As of: December 2011

AMINO ACIDS

The method for the analysis of amino acids by ion-exchange column and post-column derivatization was originally published in 1948. Its authors, Moore and Stein, were awarded the 1972 Nobel Prize in Chemistry.

Sixty years later, the analysis of a physiologic sample no longer takes two days and the resolution of the columns has been significantly improved. The method principles, however, remain the same.
One reason for this is the extraordinary insensitivity of the retention mechanism of the ion-exchange column against matrix effects, leading to a high reproducibility of retention times and quantification.

For amino acid analysis, Pickering offers the **PINNACLE PCX** and complete **Amino Acid Kits** containing eluents, reagents, diluents, columns and standards.

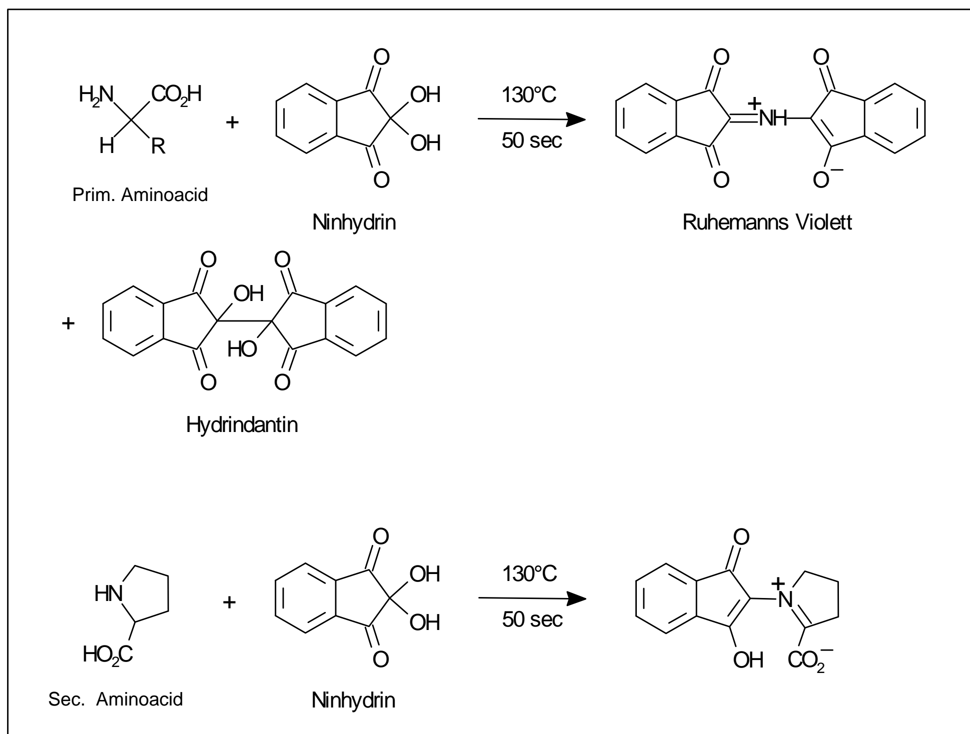
Two different types of kits are obtainable: **Sodium Kits** contain sodium citrate buffers and a sodium ion-exchange for the analysis of hydrolysates of peptides, proteins, collagens, foods and feeds.
Lithium Kits contain lithium citrate buffers and a lithium ion-exchange for the amino acid analysis of native samples. These are, for example, physiologic fluids (blood, urine) and cell tissues, but also food and drinks.

Derivatization is performed in one of two ways: either with TRIONE® (ninhydrin) or with *o*-phthalaldehyde (OPA) and Thiofluor® (2-mercaptoethanol derivative).

Derivatization of Primary and Secondary Amino Acids with Ninhydrin

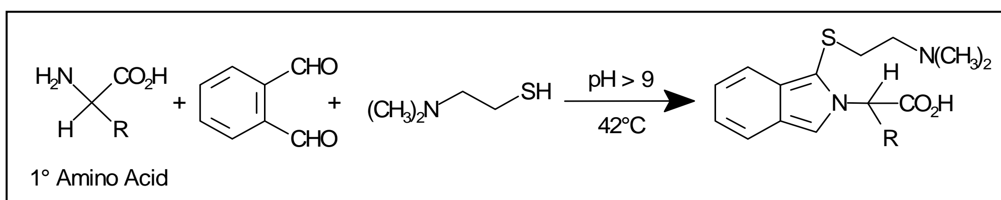
The most common reagent used for post-column derivatization of amino acids is Ninhydrin. Ninhydrin combined with primary amines generates a colored compound (Ruhemann's Purple; λ_{max}= 570 nm; ε= 20000). Hydrindantin serves as catalyst for the reaction, which is formed by reductive coupling from two molecules of ninhydrin.

A secondary amine reacts with Ninhydrin, even without the presence of hydrindantin, to form a yellow compound (λ_{max}= 440 nm).
The detection limit (570 nm) for primary amino acids is approximately 30 pmol.
Is only a 1-channel-UV/VIS-detector available, both derivatives could also be detected with a slightly lower sensitivity at 500 nm.



Derivatization of Primary Amino Acids with o-Phthalaldehyde/Thiofluor®

Primary amines can be converted with *o*-phthalaldehyde (OPA) and Thiofluor (2-mercaptoethanol derivative) in an alkaline environment (pH 9-10) to a fluorescent isoindole derivative. The detection limit of with OPA derivatized amino acids is about 5 pmol.



HPLC Conditions and Derivatization Parameters

Derivatization with Ninhydrin (TRIONE®)

HPLC	
Operational Mode	quarternary gradient
Eluant	Lithium or sodium buffer
Degassing	Helium or vacuum degassed
HPLC Column	Lithium or sodium ion-exchange columns with GARD
Flow Rate	0.3 – 0.6 ml/min (depends on the method)
Injection Volume	Up to 100 µL
Post-Column Derivatization	
Pinnacle PCX	Single-pump
Column Oven	34 – 70 °C (gradient depends on the method)
Reactor Volume	500 µL
Reactor Temperature	130 °C
Reagent	TRIONE® (Ninhydrin/Hydrindantin)
Reagent Flow	0.3 – 0.55 mL/min (depends on the method)
Detection	
Measurement	UV/VIS-Detector
UV/VIS	570 nm; primary amino acids
UV/VIS	440 nm; secondary amino acids
Flowcell	Analytical; pressure stable up to 7 bar

Derivatization with o-Phthalaldehyde/Thiofluor®

HPLC	
Operational mode	quarternary gradient
Eluent	Lithium or sodium buffer
Degassing	Helium or vacuum degassed
HPLC-column	Lithium or sodium ion-exchange column with GARD
Flow Rate	Depends on the method (0.3 -0.6 ml/min)
Injection Volume	Up to 100 µL
Post-Column Derivatization	
Pinnacle PCX	Single-pump
Column Oven	34 – 70 °C (gradient depends on the method)
Reactor Volume	150 µL
Reactor Temperature	45 °C
Reagent	o-Phthalaldehyde, Thiofluor®
Reagent Flow	0.3 mL/min
Detection	
Detection Type	Fluorescence detection
Excitation Wavelength	330 nm
Emission Wavelength	465 nm
Flowcell	Analytical; pressure stable up to 7 bar

Caution: Extreme pH-range!

