

SUBJECT : SYNTHETIC ANALYSIS OF AMINO ACIDS INDUCING METABOLIC ERRORS

INSTRUMENT: HITACHI MODEL L-8800 AMINO ACID ANALYZER

1. INTRODUCTION

Analysis of biological-fluid amino acids with an amino acid analyzer usually targets 41 kinds of amino acid components. In recent years, however, study on metabolic errors in newborn babies, etc. has advanced significantly giving rise to demand for analysis of more amino acids that induce metabolic errors.

To cope with this, a new method has been developed for simultaneous analysis of additional 12 amino acid components that concern metabolic errors besides the usual 41 components, i.e., 53 components in total. In addition, the amino acid which related to specific metabolic error is now analyzable in a short time for each case.

Amino Acid Components Inducing Metabolic Errors

No.	Abbreviation	Name	Case
1	MetSOX	Methionine sulfoxides	Hypermethioninemia
2	Pipeco	Pipecoric acid	Hyperpipecolatemia
3	HCysH	Homo cysteine	Arteriosclerosis
4	HCit	Homo citrulline	Homocitrullinuria
5	Allo-Ile	Allo isoleucine	Maple syrup urine disease
6	Saccha	Saccharopine	Saccharopinuria
7	ASA	Arginino succinic acid	Argininosuccinuria
8	Cys-HCys	Cysteine-Homocysteine Mixed disulfides	Arteriosclerosis
9	AlevA	Amino levulinic acid	Hepatoporphyrin
10	HCys	Homo cystine	Arteriosclerosis
11	ASA-Anhy1	Arginino saccinic acid Anhydrides 1	Argininosuccinuria
12	AEC	Amino ethyl cysteine	Internal standard substance

A total of 53 components, usual 41 biological-fluid amino acids and additional 12 amino acids that cause metabolic errors can be analyzed in 150 minutes.

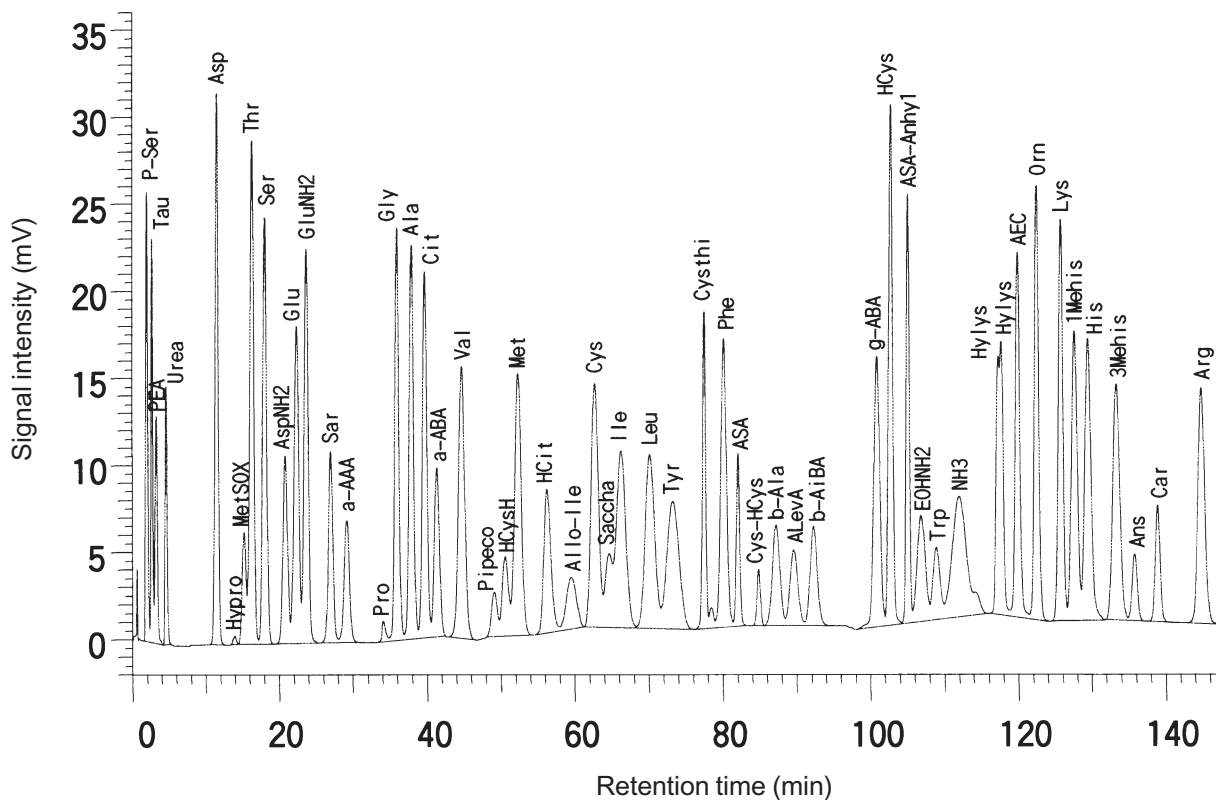


Fig. 1 Chromatogram of Amino Acid Standard Mixture (53 components, 2 nmol)

