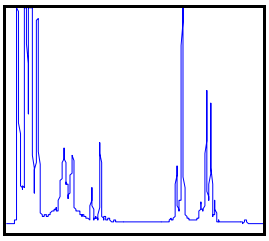

InfoMetrix®

Application Note

Characterizing Unresolved Peaks

The primary goal of chromatography is to separate the components of a mixture so as to facilitate their identification and quantitation. Ever since the practice was initially described, chromatography has been one of the most important tools in the analytical chemist's repertoire. Yet, because of the increasingly demanding needs of modern chemical analysis, the efforts to improve and enhance the quality of separations achieved by chromatography have been unceasing. As a result, remarkable improvements have been achieved in chromatographic instrumentation, encompassing detectors and column technology.

Despite the hardware advances, separation of components in a mixture is often incomplete, which demands that further effort be expended to accomplish the analytical goals. If we can be successful in extracting quantitative information even in cases where resolution is incomplete, we simplify methods development. The options for mathematically resolving coeluents are two: we can make peak shape assumptions (*e.g.*, drops to baseline, tangent skims); or, in the case of a chromatograph with a multivariate detector, we can employ factor-based chemometrics.

Pirouette's technique for mathematical resolution is based on the Self-Modeling Curve Resolution (SMCR) algorithm first described by Lawton and Sylvestre in 1971. The self-modeling portion of the name indicates that no prior knowledge of the pure component spectra and no peak-shape assumptions are required.

For an example of a tough resolution case, consider the problem of separating 1- and 2-methylnaphthalene. Because of their chemical and structural similarity, the relative retention of these analytes is similar. These compounds also have very similar UV/Vis spectra.

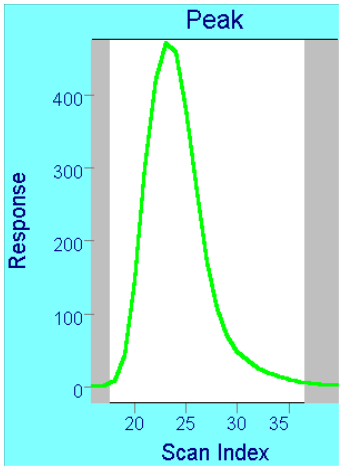

Figure 1. ***Total wavelength chromatogram of methyl-naphthalenes***

In this example, a mixture of pure standards was prepared by weighing aliquots and diluting with acetonitrile. The resulting mixture was analyzed on a C_{18} column (5 µm, 25 cm x 4.6 mm), using a mobile phase of 10% water in acetonitrile at a flow rate of 1 mL/min. Diode array data were collected at a scan rate of 1 Hz in the range of 210-400 nm and a bandwidth of 2nm. The 1- and 2-methyl naphthalenes coelute under these conditions and the resulting peak, monitored at a single wavelength, is shown on the left.

An unresolved 2-component peak

The MCR algorithm extracts the pure component profiles and their corresponding spectra strictly based on the supplied HPLC-DAD data. Despite the severe overlap (R_S = 0.28), the algorithm yields estimates of the elution profiles that, when quantited, reasonably match the known concentrations. The inferred spectra correctly identify the compounds via library search.

This tool gives us the ability to quantitate analytes even in an instance of high overlap and similar spectra.

This approach will apply equally well with data from any bilinear data system, including LC/UV, GC/MS, etc.

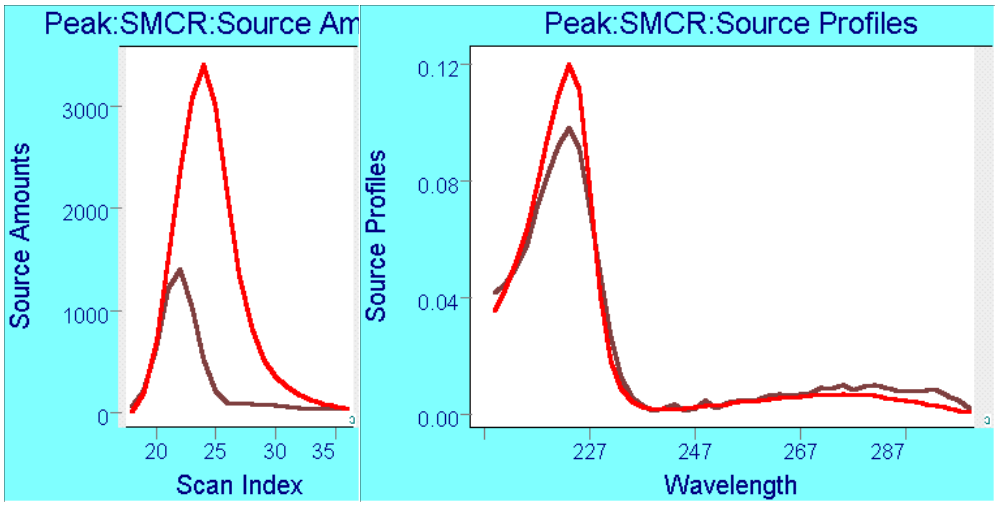

Figure 2. ***An example of resolving a mixture of 1- and 2-methylnaphthalenes, showing the elution profiles (left) and the inferred spectra (right)***

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