Estimation of endotoxin level in nanocrystal dispersion of DK-I-56-1 intended for parenteral administration

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Introduction and Aim

Estimation of endotoxin level in parenteral formulations is a prerequisite for numerous in vitro tests in preclinical studies and for future clinical development. However, the Limulus amoebocyte lysate (LAL) test in formulations containing nanoparticles could often lead to misinterpretation of results (1, 2). Therefore, we tested if endotoxins could be detected in nanocrystal dispersions by the commercial gel clot assay kit

Methods

Nanocrystals of DK-I-56-1 (7-methoxy-2-(4-methoxy-d3-phenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one) were prepared by wet-ball milling, lyophilized and reconstituted with water for injection prior experiment (3). Different dilutions of nanocrystal dispersion in LAL reagent water were prepared as well as positive and negative control.

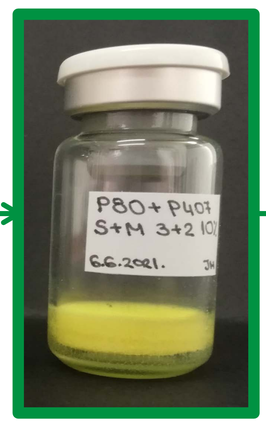
Homogenization
DK-I-56-1 (2 mg/ml)
stabilizers: polysorbate 80 and poloxamer 407 (1:1)

Wet ball milling in cell disruptor device (Disruptor Genie, Scientific Industries, USA)

Nanocrystal dispersion

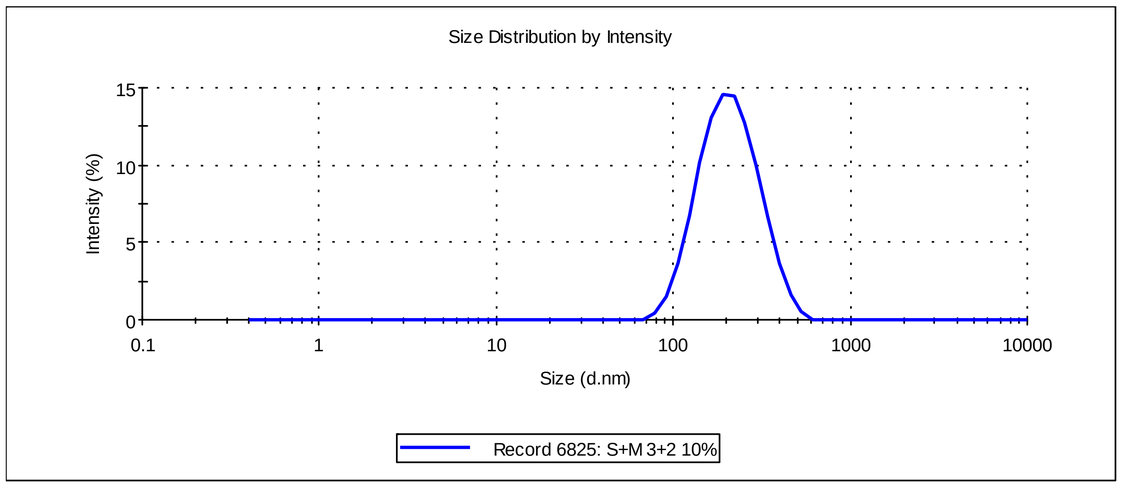
addition of sucrose 6% (cryoprotectant) and mannitol 4% (bulking agent)

Lyophilization (freezing at -50 °C (3 h), primary drying at -45 °C, 0.2 mbar (21 h), secondary drying at 20 °C (30 h))



Reconstituion with water for injection

Results



Particle size distribution

LAL test results

dilution	1:150	1:100	1:75	1:50	1:25
gel clot	-	-	+	+	+

Dilution 1:100

Dilution 1:75

Dilution 1:25

Conclusion

Despite difficulties to detect gel clots, they were visible in the sample at dilutions 1:75 and below. According to the protocol, the endotoxin limit was estimated to be 25.00 EU/ml, which corresponds to <12.50 EU/mg of DK-I-56-1. This value relates to the endotoxin limit for diazepam, with the similar dosing regimen as proposed for DK-I-56-1.

References

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