



Sensitive Analysis of Oligo/ polyethylene Glycol by Reversed- phase HPLC with ELSD

Application Note

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Introduction

Oligo/polyethylene glycol is a hydrophilic water-soluble material which has many uses in biotechnology and biomedicine as it is non-toxic and non-immunogenic. In aqueous solutions, it is heavily hydrated and can be used as an additive or shield to minimize non-specific interactions with proteins. Molecular size information and molecular size distributions can be obtained using gel permeation chromatography (GPC) which utilizes an isocratic system with refractive index (RI) detection. However, with GPC, there is insufficient resolution for the quantitation of individual oligomers. Reversed phase HPLC separates on the basis of differences in solute hydrophobicity but requires gradient elution if the analysis is to be accomplished in a reasonable time frame. This means that an RI detector cannot be used. Also oligo/polyethylene glycol does not have a UV chromophore and therefore this method of detection is unsuitable unless derivatization is first performed.

A better approach is to combine the polymeric reversed-phase HPLC column, PLRP-S 100Å, with the 385-ELSD evaporative light scattering detector. The 385-ELSD offers greater sensitivity than UV detection. Solvent peaks are absent and excellent baseline stability is present. The 385-ELSD is renowned for its rugged design and ability in delivering high performance for demanding HPLC or GPC applications. PLRP-S 100Å columns are ideally suited to the analysis of low molecular weight compounds because the small pore sizes have extremely high surface areas available to the solutes. Coupling PLRP-S columns with the 385-ELSD evaporative light scattering detector creates an excellent system for separating oligo/polyethylene glycol.



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Instrumentation

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)

Detector

Figure 1a, 1b and 3:

385-ELSD (neb = 85 °C, evap = 80 °C, gas = 1.0 SLM)

Figure 2a:

385-ELSD (neb = 85 °C, evap = 75 °C, gas = 1.0 SLM)

Figure 2b:

385-ELSD (neb = 85 °C, evap = 78 °C, gas = 1.0 SLM)

Materials and Reagents

Eluent A: Water

Eluent B: Acetonitrile

Conditions

Flow Rate: 0.5 mL/min

Gradient:

Figures 1a and 1b:

10-30% B in 15 min, 30-100% clean up 5 min

Figures 2a and 3:

30-50% B in 15 min, 50-100% clean up 5 min

Figure 2b:

40-60% B in 15 min, 60-100% clean up 5 min

Temp: 40 °C

Results and Discussion

Examples are given in Figure 1a of the separation of polyethylene glycol of 400 nominal molecular weight.

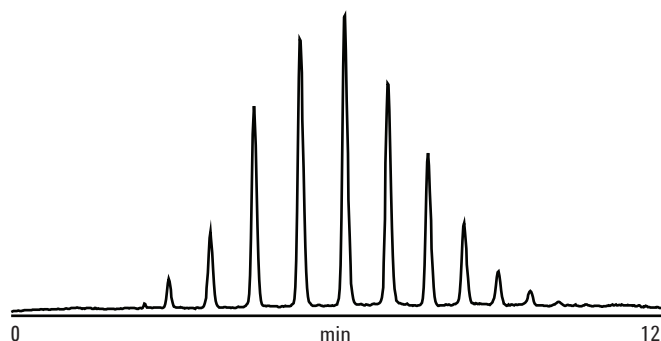


Figure 1a. PEG 400 oligomers detected by the 385-ELSD evaporative light scattering detector.

Figure 1b shows a sample with a nominal molecular weight of 600.

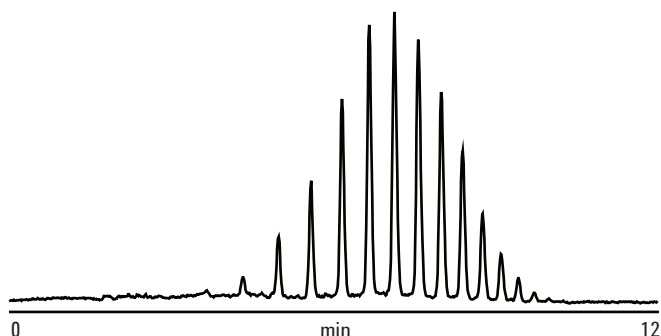


Figure 1b. PEG 600 oligomers detected by the 385-ELSD evaporative light scattering detector.