As a consequence of the alkaline regenerant (pH 13), components made from Vespel may not be present in the HPLC system. These must be exchanged for components made from pH-inert materials (Tefzel or PEEK). For advice, please contact your LC-representative or consult your technical manual.
An inert version (Titanium, PEEK) of the HPLC system is not necessary, a piston seal wash for the pump heads, however, is recommended.
To avoid corrosion of the system and contamination of the ion-exchange column with metal ions, passivating is recommended; this is especially valid for older systems. For advice, please contact your LC-representative or consult your technical manual.

Autosampler

To achieve reproducible retention times in amino acid analysis with ion-exchange columns, it is important to keep to exact time intervals between injections. Should only a manual injector be available, then the injection times should be controlled with a laboratory timer.

Amino Acid Analysis Using Sodium Ion-exchange Columns

For amino acid analysis of hydrolysates of peptides, proteins, collagens, foods and feeds we offer **Sodium Kits** with sodium citrate buffers and an ion-exchange column (type "Standard" or "High Efficiency") in sodium form.

The following kits are available:

For Pinnacle PCX Instrument (with the possibility to use a temperature gradient):

Protein-Hydrolysates (33-Minutes Chromatogram)	Trione T200 *	**0352-0058**
	Trione T100C*	**0352-0057**
	OPA*	**0352-0059**
Collagen hydrolysates (33-Minutes Chromatogram)	Trione T200	**0352-0062**
	Trione T100C	**0352-0061**
	OPA	**0352-0063**
Oxidized feed hydrolysates (33-Minutes Chromatogram)	TRIONE T200	**0352-0021**
	TRIONE T100C	**0352-0020**
	OPA	**0352-0022**

* Trione T200: shelf life: 12 months; Trione T100C: shelf life: 3 months; OPA: o-Phthalaldehyde

Method descriptions

Please use the HPLC conditions and derivatization parameters on page 3ff if there are no other parameters given.

Method: Protein- and Collagen-Hydrolysates

Protein- and Collagen-Hydrolysates: 33-minutes-chromatogram:
"High Efficiency" Ion-exchange column (1154110T); 4.6 x 110 mm; Na^+ Form

Pickering-Kit 0352-0058, 0352-0057 or 0352-0059 for protein hydrolysates
Pickering-Kit 0352-0062, 0352-0061 or 0352-0063 for collagen hydrolysates

Parameters:

Flow rates:	Eluant:	0.6ml/min
	Reagent:	0.3ml/min
Column initial temperature:		46°C for protein hydrolysates
		42°C for collagen hydrolysates

HPLC-Program:

Step	*Time*	*Interval*	*Na315 %*	*Na425 %*	*Na640 %*	*RG011 %*	*Comment*
Equil			100			0	Equilibration
1	0	0	100	0	0	0	Inject 10 µL
2	4.0	4	100	0	0	0	Isocratic
3	15.0	11	0	100	0	0	Linear gradient
4	16.0	1.0	0	0	100	0	Linear gradient
5	31.0	15.0	0	0	100	0	Isocratic
6	31.1	0.1	0	0	0	100	Step change
7	33.0	1.9	0	0	0	100	Clean-out
8	33.1	0.1	100	0	0	0	Re-equilibration
9	40	6.9	100	0	0	0	Re-equilibration

Run time 33 min
Equilibration time 7 min

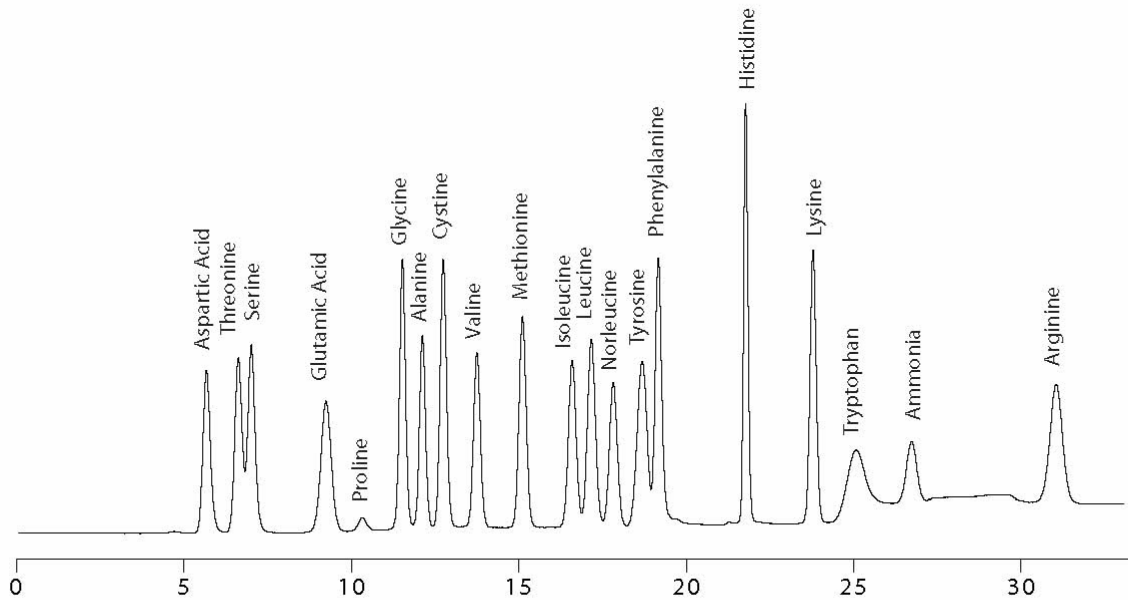
Column oven program for protein hydrolysates:

Time	Temperature, °C
0	46
4	46
9	70
32	70
33	46

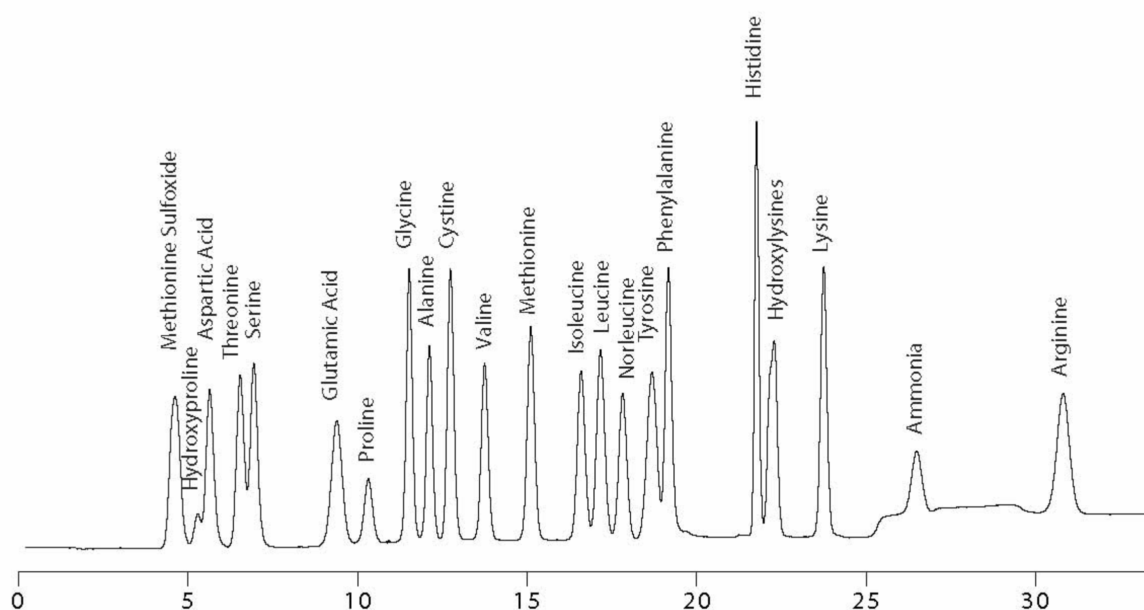
Column oven program for collagen hydrolysates:

Time	Temperature, °C
0	42
4	42
9	70
32	70
33	42

Chromatogram of an Amino Acid Standard for Protein Hydrolysates
"High Efficiency" Ion-exchange Column, 4.6 x 110 mm, Na^+ Form
Sample: Protein Hydrolysate Standard, 0.25 µmole/mL (Catalog No.: 012506H)



Chromatogram of an Amino Acid Standard for Collagen Hydrolysates
"High Efficiency" Ion-exchange Column, 4.6 mm x 110 mm, Na^+ Form
Sample: Collagen Hydrolysate Standard, 0.25 µmole/mL (Catalog No.: 012506C)



Method: Oxidized Feed Hydrolysates

Oxidized Feed Hydrolysates: 33-minutes-chromatogram
High Efficiency" Ion-Exchange-column (1154110T); 4.6 x 110 mm, Na^{+-}-Form

Pickering-Kit 0352-0021, 0352-0020 or 0352-0022 for oxidized feed samples

Parameters:

Flow rates:	Eluant:	0.6 ml/min
	Reagent:	0.5 ml/min
Column initial temperature:		55°C

HPLC-Program:

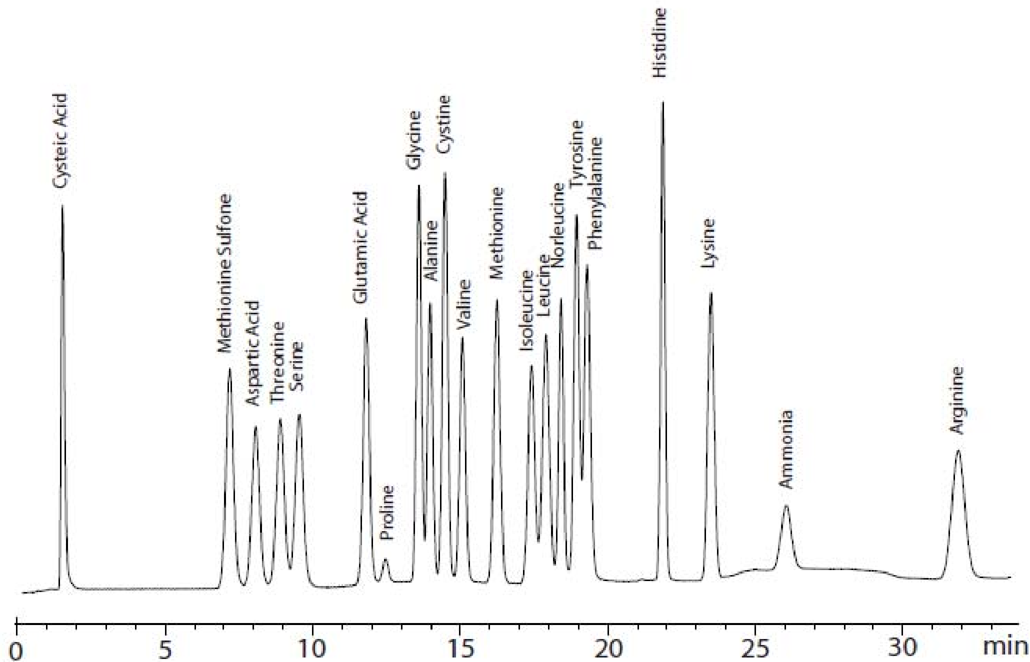
Step	***Time***	***Interval***	***Na270 %***	***Na425 %***	***Na640 %***	***RG011 %***	***Comment***
Equil.			100	0	0	0	Equilibration
1	0	0	100	0	0	0	Inject 10 µL
2	4.0	4	100	0	0	0	Isocratic
3	15.0	11	0	100	0	0	Linear gradient
4	16.0	1	0	0	100	0	Linear gradient
5	31.0	15	0	0	100	0	Isocratic
6	31.1	0.1	0	0	0	100	Step change
7	33.0	1.9	0	0	0	100	Cleanout
8	33.1	0.1	100	0	0	0	Re-equilibration
9	40	6.9	100	0	0	0	Re-equilibration

Run time 33 min
Equilibration time 7 min

Column Oven Program:

Step	***Time***	***Temperature, °C***
1	0	55
2	12	55
3	17	70
4	32	70
5	33	55

Chromatogram of an Amino Acid Standard for oxidized feed hydrolysates
"High Efficiency" Ion-Exchange Column, 4.6 mm x 110 mm, Na^+-Form (1154110T)
Sample: Oxidized Feed Hydrolysate Standard, 0.25 µmole/ML (Catalog No. 1700-0155)



Several aminoacids of interest in feed can be analyzed in a significant shorter runtime:

