SYKAM
CHROMATOGRAPHY

Amino Acid Analyzer

Advanced Technology
Classical Ninhydrin Post Column Method
Superior Reliability & Sensitivity of Amino Acid Determination

Feature

The Amino Acid Analyzer S-433H

belongs to the new generation of instruments for the determination of amino acids according to the well known reliable Ninhydrin post-column derivatisation method.

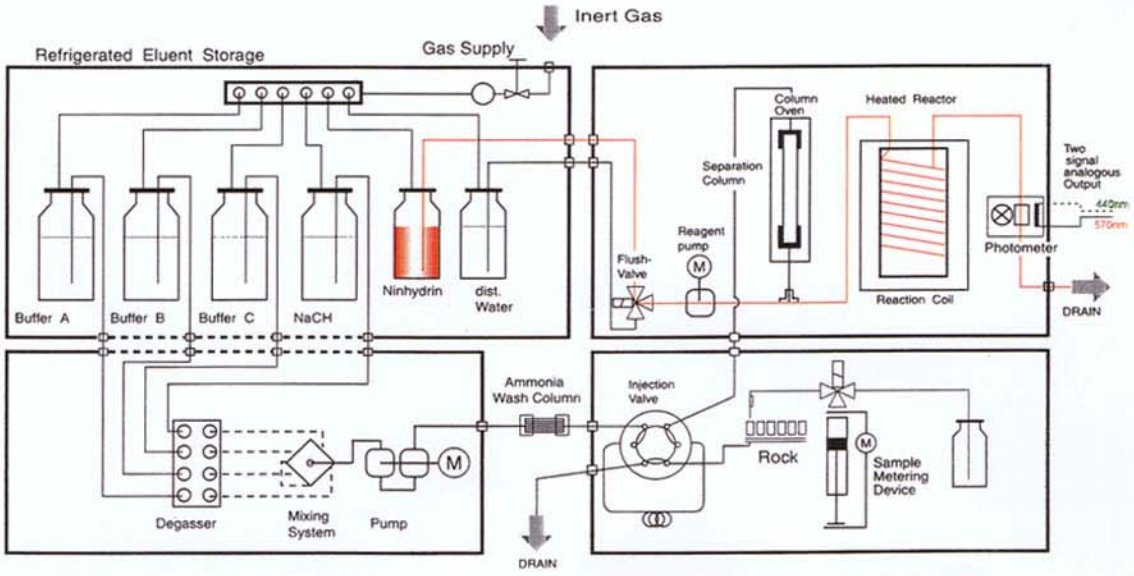
The experience of 30 years in amino acid analysis has been put into this instrument and makes this system too easy to handle stand alone system. This Amino Acid Analyzer S-433H works according to the classical ninhydrin post-column derivatisation method. It is the most accurate method for the analysis all of the basic and important amino acids both for determination of hydrolysate and physiological fluids.

The ability of this substances to react according to the environmental circumstances is used for their separation. It can be controlled completely with the help of a computer or works as a stand-alone system. With old fashioned step-elution systems, 4 or 5 buffer solutions were needed. New, the optimized buffer system, only 2 buffers for hydrolysate and 3 buffers for the physiological samples are necessary. The buffer can be adjusted individually to the samples by varying the mixture of the buffer. The resin is a special ion-exchange material, causing different interactions with the single amino acids. Therefore the different amino acids leave the column at different times. The direct determination of the separated amino acid is not possible in that range of sensitivity needed. Therefore a ninhydrin solution is continuously added to the sample leaving the column. In the heated reactor, the ninhydrin reacts with the amino acids to a substance, which is detectable via vis photometer.

The system S-433H included an autosampler with cooled sample storage and partial loop fill technique without sample loss as well as a 2 plunger buffer pump, a dual beam photometer, a column oven with temperature gradient, a ninhydrin pump, a four channel vacuum degasser and a refrigerated reagent organizer with integrated inert gas application system. It is a combination of differnt modules and includes a processor for running so system components can be upgrade to an HPLC system.