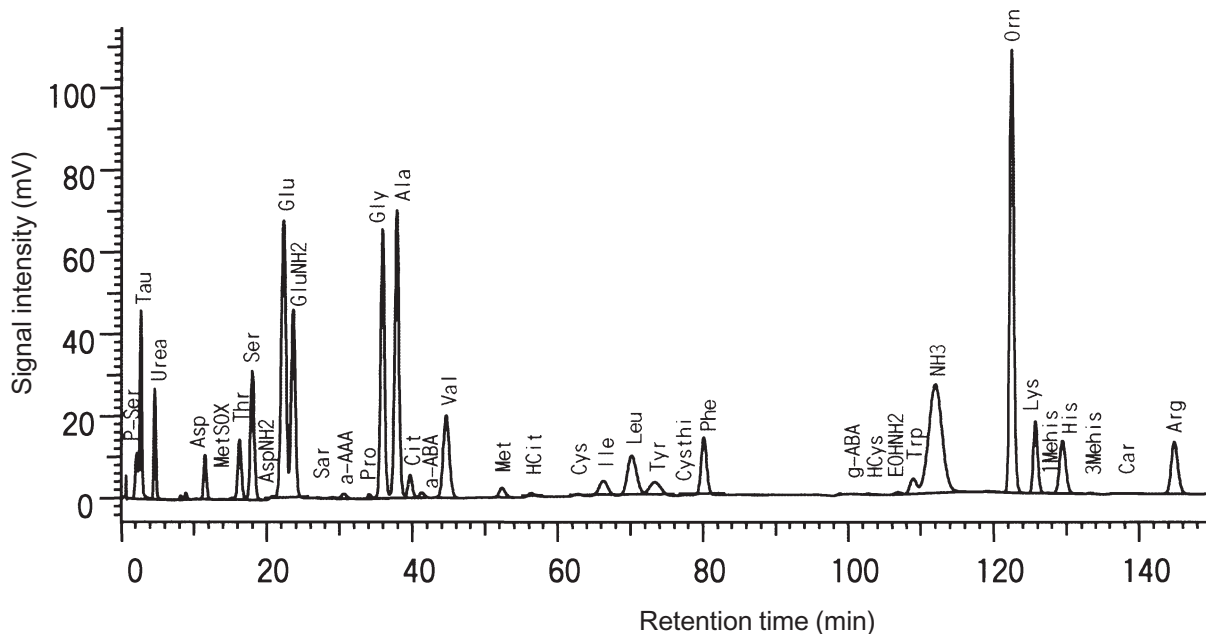


Fig. 2 Chromatogram of Normal Human Serum

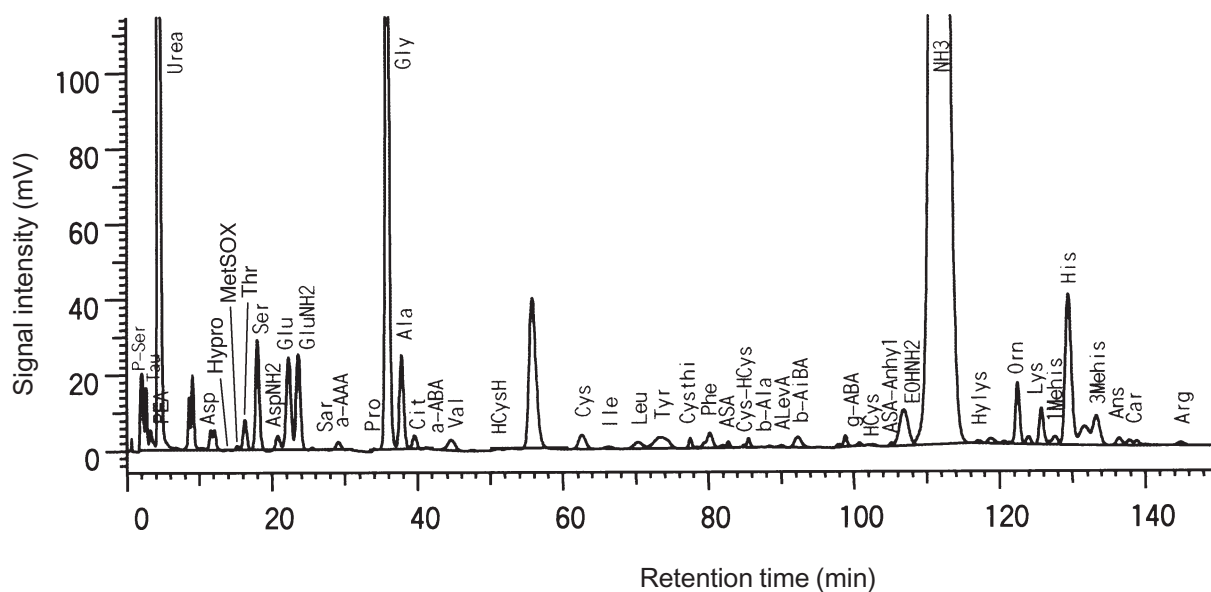


Fig. 3 Chromatogram of Normal Human Urine

<Analytical Conditions>

Column#2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column#2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit

