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Quantitation of serogroups in multivalent polysaccharide-based meningococcal vaccines: Optimisation of hydrolysis conditions and chromatographic methods

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ABSTRACT

Quantitative determination of the individual polysaccharide components in multivalent meningococcal vaccines is an important step in manufacturing and regulatory control. Current methods are complicated due to the use of multiple chromatographic setups and/or other analytical techniques for the four meningococcal serogroup polysaccharides (A, C, Y, W135). In addition, different methods are sometimes used depending on whether or not the polysaccharide is conjugated to a carrier protein. In an effort to simplify such analyses, hydrolysis conditions were determined for the optimal yield of each characteristic saccharide from the respective repeating units. One condition was identified for mannosamine-6-phosphate from MenA, one for neuraminic acid from MenC, and one for both glucose and galactose from MenY and MenW135, respectively. These conditions, initially assessed for monovalent solutions, were then confirmed for a quadrivalent solution. The monosaccharide products were separated, identified and quantitated using a single HPAEC-PAD protocol, with a customised multi-stage linear gradient eluent profile and one column setup, for determination of all four serogroup components. Comparison to calibration curves constructed from sets of monosaccharide or hydrolysed polysaccharide standards allowed for the quantitation of each characteristic serogroup monosaccharide in polysaccharide and polysaccharide-conjugate vaccines. When required, molecular size separation using a non-cellulosic centrifugal filter device effectively removed all interfering saccharide excipient without loss of serogroup polysaccharides. These methods were used to analyse multiple lots of a number of different monovalent or multivalent real polysaccharide-based vaccine products, in liquid or lyophilised powder formulations, with or without excipients. The methods were demonstrated to be highly reproducible and very useful for the evaluation of antigen content and lot-to-lot consistency of manufacture. The methods described here represent an increase in precision, level of accuracy and efficiency compared to current methods, and may be adaptable for evaluation of other types of polysaccharide-based vaccines.

1. Introduction

Neisseria meningitidis is a Gram-negative diplococcal bacterium that causes meningitis, septicemia and, rarely, pneumonia, carditis and septic arthritis. The 13 serogroups of *N. meningitidis* are classified according to the antigenic structure of the polysaccharide capsule. Six serogroups, A, B, C, Y, W135 and X, are responsible for virtually all cases of human disease [1–15]. Most meningococcal vaccines are monovalent or multivalent formulations based on polysaccharides derived from the bacterial capsules of serogroups A, C, Y and/or W135 [1–8,10–19].

The capsular polysaccharides of serogroups A, C, Y and W135 are based on specific monosaccharide and disaccharide repeat units (Table 1) [9,12,13,20,21]. Therefore, the monosaccharide repeating unit *N*-acetylmannosamine-6-phosphate (ManNAc-6-P) is characteristic of MenA. Similarly, the monosaccharide components glucose (Glc) and galactose (Gal) derived from the disaccharide repeating units are characteristic of MenY and MenW135, respectively. This allows for selective determination of these serogroups if the monosaccharide components can be completely recovered, fully separated and quantitated. However, *N*-acetylneuraminic acid

Abbreviations: ManNAc-6-P, *N*-acetylmannosamine-6-phosphate; Glc, glucose; Gal, galactose; Neu5Ac, *N*acetylneuraminic acid; HPAEC-PAD, high-pH anion-exchange chromatography with pulsed amperometric detection; PS-conj, polysaccharide-conjugate; TFA, trifluoroacetic acid; Neu, neuraminic acid; ManN-6-P, mannosamine-6-phosphate; CV, coefficient of variation; EP, European Pharmacopoeia.

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Table 1
Meningococcal serogroup capsular polysaccharides, repeating units and characteristic monosaccharides.

Serogroup	Polysaccharide	Repeating unit	Characteristic monosaccharide
MenA	Poly-α1,6-*N*-acetylmannosamine-6-phosphate (variable C3 and C4 *O*-acetylation)	α1,6-Linked ManNAc-6-P monosaccharide (ManNAc-6-P)	*N*-acetylmannosamine-6-phosphate (ManNAc-6-P); mannosamine-6-phosphate (ManN-6-P) after hydrolysis
MenC	Poly-α2,9-*N*-acetylneuraminic acid (variable C7 and C8 *O*-acetylation)	α2,9-Linked Neu5Ac monosaccharide (Neu5Ac)	*N*-acetylneuraminic acid (Neu5Ac); neuraminic acid (Neu) after hydrolysis
MenY	Poly-α2,6-glucose-α1,4-*N*-acetylneuraminic acid (variable C7 and C9 *O*-acetylation)	α2,6-Linked Glc-α1,4-Neu5Ac disaccharide (GlcNeu5Ac)	Glucose (Glc)
MenW135	Poly-α2,6-galactose-α1,4-*N*-acetylneuraminic acid (variable C7 and C9 *O*-acetylation)	α2,6-Linked Gal-α1,4-Neu5Ac disaccharide (GalNeu5Ac)	Galactose (Gal)

(Neu5Ac) is common to MenC, MenY and MenW135. Therefore, the direct determination of serogroup C in a capsular polysaccharide mixture is dependent on the selective isolation of Neu5Ac exclusively from MenC.

