Chemometrics

Application Note

ALIGNING PROCESS HPLC

Demonstration of the LineUp™ alignment technology in support of quality control

Summary

Chromatography is useful in all situations where a mixture needs to be quantified precisely. This note describes the work performed for a pharmaceutical company wanting to do quality control on injectable contrast agents used in CT scans and MRI imaging. In this particular case, an attempt was being made to move the HPLC assessment from a control laboratory to an on-line analyzer.

In general, when an analysis moves from lab to line, there is a drop in the amount of oversight that is available to the analyzer. In addition, an on-line system is less likely to have a raw data review. Chemometric alignment technology is extremely useful in eliminating the impact of retention drift.

Process Chromatograms

The problem noted in this application is that, even though there are relatively few peaks in the chromatogram, the peaks elute in clusters. At times, the elution position can be different enough to cause one analyte to be mistaken for another. In Figure 1, a collection of chromatograms is drawn as an overlay to demonstrate retention time drifting among samples.

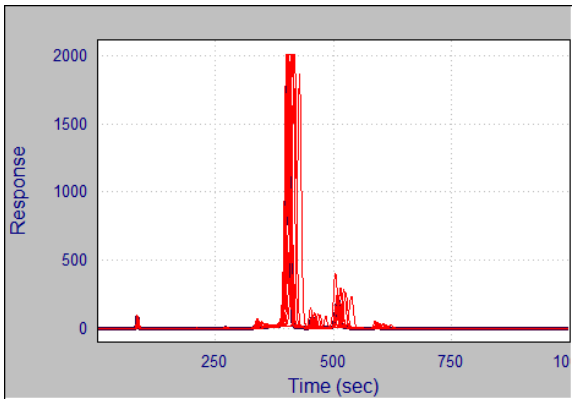

Figure 1: The original QC chromatograms for injectable CT scan and MRI contrast agents

The LineUp software is designed to handle this retention shift. The software can be run as a stand-alone program after data acquisition, or it can be integrated into the method so that it runs automatically at the end of every run. The result is that the original data is preserved and a companion trace is generated that has retention time drift minimized.

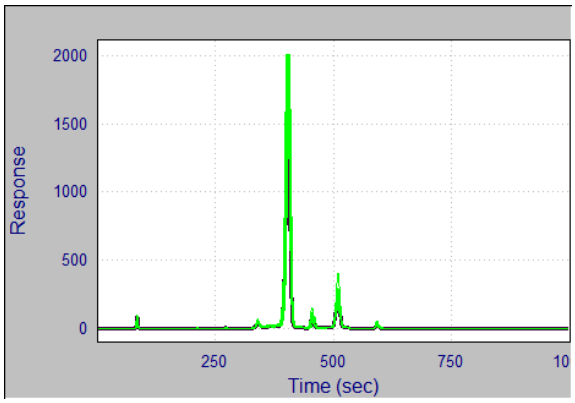

Figure 2: The same chromatographic traces after alignment with LineUp software

The LineUp files generated for the data in Figure 1 are shown in Figure 2. The black trace was used as the alignment target and all traces were aligned to the positions of peaks in this file. The alignment technology deployed is known as correlation optimized warping.

Zooming in on the early peak cluster shows the success of the LineUp approach (see Figure 3). With the alignment software turned on, none of the peaks of interest would be misidentified.

