Applikationsbericht

Determination of Molecular Weight Distribution of mPEG-OH with Xevo™ G3 QTof System

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Dies ist ein Applikationsbericht, der keinen detaillierten Abschnitt zu Versuchen enthält.

Abstract

To simplify the method development for the analysis of methoxy-capped polyethylene glycol (mPEG-OH) samples and to determine the polymer's molecular weight distribution (MWD) using the Xevo G3 QTof System.

Introduction

The biopharmaceutical industry has recently shown an increasing interest in acquiring very low polydisperse PEGs due to their wide range of applications. An important property of PEG is the molecular weight, which determines the properties of the material. For measuring the MWD of PEG biotherapeutics, high-resolution mass spectrometry (HRMS) has proven to be a suitable method of choice. The analysis of PEG sample by electrospray ionisation (ESI) is quite challenging due to the nature of polymer macromolecular ions that are formed by ESI create charge state distributions that overlap with one another. This often complicates data processing of polymers creating superimposed peaks between the molecular weight and charge state distributions that would need to be aided by deconvolution programs. To assist with the challenge of PEG analysis, a Xevo G3 QTof mass spectrometer platform coupled to an ACQUITY™ UPLC™ I-Class PLUS System, combined with waters_connect™ UNIFI™ Software, is used to analyse the MWD and polydispersity of mPEG-OH samples. The advantage of using a UPLC-HRMS system and modern software is the potential to separate and simplify data to enable interpretation with improved confidence.

The optimized approach has been shown to be efficient in this analysis for determining the molecular weight measurement and reducing the charge state distribution for three different ranges of mPEG-OH samples (30k Da, 40k Da, and 50k Da).

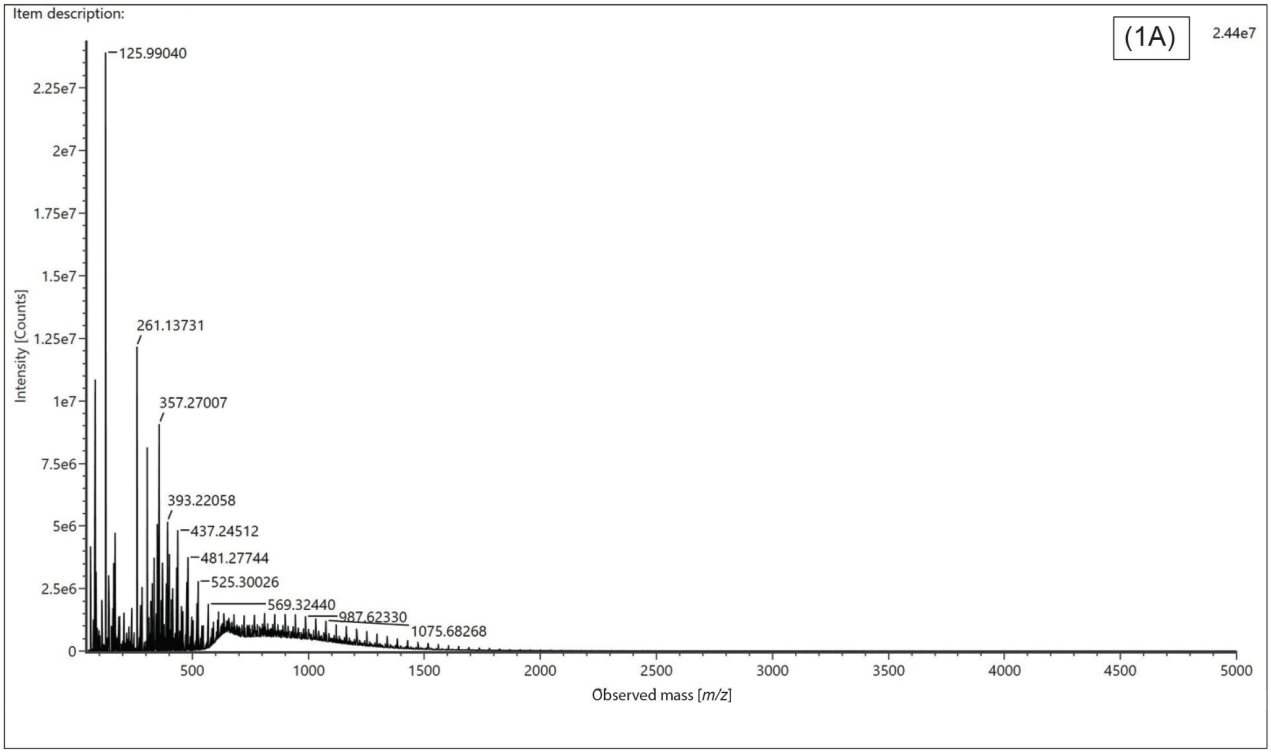


Figure 1A. Mass spectrum of mPEG-OH 30k Da before (1A) and after (1B) addition of TEA, a charge stripping agent.

