日立高速胺基酸分析儀L-8900型

1, History (from 1962)

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High-Speed Amino Acid Analyzer LA8080, the product story - YouTube

Model KLA-2

Model KLA-5

Model 835

Model L-8500

Model L-8800

Model L-8900

Model LA8080

Particle size of column	40 µm	17 µm	5 µm	3 µm		
Analysis time	22 hrs	2 hrs	50 min	30 min		
	Automation of analysis The first in Japan	Equipped with an Autosampler	Controlled by micro computer	2 NIN reagents	Controlled by PC. Reaction column method is employed.	Simple operation by the navigation function.

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關於胺基酸分析的方法

蛋白加水分解物胺基酸(PH)

游離胺基酸(PF)

預分離管柱（pre-lable）偵測法

後分離管柱（post-labing）偵測法

OPA／PITC／Acc／MFOC-CI
MBD-F　等（ODS管柱）

Nilhydrin法
（離子交換管柱）

OPA法
（離子交換管柱）

檢出下限：數pmol～數十 f mol
定量值再現性：CV值:1.5%以上
(蛋白加水分解物胺基酸、Pro除外)

檢出下限：數pmol
定量值再現性：
CV值：1.5%以下
(標準樣品、Pro除外)

檢出下限：數十 f mol
定量值再現性：
CV值：1.5%以下
(標準樣品、Pro除外)
(OPA試劑需每次調整)

後分離管柱・Ninhydrin法為胺基酸分析儀中最適合進行定量分析的方法！

Cf. Waters AAA system (Pre-column method)

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AccQTag and AccQTag Ultra for the derivitization of amino acids | Waters

Excess derivatization reagent is required.

To properly and reliably derivatize a hydrolyzed sample, the following must be conside

- Removal of particulates
- Determination of sample mass for derivatization
- Determination of whether sample neutralization is required
- Use of excess derivatization reagent based on sample mass

6.3.3 Derivatization reagent excess confirmation for AccQ•Tag derivatization

For complete derivatization of all the amino acids, 4–6x molar excess of the AccQ•Tag derivatization reagent is needed in the reaction. If there is insufficient reagent, some relatively sensitive amino acids will not be completely derivatized. The rates of derivatization for each amino acid vary based on the chemical properties of the amino acids; for example, the recovery of alanine can be significantly affected by insufficient molar excess of AccQ•Tag, while phenylalanine is more immune to these effects.

Notice for the concentration difference

6.3.1 Determination of sample amount for AccQ•Tag derivatization

In the reaction itself, the sample is typically diluted 10x, and 1 µL out of a 100 µL total derivatization volume is injected onto the column. Because not all amino acids are present in the same amount in a derivatization, the amount of sample must be sufficient for the least abundant amino acid to impact the limit of detection or quantitation. Derivatization of at least 1 pmol of the least abundant amino acid, in a maximum injection volume of 1 µL, is recommended to obtain accurate quantitation. To determine the amount of sample needed, perform the following calculation:

Warning of pH upon addition of the derivatization reagent

WARNING: *If the sample turns bright yellow upon addition of the derivatization reagent, the pH of the sample is too low. Neutralize with NaOH.*

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Cf. Agilent AAA system (Pre-column method)

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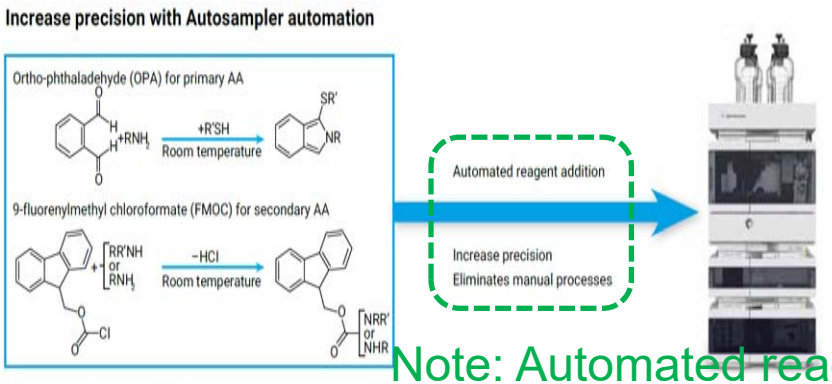


Figure 2. Online derivatization of OPA and FMOC: Separation of polar amino acids on RP-phase and detection by UV and Fluorescence

Note: Automated reagent addition

https://www.agilent.com/cs/library/applications/compendium-%20aminoacid-advancebio-5994-0033EN-us-agilent.pdf

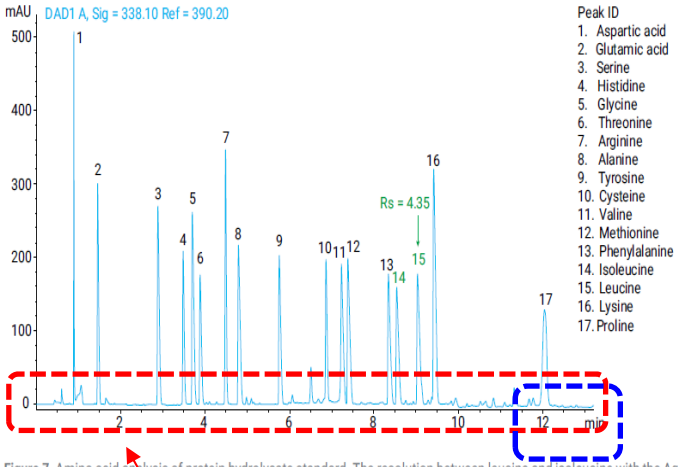
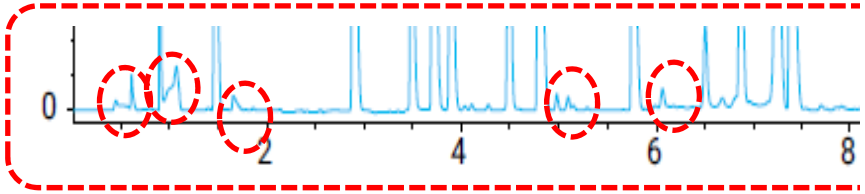


Figure 7. Amino acid analysis of protein hydrolysate standard. The resolution between leucine and isoleucine with the Agilent AdvanceBio AAA, 4.6 × 100 mm, 2.7 µm column is much higher than the reported value for system suitability requirement for this pair.

Check the baseline

Note: 13 min
(Cf. LA8080: 30 min)



There are some peaks which are not mentioned.

Derivatization reagents

Derivatization reagents (borate buffers, OPA, and FMOC) are ready-made solutions supplied by Agilent, and were transferred from their containers to autosampler vials. Precautions included:

- OPA is shipped in ampules under inert gas to prevent oxidation. Once opened, the OPA is potent for approximately 7 to 10 days. Therefore, 100 µL aliquots of OPA were transferred in microvial inserts and refrigerated. The OPA autosampler microvial was then replaced daily. Each ampule lasted 10 days (one vial/day).
- FMOC is stable in dry air, but deteriorates in moisture. Therefore, FMOC was transferred in 100 µL aliquots to microvial inserts and refrigerated. An open FMOC ampule transferred to 10 microvial inserts should last 10 days.
- Borate buffer was transferred to a 1.5 mL autosampler vial without a vial insert, and replaced every 3 days.

7 - 10 days
(Cf. LA8080: 30 days)

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Cf. Agilent AAA system (Pre-column method)

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Precision of retention time and area (n = 6)

Tables 2 and 3 summarize the average retention times and area RSDs for all the amino acids for the 100 and 1,000 pmol from six replicates of an amino acid method. The retention time RSDs for all amino acid peaks, including the early eluting peak 1 were less than 1.2%, demonstrating excellent gradient reproducibility. Peak area RSDs were less than 5 %, indicating precise sample injection. The RSD values demonstrate the robustness and precision of the amino acid method.

Table 2. Retention time and area RSD precision for amino acids (100 pmol) separated on an Agilent AdvanceBio AAA, 4.6 × 100 mm, column (n = 6).

Amino acid	RT RSD (%)	Area RSD (%)	Amino acid	RT RSD (%)	Area RSD (%)
1. Aspartic acid	1.270	1.066	13. Valine	0.084	2.47
2. Glutamic acid	0.973	1.85	14. Methionine	0.073	1.82
3. Asparagine	0.605	1.79	15. Norvaline	0.073	1.72
4. Serine	0.629	1.82	16. Tryptophan	0.054	1.57
5. Glutamine	0.470	1.56	17. Phenylalanine	0.051	1.66
6. Histidine	0.430	1.22	18. Isoleucine	0.047	1.72
7. Glycine	0.477	1.92	19. Leucine	0.03	1.7
8. Threonine	0.440	1.95	20. Lysine	0.028	1.66
9. Arginine	0.251	2.15	21. Hydroxyproline	0.021	4.13
10. Alanine	0.280	3.06	22. Sarcosine	0.026	1.15
11. Tyrosine	0.128	1.65	23. Proline	0.021	4.36
12. Cysteine	0.067	1.9			

Confidential

Reference data of Hitachi AAA
(*Area %RSD depends on method)
Official Specification: RSD 1.0%
(Gly,His) (N=5)

アミノ酸	2nmol/20μL標準液 面積 %RSD
Asp	0.16
Thr	0.16
Ser	0.16
Glu	0.20
Gly	0.18
Ala	0.17
Cys	0.19
Val	0.18
Met	0.23
Ile	0.27
Leu	0.38
Tyr	0.18
Phe	0.21
Lys	0.23
NH3	1.53
His	0.22
Arg	0.23

https://www.agilent.com/cs/library/applications/compendium-%20aminoacid-advancebio-5994-0033EN-us-agilent.pdf

