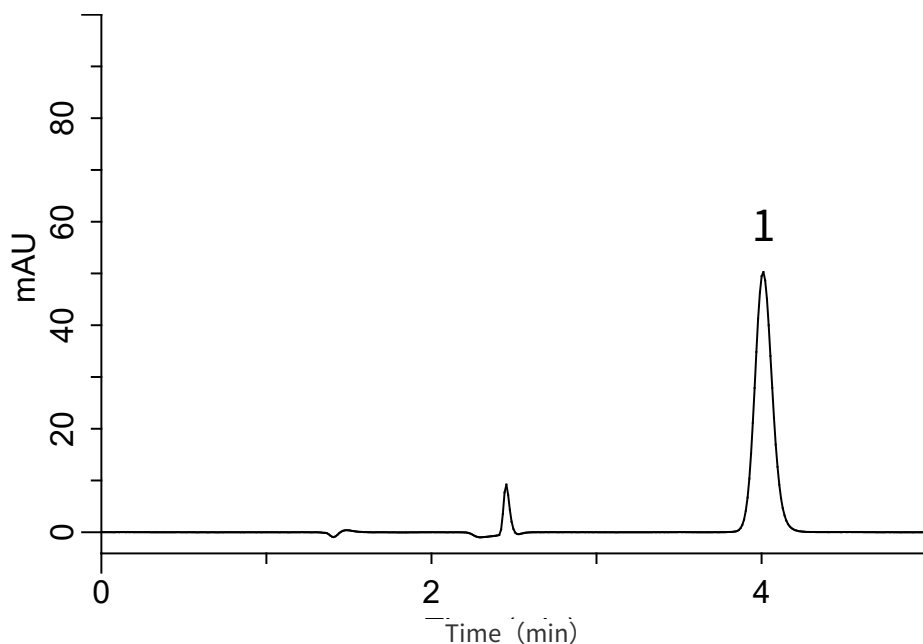


Analysis of Methotrexate

(Under the Condition of the draft for the Japanese Pharmacopoeia,
Methotrexate Tablets)

Data No. LB415-0894



Conditions

System : GL7700 HPLC system
Column : Inertsil ODS-4
 (5 μ m, 150 x 4.0 mm I.D.)
Column Cat. No. : 5020-03935
Eluent : A) CH₃CN
 B) Buffer*
 A/B = 11/89, v/v
Flow Rate : 0.85 mL/min
Col. Temp. : 25 °C
Detection : UV 302 nm (PD7752 PDA Detector)
Injection Vol. : 50 μ L
Sample : Standard

Analyte:

1. Methotrexate 2.5 mg/L
 Theoretical plate number : 6,616 ($\geq 3,000$)
 Tailing factor : 1.11 (≤ 1.5)
 RSD of the
 area (%) (n = 6) : 0.06 (≤ 1.0)

*Dissolve 27.22 g of potassium dihydrogen phosphate in 1,000 mL of water (Solution A).
 Dissolve 4.3 g of sodium hydroxide in 100 mL of water (Solution B).
 Mix 250 mL of Solution A and 29 mL of Solution B.
 Add water to make 1,000 mL.