Liquid Chromatography Problem Solving and Troubleshooting

Question

Could you explain what is meant by the term rapid-analysis HPLC and give me some suggestions about when I might consider using it in optimizing a method?

Answer

Before answering your question, it is relevant to point out a few general ideas concerning certain methods' optimization and specifically about those based on HPLC. Three important considerations in developing a useful routine assay are its specificity, ruggedness, and speed. Specificity refers to the ability of the assay to clearly distinguish and measure individual components in the sample that are free from the interference of other coadditives and contaminants that may be present; ruggedness is a measure of the method's reproducibility when it is carried out by different investigators for extended periods of use; and speed relates to the number of samples that can be assayed in a given time. The general appeal of liquid chromatographic procedures as opposed to many other approaches is their inherent specificity and the preferred use of reversed-phase conditions in combination with isocratic elution over other modes of separation that are related to simplicity and reliability. Likewise, if properly designed, an isocratic RPLC separation can be carried out quickly and automated easily.

Shown in Figure 1A is a chromatogram of a three-component mixture of aromatic acid esters obtained using a standard 4.6- × 250-mm octadecyl column, and methanol–water was used as the eluent. From the standpoint of resolution and ruggedness this would be considered to be a good separation. However, from the practical standpoint an assay based on this particular separation would be less than satisfactory because it wastes large amounts of time between the individually eluting components. In optimizing this method, several approaches could be used in order to compress the chromatogram involving either changes in the eluent, column, or both. The final goal is to eliminate as much wasted time as possible and hence develop an assay with a high-sample throughput.

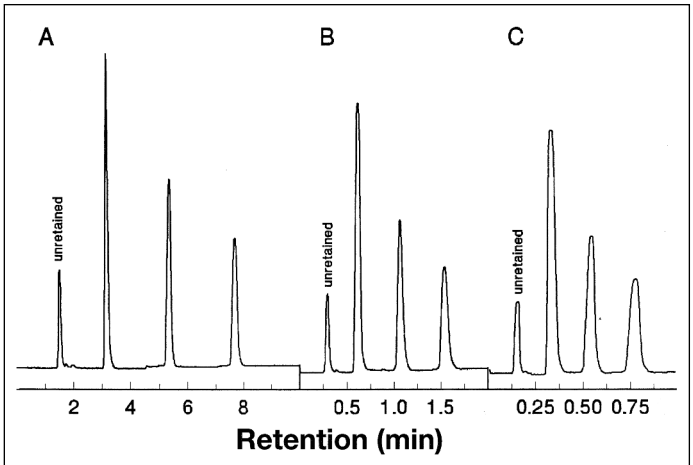

Figure 1. Three chromatograms of a three-component mixture of aromatic acid ethers.

Rapid-analysis or fast HPLC is a concept that dates back more than 20 years and is based on the idea that only one or two thousand theoretical plates are needed to carry out many routine types of separations (1,2), such as the one illustrated in Figure 1A. Thus, in contrast to modern 150- to 250-mm analytical columns that provide efficiencies equivalent to many thousands of plates, most rapid-analysis columns are much shorter. Typically, they range in length from approximately 30 to 50 mm. By using a column with the same retention properties but shorter in length and keeping other operating parameters the same (i.e., eluent composition, flow rate, and temperature), the gain in analysis speed is directly related to the reduction in length (illustrated in Figure 1B). In this example the time of the chromatographic analysis has been compressed from over 8 min to less than 2 min simply by using an equivalent octadecyl column that is 4.6 × 50 mm.

Under rapid-analysis conditions, additional gains in analysis speed are obtained by using higher eluent flow rates. This is possible with shorter columns because they have significantly reduced back pressures compared with analytical columns. This is illustrated in Figure 3C, in which the flow rate of the eluent has been doubled compared with that used to carry out the separations shown in Figures 1A and 1B. With this increase in the flow rate, the overall column efficiency is degraded from

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approximately 2300 plates (Figure 1B) to1500 plates (Figure 1C), but it is still sufficient to provide enough resolving power for the three aromatic acid esters. In more demanding separations, in which additional plate counts are needed, specially designed rapid-analysis columns are available from several manufacturers that are packed with very high-efficiency porous materials with particle diameters between 2–3 µm. Likewise, nonporous particles have been developed recently as an alternative media for carrying out highly efficient fast separations.

In terms of application, rapid-analysis HPLC has been applied to a variety of different acid, base, and neutral compounds as well as sample types (3). However, the general approach works best for assays involving single components, simple combination products, or mixtures of compounds with widely and incrementally varying retention (such as the separation illustrated in Figure 1), because these types of samples do not require the higher resolving power of conventional analytical columns.

References

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