-3100	GARD™ holder

Amino Acid Analysis of Hydrolyzed Samples

Pharmaceuticals, Foods & Feed

Amino acids analysis plays a crucial role in research and production of pharmaceuticals. A wide range of modern therapeutic agents, prophylactics and vaccines are biological products manufactured using biotechnology. The industry relies on amino acids analysis for:

- Identification of peptides and proteins by means of amino acids composition analysis;
- Determination of impurities and related substances in Active Pharmaceutical Ingredients (APIs) and intermediates;
- Single or total amino acids quantification in drug products, including markers determination in complex matrixes.
- Control of manufacturing processes for recombinant proteins.

Determining amino acid composition is also necessary to ensure the proper nutritional value of food and feedstuffs. It is especially imporant to know the amount of the essential amino acids which cannot be synthesized by the body and so must be provided by the diet. Amino acid analysis is widely used in the food and feed industries as a crucial part of the quality control of raw material and final products, monitoring of production process and nutritional research and determining market value of the feedstuffs.

Analysis of protein hydrolysates and oxidized hydrolysates are done using Pickering Laboratories Sodium columns and eluants.

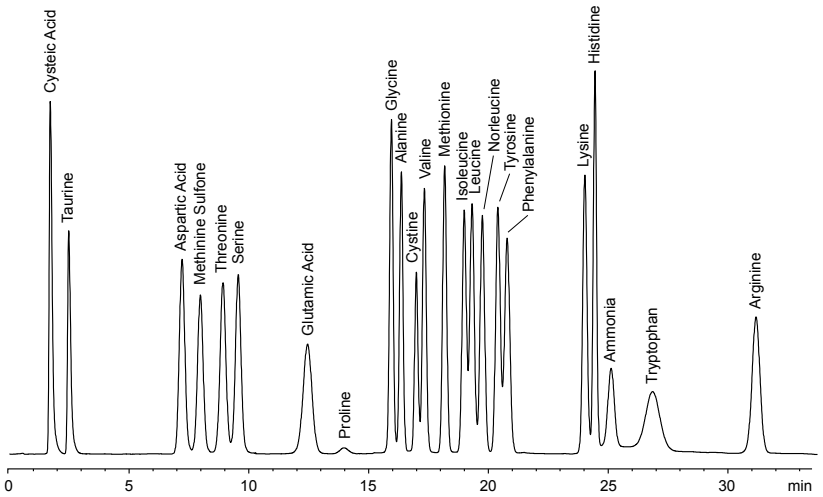


Fig 1. *Amino Acid standard. Eluants: Na270, Na425, Na640, RG011, column 1154110T, temperature gradient from 50 °C to 70 °C.*

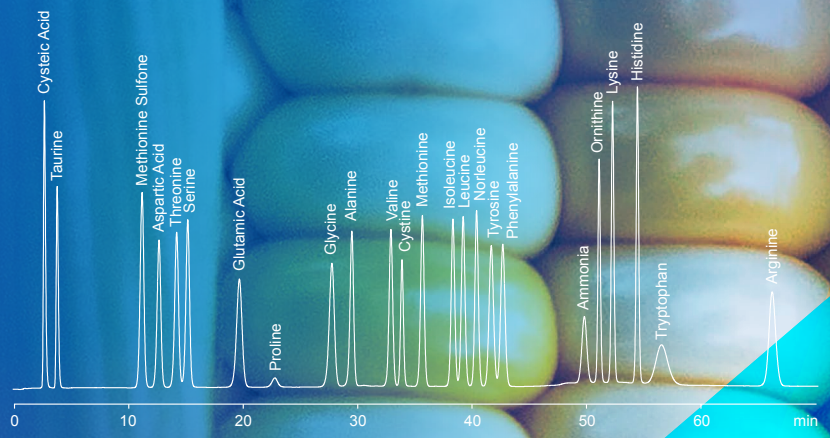


Fig 2. *Amino Acids Standard for oxidized and non-oxidized hydrolyzed samples. Eluants Na270, Na740, RG011, column 1154150T, temperature gradient from 5°C to 65 °C*

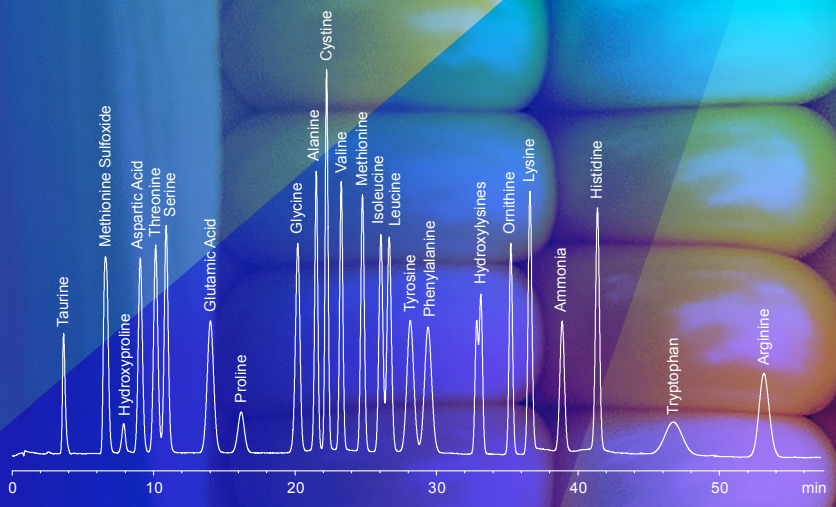


Fig 3. *Amino Acid standard, including Taurine and Ornithine. Eluants: Na315, Na740, RG011, column 1154150T, temperature 48 °C.*

