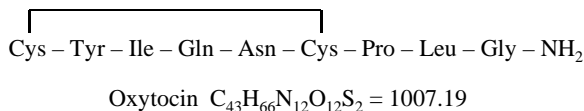


Oxytocin is a peptide composed of 9 amino acids. One of the tests performed to evaluate the quality of peptide drugs such as oxytocin is the confirmation of the amino acid composition.

Presented here is the analysis of oxytocin using the high resolution column for the Hitachi L-8900 high-speed amino acid analyzer. The amino acid composition of oxytocin was determined by using an analysis method that conforms to the Japanese Pharmacopoeia. The standard reagent oxytocin acetate was used as a model sample of the drug product.

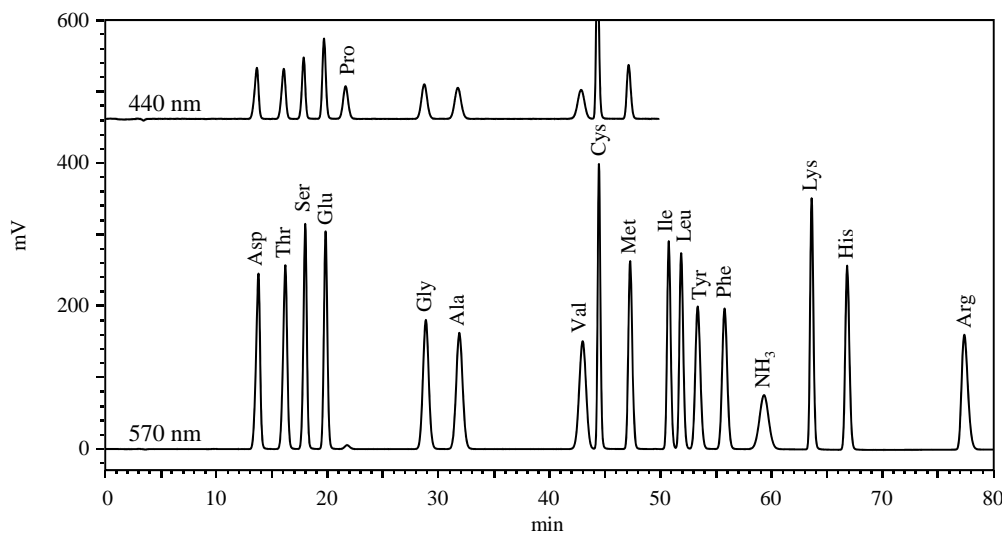


[Structural Formula of Oxytocin]



[Hitachi L-8900 High-speed Amino Acid Analyzer]

■ Analysis of Oxytocin Standard (Confirmation of System Suitability)



[Analysis of Amino Acid Standard Solution (10 nmol/20 μL each)]

(Cys in the chromatogram indicates cystine (cysteine dimer).)

<Analytical Conditions for Standard Analysis Method>

Column	: #2622 4.6 mm I.D. × 80 mm	Reaction reagent	: Ninhydrin coloring solution kit for HITACHI
Ammonia filter column	: #2650L 4.6 mm I.D. × 60 mm	Reaction reagent flow rate	: 0.3 mL/min
Eluent	: PH-Buffer-Kit	Reaction temperature	: 130°C
Flow rate	: 0.26 mL/min	Detection wavelength	: VIS 440 nm, 570 nm
Column temperature	: 57°C	Injection vol.	: 20 μL

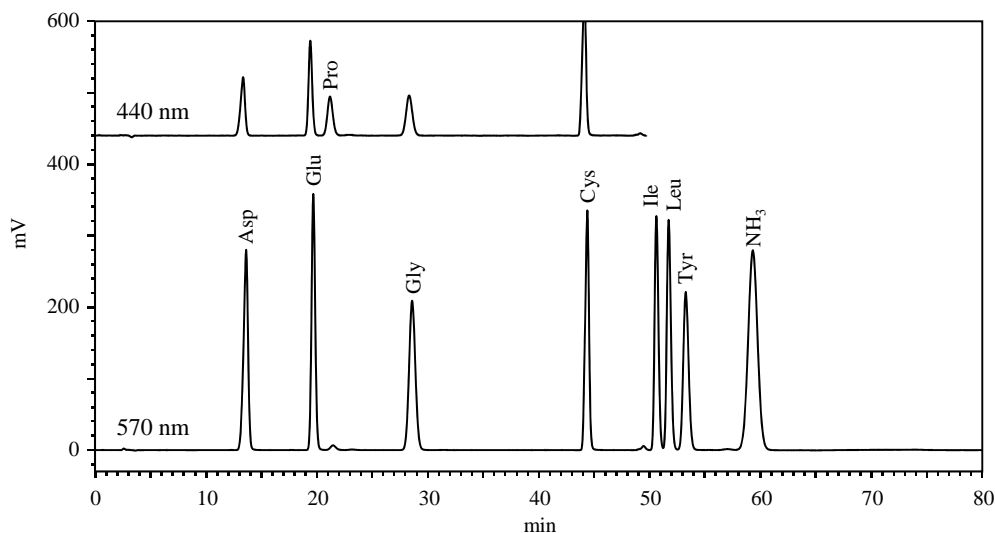
(Note: Eluent was partly adjusted according to the Pharmacopoeia.)

System Suitability Item		Specification	Analysis Result
System performance	Amino acid elution order	In the order of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine	As shown on the left
	Resolution (Thr-Ser)	Not Less Than 1.5	2.93
	Resolution (Gly-Ala)	Not Less Than 1.4	2.93
	Resolution (Ile-Leu)	Not Less Than 1.2	1.79
System reproducibility	Relative standard deviation (n=3) each for aspartic acid, proline, valine, and arginine peaks	Not More Than 2.0% for each	0.07%, 0.41% 0.37%, 0.09%

[System Suitability]

The results fully satisfy the specifications for elution order, resolution, area value, and relative standard deviation.

■ Analysis of Oxytocin (Confirmation of Constitutive Amino Acids)



[Analysis of Oxytocin]
(Cys in the chromatogram indicates cystine (cysteine dimer).)

<Confirmation of Constitutive Amino Acids>

Component name	Specification value (Molar ratio to Leu)	Analysis result
Aspartic acid (Asp)	0.95 - 1.05	0.997
Glutamic acid (Glu)	0.95 - 1.05	0.995
Glycine (Gly)	0.95 - 1.05	1.000
Cysteine (Cys)	0.80 - 1.05	0.935
Isoleucine (Ile)	0.80 - 1.10	0.963
Leucine (Leu)	Define as 1	Define as 1
Tyrosine (Tyr)	0.80 - 1.05	0.934
Proline (Pro)	0.95 - 1.05	1.007

<Sample Preparation Method (Hydrolysis)>

Sample 1 mg
| ← 1 mL of 6 mol/L hydrochloric acid
De-gas
|
Heat 115°C, 16 hr
|
Dry under vacuum
|
Dissolve in 2 mL of 0.02 mol/L hydrochloric acid
|
Filtration Pore Size 0.2 μm
|
Sample for amino acid analysis (20 μL)

The molar ratios of amino acids other than leucine to leucine (Leu) was 0.93 – 1.01, relative to the theoretical value of 1, indicating that good results were obtained.