

PINNACLE PCX  
BUFFERS  
REAGENTS  
COLUMNS  
CHROMATOGRAMS

**AMINO**  
ACIDS

AMINO ACID ANALYSIS PRODUCTS

**PICKERING**  
LABORATORIES



COMPLETE SOLUTIONS FOR ACCURATE

# AMINO ACID ANALYSIS

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 and pharmaceuticals. It offers sensitive and reliable results that have been trusted by chemists for over 50 years.

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 pre-column derivatization, 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 area and retention times. Very often the 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 the separation or derivatization of amino acids. The same method can be used for a variety of samples, from plant extract to serum or spinal fluid.

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 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.



**PINNACLE PCX**

The Pinnacle PCX is a state of the art derivatization system that combines unique features that are most important for successful Amino Acid Analysis. It reflects the ease of use, reliability and ruggedness that you have come to expect from Pickering Laboratories.

**SYSTEM DESIGN ADVANCEMENTS RESULT IN OPTIMIZED ANALYSIS**

- 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 the reagent and amino acid derivatives from oxidation. It also saves the instrument from the effects of corrosion, thereby reducing maintenance and extending system life.
- The Column oven utilizes circulating air for consistency of heating and quick cooling.

- Column oven temperature gradient programming improves separation and also lowers analysis times. The Pinnacle PCX is the only post-column system with this feature that can interface with any modern HPLC.
- Electronic valves eliminate troublesome check valves and allow automated pump flushing.
- The quick-change reactor cartridge makes replacement quick and easy.
- Up-front fluidics for easy maintenance.
- The PCX Control Software allows for precise control of the reagent flow rate and allows for conservation of reagent during the necessary column re-equilibration.

**POST-COLUMN REAGENTS FOR AMINO ACID ANALYSIS**

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**

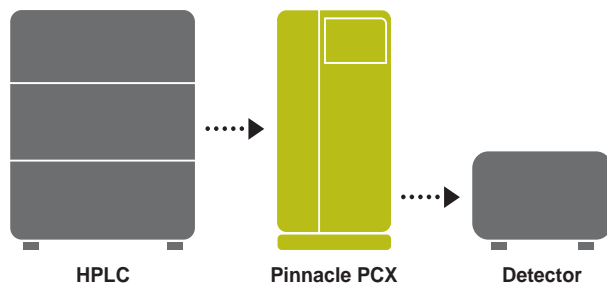
	<b>TRIONE® REAGENT</b>	<b>OPA REAGENT</b>
Products	<p><b>T100</b> – ready to use reagent; 3-month shelf life from the date of manufacture; each (950 mL/bottle)</p> <p><b>T100C</b> – ready to use reagent; 3-month shelf life from the date of manufacture; case of 4 (950 mL/bottle)</p> <p><b>T200</b> – 2-part reagent, combine and use, 12-month shelf life from the date of manufacture; to prepare case of 4 (900mL/bottle)</p>	<p><b>OD104</b> – <i>o</i>-Phthalaldehyde diluent for Amino Acid analysis; case of 4 (950 mL/bottle)</p> <p><b>O120</b> - <i>o</i>-Phthalaldehyde (OPA) crystals; Each (5g/bottle, makes 16x950 mL of reagent)</p> <p><b>3700-2000</b> - Thiofluor™; Each (10g/bottle, makes 5x950 mL of reagent)</p> <p>All three products are necessary to prepare OPA reagent for amino acid analysis</p>
Analytes	Primary and secondary amino acids	<p>Primary amino acids.</p> <p>To detect secondary amino acids, an oxidation step is required prior to reaction with OPA. Sensitivity of detection of primary amino acids decreases when oxidation step is used.*</p>
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
Pinnacle PCX Configuration	Single-pump Pinnacle PCX with 0.5 mL reactor	<p>Single-pump Pinnacle PCX with 0.15 mL reactor.</p> <p>* Two-pump Pinnacle 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.</p>



### STANDARD CONFIGURATION

The Pinnacle PCX for Amino Acid Analysis will connect with any HPLC and detector. Pickering Laboratories offers everything you need for a variety of Amino Acid Analysis methods:

- ➔ COLUMNS
- ➔ ELUANTS
- ➔ AMINO ACID STANDARDS
- ➔ METHODS AND SUPPORT



*Standard configuration between any HPLC and detector*

### COLUMNS AND ELUANTS

We manufacture a wide range of cation-exchange columns and eluants to suit your analytical needs. Sodium cation-exchange chromatography is used for fast analysis of amino acids commonly found in hydrolyzed protein samples or simple formulations. Lithium cation-exchange is a slower technique with higher resolution 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 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 (3.0 x 250 mm), despite having the longest run times, can be useful due to unique selectivity that 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 fouling the analytical column. Pickering Laboratories offers conventional guard columns that use the same material as analytical columns and must be matched to analytical column type, as well as a novel GARD™ column protection system that can be used with any cation-exchange column for amino acid analysis.