Simultaneous 53-Component Analysis Program

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	100.0	0.0	0.0	0.0	0.0	34	0.36	50.0	50.0	0.0	0.30
2.0	100.0	0.0	0.0	0.0	0.0	31					
20.0	100.0	0.0	0.0	0.0	0.0						
20.1	80.0	20.0	0.0	0.0	0.0	56					
45.0	80.0	20.0	0.0	0.0	0.0	62					
55.0						34					
73.0						66					
84.0	15.0	75.0	10.0	0.0	0.0						
85.0						59					
92.0	15.0	75.0	10.0	0.0	0.0						
92.1	0.0	60.0	40.0	0.0	0.0						
105.0						66					
117.0	0.0	20.0	0.0	80.0	0.0						
117.1	0.0	25.0	0.0	75.0	0.0						
130.0	0.0	25.0	0.0	75.0	0.0						
130.1	0.0	0.0	0.0	100.0	0.0						
145.0	0.0	0.0	0.0	100.0	0.0						
145.1	0.0	0.0	0.0	0.0	100.0						
149.0								50.0	50.0	0.0	
149.1								0.0	0.0	100.0	
153.0	0.0	0.0	0.0	0.0	100.0						
153.1	100.0	0.0	0.0	0.0	0.0						
155.0						34					
159.0								0.0	0.0	100.0	
159.1								50.0	50.0	0.0	
178.0	100.0	0.0	0.0	0.0	0.0						

Adding new 9 amino acid components related with metabolic errors to usual 41 biological-fluid amino acid components, a total of 50 components are analyzable in only 120 minutes.

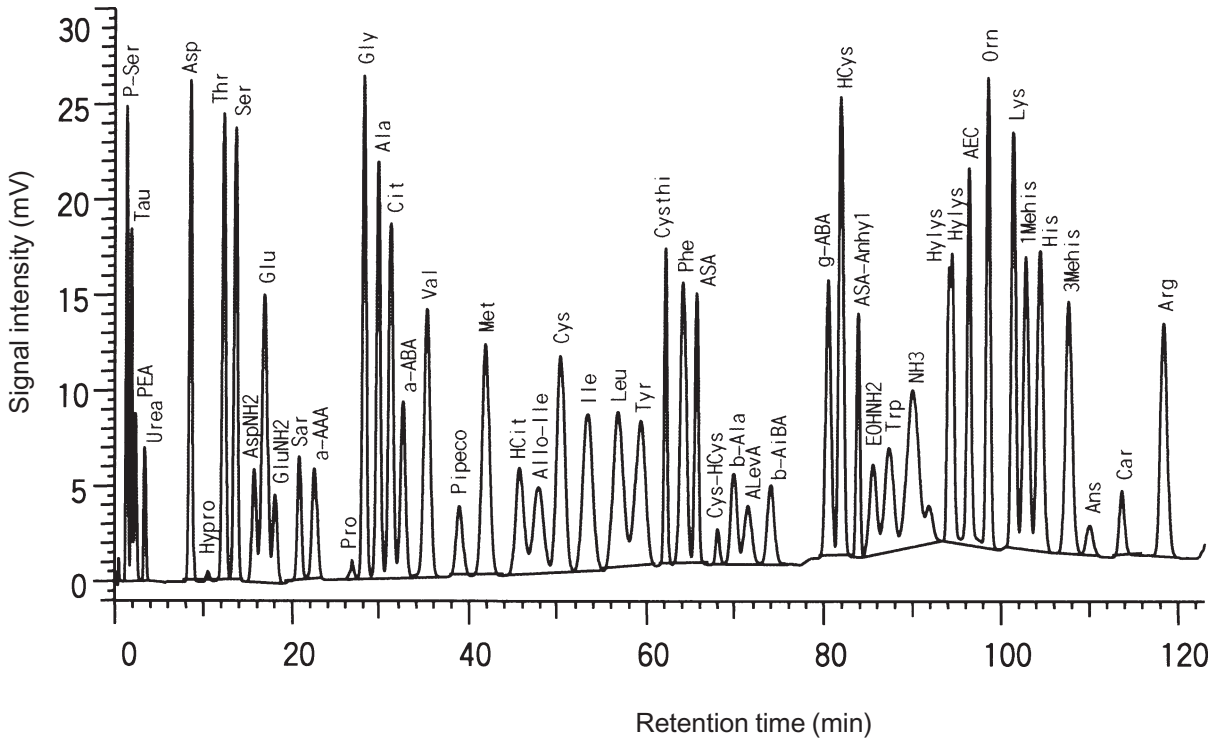


Fig. 4 Chromatogram of Standard Mixture

<Analytical Conditions>

Column#2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column#2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit
 Injection amount 2 nmol

