

An Executive Summary

Reversed-Phase Liquid Chromatography Using Surrogate (Additional) Stationary Phases



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Introduction

Liquid chromatography (LC) has several uses in the pharmaceutical world: to identify an analyte or active pharmaceutical ingredient (API), to quantitate the amount of an analyte or API (assay), and as a preparative method for separating the analyte or API from their related substances. Techniques that can increase the resolution between related substances and the API are vitally needed when establishing the identification, assay, or preparative output. This manuscript discusses the use of quaternary ammonium salts that are bound to C8 or C18 reversed-phase media as surrogate or additional stationary phases, and their effect on the resolution parameters such as retention factor (k'), efficiency factor (i.e., the number of theoretical plates [N]), tailing factor (i.e., peak asymmetry), and the selectivity factor (α).

Chromatographic Method Objectives and Resolution Basics

In general, chromatography objectives can be separated into four categories: identification (ID), purity, assay, and preparative isolation. Almost all API specifications have an ID test that correlates the analyte's retention time with that of its reference standard. A purity or high-performance liquid chromatography (HPLC) method that separates the API from its impurities and quantitates each impurity by area percentage is also often required. Over the years, in fact, HPLC methods for the quantitative analysis of an API have largely replaced less-specific methods like titration or colorimetric assays. HPLC is also often used for preparative purposes by separating a compound from its impurities on the gram to kilogram scale.

Often, the first separation technique chromatographers are exposed to is thin-layer chromatography (TLC). In TLC, the mobile-phase composition is adjusted to get the sample of interest to move in the "retardation factor" (R) range of 0.3–0.7 for optimal component separation. Likewise, the mobile-phase composition in HPLC is adjusted to a k' of three to 10 for an optimal separation. Similar to a non-absorbed compound that moves to the solvent front in TLC, a non-absorbed compound in HPLC elutes in the void volume. In that way, column chromatography can be thought of as TLC (or planar chromatography) in three dimensions.

The "resolution equation" expresses the separation between any two components in a mixture, as illustrated in **Figure 1**. Resolution is affected by three things: retention, efficiency, and selectivity. The **retention factor** (k or k') is a means of measuring the retention of an analyte on the chromatographic column. It can be defined in terms of an analyte's elution time relative to that of an unretained compound (see **Figure 2**). A high k or k' indicates that the compound is highly retained and has spent a significant amount of time interacting with the stationary phase.

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