Chromatographic retention prediction and octanol–water partition coefficient determination of monobasic weak acidic compounds in ion-suppression reversed-phase liquid chromatography using acids as ion-suppressors

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ABSTRACT

Although simple acids, replacing buffers, have been widely applied to suppress the ionization of weakly ionizable acidic analytes in reversed-phase liquid chromatography (RPLC), none of the previously reported works focused on the systematic studies about the retention behavior of the acidic solutes in this ion-suppression RPLC mode. The subject of this paper was therefore to investigate the retention behavior of monobasic weak acidic compounds using acetic, perchloric and phosphoric acids as the ion-suppressors. The apparent octanol–water partition coefficient (K_{ow}'') was proposed to calibrate the octanol–water partition coefficient (K_{ow}) of these weak acidic compounds, which resulted in a better linear correlation with log k_w, the logarithm of the hypothetical retention factor corresponding to neat aqueous fraction of hydroorganic mobile phase. This log K_{ow}'' − log k_w linear correlation was successfully validated by the results of monocarboxylic acids and monohydrating phenols, and moreover by the results under diverse experimental conditions for the same solutes. This straightforward relationship not only can be used to effectively predict the retention values of weak acidic solutes combined with Snyder–Soczewinski equation, but also can offer a promising medium for directly measuring K_{ow} data of these compounds via Collander equation. In addition, the influence of the different ion-suppressors on the retention of weak acidic compounds was also compared in this RPLC mode.

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1. Introduction

Organic modifiers and water are the most common mobile phase components for reversed-phase liquid chromatography (RPLC). For the RPLC separation of weakly ionizable compounds, conventionally, acids, bases or buffers are added in the aqueous mobile phase according to nature of analytes. The functions of these additives are suppressing dissolution of ionizable solutes, covering active and polar sites of stationary phase surface, and/or buffering mobile phase to adjust chromatographic retention on lipophilic stationary phase, to amend peak shape, and to improve resolution of ionizable solutes [1–4].

Actually, most environmental and biomedical molecules are ionizable more or less. The theories and practices on separation of weakly ionizable solutes in RPLC using buffers as ion-suppressors have been extensively studied. However, most buffers used as ion-suppressors in RPLC are prone to isolating from mobile phase and clogging in the connecting tube of chromatographic instrumenta-

tion, especially micro- or nano-scale LC tubing, owing to the low solubility in organic modifiers such as methanol and acetonitrile, and therefore a time-consuming redevelopment of the separation is often required. On the other hand, liquid chromatography–mass spectrometry (LC–MS) has become a popular technique that can provide excellent specificity instead of LC with UV–vis detection. At the same time, many buffer constituents such as metal salts and phosphates are nonvolatile, causing fouling and subsequent failure of ionization sources [2,5,6]. The incompatibility of many buffers with mass spectrometry limits their applicability in LC–MS. Then postcolumn removal of nonvolatile buffer components has been developed recently in hybrid LC–MS for separation and analysis of weakly ionizable compounds [2]. Though this technique is a potential solution, the more convenient practice is still to substitute simple acids or bases as mobile phase additives for nonvolatile buffers. The primary purpose of this paper is to investigate the RPLC retention behavior of weakly ionizable monobasic acidic compounds using acetic, perchloric and phosphoric acids as ion-suppressors.

It is well known that retention prediction before experimental separation is a subject of fundamental studies to allow fast optimization of chromatographic conditions, to improve peak

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resolution and to decrease analysis time. There are some useful systematic approaches to aid selectivity optimization, such as chemometrics, computer-assisted optimization, and quantitative structure–retention relationships (QSRR). Among these above-mentioned methods, however, only QSRR relates chromatographic retention to molecular structure of solutes [7,8].

Nowadays, the influence of chromatographic variables such as mobile phase type and composition on the retention of neutral molecules has been well understood [9,10]. For weakly ionizable compounds, there have been some reports describing their RPLC retention behaviors [11–13]. Roses and Bosch's group has systematically and deeply studied the retention of weakly ionizable compounds on RPLC which was summarized in a review [14]. Recently, they deduced a general equation for prediction of the retention of weak acids and bases as a function of mobile phase pH and column temperature based on buffer solutions and organic modifiers as the eluents [15]. They also established a model that relates the chromatographic hydrophobicity index (CHI) of ionzable solutes to mobile phase pH as well [16]. However, all of above scientists paid their primary attention on buffers as ion-suppressors. This is mainly because the study about buffer solutions was thought easy to perform due to the constant pH and ion strength during chromatographic process. Another probable reason is that there were not contemporary stationary phases capable of enduring extremely acidic or alkaline mobile phases. On the other hand, these retention prediction models generally include sets of rigorous equations, respectively, composed of odd parameters that are often difficult to obtain, having the prediction a little bit discommodious and therefore unpractical.