Simultaneous 50-Component Analysis Program

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	100.0	0.0	0.0	0.0	0.0	35	0.47	50.0	50.0	0.0	0.40
1.5	100.0	0.0	0.0	0.0	0.0	32					
16.0	100.0	0.0	0.0	0.0	0.0						
16.1	80.0	20.0	0.0	0.0	0.0	57					
36.0	80.0	20.0	0.0	0.0	0.0	60					
45.0						32					
57.0						70					
68.0	15.0	75.0	10.0	0.0	0.0						
69.0						58					
73.0	15.0	75.0	10.0	0.0	0.0						
73.1	0.0	60.0	40.0	0.0	0.0						
86.0						65					
95.0	0.0	20.0	0.0	80.0	0.0						
95.1	0.0	25.0	0.0	75.0	0.0						
109.0	0.0	25.0	0.0	75.0	0.0						
109.1	0.0	0.0	0.0	100.0	0.0						
119.0	0.0	0.0	0.0	100.0	0.0						
119.1	0.0	0.0	0.0	0.0	100.0						
123.0								50.0	50.0	0.0	
123.1								0.0	0.0	100.0	
125.0	0.0	0.0	0.0	0.0	100.0						
125.1	100.0	0.0	0.0	0.0	0.0						
127.0						35					
128.0								0.0	0.0	100.0	
128.1								50.0	50.0	0.0	
142.0	100.0	0.0	0.0	0.0	0.0						

Homosysteine (HCysH) is now drawing attention as a substance that induces homocysteinuria, arteriosclerosis and thrombosis. Three components including internal standard substance, norvaline (n-Val) can be analyzed in only 11 minutes.

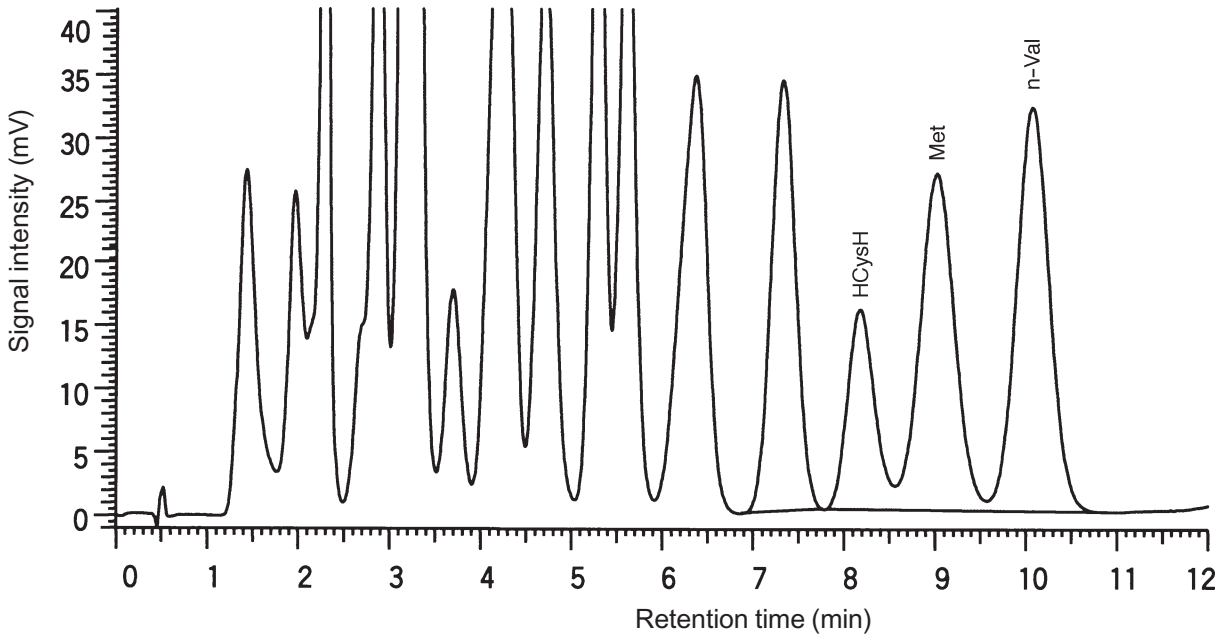


Fig. 5 Chromatogram of Standard Mixture

<Analytical Conditions>

Column#2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column#2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit (PF2 prepared without adding ethanol)
 Injection amount 2 nmol

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	15.0	85.0	0.0	0.0	0.0	57	0.45	50.0	50.0	0.0	0.40
7.8	15.0	85.0	0.0	0.0	0.0						
7.9	0.0	0.0	0.0	100.0	0.0						
8.0	0.0	0.0	0.0	100.0	0.0						
8.1	0.0	0.0	0.0	0.0	100.0						
11.0					100.0			50.0	50.0	0.0	
11.1	0.0	0.0	0.0	0.0	100.0			0.0	0.0	100.0	
12.0	0.0	0.0	0.0	0.0	100.0						
12.1	15.0	85.0	0.0	0.0	0.0						
13.0								0.0	0.0	100.0	
13.1								50.0	50.0	0.0	
23.0	15.0	85.0	0.0	0.0	0.0						

In only 26 minutes, it is possible to analyze 8 components that concern arteriosclerosis, maple syrup urine disease (MSUD), phenylketonuria (PKU), hypermethioninemia and cystathioninuria, and an internal standard substance, acetyl-lysine (Acetyl-Lys).