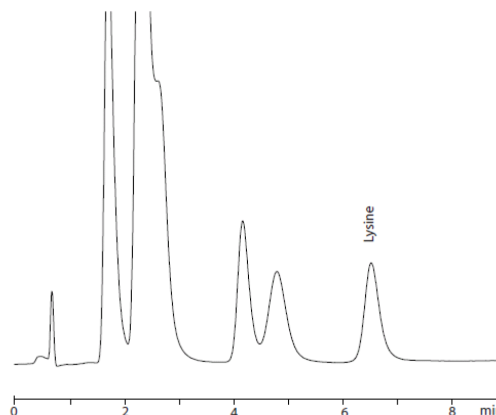
- **Method for accelerated Lysine analysis (9min)**

Parameters:

Flow rates: Eluant: 0.6ml/min
Reagent: 0.5ml/min
Column temperature: 60°C (no temperature gradient necessary)

HPLC-Program:

Time , min	*% Na640*	*% RG011*
0	100	0
6	100	0
6.1	0	100
9	0	100
9.1	100	0
15	100	0



Run time: 9 min
Equilibration time: 6 min

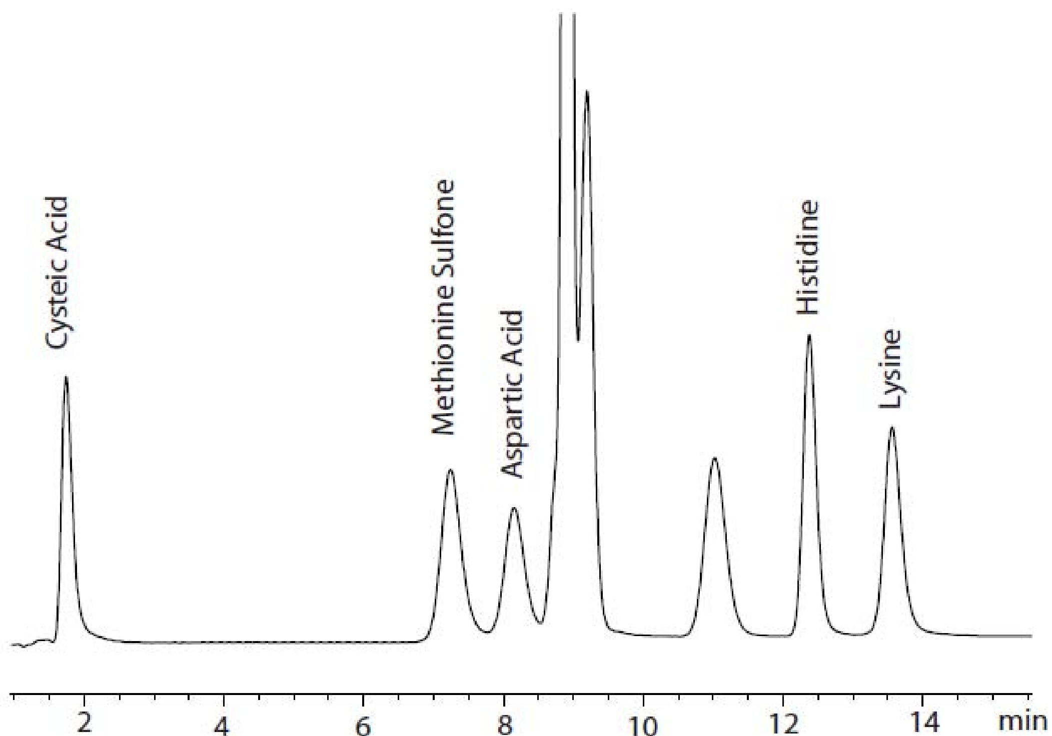
- **Method for accelerated analysis of Cysteic Acid, Methionine Sulfone and Lysine (16 min)**

Parameters:
Flow rates: Eluant: 0.6ml/min
Reagent: 0.5ml/min
Column temperature: 55°C (no temperature gradient necessary)

HPLC-Program:

Time , min	% Na270	% Na640	% RG011
0	100	0	0
5	100	0	0
5.1	0	100	0
12	0	100	0
12.1	0	0	100
16	0	0	100
16.1	100	0	0
23	100	0	0

Run time 16 min
Equilibration time 7 min



Amino Acid Analysis Using Lithium Ion-Exchange Columns

For amino acid analysis of native samples, e.g. blood, urine and tissues, and also foods and beverages, we offer **Lithium Kits** with lithium citrate buffers and an ion-exchange column in lithium form.
The following kit is available:

For Pinnacle PCX with the possibility to use a temperature gradient:

Method		Order Number
Native Samples (70-minutes-chromatogram)*	TRIONE® T200	**0352-0007**
	TRIONE® T100C	**0352-0006**
	OPA	**0352-0008**

* It is not possible to use Norleucine as internal standard; we suggest to use for example Glucosaminic Acid

Method: Physiologic Fluids and Native Samples

Physiological Fluids: 70-minutes-chromatogram
"High Efficiency" Ion-exchange column (0354675T); 4.6 x 75 mm; Li^+ Form

Pickering-Kit 0352-0006, 0352-0007 or 0352-0008 for physiological fluids

Parameters:
Flow Rate: Eluant: 0.55 ml/min
Column initial temperature: 34°C

HPLC program:

