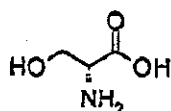


Specification and Testing Method of L-Serine

This Testing Method is based on The Japanese Pharmacopoeia Fourteenth Edition (JP 14). Please refer to The Japanese Pharmacopoeia for the method of general tests, reagents, making test solutions (TS) and standard solutions for volumetric analysis (VS).

• *The Japanese Pharmacopoeia (English Version) HP* : <http://jpdh.nhs.go.jp/jp14e/>

L-Serine



$C_3H_7NO_3$: 105.09

(2*R*)-2-Amino-3-hydroxypropanoic acid

pH Dissolve 5.0 g in 50 mL of water: the pH of this solution is between 5.2 and 6.2.

Specific Rotation $[\alpha]_D^{20}$: -14.4 - -15.7° Weigh accurately about 1.0 g of L-Serine, previously dried, and dissolve in 2 mol/L hydrochloric acid TS to make exactly 10 mL, and determine the optical rotation of the solution in a 100-mm cell.

Chloride—Perform the test with 0.7 g of L-Serine. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.02%).

Sulfate—Perform the test with 1.2 g of L-Serine. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.02%).

Ammonium—Perform the test with 0.25 g of L-Serine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

Heavy metals—Proceed with 2.0 g of L-Serine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Arsenic—Dissolve 2.0 g of L-Serine in 10 – 15 mL of water by heating, and perform the test with this solution as the test solution using Apparatus B (not more than 1 ppm).

Other amino acids—Dissolve 0.20 g of L-Serine in water to make accurately 10 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 10mL. Pipet 1 mL of this solution, add water to make exactly 50mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μ L

each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the mixture of 1-butanol, water, ethanol (99.5), ammonia solution (28) (8:3:2:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Transmittance

1) Weigh 2.5 g of L-Serine; dissolve in water to make 50 mL (the sample solution). Perform the test and measure the percent transmission (*T*) of this solution as directed under the Ultraviolet-visible Spectrophotometry (wavelength : 430 nm, length of the layer of the solution : 1 cm): it shows more than 98.0%.

2) Weigh 5.0 g of L-Serine, dissolve in water to make 50 mL (the sample solution). Perform the test and measure the percent transmission (*T*) of this solution as directed under the Ultraviolet-visible Spectrophotometry (wavelength : 430 nm, length of the layer of the solution : 1 cm): it shows more than 96.0%.

Loss on drying Not more than 0.20 % (2 g, 105°C, 3 hours).

Residue on ignition Not more than 0.10 % (2 g).

D-Serine D-Serine contained in L-Serine is analyzed by HPLC using chiral column under following conditions.

Sample Making

Dissolve 10 mg of L-Serine in 20 mL of water, and perform HPLC with this solution.

HPLC Condition

Column : TSK-GEL Enantio L1 ϕ 4.6mm \times 250mm (TOSOH)
 Mobile Phase : 0.5 mM Copper(II) sulfate
 Flow Rate : 1.0 mL/min
 Wavelength : 254 nm
 Column Temperature : 50°C
 Injection Volume : 3 μ L

Purity Weigh accurately about 0.2 g of L-Serine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
 = 10.509 mg C₃H₇NO₃