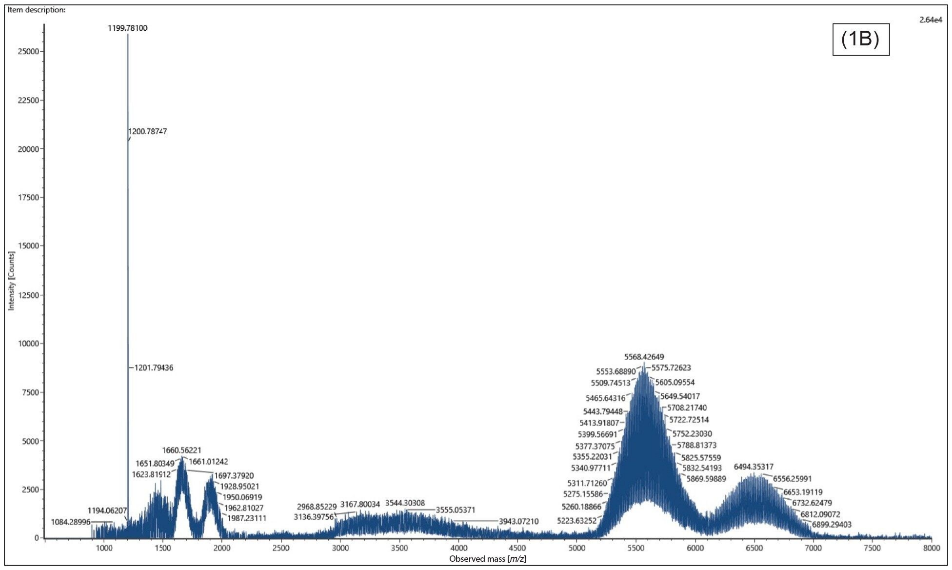


Figure 1B. Addition of TEA, a charge stripping agent.

Experimental

An UPLC-MS method for the determination of molecular weight distribution and polydispersity under less than three minutes using isocratic conditions with post-column addition was acquired in ESI positive mode for the PEG samples. The instrument was calibrated before the sample analysis using positive mode calibration solutions. The instrument parameters, chromatographic conditions and ion source settings were optimized using representative PEG samples. The samples were eluted on an ACQUITY UPLC BEH 300 Å C_4 2.1 x 50 mm, 1.7 µm Column using mobile phase (A) 0.1% TFA in water and (B) 0.1% TFA in acetonitrile at a flow rate of 0.1 mL/min and mixed with post-column addition of a charge stripping agent, triethylamine (TEA). This was accomplished by infusing 2% TEA prepared in 50:50 acetonitrile:water using the on-board fluidics system of the Xevo G3 QTof mass spectrometer. An appropriate flow of TEA concentration was controlled by adjusting the events setup controlled by the software. Figure 1 represents the effect of charge stripping with and without TEA. The obtained raw data were processed using waters_connect UNIFI platform which incorporates defined steps with an automated workflow. This approach facilitates an efficient and accesible approach in the determination of MWD by defining the deconvolution parameters and custom fields.

Mass distributions representing the different monomer repeat units of ethylene glycol (44 Da) and varying degrees of polymerization of PEG were easily seen in the deconvoluted spectrum. The impact of increasing the concentration of TEA solutions results in well distributed charge states of mPEG-OH. Each charge state represents the molecular weight distribution of the samples. Figure 2 shows a representative multiply charged and deconvoluted mass spectrum of 30k Da mPEG-OH sample.

The experimental data was further processed using waters_connect UNIFI software to determine the number average molecular weight (Mn), weight average molecular weight (Mw) and polydispersity (PDI or Đ) of mPEG-OH samples. The value of Mw/Mn is usually used as an index of the polydispersity of a polymer. Table 1 illustrates the details of Mn, Mw, and PDI calculations for all three samples. The monofunctional linear mPEGs with the narrow distribution generally have Mw/Mn values of approximately 1.02-1.05. The calculated polydispersity values of the mPEG-OH samples being nearly close to a value of 1.00 (ca. PDI of 1.0009-1.0018) clearly demonstrates that it has a significantly high purity of its degree of polymerization.

