Fast Hydrolysis for Determination of Lipopeptides on Solid Surface

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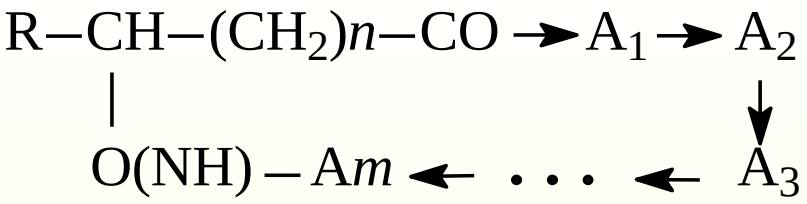
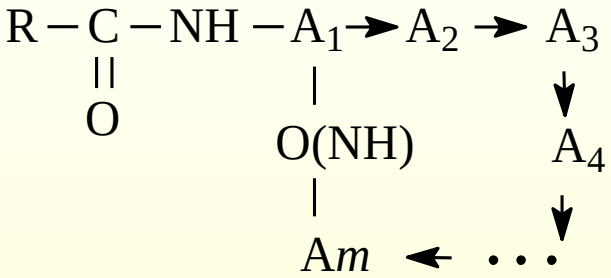
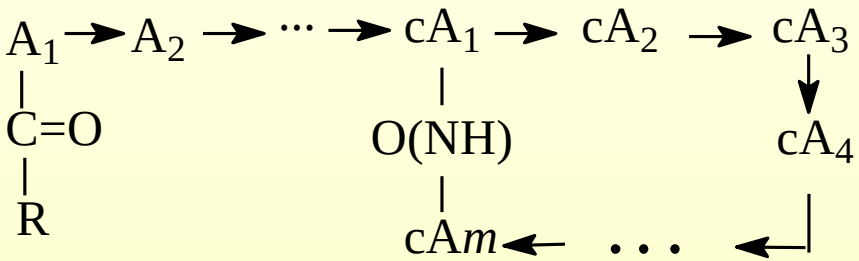
1 Introduction