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Cf. Nexera AAA system (OPA method)

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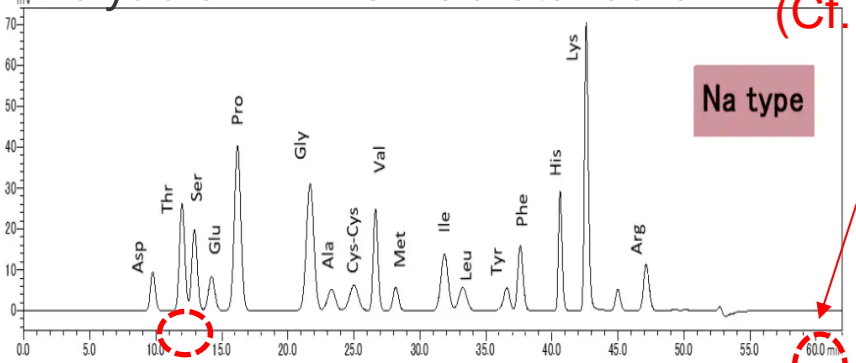
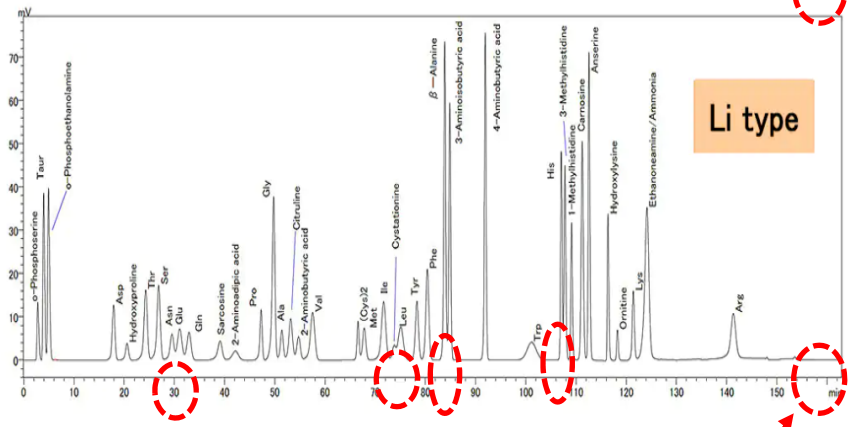
Nexera Amino Acid Analysis System - Features : SHIMADZU (Shimadzu Corporation)

Analysis of Amino Acid Standard

60 min
(Cf. LA8080: 30min)



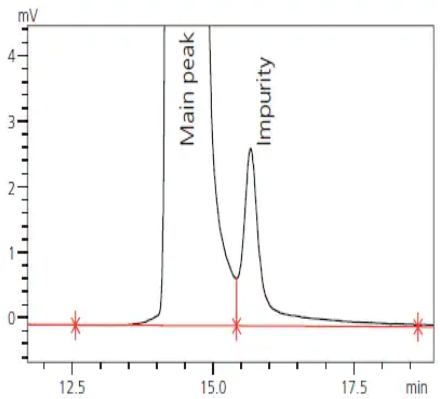


160 min
(Cf. LA8080: 110min)

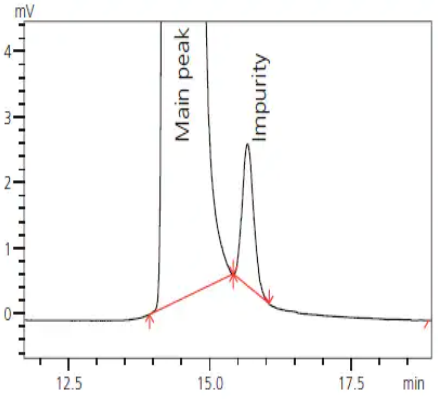
There is a description of peak splitting

Consistent Peak Splitting

Manually setting the baseline for fused peaks is time-consuming, and the peaks may be split differently depending on the user. The i-PeakFinder has adjustable parameters for different analyses, allowing you to consistently apply the best baseline in each situation.



Baseline not set



Setting a baseline provides complete separation

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Cf. Nexera AAA system (OPA method)

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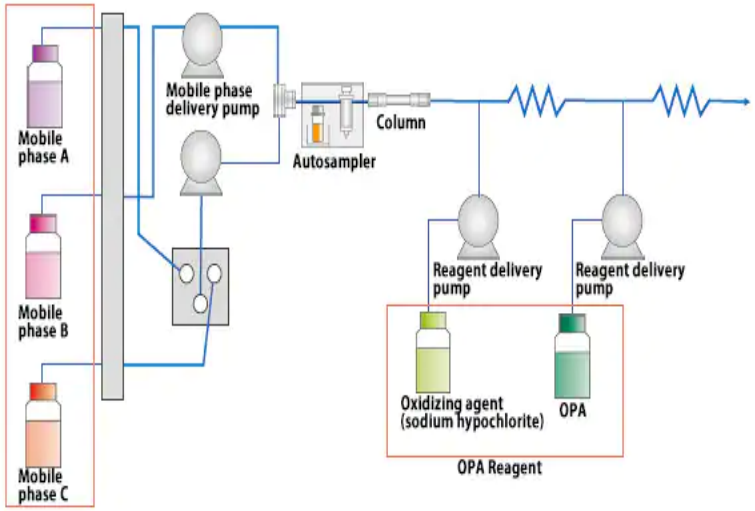
Ready-to-use Special Reagent Kit

In terms of the mobile phase, the pH must be carefully prepared in order to obtain separation repeatability in amino acid analysis. To avoid this labor-intensive process and the risk of preparation mistakes, the mobile phase and reaction reagent used in this system are available as a special reagent kit that can be used immediately after opening.



Mobile Phase Kit for Amino Acid Analysis System

Amino Acid Mobile Phase Kit

OPA reagent kit

(Cf. LA8080: 30 days)

*OPA reagent is easy to be oxidated and degraded.

https://www.shimadzu.com/an/products/liquid-chromatography/hplc-system/amino-acid-analysis-system/features.html

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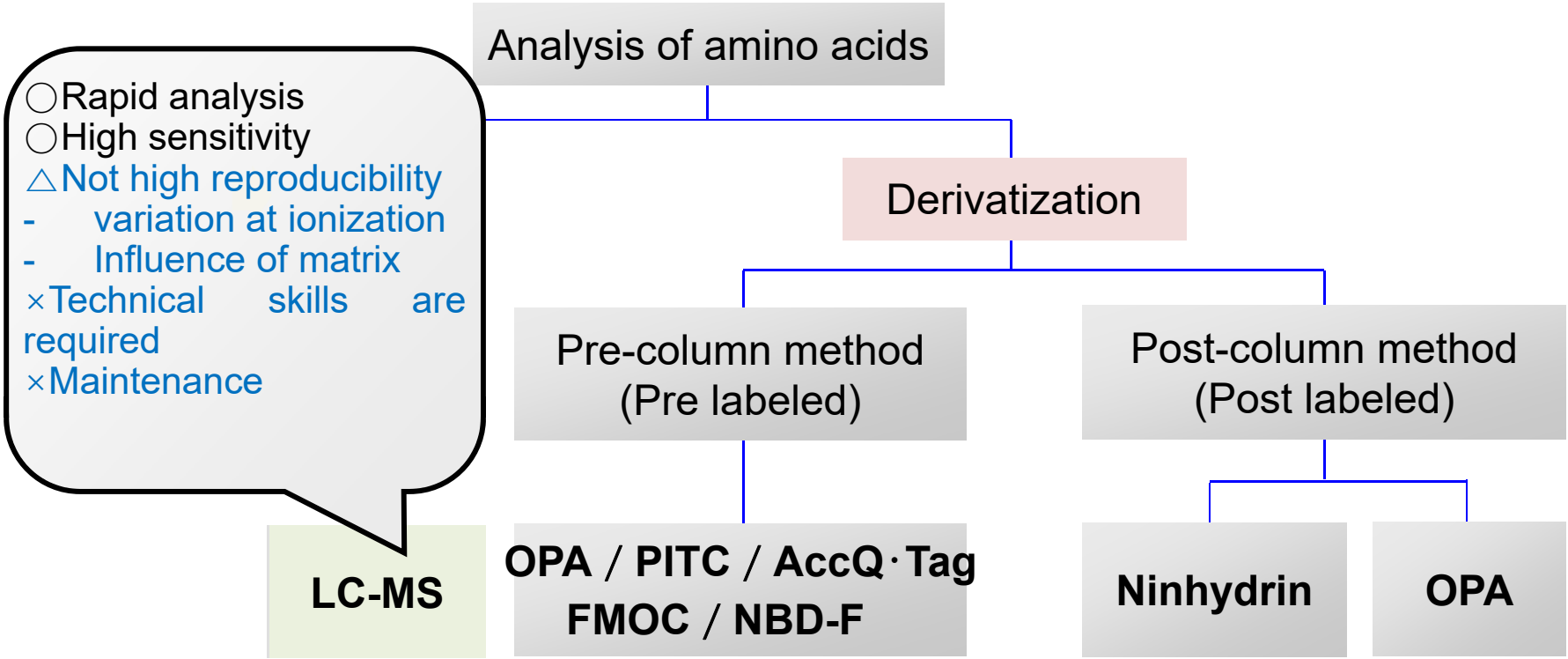
2-2, Derivatization of amino acids

Since most amino acids have low UV absorption and no fluorescence, they are analyzed by LC-MS or are detected after the derivatization by some reagents.