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

**TABLE 2. SODIUM AMINO ACID ANALYSIS**

ANALYTICAL COLUMN	GUARD COLUMN	SAMPLES/ELUANTS	RUN TIME
1154110T (4.0 x 110 mm)	GARD™ column protection system	For protein and collagen hydrolysates with temperature gradient: Na315, Na740, RG011  For oxidized feed hydrolysates with temperature gradient: Na270, Na740, RG011  Internal Standard: Norleucine	30 min
1154150T (4.0 x 150 mm)	GARD™ column protection system or 1193020 (3.0x20 mm) guard column	For protein and collagen hydrolysates with constant column temperature: Na315, Na740, RG011  For oxidized feeds samples with constant column temperature: Na270, NA740, RG011  Internal Standard: Norleucine	55 min  60 min
1193250 (3.0 x 250mm)	GARD™ column protection system or 1192020 (2.0x20 mm) guard column	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/EUANTS	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	120 min
	or 0352020 (2.0x20 mm) guard column	For physiological samples with temperature gradient: 1700-1125, Li365, Li375, RG003 Internal Standards: Glucosaminic Acid	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
	or 0392020 (2.0x20 mm) guard column		



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**

Determining amino acid composition is necessary to ensure the proper nutritional value of food and feedstuffs. It is especially important 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 industry as a crucial part of quality control of the raw material and final products, monitoring the production process and nutritional research, and determining the market value of the feedstuffs.

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

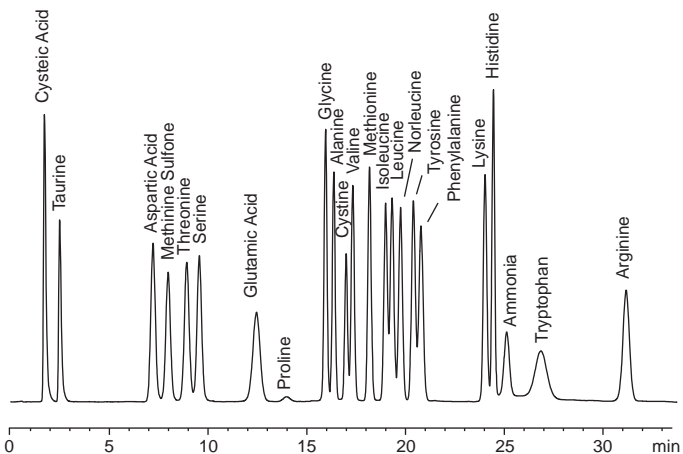
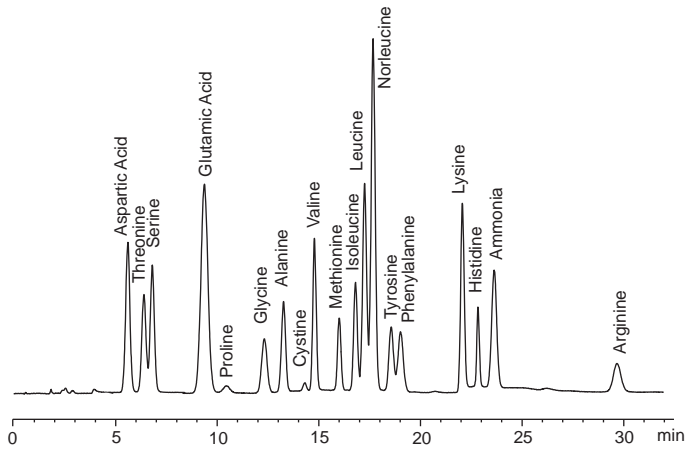
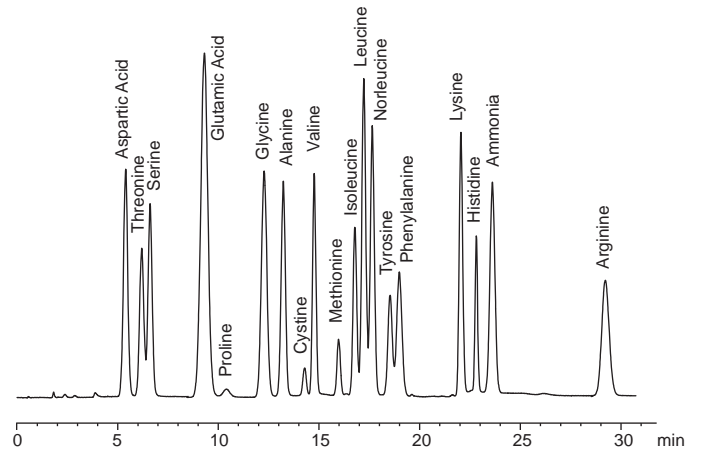