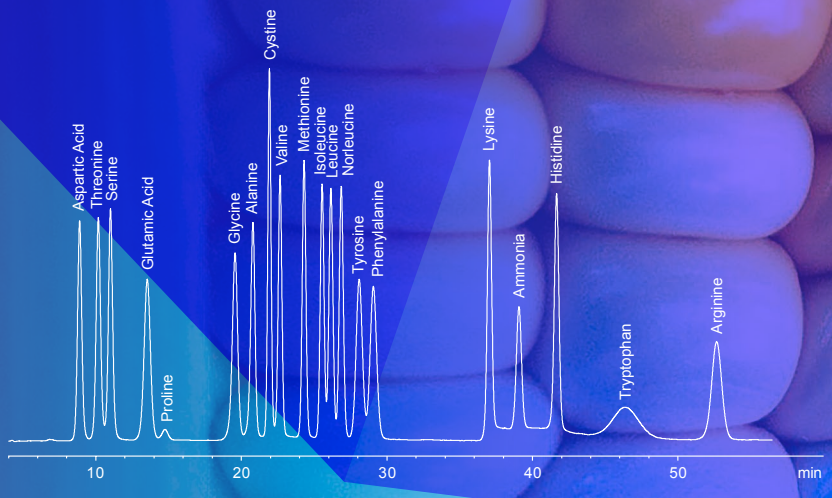


Fig 4. *Amino Acid standard, including Norleucine as Internal Standard. Eluants: 1700-0112, Na740, RG011, column 1154150, temperature 48 °C.*

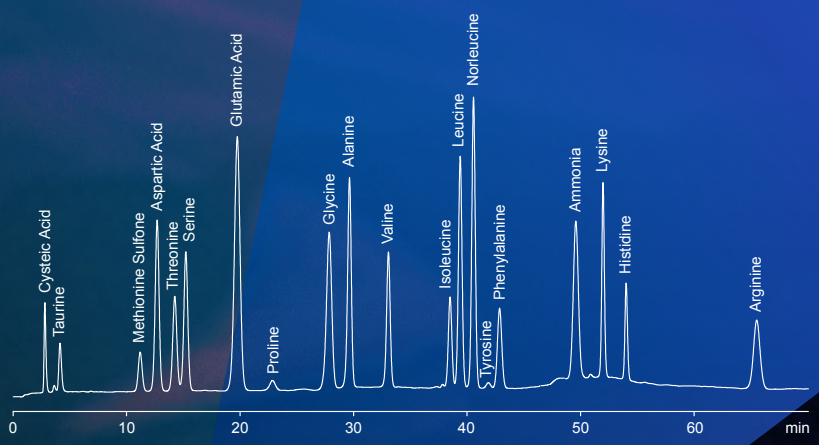


Fig 5. *A feed sample after oxidative hydrolysis. Eluants Na270, Na740, RG011, column 1154150T, temperature gradient from 55 °C to 65 °C.*

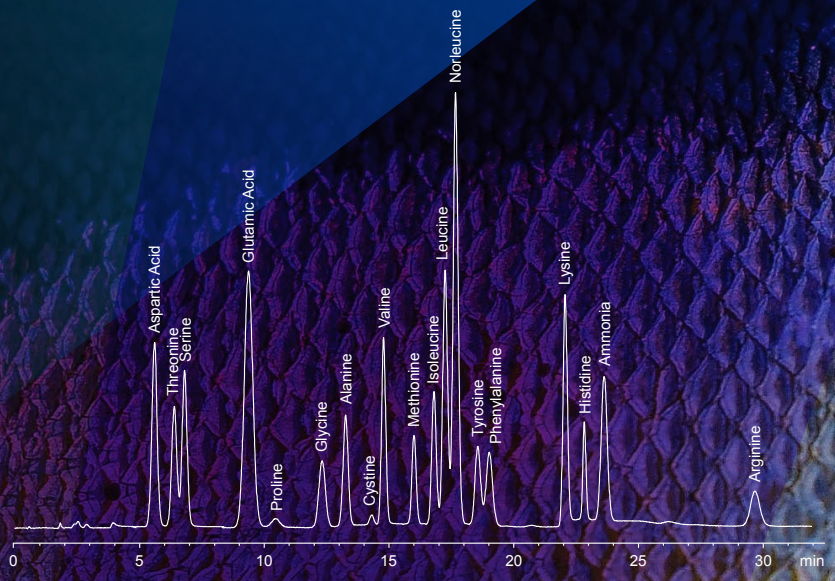


Fig 6. *A sample of hydrolyzed NIST formula. Eluants: Na315, Na425, Na640, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C.*

