

melts between 174°C and 179°C. With the residue, proceed as directed in the Identification (1) and (2) under Phenobarbital.

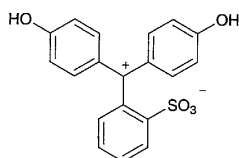
**Assay** Weigh accurately about 10 g of 10% Phenobarbital Powder, transfer to a glass-stoppered flask, and add exactly 100 mL of a mixture of chloroform and ethanol (95) (10:1). Stopper tightly, shake, and allow to stand for 30 minutes. Transfer the mixture to a glass-stoppered centrifuge tube, and centrifuge. Measure exactly 50 mL of the supernatant liquid, evaporate on a water bath to dryness, dissolve the residue in 50 mL of *N,N*-dimethylformamide, and proceed as directed in the Assay under Phenobarbital.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS  
= 23.224 mg of  $C_{12}H_{12}N_2O_3$

**Containers and storage** Containers—Well-closed containers.

## Phenolsulfonphthalein

フェノールスルホンフタレイン



$C_{19}H_{14}O_5S$ : 354.38  
2-[Bis(4-hydroxyphenyl)methylumyl]benzenesulfonate  
[143-74-8]

Phenolsulfonphthalein, when dried, contains not less than 98.0% of  $C_{19}H_{14}O_5S$ .

**Description** Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.

It is very slightly soluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS.

**Identification (1)** Dissolve 5 mg of Phenolsulfonphthalein in 2 to 3 drops of sodium hydroxide TS, add 2 mL of 0.05 mol/L bromine VS and 1 mL of dilute sulfuric acid, shake well, and allow to stand for 5 minutes. Render the solution alkaline with sodium hydroxide TS: a deep blue-purple color develops.

**(2)** Dissolve 0.01 g of Phenolsulfonphthalein in diluted sodium carbonate TS (1 in 10) to make 200 mL. To 5 mL of this solution add diluted sodium carbonate TS (1 in 10) to make 100 mL. Perform the test with this solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Insoluble substances—To about 1 g of Phenolsulfonphthalein, accurately weighed, add 20 mL of a solution of sodium hydrogen carbonate (1 in 40). Allow the mixture to stand for 1 hour with frequent shaking, dilute with water to 100 mL, and allow to stand for 24 hours. Collect the insoluble substances using a tared glass filter (G4), wash

with 25 mL of a solution of sodium hydrogen carbonate (1 in 100) and with five 5-mL portions of water, and dry at 105°C for 1 hour: the mass of the residue is not more than 0.2%.

**(2)** Related substances—Dissolve 0.10 g of Phenolsulfonphthalein in 5 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Pipet 0.5 mL of this solution, add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10  $\mu$ L each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of *t*-amyl alcohol, acetic acid (100) and water (4:1:1) to a distance of about 15 cm, and air-dry the plate. After allowing the plate to stand in an ammonia vapor, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** Not more than 1.0% (1 g, silica gel, 4 hours).

**Residue on ignition** Not more than 0.20% (1 g).

**Assay** Weigh accurately about 0.15 g of Phenolsulfonphthalein, previously dried, transfer to an iodine flask, dissolve in 30 mL of a solution of sodium hydroxide (1 in 250), and add water to make 200 mL. Add exactly measured 50 mL of 0.05 mol/L bromine VS, add 10 mL of hydrochloric acid to the solution quickly, and stopper immediately. Allow the mixture to stand for 5 minutes with occasional shaking, add 7 mL of potassium iodide TS, stopper again immediately, and shake gently for 1 minute. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS  
= 4.430 mg of  $C_{19}H_{14}O_5S$

**Containers and storage** Containers—Well-closed containers.

## Phenolsulfonphthalein Injection

フェノールスルホンフタレイン注射液

Phenolsulfonphthalein Injection is an aqueous solution for injection. It contains not less than 0.54 w/v% and not more than 0.63 w/v% of phenolsulfonphthalein ( $C_{19}H_{14}O_5S$ : 354.38).

### Method of preparation

Phenolsulfonphthalein	6 g
Sodium Chloride	9 g
Sodium Bicarbonate (or Sodium Hydroxide)	1.43 g (0.68 g)
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above in-

redients.

**Description** Phenolsulfonphthalein Injection is a clear, orange-yellow to red liquid.

**Identification** To 1 mL of Phenolsulfonphthalein Injection add 2 to 3 drops of sodium hydroxide TS, and proceed as directed in the Identification (1) under Phenolsulfonphthalein.

**pH** 6.0 – 7.6

**Sensitivity** To 1.0 mL of Phenolsulfonphthalein Injection add 5 mL of water. To 0.20 mL of this solution add 50 mL of freshly boiled and cooled water and 0.40 mL of 0.01 mol/L sodium hydroxide VS: a deep red-purple color develops, and it changes to light yellow on the addition of 0.40 mL of 0.005 mol/L sulfuric acid VS.

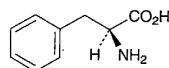
**Assay** Pipet 5 mL of Phenolsulfonphthalein Injection, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.03 g of phenolsulfonphthalein for assay, previously dried in a desiccator (silica gel) for 4 hours, and dissolve in a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances,  $A_T$  and  $A_S$ , of the sample solution and the standard solution at 559 nm as directed under the Ultraviolet-visible Spectrophotometry.

$$\begin{aligned} & \text{Amount (mg) of phenolsulfonphthalein (C}_{19}\text{H}_{14}\text{O}_5\text{S)} \\ &= \text{amount (mg) of phenolsulfonphthalein for assay} \\ & \quad \times \frac{A_T}{A_S} \end{aligned}$$

**Containers and storage** Containers—Hermetic containers.

## L-Phenylalanine

L-フェニルアラニン



$\text{C}_9\text{H}_{11}\text{NO}_2$ ; 165.19

(2S)-2-Amino-3-phenylpropanoic acid [63-91-2]

L-Phenylalanine, when dried, contains not less than 98.5% of  $\text{C}_9\text{H}_{11}\text{NO}_2$ .

**Description** L-Phenylalanine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

**Identification** Determine the infrared absorption spectrum of L-Phenylalanine, previously dried, as directed in the potassium bromide disk method under the Infrared Spec-

trophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation**  $[\alpha]_D^{20}$ :  $-33.0 - -35.5^\circ$  (after drying, 0.5 g, water, 25 mL, 100 mm).

**pH** Dissolve 0.20 g of L-Phenylalanine in 20 mL of water: the pH of this solution is between 5.3 and 6.3.

**Purity (1)** Clarity and color of solution—Dissolve 0.5 g of L-Phenylalanine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride—Perform the test with 0.5 g of L-Phenylalanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate—Perform the test with 0.6 g of L-Phenylalanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

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

(5) Heavy metals—Dissolve 1.0 g of L-Phenylalanine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic—Dissolve 1.0 g of L-Phenylalanine in 5 mL of dilute hydrochloric acid and 15 mL of water, and perform the test with this solution as the test solution using Apparatus B (not more than 2 ppm).

(7) Other amino acids—Dissolve 0.10 g of L-Phenylalanine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5  $\mu\text{L}$  each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°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.

**Loss on drying** Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** Not more than 0.10% (1 g).

**Assay** Weigh accurately about 0.17 g of L-Phenylalanine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L perchloric acid VS} \\ &= 16.519 \text{ mg of } \text{C}_9\text{H}_{11}\text{NO}_2 \end{aligned}$$

**Containers and storage** Containers—Tight containers.