

# **Elite-AAK Amino Acids Analysis System User's Guide**

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# Introduction

## **Fast easy & accurate reliable analysis system**

Elite-AAK amino acids analysis system contains a common binary high-pressure gradient system. By chromatography workstation, it can guarantee precise flow rate and mobile phase components.

Adopting DNFB pre-column derivation and a special column, 18 kinds of amino acids (as shown in Table 1) can be separated distinguishably in 40mins and detected by ultraviolet detector.

The system provides excellent reproducibility, high sensitivity and good resolution of amino acids analysis.

## **Technical Specifications**

The resolution of some amino acids which are hard to separate is more than 1.0, better than the traditional amino acids analyzer.

The excellent reproducibility and stability of the analytical method can be achieved, guarantee the reliable result of qualitative and quantitative analysis.

The detection limit of amino acids is up to pmol degree, satisfying the requirement of trace analysis.

18 kinds of amino acids are separated in 40 minutes, which greatly increase the efficiency.

Multipurpose system, it can also be used for standard HPLC analysis.

Buffer and organic solvent, used as mobile phase, no need of special reagent, which is economical.

Expert in chromatographic instruments and analytical chemistry maintain vigorous technical support and guarantee.

Tab.1 the information of 18 kinds of amino acids

No.	Name	Shorten form	structural formula	M.W.	I.P.
	Aspartic	Asp	HOOC-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	133.10	2.77
	Glutamic Acid	Glu	HOOC-(CH <sub>2</sub> ) <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	147.13	3.22
	Serine	Ser	HO-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	105.09	5.68
	Glycine	Gly	NH <sub>2</sub> -CH <sub>2</sub> -COOH	75.07	5.97
	Threonine	Thr	CH <sub>3</sub> -CH(OH)-CH(NH <sub>2</sub> )-COOH	119.12	5.64
	Arginine	Arg	HN=C(NH <sub>2</sub> )-NH-(CH <sub>2</sub> ) <sub>3</sub> -CH(NH <sub>2</sub> )-COOH	174.20	11.15
	Proline	Pro	NH-(CH <sub>2</sub> ) <sub>3</sub> -CH-COOH 	115.13	6.30
	Alanin	Ala	CH <sub>3</sub> -CH(NH <sub>2</sub> )-COOH	89.09	6.00
	Valine	Val	(CH <sub>3</sub> ) <sub>2</sub> -CH-CH(NH <sub>2</sub> )-COOH	117.15	5.96
	Methionine	Met	CH <sub>3</sub> -S-(CH <sub>2</sub> ) <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	149.21	5.74
	Isoleucine	Ile	CH <sub>3</sub> -CH <sub>2</sub> -CH(CH <sub>3</sub> )-CH(NH <sub>2</sub> )-COOH	131.17	5.94
	Leucine	Leu	(CH <sub>3</sub> ) <sub>2</sub> -CH-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	131.17	5.89
	Tryptophan	Trp	Ph-NH-CH=C-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH 	204.23	5.89
	Phenylalanine	Phe	Ph-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	165.19	5.48
	Histidine	His	NH-CH=N-CH=C-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH 	155.16	7.47
	Lysine	Lys	H <sub>2</sub> N-(CH <sub>2</sub> ) <sub>4</sub> -CH(NH <sub>2</sub> )-COOH	146.19	9.59
	Tyrosine	Tyr	HO-p-Ph-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	181.19	5.66
	Cysteine	Cys	HS-CH <sub>2</sub> -CH(NH <sub>2</sub> )-COOH	121.15	5.02

# Unpacking

Carefully unpack the product and accessories from the shipping container and inspect both the unit and packing for any signs of damage. If any damage is noted, contact the shipping company immediately.

Carefully check the packing list. If you find anything is missing, please contact manufacturer or your dealer.