Data System

Fluorescence Detector



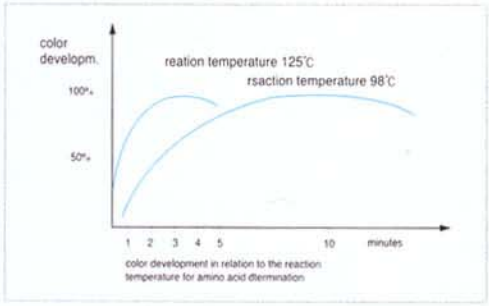
< Schematic Diagram Amino Acid Analyzer S-433H>

1. Reagent Organizer
with cooling unit

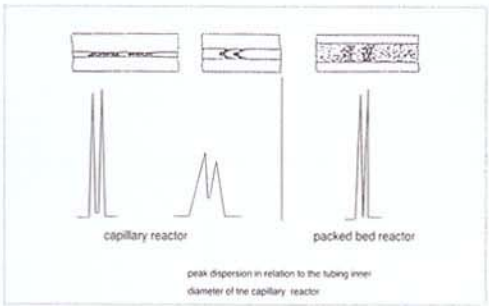
2. Gas controller

3. Amino acid reaction module

1) 440nm, 570nm photometer
2) Column oven
3) Ninhydrin pump
4) Reaction unit



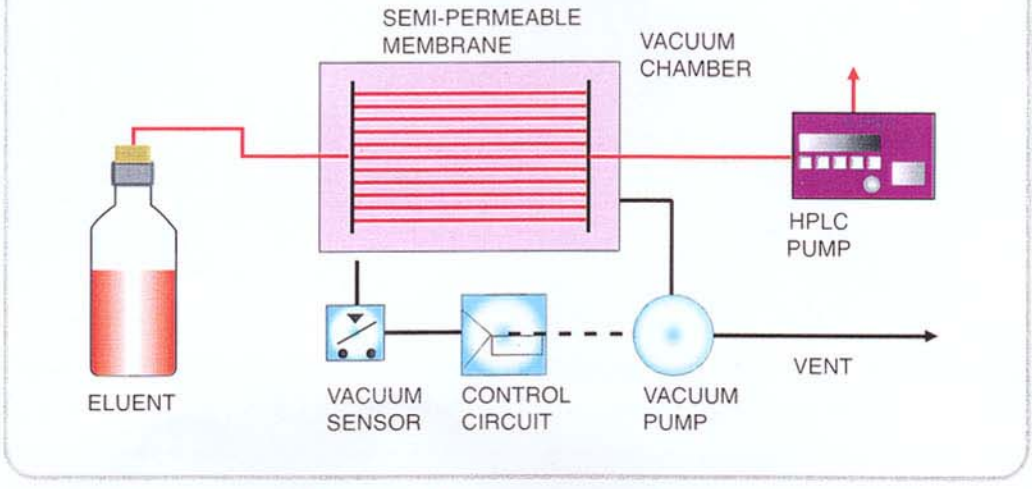
color development in relation to the reaction temperature for amino acid determination



peak dispersion in relation to the tubing inner diameter of the capillary reactor

4. Buffer pump unit
with 4 CH Vacuum Degasser

5. Auto-sampler with cooling unit



< SYKAM Vacuum Degasser System>

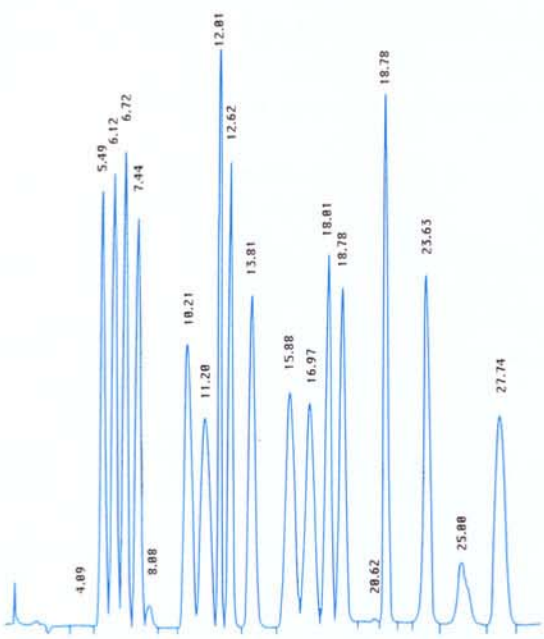
Hydrolysate Program

Analysis time is directly influenced by resolution of column and pH-gradient of buffer. As SYKAM S-433H produces pH-gradient in the buffer pump, analysis time is controlled automatically by user programmable pH-gradient. So users can make their own buffer by themselves and there is no need to buy a buffer from makers. This pH-gradient programming function is quite convenient because it eliminates frequent change of column and buffer to make the best resolution.