Facile techniques to analyse and quantitate the polysaccharide components of bacterial polysaccharide-based vaccines are essential for the evaluation of antigen content and lot-to-lot consistency of manufacture, with the associated implications for efficacy and safety. Current methods are often polysaccharide-specific and complicated due to the use of multiple preparative and chromatographic setups and/or other analytical techniques, which variably require specialised or proprietary reagents, multiple techniques, column/eluent changes, *etc.* [22–40]. In addition, different methods are sometimes used whether the polysaccharide is unconjugated or conjugated to a carrier protein. Here are reported widely applicable deformulation, acid-hydrolysis and chromatographic methods for the quantitative analysis of the four serogroup polysaccharides in meningococcal polysaccharide-based vaccines.

2. Materials and methods

2.1. MenACYW135 polysaccharide-conjugate sample preparation and optimisation of acid hydrolysis conditions

Six conditions were assessed for the acid hydrolysis of meningococcal serogroup ACYW135 polysaccharides, each alone in a monovalent solution, as well as in a combined quadrivalent solution. Due to constraints on article length, complete details for determination of the optimal acid hydrolysis condition for each serogroup polysaccharide may be found in the Supplementary material.

2.2. Standard preparation

Meningococcal polysaccharide hydrolysis products were identified and quantitated using calibration curves constructed for each characteristic monosaccharide for serogroups A, C, Y and W135. Complete details for standard preparation may be found in the Supplementary material.

2.3. HPAEC-PAD chromatography and quantitation

Monosaccharide analysis by high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) chromatography using the appropriate calibration curves allowed for determination of the serogroup components in monovalent and multivalent solutions, "mock" vaccines and real polysaccharide-based meningococcal vaccines. Complete details for HPAEC-PAD chromatography, construction of calibration curves, and quantitation calculations may be found in the Supplementary material.

2.4. Excipient removal method assurance and application

Disaccharide excipient was removed from samples in which it would directly interfere with determination of polysaccharides. This was accomplished by reiterative size-exclusion centrifugal filtration. This approach was first developed using bivalent AC and quadrivalent ACYW135 polysaccharide-conjugate (PS-conj) "mock" vaccine constructs assembled from monovalent Men PS-conj liquid bulk samples to mimic vaccines with and without lactose. The mock vaccine samples were either filtered to remove excipient and analysed by acid hydrolysis/HPAEC-PAD, or analysed directly without prior filtration. Complete details for the development, qualification and application of reiterative centrifugal filtration for excipient removal may be found in the Supplementary material.

2.5. Serogroup polysaccharide hydrolysis and sample preparation

The meningococcal serogroup polysaccharide components in multiple lots of real monovalent and multivalent PS-conj and polysaccharide vaccines obtained from the manufacturers were determined. Full sample information and complete details for sample preparation, processing and cleanup may be found in the Supplementary material.

3. Results and discussion

3.1. Determination of optimal hydrolysis condition for each serogroup polysaccharide

Six acid-hydrolysis conditions were assessed for each of the four PS-conj samples separately and in a quadrivalent mixture. The optimum hydrolysis condition for each serogroup is a multivariate of trifluoroacetic acid (TFA) concentration, temperature and time that provides the best compromise between complete hydrolysis of the serogroup polysaccharides and the subsequent degradation of the resulting monosaccharide products. Acid hydrolysis under the optimal conditions is expected to result in essentially complete de-*O*- and de-*N*-acetylation of the target monosaccharides.

Identification of peaks was by retention time comparison with standards. Monosaccharide standards for the determination of serogroups C, Y and W135 were not subjected to polysaccharide hydrolysis conditions since they were nearly totally destroyed when exposed to these conditions for the full time of the hydrolysis (data not shown). Glucose and galactose are unique to MenY and MenW135, respectively, and were used for the determination of these serogroup polysaccharides. Neuraminic acid (Neu), the expected de-acetylated hydrolysis product of MenC is not commercially available. Efforts to produce Neu from Neu5Ac using the mild acid hydrolysis methods described here resulted in some destruction of the monosaccharide and loss of yield. Therefore, Neu5Ac was used as the standard since it also co-elutes with

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