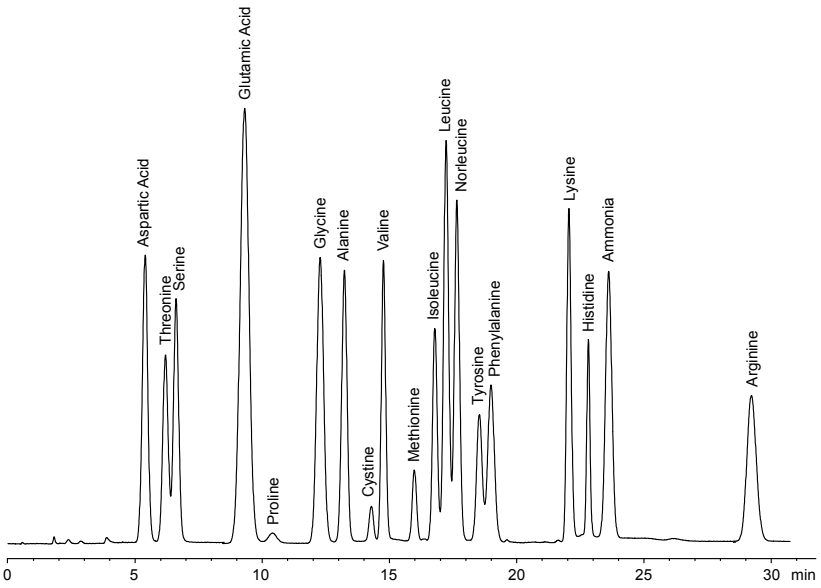


Fig 7. *A sample of hydrolyzed soy meal. Eluants: Na315, Na425, Na640, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C.*

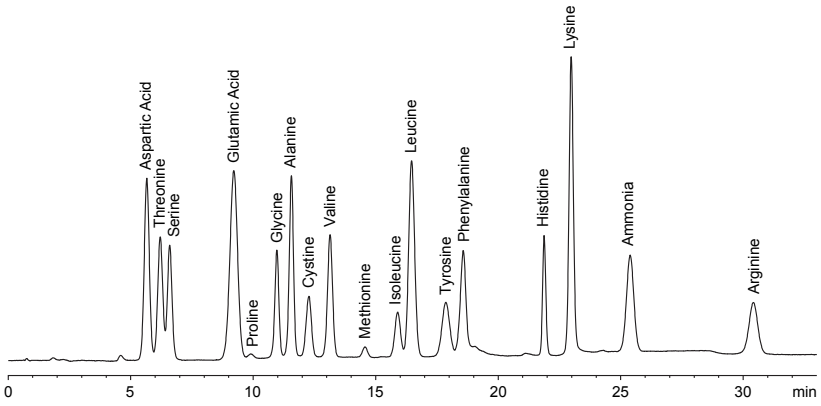


Fig 8. *A sample of hydrolyzed IL-16F monoclonal antibody. Eluants: Na315, Na425, Na640, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C.*

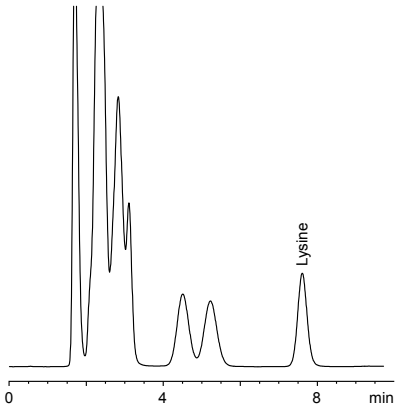


Fig 9. *Accelerated analysis of Lysine. Eluants: Na270 or Na315, Na740, RG011, column 1154110T, temperature 55 °C.*

Amino Acids According to European Pharmacopeia

A Complete Set of Solutions

The European Pharmacopoeia (Ph. Eur.) defines requirements for the qualitative and quantitative composition of medicines, as well as the tests to be carried out on both medicines and the substances and materials used in their production.

It covers active substances, excipients and preparations of chemical, animal, human or herbal origin, homoeopathic preparations and homoeopathic stocks, antibiotics, as well as dosage forms and containers. The European Pharmacopoeia and its requirements are legally binding in the member states of the European Pharmacopoeia Convention and the European Union, so all manufacturers of medicines or substances for pharmaceutical use therefore must apply the Ph. Eur. quality standards in order to market and use these products in Europe.

Ph. Eur. monographs have officially introduced the Amino Acid Analysis Method with Post-column Ninhydrin Derivatization as the analytical procedure required for the determination of the Ninhydrin-positive substances.

