



# Retention of ionisable compounds on high-performance liquid chromatography XVIII: pH variation in mobile phases containing formic acid, piperazine, tris, boric acid or carbonate as buffering systems and acetonitrile as organic modifier

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## ABSTRACT

In the present work dissociation constants of commonly used buffering species, formic acid, piperazine, tris(hydroxymethyl)–aminomethane, boric acid and carbonate, have been determined for several acetonitrile–water mixtures. From these  $pK_a$  values a previous model has been successfully evaluated to estimate pH values in acetonitrile–aqueous buffer mobile phases from the aqueous pH and concentration of the above mentioned buffers up to 60% of acetonitrile, and aqueous buffer concentrations between 0.005 (0.001 mol L<sup>-1</sup> for formic acid–formate) and 0.1 mol L<sup>-1</sup>. The relationships derived for the presently studied buffers, together with those established for previously considered buffering systems, allow a general prediction of the pH variation of the most commonly used HPLC buffers when the composition of the acetonitrile–water mobile phase changes during the chromatographic process, such as in gradient elution. Thus, they are an interesting tool that can be easily implemented in general retention models to predict retention of acid–base analytes and optimize chromatographic separations.

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## 1. Introduction

pH in mobile phases plays an outstanding role in the chromatographic retention of analytes with acid/base properties, because it affects the ionisation degree of the ionisable analytes. In fact, slight variations in the mobile phase pH when it is close to the  $pK_a$  of the analytes, may cause notable changes in retention times [1–5]. In previous works a model has been developed to estimate the pH variation in acetonitrile– [6,7] and methanol–water [8] buffered mobile phases from the aqueous pH value and concentration of the buffer, and from the organic solvent content of the mixture. The buffering systems studied in these works were acetic, phosphoric and citric acids, and ammonia. In the present work this model has been extended to acetonitrile–water mobile phases containing formic acid, piperazine, tris(hydroxymethyl)–aminomethane, boric acid or carbonate as buffering systems.

Our aim is set up relationships between pH, on one hand, and buffer concentration and solvent composition, on the other hand, for the most common HPLC buffers. The ultimate purpose is to implement these relationships in general models predicting retention of acid–base analytes from mobile phase composition and pH

[9,10] which can be useful to optimize chromatographic separations. We think that our equations can be especially useful when mobile phase composition, and thus buffer pH changes during the elution process, such as in gradient elution.

## 2. Experimental

### 2.1. Apparatus

Potentiometric measurements were taken with a Crison (Barcelona, Spain) 5014 combination electrode (glass electrode and a reference electrode with a 3.0 mol L<sup>-1</sup> KCl solution in water as salt bridge) in a Crison GLP22 pH meter with a precision of  $\pm 0.1$  mV ( $\pm 0.002$  pH unit). All solutions were thermostated externally at  $25 \pm 0.1$  °C. Titrations were carried out using an autoburette Metrohm (Herisau, Switzerland) 665 Dosimat.

### 2.2. Chemicals

Acetonitrile was RP HPLC gradient grade from Merck (Darmstadt, Germany) and water purified by the Milli-Q plus system (to 18 M $\Omega$ ) from Millipore (Bedford, MA, USA). The studied buffers were prepared from tris(hydroxymethyl)aminomethane (Aldrich, Steinheim, Germany; 99.9+%), piperazine anhydrous (Fluka, The Netherlands;  $\geq 99\%$ ), sodium carbonate (Merck, for analysis), formic

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