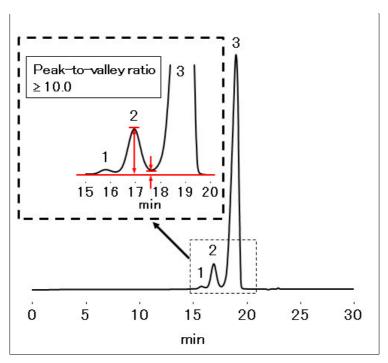
Analysis of Insulin According to USP Method (KW-802.5)

According to USP 39 method, high molecular weight proteins in insulin should be analyzed using a column packed with L20. It is necessary for the system suitability to satisfy retention times (between 13 and 17 min for the polymeric insulin complexes, about 17.5 min for the covalent insulin dimer, and between 18 and 22 min for the insulin monomer), and peak-to-valley ratio which is the ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak \geq 2. It was confirmed that the all conditions were satisfied when they were analyzed using PROTEIN KW-802.5.

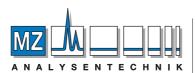


Sample: 100 µL

- 4.0mg/mL of Insulin (beef) containing dimer (in 0.01N HCl aq.)
- 1. High molecular weight proteins
- 2. Insulin dimer
- 3. Insulin monomer

Column : Shodex PROTEIN KW-802.5 (8.0mmI.D. x 300mm) Eluent : 0.1wt% L-Arginine aq./CH3CN/CH3COOH=13/4/3

Flow rate : 0.5 mL/min Detector : UV(276nm) Column temp. : $25\,^{\circ}C$



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