# Installation

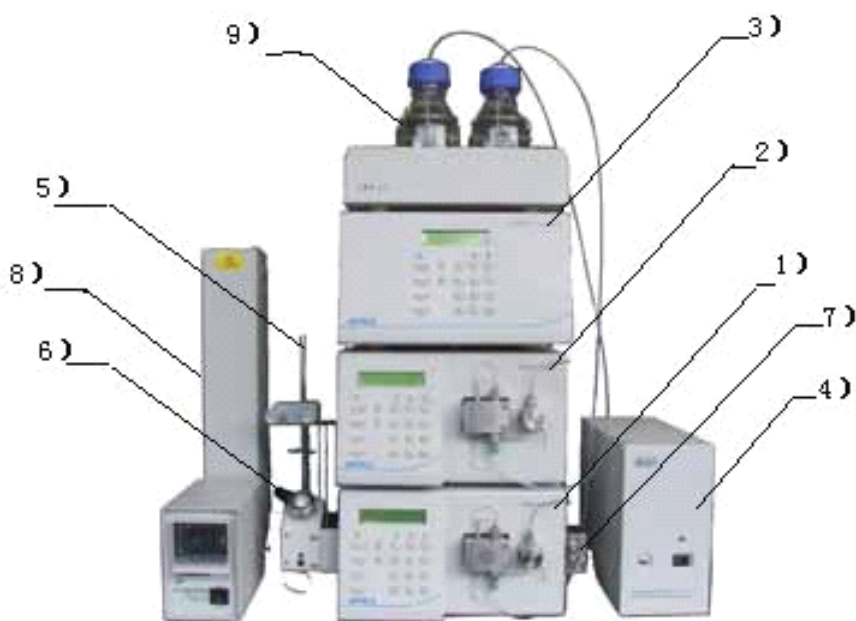
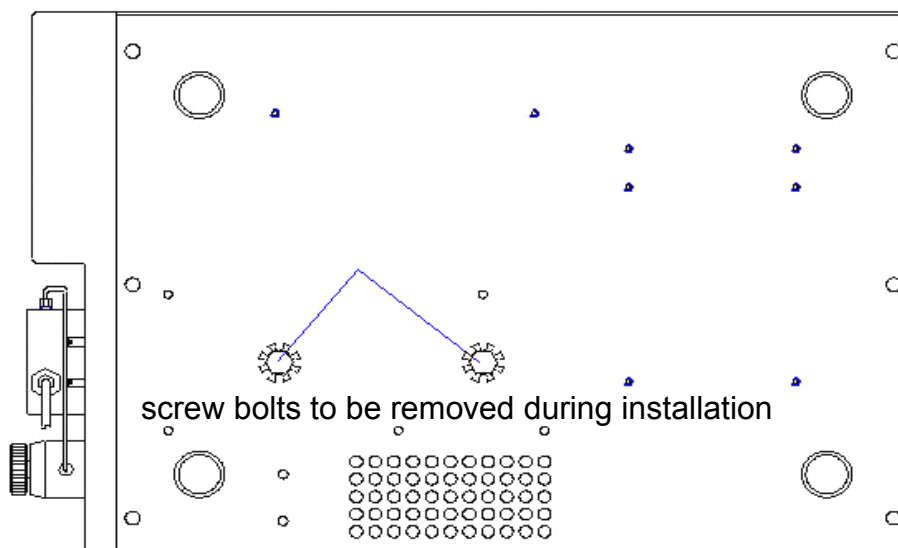


Fig.1 The configuration of Elite-AAK amino acids analysis system

- 1) P230 pump A;
- 2) P230 pump B;
- 3) UV230+ detector;
- 4) EC2000 Chromatography workstation;
- 5) ZJ-1 valve bracket;
- 6) 7725i manual injection valve;
- 7) Gradient mixer;
- 8) ZW column temperature control system;
- 9) mobile phase reservoir

## P230 pump A&B

Remove the two screw bolts from the under plate of P230 pump shown as the following Fig.2. The purpose of the screws is to secure the pump during transportation. If the screws are not removed, audible “cracking” might be appeared during operation. Please keep the screws for future to screw them back in case of return.



*Fig.2: the under plate*

Fig.1 illustrates the instrument configuration for binary gradient system. Place Pump A over Pump B.

## UV230+ UV-Vis detector

Place UV230+ over P230 pump B.

## EC2000 Chromatography workstation

Place chromatography workstation between the pumps and the computer. For further details, please refer to EC2000 chromatography workstation installation manual.

## **ZJ-1 valve bracket**

Fix ZJ-1 valve bracket beside P230 pump A.



*Fig.3: ZJ-1 valve bracket and manual injection valve*

## **7725i manual injector**

Install 7725i manual injection valve on ZJ-1 valve bracket.

## **Gradient mixer**

Fix the mixer on the other side of P230 pump A.