As the molecular weight increases, it is no longer possible to separate the individual oligomers, as shown in Figures 2a and 2b.

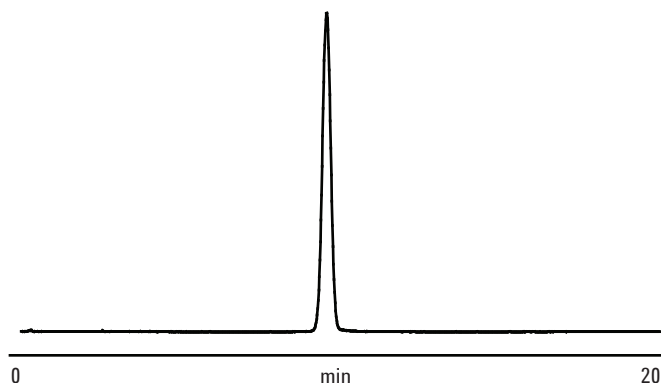


Figure 2a. No separation of oligomers in PEG 4,120.

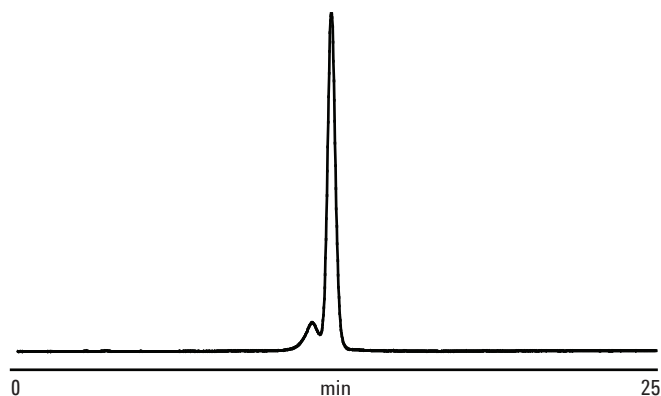


Figure 2b. PEG 22,800 oligomers not visible.

Reversed-phase HPLC with ELS detection can also be used to look for contaminants in polyethylene glycol samples which have slightly different hydrophobicities.

Figure 3 shows the separation of a sample of mono-hydroxyl polyethylene glycol containing a small amount of the unwanted diol derivative.

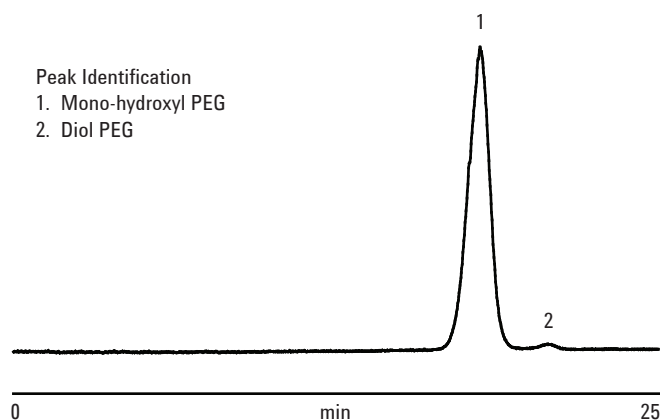


Figure 3. The presence of unwanted diol derivative of PEG revealed by the 385-ELSD evaporative light scattering detector.

Conclusion

Reversed-phase HPLC with the 385-ELSD evaporative light scattering detector and PLRP-S columns successfully separated oligo/polyethylene glycols and revealed the presence of unwanted derivatives.

PLRP-S columns are the preferred choice for the analysis of many small molecules. These columns are more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and therefore even polar molecules may be retained for much longer, resulting in greater resolution.

A PLRP-S column used with the 385-ELSD detector is an ideal combination for the separation of low molecular weight compounds that have no chromophores.

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Published in UK, May 20, 2011

5990-8197EN



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