

Retention of ionisable compounds on high-performance liquid chromatography

XVI. Estimation of retention with acetonitrile/water mobile phases from aqueous buffer pH and analyte pK_a

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Abstract

In agreement with our previous studies and those of other authors, it is shown that much better fits of retention time as a function of pH are obtained for acid–base analytes when pH is measured in the mobile phase, than when pH is measured in the aqueous buffer when buffers of different nature are used. However, in some instances it may be more practical to measure the pH in the aqueous buffer before addition of the organic modifier. Thus, an open methodology is presented that allows prediction of chromatographic retention of acid–base analytes from the pH measured in the aqueous buffer. The model presented estimates the pH of the buffer and the pK_a of the analyte in a particular acetonitrile/water mobile phase from the pH and pK_a values in water. The retention of the analyte can be easily estimated, at a buffer pH close to the solute pK_a , from these values and from the retentions of the pure acidic and basic forms of the analyte. Since in many instances, the analyte pK_a values in water are not known, the methodology has been also tested by using Internet software, at reach of many chemists, which calculates analyte pK_a values from chemical structure. The approach is successfully tested for some pharmaceutical drugs.

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1. Introduction

Reproducible and successful chromatographic studies of ionisable compounds require a proper pH measurement. As we have extensively discussed in previous works [1–8], there are three ways of measuring the pH for a chromatographic system. Commonly [9], the pH is measured in the aqueous buffer before mixing it with the organic modifier (w pH scale [10]). However, we recommend measuring the pH in the mobile phase after mixing aqueous buffer and organic modifier. The electrode system can be calibrated with buffers of known pH in the same organic–water mixture used as mobile phase [11], s pH scale [10], or with commercial aqueous pH standards in water [12], and thus the pH readings directly provide the s pH values [10] of the mobile phase (i.e. the pH value in the hydroorganic

solvent (s) relative to water (w) as standard state solvent [10]).

The shortcomings of measuring the pH variation in the aqueous buffer are clear: the pH variation when adding methanol or acetonitrile to the aqueous buffer depends on the particular buffered system, on its concentration, and on the fraction of organic solvent in the mixture [11–13]. Buffered solutions prepared from anionic and neutral (uncharged) acids (e.g. HAc/Ac⁻, H₂PO₄⁻/HPO₄²⁻ buffers) increase their pH value when acetonitrile or methanol is added, whereas buffers from cationic acids (e.g. NH₄⁺/NH₃ buffers) show the reverse trend. The pK_a variation of analytes follows similar tendencies. Thus, fits of retention to aqueous pH may show a big disparity when buffers of different nature are used, and the pH of the inflection point does not agree with the pK_a of the analyte [9]. In this paper we demonstrate this effect and evaluate its importance for some common buffers (acetic, phosphoric, citric and ammonium buffers) in acetonitrile/water mobile phases.

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