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Vaccine 24S2 (2006) S2/70–S2/71
Vaccine
www.elsevier.com/locate/vaccine

High-performance reverse phase chromatography with fluorescence detection assay for characterization and quantification of pneumococcal polysaccharides

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Available online 6 February 2005

Abstract

A methods using high-performance reverse phase (RP) chromatography with fluorescence detection, has been developed to determine the composition and identity of *Streptococcus pneumoniae* capsular polysaccharide used in formulating conjugate vaccine for prevention of pneumococcal infection. For the monosaccharide composition, the polysaccharides were subjected to hydrofluoric acid (HF) hydrolysis followed by trifluoroacetic acid (TFA). After acid hydrolysis, the released monosaccharides were re-*N*-acetylated and labeled with 2-aminobenzamide (2AB) by reductive amination reaction. High-performance RP chromatography was performed on C18 TSKODS 120T column. Nuclear magnetic resonance was used to confirm chemical structure and purity of pneumococcal capsular polysaccharides.

Keywords: Pneumococcal capsular polysaccharides; *Streptococcus pneumoniae*

1. Introduction

For the polysaccharide used in vaccines, specifications include serotype identity, molecular size, and purity of the component polysaccharide preparation. For the conjugates, concentration of the polysaccharides should be determined at every stage of the process and at least the polysaccharide/protein ratio has to be determined. Routine characterization of the composition, concentration and chemical identity of the polysaccharide preparations has been confined to colorimetric assays specific for selected types of monosaccharides or substituents which have been identified in the polysaccharide repeating unit structure. A major improvement in carbohydrate analysis, the introduction of high-performance reverse phase (RP) chromatography with fluorescence detection permits the characterization and detection of pmole amounts of constituent monosaccharides in formulating conjugate vaccine.

2. Materials and methods

For monosaccharide composition, PnPS19F; PnPS23F; PnPS14, PnPS6B, and PnPS18C polysaccharides were subjected to sequential 48% hydrofluoric acid (HF), and 2M trifluoroacetic acid (TFA) hydrolysis [1]. After acid hydrolysis, the released monosaccharides were re-*N*-acetylated and labeled with 2-aminobenzamide (2AB) by reductive amination reaction in dimethyl sulphoxide/acetic acid [2]. For polysaccharide quantification, a monosaccharide reference mixture (Gal, Glc, Rha, ManNAc, GlcNAc, and GalNAc) labeled with 2-AB ($\lambda_{ext} = 250$ nm and $\lambda_{em} = 428$ nm) was used as standard for routine analysis. Reverse phase high-performance liquid

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doi:10.1016/j.vaccine.2005.01.128

chromatography was performed on a 7.8 mm × 300 mm C 18 TSK ODS120T column. ^{1}H nuclear magnetic resonance (NMR) was used to confirm chemical structure and polysaccharide purity.

3. Results

The fluorescence response to varying concentrations of Rha, Gal, Glc, ManNAc, GlcNAc, and GalNAc monosaccharides conjugated to 2-AB is linear, but dependent on monosaccharide structure. For quantification linear calibration curves were obtained for each monosaccharide. The recovery of Gal, Glc and ManNAc for the pneumococcal polysaccharides under study was close to the expected values. Whereas, Rha recovery was very efficient for PnPs 23F, it was inefficient for 19F, 18C, and 6B polysaccharides with values under 50%. GlcNAc was also lower than expected for PnPs14 with only 54% of the theoretical value.

4. Discussion

This method is rapid, very sensitive (only 2 μg of polysaccharide are required), and the presence of carrier proteins will not interfere in the analysis. The method may replace advantageously the currently colorimetric assays used for composition characterization, and chemical identity of polysaccharides produced for multivalent conjugate vaccine preparations.

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