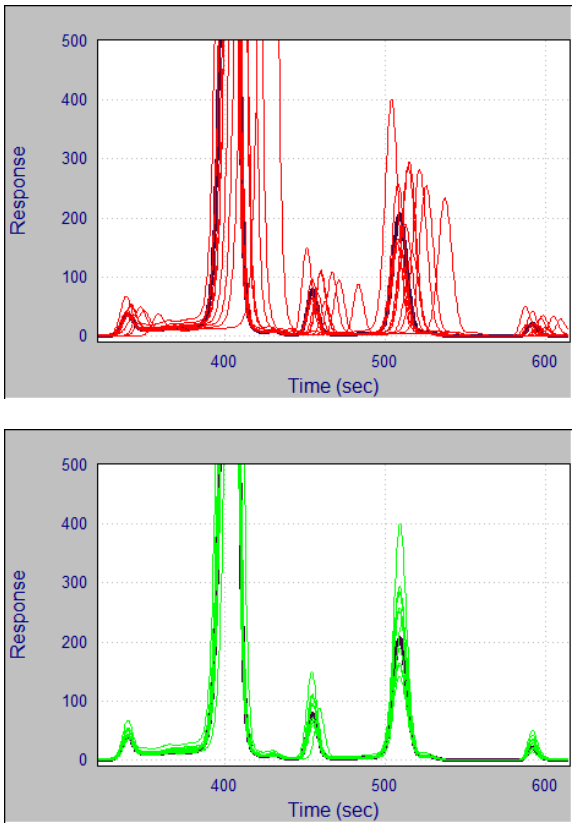

Figure 3: Zooming in on the major peaks of interest (original in red, aligned traces in green)

Principal Component Analysis

Ultimately, we would like to automate the quality assessment for these contrast agents. One of the most powerful techniques is to use chemometric fingerprinting of a series of good batches, using these data to construct a model. Any subsequent batches can then be compared to this experience set of good batches to qualify or disqualify the new samples.

Principal Component Analysis (PCA) is the most appropriate technology to model data as seen here. PCA allows the user to visualize the relative similarity of each batch in a simple plot, but has powerful diagnostics that can trigger a review of the data if needed. Figure 4 below shows the PCA scores of the chromatograms before and after alignment. Each point in the figure represents an entire chromatogram and the relative similarity between two chromatograms will be reflected by the distance between the two points: the closer the points, the more similar.

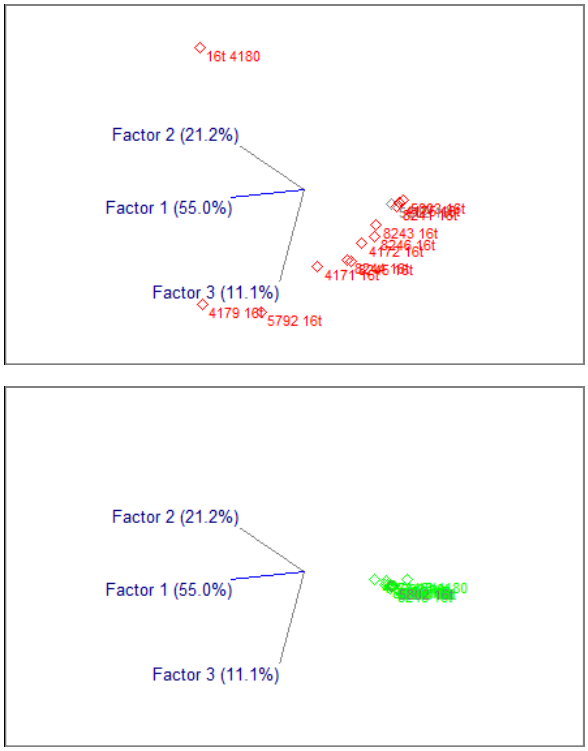

Figure 4: The PCA scores of the original chromatograms (red) and after alignment (green). The target chromatogram is represented by the black point.

We can use the scores plot of PCA to visualize more about the improvement in consistency generated by the LineUp technology. In the case of Figure 4, the scaling applied to both plots is the same. As can be seen, the vast majority of the variance in the data is driven by retention time variability. Eliminating this source of variance reveals the true chemical differences among the batches .

If we expand the PCA scores view of the aligned data, the relative similarities among these batches are quite clear. Shown in Figure 5, most of the batches are consistent, with two batches (4171 and 4180) showing unusual chromatographic patterns.

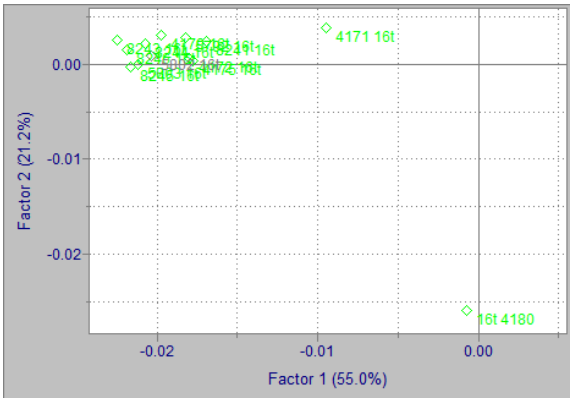

Figure 5: The PCA scores of the aligned chromatograms accentuating the true chemical differences among the batches