Performance PerSPECtives

Waters® Millennium®32 Software

Chromatographic Pattern Matching

Is the chromatogram from sample 'A' different from the chromatogram from sample 'B'? Or are they the same? If you have ever asked yourself these questions, then Chromatographic Pattern Matching could be a valuable tool for your laboratory. Comparing two chromatograms has traditionally been a manual process, often involving a light box and a keen eye. Overlays of chromatograms cannot take into account common run-to-run variations such as retention time offset, baseline offset and drift, and change in concentration (Figures 1A,B,C).

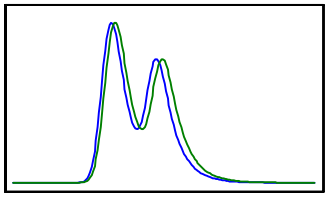


Figure 1A-Retention time offset

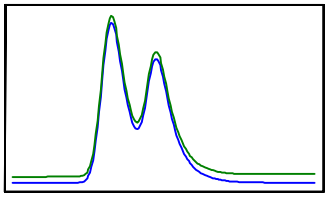


Figure 1B-Baseline offset

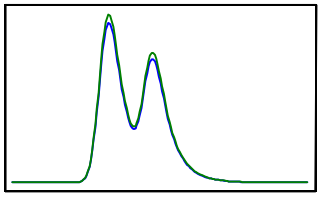
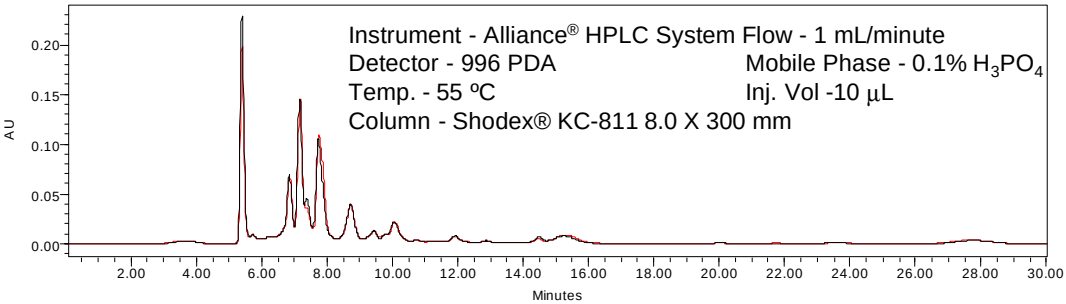


Figure 1C-Concentration change

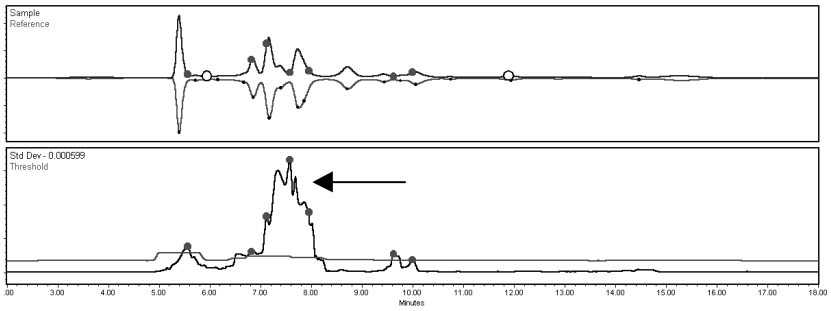
Chromatographic Pattern Matching is a Millennium32 software option that utilizes a patented "chromatographic alignment" algorithm. Rather than integrating each individual peak, Pattern Matching Software compares the data directly and identifies regions containing significant pattern differences. The alignment algorithm removes run-to-run system variations between compared regions, so only only those differences due to the sample are reported. To illustrate Chromatographic Pattern Matching, two different types of California white wine were analyzed for organic acids (Figure 2).

Figure 2 - Overlay of a Chablis and a Sauvignon Blanc, at 214 nm.



This overlay plot contains subtle differences that could easily be missed with traditional visualization techniques. Figure 3 displays the result of a Chromatographic Pattern Matching analysis, which reveals regions containing significant differences (indicated by the dots) between the two chromatograms.

Figure 3 - Mirror and Threshold plots comparing two different white wine samples



In Figure 3, the mirror (top) plot compares the chromatograms. The threshold (bottom) plot shows the magnitude of the pattern difference between corresponding intervals in the chromatograms. The region of maximum difference is indicated with the arrow.