1.1 lipopeptides

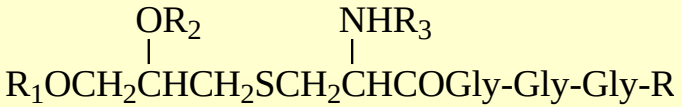


Cyclic ones

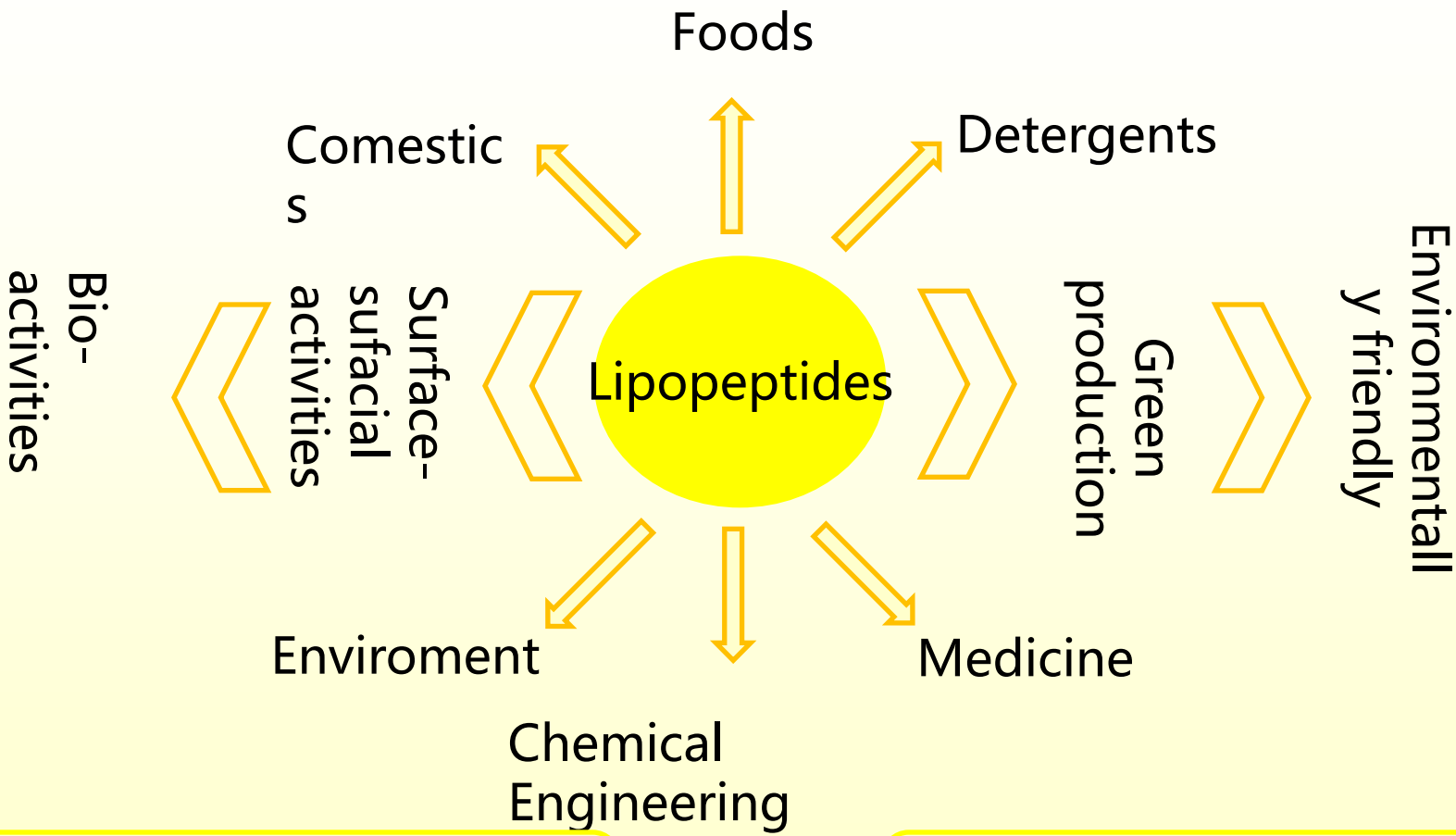




Linear ones



1 Introduction



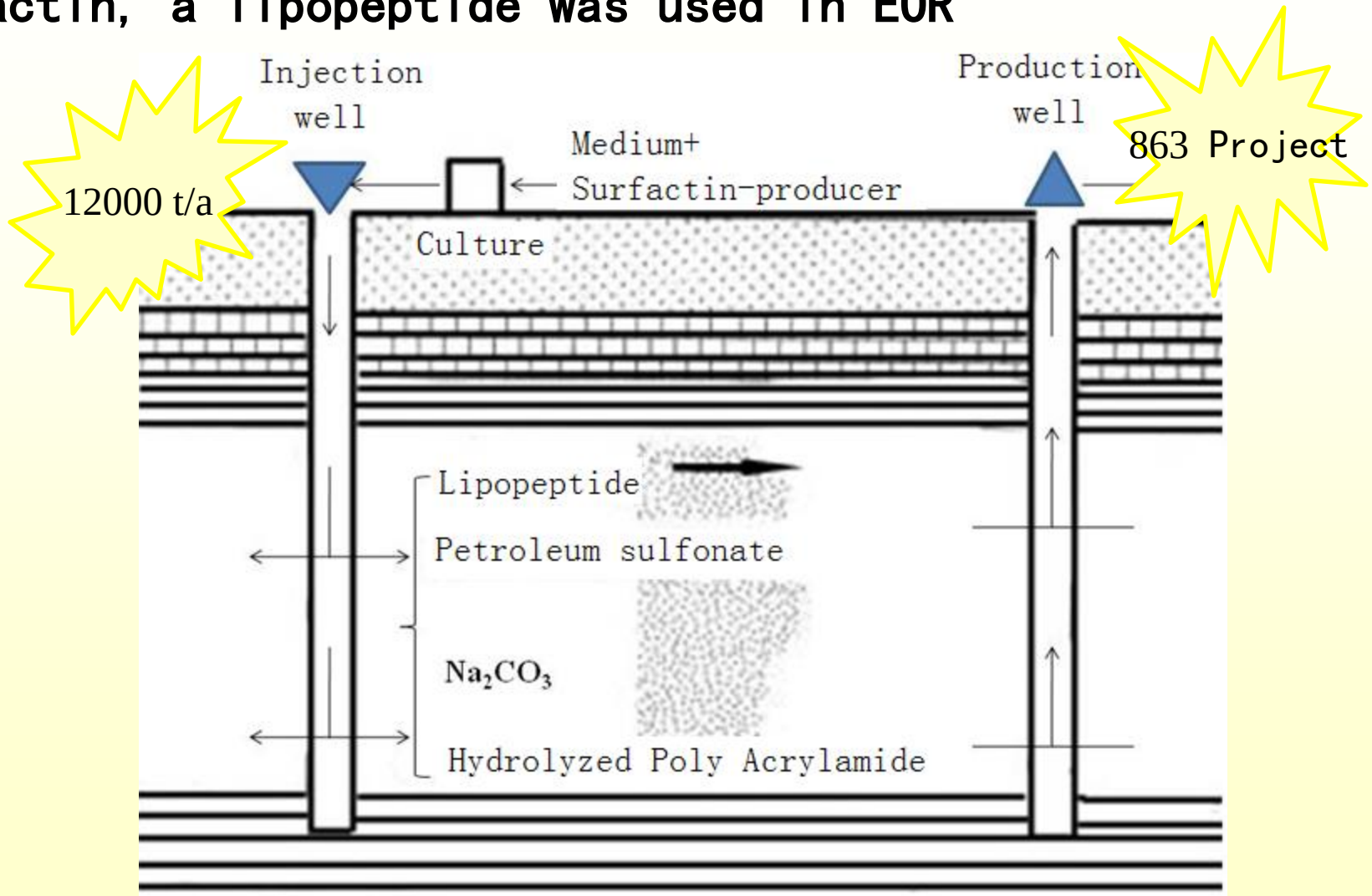
Low contents in samples

Mixture

No sensitive methods

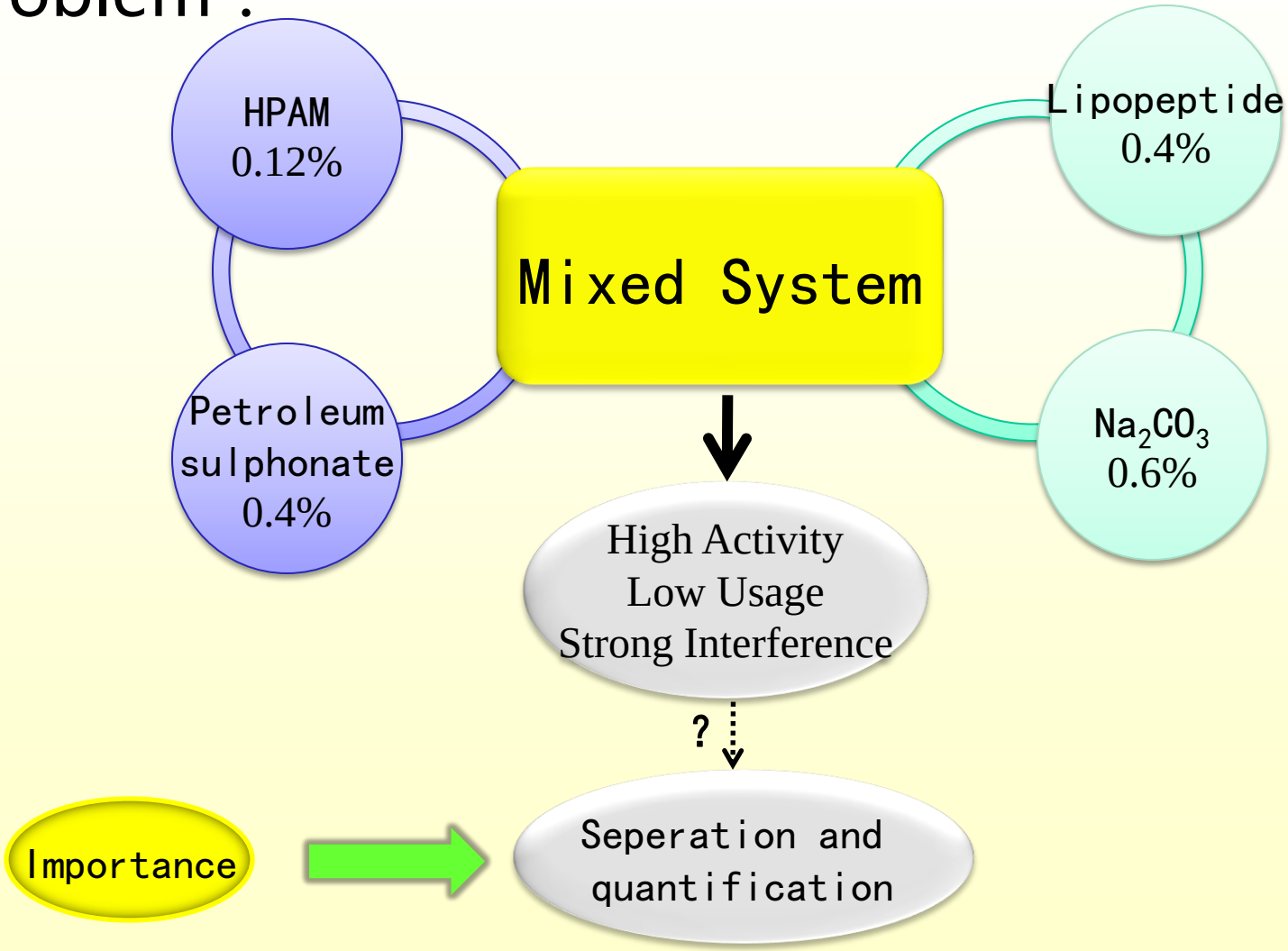
1 Introduction