*Fig.4: gradient mixer and pump A*

## **ZW column oven**

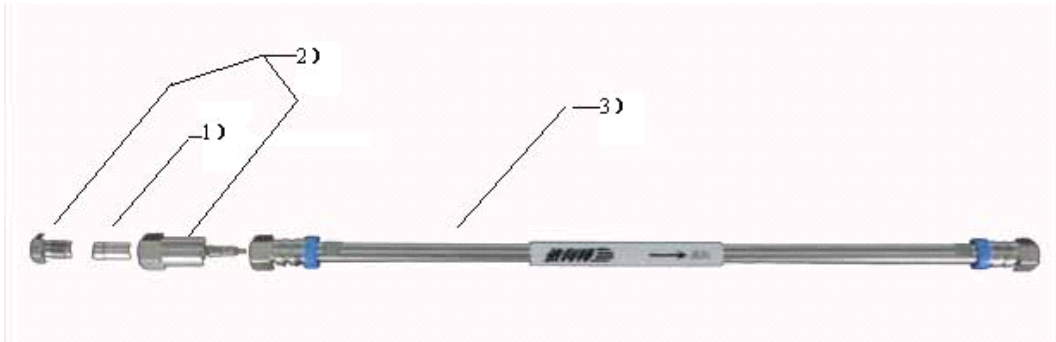
Place ZW column oven on the left of P230 pump A. For further details, please refer to ZW column temperature control system operator's manual.

## **Elite-AAK column**

## **UNIGUARD™ Direct-Connect Drop-in Guard Cartridge Holder**

## **BioBasic 8 Drop-in Guard Cartridges**





*Fig.5: Elite AAK column and Guard column*

- 1) BioBasic 8 Drop-in Guard Cartridges; 2) UNIGUARD™ Direct-Connect Drop-in Guard Cartridge Holder; 3) Elite-AAK column

Put the column and guard into the ZW column oven

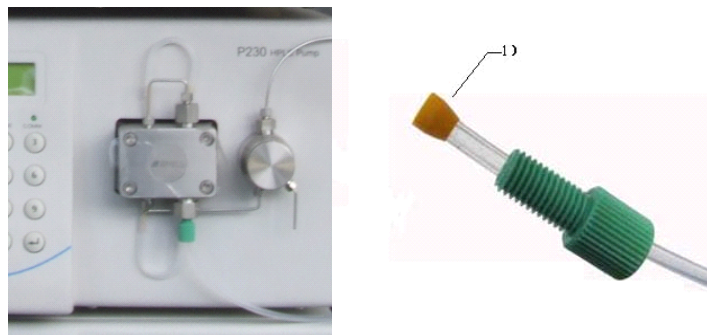
**NOTE: Attention to the direction of column.**

## Tubing Connection

### Connect mobile phase reservoir to P230 pump

Place the bottle-head assembly into mobile phase solvent reservoir. Connect the bottle-head assembly to the inlet of P230 pump with Teflon solvent tubing. Each P230 pump should have an individual solvent reservoir and dedicated bottle-head assembly. For details, please refer to P230 pump operator's manual.

**NOTE: Attention to the direction of Flangeless Ferrule.**

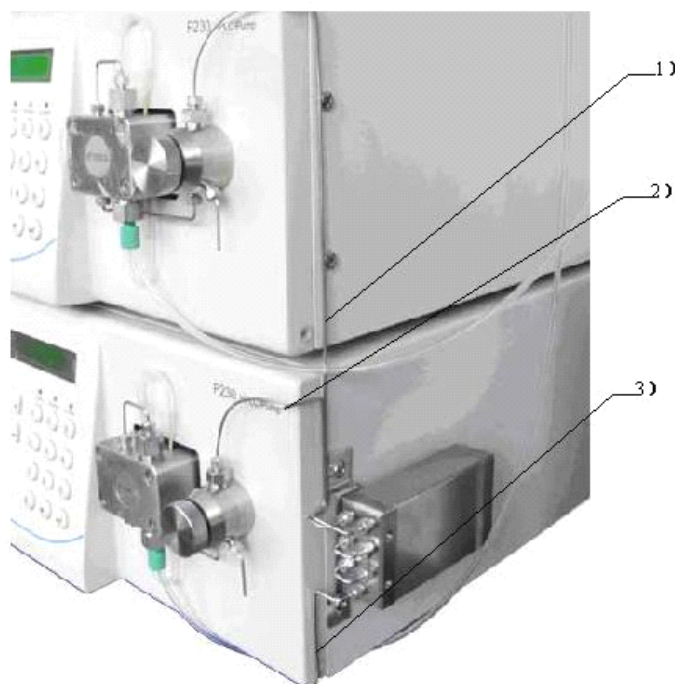


*Fig.6: the inlet of P230 pump*

- 1) Flangeless Ferrule 1/8 in

## Connection between Gradient mixer and Pumps

Connect the outlets of P230 pump A&B with the top left entrance and the top right entrance of gradient mixer respectively by using stainless steel capillary tube.