Pickering Laboratories offers a complete solution for amino acids analysis according to European Pharmacopoeia. This includes the Onyx PCX post-column derivatization instrument, analytical columns and GARDs, buffers and Trione® Ninhydrin reagent. The methods were optimized to comply with the system suitability requirements of Pharmacopoeia monographs.

For all amino acids, except Cysteine, both Sodium-based and Lithium-based methods are available. For Cysteine analysis, only Lithium-based methods are suitable. Sodium-based methods have shorter run times and are preferable for all amino acids except Cysteine. Examples of chromatograms obtained following European Pharmacopeia 8.0 requirements are shown Fig 10 – Fig 14.

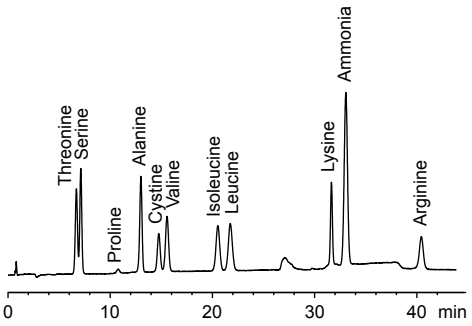


Fig 10. *Chromatogram of amino acids analyzed according to Pharmacopeia methods (3 ug/mL each). Eluants: Na315, Na425, Na640, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C*

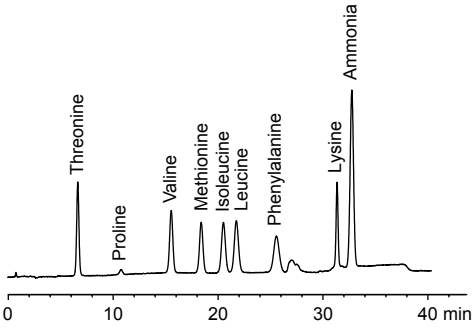


Fig 11. *Chromatogram of an alternative set of amino acids analyzed according to Pharmacopeia methods (3 ug/mL each). Eluants: Na315, Na425, Na640, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C*

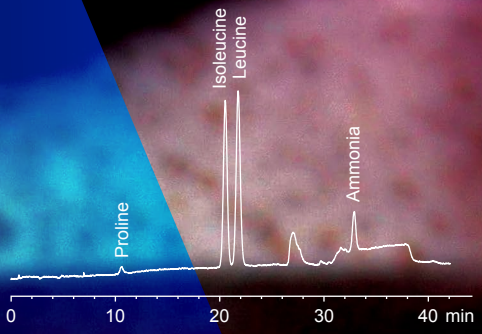


Fig 12. *System suitability check chromatogram for Pharmacopeia methods. Proline 1.2 ug/mL, Isoleucine 3 ug/mL, Leucine 3 ug/mL. Eluants: Na315, Na425, Na640, RG01T, column 1154110T, temperature gradient from 46 °C to 70 °C*

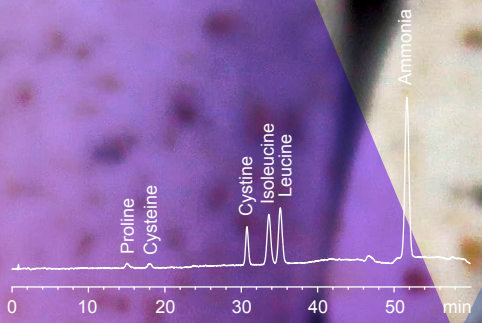


Fig 13. *Lithium Chromatogram of amino acids used a reference solution for Cysteine analysis according to Pharmacopeia (3 ug/mL each). Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

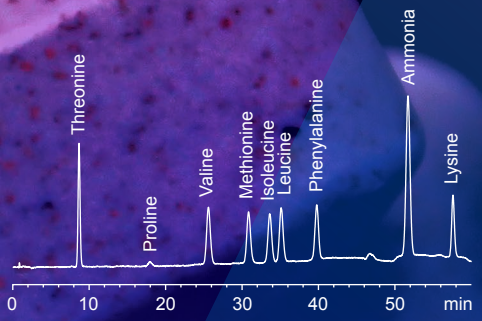


Fig 14. *Lithium Chromatogram of amino acids analyzed using Pharmacopeia methods (3 ug/mL each). Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

Amino Acid Analysis of Native Samples

Full Amino Acids Profiles and Accelerated Methods

Free amino acids present in physiological fluids are key indicators of health, nutritional status and metabolism of living organisms. Amino acids serve as effective markers for metabolic and cardiovascular diseases, different types of cancer, organ dysfunction and other health problems. Amino acid analysis is used in both diagnostics and treatment monitoring.

Other important matrices regularly analyzed for free amino acids include certain foods and drinks, plant extracts and cell culture media.

Pickering Laboratories offers methods for full amino acid profiles of native samples as well as accelerated screening methods for specific amino acids.

