NEW METHOD OF SIMULTANEUOS DATERMINATION OF pK_a AND $log\ k_w$ EMPLOYING *pH*-GRADIENT HPLC

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pH gradient reversed-phase HPLC consists of a programmed increase during the chromatographic run of the eluting power of the mobile phase with regard to ionizable analytes. On the analogy of the conventional organic modifier gradient RP HPLC, in the *pH* gradient mode, the eluting strength of the mobile phase increases due to its increasing (with acid analytes) or decreasing (with basic analytes) *pH*, whereas the content of organic modifier is kept constant. A strict theoretical model is proposed to determine pK_a values based on the retention data employing a *pH* gradient RP HPLC run. The pK_a data so obtained are discussed in relation to the concentration of methanol in the mobile phase, the type of stationary phase, and the duration of the gradient.

The approach applied is demonstrated to provide, along with the pK_a data, also the chromatographic lipophilicity parameter, $log\ k_w$. The pK_a values determined by the *pH* gradient method are related to the respective data obtained conventionally in a series of isocratic experiments. A close similarity of the two types of chromatographically determined pK_a data is demonstrated. The HPLC-derived pK_a parameters correlate to the literature pK_a values ($^{w}{}_{w}pK_a$) determined by titrations in water. The chromatographically derived and the reference pK_a values are not identical, however. That is probably due to the effects on the chromatographic pK_a of the specific sites of interactions with analytes on the surfaces of the HPLC stationary phases. Nonetheless, the proposed *pH* gradient HPLC method may supply in a fast and convenient manner comparable acidity parameters for larger series of drug candidates, including those available in only minute amounts, without need of their purification, and also when the compounds are provided as complex mixtures, like those produced by combinatorial chemistry.