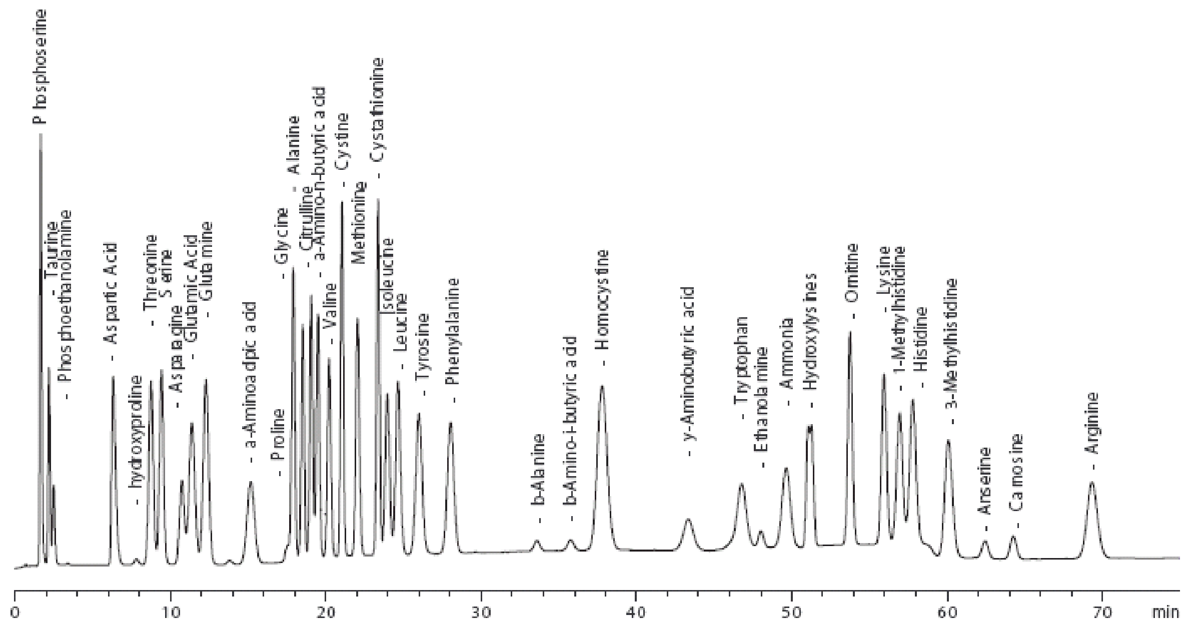
Step	Time	1700-1125 %	Li365 %	Li375 %	RG003 %	Comment
0	0	100	0	0	0	Inject
1	10	100	0	0	0	Isocratic
2	19	40	60	0	0	Linear Gradient
3	32	0	100	0	0	Linear Gradient
4	43	0	100	0	0	Isocratic
5	43.1	0	0	100	0	Step Change
6	57	0	0	100	0	Isocratic
7	57.1	0	0	70	30	Step Change
8	72	0	0	70	30	Isocratic
9	72.1	100	0	0	0	Step Change
10	88	100	0	0	0	Re-equilibration

Run time: 72 min
Equilibration time: 16 min

Column oven program:

Time, min	Tempera-ture, °C
0	34
6	34
17	65
25	70
70	70
71	34

Chromatogram of an Amino Acid Standard for Native Samples
"High Efficiency" Ion-exchange column, 4.6 x 75 mm, Li^+ Form
Sample: Lithium Calibration Standard, 0.25 µmole/mL (Catalog No. 1700-0170)



Chemicals and Columns

Post-Column Derivatization Unit

Order Number	Description
1153-1022	PINNACLE PCX; single-pump, 500 µL reactor for TRIONE®
1153-1012	PINNACLE PCX; single-pump, 150 µL reactor for OPA

Kits for the Analysis of Hydrolysates

For all mentioned kits below, it is necessary to use a temperature gradient of the column:

Order Number	Description	
0352-0057	**30-Min High Efficiency Kit for Protein Hydrolysates**	
1154110T	Na+ Ion-Exchange Column	4.6 x 110 mm
1700-3102	Kit, GARD Holder & Cation GARD	2 units
Na315	Na+ Eluent, pH 3.15	4 x 950 mL
Na425	Na+ Eluent, pH 4.25	4 x 950 mL
Na640	Na+ Eluent, pH 6.40	4 x 950 mL
RG011	Na+ Column Regenerant	950 mL
T100C	TRIONE® Ninhydrin Reagent, ready to use (max 3-month shelf life from date of production)	4 x 950 mL
Na220	Na+ Diluent, pH 2.20	4 x 250 mL
012506H	Na+ Calibration Standard for protein hydrolysate, 0.25 µmol/mL	5 mL
0352-0061	**30-Min High Efficiency Kit for Collagen Hydrolysates**	
Kit identical to 0352-0057, but replaces the standard with:		
012506C	Na+ Calibration Standard for collagen hydrolysate, 0.25 µmol/mL	5 mL
0352-0058	**Kit identical to 0352-0057 with T200 replacing T100C**	
0352-0062	**Kit identical to 0352-0061 with T200 replacing T100C**	
T200	TRIONE® Two-part Ninhydrin Reagent, to prepare	4x 900 mL
0352-0059	**Kit identical to 0352-0057 with items below replacing T100C**	
0352-0063	**Kit identical to 0352-0061 with items below replacing T100C**	
O120	*o*-Phthalaldehyde (OPA),	5 g
OD104	OPA Diluent,	4 x 950 mL
3700-2000	Thiofluor™ (2 each per kit)	10 g

0352-0021	33-Min High Efficiency Kit for oxidized feed hydrolysates	
1154110T	Na+ Ion-Exchange-column	4.6 x 110 mm
1700-3102	Kit, Gard Holder & Cation GARD	2 pieces/pack
Na270	Na+ Eluant, pH 2.70	4 x 950 mL
Na425	Na+ Eluant, pH 4.25	4 x 950 mL
Na640	Na+ Eluant, pH 6.40	4 x 950 mL
RG011	Na+ Column Regenerant	950 mL
T100C	TRIONE® Ninhydrin Reagent, ready to use (max 3-month shelf life from date of production)	4 x 950 mL
Na220	Na+ Diluent, pH 2.20	4 x 250 mL
1700-0155	Na+ calibration standard for oxidized feed hydrolysates; 0.25 µmol/mL	5 mL
0352-0020	**Kit identical to 0352-0021 with T100C replacing T200**	
T200	TRIONE® Two-part Ninhydrin Reagent, to prepare	4x 900 mL
0352-0022	**Kit identical to 0352-0021 with items below replacing T100C**	
O120	*o*-Phthalaldehyde (OPA),	5 g
OD104	OPA Diluent,	4 x 950 mL
3700-2000	Thiofluor™ (2 each per kit)	10 g

Kits for the Analysis of Physiologic Fluids and Native Samples

Order Number	Description	
0352-0006	**70-min Physiologic Fluid/Native Sample Analysis Kit**	
0354675T	Li+ Ion-Exchange Column & 1700-0070 AA test mix	4.6 x 75 mm
1700-3102	Kit, GARD Holder & Cation GARD	2 units
1700-1125	Li+ Eluant, pH 2.80	4 x 950 mL
Li365	Li+ Eluant, pH 3.65	4 x 950 mL
Li375	Li+ Eluant, pH 3.75	4 x 950 mL
RG003	Li+ Column Regenerant	950 mL
T100C	TRIONE® Ninhydrin Reagent, ready to use	4 x 950 mL
Li220	Li+ Diluent, pH 2.20	4 x 250 mL
SP100	SERAPREP™	250 mL
UP100	URIPREP™	250 mL
1700-0170	Li+ Calibration Standard, without Norleucin and alpha-Amino-ß-guanidinopropionic acid, Physiologic fluid, 0.25 µmole/mL	5ml
0352-0007	**Kit identical to 0352-0006 with T200 replacing T100C**	
T200	TRIONE® Two-part Ninhydrin Reagent, to prepare	4 x 900 mL
0352-0008	**Kit identical to 0352-0006 with items below replacing T100C**	
O120	*o*-Phthalaldehyde (OPA)	5 g
OD104	OPA Diluent	4 x 950 mL
3700-2000	Thiofluor™ (2 each per kit)	10 g

Caution-Exchange Columns for Amino Acid Analysis

Each amino acid column is tested by applying an amino acid calibration standard and running it according to a specific gradient elution protocol. After a column passes this rigorous QC test, it receives a serial number and is packaged with its test chromatogram. Pickering Laboratories specializes in the manufacture of cation-exchange columns for amino acid analysis. These are described in more detail below. Although pre-column derivatization followed by reversed-phase chromatography is popular with some HPLC users, its successful application is limited to protein hydrolysates due to matrix interference with native samples. Without extensive pre-treatment, the carbohydrates and organic acids in wine, the fats in tobacco, and the macromolecules in human serum will cause the amino acids to elute at a different time for each unique matrix.

