

April 29, 2010

The following is a document summarizes retention time evaluation in SEC data processing applications.

## **Evaluating Retention Time Consistency**

The following is a discussion of our general approach to accepting or rejecting a series of GPC chromatograms. While some laboratories engage a retention volume marker as a flow rate indicator, Jordi Labs prefers using multiple injections of standard solutions which bracket the sample runs.

Jordi's approach allows us to identify when shifts in flow rate are occurring within a run. If a shift is observed within a series of injections, the entire run will be discarded to avoid the risk of introducing errors in molecular weight.

To ensure that no shifts have occurred Jordi overlays standard chromatograms from before and after sample injections. The retention time of the standards are compared and evaluated. If a shift is observed between the resultant chromatogram of the same standard solution before and after the runs, this is considered an indication of a flow rate shift, and the entire sample series will be discarded and the sample set (along with standards) will be reanalyzed. Figures I through III on the following pages demonstrate "good overlap" (run accepted) and a "shift in retention time" (run rejected) examples.

In addition, it is our standard practice to use the overlap of the solvent peaks as a secondary indication of consistent flow. Note that no shifts in standard, sample or solvent peak occur in **Figures I** and **II**. A retention time shift is shown in **Figure III** neither the standard nor the solvent peaks overlap.

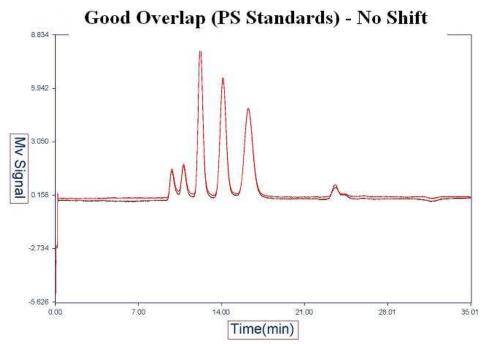


Figure I: Overlay of Polystyrene Standards, no shift

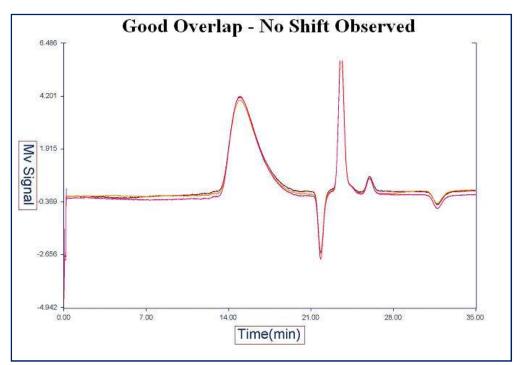


Figure II: Overlay of Polymer Samples, no shift

## Shift in Retention time

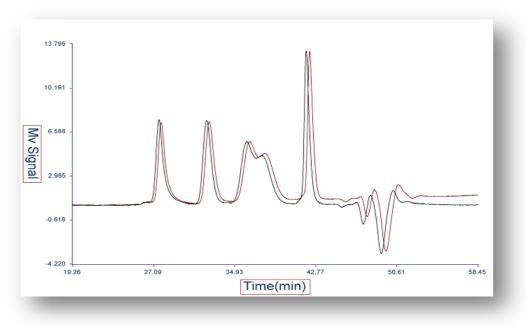


Figure III: Overlay of Polystyrene Standards, shift within run

Jordi's approach does not apply a correction factor for flow shifts across a chromatogram. The risk of utilizing this approach is that it assumes a constant flow rate throughout the entire run although retention times have shifted at unidentified times within the series. This could result in an error being introduced to each run in the series.