Sufactin, a lipopeptide was used in EOR



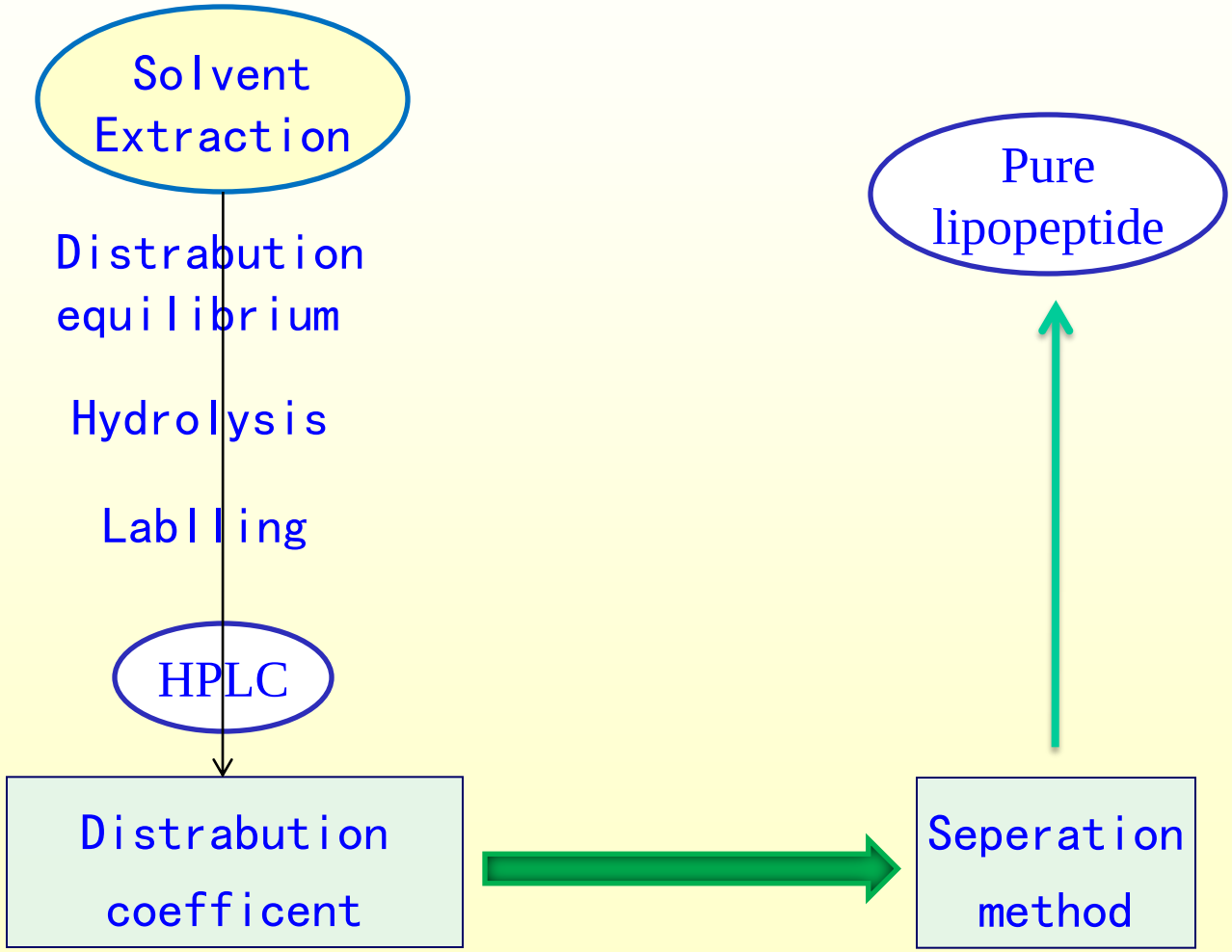
1 Introduction

Problem :



1 Introduction

Seperation method was established



1 Introduction

1.2 Lipopeptide determination

Seperation

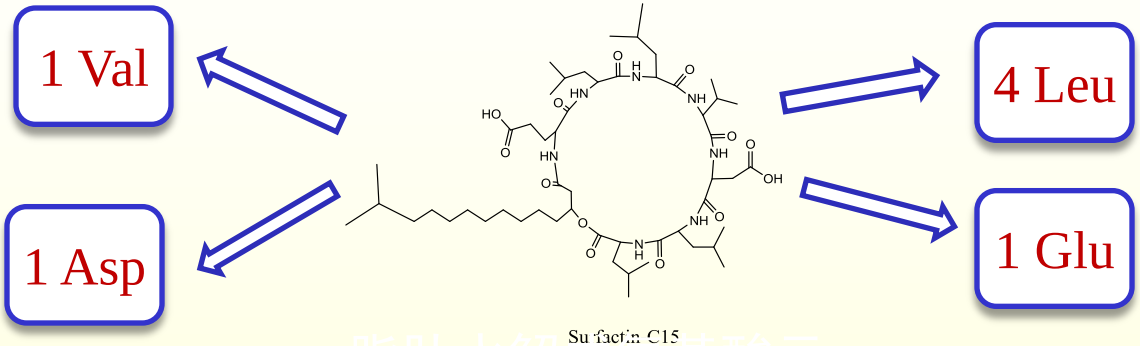
- Acid precipitation
- Sovent extraction
- Chromatography