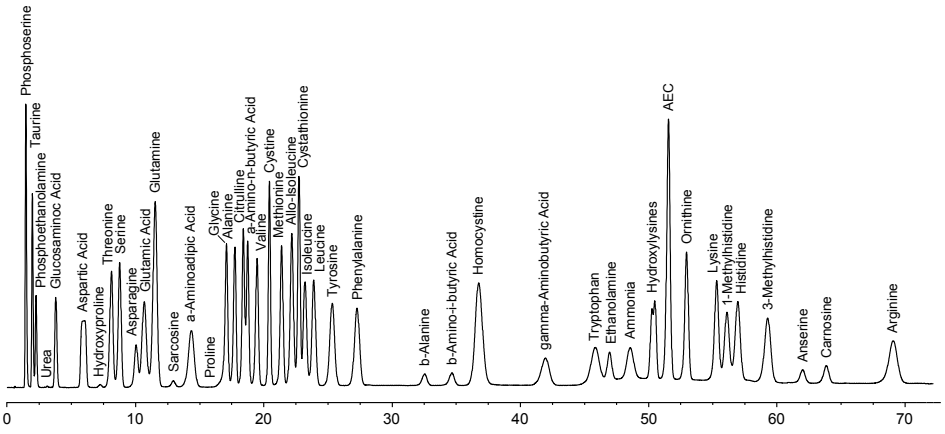


Fig 15. *Amino Acids standard for physiological fluids. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

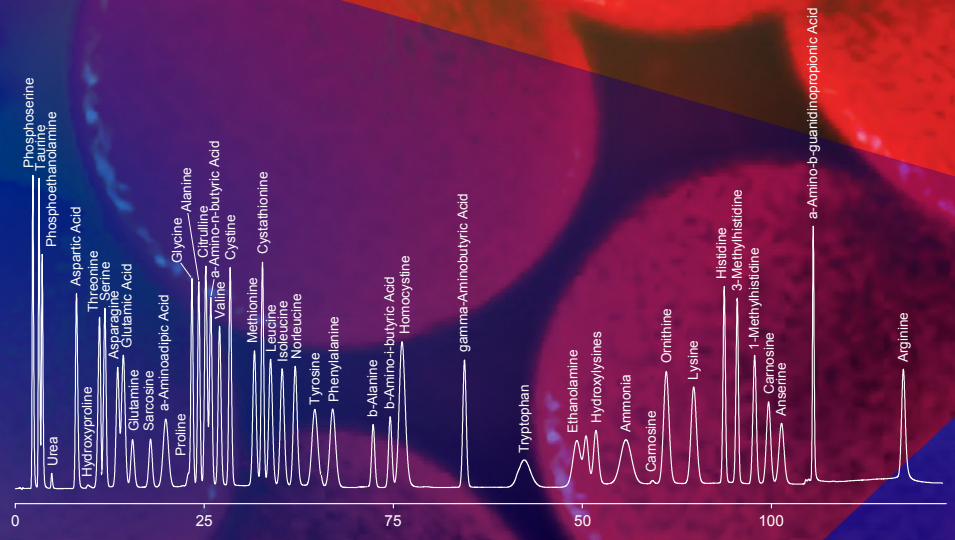


Fig 16. *Amino Acids standard for physiological fluids. Eluants: Li275, Li750, RG003, column 0354100T, temperature 37 °C*

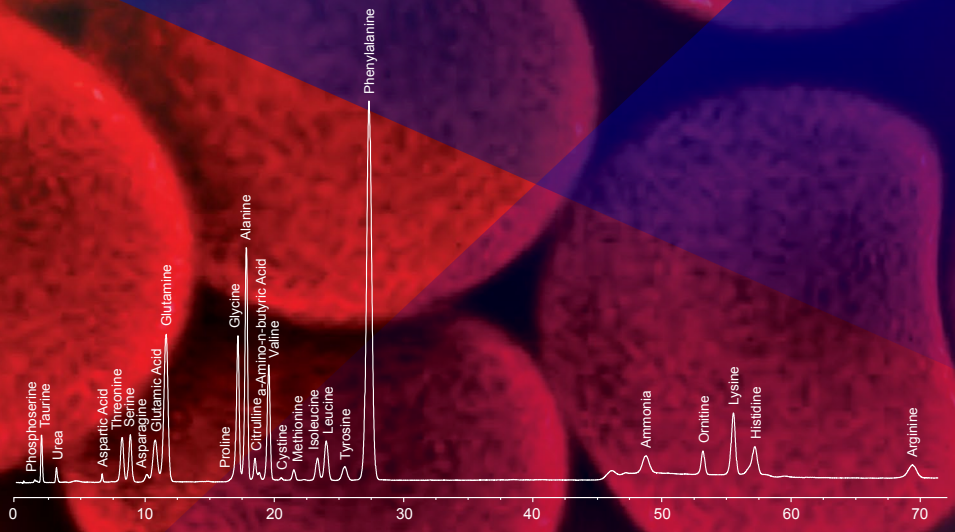


Fig 17. *A plasma sample of patient with PKU. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

