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MP 562

Determination of Methyl pentose in Pneumococcal Polysaccharide hydrolysates derived from serotype 18C and 23F using LCMS-8045RX

Nitish Ramchandra Suryawanshi[1], Chetan Dhatunde[2], Swapnil Sawant[2], Uma Raghuram[2], Samruddha Chavan[1], Nitin Shukla[1], Devika Tupe[1], Siddhesh Ghadi[1], Ramesh Manigiri[1], Shalu Nair[1], Satyajit Gavhane[1], Pratap Rasam[1] and Jitendra Kelkar[1]
[1]Shimadzu Analytical (India) Pvt. Ltd. [2]Spinco Biotech Pvt. Ltd.

1. Introduction

Pneumococcal polysaccharide vaccine (PPSV) is a mixture of several serotypes of polysaccharides, used to kill bacteria *Streptococcus pneumoniae*. The use of standard 23- valent vaccine, also known as pneumovax 23 or PPSV-23, is an important landmark in medical history. Since 1983, PPSV- 23 has saved millions of lives, especially of those patients with HIV/AIDS diseases. It was reported that the vaccine has decreased the incidence of invasive pneumococcal disease from 768/100,000 patient-years to 244/100,000 patient-years. Traditional European Pharmacopeia (EP) methods utilize UV/Vis spectrophotometry to analyze derivatized monosaccharides such as Methyl pentose (L-Rhamnose) [Figure 1], hexosamine, and hexonic acid in PPSV hydrolysates.[1]

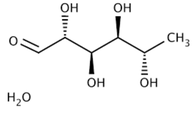

Figure 1: Structure of Methyl pentose

Analyzing a single serotype of PPSV is time-consuming, often exceeding 12 hours due to a labor-intensive derivatization step that takes over 4 hours per monosaccharide. Additionally, improved detection selectivity and measurement precision are needed for reliable quantitation. This application note presents a partially validated method for accurate quantitation of Methyl pentose in Pneumococcal Polysaccharide hydrolysates from serotypes 18C and 23F using the Shimadzu LCMS-8045RX triple quadrupole system. Leveraging the sensitive UFMS platform and an optimized hydrolysis protocol, the method eliminates the need for derivatization and improves stability and accuracy. The method's validity is evaluated using theoretical composition data from WHO Technical Report Series No. 977 (2013), which outlines expected component percentages for various serotypes. (Table 1) [2]
This application note uses these values to assess the accuracy of the developed method.

Table 1: Theoretical concentration of Methyl pentose in pneumococcal polysaccharides.

Serotype	Methyl pentose (%)
18 C	15.96 (≥14)
23 F	40.77 (≥37)

2. Methods

2-1. Method development

Methyl pentose standard was sourced from Sigma-Aldrich, while hydrolysed Pneumococcal Polysaccharide samples from serotypes 18C and 23F were obtained from American Type Culture Collection (ATCC). LC-MS/MS method development involved precursor ion selection, MRM tuning, and optimization of collision energy, voltages, and ion source parameters using Shimadzu's "Optimization for Method" feature.

MRMs with optimized voltages and CEs were finally used for quantitation. An LC method (Table 2) was developed using HPLC column (Shim-pack Scepter Diol HILIC-120, 150 mm x 4.6 mm I.D., 5 µm) to separate Methyl pentose from any unwanted interferences from sample matrix. Sample preparation steps such as acid concentration and incubation time were optimized to achieve accurate and reproducible results.

2-2. Quantitation

For quantitation, a seven-point calibration curve was plotted by analysing the linearity standards of Methyl pentose ranging from 5.0, 10.0, 20.0, 50.0, 100.0, 150.0 & 200.0 ppb. The limit of quantitation (LOQ) was established at 5.0 ppb, based on a signal-to-noise ratio exceeding 10 and a repeatability of less than 10% RSD.

