

## AMINO ACID ANALYZER SYSTEM **S 433**

PROTEIN HYDROLYSATES
 PHYSIOLOGICAL FLUIDS

♦ BIOGENIC AMINES



# AMINO ACID ANALYZER S 433

The innovative automatic *Amino Acid Analyzer S 433* combines the advantages of the classical ion exchange separation method with the modern technique of high performance liquid chromatography. The complete package of sophisticated instrumentation, a wide variety of prepacked and tested separation columns, combined

with optimized ready-to-use buffer solutions and chemicals, creates the right answer for any routine or research problem in amino acid determination. More than 30 years experience in developing and operating sophisticated amino acid analyzers results in unmatched performance.

With old fashioned step-elution systems, 4 and/or 5 buffer solutions were needed. Now, due to the optimized buffer system, only 2 buffers for hydrolysates and 3 for the physiological sample are necessary. The buffer can be adjusted individually to the samples by varying the mixture of the buffer.

pulsation-reduced solvent and Ninhydrine delivery through two independent completely inert dual-piston pumps



### **Multistep Separation**

Only two or three buffer solutions have to be combined to form the best optimized buffer profile at any part of the separation program. No more compromises by the limitation to four or five buffer changes.

### **Cooled Reagent Storage**

All buffer solutions, as well as the Ninhydrine reagent are stored under inert gas pressure in a refrigerated cabinet to avoid oxidation and air contamination independent of the ambient environment.

### **Integrated Autosampler**

with a capacity of 120 vials in a cooled sample tray. Volumes from 1µl to 100 µl can be injected without any loss of sample. The injected volume is adjusted by a high precision syringe, driven by a stepper motor with a resolution of 17 steps per µl. A programmable wash program will flush the entire injection system to avoid cross contamination of the sample.

### Integrated Vacuum Degasser

avoids the interruption of the buffer pump by air bubbles without the need of bubble traps with varying volumes,



causing changes in retention times of the different amino acids.

### Separation Column Oven

integrated solid state column oven with fast heating and

cooling capability with a temperature range from  $+20^{\circ}$ C to  $+99^{\circ}$ C independent of the ambient temperature. Up to twelve programmable temperature steps can be used during one separation program.



### **High-Temperature Reactor**

with a programmable temperature range from ambient to 180°C with a coiled capillary for the color reaction of the amino acid-ninhydrine complex. Automatic flushing of the reactor coil with a washing solution after each run prevents the blockage of the capillary.

### Integrated Reagent Dosing Pump

for Ninhydrine delivery and flushing of the reaction coil after each run. Programmable flow rate from 0.01 to 2.0 ml/min.

### **Complete Inert Design**

All materials coming into contact with the buffer solutions and reagents are made of inert materials as PEEK, PTFE, PVDF etc. Therefore, there is no need for using special non-corrosive buffer compositions or reagents.

### **Integrated Dual-Channel Photometer**

Working principle of photometer

for the amino acid detection at 440 nm and 570 nm wavelengths. Summing option for both channels, for single channel integration of all amino acids.

### **Safety Devices**

control pump pressures, temperatures and leakages.

### **Optional Application**

- Post column derivatisation with OPA (needs an optional Fluorescence Detector)

# MODULAR SYSTEM DESIGN

The modular system design of the S 433 enables the budget-minded buyer to use several components as parts for a standard HPLC system without further costs. Another solution is our low-cost Amino Acid Analyzer S 430 with manual injection valve and ambient temperature reagent organizer, consisting of the same Amino Acid Reaction Module and Quaternary Gradient Pump as the S 433.



Sample Racks for up to 120 samples



Exchangeable Sample Loop made of PEEK

#### **Autosampler**

- only inert materials come in contact with buffer solutions & samples (except sample needle)
- exchangeable sample loop (PEEK)
- fixed and variable volume injection mode
- integrated sample cooling
- up to 120 samples
- also usable as standard HPLC Autosampler
- optionally with pre-column derivatisation mode



#### SYKAM S 7130 AMINO ACID REAGENT ORGANIZER





Exchangeable Dosing Syringe



#### Consumables

- ready made buffer solutions (each lot tested individually)
- separation columns for a variety of applications with long term stability
- ready made Ninhydrine solution
- a variety of standard solutions

### **Reagent Organizer**

• inert gas (N2) supply with adjustable pressure to prevent buffer/reagent oxidation and contamination

integrated cooling

#### **Amino Acid Reaction Module**

• only inert materials come in contact with buffer solutions & samples

• integrated 2-plunger reagent dosing pump with a flow rate from 0.01

- to 2.0 ml/min
- programmable reactor temperature

• integrated column oven with programmable temperature gradient and leakage sensor

• automatic washing of reactor coil with washing solution after the last sample

• integrated dual-channel photometer for the detection of amino acids at 440 and 570 nm.

