Quantifying Uncertainty in Determination of Polysaccharides in Glycoconjugate Vaccines

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Introduction

As Italian OMCL (Official Medicines Control Laboratories), ISS (National Institute of Health) provides analytical capability for AIFA (Italian Medicines Agency) not only in the area of Official Control Authority of Batch Release (OCABR) but also in a number of activities including testing of chemicals and biological drugs sampled for defects, recalls, complaints, etc. In this context, our Bacterial Vaccines Unit, in accordance to UNI CEI EN ISO/IEC 17025, has determined the uncertainty in quantifying the polysaccharide content in glycoconjugate vaccines, such as anti *Haemophilus influenzae type b* and anti *Meningococcal type C*. As envisaged by OCABR OF HUMAN BIOLOGICALS 2010, EDQM (European Directorate for the Quality of Medicines) total and free saccharide have to be determined. Here, the study of uncertainty determination in quantifying total saccharide (C_{STOT}) by concentration of sialic acid in an anti *Meningococcal type C* vaccine is reported. A validation study of a new in-house analytical method to determine concentration of sialic acid by HPAEC-PAD (High Pressure Anion Exchange Chromatography – Pulsed Amperometric Detection) was used. The glycoconjugate vaccine chosen was a MenC-CRM type, where a MenC oligosaccharide, an antigen made of N repeat units of sialic acid, is covalently linked to a protein as CRM-197, a non-toxic mutant of diphtheria toxin. The specification of this product is 18-30 µg/ml of total saccharide assayed as sialic acid content.

Materials

- Sialic acid (N-Acetylneuraminic acid) provided by Fluka Biochemica (Buchs, Switzerland); Batch 334332/1 of certified purity of 100%.
- MenC polysaccharide (poly N-Acetylneuraminic acid) kindly provided by Novartis Vaccines and Diagnostics srl (Italy); Batch 118 of declared purity of 80.8%. *No certified reference material for MenC polysaccharide is available.*
- MenC-CRM Vaccine, Batch XXXXXX, kindly provided by Novartis Vaccines and Diagnostics srl.

Method

In order to determine the total MenC polysaccharide content (C_{STOT}) on a single dose of MenC-CRM vaccine, an acid hydrolisys was performed on a pool of 5 doses. It released sialic acid, a monosaccharide quantified by HPAEC-PAD after basification with NaOH treatment.

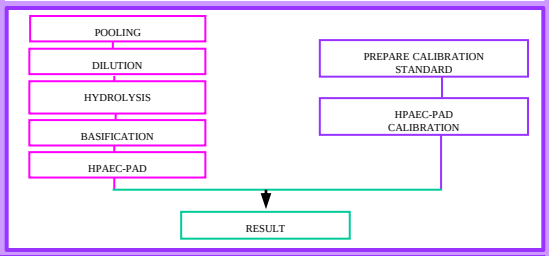
Data

Data from in-house validation study were collected following ICH guidelines. Studies of Linearity, Repeatability, Intermediate Precision, Trueness were performed. In each study samples were run in batches including a calibration set and two recovery check samples: sialic acid sample prepared independently from calibration set to control the overall bias, excluded that from hydrolysis, and MenC polysaccharide sample to control bias from hydrolysis. All samples were analysed in duplicate to check precision in the range between 0.1 – 2.0 µg/mL.

Goal

To quantify uncertainty of an in-house method for the determination of total MenC polysaccharide C_{STOT} in anti meningococcal glycoconjugate vaccines as MenC-CRM by a validation study.

Measurement Procedure

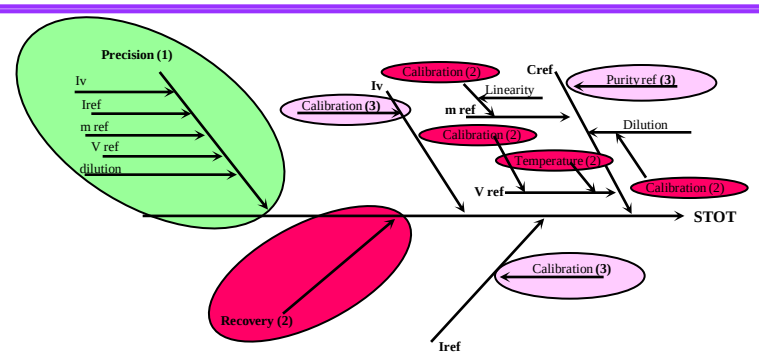


Measurand

$$C_{STOT} = \frac{Iv \cdot Cref}{Iref \cdot Rec} \quad \mu g/ml$$

C_{STOT} = Total Saccharide present in Vaccine sample [µg/ml]
Iv = Peak intensity of sialic acid in vaccine sample
Cref = Mass concentration of the sialic acid Reference Standard [µg/ml]
Iref = Peak intensity of sialic acid Reference Standard
Rec = Recovery