Sample details	Observed mass	Mn	Mw	PDI
30k Da mPEG-OH	32578.1508	32814.57	32845.40	1.00
40k Da mPEG-OH	39407.1415	39147.98	39220.27	1.00
50k Da mPEG-OH	49157.6304	49121.66	49168.62	1.00

Table 1. Mn, Mw, and PDI calculations for all the three samples.

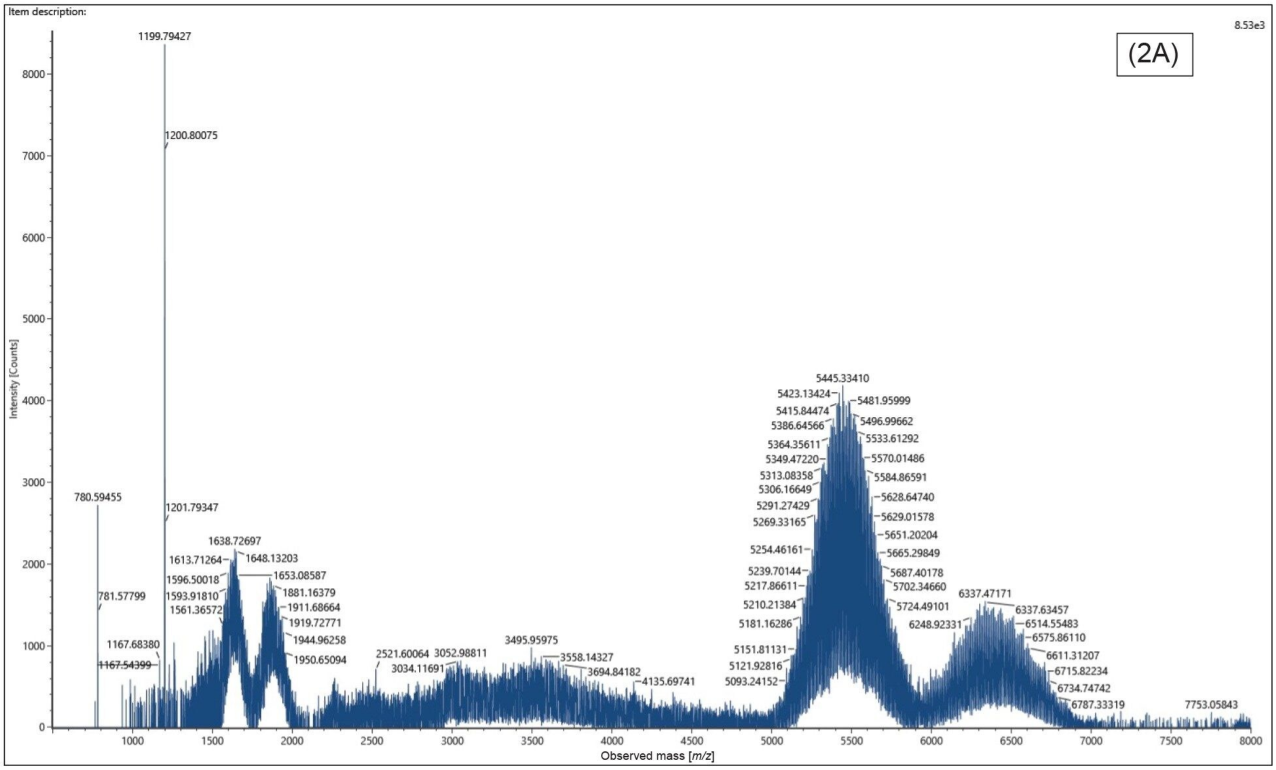


Figure 2. Mass spectrum of 30k Da mPEG-OH after post column addition of TEA (2A) and (2B) after deconvolution. Mass spectrum was processed using waters_connect UNIFI software to obtain (2C) Mn, Mw and PDI.