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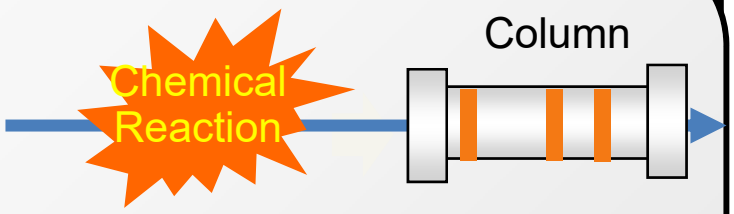
2-2, Derivatization of amino acids

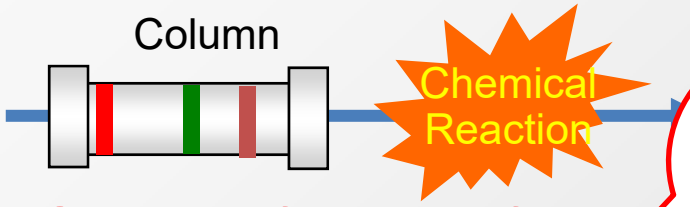
Derivatization

Pre-column method (Pre labeled)



○Simple System
○Fast analysis
△Low Reproducibility
△Undesired influence of impurities during reaction

Post-column method (Post labeled)



Good Quantification

◎Little Influence of impurities during reaction
◎Precise quantification
◎High Reproducibility
◎Many separable amino acids

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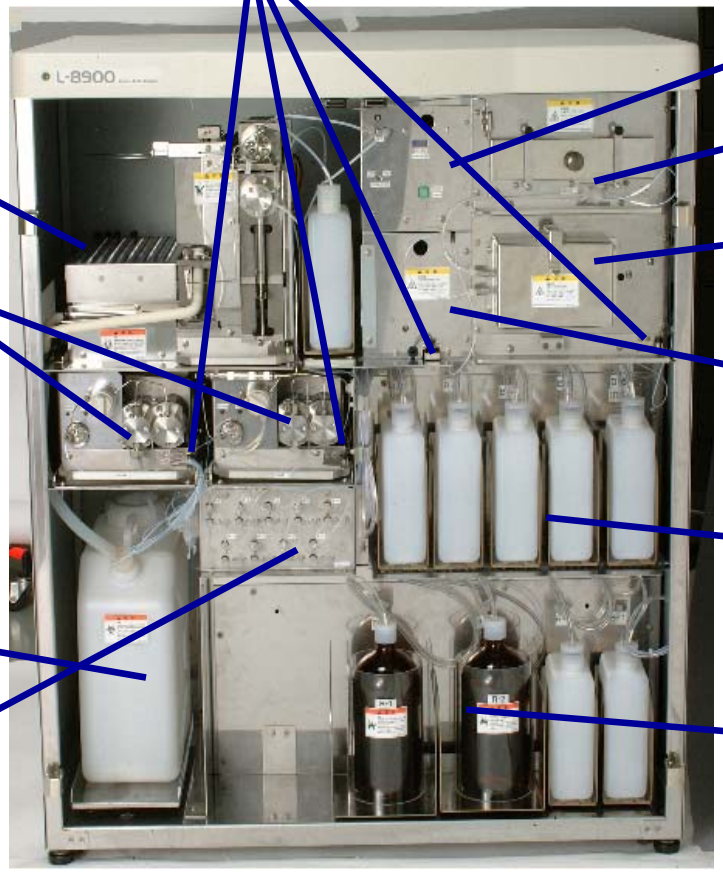
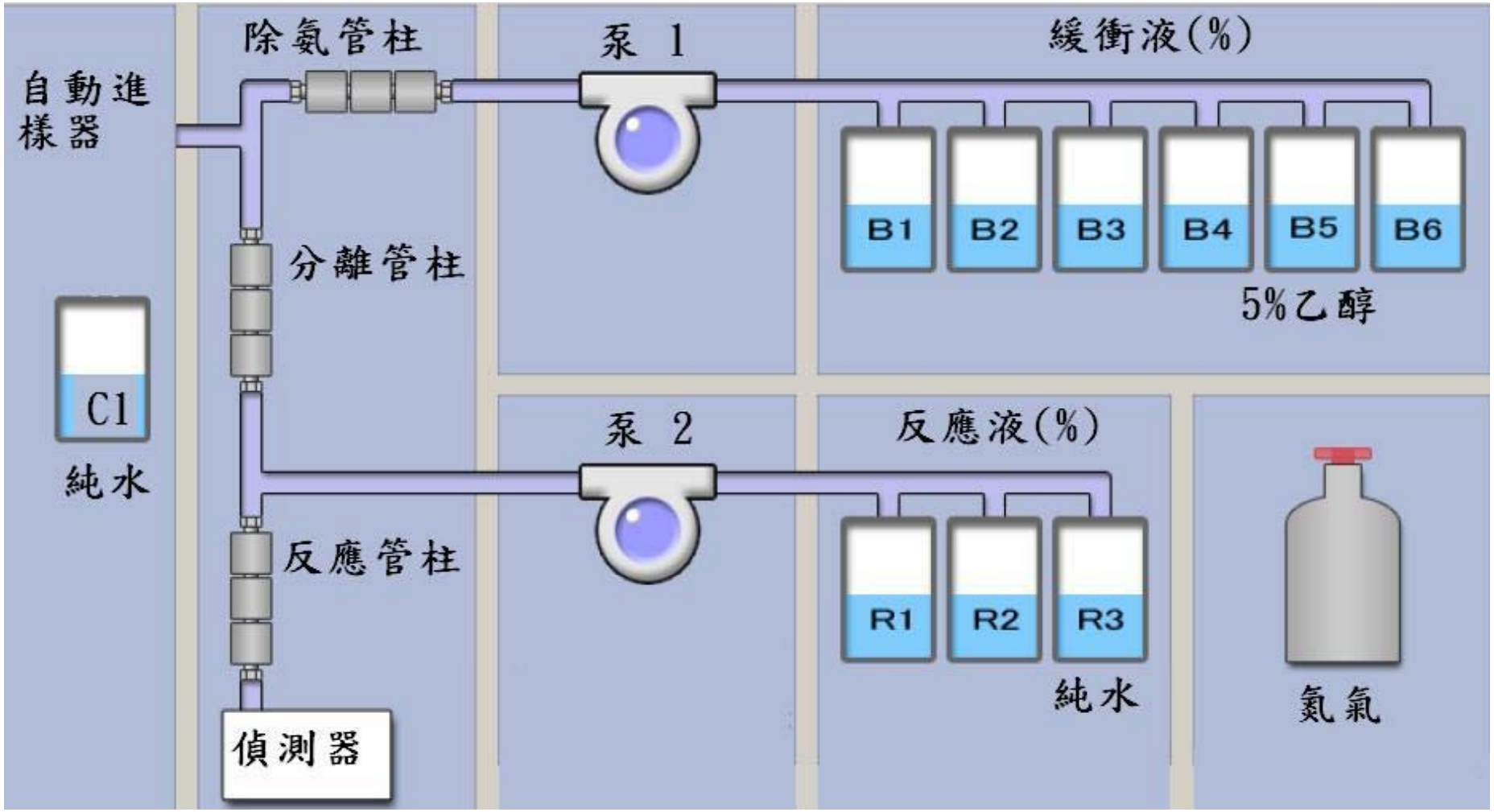
L-8900內部照片（正面）

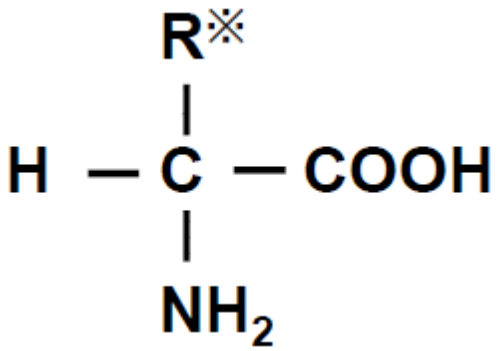


胺基酸分析管路圖

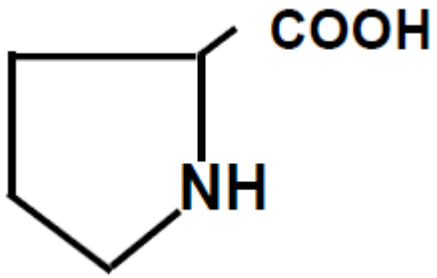

自動進樣器
除氨管柱
泵 1
緩衝液(%)
B1
B2
B3
B4
B5
B6
5%乙醇
分離管柱
C1
純水
泵 2
反應液(%)
反應管柱
R1
R2
R3
純水
氮氣
偵測器

胺基酸

組成蛋白質的20種胺基酸除甘胺酸外，都有一個不對稱碳原子，即α碳。 α碳有四個不同取代基：羧基、胺基、氫原子和R基團，不同胺基酸的R基團不同。每種胺基酸有D-form和L-form，蛋白質中的胺基酸都是L-form。



General structural formula of amino acids



Structure of Proline (Pro)

