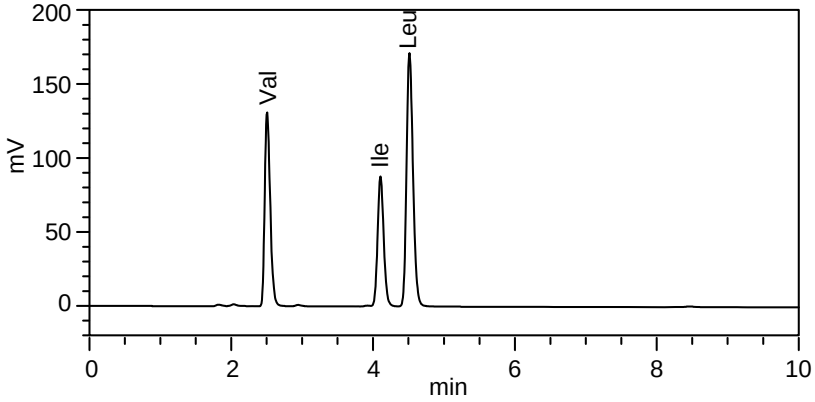
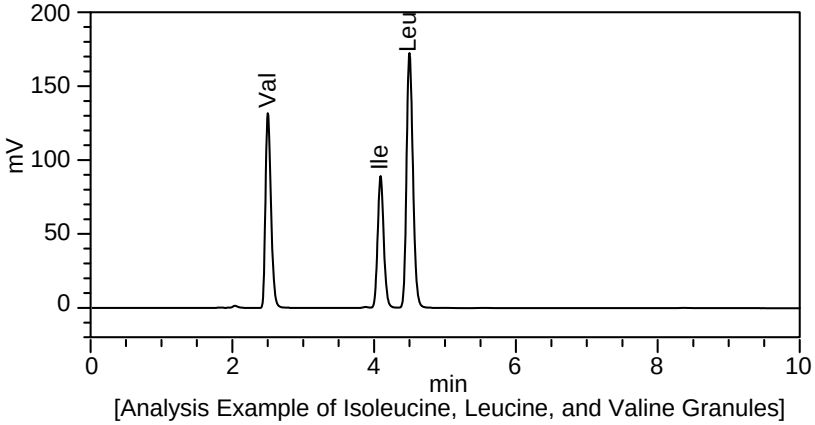
■ Analysis Example for a Drug Listed in the Japanese Pharmacopoeia Sixteenth Edition (Isoleucine, Leucine, and Valine Granules)

The Japanese Pharmacopoeia Sixteenth Edition, effective as of April 2011, specifies that HPLC analysis with a conventional column is to be used for the identification of isoleucine, leucine, and valine granules. It also specifies that the post-column ninhydrin method should be used for the assay.
In this publication, the Chromaster, a Hitachi high speed liquid chromatography instrument, and the Hitachi L-8900 amino acid analyzer were used to perform the identification test and assay under the analysis conditions conforming to the Pharmacopoeia. The specified values and measured values related to the system suitability, etc. were compared.

■ Identification by Chromaster



[Analysis Example of Standard]



[Analysis Example of Isoleucine, Leucine, and Valine Granules]

<Concentration of Each Component>

Component	Concentration (mg/mL)
Val : Valine	1.10
Ile : Isoleucine	0.92
Leu : Leucine	1.84

<Analysis Conditions>
Column : HITACHI LaChrom C18 (3 μm) 4.6 mm I.D. × 150 mm
Eluent : (A) / acetonitrile = 970/30 (v/v)
Flow rate : 0.9 mL/min
Column temperature : 40°C
Detection wavelength: UV 210 nm
Injection volume : 20 μL

*(A) : Add 31.2 g of sodium dihydrogen phosphate dihydrate to 1000 mL of water to dissolve and then add phosphoric acid to adjust the pH to 2.8.
* Adjust the flow rate so that the retention time of valine is about 2.5 minutes.

<Sample Preparation Method>
Weigh 92 mg of L-isoleucine, dissolve in eluent, and adjust volume to 100 mL.
The solution is filtered through a 0.45 μm filter prior to injection.

[Identification]
Shown below are the system suitability requirements for the identification of isoleucine, leucine, and valine, as well as the analytical results. This comparison shows that the results meet the requirements for "peak retention times that are the same."