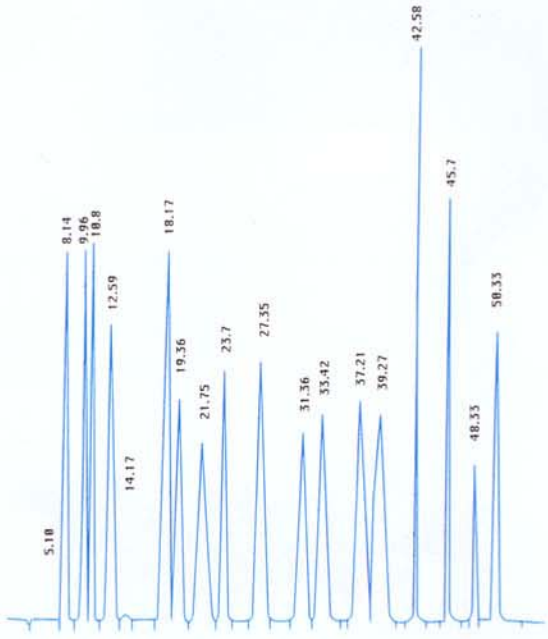
For the determination of amino acids in Hydrolysate fluids two different buffers are necessary; The different amino acids are separated due to the pH-values of the buffers during the run.

- ▶From ASP to Cys buffer A is active.
- ▶From Val to Phe a mixture between buffer
- ▶A and B is used.
- ▶From His to Arg only Buffer B is working.

	buffer A	buffer B	Regene solution	Diluting buffer
pH-value	3.43	10.85		2.20
Normality	0.12	0.12	0.3	0.12
Na Citrate	11.9g	11.9g		11.9g
Citric acid	6g			
NaOH		2.5g	12.0g	
Monomethy Ether	65ml			
Thiodiethanol(25%)				20ml
EDTA			1g	
Phenal	1g	1g		
HCl(37%)	6ml			14ml
Boric acid		5.0g		
Volume	1L	1L	1L	1L



Example of protein Hydrolysate Analysis
- 30min High Resolution



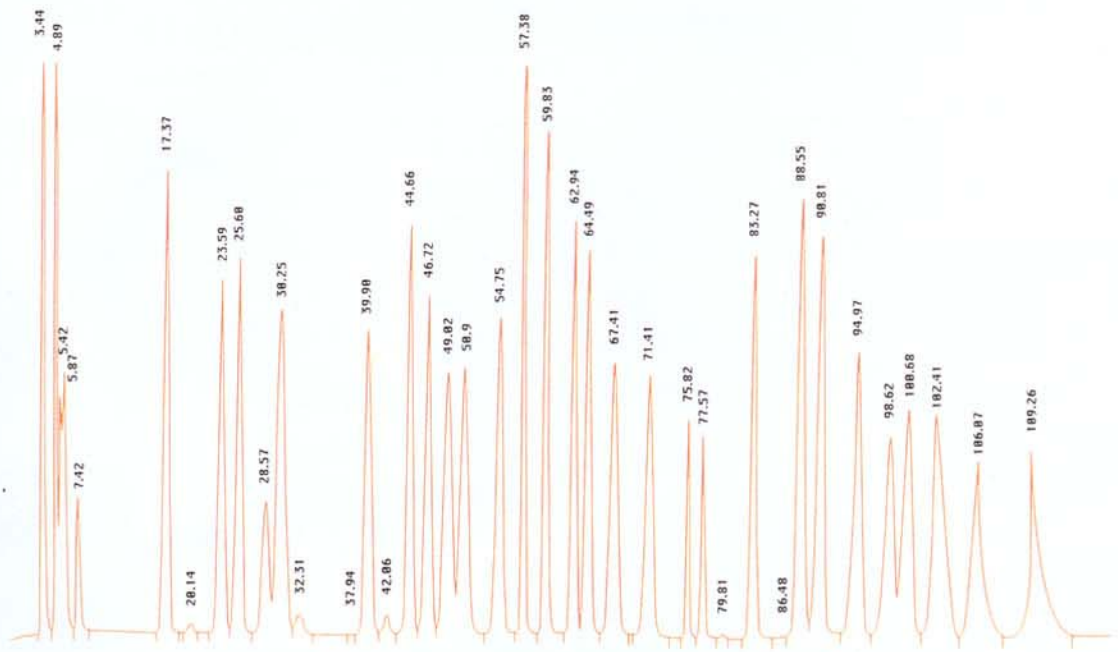
Example of protein Hydrolysate Analysis
- 50min Run time (feed samle Analysis)