### **Quaternary Gradient Pump**

- only inert materials come in contact with buffer solutions & samples
- flow rate of 0.01 to 10.0 ml/min
- integrated 4-channel vacuum degasser
- more than 100 programmable gradient steps with a resolution of 0.1%
- two-plunger pump for smooth eluent delivery
- also usable as HPLC gradient pump





Reagent Organizer Pressure Control



Integrated Vacuum Degasser



## **CLARITY AMINO**

The S433 control and data handling software is based on the DataApex Clarity chromatography data station (CDS). Although the user is free to use any other CDS, the control software stores at all times the analysis, including all system parameters of the separation program including the actual buffer pressure, reagent pressure and column temperatures during the run. The S433 control and data handling software is FDA 21 CFR part 11 compliant for use in GxP regulated laboratories. See some examples of the control software below.







All method parameters are stored within the chromatogram datafile. Buffer gradient, column temperature gradient, integration and calculation parameters as well as the actual buffer- and reagent pressure and actual column temperature are recorded during the run. The buffer gradient program is shown here as one of the tabs of the method screen.

The column temperature program.

Help

ethod Setup PhysioC-4PH+Sigr

Time [min]

Initia 100,0

10,00 11,00 30,00 41,00 63.00

0,4

0.0

No.

na (MODIFIE

C-4/Li D Reg L [%] [%]

0,0

0,0 0,0 0,0

Flow [mL/min]

0,450

0 450 0,450 0,450

0.450

[%]

-80

n

Cancel

- Ioy

Apply

Gradient Table

0,0 21,0 21,0 38,0 100,0

A-1/Li B-1/Li C-4/Li D Reg Li

60 80 100 120 [min.]

Time

OK

A-1/Li B-1/Li [%] [%]

79,0 79,0 79,0 62,0

0.0 100.0 0.0





The data acquisition screen with the actual gradient visualized with a different color for each buffer as background of the running chromatogram.



Example of a result screen.

# AMINO ACID APPLICATIONS

The Amino Acid Analyzer offers a high range of analysis profile from a variety of applications.

- Sample Matrices
  - Protein Hydrolysates
  - Physiological Fluids (Serum/Urine)
  - Pharmalogical Sample
- Applications / Features
  - Feedstuff
  - Foodstuff
  - Biogenic Amines
  - Pharma Quality Control
  - Pharma Conformity (Pharmacopoea)





Pharmacopoea Requirements for Amino Acid Analysis



### Protein Hydrolysates



Standard Hydrolysate analysis program. Retention time to Arginine 42 minutes. Injection to injection time 58 minutes.



Standard Oxidized Hydrolysate analysis program. Retention time to Arginine 46 minutes. Injection to injection time 59 minutes.



Oxidized hydrolysate program based on Lithium buffer system. Enabling to run hydrolysate and physiological programs without changing any buffers or columns. Retention time to Arginine 57 minutes. Injection to Injection time 77 minutes.



Oxidized hydrolysate program, showing 440nm channel for Hydroxyproline and proline. Additional amino acid B-Alanine eluting after Phenylalanine. Retention time to Arginine 68 minutes. Injection to injection time 88 minutes.



## Physiological Fluids



Physiological standard. Sigma Acidics/Neutrals/Basics. Routine program with Arginine eluting at 103 minutes. Injection to injection time 128 minutes.



Same as above showing p-Ser, Tau, p-ethanolamine, Asp, OH-Pro, Thr, Ser, Asn, Glu, Sar, alpha-Aminoadipic acid, Pro.



Extended Physiological – extended – program with separation of additional amino acids. Internal standards: d-Glcosaminic acid, Norleucine, Aminoethylcystein. Additional amino acids: Pipecolic acid, Sacharopine, Homocitrulline, allo-Isoleucine, Arginine succinic acid, Cystine-homocysteine-disulfide, d-Amino-levolinic acid, 3-hydroxy-kynurenine, Kynurenine, Homocarnosine. Retention time to Arginine 158 minutes. Injection to injection time 184 minutes.