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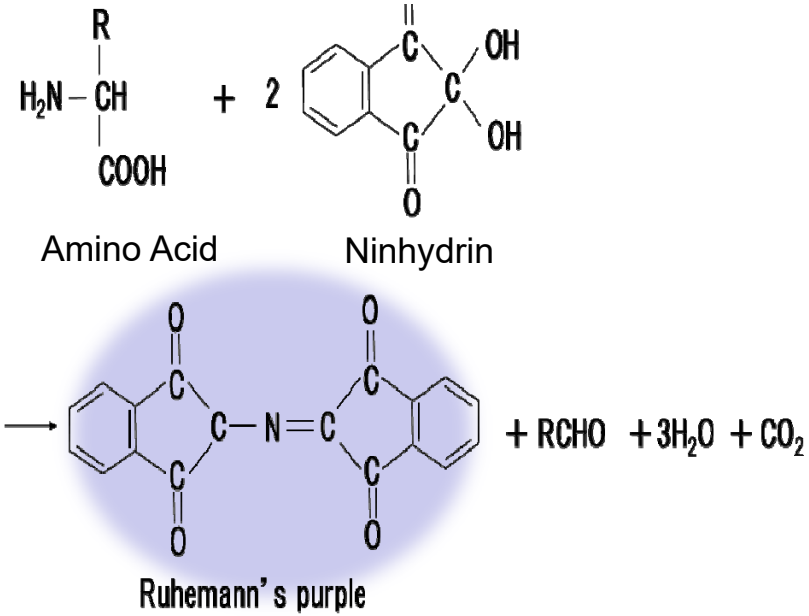
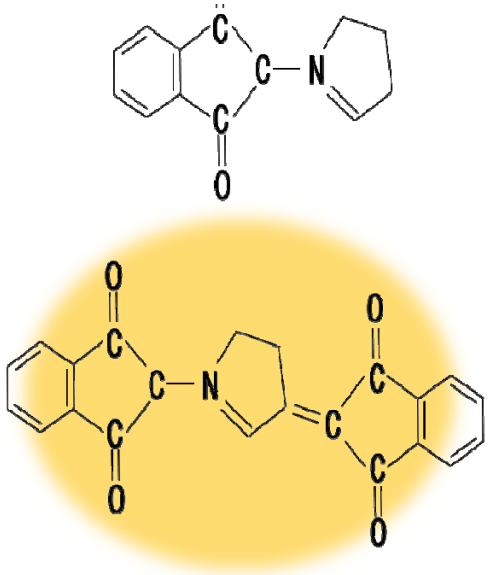
2-2, Derivatization of amino acids

Ninhydrin reaction



Absorb the light of 570nm

Reaction product of Proline



Absorb the light of 440nm

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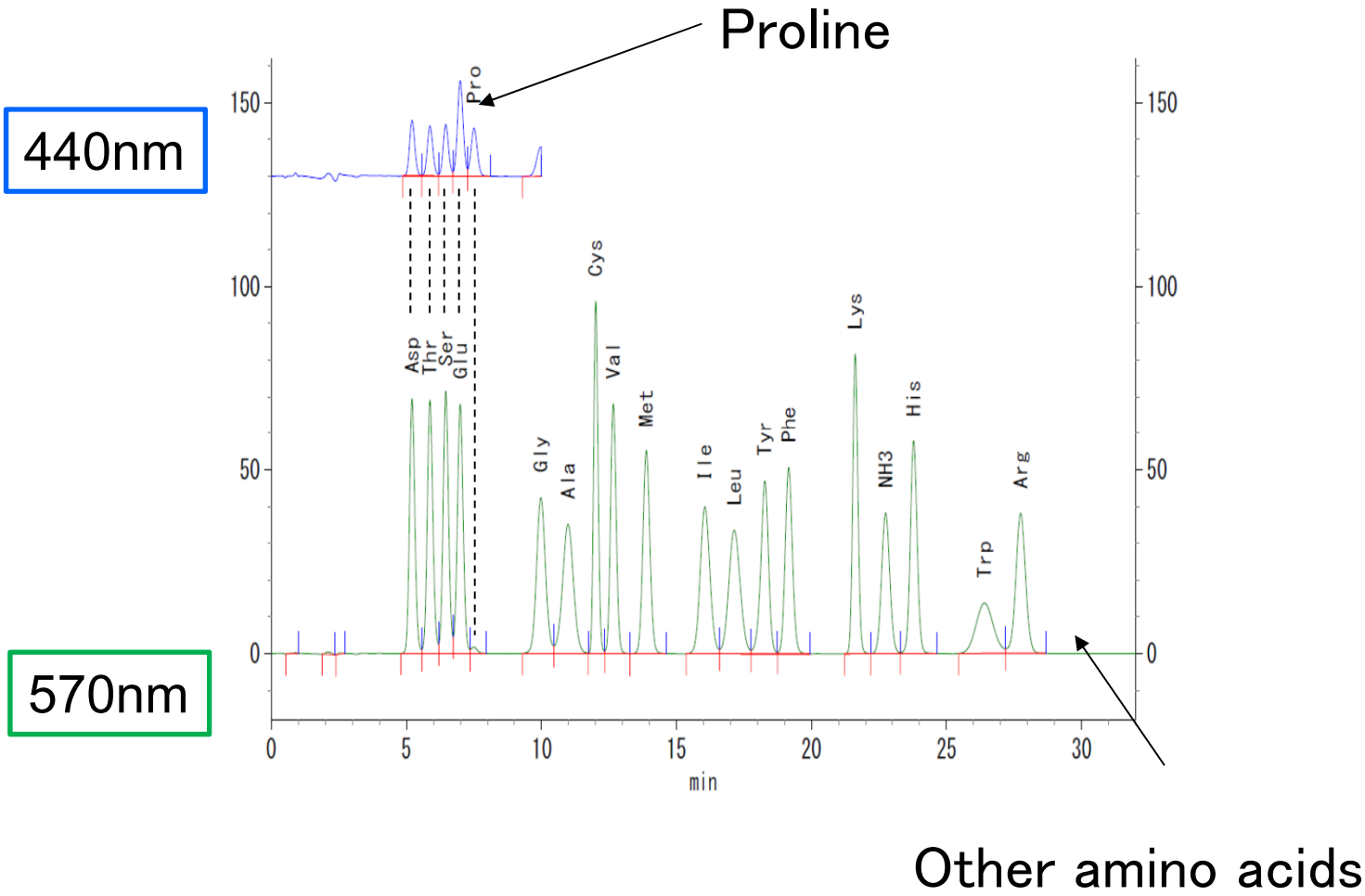
2-2, Derivatization of amino acids



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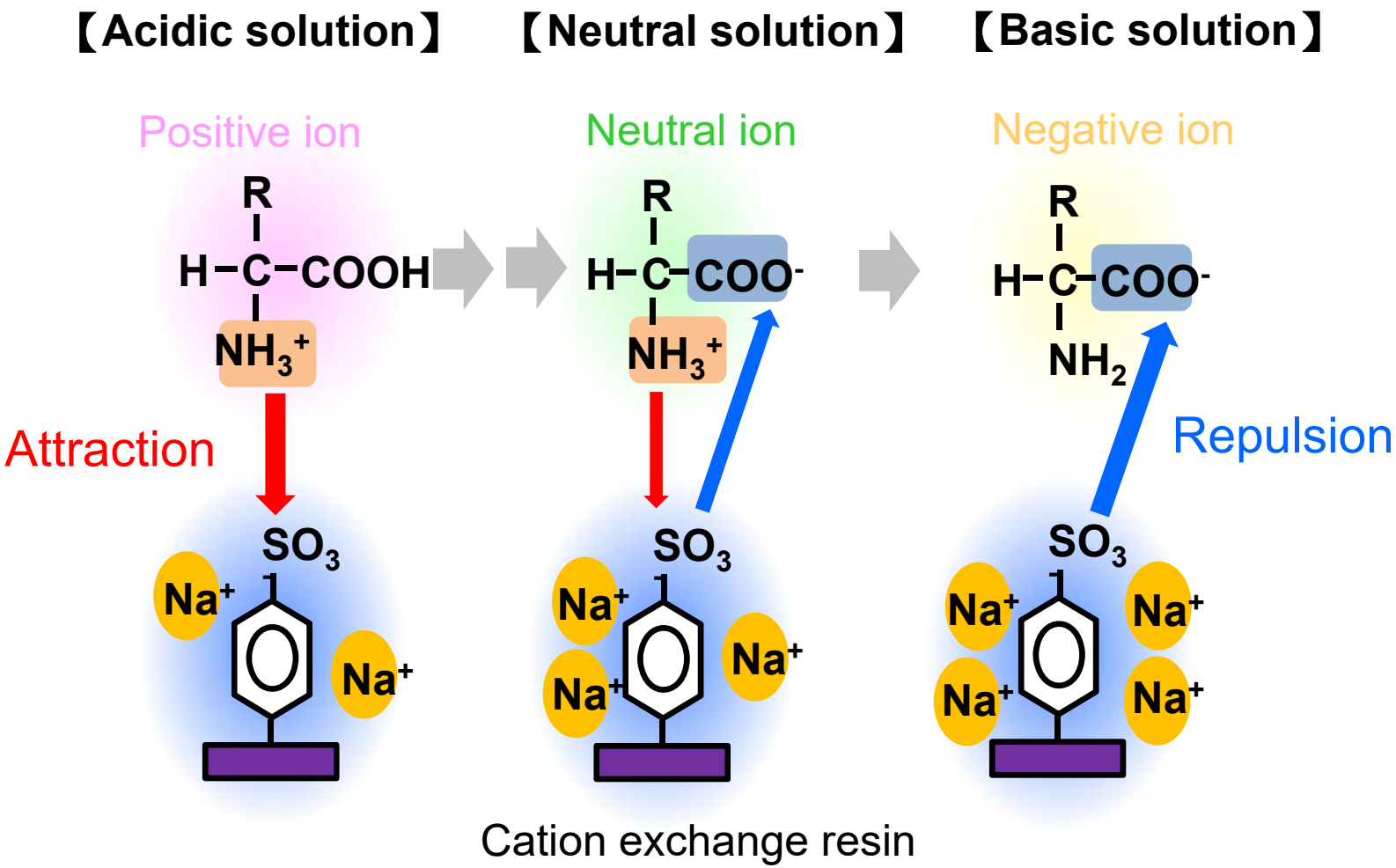
2-3, Separation of amino acids



