SEPARATION OPTIMIZATION OF O-PHTHALALDEHYDE DERIVATIVES OF AMINO ACIDS DURING REVERSED-PHASE LIQUID CHROMATOGRAPHY AT DIFFERENT pH VALUES

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The present study reports the effects of different mobile phase pH values (2.5, 5.05 and 7.0) on reversed-phase HPLC separation of eighteen o-phthalaldehyde (OPA) derivatives of amino acids. A limited number of four gradient experiments at each mobile-phase pH value are arranged and the retention behavior of each solute is modeled by an adequate fitting algorithm using the following equation for dependence of the solute retention factor, k, on the concentration of the organic modifier, φ, in the mobile phase [1]:

\[ \ln k = a - cφ / (1+bφ) \]

This retention model is first validated against experimental gradient elutions at each pH value and then it is used in an optimization algorithm for the search of an optimum separation at pHs 2.5, 5.05 and 7.0. The algorithms used in this study, for fitting and optimization, are those proposed elsewhere [2] and they are a repeated application of the Levenberg-Marquardt method for fitting (R_LM) and a modified descent algorithm for optimization (RND_D).

The effect of mobile phase pH on detection of OPA derivatives of amino acids by using on-line fluorescence and electrochemical detection is also examined.