Same as above showing middle part of chromatogram. alfa-Amino-adipic-acid, Pro, Gly, Ala, Cit, alpha-Aminobutyric acid, Val, Pipecolic acid, Cystine, Sacharopine, Homocitrulline, Met, allo-Isoleucine, Isoleucine, Arginine-succinic acid, Leucine, Cystine-homocystine-disulfide, Nle, Tyr, B-Ala, Phe. Cystathionine (not present) elutes between allo-Isoleucine and Isoleucine.





Same as above, showing end part of chromatogram. Phe, ß-AIBA, Homocystine, d-Amino-levolinic acid, GABA, Hydroxykynurenine, Kynurenine, His, 1M-His, 3M-His, Trp, Car, Homocarnosine, Ans, Aminoethylcysteine (ISTD), Hydroxylysine, Orn, Lys, NH3, Ethanolamine, Arginine. *Note*: Tryptophan elutes after the histidines.



Example of a short program containing Argininosuccinic acid (RT 8.0 min), Homocysteine, Methionine, allo-Isoleucine (RT 14.4 min.), Leucine, Tyrosine, Phenylalanine and Homocystine. Norleucine (ISTD, not present) elutes between Leucine and Tyrosine. Time from injection to injection 44 minutes.

# TECHNICAL SPECIFICATIONS

### S 7130 Reagent Organizer

- for storing all reagents, buffers and wash solutions.
- front side operated
- special valves for applying inert gas for oxygen-free stora

### S 5200 Autosampler

- for automatic injection of samples. All parts which come in contact with liquids are chemically inert and biocompatible (PEEK or PTFE).
- sampling system operating in x, y, and z-axis
- variable sample dosage without any sample loss
- loop overfill mode
- $\bullet\,$  reproducibility less than 1 % upon injection of 10  $\mu I$  variable volume.
- memory effect less than 0.01 % depending on the selected washing procedure
- large graphical display and keyboard for easy control
- injection volume programmable in 1  $\mu I$  increments
- temperature controlled sample compartment (5 70° C)
- programmable washing procedure with selectable volumes
- programmable port for purging
- programmable sample sequence
- optional: pre-column derivatisation mode

### S 2100 Solvent Delivery System

- Quaternary Pump for the reliable and reproducible mixing of the buffer solutions. All parts coming into contact with the buffers are chemically inert (PEEK or PTFE).
- dual plunger pump with special design for low pulsation (less than 1 %)
- flow range depending on installed pump head
- (0.01 to 10.00 ml/min)
- maximum pressure up to 350 bar (%000 PSI)
- battery buffered programs stored for the amino acid

determination (hydrolysates and physiological fluids)

- graphic display of gradient profile A, B, C, and D
- programmable mixing cycle for the buffer solution
- integrated 4-channel vacuum degasser
- programmable flushing procedure

• extended diagnostic features (e.g. high pressure control, low pressure control)

### S 4300 Amino Acid Reaction Module

- integrated 2-plunger reagent dosing pump with adjustable flow rate (0.01 to 2.0 ml/min)
- built-in dual filter photometer (440 and 570 nm) with
- constant signal output and signal summary option
- programmable signal offset
- three different risetimes selectable
- temperature controlled column oven (20 to 99° C  $\pm$ 1° C) with active cooling capability
- temperature controlled post-column derivatisation reactor (up to 180° C  $\pm 1^\circ$  C)
- automatic valve for coil flushing
- display of the actual system pressure
- safety features (e.g. leakage of reactor and column, high pressure)

# ORDER INFORMATION

### Amino Acid Analyzer S 433

Catalog No	Description	Dimensions
S001570	Amino Acid Analyzer S 433 - Hydrolysates	S 433 for analysis of protein hydrolysates
S001638	Amino Acid Analyzer S 433 - Physiological	S 433 for analysis of physiological fluids



# SYKAM PRODUCTS

## S 500 Series HPLC Systems S 600 Series HPLC Systems



## S 150 Plus IC Systems



## Sykam OEM Solutions





### S 6000 Valve Series



## Sykam Preparative Solutions





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