Physiological Program

For the determination of amino acids in physiological fluids three different buffers are necessary; The different amino acids are separated due to the pH-values of the buffers during the run.

- ▶ From Phosphoserine to Glutamine buffer A is active.
- ▶ From alpha-Aminoadipic acid to alpha-Aminobutyric acid a mixture between buffer A and B is used.
- ▶ From Valine to Tyrosine buffer B is working.
- ▶ From Phenylalanine to Tryptophan a mixture between buffer B and C is used.
- ▶ From Ammonia to Arginine only buffer C is working

	buffer A	buffer B	buffer C	Regene solution	Diluting buffer
pH-value	2.85	4.50	3.30		2.20
Normality	0.12	0.12	1.40	0.3	0.15
Li Citrate	14.1g	14.1g	18.8g		14.1g
Li Chloride			50.7g		
Li hydroxide				12.8g	
Citric acid	7.00g	7.00g			
Ethylene Gly					20ml
Methanol	50ml				
HCl(37%)	9ml	6ml	10ml		15ml
Volume	1L	1L	1L	1L	1L



Example of physiological Analysis program
- 120min run time

Order Numbers Referencials

Cat. No	Description	Cat. No	Description
1120 001	Amino Acid Analyzer S433H high resolution	6001 005	1000ml Buffer A0.12N ph3.45
1120 002	Amino Acid Analyzer S433S s'td type	6001 006	1000ml Buffer B0.20N ph10.85
1120 003	Amino Acid Analyzer S430 manual version	6003 001	1000ml Regeneration Solution 0.3N
1120 004	Amino Acid Analyzer S430D	6004 003	Hydrolysate Kit I
			3×A, 2×B and Regeneration
1041 002	Amino Reaction Module S4300	6002 006	1000ml Buffer A0.12N ph2.85
	included of: column oven, photometer	6002 007	1000ml Buffer B0.12N ph4.20
	reactor	6002 008	1000ml Buffer C1.40N ph3.30
1050 001	Automatic sample Injector S5200	6004 004	physiological Fluid Kit I
1090 087	Fluorescence detector S 3350		3×A, 2×B and Regeneration
	pulsed xenon lamp, 200 to 650nm	6005 001	1000ml Solium Acetate Buffer 4N; ph5.51
107 1003	SYKAM Vacuum degasser	6005 002	1000ml Lithium Acetate Buffer 4N; ph5.20
5112 007	Cation Setaration Column LCA K06/Na	6006 001	Standard Solution
	4.6×150mm column, particle size 7um		18 amino acids, 2.5umol/ml
5112 008	Cation Separation Column LCA K07/Li	6006 002	Standard Solution
	4.6×150mm PEEK column, particle size 7um		35 amino acids, 1µmol/ml
5112 005	Cation Ammonia Filter Resin 5gr	5090 003	1000ml Ninhydrine Regent
5112 017	Cation Separation Column LCA K08	5100 001	Column packed Kit
	4.6×150mm PEEK column, particle size 5um	8100 005	Sample preparation Kit
5112 018	Cation Separation Column LCA K09	9100 009	Start up Kit
	4.6×150mm PEEK column, particle size 5um		<Data system>
5112 009	Ammonia filter column LCA K04/Na	900-001	Pyramid Chromatography manager P1
5112 010	Ammonia filter column LCA K05/Li	900-002	Pyramid Chromatography manager P2
		900-003	Peaksimple Chromatography manager