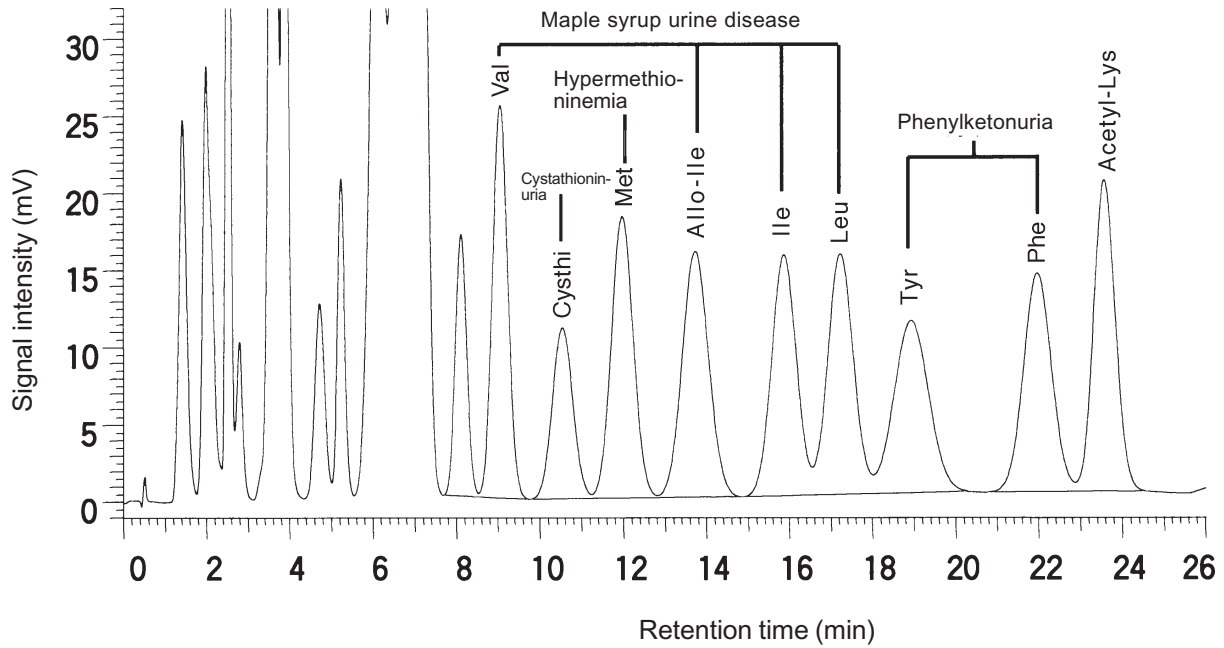


Fig. 6 Chromatogram of Standard Mixture

<Analytical Conditions>

Column #2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column #2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit
 Injection amount 2 nmol

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	27.0	73.0	0.0	0.0	0.0	45	0.45	50.0	50.0	0.0	0.40
2.0	27.0	73.0	0.0	0.0	0.0						
8.0	27.0	73.0	0.0	0.0	0.0						
13.0						40					
18.0	0.0	60.0	40.0	0.0	0.0						
20.0						45					
22.0	0.0	60.0	40.0	0.0	100.0						
22.1	0.0	0.0	0.0	100.0	0.0						
23.0	0.0	0.0	0.0	100.0	0.0						
23.1	0.0	0.0	0.0	0.0	100.0						
25.0	0.0	0.0	0.0	0.0	100.0			50.0	50.0	0.0	
25.1	0.0	0.0	0.0	0.0	100.0			0.0	0.0	100.0	
27.0	0.0	0.0	0.0	0.0	100.0						
27.1	27.0	73.0	0.0	0.0	0.0						
28.0	27.0	73.0	0.0	0.0	0.0	45		0.0	0.0	100.0	
28.1	27.0	73.0	0.0	0.0	0.0			50.0	50.0	0.0	
38.0	27.0	73.0	0.0	0.0	0.0						

In only 26 minutes, 9 components that concern arteriosclerosis, maple syrup urine disease (MSUD) and phenylketonuria (PKU) can be analyzed.