*Fig. 7: the connection between Gradient mixer and Pumps*

- 1) Connect the outlet of P230 pump B with the entrance of gradient mixer;
- 2) Connect the outlet of P230 pump A with the entrance of gradient mixer;
- 3) Connect the mixer outlet with the manual injection valve inlet port #2

## Connection between Gradient mixer to injector

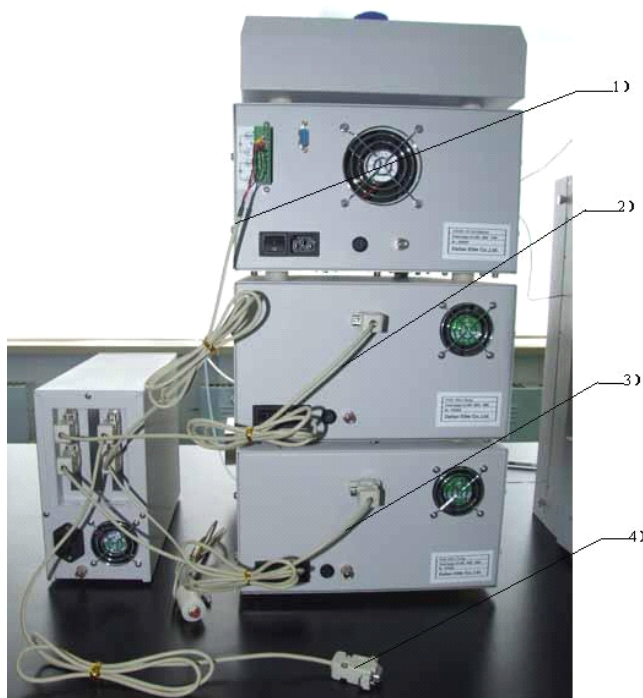
Connect the mixer outlet with the 7725i manual injector inlet port #2.

## Column connection

Remove the column seal from the end of the column. Connect the inlet of the column to the port #3 outlet of the manual injector. There should be an arrow on the column indicating the mobile phase flow direction.

# Electrical Connection

Chromatography Workstation connection



*Fig.8: Electrical connection for chromatography workstation*

## Connect to detector

1) Input signal transmission cable from detector

## Connect to pump

- 2) Control cable of P230 pump B
- 3) Control cable of P230 pump A

## Connect to computer

- 4) Output signal transmission cable should connect to computer (RS232 or USB).

**NOTE: Please carefully read this entire section and refer to the rear panel diagram below before making any electrical connection. Do not attach the system's power cord until all the other connections have been made.**

# Power connection

After you've made all the necessary connections, you're ready to attach the power cord and turn the power on.

# Software installation

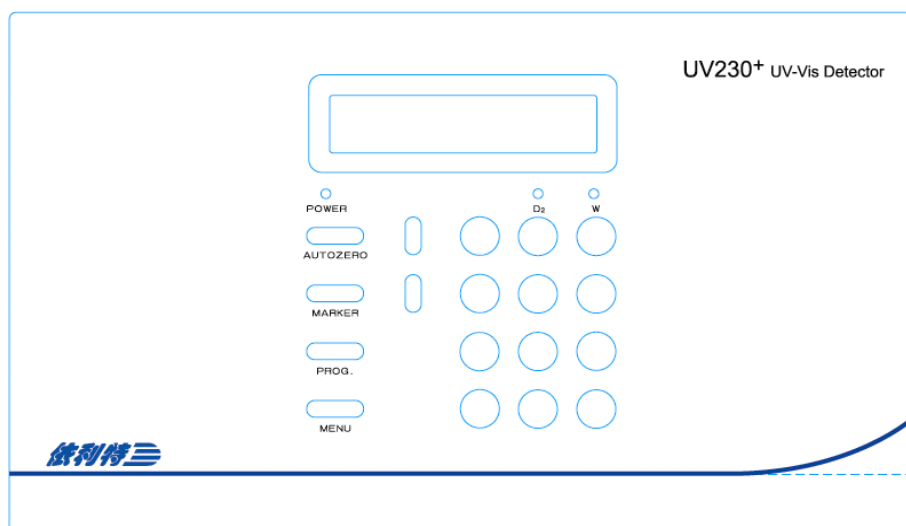
Turn on the computer, shut off all the Windows application procedures.