System Control Software

Efficient Peak Finding and Baseline Editing

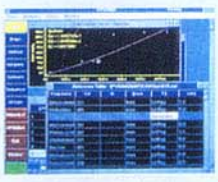
PYRAMID helps you find and quantitate component peaks quickly and accurately, saving all baseline modifications for immediate recall.

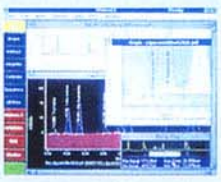
- Identify peaks reliably with dynamically-referenced retention times, and separate fused peaks with skim, tangent or exponential skim logic—*observe results on-screen in user-definable Peak Info windows!*
- Determine optimal integration algorithm automatically for any separation using powerful, graphical Measure functions—*use "drag-and-drop" zone codes to customize processing of any chromatogram!*
- Draw baseline edits, with automatic reintegration and unique computer-assisted code adjustment as you work—*undo individual trial edits instantly, without recomputing the entire chromatogram!*
- Check analysis of batched runs effortlessly with Browse reprocessing, as the system "steps" through each action, giving you the opportunity to make corrections—*eliminate integration and reporting errors!*

Unprecedented Calibration Versatility

PYRAMID gives you more control over calibrations—and their interpretation and validation—than ever before.

- View **all** selected calibration information from **all** standards for each peak on one screen, with instant access to any peak's data—*use the interactive Plot window to graphically identify points and fat outliers!*
- Select external or internal standardization for individual peaks in any run—set calibration basis, multilevel curve fit, weighting, normalization, group reporting, and other options *for each peak independently!*
- Evaluate and optimize calibrations quickly, using programmable Statistics window to immediately see the impact of changing calibration strategy—*superimpose fit or weighting plots to judge effects visually!*
- Bracket calibrations in batch runs, with innovative "time-of-run weighting"—*automatically update peak retentions, track calibration history, and monitor inter- or intra-batch response variance!*





Incomparable Full-Screen Graphics

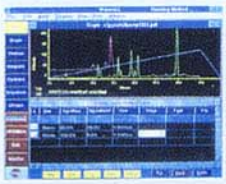
PYRAMID opens windows on your chromatography like no other system, with single-button access to any program or channel at all times.

- View real-time signal inputs from multiple time bases on one screen, while processing saved chromatograms in the editing time base—*even overlay reference chromatograms with new traces during acquisition!*
- Compare up to sixteen chromatograms in one or more windows, during real time operation or while idle—reposition and offset chromatograms, or align points or peaks, *by simple graphical "drag and drop"!*
- Change scaling, scale units, colors, annotation, shading, or overlay mode instantly in any window—zoom or pan at constant scaling, to see any part of a chromatogram, *even while it is being acquired!*
- Plot gradient, temperature, detection wavelength, and other timed events along with chromatograms—*monitor input signals, time, and other parameters you select via movable, resizable Status window!*

Total Method Automation For Any Laboratory

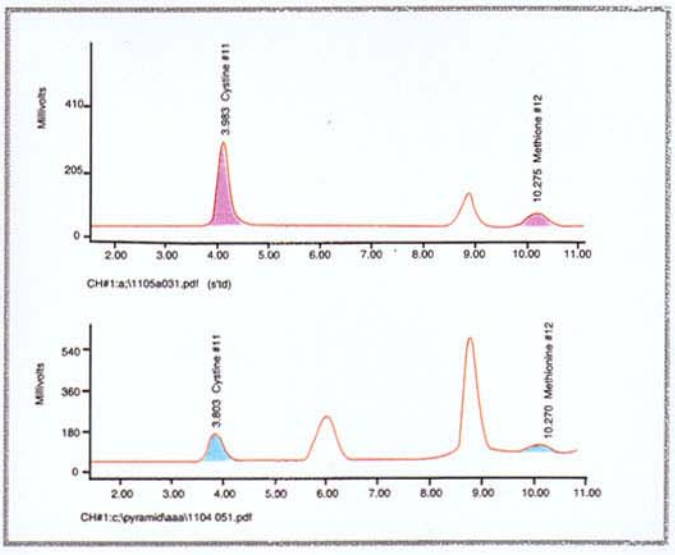
PYRAMID provides a single, common software interface for all your chromatographs, regardless of techniques and range of operator skills.