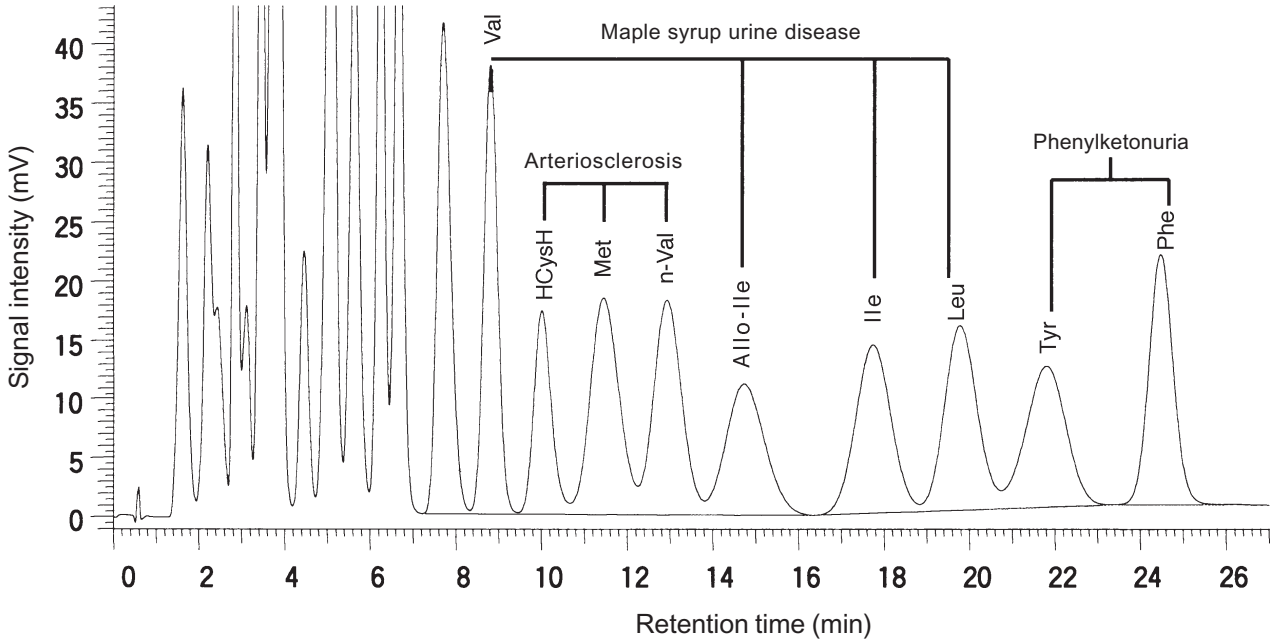


Fig. 7 Chromatogram of Standard Mixture

<Analytical Conditions>

Column#2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column#2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit (PF2 prepared without adding ethanol)
 Injection amount 2 nmol

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	15.0	85.0	0.0	0.0	0.0	55	0.40	50.0	50.0	0.0	0.35
8.0						30					
10.0	15.0	85.0	0.0	0.0	0.0						
18.0	0.0	40.0	60.0	0.0	0.0						
20.0						55					
23.4	0.0	40.0	60.0	0.0	0.0						
23.5	0.0	0.0	0.0	100.0	0.0						
23.6	0.0	0.0	0.0	100.0	0.0						
23.7	0.0	0.0	0.0	0.0	100.0						
27.0	0.0	0.0	0.0	0.0	100.0			50.0	50.0	0.0	
27.1	0.0	0.0	0.0	0.0	100.0			0.0	0.0	100.0	
28.0	0.0	0.0	0.0	0.0	100.0						
28.1	15.0	85.0	0.0	0.0	0.0						
29.0	15.0	85.0	0.0	0.0	0.0	55		0.0	0.0	100.0	
29.1	15.0	85.0	0.0	0.0	0.0			50.0	50.0	0.0	
40.0	15.0	85.0	0.0	0.0	0.0						

Tyrosine and phenylalanine are analyzable in only 10 minutes.