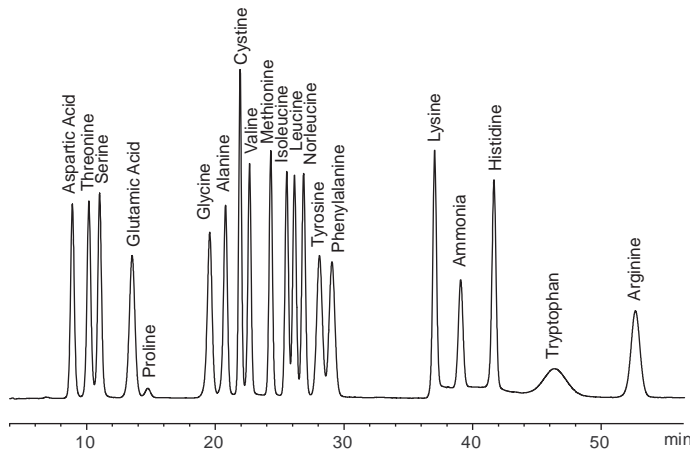
Fig 1. Amino Acid standard. Eluants: Na270, Na740, RG011, column 1154110T, temperature gradient from 50 °C to 70 °C.



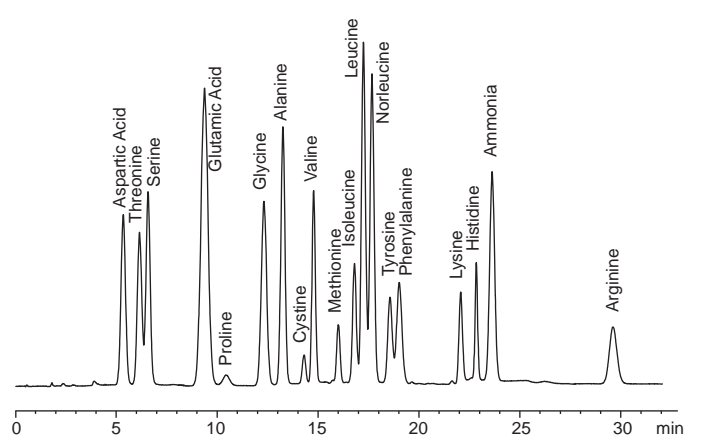
**Fig 2. A sample of hydrolyzed NIST formula. Eluants: Na315, Na740, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C**



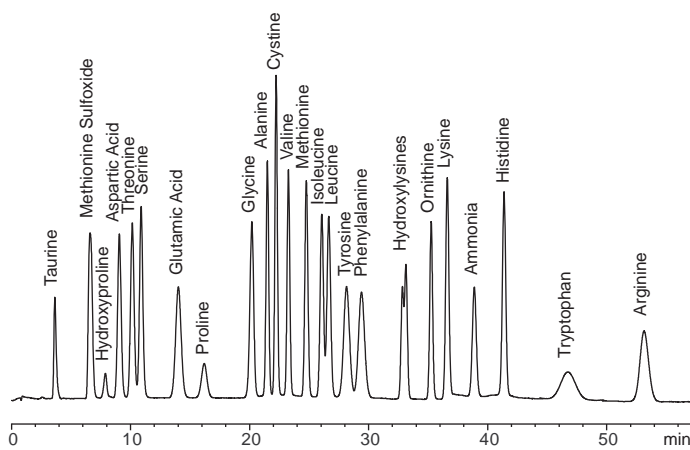
**Fig 5. A sample of hydrolyzed soy meal. Eluants: Na315, Na740, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C**



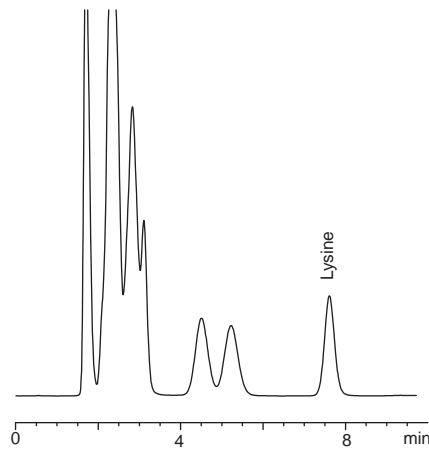
**Fig 3. Amino Acid standard, including Norleucine as Internal Standard. Eluants: 1700-0112, Na740, RG011, column 1154150, temperature 48 °C.**



