Journal of Chromatography A

Volume 1498, 19 May 2017, Pages 163-168

Development of a dose assay for a *Clostridium difficile* vaccine using a tandem ion exchange high performance liquid chromatography method

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https://doi.org/10.1016/j.chroma.2017.03.044

Highlights

- A novel dose assay was developed for a tetravalent *C. difficile* vaccine.
- Cation-anion exchange columns connected in series are used for the dose assay.
- Protein separation is achieved at two different pHs with salt gradient elution.
- The new method can also be used as a stability indicating assay for the final vaccine.

Abstract

Clostridium difficile is a gram-positive intestine bacterium that causes a severe diarrhea and could eventually be lethal. The main virulence factor is related to the release of two major exotoxins, toxin A (TcdA) and toxin B (TcdB). Recent *C. difficile*-associated disease (CDAD) outbreaks have been caused by hypervirulent strains which secrete an additional binary toxin (CDTa/CDTb). Vaccination against these toxins is considered the best way to combat the CDAD. Recently, a novel tetravalent *C. difficile* vaccine candidate containing all four toxins produced from a baculovirus expression system has been developed. A dose assay to release this tetravalent *C. difficile* vaccine was developed using tandem ion-exchange

HPLC chromatography. A sequential weak cation exchange (carboxyl group) and weak anion exchange (tertiary amine group) columns were employed. The four *C. difficile* vaccine antigen pIs range from 4.4 to 8.6. The final optimized separation employs salt gradient elution at two different pHs. The standard analytical parameters such as LOD, LOQ, linearity, accuracy, precision and repeatability were evaluated for this method and it was deemed acceptable as a quantitative assay for vaccine release. Furthermore, the developed method was utilized for monitoring the stability of the tetravalent *C. difficile* vaccine in final container.

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Keywords

Clostridium difficile infection; Vaccine; TcdA/TcdB; Binary toxin; Ion-exchange HPLC; Dose assay

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