Mutual peak resolution and matching in a series of HPLC/DAD mixture analyses

Abstract
One of the largest challenges in chromatographic method development is the necessity for tracking the movement of peaks as separation conditions are changed. Peak increments are then used to determine the number of components in an optimization circuit. Method optimization for an unknown mixture is, moreover, complicated by the absence of a priori information on component properties and retention times when direct signal assignment is not possible. On the contrary, the achievement of maximum separation becomes an important factor for successful identification or quantification. In this case, the optimization may be based on the assumption that all of them belong to the same component. In other words, mutual peak matching between the HPLC runs is required.

A new method for mutual peak matching in a series of HPLC/DAD analyses of the same unknown mixture acquired at varying separation conditions has been developed. The approach does not require any prior knowledge of the mixture composition. Applying aFA (Abstract Factor Analysis) and BFA (Iterative Key Set Factor Analysis) on the augmented data matrix, the algorithm detects the number of mixture components and calculates the retention times of every individual component in each of the input chromatograms. Every candidate peak is then validated by target testing. In each run, the HPLC data are transformed to provide quantitative criteria for the detection of “missing” peaks and non-analyte components as well as confirming successful matches. The matching algorithm by itself does not perform full curve resolution. However, its output may serve a good initial estimate for the further modeling. A common set of UV spectra of pure components can be obtained, as well as their corresponding concentration profiles in separate runs, by means of ALS MCR (Alternating Least Square Multiple Component Regression), resulting in reconstruction of original peaks.

The algorithms were programmed in MATLAB and tested on a number of simulated datasets. Possible ways to improve the stability of results, reduce calculation time, and minimize operator interaction are discussed. The technique can be used to optimize HPLC analysis of a complex mixture without preliminary identification of its components.

Keywords: HPLC, DAD, mutual peak matching, AFA, BFA, ALS MCR

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4. Compiling the table of peaks
5. Curve resolution
6. Curve resolution (optional)
7. Conclusion

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Figure 2: Skyline chromatograms
Figure 3: Spectra of components
Figure 4: Set of 11 spectra of the augmented data matrix
Figure 5: Key set spectra 10 and 11
Figure 6: Target set results of component 3: successful (step 2) and failed (step 1)
Figure 7: Resulted spectra and concentration profiles (normalized of experiment 1)