Insert the EC2000 Chromatography Workstation Software CD into the CD-ROM driver, and then proceed the installation procedures according to the screen indications. After the installation, there will be a shortcut menu of "E2000 Data System" and "EC2000 GPC system" in the desktop of Windows 2000/XP.

Till now, all the Installation of the P230 HPLC Binary Gradient System completed. Please read the operation manual of each HPLC unit thoroughly before your operation, keep the manuals for future reference. If there are any questions, please contact Dalian Elite Analytical Instruments Co., Ltd.

# Operation

## Detector setting



*Fig.9: the front panel of UV230+ detector*

Turn on the power of detector. The green LED on the front panel indicates that a Deuterium (D2) is lit. Select the wavelength at UV360nm.

**NOTE: Do not try to go below 190 nm or over 720 nm. The wavelength drive may be damaged as a result.**

## Pump setting

Select external control model.

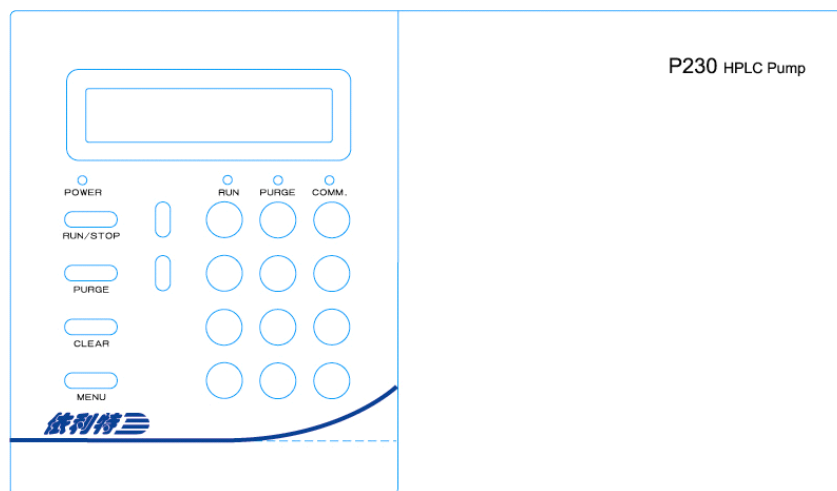
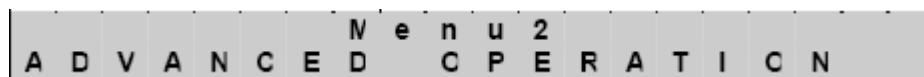


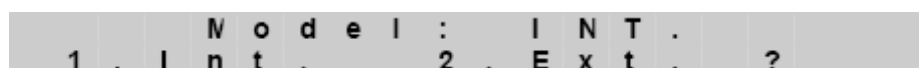


Fig.10: the front panel of P230 pump

Press the “MENU” key, select “Menu 2”;



Press “” key, select “2”, external model. Then press “”. Be sure the “COMM” light is lit.



## ZW column Oven setting

Column temperature: 27°C

## Workstation setting

Set the gradient profile as Tab.2.

*Tab.2: Gradient profile for the analysis*

#	Time(mi n)	Flow rate(mL/min)	Pump A(%)	Pump B(%)	Shape(s)
1	0	1.20	16	84	0
2	0.3	1.20	16	84	1
3	4	1.20	31	69	1
4	9.5	1.20	36	64	1
5	17	1.20	55	45	1
6	28	1.20	65	35	1
7	34	1.20	100	0	0
8	36	1.20	100	0	1
9	38	1.20	16	84	0

**NOTE: For more information about how to operate, please read the operation manual of each HPLC unit thoroughly. If there are any questions, please contact Dalian Elite Analytical Instruments Co., Ltd.**

# Experiment

## Reagents

### Derivatization reagent



*Fig. 11: derivatization reagent*

**NOTE: Toxic. Ingestion, Inhalation or skin contact may be harmful to health even cause cancer.**

### Amino acids standard

This standards contains 18 amino acids shown as Tab.1. For details, refer to Contents of 18 standard amino acids. Dilute the standard to 100mL with water in a volumetric flask.