quantification

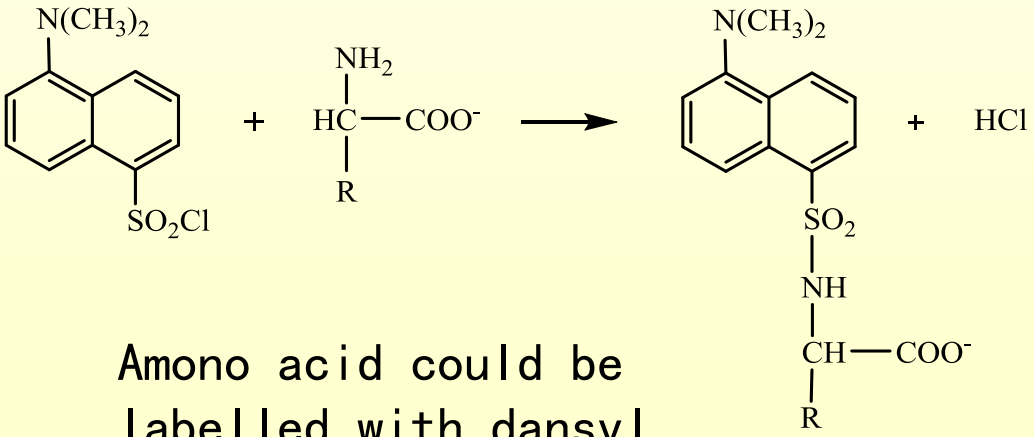
- **HPLC**
  - Natuaral ultraviolet absorbence
  - Low sensitivity
  - Needing pure standard
- **Spectrometry of Amino acid**
  - Solvent seperation
  - Hydrolysis
  - Total gross
- **GC-MS**
  - Hydrolysis
  - Trimethylsilylation

1 Introduction

Object

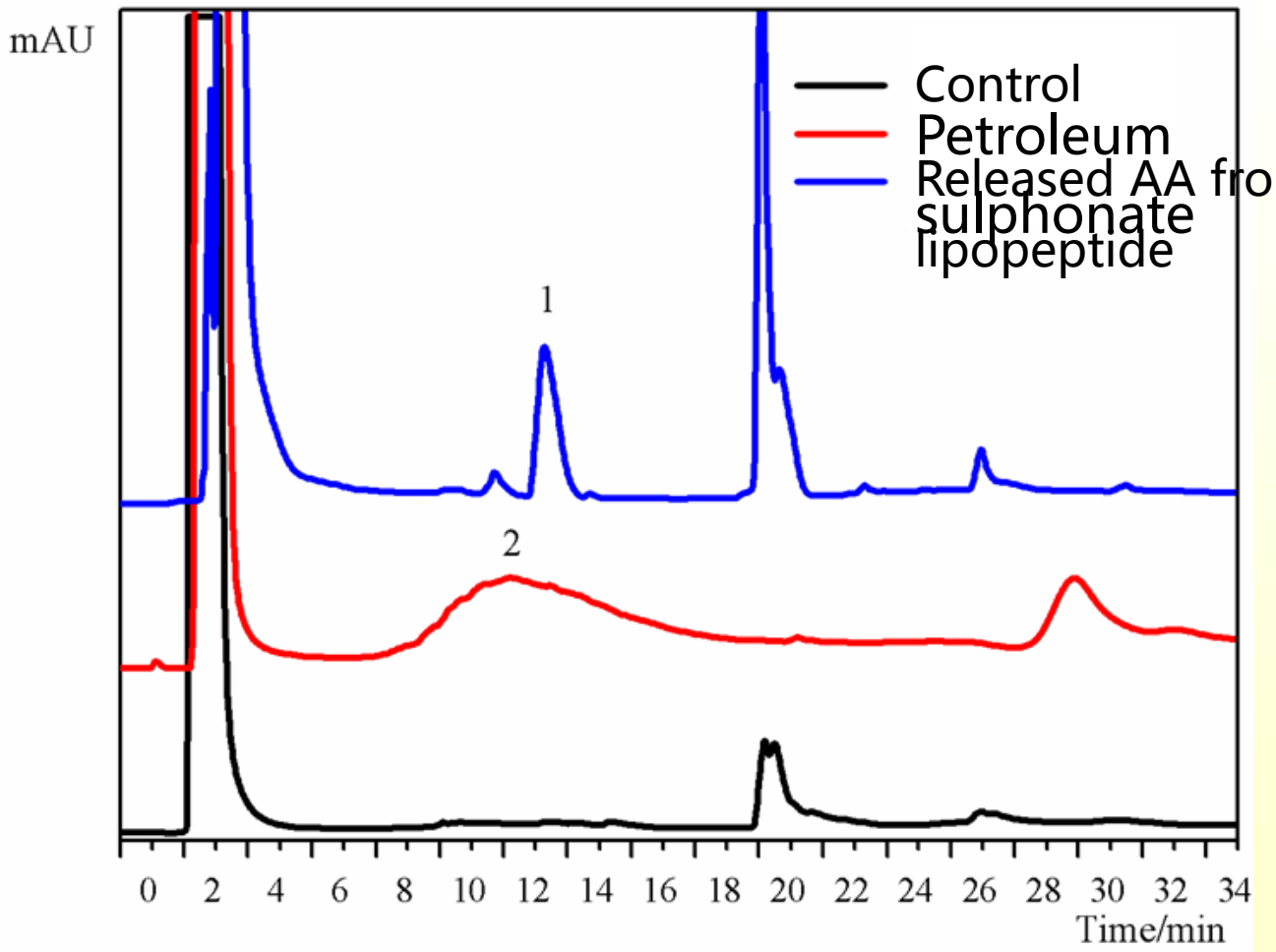


脂肽水解成氨基酸示意图



Amono acid could be labelled with dansyl

1 Introduction



HPLC of Lablled Amino Acids Released by Hydrolysis of Lipopeptide

1 Introduction

1.3 Lipopeptide Hydrolysis

Tranditional method stems from protein hydrolysis

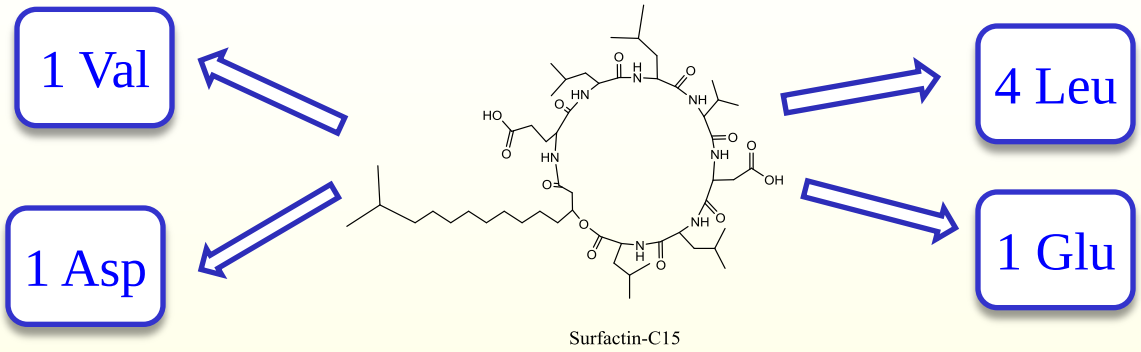
Hydrolysis Condition: in 6 M HCl at 110°C for 24 h