**Fig 6. A sample of hydrolyzed dry distillers grain (DDG). Eluants: Na315, Na740, RG011, column 1154110T, temperature gradient from 46 °C to 70 °C**



**Fig 4. Amino Acid standard, including Taurine and Ornithine. Eluants: Na315, Na740, RG011, column 1154150T, temperature 48 °C.**



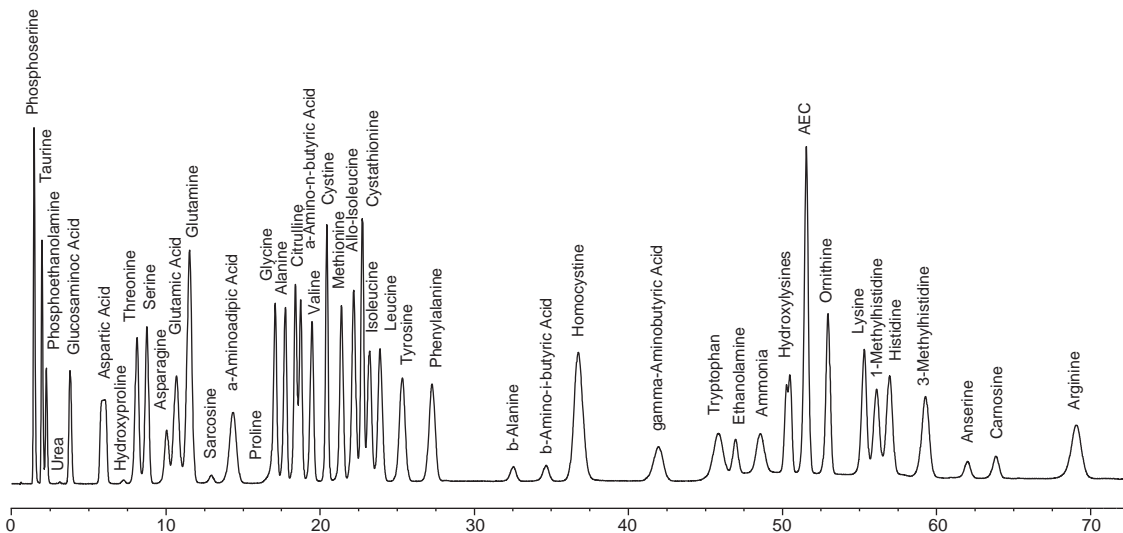
**Fig 7. Accelerated analysis of Lysine. Eluants: Na270 or Na315, Na740, RG011, column 1154110T, temperature 55 °C.**

**AMINO ACID ANALYSIS OF NATIVE SAMPLES**

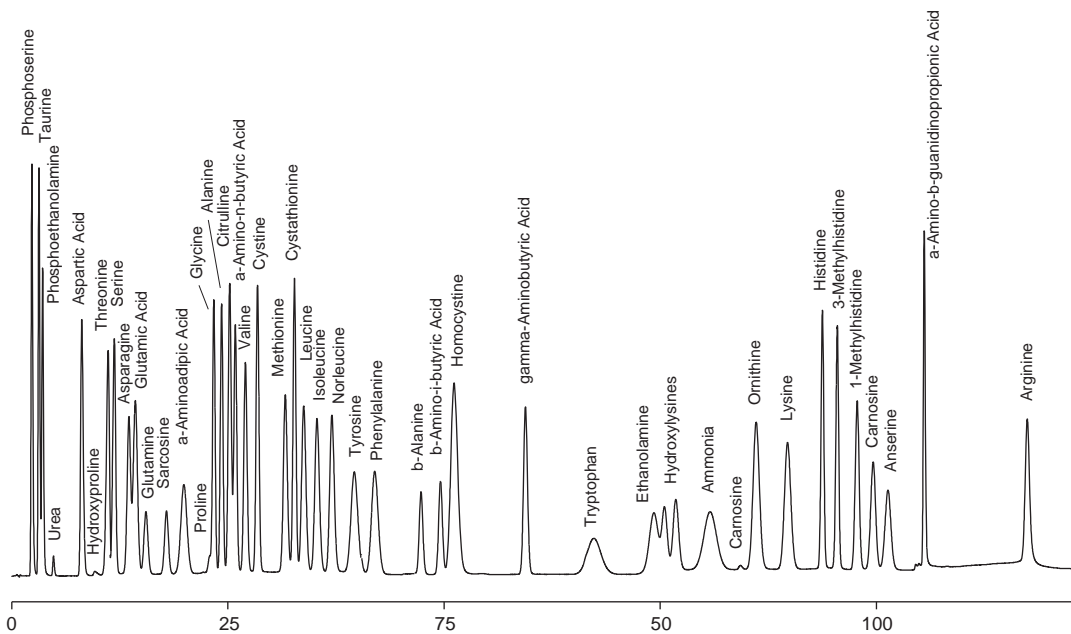
Free amino acids present in physiological fluids are key indicators of health, nutritional status and metabolism of living organisms. Amino acids serve as effective markers for metabolic and cardiovascular diseases, different types of cancer, organ dysfunction and other health problems. Amino Acid Analysis 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.