In the reversed-phase environment all species in solution compete simultaneously for residency in the stationary phase. The retentiveness of any particular species will be influenced by the concentration of all other solutes present.
In contrast, ion-exchange chromatography followed by post-column derivatization is intrinsically more rugged and repeatable than pre-column analysis. As chromatography and derivatization represent two separate and distinct events, each can be optimized independently.

The retention mechanism in ion-exchange chromatography is almost completely matrix-insensitive. Since the sulfonated divinylbenzene polymer comprising the stationary phase has a high ion-exchange capacity, the positively charged amino acids exhibit a strong affinity for these fixed negative sites. Consequently, all amino acids, except for the very acidic, are fixed in a narrow band in the guard column, while the sample matrix moves on. Thus the same program used by one laboratory for the analysis of wine can be employed by another to analyze human urine.

The most important difference between ion-exchange resins and reversed-phase silicas is in chemical selectivity. Bonded reversed-phase silicas are manufactured to exhibit monotypical chromatographic behavior, e.g., partitioning.

In contrast ion-exchange resins are polytypical. Separation is based not only upon ion-exchange, but also partitioning, adsorption, ion exclusion, and more. Because of the involvement of so many retention mechanisms, a single change in any operational parameter—cation concentration, pH, flow rate, column temperature—can result in multiple changes in peak position. This flexibility is particularly advantageous, for example, when developing a method to optimize the separation of a few amino acids of interest at the expense of the others, as in the PKU method and other rapid screens.

Pickering also offers also a new guard column, the cation exchange column **GARD™**:

The new GARD™ guard column is recommended to protect the HPLC column from contamination by the matrix. This will avoid significant costs. The GARD™ prolongs the life time of your HPLC column without band spreading or additional pressure.
The GARD™ precolumn is very easy to use and also more cost-effective for your laboratory and can be used for almost all cation-exchange applications.

Cation-Exchange Columns for Analysis of Hydrolysates

- Constant Column Temperature or Temperature Gradient
- “High Efficiency“ 30-Minute (with temperature gradient) or 55-Minute (without temperature gradient) Analysis

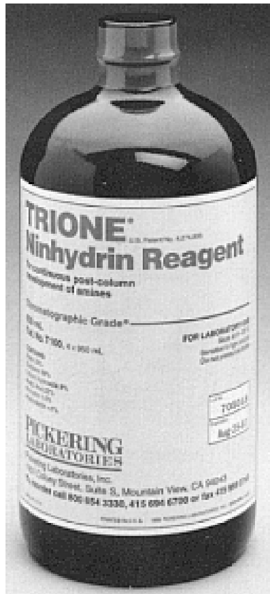
Catalog No.	Description
1154110T	“High efficiency“ Analytical Column (30 minutes) for the analysis of protein and collagen hydrolysates or sulfur containing amino acids in oxidized feed hydrolysates; cation-exchange, Na^+ form, 4.6 x 110 mm (no guard column available)
1193250	Standard Analytical Column (60 minutes) for the analysis of protein and collagen hydrolysates; cation-exchange, Na^+ form, 3 x 250 mm
1154150T	“High efficiency” Analytical Column (55 minutes) for the analysis of protein and collagen hydrolysates; cation-exchange, Na^+ form, 4 x 150 mm
1700-3102	GARD: consists of holder and 2 columns

Cation-Exchange Columns for the Analysis of Native Resp. Physiologic Samples

- Constant Column Temperature or Temperature Gradient
- ”High Efficiency“ 70-Minutes Analysis or “High Efficiency” 95–Minute Analysis (both with temperature gradient)

Order Number	Description
0354675T	“High Efficiency” Analytical Column (70 minutes) for the analysis of native resp. physiologic samples; cation-exchange, Li+ form, 4.6 x 75 mm (no guard column available)
0354100T	”High Efficiency“ Analytical Column (95 minutes or 120 minutes) for the analysis of native resp. physiologic samples; cation-exchange, Li^+ form, 4.0 x 100 mm
1700-3102	GARD: Kit consists of holer and 2 columns

TRIONE® Ninhydrin Reagent



1) Stored at room temperature
2) Superb signal/noise-ratio
3) Superb signal/noise-ratio, thereby high detection sensitivity

TRIONE is a Ninhydrin reagent especially formulated for amino acid analysis. It is so stable that it does not require refrigeration, either in shipment, storage, or in the reservoir. Quantitation is consistent from the first to the last milliliter, eliminating waste. The high signal-to-noise ratio of TRIONE, when compared to DMSO-containing reagents, permits detection sensitivity to be increased with minimum increase in background noise - a feature particularly appreciated at sample amounts of <50 picomoles.
TRIONE Ninhydrin Reagent is a patented formulation containing Ninhydrin, Hydrindantin (reduced Ninhydrin), a Lithium Acetate buffer, and sulfolane, a water-miscible organic solvent. The solvent is necessary to maintain the solubility of both the Hydrindantin and the primary amine product, Ruhemann's Purple. The buffer is required because the reaction is pH dependent. The active ingredients - Ninhydrin and Hydrindantin - are required for proper development of secondary and primary amines, respectively.