Cause and effect diagram after rearrangement with data from validation study



(1) Contribution included in Precision component

(2) Contributions included in Bias Component

(3) Contributions included in Other Sources of Uncertainty Component

Evaluation of Uncertainty Components

(1) Precision Component

Precision component was investigated for MenC and C_{STOT} by two determinations per analytical session on six different days. Here, only the analysis of variance for C_{STOT} is reported. The ANOVA for C_{STOT} was significant and a pooled standard deviation was used to give a more realistic estimate of the variability of the measurements in our laboratory.

Table 1. ANALYSIS OF VARIANCE for STOT

Source of variability	Sum of Squares (µg/mL)²	Degrees of freedom	Mean Square (µg/mL)²	F-Ratio	Significance (p value)	F critical
BETWEEN	6,605	5	1,321	8,210	0,012	4,387
WITHIN	0,965	6	0,161			
TOTAL	7,570	11	0,688			

(2) Bias Component

The overall bias component of the analytical procedure was investigated from recovery. We evaluated the two following different recoveries:

- **Recovery from sialic acid spiked vaccine samples.** Student's *T* Test was performed from six measurements to determine whether the mean recovery were significantly different from 1.0. The bias component resulted negligible and no correction factor was considered (see Tab. 2). This is in accordance with the recovery of the sialic acid check sample that was run in each analytical session of the validation study. Similarly, the *T* test resulted not significant.
- **Recovery from hydrolysed MenC samples.** From ten measurements on five different days, the Bias component from Hydrolysis was quantified by recovery data. This source of uncertainty was calculated separately because it was not included in the above mentioned recovery study. The bias component resulted not negligible (see Tab. 2).

A correction factor (1/ "Mean Recovery") of 1,05 has to be applied while the bias standard uncertainty has been considered in the quantification of the combined uncertainty.

(3) Other Sources Component

The purity of the sialic acid reference standard reported in the Certificate of Analysis was ≥ 98%. The contribution was so small that is clearly safe to neglect it.

Linearity of response to sialic acid within the given concentration range was established during validation study and no allowance was required.

All the other sources of uncertainties, including balance and volumetric measuring devices, were considered within precision and recovery studies.

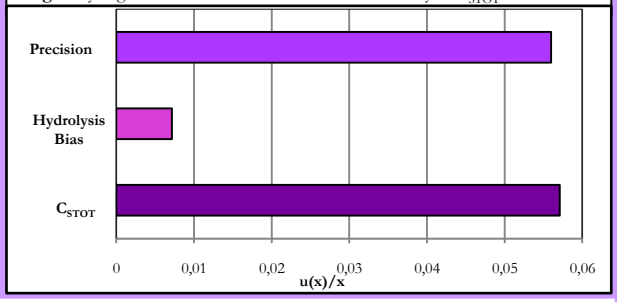
Quantification of Expanded Uncertainty

In Table 2, the overall uncertainties in C_{STOT} analysis are reported. The combined relative standard uncertainty for C_{STOT} was obtained combining the two significant contributions of Precision and Hydolysis Bias, according to the law of propagation of uncertainty. In Fig. 1, the sizes of the two major contributions of the relative standard uncertainties, together with the combined uncertainty, are shown diagrammatically. As expected, the measurement uncertainty is clearly dominated by the Precision contribution.

Table 2. Overall Uncertainty Estimate

Description	Value x	Standard Uncertainty u(x)	Relative Standard Uncertainty u(x)/x	Comments
PRECISION	21,5	1,22	0,0567	Evaluated by ANOVA and calculated by *"between and within" Pooled Standard Deviation*
TRUENESS: BIAS (Method)	0,995	0,0274	0,0275	The result of the Student's T Test was Not Significant ($p = 0,673$); the contribution was not included in the calculation
Hydrolysis Bias	0,953	0,0069	0,0072	The result of the Student's T Test was Significant ($p < 0,001$); the contribution was included in the calculation
C_{STOT}	--	--	0,0572	Combined Relative Standard Uncertainty

Fig 1. Hystogram of the contribution to the Uncertainty in C_{STOT}



Expanded Uncertainty $U_{STOT} = k \cdot 0,0572 \cdot C_{STOT} = 0,11 \cdot C_{STOT}$ with k=2: coverage factor in order to obtain a level of confidence of approximately 95%.

In order to eventually compare the uncertainty of this method among OMCL's, the Expanded Relative Uncertainty for MenC polysaccharide was calculated. It resulted of 0,08