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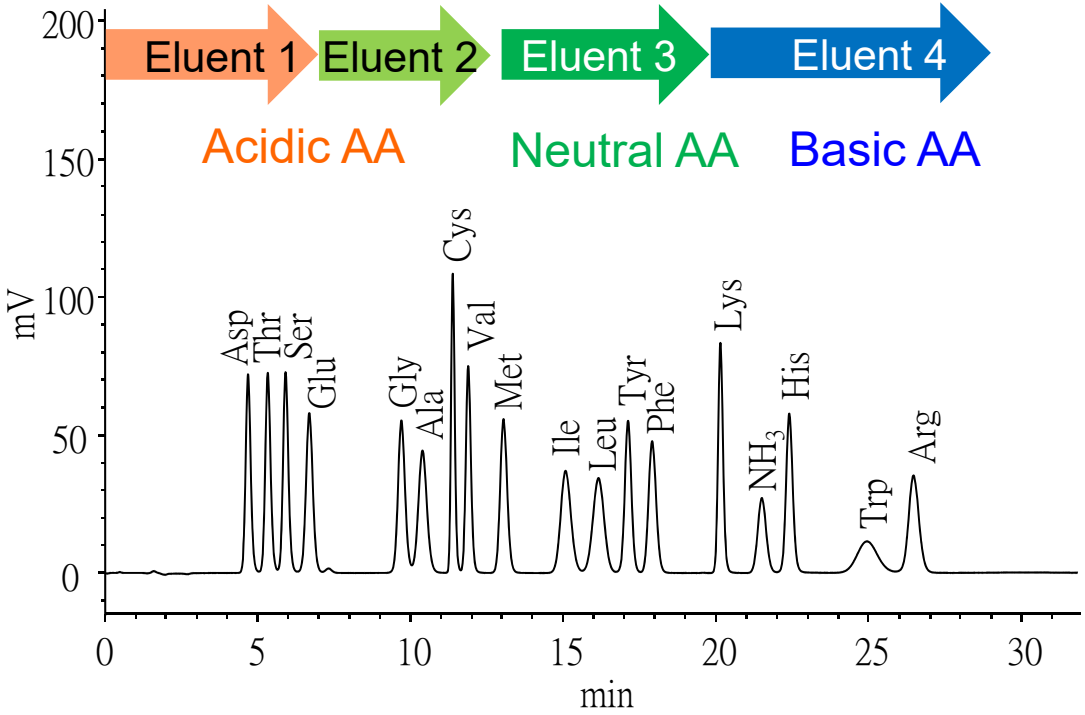
2-3, Separation of amino acids



Necessary elements for amino acid analysis buffer

Condition	Chemicals
pH	Citric acid
Ionic strength	NaCl, LiCl
Buffer capacity	Sodium citrate, Lithium citrate

Analysis column: # 2622 PH 4.6 mm x 60 mm
Ammonia filter column: # 2650 L 4.6 mm x 40 mm
Eluent: PH-kit for L-8500 (Mitsubishi Chemical)

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3,Applications

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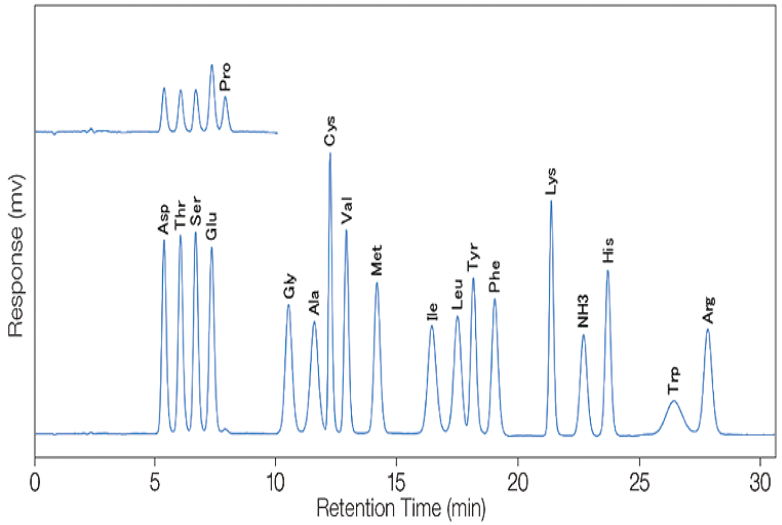
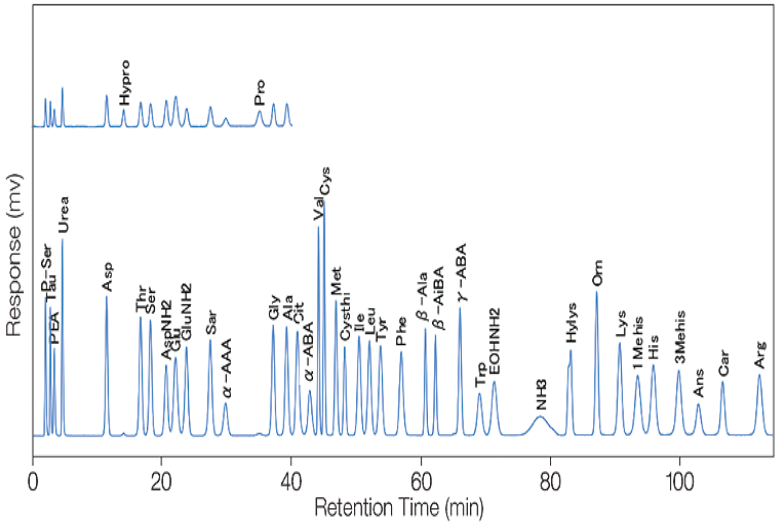
PH analysis method (30 min)



STANDARD ANALYSIS COLUMN (Part No. 855-4506)
Basic method for the simultaneous analysis of 18 protein-constituting amino acids.

PF analysis method (110 min)



PHYSIOLOGICAL ANALYSIS COLUMN (Part No. 855-4507)
42 components including the protein-constituting amino acids can be simultaneously analyzed.
Suitable for the analysis of free amino acids (amino acids found in physiological samples).

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生體液分析法的前處理(PF)

主要目的是做胺基酸成份的萃取,除去蛋白質以及不溶性物質

a. 血液

(1)把血液用7,000~10,000rpm離心分離15分鐘
(2)在澄清液中加入5~10%三氯醋酸.稀釋2~3倍
(3)用7,000~10,000rpm離心分離15分鐘
(4)用澄清液作為樣品

注意事項

(1)澄清液有混濁時,要不提高離心分離的轉速,要不進一步使用過濾器
(2)雖然三氯醋酸的濃度根據蛋白質的量不一樣,通常把添加後的濃度做為3%左右
(3)將血液就這樣做除蛋白操作,回收率很低,特別是Arg的消失明顯
(4)最終樣品量做成0.05~0.1ml

b. 濾紙萃取血液(直徑10mm的點約30μl,最少也是直徑3mmx5片)

(1)剪開濾紙,沿著離心管的管壁放入

(2)加入0.5ml的1%三氯醋酸

(3)用超音波震盪器震盪2分鐘

(4)用7,000~10,000rpm離心分離15分鐘

(5)用0.45μm的過濾器將澄清液過濾,裝入樣品中進行分析

注意事項

樣品很混濁的時候,不要使用三氯醋酸,請使用水/乙醇萃取,待減壓乾燥後,用0.02N-HCl稀釋的話,分離管柱的污染將減少

c. 尿

(1)在原尿中加入1%三氯醋酸再稀釋2~5倍(由於異常的尿有大量的胺基酸出現所以要提高稀釋倍率)

(2)有混濁時,進行離心分離及過濾

(3)把得到的液體的0.05~0.1ml作為樣品

注意事項

檢測微量成份的良好靈敏度時,在除蛋白並減壓乾燥後,用稀釋溶解,濃度就和原液吻合

d. 醬汁,(調味汁),果汁等含有蔬果原料食品類

(1)用1~5%三氯醋酸溶液稀釋10倍
(2)用7,000~10,000rpm離心分離15分鐘
(3)把澄清液作為樣品

e. 醬油,酒等沒有沉澱的水系食品

(1)以0.02N-HCl稀釋樣品

注意事項:食醋等雖然在低稀釋度即可,但醬油等由於胺基酸濃度很高,要稀釋200~300倍左右再進行分析

f. 從植物葉的萃取

(1)研磨並剁碎樣品

(2)使其分散在20~30%的乙醇水溶液中(1g樣品/3g乙醇水溶液)

