

*Title:* **Characterization of HILIC systems: effect of the organic solvent on the mobile phase pH**

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*Date:* July 2020

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Hydrophilic Interaction Liquid Chromatography (HILIC) is a particularly useful chromatographic method in the determination of polar analytes from biological samples and in the analysis of drugs in cases where their retention and solubility are insufficient in reverse phase mode and normal phase, respectively. The mobile phases consist of buffered hydro-organic mixtures with a high content of organic solvent (usually acetonitrile) and use polar stationary phases such as silica, zwitterions, short chain alkanes with amino, cyano and other groups. In HILIC, the main retention mechanism is based on the partitioning of solutes between the mobile phase and an aqueous layer absorbed in the hydrophilic stationary phase.

In this work, it is proposed to determine the acidity constant ( $pK_a$ ) of formic and acetic acids suitable for HILIC by means of acid-base potentiometric titrations at concentrations between 70-90% acetonitrile. The determined  $pK_a$  values will allow to calculate the pH of any mobile phase from the initial pH and the concentration of the aqueous buffer, and thus, to deduce the corresponding model for the variation of the pH. For neutral acid buffers such as acetic acid or formic acid, the addition of the organic solvent results in a shift of the maximum buffering capacity to higher  $s_w$ pH values. Changes in the buffer capacity of the acetic/acetate and formic/formate buffered mixtures are observed.

**Keywords:** Hydrophilic interaction liquid chromatography, acetonitrile,  $pK_a$ , pH, buffered mobile phase, potentiometry, buffer capacity.