

Accepted Manuscript

Title: Retention prediction and separation optimization of ionizable analytes in reversed-phase liquid chromatography by organic modifier gradients in different eluent pHs

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PII: S0021-9673(13)01029-7
DOI: <http://dx.doi.org/doi:10.1016/j.chroma.2013.07.009>
Reference: CHROMA 354489

To appear in: *Journal of Chromatography A*

Received date: 4-4-2013
Revised date: 2-7-2013
Accepted date: 3-7-2013

Please cite this article as: S. Fasoula, Ch. Zisi, P. Nikitas, A. Pappa-Louisi, Retention prediction and separation optimization of ionizable analytes in reversed-phase liquid chromatography by organic modifier gradients in different eluent pHs, *Journal of Chromatography A* (2013), <http://dx.doi.org/10.1016/j.chroma.2013.07.009>

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Retention prediction and separation optimization of ionizable analytes in reversed-phase liquid chromatography by organic modifier gradients in different eluent pHs

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Abstract

The influence of eluent pH on retention of ionizable analytes during organic modifier gradients in reversed-phase high performance liquid chromatography is studied. Two approaches are examined for the retention prediction of solutes under organic modifier gradient conditions at any constant mobile phase pH: In the first approach an analytical solution of the fundamental equation of gradient elution in linear organic modifier gradients for monoprotic acids/bases under certain assumptions is proposed. The second approach is based on an empirical model arising from the evaluation of the gradient retention data. Both approaches were successfully applied to describe the retention behavior of 16 OPA derivatives of amino acids obtained in 19 simple mono-linear organic modifier gradient runs performed between two given acetonitrile contents with different gradient duration and at different eluent pHs, in a particular pH range where amino acids behave as weak monoprotic acids. Further, this study provides a reliable way for optimizing gradient separations of ionogenic compounds with respect to mobile phase pH.

Keywords: Reversed-phase liquid chromatography; retention prediction; organic modifier gradients in different eluent pHs; amino acid derivatives separation optimization.

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