### Buffer A

Dilute 4.2g Buffer A to 100mL with water in a volumetric flask.

### Buffer B

Dissolve 3.4g Buffer B in about 200mL water, then add 145.5mL of NaOH solution (0.1M).

Dilute the solution above to 500mL with water in a volumetric flask.

### **Eluent A**

ACN : H<sub>2</sub>O (50 / 50)

### **Eluent B**

Dissolve 8.2g solid B in about 1800mL water. Adjust pH to 6.4 with glacial acetic acid. Then add 20mL DMF.

Dilute the solution above to 2000mL with water in a volumetric flask.

**NOTE: Eluent B should be filtrated by 0.45µm filtration membrane.**

**NOTE: Eluent A & B should be vibrated by Ultrasonic cleaner for 10~15 mins before being used.**

### **Sample pretreatment**

Add 5mL sample of the solution of amino acids with 5mL of buffer A into a 50mL volumetric flask. Then add 5mL of derivatization reagent and surge it.

Keep the reaction in a 60°C water bath for 60mins, keep dark.

Let the flask stand in room temperature for 30mins.

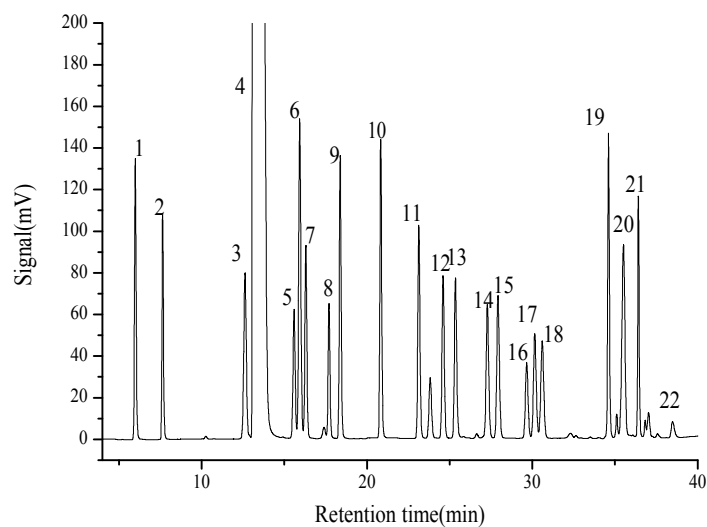
Dilute the solution to 50mL with Buffer B in volumetric flask.

Inject 20µL sample of derivated amino acids onto the column of Elite-AAK system.

**NOTE: Sample must be filtrated by 0.45µm filtration membrane before been injected.**

**NOTE: Make sure that you wear glove and glass during this operation.**

## Chromatogram



*Fig.12 the standard chromatogram of 20 kinds of amino acids*

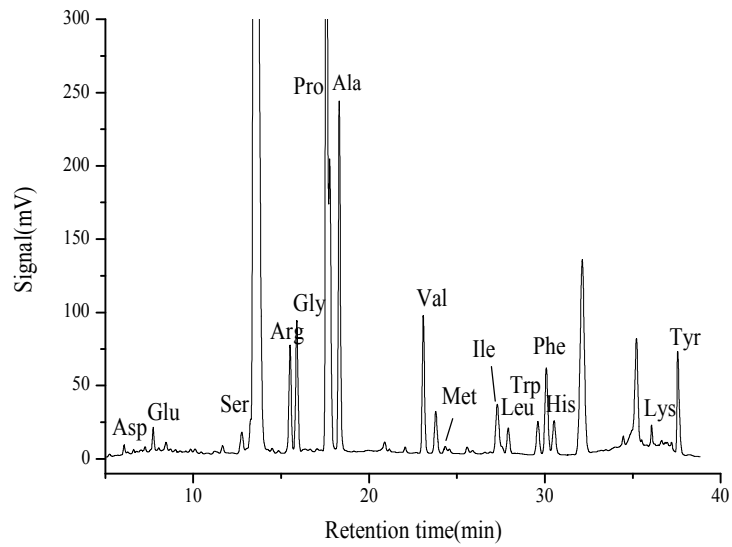
Asp; 2. Glu; 3. Ser; 4. DNP-OH; 5. Arg; 6. Gly; 7. Thr; 8. Pro; 9. Ala; 10.  $\gamma$ -AB; 11. Val; 12. Met; 13. Cys; 14. Ile; 15. Leu; 16. Trp; 17. Phe; 18. His; 19. Orn; 20. DNFB; 21. Lys; 22. Tyr