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The data shown in Figure 3 is displayed below as an overlay plot (Figure 4A). Figure 4C zooms in on the region containing the largest pattern difference, to confirm the presence of pattern dissimilarity at 7.5 minutes. The white marker at 5.9 minutes in the overlay plot indicates the presence of a new sample peak. Figure 4B zooms in on the region containing the white marker to confirm that the peak is present in the sample chromatogram, but not in the reference. These Pattern Match Results can also be reported in tabular form (Figure 5.)

Figure 4A- Overlay plot and Threshold plot comparing two different white wine samples

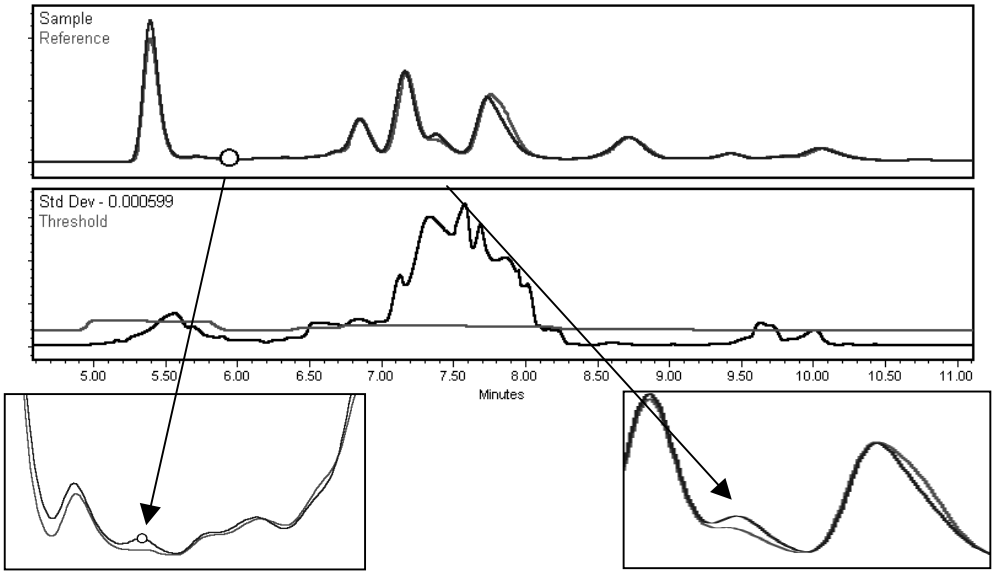


Figure 4B- New sample peak at 5.9 min.

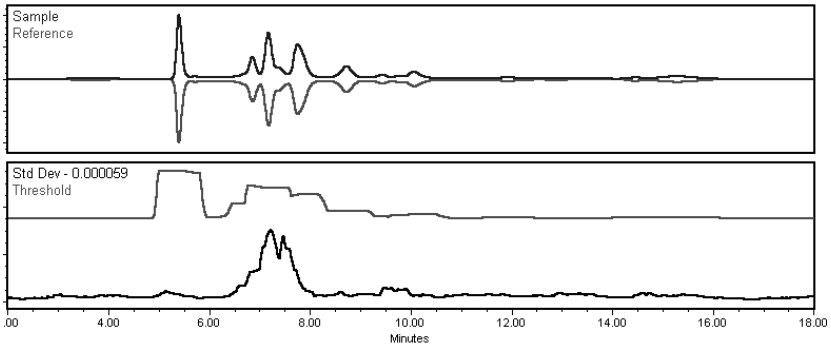
Figure 4C-Pattern dissimilarity at 7.5 min.

Figure 5-Pattern Match results in tabular form can be included in reports and exported for further analysis.

	Condition	Time	Std Dev	Std Dev Threshold
1	Std Dev above threshold	5.568	0.00077	0.00058
2	Sample peak apex not in reference	5.952	0.00014	0.00036
3	Std Dev above threshold	6.827	0.00061	0.00049

To confirm that the pattern matching algorithm is detecting genuine sample differences, replicate injections of the reference are compared (Figure 6). As shown in Figure 6, the magnitude of differences lies below the red threshold line. At 7.5 min, the magnitude of pattern difference is about 10 times smaller between the replicates than between the sample and the reference.

Figure 6- Comparison of replicate injections of Chablis reference.



Traditional techniques for comparing two chromatograms can be tedious and may miss slight differences. Millennium[32] software with Chromatographic Pattern Matching can be a valuable tool to detect differences between two very similar chromatograms.