The octanol–water partition coefficient (K_{ow} or P_{ow}) expressing compound hydrophobicity has been widely accepted as providing a good indication of the distribution of analytes into biological membranes. This has made it one of the most commonly reported physical properties of drugs, pesticides and other chemicals [17,18]. To measure K_{ow} by RPLC, in the past, there was significant interest in correlating the logarithm of K_{ow} with the logarithm of retention factor k of the solute under a specific chromatographic condition [19–21]. As many commercial software products can be employed to compute the K_{ow} value with acceptable accuracy, the linear relationship between log K_{ow} and log k has become useful for the prediction of RPLC retention [22]. It was further suggested that log k_w, the logarithm of the retention factor obtained by extrapolating to neat aqueous fraction of binary mobile phase, gives a more accurate correlation with log K_{ow} [23]. However, in general ion-suppression RPLC mode with pure acids as the additives, eluents were not acidic enough to absolutely suppress the ionization of the analytes for protecting stationary phase in practical operation. Actually, k reflected the average behavior of all existing species of a solute including the molecule and its ions [11]. Thus, it is inappropriate to ignore the hydrophobicity difference between the molecule and its ions [24]. The K_{ow} value of a solute, only revealing the lipophilicity of the unionized species, lost their predictive capability for explaining the chromatographic behavior of ionizable ones. In our previous paper [25], apparent octanol–water partition distribution coefficient (K''_{ow}) reduced from experimental octanol–water partition coefficient (K'_{ow}, that is, octanol–water distribution coefficient D_{ow}) [24] was put forward to calibrate K_{ow} values of weakly ionizable solutes, and the introduction of K''_{ow} was primarily considered to cause a better correlation with log k_w than K_{ow}.

In this paper, an in-depth experimental study was made in order to investigate retention behavior of weakly ionizable monobasic acidic compounds using monocarboxylic acids (derivatives of benzoic acid) and monohydrating phenols (derivatives of phenol) as the model compounds in RPLC employing acetic, perchloric and phosphoric acids as ion-suppressors, respectively. The K_{ow} of these

weakly ionizable monobasic acidic compounds was calibrated to corresponding K''_{ow} to obtain good linear relationship between log K''_{ow} and log k_w. This correlation was further validated by the experiments under different column parameters in different HPLC instrumentation. This log K''_{ow} – log k_w linear relationship can be applied to immediately predict the retention behavior of weakly ionizable acidic solutes combined with Snyder–Soczewinski equation in this ion-suppression RPLC with simple acids as the additives. This correlation also offered a reliable protocol for verifying the K_{ow} values reported or software-computed, and even for forecasting unknown K_{ow} values for weak acidic compounds.

2. Theory

In liquid chromatography, general Snyder–Soczewinski equation relating k to volume fraction of organic modifier in binary aqueous mobile phase, C_B, has the form [9]:

$$\log\ k = \log k_w - SC_B \tag{1}$$

where S, the regression coefficient of C_B, is a constant for a given solute–eluent combination.

The linear relationship between log K_{ow} and log k_w, known as Collander equation, has been illustrated experimentally and theoretically [19]

$$\log\ K_{ow} = m \log k_w + n \tag{2}$$

This correlation concerned by most works was limited to neutral solutes when the mobile phase was usually near neutral [10,21,26]. In that situation, the k_w value was certainly the retention factor of neutral species of the analyte. It was reasonable to compare log k_w with log K_{ow} for unionizable solutes, but not just so for the substituted benzoic acids and phenols studied in this ion-suppression RPLC.

On one hand, K_{ow} or P_{ow} of a solute employed in most studies refers to hydrophobicity of the neutral form of the solute, which is completely different from that of its ionic forms ($K_{ow}^{+ or -}$ values) [27]. The hydrophobicity of the tested weakly acidic compound should be expressed by K''_{ow} (or D_{ow}) [24] that takes into account the amount of compound in its molecular and ionic states combining K_{ow} and $K_{ow}^{+ or -}$ values. The K''_{ow} of a monobasic acidic solute is described by

$$K'_{ow} = \frac{[HA]_o + [A^-]_o}{[HA]_w + [A^-]_w} \tag{3}$$

The subscripts o and w refer to the octanol (or oil) and the aqueous phase, respectively. Using the expression of dissociation constant K_a, the K''_{ow} can be trivially formulated as

$$K'_{ow} = \frac{K_{ow} + K_{ow}^-(K_a/[H^+])}{1 + (K_a/[H^+])} \tag{4}$$

in which, K_{ow}^- is octanol–water partition coefficient of the A$^-$ anionic form. Usually K_{ow}^- is thought to be very small when compared to K_{ow} of its neutral form. The approximation of K'_{ow} was expressed by K''_{ow} [25].

$$K''_{ow} = \frac{[HA]_o}{[HA]_w + [A^-]_w} = \frac{K_{ow}}{1 + K_a/[H^+]} \tag{5}$$

On the other hand, the k of an acidic solute at a certain mobile phase pH was given as a weighted average of the retention factors of all neutral and ionic species of the solute [11]. Although it has been reported that when the pH of mobile phase was adjusted to 3 pH units lower than pK_a of the solute [26], the neutral species of an acidic solute played dominant role of the weighted average of k and the contribution of all ionic species to overall retention of the solute could be neglected, the actual pH value of the mobile phase could