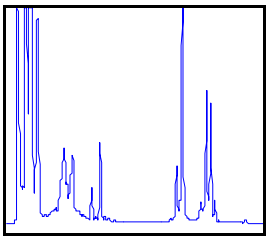

InfoMetrix®

Product Note

Aligning Chromatograms

The Infometrix software Pirouette® and InStep™ facilitate chromatographic pattern matching with minimal customization. These products build on experience gained from the development of custom systems for analysis of GC, HPLC, HPCE, TLC and gel electrophoresis data. Customers who have utilized these solutions include the FDA, the CDC, and several food and petroleum companies, automating interpretation for instruments from Agilent, Beckman, PerkinElmer, Shimadzu, Waters and others.

In the past, chromatographic pattern matching using Infometrix products has been based on extracting tabulated results (area, height) from processed chromatograms. This approach works well on closed systems with a small number of peaks (<100). However, if the chromatograms to be matched have many different peaks or have few peaks in common, it may be better to process the whole chromatographic profile. Peaks with incomplete resolution or peaks from unexpected components are routinely characterized with whole profiles but would be overlooked with peak table data. A primary problem in working with whole chromatograms is the lack of retention time stability, which affects multivariate data processing. Pirouette 3.10 includes a mechanism to align chromatographic traces; each chromatogram is aligned to marker peaks in a designated sample.

Data Preparation

To exploit the alignment feature requires import of whole profiles into Pirouette. This is accomplished most easily by export from the chromatography system of an AIA format file (in the case of the Agilent ChemStation, which does not store marker information in the AIA file, a special macro will save a Pirouette ASCII file). Suitable alignment markers in each file are entered into the Pirouette spreadsheet before processing. Marker identification and time entry can be automated by including marker peaks in the peak ID table of the chromatographic method. Different scenarios for processing chromatograms include:

Using native peaks as alignment markers - If you have compounds that appear in all of your chromatograms, you can use them as alignment markers.

Using internal standards as alignment markers - If suitable markers cannot be designated among native peaks, particularly if no common peaks appear in all chromatograms, incorporate internal standards.

Using external standards as surrogate markers - When native markers are not found and peak density precludes the use of internal standards, an external standard approach may be required. In this case, you usually analyze a QC sample that contains appropriate alignment markers, followed by analysis of one or more analytical samples.

Alignment Processing

Pirouette uses the marker retention times (in scan units) to perform interpolative alignment, modifying the retention time of intervening peaks. In each case, Pirouette aligns sample chromatograms to a designated target chromatogram. The actual times used by Pirouette in the alignment step are derived by:

- Estimating peak tops for markers in all samples. True peak tops are found via a polynomial fit on the tallest peak around each marker retention time, for every sample.
- Estimating peak tops for markers from the target. Marker times for samples other than the target can also be set to zero. In this case, Pirouette will use the target's marker times in each sample that has these zero values. It will then proceed as above to find true peak tops.
- To handle external standards, you assume there is negligible retention time drift between samples. In this case, the Pirouette option for alignment window size is set to zero and no fit is made to find true peak tops. Rather, the explicit time entered for each marker peak is used for all samples. If a marker value is zero, the target's marker time is used.

Infometrix, Inc., 10634 E. Riverside Dr., Bothell, WA 98011 Phone: (425) 402-1450, Fax: (425) 402-1040, email: info@infometrix.com