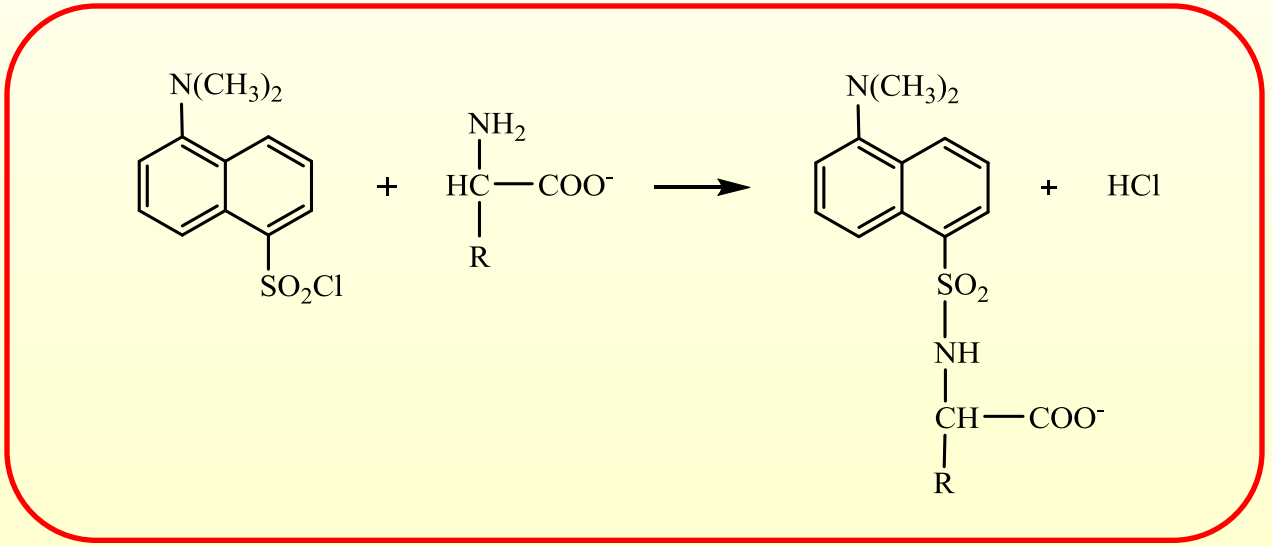
It has been improved to

Hydrolysis Condition: in 6M HC at 90°C for 24 h

At relative low temperature, but for **long time** and needing **post treatment** of evaporating the acid water solution