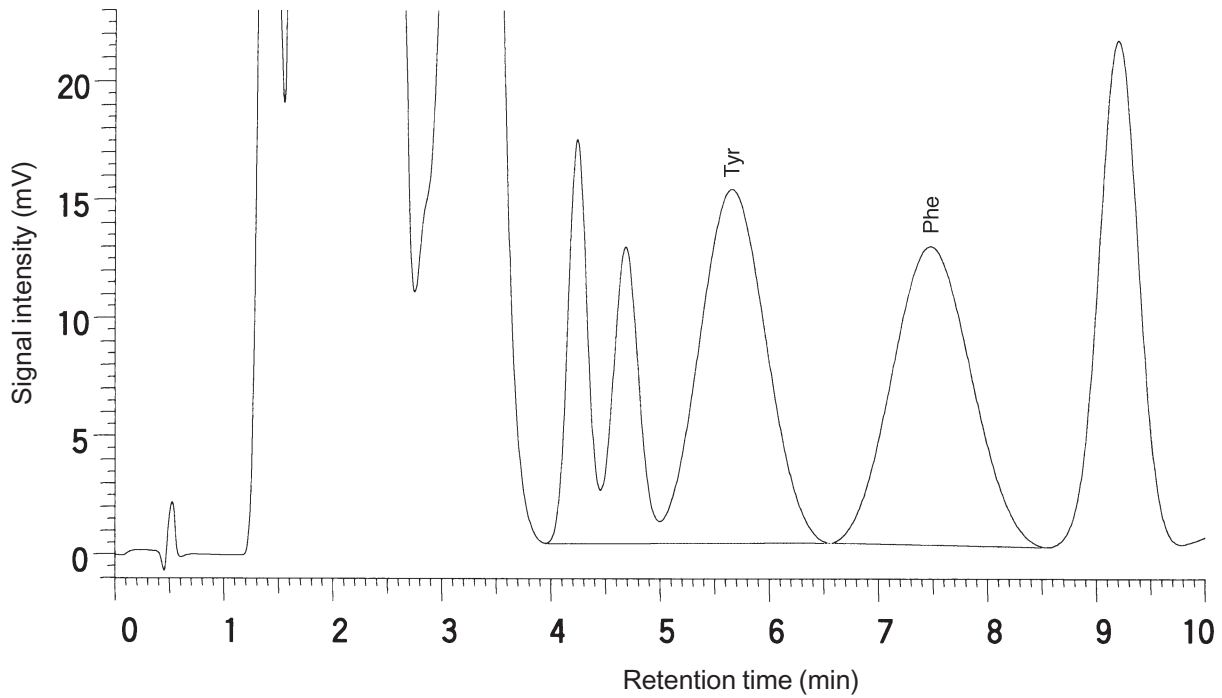


Fig. 8 Chromatogram of Standard Mixture

<Analytical Conditions>

Column#2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column#2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit
 Injection amount 2 nmol

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	0.0	0.0	0.0	100.0	0.0	30	0.45	50.0	50.0	0.0	0.40
6.0	0.0	0.0	0.0	100.0	0.0						
6.1	0.0	0.0	0.0	0.0	100.0						
9.0								50.0	50.0	0.0	
9.1								0.0	0.0	100.0	
10.0	0.0	0.0	0.0	0.0	100.0						
10.1	0.0	0.0	0.0	100.0	0.0						
11.0								0.0	0.0	100.0	
11.1								50.0	50.0	0.0	
21.0	0.0	0.0	0.0	100.0	0.0						

Asparagine (AspNH₂), glutamic acid (Glu), and glutamine (GluNH₂) can be analyzed in 25 minutes, which would otherwise be inseparable by conventional biological-fluid analytical methods.

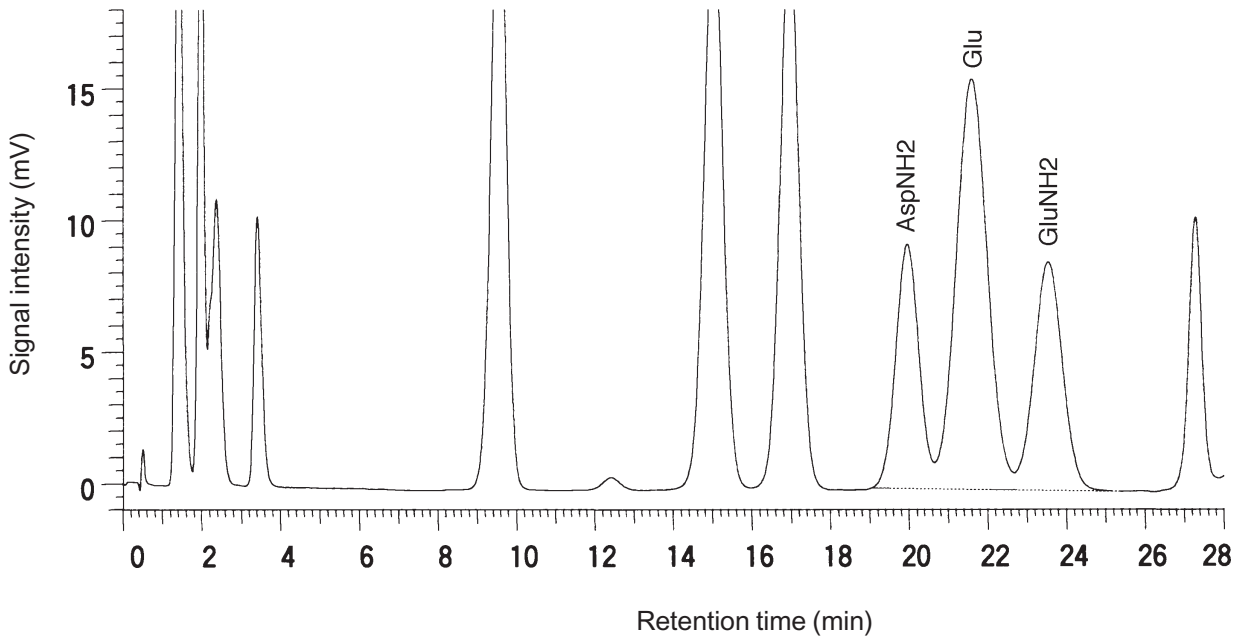


Fig.9 Chromatogram of Standard Mixture

<Analytical Conditions>

Column#2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column#2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit (PF1 prepared by adding 30% of water)
 Injection amount 2 nmol