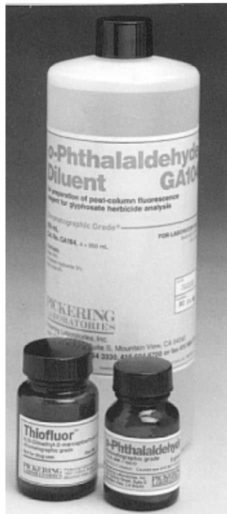
Two preparations are available to suit your usage and storage requirements:

T100C
1) Pour into your reservoir and use; the ultimate in convenience with a minimum of handling
2) Three-month shelf life

T200
1) Combine two solutions, swirl and use
2) Twelve-month shelf life before mixing; three months in the reservoir

Order Number	Description
T100C	TRIONE Ninhydrin Reagent, 4 x 950 mL
T200	TRIONE Ninhydrin, two solutions for mixing, gives 4 x 950 mL

o-Phthalaldehyde Reagent



Primary amines such as amino acids form highly fluorescent compounds when reacted with *o*-phthalaldehyde (OPA) and a mercaptan under basic conditions. At a pH >9 and ambient temperature, reaction is generally complete within 1 - 30 seconds. The products of this reaction, 1-alkyl-2-alkylthio-substituted isoindoles, exhibit optimal excitation at 330 nm and maximal emission at 465 nm.
For an oxygen-sensitive reagent like OPA to remain stable for days instead of hours, it is important to start with the purest and most stable ingredients available, and to store and use the reagent under anaerobic conditions.
Using the chemicals described below, a long-lasting (up to ten days) OPA reagent for post-column derivatization of primary amines can be prepared. Each chemical is accompanied by clear instructions for formulating the reagent in your laboratory within minutes.

For the preparation of *o*-phthalaldehyde reagent PICKERING offers:

1) Ultra-pure, crystalline OPA
2) Borate diluent; free of heavy metals, particulates and amines
3) Thiofluor; crystalline substitute for 2-mercaptoethanol

o-Phthalaldehyde

Order Number	Description
O120	*o*-Phthalaldehyde (OPA), *"Chromatographic Grade™"*, 5 g

o-Phthalaldehyde Diluent

For the amino acid analysis Pickering offers a sodium borate buffer with pH 10.4 capable to buffer acidic eluents (pH 2).

Order Number	Description
OD104	OPA Diluent, *"Chromatographic Grade™"*, 4 x 950 mL

Thiofluor®

Pickering's Thiofluor, a solid, nearly odorless nucleophile, is a superior substitute for 2-mercaptoethanol in the preparation of OPA reagents. It forms a more stable and longer-lasting fluorophore with OPA than does 2-mercaptoethanol, while possessing the same fluorescence properties.
Unlike the volatile 2-mercaptoethanol, Thiofluor will not migrate through the gas manifold and regulator of the OPA reagent pressurization system.

Order Number	Description
3700-2000	Thiofluor®, *"Chromatographic Grade™"*, 10 g

Eluent Buffers and Regenerants

1) *"Chromatographic Grade™"*
2) Guaranteed pH stability at ambient conditions
3) Negligible amine contamination for optimum signal-to-noise characteristics
4) Cost-effective: long shelf life; can be used to the last milliliter
5) Consistent elution profiles, bottle-to-bottle, lot-to-lot
6) No refrigeration required

There are two dominant mechanisms for effecting separation in ion-exchange chromatography. One mechanism is *titration*, or increasing the pH. As pH increases, the amino acids go from a positive charge to a neutral state, and so are released from the fixed anionic sites in the resin (stationary phase).

The other mechanism is *competitive ion-exchange*. The positively charged amino acids show a higher affinity for the ion-exchange sites than do the eluting cations (H^+/Li^+ or Na^+) and so they are bound strongly to the anionic sites. They are eluted by the continuous flow of cation or by increasing cation concentration developed by gradient formation.
The earliest protocols for ion-exchange of amino acids were based on step gradients—isocratic procedures that step through a set of 3–6 solutions of varying pH and cation concentration. Both titration and elution adjustments are made with each eluant step. This technique persists today in the design of dedicated amino acid analyzers.

The modern liquid chromatographic (HPLC) techniques for ion-exchange use three solutions and continuous gradients. Gradient capability allows for easier methods development and results in flatter baselines—especially important at high sensitivity.
As with dedicated analyzers, the eluants are either sodium- or lithium-based. The need for two cation systems derives from the two fundamental types of samples encountered in amino acid analysis: hydrolyzed and non-hydrolyzed ("native") samples. Hydrolyzing the sample results in a smaller array of amines (typically up to 21) than is present in native samples (typically 40–60). The wider range of amines in native samples requires a more discriminating or weaker eluant to fully resolve them. A lithium ion-based eluant provides the necessary resolution.

If the sample is hydrolyzed, a sodium system can be used for a shorter run time.
Both Na^+ and Li^+ eluants for gradient HPLC are designed in the same way. The first, low-pH eluant is a buffer with low cation concentration. The second eluant has no buffering capacity and a high cation concentration. The third, high-pH eluant (usually referred to as the column regenerant) is based on the cation hydroxide and is of comparable cation

concentration to the first eluant. These solutions allow for titration relatively independent of changes in eluant strength, or allow for increasing eluant strength independent of pH.

Pickering's Lithium and Sodium eluants are not sensitive to oxidation and do not need refrigeration, either in storage or use. Degassing is not required. However, they should be protected from air to prevent contamination. Ambient air actually contains amines and amino acids that will dissolve in the low-pH eluants and will appear in the chromatograms. All buffers are packaged in cases of four 950 mL polyethylene bottles. The cation hydroxide regenerants are packaged in single 950 mL bottles because of the small volumes consumed during each analysis. Sample diluents are packaged in cases of four 250 mL borosilicate glass bottles.

Order Number	Description
Sodium buffers for hydrolysates	
Na270	Sodium Eluent, pH 2.70, 4 x 950 mL
Na315	Sodium Eluent, pH 3.15, 4 x 950 mL
Na328	Sodium Eluent, pH 3.28, 4 x 950 mL
Na425	Sodium Eluent, pH 4.25, 4 x 950 mL
Na640	Sodium Eluent, pH 6.40, 4 x 950 mL
Na740	Sodium Eluent, pH 7.40, 4 x 950 mL
1700-0112*	Sodium Eluent, pH 3.15, 4 x 950 mL, with 5 % sulfolane
1700-0114*	Sodium Eluent, pH 3.15, 4 x 950 mL, with 2.5 % sulfolane
RG011	Column regenerant, Na^+ Form, 950 mL
* for use with 1154150 with Serial No. above 1314	
Lithium buffer for native and physiologic samples	
Li275	Lithium Eluent, pH 2.75, 4 x 950 mL
1700-1125	Lithium Eluent, pH 2.80, 4 x 950 mL
Li280	Lithium Eluent, pH 2.80, 4 x 950 mL
Li292	Lithium Eluent, pH 2.92, 4 x 950 mL
Li357	Lithium Eluent, pH 3.57, 4 x 950 mL
Li365	Lithium Eluent, pH 3.65, 4 x 950 mL
Li375	Lithium Eluent, pH 3.75, 4 x 950 mL
Li750	Lithium Eluent, pH 7.50, 4 x 950 mL
RG003	Column regenerant, Li^+ Form, 950 mL

Sample Diluents

Use of these sample diluents is essential to ensuring reproducibility from injection to injection. They establish a uniform pH and ion concentration at the outset, regardless of the source and pre-treatment of the sample. The sample is maintained buffered and at optimum pH for sample storage and analysis. Use Na220 for hydrolysates and Li220 for native amino acid samples.

