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Endotoxin Quantification by the Chemical Instrumental HPLC-Kdo-DMB Assay

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Summary

Bacteria are ubiquitous in the environment, leading to the constant risk of endotoxin contamination in the pharmaceutical and the biotechnology industries. Endotoxins are molecules integrated into the outer membrane of Gram-negative bacteria and released constantly in large quantities. They are strong immunostimulants, even quantities as low as pg mL^{-1} present in the human bloodstream, trigger severe reactions such as fever, sepsis, or potentially fatal organ failure. Consequently, strict quality controls of these contaminants are requested by health authorities.

Endotoxin testing employs biological assays such as the rabbit pyrogen test, monocyte activation test, or the Limulus Amoebocyte Lysate (LAL) assay, considered inhere as the gold standard. While highly sensitive, all these assays have significant drawbacks. They are all susceptible to strong sample matrix interferences, leading to a phenomenon known as “low endotoxin recovery” which endangers patient health. Moreover, the LAL assay is validated with endotoxin recovery values of 50 – 200%, and a measurement accuracy of 25% leading to large experimental errors.

Recognizing these limitations which come from the enzymatic nature of these assays, a chemical assay presents a viable solution. Our HPLC-Kdo-DMB assay [1] uses the rare sugar acid Kdo, present in each endotoxin molecule as endotoxin marker. Kdo is released quantitively by mild acidic hydrolysis. Sensitive detection is obtained by Kdo derivatization with the fluorophore DMB. Matrix effects are minimized by the separation of Kdo-DMB by RP-(U)HPLC from potential interfering matrix compounds. This chemical endotoxin quantification approach significantly reduces the likelihood of “low endotoxin recovery”. Its current limit of quantification is at 30 EU mL^{-1}, with ongoing research efforts to reduce it to 0.25 EU mL^{-1}, a health authority requirement for pharmaceutical applications.

The novel assay has been employed to monitor endotoxin release in bioreactor cultivations [2], assess the efficacy of downstream process filtrations, and analyze high protein load matrixes.

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References

[1] B. Bucsella, A. Hoffmann, M. Zollinger, F. Stephan, M. Pattky, R. Daumke, F.J. Heiligtag, B. Frank, M. Bassas-Galia, M. Zinn, F. Kalman, Novel RP-HPLC based assay for selective and sensitive endotoxin quantification, Anal. Methods 12 (2020) 4621–4634.

[2] A. Hoffmann, K. Pacios, R. Mühlemann, R. Daumke, B. Frank, F. Kalman, Application of a novel chemical assay for the quantification of endotoxins in bacterial bioreactor samples, J. Chromatogr. B 1228 (2023) 123839.