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	100.0	0.0	0.0	0.0	0.0	35	0.45	50.0	50.0	0.0	0.40
2.0						32					
23.0	100.0	0.0	0.0	0.0	0.0						
23.1	0.0	0.0	0.0	100.0	0.0						
24.0	0.0	0.0	0.0	100.0	0.0						
24.1	0.0	0.0	0.0	0.0	100.0						
27.0								50.0	50.0	0.0	
27.1								0.0	0.0	100.0	
28.0	0.0	0.0	0.0	0.0	100.0						
28.1	100.0	0.0	0.0	0.0	0.0						
29.0						35		0.0	0.0	100.0	
29.1								50.0	50.0	0.0	
39.0	100.0	0.0	0.0	0.0	0.0						

Measurement of 3-methyl histidine (3Mehis) makes it possible to determine the health condition of a fluid-trans-fused patient.

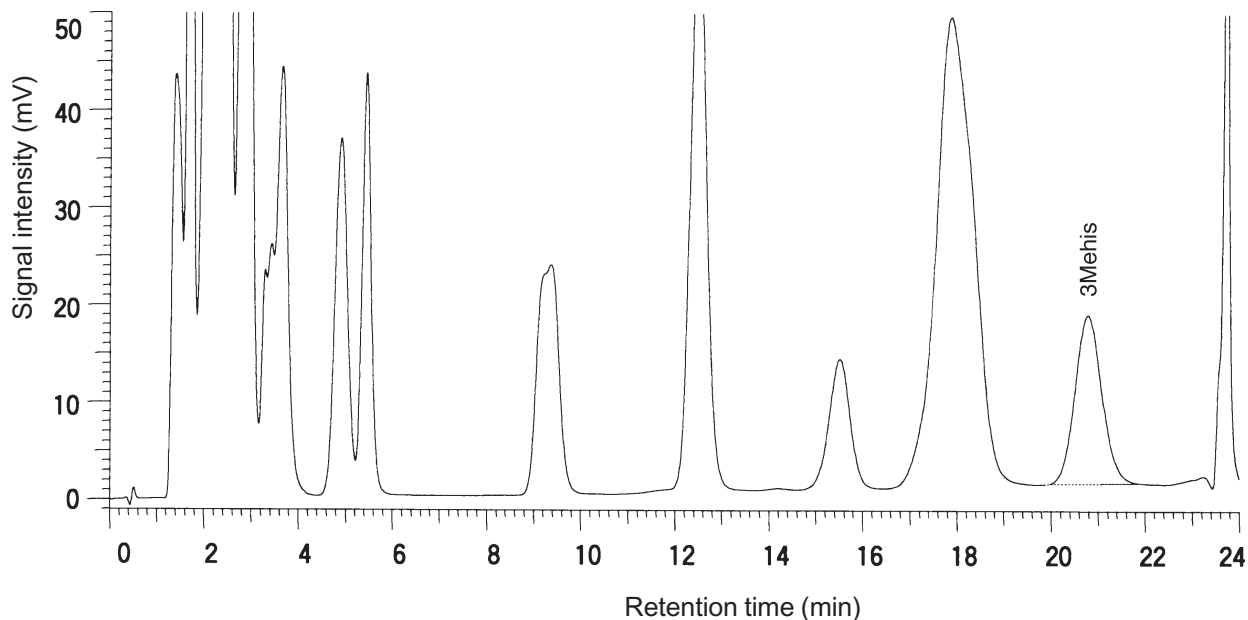


Fig. 10 Chromatogram of Standard Mixture

<Analytical Conditions>

Column#2622 (HITACHI) 4.6 × 60 mm
 Ammonia trap column#2650L (HITACHI) 4.6 × 40 mm
 Eluent L-8500 PF-Kit
 Injection amount 2 nmol

Time (min)	%B1	%B2	%B3	%B4	%B5	Temperature (°C)	Flow rate 1 (mL/min)	%R1	%R2	%R3	Flow rate 2 (mL/min)
0.0	0.0	0.0	0.0	100.0	0.0	70	0.45	50.0	50.0	0.0	0.40
19.0	0.0	0.0	0.0	100.0	0.0						
19.1	0.0	0.0	0.0	0.0	100.0						
21.8								50.0	50.0	0.0	
21.9								0.0	0.0	100.0	
22.0	0.0	0.0	0.0	0.0	100.0						
22.1	0.0	0.0	0.0	100.0	0.0						
23.0								0.0	0.0	100.0	
23.1								50.0	50.0	0.0	
30.0	0.0	0.0	0.0	100.0	0.0						

Keywords: Analysis biological-fluid amino acids, Free amino acid, Amino acid metabolic error

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