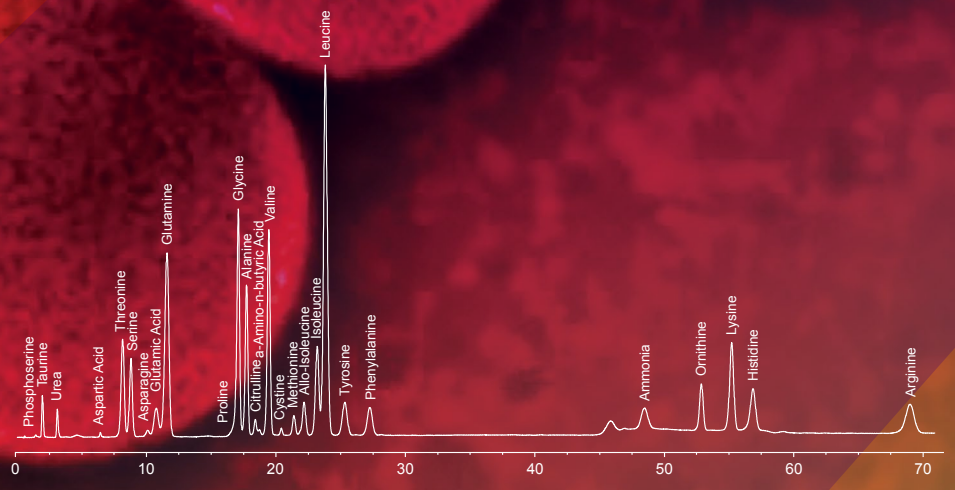


Fig 18. *A plasma sample of patient with MSUD. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

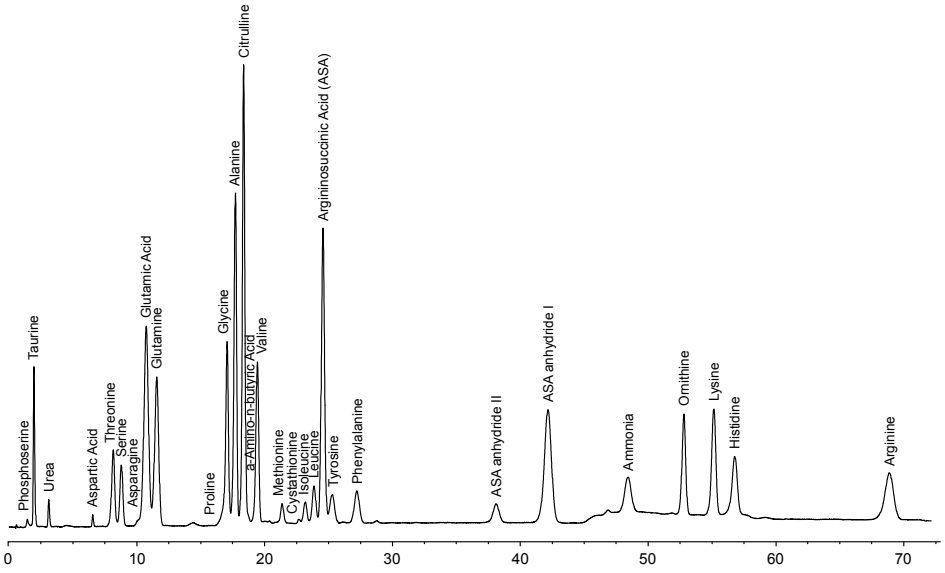


Fig 19. *A plasma sample of patient with Argininosuccinic aciduria (ASA). Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

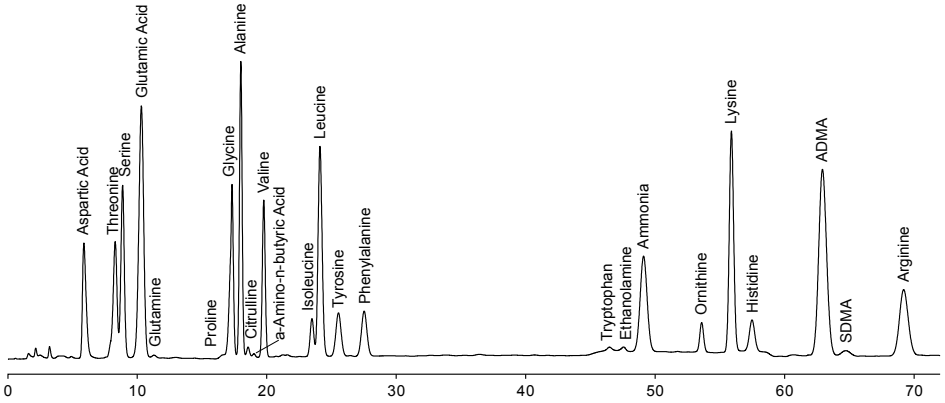


Fig 20. *A human serum sample spiked with sym-Dimethylarginine (SDMA) and asym-Dimethylarginine (ADMA). Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