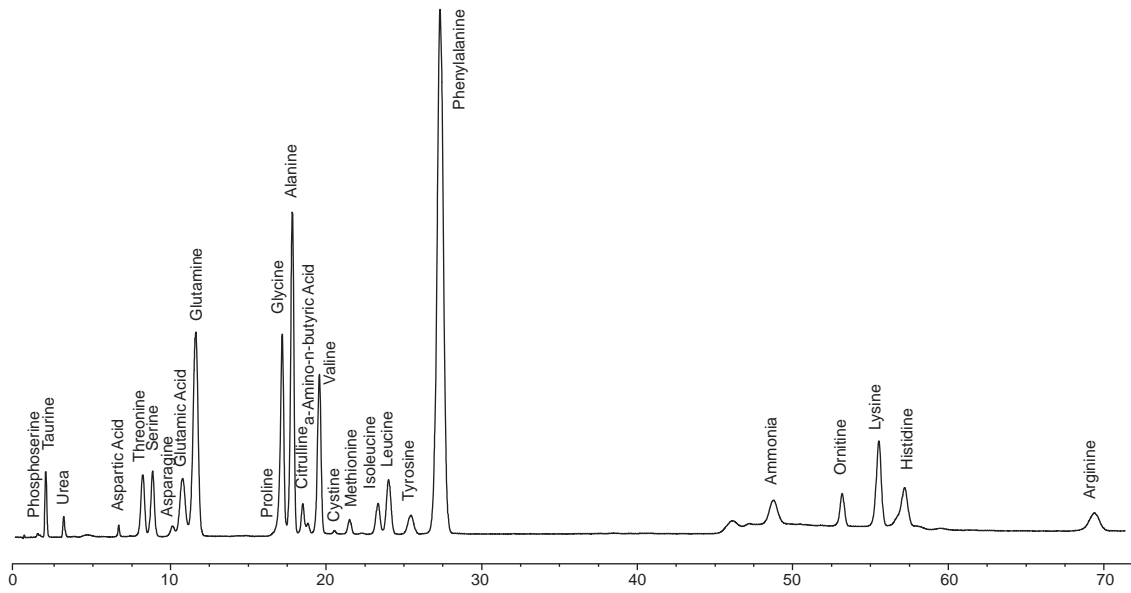
Pickering Laboratories offers methods for full amino acid profiles of native samples as well as accelerated screening methods for specific amino acids.



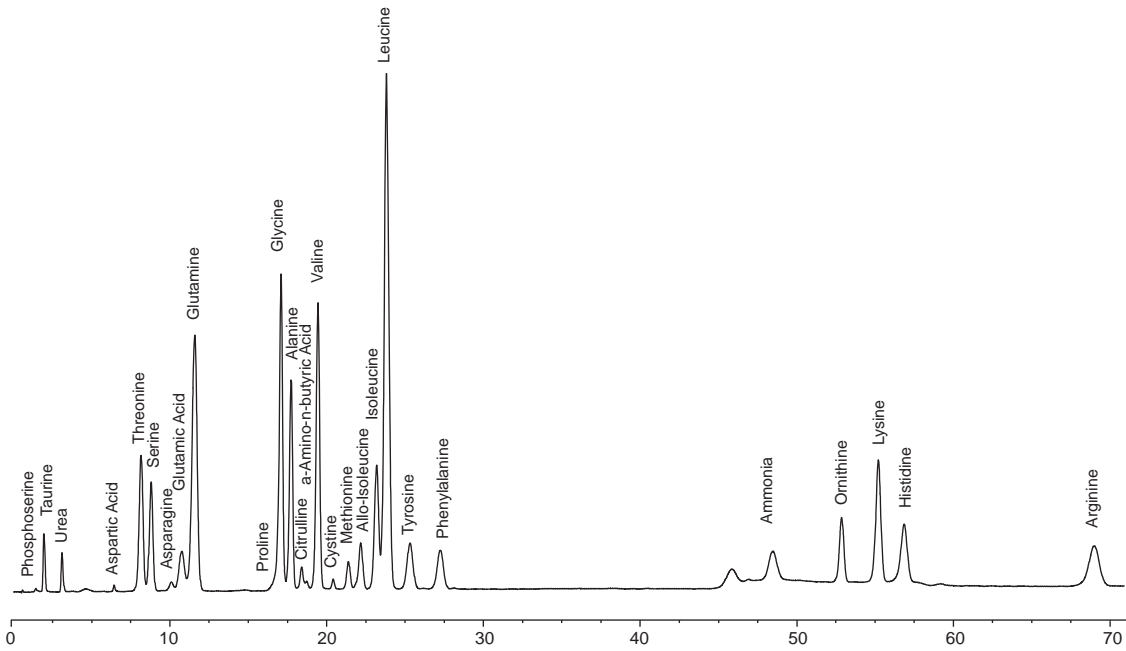
**Fig 8. Amino Acids standard for physiological fluids. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C**