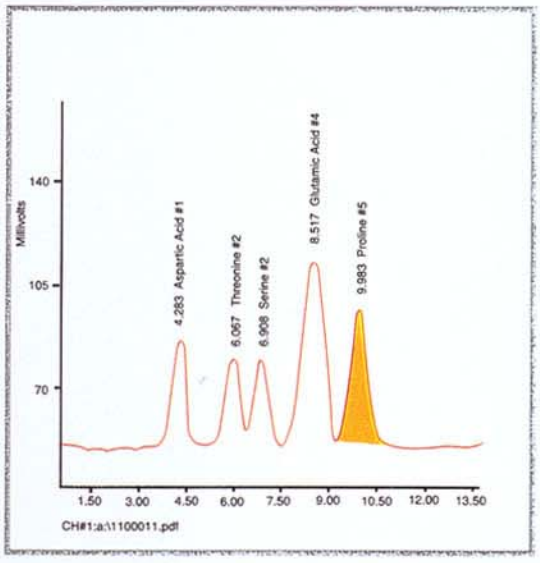
- Control your HPLC, GC, IC, SFC, or CZE instruments, in any combination of models or brands—*configure all screens, menus, computations, and reports to match each channel's application!*
- Program unique automatic file saving, integration algorithm, calibration, and reporting modes for each of up to four detectors per time base—*change **any** parameter "on-the-fly" in a running Method or Sequence!*
- Validate results on-line automatically via user-definable profile of system suitability, calibration statistics, and system checks—*set to accept/flag, repeat, or reject results from out-of-tolerance runs!*
- Ensure GLP/GMP compliance through multilevel operator security system, raw data and program file protection, and auto change logging—*define exact contents of file audit trails to meet **your** requirements!*



Data Analysis

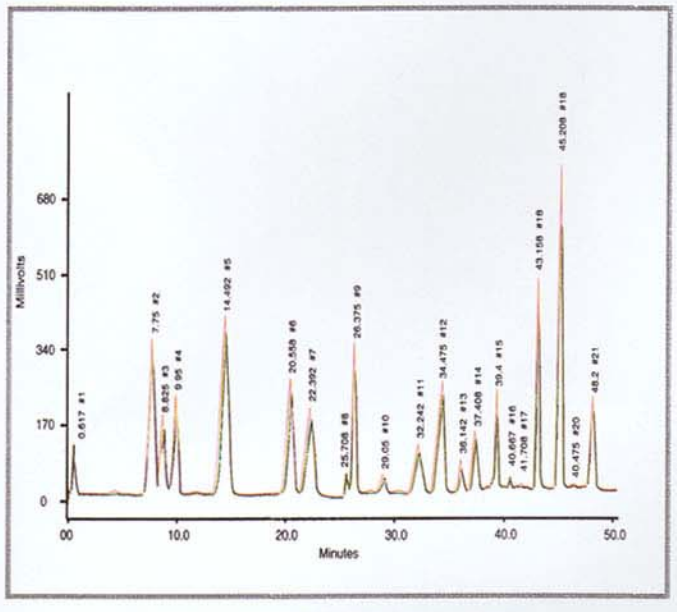
In most cases the acid Hydrolysis with 6N HCl has to be applied in the high purity N20 gas. As sulfur containing amino acid (Met, His) keeps unstable status, performic acid should be added.