(3)以80℃的水浴鍋加熱萃取20分鐘以上

(4)過濾收集澄清液,並將殘渣重複萃取三次

(5)將剩下的殘渣以乙醇清洗

(6)將(4)(5)收集的液體,以水浴加熱的方式除去乙醇

(7)把殘留下來的液體加入乙醚萃取後,保留水層

(8)將水層減壓乾燥後,以0.02N-HCl調整體積

生理液樣品的處理

生理液的樣品首先要除去樣品中的蛋白質，獲得游離胺基酸。除去蛋白質化學方法為：

1、苦味酸法

用1%的苦味酸沉澱，然後過柱子除苦味酸，在把氨基酸從過濾柱上沖提下來進行分析。

2、三氯醋酸法

生物鹼沉澱劑醫院臨床生化、常用此類試劑沉澱血漿中的蛋白質。

3、乙醇沉澱法

當乙醇加入含蛋白質的水溶液中，可使蛋白質表面失去水膜，並增大顆粒間的引力，引起蛋白質沉澱。

4、磺基水楊酸法（常用法）

用4%-10%的磺基水楊酸，按1:3的比例與樣品混合離心去蛋白，轉速2萬轉以上離心10分鐘或更長一些時間。取上清液用樣品稀釋液稀釋後上機測定。（建議進樣前用c18預處理過濾柱處理一下較好）

游離胺基酸樣品的前處理

游離胺基酸的樣品在分析前必需進行磨碎、脱脂、萃取、脱塩、去蛋白、脱色等處理。在進行分析前建議使用C18過濾柱處理一下較好。

4. Measurement of Amino Acids in Saury

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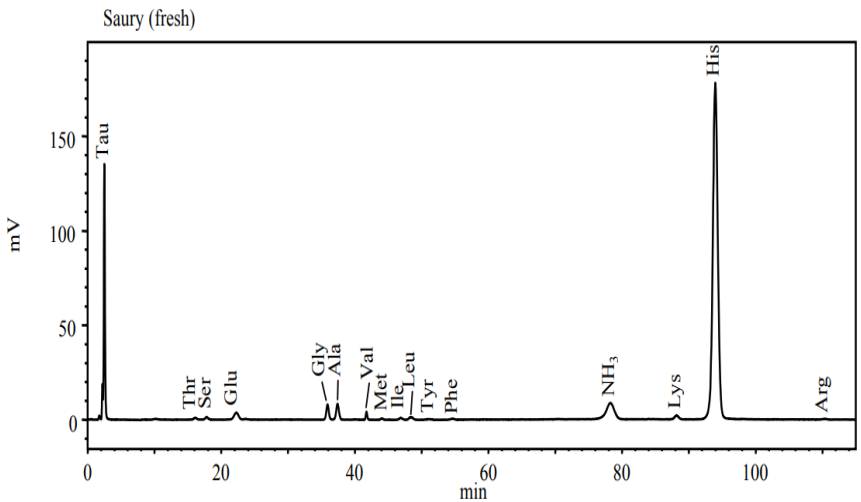
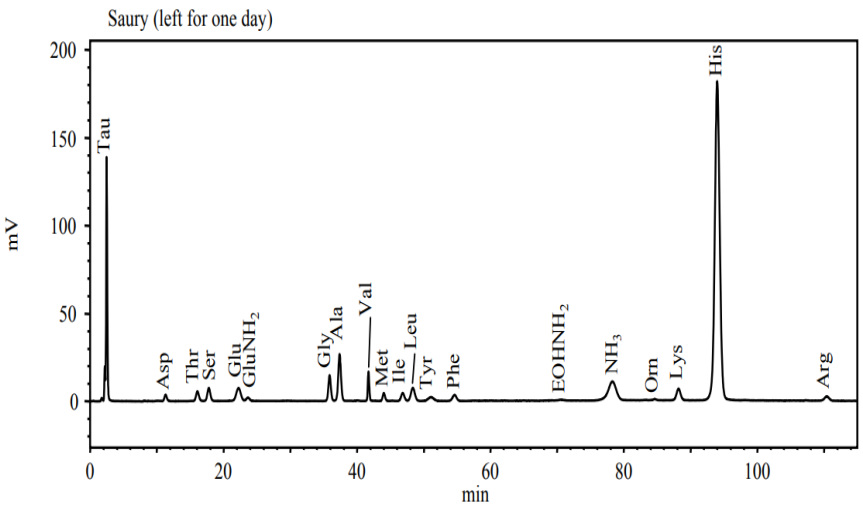
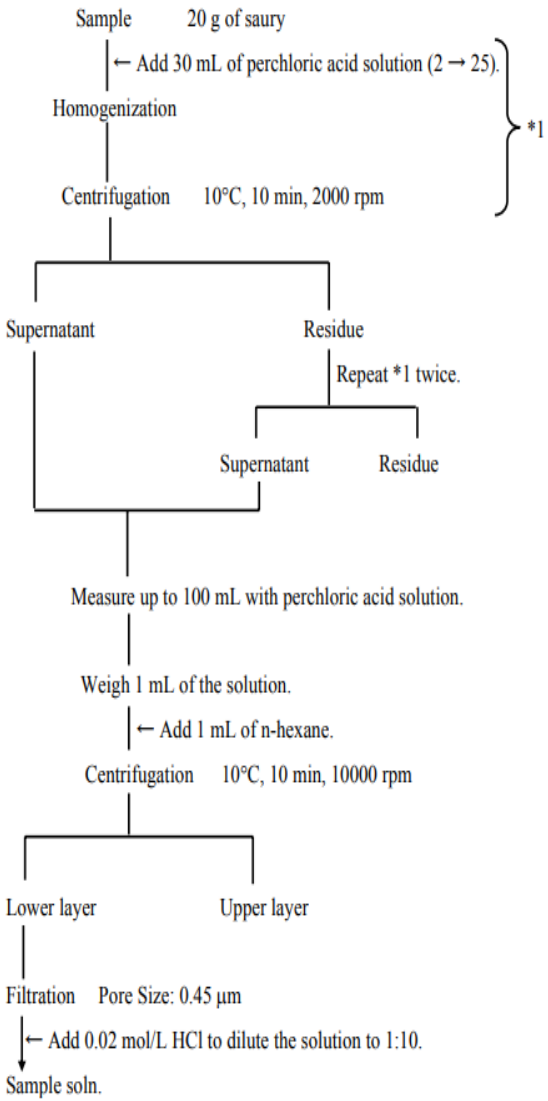
Amino acid analysis in Saury (PF method)





[Sample preparation] [LC060112-03]



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4. Measurement of Amino Acids in Salmon

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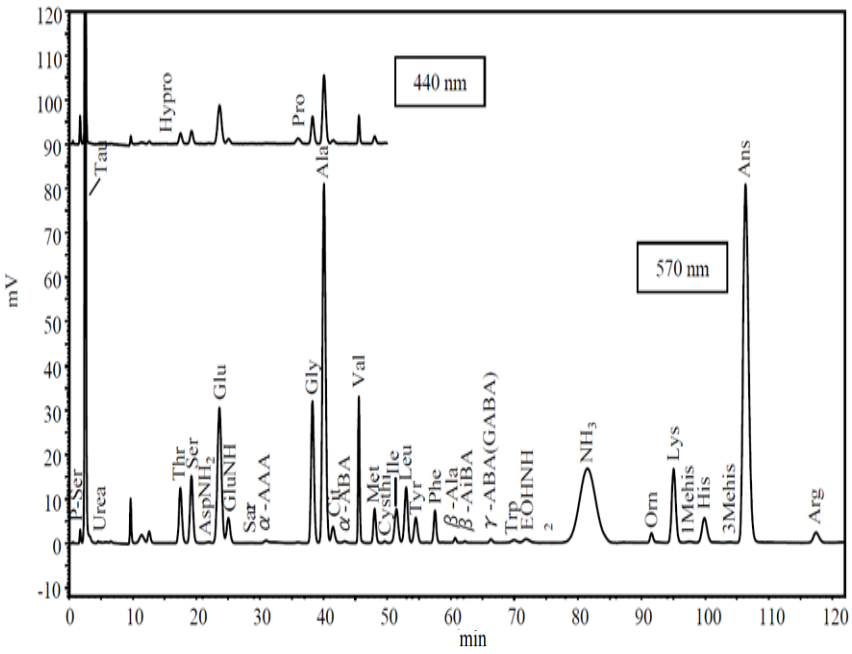
Amino acid analysis in Salmon (PF method)



[Sample preparation] [LC090018-02]

Sample Appx. 5 g of salmon shreded by a food processor

← 0.02 mol/L HCl 15 mL

Homogenize 1 min

Make up the volume to 50 mL

Centrifuge 3,000 rpm, 10 min

Supernatant 0.5 mL

← Add 0.5 mL of 10 % trichloroacetate and mix

Let it stand for 15 to 30 min in a refrigerator

Centrifuge 10,000 rpm, 10 min

Supernatant 0.5 mL

← Add 0.5 mL of hexane and mix

Centrifuge 10,000 rpm, 10 min

Bottom layer

Filter Pore Size 0.2 μm

↓

Sample for injection

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4. Measurement of Amino Acids in Tuna