**Fig 9. Amino Acids standard for physiological fluids. Eluants: Li275, Li750, RG003, column 0354100T, temperature 37 °C**

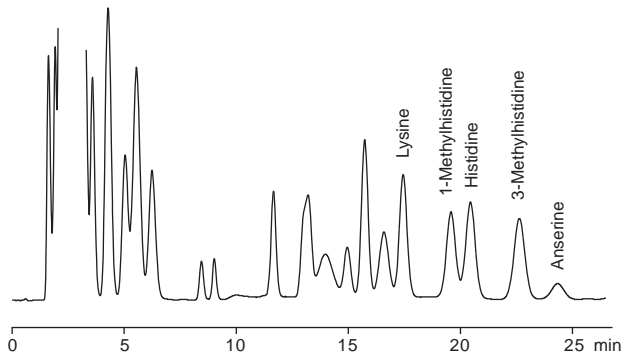


**Fig 10.** A plasma sample of patient with PKU. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C

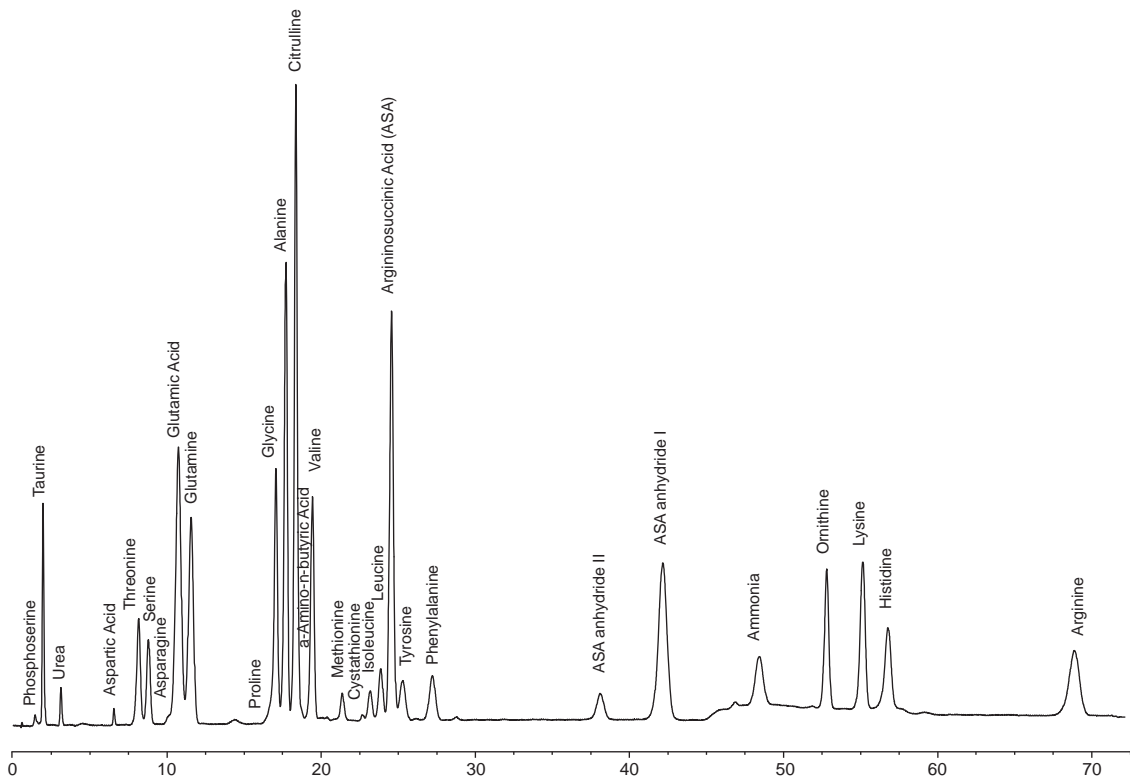