Figure 2: Shimadzu Nexera™ X3 UHPLC coupled with an LCMS-8045RX Triple quadrupole mass spectrometer

2-3. Analytical conditions

Table 2. Instrument parameters for LC-MS/MS

HPLC System	: Nexera™ X3
Column	: Shim-pack Sceptor Diol HILIC-120, 150 mm ID x 4.6, 5 µm
Column Temp.	: 45 °C
Mobile Phases	: MP-A: 0.02 % formic acid and 5 mM ammonium formate in water. MP-B: 100 % acetonitrile.
Flow Rate	: 0.45 mL/min
Gradient Program (B%)	: 0.0-1.0 min → 90 (%); 1.0-1.01 min → 90-10 (%); 1.01-8.0 min → 10 (%); 8.0-8.01 min → 10-90 (%); 15.0 min → STOP
Injection Volume	: 15 µL
LC-MS System	: LCMS™-8045RX
Ionization Source	: CoreSpray (ESI)
Interface Temp.	: 250°C
Nebulizing Gas	: 3.0 L/min
Drying Gas	: 12.0 L/min
Acquisition	: MRM
MRM	: 209.1>58.9

2-4. Sample preparation

Pneumococcal Polysaccharide hydrolysate samples were prepared by following steps mentioned in the figure 3 and were subjected to LC-MS/MS analysis. Serotype 23F, 18C and mixture of both were processed in triplicate and were analyzed using LC-MS/MS

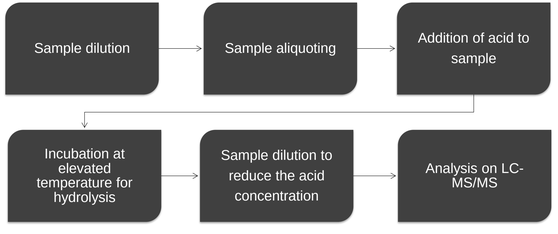

Figure 3: Sample preparation protocol

3. Results

Figure 4 depicts the chromatographic peak detection at LOQ level (5.0 ppb) and calibration curve for linearity ranging from 5.0-300.0 ppb.

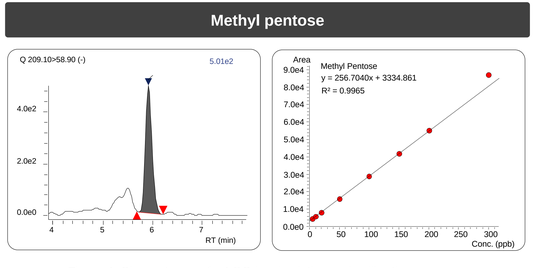

Figure 4: Chromatogram for LOQ level solution and calibration curve

Pneumococcal polysaccharides consist of polymeric saccharides made up of various monomeric sugar units, including Methyl pentose, hexose, hexosamine, and hexonic acid. The precision of this MRM-based method was established by comparing the measured concentration of Methyl pentose to the theoretical values outlined in the WHO Technical Report Series No. 977 from 2013. According to this report, the expected concentrations of methyl pentose in serotype 23F and 18C are 40.77 % and 15.96 %, respectively.

The concentration values obtained from the polysaccharide hydrolysate was compared with the WHO reference and the accuracies were calculated by using the formula mentioned below. (Table 3)

Theoretical conc. of Methyl pentose in aliquoted serotype sample: (Spiked conc. of serotype in sample/100) x 15.96 = (300/100) x 15.96 = 47.88 ppm

% Accuracy: Actual conc. of methyl pentose found in serotype sample/Theoretical conc. of Methyl pentose in aliquoted serotype sample x 100 = (33.89/47.88) x 100 = 71 %

Table 3: Accuracy summary of Methyl pentose in serotype 23F & 18C

Recovery summary		
	Type 18C	**Type 23F**
Composition of Methyl pentose as per WHO label claim (%)	15.96	40.77
Spiked conc. of serotype in sample (ppm)	300	300
Theoretical conc. of methyl pentose present in serotype (ppm)	47.88	122.31
Calculated conc. of Methyl pentose	33.89	94.69
% Accuracy w.r.t. theoretical concentration	71	77

4.Conclusion

- ✓ A streamlined, non-derivatized sample preparation method has been developed, offering a significantly faster and more reliable alternative to conventional UV/Vis methodologies.
- ✓ The concentrations of methyl pentose obtained from both individual serotypes and their mixtures align closely with the theoretical values outlined in the WHO Technical Report Series No. 977, 2013.
- ✓ The percentage accuracy for the concentrations measured against the WHO label claim for individual serotypes falls well within the acceptable range of 70-130 %.
- ✓ Key factors, such as the freshness of sample preparation, acid concentration, and processing time, are crucial for achieving precise results.
- ✓ LCMS enables simultaneous and selective detection of monosaccharides in complex PPSV samples.

Reference
1) Journal of Pharmaceutical and Biomedical Analysis Volume 155, 5 June 2018, Zhen Long et al.
2) Annex 3, Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines, Replacement of WHO Technical Report Series, No. 927, Annex 2.