Order Number	Description
Na220	Sodium Diluent, pH 2.20, 4 x 250 mL, *“Chromatographic Grade™”*
Li220	Lithium Diluent, pH 2.36, 4 x 250 mL, *“Chromatographic Grade™”*

SERAPREP® und URIPREP®

1) *“Chromatographic grade™”*
2) For the preparation of native samples
3) Ensures reproducibility of early eluting peaks
4) Accommodates sample buffering capacity
5) Little to no pH adjustments
6) Mix, spin, filter, inject

Native amino acids are those found “free” in samples such as serum, urine and other physiologic fluids, plant extracts, foods and beverages. Although preparation of these samples for amino acid analysis is much simpler and less time-consuming than protein hydrolysis, control of pH and normality, and removal of soluble protein are critical factors which can affect the chromatography.
The early-eluting amino acids - taurine, urea, aspartic acid, threonine, serine, etc. - are particularly sensitive to pH and normality. Accordingly, the samples must be held to a narrow pH range between 2.1 and 2.5, and at the proper Lithium ion concentration to ensure reproducibility in the early part of the chromatogram. The later-eluting compounds are more tolerant of initial sample conditions, and their retention times are not as likely to be affected.
SERAPREP® and URIPREP® replace commonly used protein precipitation reagents such as acetonitrile, trichloroacetic acid and picric acid, and eliminate the need for dialysis, ultrafiltration, and repeated centrifugation steps, followed by pH adjustment.
Use SERAPREP® for preparing serum and URIPREP® for preparing urine and other samples with low buffering capacity, such as orange juice or wine. The efficiency of protein precipitation and need for post-centrifugation pH adjustment of the sample determine which reagent is best for your particular sample.

In a microcentrifuge tube, thoroughly mix equal portions of sample and SERAPREP® or URIPREP®.

1. Let stand for 5 minutes. Centrifuge the mixture at 13.000 rpm for 5 minutes. Check the supernatant pH to ensure that the range is pH 2.3 ±0.2. Adjust the initial mixing ratio as necessary.
2. Filter the supernatant with a syringe filter (0.2 or 0.45 µm). The filtrate is ready to be injected into an auto-sampler vial for amino acid analysis.
3. If further dilution is needed, use Li 220 to adjust the concentration of analyte.

Order Number	Description
SP100	SERAPREP®, Sample Preparation for Serum, 250 mL
UP100	URIPREP®, Sample Preparation for Urine, 250 mL

Amino Acid test Mixture (1700-0070)

L-Cysteic Acid
L-Threonine
L-Serine

This test mixture is in 0.01 N HCl at a concentration of 0.25 µmol/mL. The three amino acids are eluted isocratically with the first eluant (Na328, Na315, Li275 or Li280). Since cysteic acid is not retained by the stationary phase, its retention time may be used to calculate the system void volume. The resolution between threonine and serine indicates the degree of band-spreading due to system leakages or excessive dead volumes.
The Test Mixture is a convenient tool for rapid, routine monitoring of the performance of either a sodium or lithium amino acid analysis system.
Test mixture should not be used for quantitation

Amino Acid Standards

1) Quantitative
2) Chromatographically-pure starting components
3) Each lot tested by amino acid analysis

Pickering's amino acid standards have a reputation worldwide for quality and reliability in all post-column systems and methods. Six amino acid mixtures are available for a variety of applications. Except for the three-component test mixture (1700-0700) the standards are in 5 mL vials, in an appropriate citrate buffer.
Although they are stored frozen at the factory, the Pickering calibration standards will remain stable when shipped at ambient temperatures. Upon receipt, however, it is important to place them into a freezer immediately and store frozen until ready for use.

CALIBRATION STANDARDS FOR AMINO ACID ANALYSIS									
CONSTITUENTS	1700-0180*	1700-0175*	011006P	012006P	1700-0150	012506C	012506H	1700-0155	1700-0170
Beta-Alanine	•		•	•					•
Alanine	•		•	•		•	•	•	•
D,L-a-Amino-adipic acid	•		•	•					•
Gamma-Amino butyric acid		•	•	•					•
Alpha-Amino-n-butyric acid	•		•	•					•
D,L,b-Amino-i-butyric acid	•		•	•					•
Alpha-Amino-Beta-guanidinopropionic acid			•	•					
Ammonia		•	•	•		•	•	•	•
Anserine		•	•	•					•
Arginine		•	•	•		•	•	•	•
Asparagine	•		•	•					•
Aspartic acid	•		•	•		•	•	•	•
Carnosine		•	•	•					•
Citrulline	•		•	•					•
Creatinine		•	•	•					•
Cystathionine	•		•	•					•
Cystine	• (1.25)		•	•		•	•		•
Cysteic acid								•	
Ethanolamine		•	•	•					•
Glutamic acid	•		•	•		•	•	•	•
Glycine	•		•	•		•	•	•	•
Histidine		•	•	•		•	•	•	•
D,L-Homocystine		•	•	•					•
L,L & allo-Hydroxylysine		•	•	•		•			•
4-trans-L-Hydroxyproline	•		•	•		• (1.25)			•
Isoleucine	•		•	•	•	•	•	•	•
Leucine	•		•	•	•	•	•	•	•
Lysine		•	•	•		•	•	•	•
Methionine	•		•	•	•	•	•		•
Methionine-D,L-sulfoxide						•			
Methionine-D,L-sulfone								•	
1-Methyl-histidine		•	•	•					•
3-Methyl-histidine		•	•	•					•
Norleucine			•						
Ornithine		•	•	•					•
Phenylalanine	•		•	•	•	•	•	•	•
Phosphoethanolamine	•		•	•					•
Phosphoserine	•		•	•					•
Proline	•		•	•		• (1.25)	•	•	•
Sarcosine	•		•	•					•
Serine	•		•	•		•	•	•	•
Taurine	•		•	•					•
Threonine	•		•	•		•	•	•	•
Tryptophan		•	•	•			•		•
Tyrosine	•		•	•	•	•	•		•
Urea	•		•	•					•
Valine	•		•	•		•	•	•	•

NOTE: Concentration of all the constituents in the Amino Acid standards is 0.25 µmole/mL unless otherwise specified.
*Concentration of all the constituents is 2.5 µmole/mL unless otherwise specified