2 Experiment





Anino acids will be labled by dansyl

2 Experiment

Stainless Steel Reaction Kettle

lined PTFE

Sample was stained

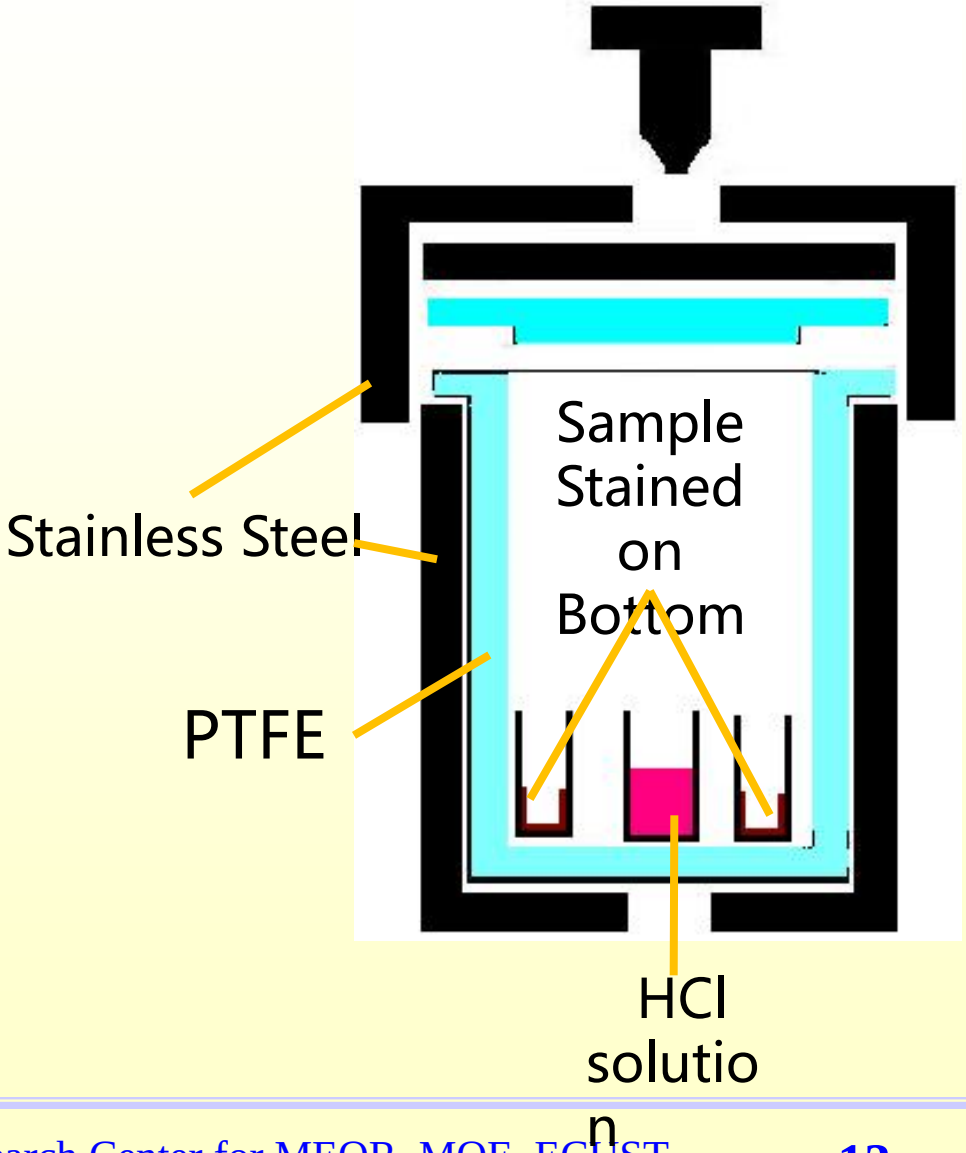
HCl solution was filled seperately

Sealed

Heated

Lablled directly

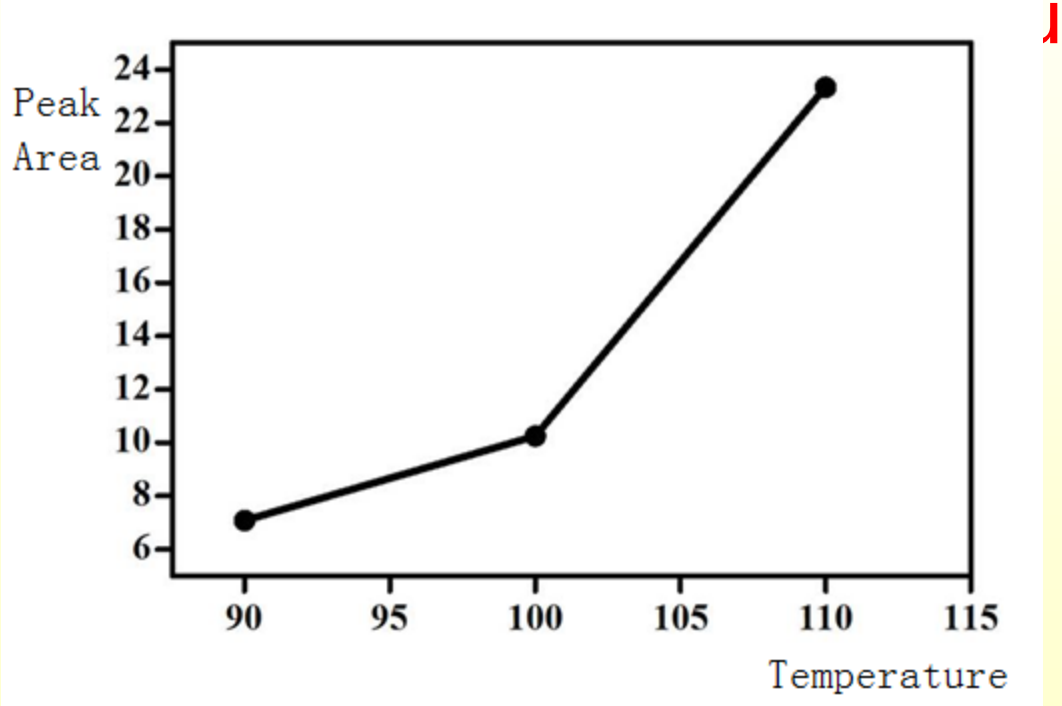
Determined



3 Results and Discussion

Orthogonal experimental design for optimal condition

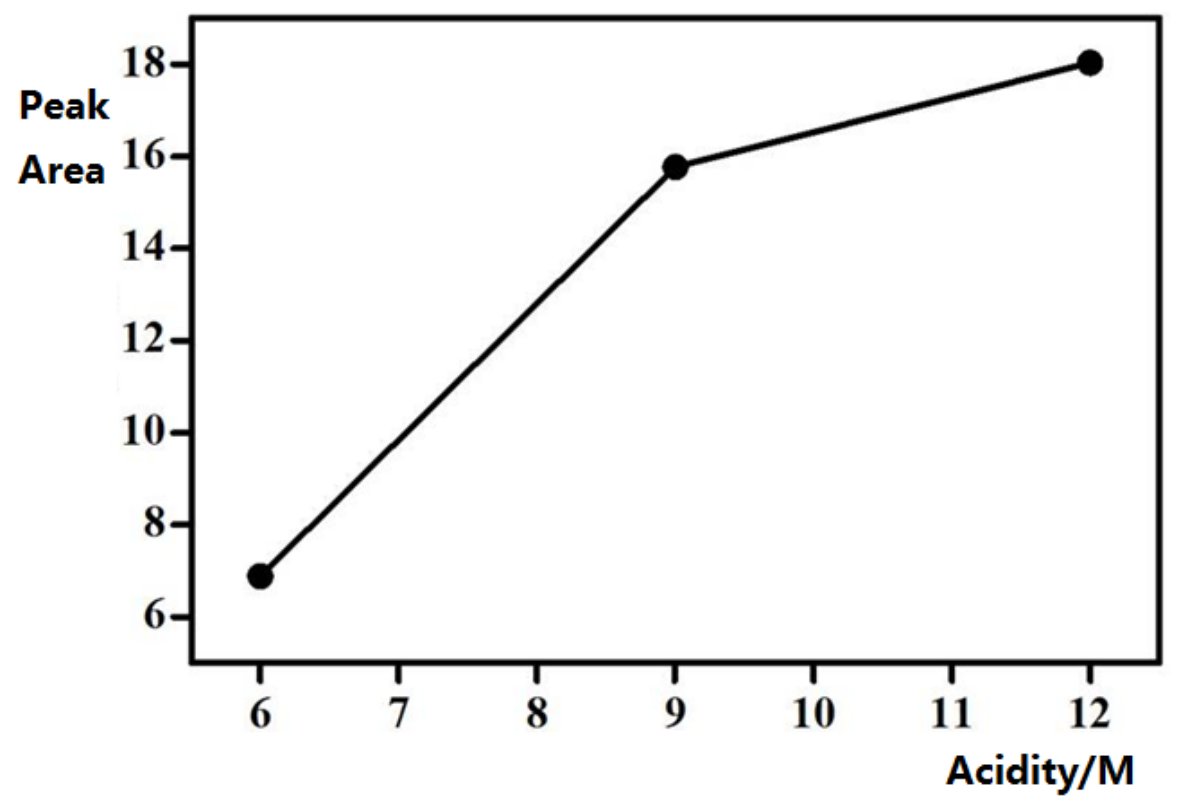
3.1 Effe ures



Index increased sharply with temperature increase

3 Results and Discussion

