JP XIV

25°C.

Reaction coil: A column 0.25 μm in inside diameter and 5
m in length.

Mobile phase: Dissolve 28.41 g of anhydrous sodium sul-
fate and 5.23 g of sodium 1-pentane sulfonate in 900 mL of
water, add 1 mL of acetic acid (100), and add water to make
exactly 1000 mL.

Reagent: To 500 mL of boric acid-potassium chloride-sodi-
um hydroxide buffer solution, pH 10.0, add 5 mL of a so-
lution of o-phthalaldehyde in ethanol (95) (2 in 25), 1 mL of
2-mercaptoethanol and 2 mL of a solution of
lauramacrogol (1 in 4).

Reaction temperature: A constant temperature of about
45°C.

Flow rate of the mobile phase: About 0.6 mL per minute.
Flow rate of the reagent: About 0.5 mL per minute.

System suitability—

System performance: Dissolve 2 mg of Gentamicin B in
10 mL of the standard solution. When the procedure is run
with 5 μL of this solution under the above operating condi-
tions, isepamicin and gentamicin B are eluted in this order
with the resolution between these peaks being not less than
1.0.

System repeatability: When the test is repeated 5 times
with 5 μL of the standard solution under the above operat-
ing conditions, the relative standard deviation of the peak
areas of isepamicin is not more than 3%.

Containers and storage Containers—Tight containers.

L-Isoleucine

L-イソロイシン

C₉H₁₃NO₂: 131.17
(2S,3S)-2-Amino-3-methylpentanoic acid [73-32-5]

L-Isoleucine, when dried, contains not less than
98.5% of C₉H₁₃NO₂.

Description L-Isoleucine occurs as white crystals or crystal-
line 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-Isoleucine, previously dried, as directed in the potas-
sium bromide disk method under the Infrared Spectrophot-
ometry, and compare the spectrum with the Reference Spec-
trum: both spectra exhibit similar intensities of absorption
at the same wave numbers.

Optical rotation [α]D₂⁰: +39.5 - +41.5° (after drying, 1 g,
6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH Dissolve 1.0 g of L-Isoleucine in 100 mL of water: the
pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g
of L-Isoleucine 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-Isoleu-
cine. 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-Isoleucine.
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-
Isoleucine. Prepare the control solution with 5.0 mL of Stan-
dard Ammonium Solution (not more than 0.02%).

(5) Heavy metals—Dissolve 1.0 g of L-Isoleucine 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 solu-
tion 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—Prepare the test solution with 1.0 g of L-
Isoleucine according to Method 2, and perform the test us-
ing Apparatus B (not more than 2 ppm).

(7) Other amino acids—Dissolve 0.10 g of L-Isoleucine
in 25 mL of water, and use this solution as the sample sol-
tion. 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 μL 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 the plate with a solution
of ninhydrin in acetone (1 in 50), 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 0.13 g of L-Isoleucine,
previously dried, and dissolve in 3 mL of formic acid, add
50 mL of acetic acid (100), and titrate with 0.1 mol/L per-
chloric acid VS (potentiometric titration). Perform a blank
determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.117 mg of C₉H₁₃NO₂

Containers and storage Containers—Tight containers.

Isoniazid

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