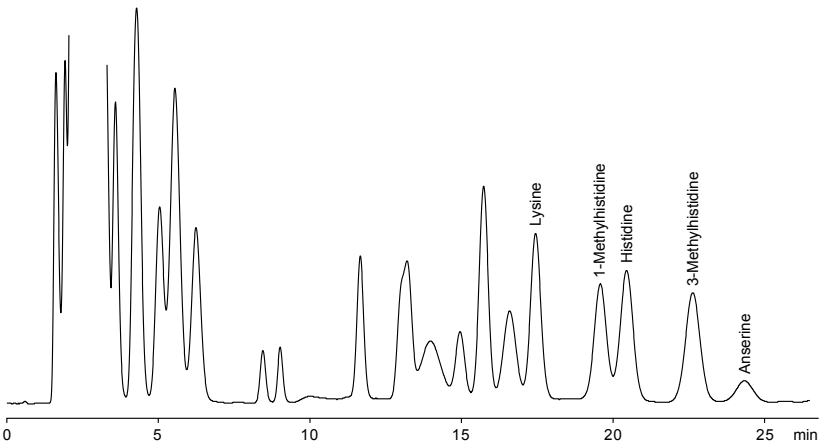


Fig 21. *Accelerated method for analysis of Methyl-histidines in physiological samples. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature 70 °C*

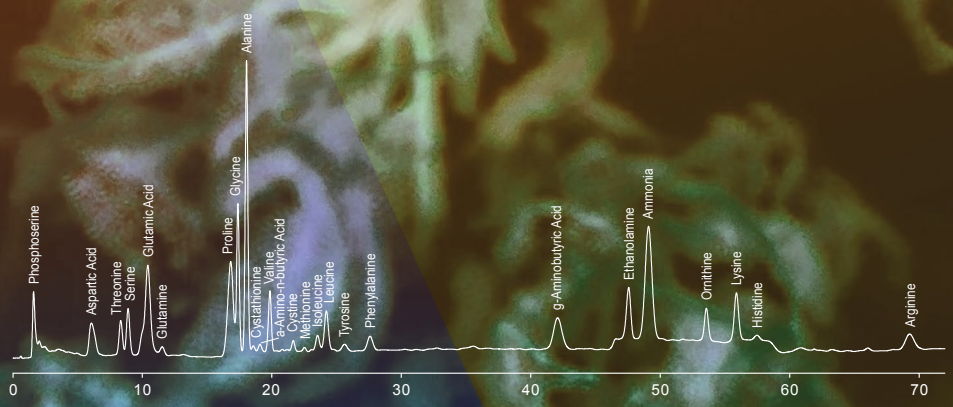


Fig 22. *A red wine sample. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

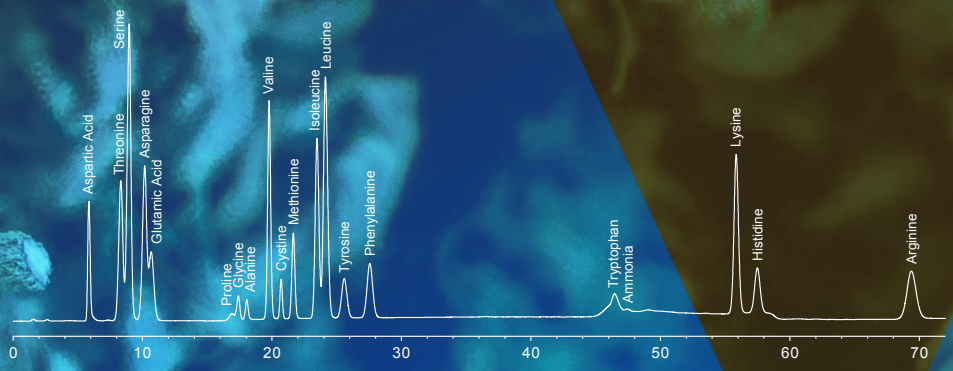


Fig 23. *A cell culture media sample. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C*

Calibration Standards for Amino Acid Analysis

Standard & Custom Solutions

As part of Pickering Laboratories' complete support for amino acid analysis, we offer a wide range of calibration standard solutions that are suitable for the analysis of native and hydrolyzed samples. Custom calibration solutions are also available.