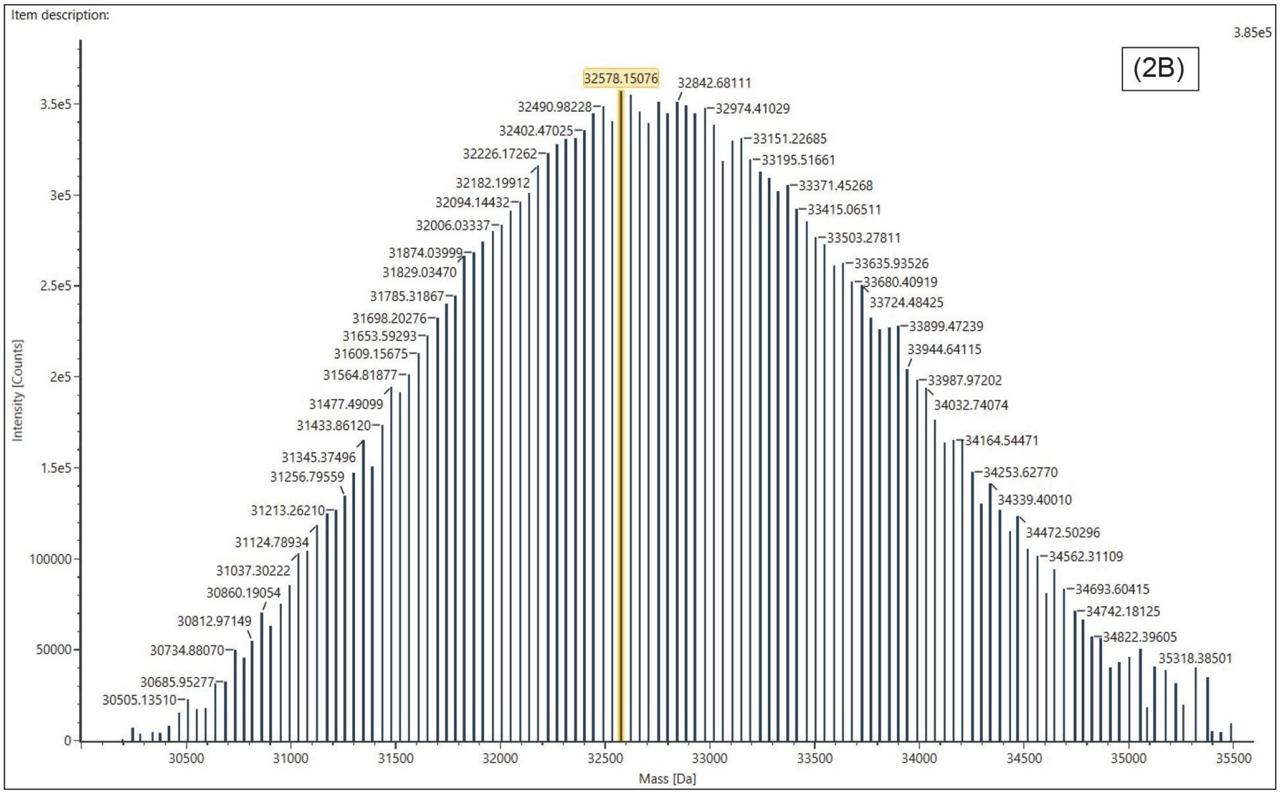
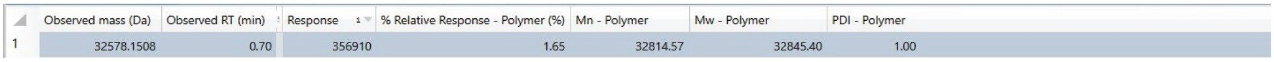


Figure 2. *Mass spectrum of 30k Da mPEG-OH after post column addition of TEA (2A) and (2B) after deconvolution. Mass spectrum was processed using waters_connect UNIFI software to obtain (2C) Mn, Mw and PDI.*

(2C)



	Observed mass (Da)	Observed RT (min)	Response	% Relative Response - Polymer (%)	Mn - Polymer	Mw - Polymer	PDI - Polymer
1	32578.1508	0.70	356910	1.65	32814.57	32845.40	1.00

Figure 2. *Mass spectrum of 30k Da mPEG-OH after post column addition of TEA (2A) and (2B) after deconvolution. Mass spectrum was processed using waters_connect UNIFI software to obtain (2C) Mn, Mw and PDI.*

Conclusion

The present data has provided a rapid method that demonstrates the enhanced utility of the Xevo G3 QTof platform coupled to an ACQUITY UPLC I-Class PLUS Ssystem using waters_connect UNIFI software for routine analysis of PEG samples.

- The workflow combination has been shown to excel for this complex sample analysis approach
- In addition to rapid method solutions, the Xevo G3 QTof platform also achieved improvement in deconvoluted mass spectrum distribution pattern with reduction in spectral complexity
- The waters_connect UNIFI platform has enabled efficient determination of Mn, Mw, and PDI values

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