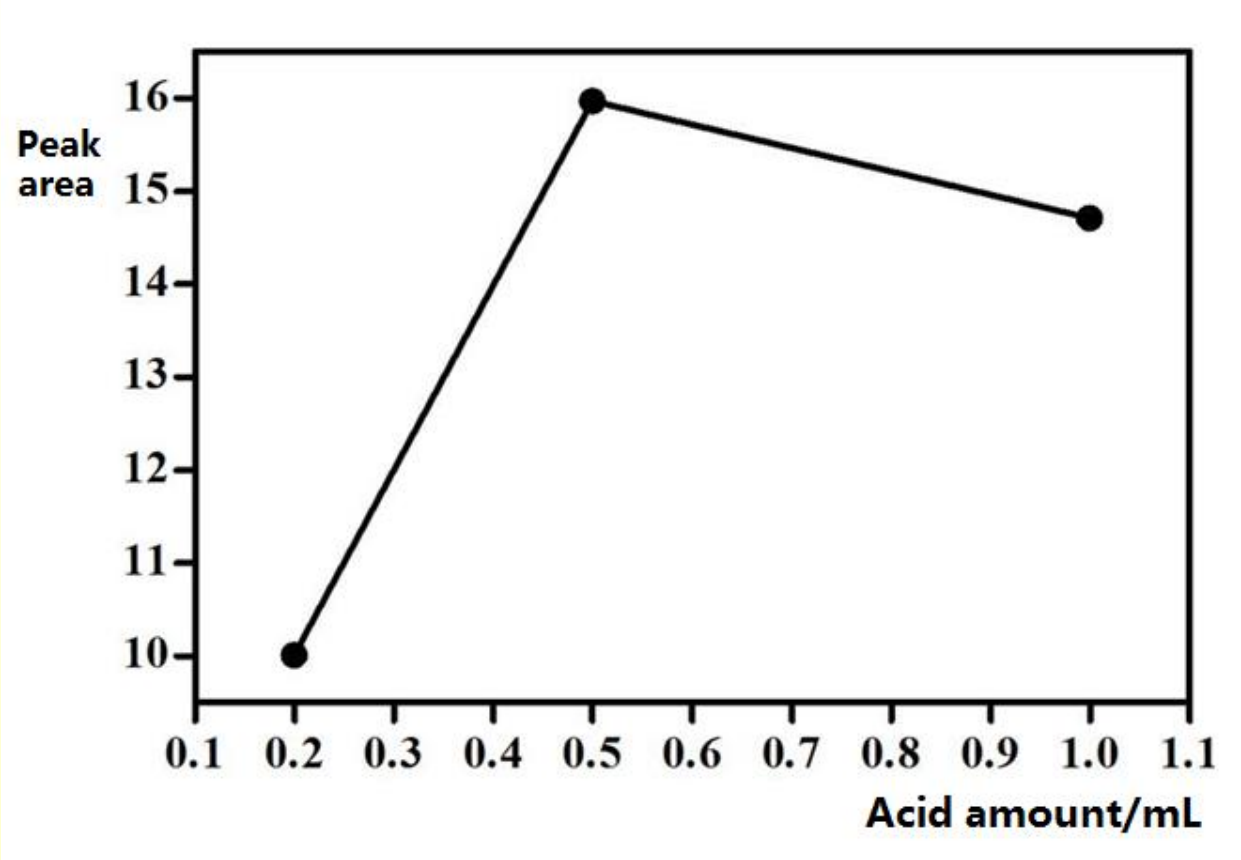
3.2 Effect trend of acid concentrations



Index increased sharply then slowly with acidity increase

3 Results and Discussion

3.3 Effect trend of acid quanlities



Index increased sharply then dicreased slowly with acid amount

3 Results and Discussion

The deviations were 18, 12 and 6 for temperature, acidity and acid amount, respectively.

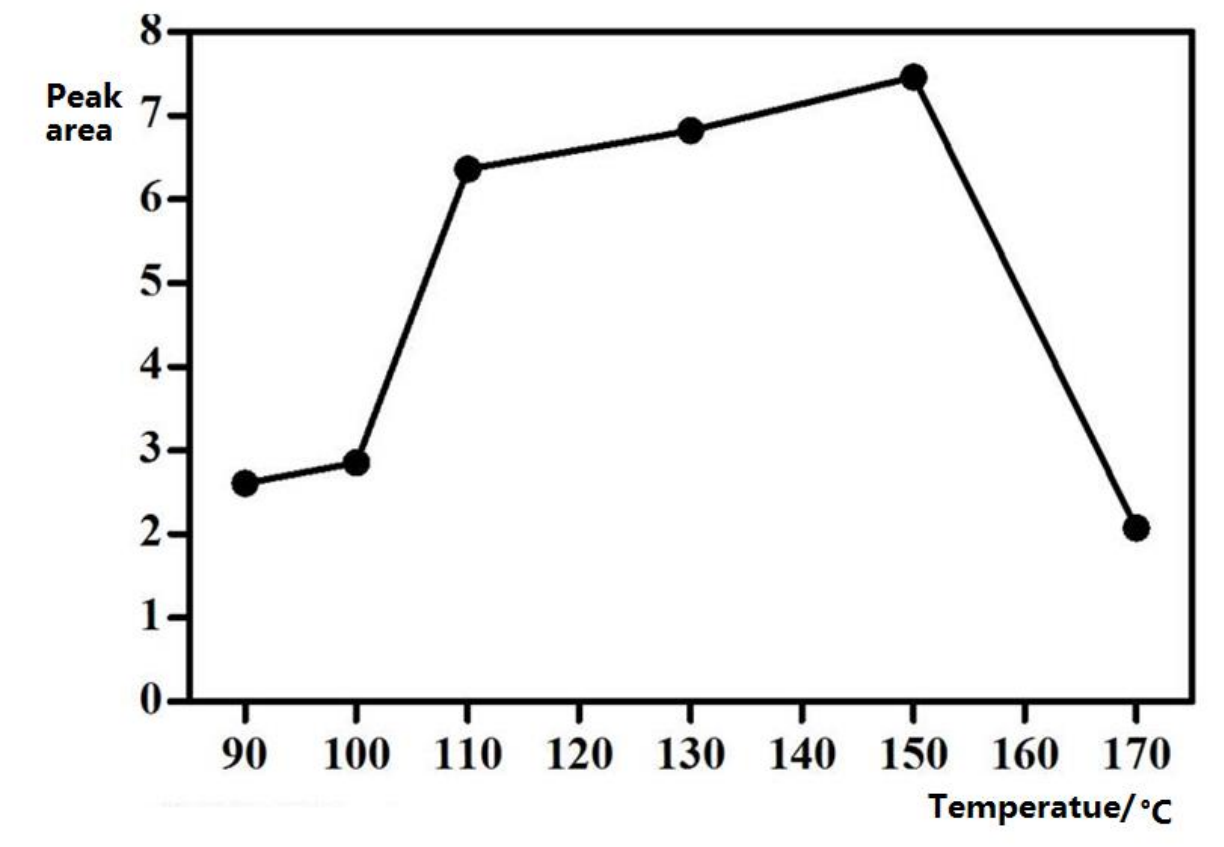
The acidity was 12 M, could not increase.

The acid amount should be fixed at 0.5 mL.

