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Using the fundamentals of adsorption to understand peak distortion due to strong solvent effect in hydrophilic interaction chromatography

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ABSTRACT

The peak distortion observed in hydrophilic interaction chromatography (HILIC) may be caused by the sample diluent to mobile phase mismatch. The United States Pharmacopeia (USP) method for organic impurities in cetirizine HCl tablets calls for such a mismatch, having a higher concentration of strong solvent in the sample diluent than in the mobile phase. A significant peak deformation is reported for cetirizine (a second-generation antihistamine) when it is purified on a Ethylene Bridged Hybrid (BEH) HILIC column (4.6 mm × 100 mm, 2.5 μm particles) using an acetonitrile–water eluent mixture and a sample diluent containing 7% and 9% water (in volume), respectively. The mechanism and physical origin of such peak distortion are related to (1) the diluent-to-eluent excess of water that propagates along the column at a velocity similar to that of the analyte, (2) the significant drop of the Henry's constant of the analyte upon increasing water concentration in the eluent, (3) the sample volume injected, and (4) to the pre-column sample dilution factor that depends on the characteristics of the LC instrument used.

This proposed mechanism is validated from the calculation of the concentration profiles of cetirizine and water by using the equilibrium-dispersive (ED) model of chromatography. The observed distortion of cetirizine peaks is successfully predicted from the measurement of (1) the excess adsorption isotherm of water from acetonitrile onto the BEH HILIC adsorbent, (2) the retention factor of cetirizine as a function of the volume fraction (7, 8, and 9%) of water in the mobile phase, and (3) of the pre-column sample dispersion related to the instrument used (HPLC or UHPLC). The results of the calculations enables the user to anticipate the impacts of the diluent-to-eluent mismatch in water content, the injection volume, the analyte retention under infinite dilution, and of the pre-column sample dispersion on the amplitude of peak distortion in HILIC. Appropriate and permitted alterations of the USP method are then suggested based on a sound physico-chemical approach.

1. Introduction

HILIC is particularly well suited for the retention and separation of polar analytes which are barely retained in the RPLC retention mode [1–5]. The popularity of HILIC has increased with the development of polar drugs within the pharmaceutical industry and with the growing field of metabolomics and proteomics, which both produce primarily polar molecules [6–8]. The retention mechanism in HILIC has been studied in depth and revealed the existence of a water-rich layer at the surface of the HILIC adsorbent [9–18]. This was confirmed from thermodynamic data based on dynamic chromatographic methods (minor disturbance method [17,19–21]) regarding the measurement of the excess (or deficit) of adsorption of the solvent molecules in HILIC [17]. Mechanistically, the polar analytes partition between the acetonitrile-rich eluent and the water-rich layer and/or adsorb from the water-rich layer onto the surface of the HILIC adsorbent [22–28].

Among the many polar pharmaceutical compounds analyzed by HILIC methods, cetirizine belongs to the second generation of antihistamine, which are used in the treatment of hay fever, urticaria, angioedema and various allergies. The USP method designed to quantify the organic impurities from cetirizine HCl tablets recommends using either a 4.0 × 250 mm column packed with porous silica particles (1.5–10 μm) or a silica monolithic rod (same dimension) under isocratic elution conditions [29]. The USP general chapter 621 allows the users to apply certain alterations to USP methods such as modifying the particle size and/or the column

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length as long as the ratio of the column length to the particle size remains constant or within the range from −25% to +50% of the original column specified [30]. Simple rules of thumb apply for the selection of the modified flow rate (to achieve similar column efficiency) and injected sample volume (to maintain the same loading factor). Remarkably, in the case of cetirizine, the USP method recommends a slightly different composition for the sample diluent (9% water in volume) relatively to that of the mobile phase (7% water). This diluent-to-eluent mismatch could cause serious peak distortion in HILIC. However, USP methods have a requirement for the tailing factor of cetirizine, so severe peak distortion would have been a problem. So, peak distortion was not observed originally likely due to the use of a higher dispersion LC system and/or a column with specific retention properties.

However, with current LC instruments and columns, the peak shape of cetirizine happens to be severely distorted under the chromatographic conditions recommended by USP. The experimenter is then facing a complex problem of method alteration/optimization by adjusting a very large number of experimental parameters such as column length, column diameter, particle size, flow rate, injection volume, LC instrument, eluent and diluent composition, temperature, etc. The new developed and successful method requires a deep understanding of the phenomenon of peak distortion in HILIC and should remain within the USP guidelines.

