APPLICATION NOTE

AN1304: Molecular weight analysis of low-molecular-weight heparin by SEC-MALS

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Low-molecular-weight heparins (LMWHs), a class of anti-coagulant medications used in the prevention blood clots, are obtained by fractionation or depolymerization of natural heparins. A heparin solution is considered 'LMWH' if the weight-average molar mass M_w is less than 8000 g/mol, and at least 60% of the total mass is also below 8000 g/mol.

The US Pharmacopeia (USP) monograph for LMWH specifies single-detector SEC with the usual column calibration in order to verify that it has been properly fractionated. The European Pharmacopeia (EP) monograph for LMWH specifies characterization of molar mass via size-exclusion chromatography (SEC) coupled with downstream detection by tandem 234 nm ultraviolet (UV) and refractive index (RI) detectors, known as the Nielsen method. This method is complex in comparison to typical SEC calibration since it combines calibration with known low-molar-mass heparin standards and analysis of the UV/RI ratio. While the USP and EP monographs provide a known, commercially available calibration standard, the need to purchase the special standard, perform the calculations (in the case of EP) and recalibrate the column on a regular basis adds cost, time and labor to the procedure.

A more convenient, absolute method for LMWH characterization involves the use of multi-angle light scattering (MALS), which does not require any standards or periodic calibration. We have developed a SEC-MALS method and found it to be very suitable for the analysis of LMWHs. In order to cross-validate the SEC-MALS method against the EP method, we implemented the UV-RI method described in the EP monograph and compared the molecular weight results generated for LMWH using each analytical technique.

Both methods were run on an Agilent LC-1200 series HPLC. Mobile phase was 0.2M sodium sulfate pH 5.0 and a Tosoh TSK-gel G2000 SWxl column was used with Tosoh TSK-gel Guard SWxl.

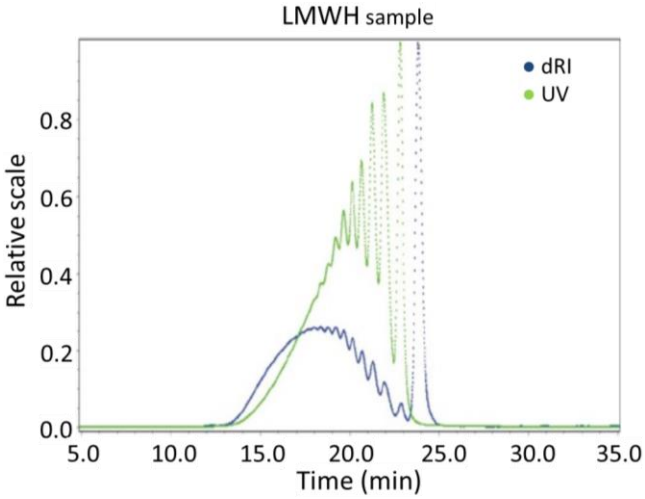


Figure 1. Examples of UV and RI traces for an LMWH sample.

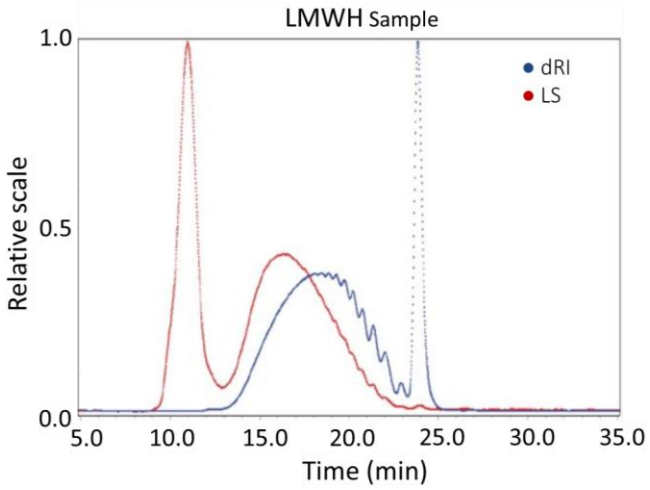
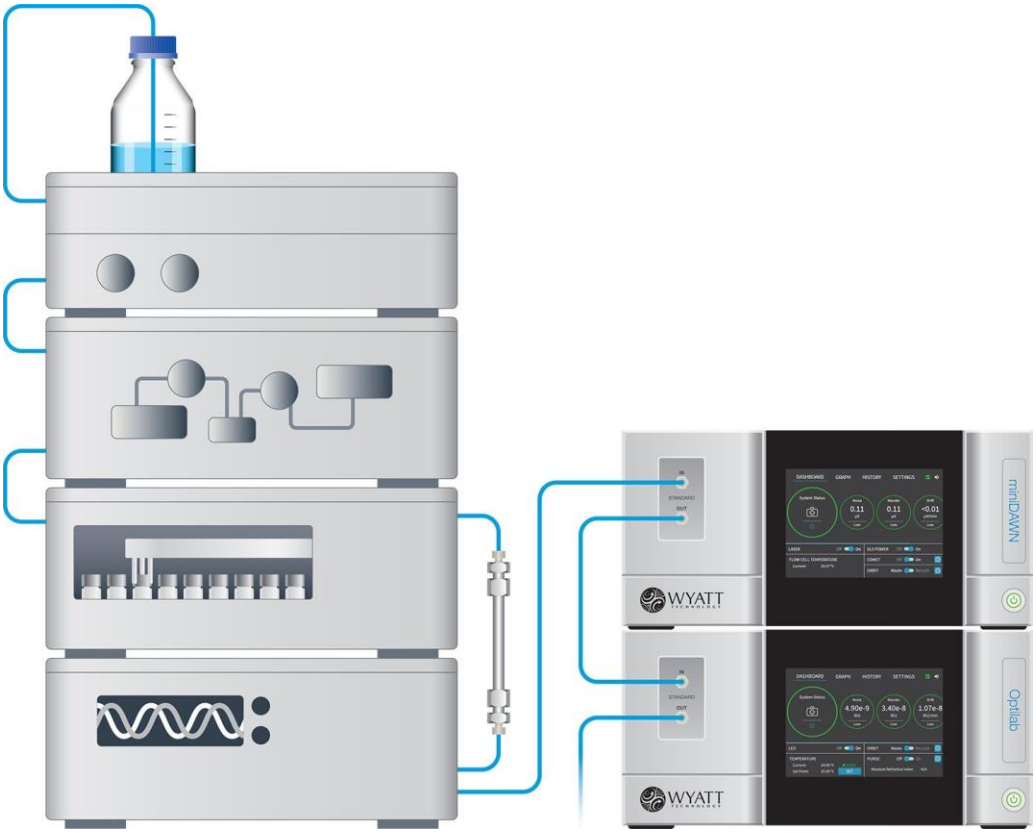


Figure 2. Examples of UV and RI traces for an LMWH sample.

For UV/RI analysis, a Waters 2487 dual wavelength UV detector was combined with a Wyatt Optilab® refractive index detector. For MALS analysis the UV detector was replaced with a Wyatt miniDAWN® MALS detector.

The results indicated that both detection types are suitable and acceptable for the analysis of LMWHs. The molecular weight and distribution results generated using each detection type are quite comparable. Hence a SEC-MALS method could readily be adopted in place of the SEC/UV-RI method currently required by the EP monograph, reducing time and cost because it obviates the need for periodic recalibration and the purchase of low-molar-mass heparin standards.



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