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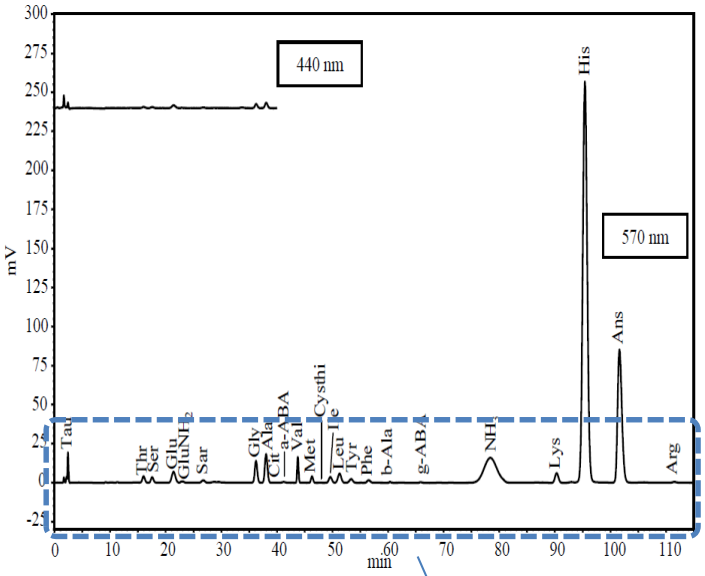
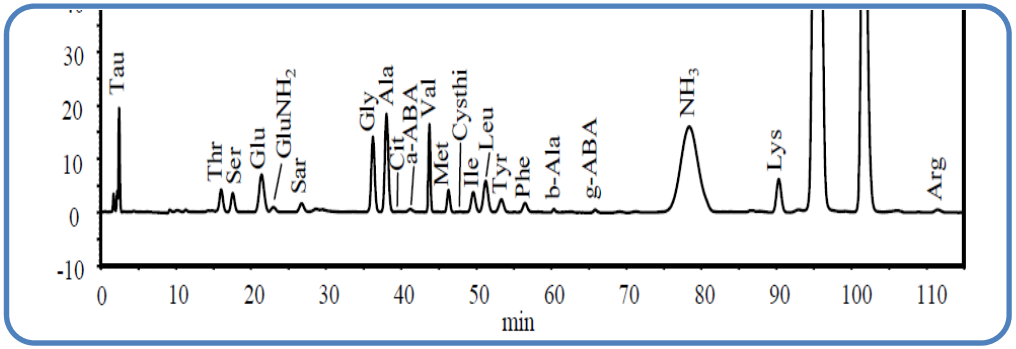
Amino acid analysis in Tuna (PF method)





[Sample preparation] [LC070039-07]

Sample 10 g of tuna or beef

← Pure water 10 mL

Homogenize 1 mi

Make the volume to 100 mL

Centrifuge 3000 rpm, 10 min

Supernatant 1 mL

← Add 10% trichloroacetate and mix

Centrifuge 10000 rpm, 10 min

Supernatant 1 mL

← add 1 mL of n-hexane and mix

Centrifuge 10000 rpm, 10 min

Aqueous layer

Filtration Pore Size 0.2 μm

Sample for injection *

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蛋白質的水解(PH)：

用於全胺基酸測定的樣品，凡是以蛋白質形式存在的都要進行水解處理，水解方法有三種：

1、酸水解法：標準水解法，是普遍採用的水解方法，此方法的特點是水解徹底，但色胺酸遭破壞。（6N HCL、110℃真空水解24小时）

優點：HCL本身加熱可以蒸發除掉。

缺点：溶液顯黑褐色、與含醛基化合物作用的結果。

2、鹼水解法：用NaOH(LiOH、Ba(OH)2)作為水解劑，色胺酸不被破壞，但有消旋作用，絲胺酸、酥胺酸、精胺酸、胱胺酸遭不同程度的破壞。

優點：水解液澄清。

缺點：放出氨氣和硫化氫.

3、酶水解法：酶是有機催化劑，它不需要高温高壓，而是在常温常壓下即可催化有機物質的合成與分解。

特点：水解條件温和，無需特殊設備，胺基酸不受破壞；產物中除胺基酸外尚有較多胜肽；此方法主要用於生產水解蛋白及蛋白肽。

缺点：水解時間長、而且不易水解完全

附：飼料行業樣品前處理具體方法

酸水解法：稱取蛋白質樣品適量（100mg左右為宜）放入水解管中加10ml、6NHCL，置液氮或乾冰中冷凍，然後抽真空至7Pa後封口，將水解管放在110度恒温乾燥箱内水解22小時。完畢冷卻後，開管、定容、過濾、取適量的濾液置旋轉蒸發器或濃縮器中60度抽真空蒸發至乾，必要時可加少許水重複蒸乾1—2次，加入樣品稀釋液將樣品稀釋到所需濃度、摇均、過濾待用。

氧化水解法：是將含硫胺基酸如：胱胺酸、半胱胺酸和甲硫胺酸用過甲酸氧化變成穩定物質，然後再進行水解。過甲酸加入量2ml，現用現配（甲酸與過氧化氫按9比1）在室溫下反應1小時

鹼水解法：稱取蛋白質樣品適量（100mg左右為宜）置於聚氟乙烯襯管中加1.5mlLiOH(4mol濃度的），於液氮乾冰中冷凍，然後將襯管插入水解管中，抽真空至7Pa或充氮氣5分鐘以上封管，然後將水解管放入110度恒溫乾燥箱，水解20小時。取出水解管冷卻至室溫、開管加入1ml6mol/L的塩酸中和，用樣品稀釋液定容（25ml容量瓶）稀釋所需濃度、搖均、過濾待用。

5, Analysis of Amino Acids in Chicken Feed

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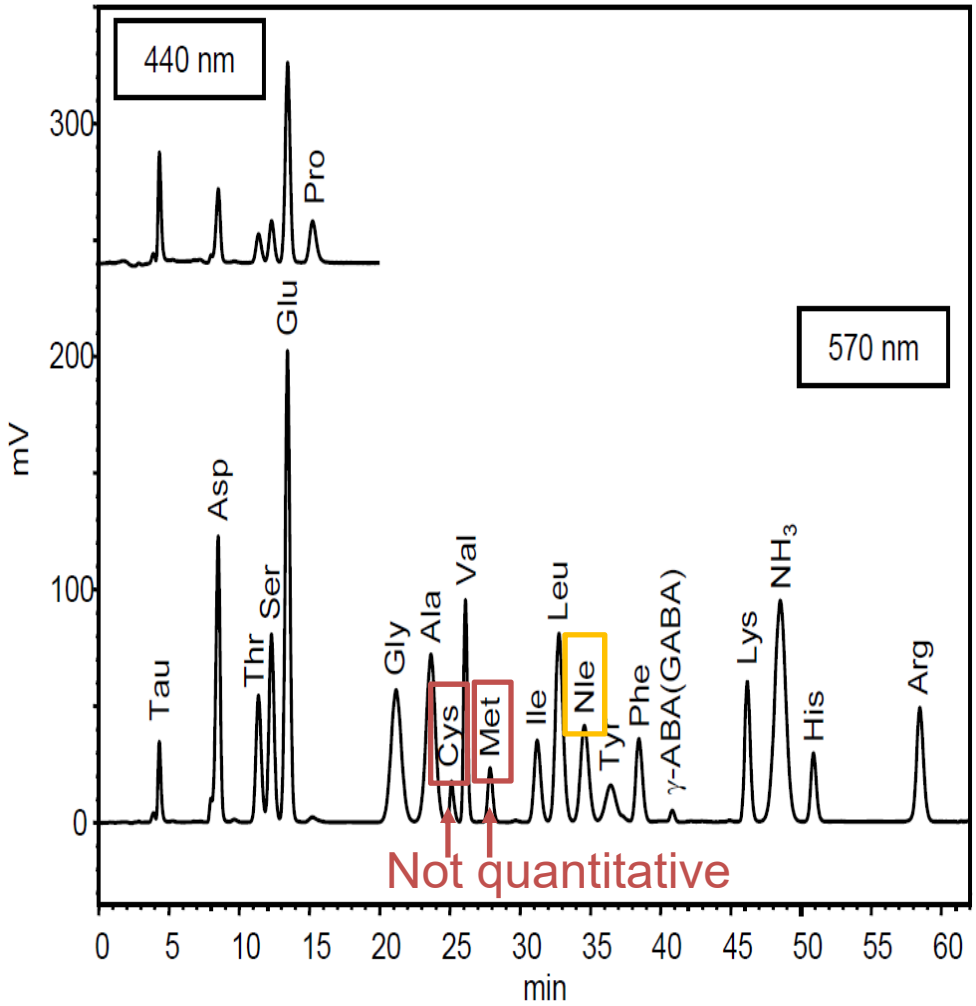
1) Hydrochloric Acid Hydrolysis



[Preparation Method]

Weigh out 6 mg of the pulverized sample and place in a hydrolysis tube.

← Add 1 mL of 6 mol/L HCl

← Add 0.5 mL of 0.4 μmol/mL Nle (as the internal standard)

Degas by an aspirator for 15 min

110°C, 22 hrs

Dry under vacuum

← Add 2 mL of 0.02 mol/L HCl

Dissolve

Centrifuge at 10,000 rpm for 10 min

Filter the supernatant through a 0.2 μm filter

↓

Sample for injection

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5, Analysis of Amino Acids in Chicken Feed