<System Suitability for Standard>

Item		Requirement for system suitability	Measurement result
System performance	Elution order	In order of Val, Ile, and Leu	OK
	Resolution for Ile -Leu	NLT 1.5	2.55
System repeatability	Relative standard deviation of each peak retention time (n = 6)	NMT 1.0 %	0.05 % (Val) 0.09 % (Iie) 0.09 % (Leu)

<Result for Identification>

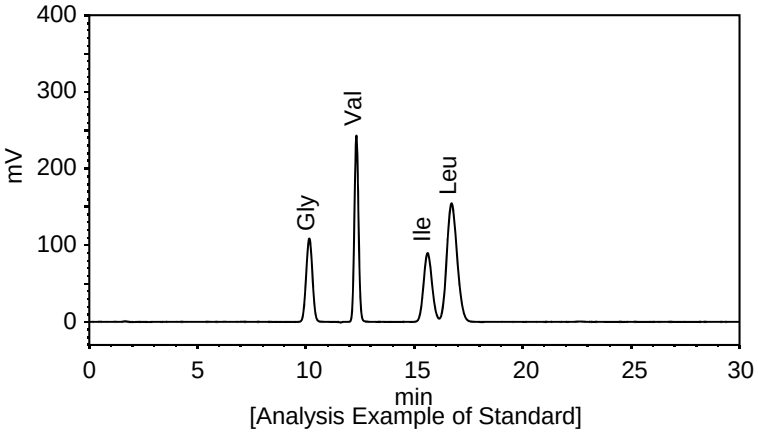
Component	Requirement	Retention time of standard (min)	Retention time of sample (min)
Val : Valine	Peak retention times are the same	2.51	2.50
Ile : Isoleucine		4.11	4.09
Leu : Leucine		4.51	4.50

*The sample for this analysis was provided by Division of Physical Pharmaceutical Chemistry, Faculty of Pharmacy at Keio University.

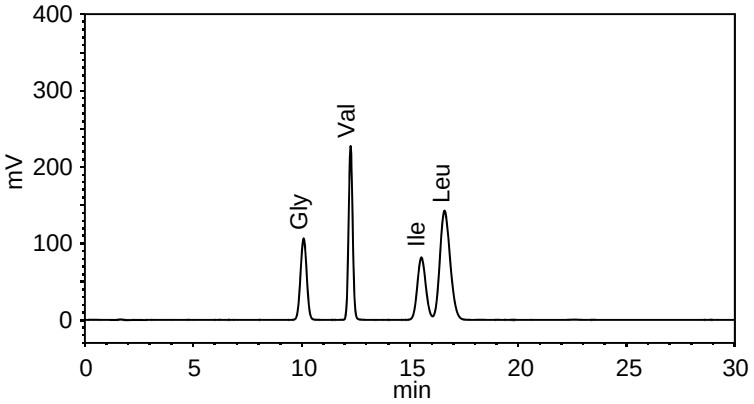
Main components of instrument: Chromaster, 5110 pump, 5210 autosampler, 5310 column oven, 5410 UV detector

■ Analysis Example for a Drug Listed in the Japanese Pharmacopoeia Sixteenth Edition (Isoleucine, Leucine, Valine Granules)

■ Assay Method by L-8900



[Analysis Example of Standard]



[Analysis Example of Isoleucine, Leucine, Valine Granules]

[Assay]
Shown below are the system suitability requirements for the assay as well as the analytical results, which meet the requirements.

<Concentration of Each Component>

Component	Concentration in the sample for injection (ng/20 μL)
Gly : Glycine (internal standard)	400
Val : Valine	960
Ile : Isoleucine	800
Leu : Leucine	1600

<Analysis Conditions for Standard Analysis Method>

Column : #2622PH 4.6 mm I.D. × 60 mm
Ammonia filter column: #2650L 4.6 mm I.D. × 40 mm
Eluent : PH Buffer Kit
Flow rate : 0.4 mL/min
Column temperature : 57°C
Reaction reagent : Ninhydrin coloring solution kit for HITACHI
Reaction reagent flow rate: 0.35 mL/min
Reaction temperature : 130°C
Detection wavelength : VIS 570 nm
Injection volume : 20 μL

<Sample Preparation Method>
Weigh 0.95 g of L-isoleucine. Add 10 mL of the internal standard solution (glycine) (*), dissolve in 0.1 mol/L hydrochloric acid reagent, and adjust volume to 250 mL. Dilute 2 mL of the above solution to a volume of 200 mL with 0.02 mol/L hydrochloric acid. Filter the solution through a 0.2 μm filter.
(*) Internal standard solution: Glycine in 0.1 mol/L hydrochloric acid reagent solution (1 g/ 20 mL)

<System Suitability for Standards>

Item		Requirement for system suitability	Measurement result
System performance	Elution order	In order of Val, Ile, and Leu	OK
	Resolution for Ile -Leu	NLT 1.2	1.37
System repeatability	Relative standard deviation for area ratio of the internal standard (Gly) peak to each peak (n = 6)	NMT 1.0 %	0.09 % (Val) 0.17 % (Ile) 0.09 % (Leu)

<Assay Result>

Component	Content / Label claim × 100 (%)	
	Required value	Measurement result
Val : Valine	93.0 - 107.0	100.3
Ile : Isoleucine		98.4
Leu : Leucine		100.4

* The sample for this analysis was provided by Division of Physical Pharmaceutical Chemistry, Faculty of Pharmacy at Keio University.

Main component of instrument: Hitachi L-8900 amino acid analyzer

Note: The data here is shown as an example of the analysis and does not warrant the performance of the instrument.