**NOTE: The Fig.12 contains 20 amino acids.  $\gamma$ -AB and Orn are not mentioned in Tab.1.**

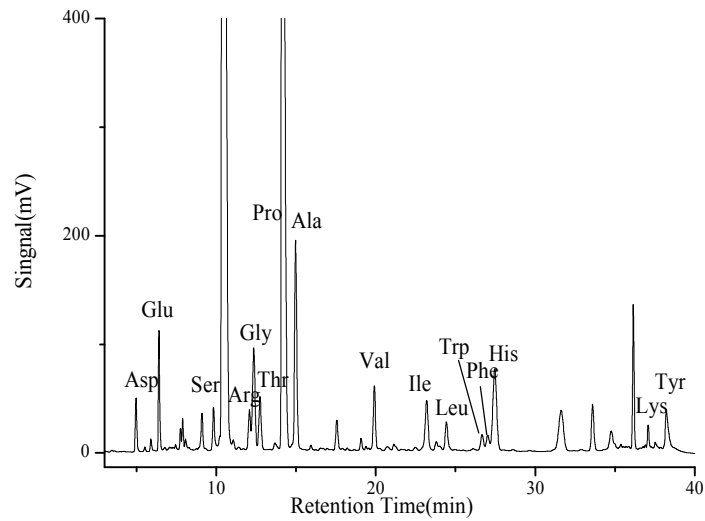
**NOTE: When the experiment ends every day, the column should be eluted by Eluent A for at least 30mins.**

# Applications

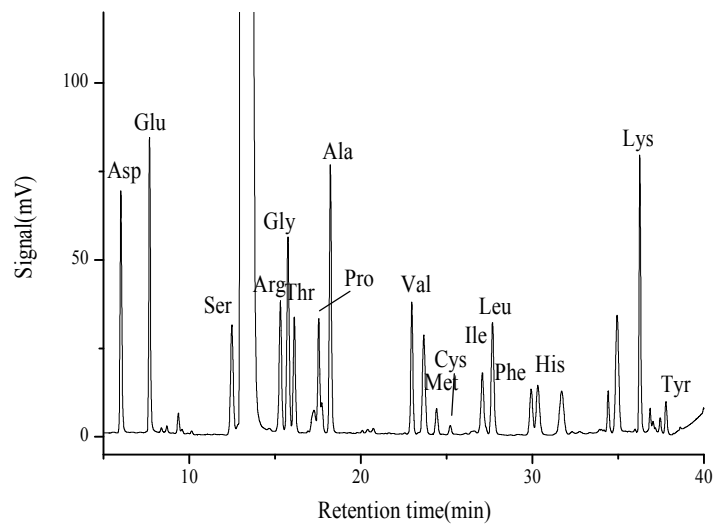
## Beer



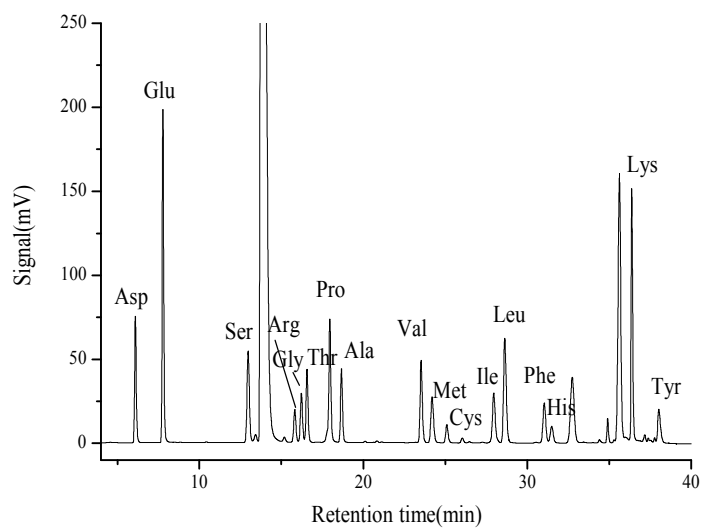
## Wine



## Cordyceps Sinensis



## Milk



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