**Fig 11.** A plasma sample of patient with MSUD. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C

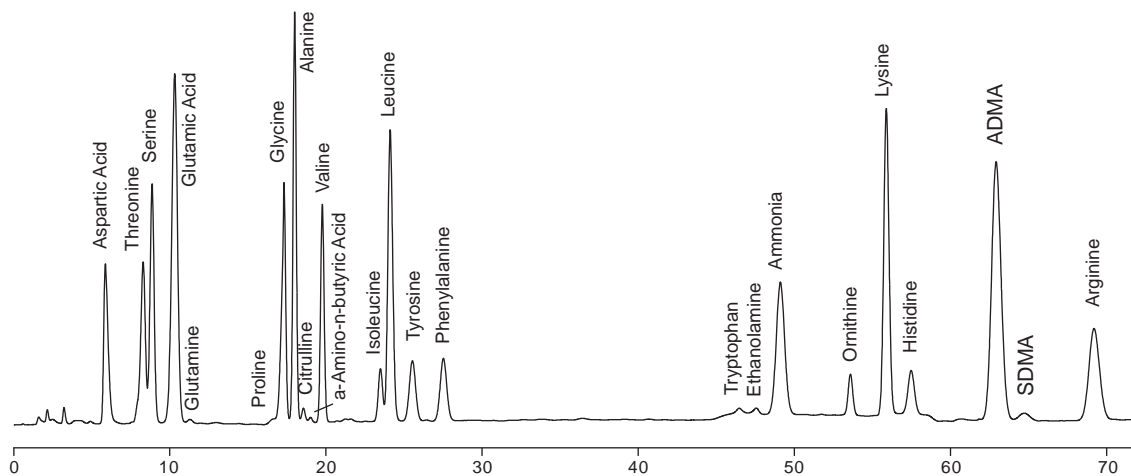




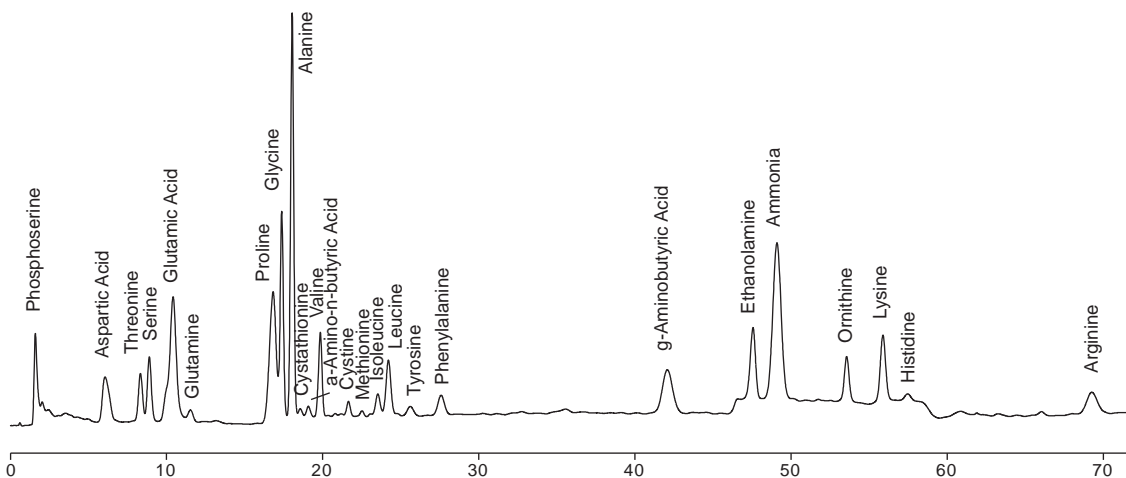
**Fig 12. Accelerated method for analysis of Methylhistidines in physiological samples. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature 70 °C**



