Novel RP-HPLC based assay for selective and sensitive endotoxin quantification

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Endotoxin testing in pharmaceuticals and injectables is a crucial requirement for patient safety. This paper presents a novel instrumental analytical endotoxin quantification assay.[1] It uses common analytical laboratory equipment (HPLC-FLD) and allows quantifying endotoxins in different matrices from about 10^9 EU / mL down to about 40 EU / mL (RSE based). Test results are obtained in concentration units (*e.g.* ng endotoxin / mL), which can then be converted to commonly used endotoxin units (EU / mL) in case of known pyrogenic activity. During endotoxin hydrolysis, the endotoxin specific rare sugar acid KDO is obtained quantitatively. After that, KDO is stoichiometrically reacted with DMB, which results in a highly fluorescent derivative. The mixture is separated using RP-HPLC followed by KDO-DMB quantification with a fluorescence detector. Based on the KDO content the endotoxin content in the sample is calculated. The novel chemical endotoxin assay is economic and has a small error. Its applicability was demonstrated in applied research. Endotoxins were quantified in purified bacterial biopolymers, which were produced by Gram-negative bacteria. Results were compared to LAL results obtained for the same samples. A high correlation was found between the results of both methods. Further, the new assay was successfully utilized for the development of novel endotoxin specific depth filters, which allow efficient, economic and sustainable endotoxin removal e.g. during DSP. Further, endotoxin content development was monitored in the supernatant of *E. coli K12* and *P. putida* bioreactors from inoculation until harvest. With that an easy to install tool is available to optimize reactor conditions with respect to the development of the endotoxin content during cultivation, a task difficult to achieve using the LAL assay. Those examples demonstrate that the new chemical endotoxin assay has the potential to complement the animal-based biological LAL pyrogenic quantification tests, which are accepted today by the major health authorities worldwide for the release of commercial pharmaceutical products and bring the endotoxin assay to the 21st century.

[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, *Anal. Methods* **2020**, 12, 4621–4634.