The overall goal of this work is to provide separation scientists with some fundamental understanding regarding the development and optimization of HILIC methods. More specifically, it aims at describing from a physico-chemical viewpoint the reproducible phenomenon of peak distortion encountered in HILIC. Peak distortion can be observed when the diluent composition only slightly differs from that of the eluent composition. The problem is illustrated in this work with the separation of the pharmaceutical compound cetirizine from organic impurities. The USP method recommends applying a slight diluent-to-eluent mismatch in terms of volume fraction of water (9% *versus* 7%). The impacts on peak distortion of (1) the diluent-to-eluent mismatch in water content (9 to 8%, 9 to 7%, and 9 to 9% match), (2) the column length (10 and 25 cm, 4.6 mm i.d.) and particle size (5 and 2.5 μm), (3) the injection volume (1 to 40 μL), (4) the amount of pre-column sample dispersion that depends on the LC instrument (HPLC and UHPLC), and of (5) the retention of the analyte relative to that of the water perturbation are all investigated from both experimental and theoretical viewpoints. The experimental concentration profiles of cetirizine are predicted by using the equilibrium-dispersive (ED) model of liquid chromatography. Experimental and calculated peak profiles are compared, discussed, and used to propose a sound mechanism of peak distortion in HILIC. The predicted results are used to enable the users to properly develop and optimize HILIC methods.

2. Theory

2.1. Minor disturbance method: unique excess adsorption isotherm of water onto HILIC adsorbents

When the components of the eluent mixture are weakly adsorbed onto the chromatographic adsorbent, the composition of the adsorbed phase barely differs from that of the bulk phase. As a result, the total number of moles adsorbed is strongly dependent on the location of the Gibbs dividing surface, e.g., on the arbitrary delimitation between the stationary and mobile phase or on the determination of the chromatographic hold-up volume V_0 [19]. Frontal analysis is no longer an accurate method for the measurement of adsorption isotherms of weakly adsorbed compounds due to the determination uncertainty of V_0 [31–33]. In contrast, the minor disturbance method (MDM) using refractive index (RI) detection is very suitable to detect the perturbation of the equilibrium plateau of binary [34,35] or ternary eluents [36] enabling the unambiguous measurement of the thermodynamic dead volume V_M of a chromatographic column. Such pulse techniques have been used abundantly in the past to measure the excess adsorption isotherms of the eluent components in the RPLC retention mode [19–21,37–41].

In this work, the solid adsorbent is underivatized porous hybrid organic/inorganic particles [8] and the mobile phases are binary mixtures of water and acetonitrile. These mixtures are assumed to be ideal (no volume expansion or contraction upon mixing, so, the partial molar volumes are equal to the molar volumes of the pure solvents) and incompressible. x is the volume fraction of water in the binary eluent mixture. y is the volume fraction of water in the column volume V_M (or thermodynamic dead volume) in equilibrium with the bulk eluent of composition x. Assume a small perturbation of the entering eluent from composition x to $x + dx$. The equilibrium composition of the column volume V_M becomes $y + dy$. From the mass conservation of water inside the column, Knox and Kaliszan have shown that the elution volume, $V_R(x)$, of the eluent perturbation propagating along the column is given by [19]:

$$[(x + dx)V_R(x)]_{z=0} - [xV_R(x)]_{z=L} = [(y + dy)V_M]_{t=V_R(x)/F_v} - [yV_M]_{t=0} \quad (1)$$

or

$$V_R(x)dx = V_M dy \quad (2)$$

Integration of Eq. (2) between the volume fractions $x = y = 0$ (pure acetonitrile) and $x = y = 1$ (pure water) provides the thermodynamic column volume V_M [19]:

$$\int_0^1 V_R(x)dx = V_M \int_0^1 dy = V_M \quad (3)$$

The excess number of moles, $n^e(x)$, of adsorbed water is defined as the equilibrium number of moles of water present in the volume V_M minus the number of moles of water in the same volume if the adsorbent would not preferentially adsorb any of the eluent components (e.g., $y(x) = x$ for a virtual inert adsorbent). Accordingly, by definition:

$$n^e(x) = \frac{y(x)V_M}{v^*} - \frac{xV_M}{v^*} = \frac{V_M}{v^*}[y(x) - x] \quad (4)$$

where v^* is the molar volume of pure water.

It is important to note that $n^e(x)$ is unique and unambiguously measured by the MDM on a series of equilibrium plateaus [19–21]. Combining Eqs. (2) and (4), $n^e(x)$ is measured from the integral:

$$n^e(x) = \frac{1}{v^*}\int_0^x [V_R(u) - V_M]du \quad (5)$$

where u is a dummy variable.

2.2. Arbitrary total adsorption isotherm of water onto HILIC adsorbents

The difference between absolute (or total) and excess isotherms has been extensively explained by Gibbs and Everett in the field of solid-liquid adsorption [42]. In particular, the same notions of total and excess adsorption were applied in liquid chromatography [19–21,34,39]. The excess adsorption isotherm is unique and measurable: it represents the excess of mass actually present in the system with respect to the system mass that would be observed if the solid phase was ideally inert to all the liquid components. In contrast, the absolute or total adsorption isotherm is arbitrary because it depends on the convention for the physical delimitation