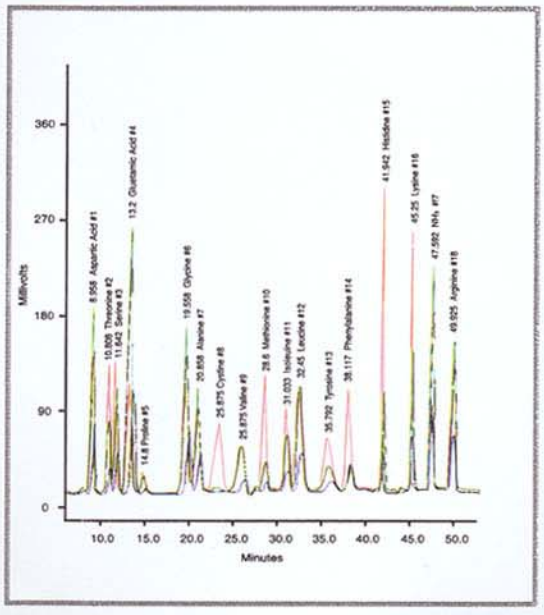


<Sulfur Containing Amino Acid>

The accuracy of pH-gradient is the critical factor in Amino Acid Analysis. SYKAM S-433H produces precise pH-gradient, thus RT of peak is very stable and accuracy of data is excellent.



<Comparative of Retention Time>



In-A:a:/0127b751.pdf standard
a:/0127b731.pdf white rice
In-B:a/0127b761.pdf black rice

Technical Data (standard type)

1. Column unit

Heating and cooling is effected by peltier elements	
Temperature gradient	
Temperature gradient programmable	20 to 99℃
Accuracy	0.1℃
Column	PEEK, 4.6 x 150mm, 5㎛, 10% cross
Overheat security dev.	safety fuse

2. Auto sampler

Injection modes	Variable volume 100ul sample loop
Injection volume	Variable ; 1ul - 5000ul in 0.1ul increments
Sample processing	3 reagents each one 1ul to 5000ul indepenty programmable
	3 reaction times independently programmable
Sample Diluting	up to 5000ul diluting solvent in 1ul increments
Reproducibility	1% of 10ul variable volume
Injection / vial	1 to 9 different volumes programmable for each injection
Sample trays	2 PCs. for 60 vails/ each total 120 for 1.5ml glass vials
Temperature control	+5℃(±1℃) to +70℃(Peltier cooling and heating)

3. Analysis Time

Protein hydrolysate analysis	30 - 50min automatical time control
Physiological fluid analysis	90 - 180min automatical time control

4. Reagent organizer with Peltier cooling unit

Gradient for 6 ea buffer and one regeneration solution	
Temperature range	4℃
Built-in refrigerator with temperature control containing	

5. Buffer pump System

Piston	Dual piston
Rinsing (Plunger washing for extended seal life) Yes	
Flow rate	0.01 ml/min to 2.00ml/min
Pressure Pulsation	0.1 %
Maximum pressure	40 MPa (400 bar)
Operating mode	constant flow, constant pressure
Gradient mixing chamber	100 to 500 ㎕
Program storage	20 gradient programs
Materials	PEEK,

6. Detection System

Number of wavelength monitored	570 nm, 440 nm
Flow cell volume	8 ul
Pathlength	15 mm
Detection limit . Ninhydrin	10 pmol
Fluorescence	3 pmol

7. High temperature post column reactor

Configuration	T-pieces, pump, reaction coil
Temperature range	ambient up to 199℃
Standard reactor	1.5 mm x 0.3 mm x 15m
Dosage pump Flow rate	0.1 to 6.0 ml/min
Safety device	overheating,
Temperature accuracy	0.1℃

8. System control and data handling system

(2 system 4 channel two interface boards)
User-configurable workstation for data acquisition and system control.
Multitasking system under windows 98 in combination with EXCEL 7.0 for report configuration.
Free selectable configuration of the result printout through EXCEL macros.
Automatic calculation of the peak parameters and statistic values according to USP and ESP standard.
Up to 200 different method files
Automatically reset base line to a predetermined level
Resetting of baseline can take place at any time and any number of times during the programme.
Fault diagnosis system . High/ low buffer pressure and flow rate
- High/ low nitrogen pressure
- reactor coil high/low pressure
- Autostart faults

Resolutio	: 32 bits at 2 Hz.
Sampling rate	: 1, 2, 5, 10, 20, 30, 100Hz

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