Table 6. Calibration Standards for Amino Acid Analysis

Catalog Number	Product Description
O11006P	Native Sample Standard with Norleucine in Lithium Citrate Buffer, 0.25 µmole/mL, Pack (5x1 mL)
O12006P	Native Sample Standard without Norleucine in Lithium Citrate Buffer, 0.25 µmole/mL, Pack (5x1 mL)
O12506C	Collagen Hydrolysate Standard in Sodium Citrate Buffer, 0.25 µmole/mL, Proline and Hydroxyproline 1.25 µmole/mL, Pack (5x1 mL)
O12506H	Protein Hydrolysate Standard, in Sodium Citrate Buffer, 0.25 µmole/mL, Pack (5x1 mL)
1700-0155	Oxidized Feed Hydrolysate Standard in Sodium Citrate Buffer, 0.25 µmole/mL, Pack (5x1 mL)
1700-0170	Native Sample Standard Without Norleucine & Alpha-Amino-Beta-Guanidinopropionic Acid in Lithium Citrate Buffer, 0.25 µmole/mL, Pack (5x1 mL)
1700-0175	Native Sample Standard, Basics, in 0.1 N HCl, 2.5 µmole/mL, Pack (5x1 mL)
1700-0180	Native Sample Standard, Acidics and Neutrals, in 0.1 N HCl, 2.5 µmole/mL, Cystine 1.25 µmole/mL, Pack (5x1 mL)
1700-0165	Sodium Amino Acid Standard with Norleucine, 0.25 µmole/mL, Pack (5x1 mL)

Sample Preparation

Saving Time and Money

Sample preparation is a very important step of amino acid analysis. Improperly done, it will adversely affect the results and shorten column lifetime. The choice of procedure depends on the sample type and on the amino acids of interest.

Proteins and peptides must be hydrolyzed prior to analysis. This hydrolysis step can be the most challenging and time-consuming part of the whole process. Acidic hydrolysis with HCl is the most popular technique, but basic and enzymatic hydrolysis are also used. Some amino acids can be destroyed or converted to a different form during the hydrolysis step, so the procedure should be carefully considered. AOAC Official Methods 994.12 and 988.15 describe hydrolysis protocols suitable for analyzing a variety of matrices.

It is recommended that after HCl hydrolysis the acid be removed and the residual amino acids be reconstituted in Na220L diluent to control pH and normality. Incorrect pH of the sample causes poor separation and can shift retention times, making it difficult to identify and quantify the compounds of interest. Hydrolyzed samples are analyzed using a Sodium-based column and buffers.

Native samples, such as physiological fluids, plant extracts, foods and beverages, contain free amino acids so hydrolysis is not required. But it is necessary to 1) remove proteins to avoid fouling the cation-exchange column and 2) adjust the pH and normality of the sample to ensure reproducibility during the early part of the chromatogram.

Pickering Laboratories' Seraprep™ and Uriprep™ replace commonly used protein precipitation reagents such as Acetonitrile, Perchloric acid and Picric acid, thereby eliminating the need for dialysis, ultrafiltration and pH adjustment. Just mix equal parts of sample with the sample preparation solution, centrifuge, filter and inject.

Table 7. Reagents and Diluents for Sample Preparation of Amino Acids	
Product	**Suggested Use**
Seraprep™	Used for preparation of serum and other samples with high buffering capacity, for example sardine oil
Uriprep™	Used for preparation of urine and samples with low buffering capacity such as beers, wines and fruit juices
Li220	Used for diluting samples and standards for analysis with Lithium columns and buffers
Na220L	Used for diluting samples and standards for analysis with Sodium columns and buffers

Amino Acid Analysis Kits

It's Everything You'll Need

For customers initially setting up a method, Pickering Laboratories offers application-specific chemistry kits. A chemistry kit has everything you need to start running the analysis – columns, eluants, reagents and standards. For each application, multiple kits with different choices of reagents are available (Table 8).

All the kit components can be purchased separately as required.

Table 8. Reagents and Diluents for Sample Preparation of Amino Acids				
Description	**Analytical Column**	**Kit with T100C**	**Kit with T200**	**Kit with OPA**
Sodium Amino Acid Analysis				
30-min high-efficiency protein hydrolysate kit	1154110T	0352-0057	0352-0058	0352-0059
30-min high-efficiency collagen hydrolysate kit	1154110T	0352-0061	0352-0062	0352-0063
30-min high-efficiency oxidized feed hydrolysate kit	1154110T	0352-0020	0352-0021	0352-0022
55-min high-efficiency protein hydrolysate kit	1154150T	AT31FH	0352-0031	AO31FH
55-min high-efficiency collagen hydrolysate kit	1154150T	AT32FC	0352-0032	AO32FC
60-min oxidized feed hydrolysate kit	1154150T	0352-0018	0352-0017	0352-0019
60-min standard protein hydrolysate kit	1193250	AT30SH	0352-0030	AO30SH
Lithium Amino Acid Analysis				
70-min high-efficiency physiologic fluids/native samples kit	0354675T	0352-0006	0352-0007	0352-0008
120-min high-efficiency physiologic fluids/native samples kit	0354100T	0352-0015	0352-0011	0352-0012
90-min temperature gradient physiologic fluids/native samples kit	0354100T	0352-0013	0352-0014	0352-0016
185-min standard physiologic fluids/native samples kit	0393250	AT33SP	0352-0033	AO33SP

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