The hydrolysis temperature and time will be modified for good results

3 Results and Discussion

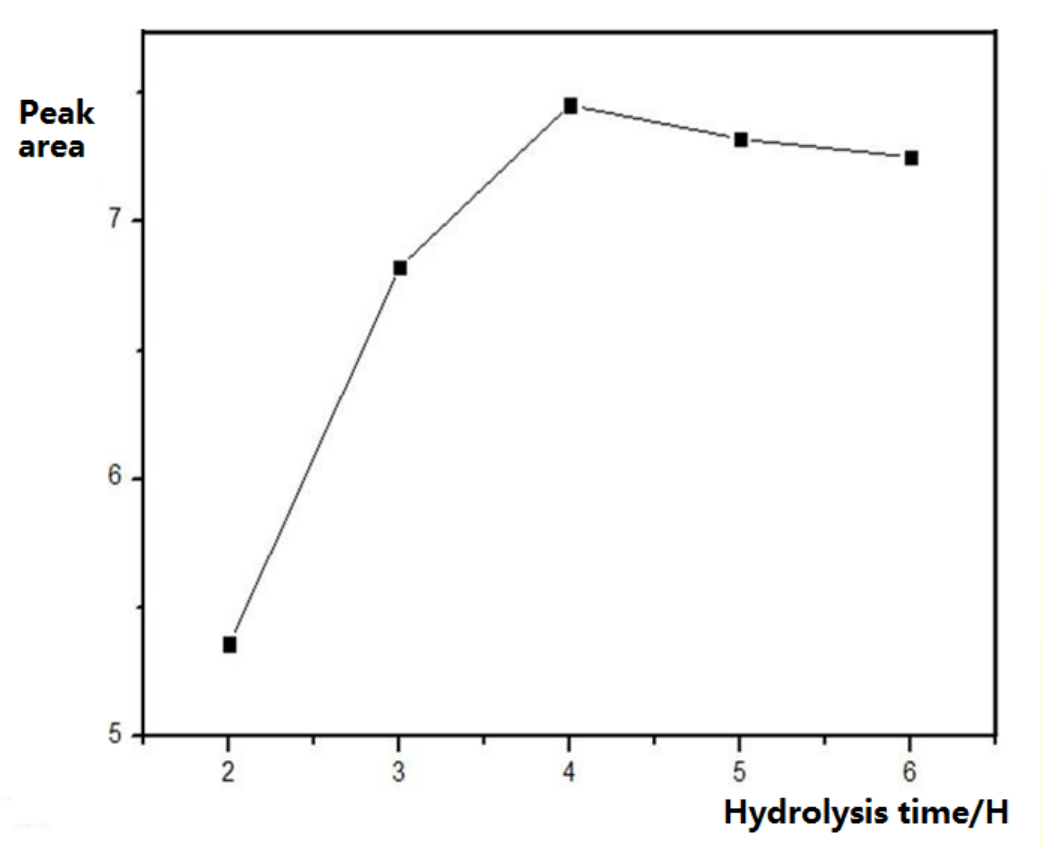
At defferent temperature using 0.50 mL 12 M HC for 4 H



Index was allmost stable between 110-150 celsius degree

3 Results and Discussion

For defferent time at 150 °C using 0.50 mL 12 M HC

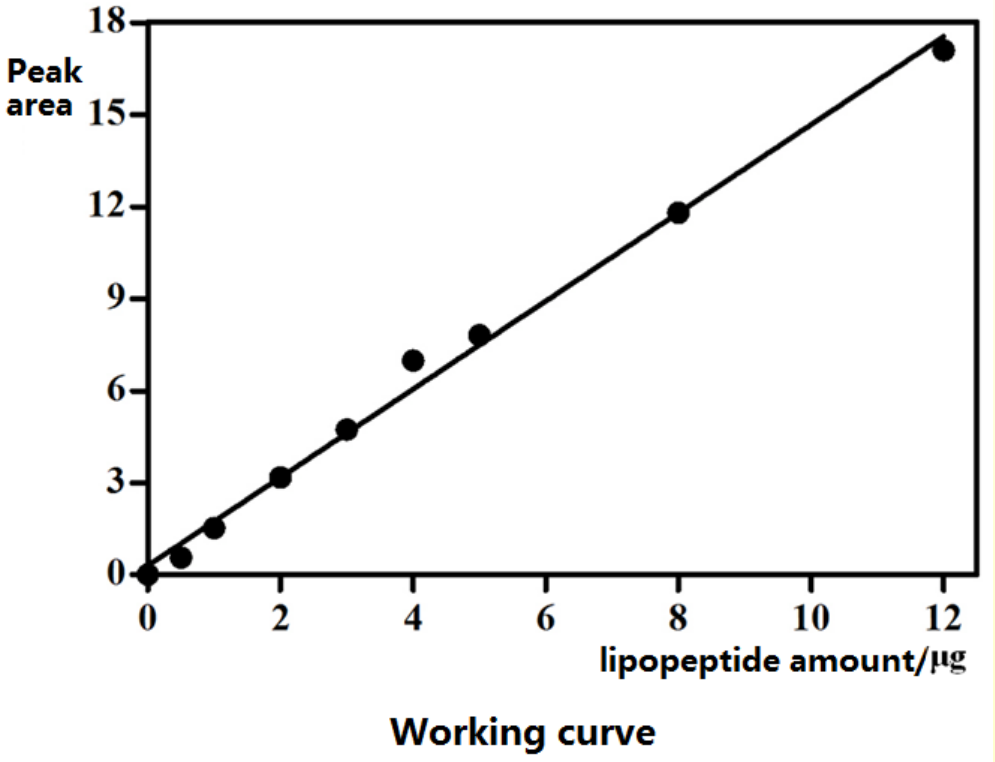


Index was allmost stable after 4 h hydrolysis

3 Results and Discussion

3.4 good results at optimal condition

0.5 mL of 12 M HCl , hydrolysis at 150°C for 4 h



Working curve

Good linear relationship could be obtained

Linear equation: y=12.15c

Linear coefficient: r^2=0.9940

3.5 Results of Ddetermining Samples

Hydrolysis was applied in determining samples

Determination results using hydrolysis method

Parallel samples	*n*=5
Mean value	7.05
S	0.47
RSD(%)	6.63
Working curve	y=12.15c
Recovery (%)	92.1

4 Conclusion

Acidity, acid amount, temperature and time were evaluated through orthogonal experimental design to resolve the long time and post-treatment of lipopeptide hydrolysis

The optimal codition of hydrolysis was determined: at 150ºC and 0.50 mL 12 M HCl for 4 h, conducted on gas-solid suface

The hydrolysis time of lipopeptides was shortened from 24 to 4 h

The hydrolysis post-treatment, such as evaperating solvent, was elimilated

Thank You!

敬请指正！