Molecular Weight Determination of Unknown Proteins and Protein Mixtures by Size-Exclusion (SEC) HPLC

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Purpose
There is an increasing need for characterization of complex biological molecules, particularly molecular weight distribution during initial characterization of biologics and for stability studies. Each analytical technique (mass spec characterization, PAGE electrophoresis, capillary electrophoresis, etc.) has its own advantage and disadvantage. This study describes the development of size exclusion (SEC) HPLC method for the determination of molecular weight in various biological formulations. The method covers molecular weight range from 1.3 kDa to 66 kDa.

Methods
The method does not involve special sample preparation and utilizes a direct injection of protein with unknown molecular weight to SEC-HPLC column (injected amount 10 ng - 1 μg). Control proteins with known molecular weights are injected during the same run to obtain a calibration curve that will be used for molecular weight determination of unknown compounds. SEC-HPLC run was carried out on an Acquity UPLC BEH SEC 200Å, 100 mm x 4.6 mm, 1.7 μm column (30°C) at a flow rate of 0.3 mL/min with an analysis time of 10 minutes. Mobile phase was 10 mM Phosphate Buffer containing 250 mM Sodium Chloride, pH 7.4. Detection was performed at 280 nm. Proteins of molecular weight from 67 kDa (high range) to 1.3 kDa (low range) eluted from 4.4 min to 6.5 min.

Results
No blank interference was observed across the elution window of proteins, indicating the specificity of the method. Linear curve was plotted as the retention time of known proteins (X axis) vs logarithm of molecular weight (Y axis). Seven proteins with known molecular weight were used for linear curve. The linearity of the method has been demonstrated across the range of 1.3 kDa (Vitamin B12) to 67 kDa (Bovine Serum Albumin). The precision of the method was demonstrated by injections of individual sample preparations multiple times (n=6). The accuracy of the method was demonstrated by injecting protein with known molecular weight and comparing calculated value to the known value.

Conclusion
The test method for determination of molecular weight of proteins of interest by size-exclusion (SEC) HPLC was developed. The method has been shown to be specific, linear, precise and accurate.