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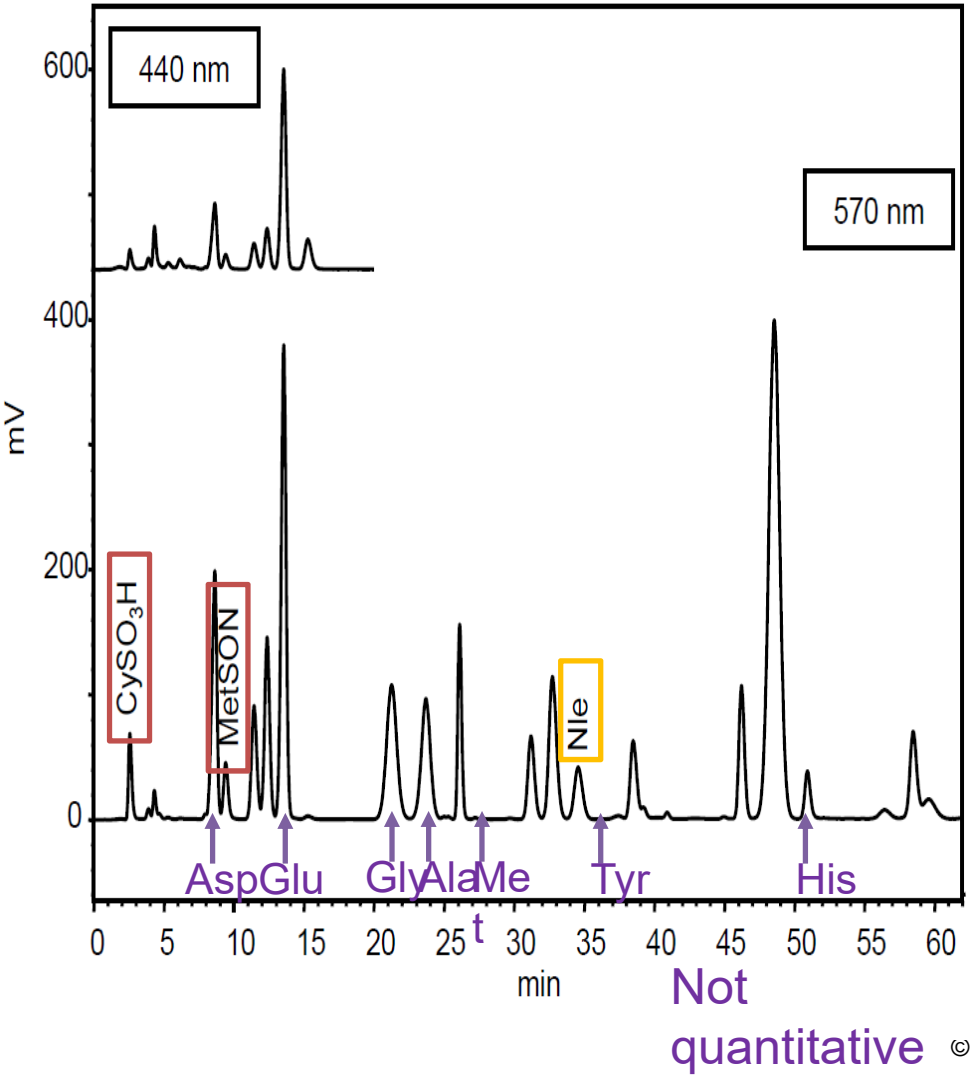
2) Oxidation with Performic Acid + Hydrochloric Acid Hydrolysis



Not quantitative

[Preparation Method]

Weigh out 6 mg of the pulverized sample and place in a round bottom flask

← Add 2 mL of performic acid reagent

← Add 0.5 mL of 0.4 μmol/mL Nle (as the internal standard)

Let it stand in a refrigerator for about 20 hrs

← Add 0.3 mL of 48% HBr

Dry under vacuum

← Add 1 mL of 6 mol/L HCl

Transfer into a hydrolysis tube

Degas by an aspirator for 15 min

110°C, 18 hrs

Dry under vacuum

← Add 2 mL of 0.02 mol/L HCl

Dissolve

Centrifuge at 10,000 rpm for 10 min

Filter the supernatant through a 0.2 μm filter

↓

Sample for injection

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5, Analysis of Amino Acids in Chicken Feed

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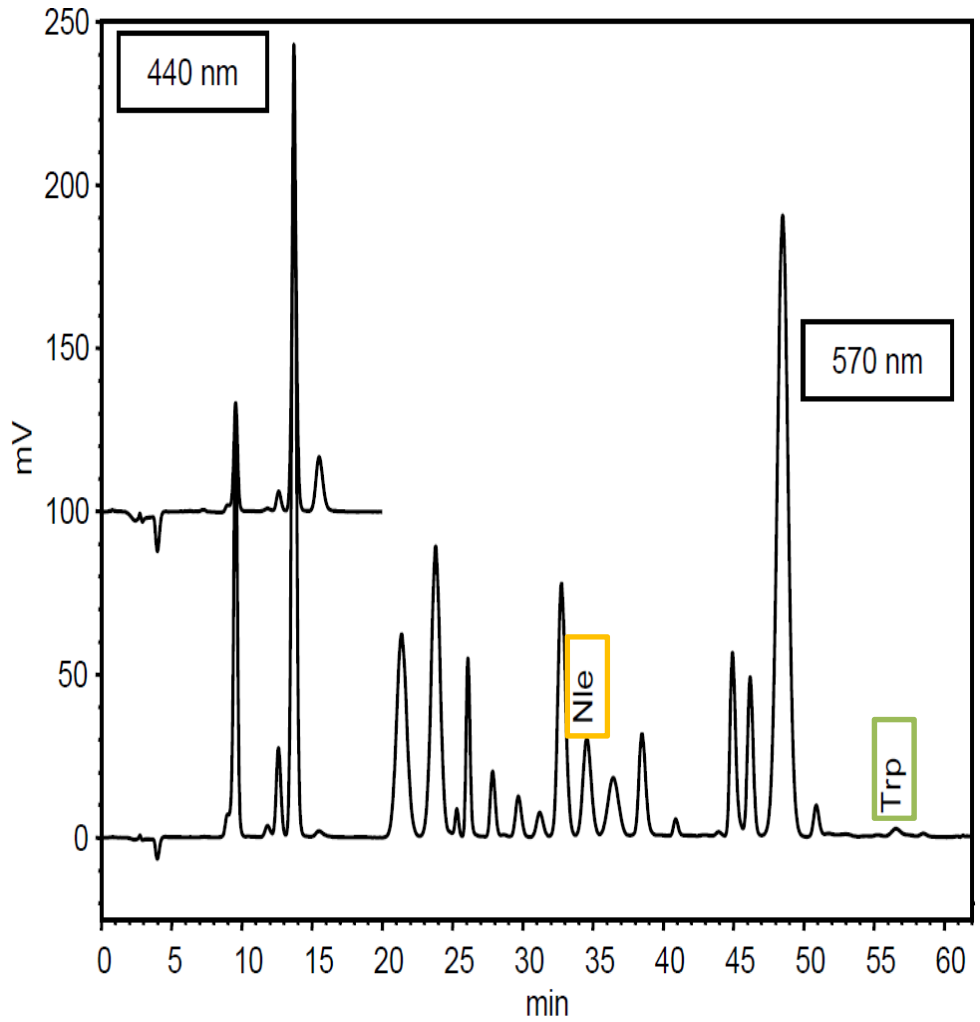
3) Basic Hydrolysis

【Technical sheet: LC090033】



[Preparation Method]

Weigh out 60 mg of the pulverized sample and place in a hydrolysis tube

← Add 1.5 mL of 5 mol/L NaOH

← Add 0.5 mL of 4 μmol/mL Nle (as the internal standard)

Degas by an aspirator for 15 min

110°C, 16 hrs

Centrifuge at 3,000 rpm for 10 min

Collect 0.5 mL of the supernatant

← Add 0.5 mL of 6 mol/L HCl

← Add 4 mL of purified water

Mix

Centrifuge at 10,000 rpm for 10 min

Filter the supernatant through a 0.2 μm filter

↓

Sample for injection

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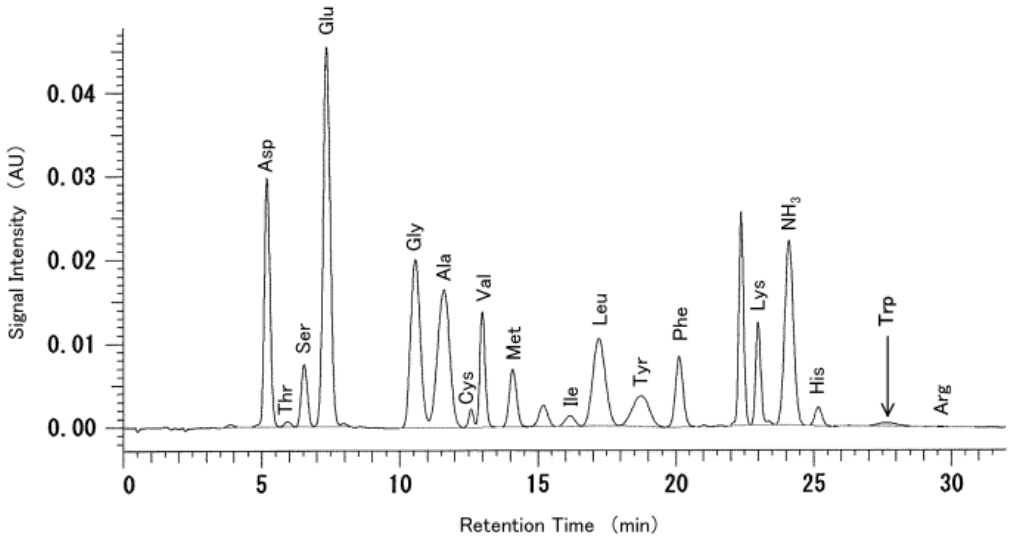
稻米



Sample preparation :

five grains of rice
|
remove rice chaff
|
crush with metal pincers
|
pound into a powder
|
put in a decomposition tube
|
← deaerate with 5 mol/L NaOH aq. 2000 μL
|
reaction at 110 ℃ for 28 hours
|
put the decomposed substance into 500 μL test tube.
|
← add 6 mol/L HCl 500 μL
|
← add 4mL pure water and centrifuge at 7000 rpm for 10 minutes
filter with a 0.45 μm filter
↓
Sample solution

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END

Amino acid analysis

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