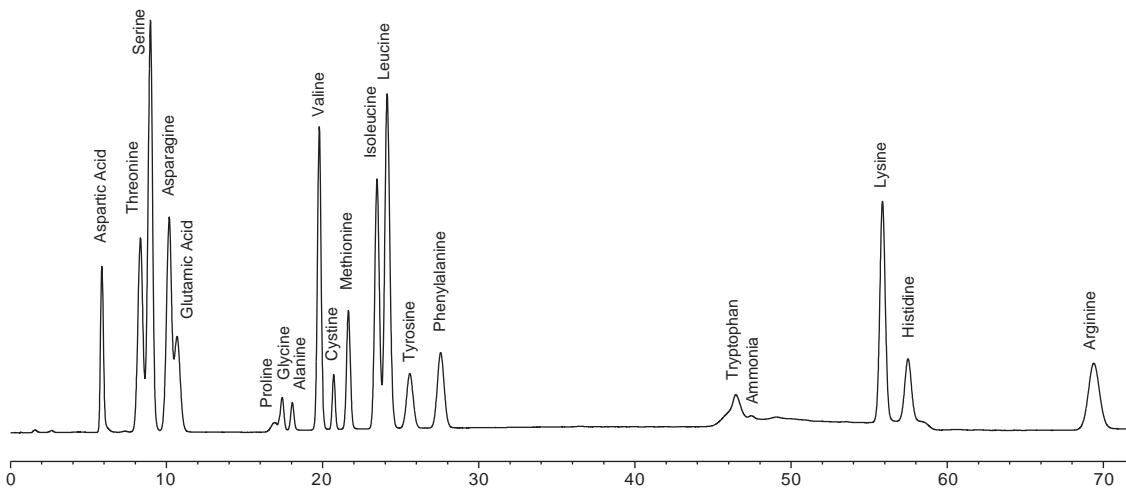
**Fig 13. A plasma sample of patient with Argininosuccinic aciduria (ASA). Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C**



**Fig 14. 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 15. A red wine sample. Eluants: 1700-1125, Li365, Li375, RG003, column 0354675T, temperature gradient from 34 °C to 70 °C**



**Fig 16. 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

As part of Pickering Laboratories' complete support for Amino Acid Analysis we offer a wide range of standard solutions suitable for analysis of native and hydrolyzed samples. Custom calibration solutions are also available.

TABLE 6. CALIBRATION STANDARDS FOR AMINO ACID ANALYSIS

CATALOG NUMBER	PRODUCT DESCRIPTION
011006P	Native Sample Standard with Norleucine and $\alpha$ -Amino- $\beta$ -guanidinopropionic acid, in Lithium citrate buffer, 5 mL
012006P	Native Sample Standard with $\alpha$ -Amino- $\beta$ -guanidinopropionic acid, in Lithium citrate buffer, 5 mL
012506C	Collagen Hydrolysate Standard, in Sodium citrate buffer, 5 mL
012506H	Protein Hydrolysate Standard, in Sodium citrate buffer, 5 mL
1700-0155	Oxidized Feed Hydrolysate Standard in Sodium citrate buffer, 5 mL
1700-0170	Native Sample Standard in lithium citrate buffer, 5 mL
1700-0175	Native Sample Standard, basics, in 0.1 N HCl, 5 mL
1700-0180	Native Sample Standard, acidics and neutrals, in 0.1 HCl, 5 mL

## SAMPLE PREPARATION

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 into 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 Na220 diluent in order 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 column and buffers.

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

Pickering Laboratories' SERAPREP™ and URIPREP™ replace commonly used protein precipitation reagents such as home-made solutions of Sulfosalicylic acid, Acetonitrile, Perchloric acid and Picric acid, and eliminate 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

CATALOG NUMBER	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
Na220	Used for diluting samples and standards for analysis with Sodium columns and buffers

## AMINO ACID ANALYSIS KITS

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 components of the kit can be purchased separately as well. Please refer to our catalog for individual components.

TABLE 8. REAGENTS AND DILUENTS FOR SAMPLE PREPARATION OF AMINO ACIDS

DESCRIPTION	ANALYTICAL COLUMN	KIT WITH T100	KIT WITH T200	KIT WITH OPA
<b>SODIUM AMINO ACID ANALYSIS</b>				
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
<b>LITHIUM AMINO ACID ANALYSIS</b>				
70-min high-efficiency physiologic fluids/native samples kit	0354675T	0352-0006	0352-0007	0352-0008
90-min temperature gradient physiologic fluids/native samples kit	0354100T	0352-0013	0352-0014	0352-0016
120-min high-efficiency physiologic fluids/native samples kit	0354100T	0352-0015	0352-0011	0352-0012
185-min standard physiologic fluids/native samples kit	0393250	AT33SP	0352-0033	AO33SP



CATALYST FOR SUCCESS

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