投 稿 ▶技術報告

デキストラン70のエンドトキシン試験の条件検討

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Study of Endotoxin Test Conditions for Dextran 70

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Summary

Endotoxin, a type of pyrogen that can cause fever and shock when injected into the bloodstream, is a common pharmaceutical contaminant. The bacterial endotoxins test (BET) is listed in the Japanese Pharmacopoeia (JP) for detecting endotoxins. Although the JP also lists a rabbit pyrogen test, this is being replaced by the BET in order to reduce false negatives and in response to concerns about animal welfare. Supplement I to the JP Eighteenth Edition introduced a BET for dextran 70 with an endotoxin limit of 4.2 endotoxin units per gram (EU/g). However, this limit is stricter than those in the European Pharmacopoeia (16 EU/g) and the United States Pharmacopeia (8.33 EU/g), necessitating further examination of the JP BET for dextran 70. This study evaluated the gel-clot technique for the BET of dextran 70 by conducting the "test for interfering factors." According to the JP, the sample solution's pH should be between 6.0 and 8.0; however, dextran 70 initially had a pH of 5.6-6.0. Therefore, the pH was adjusted to 6.8-6.9 using a tris (hydroxymethyl) aminomethane-hydrochloride buffer. It was also found that the lysate reagent could appropriately buffer the solution. Using a lysate reagent with a labeled sensitivity λ of 0.03 EU/mL, the BET for dextran 70 at pH 6.8-6.9 showed an endotoxin endpoint concentration of 0.030-0.036 EU/mL (1-1.2 λ). This lies within the 0.5-2 λ range indicating no interfering factors as described in the JP. Moreover, consistent test results were obtained even when the pH of dextran 70 sample solutions was 5.6-6.0 and 8.3-8.4. This indicates that the BET for dextran 70 is robust across a broader pH range than the 6.0-8.0 range described in the JP. These results confirm that the BET for dextran 70 can detect endotoxin exceeding the JP limit of 4.2 EU/g when the pH is adjusted to the 6.0-8.0 range using an appropriate buffer, as described in the JP.

Key words

Japanese Pharmacopoeia, Bacterial Endotoxin Test, Dextran 70, Gel-clot technique, Test for interfering factors