Title: Optimization of reversed-phase chromatography methods for peptide analytics

Author: Rushd Khalaf Daniel Baur David Pfister

PII: S0021-9673(15)01670-2
DOI: http://dx.doi.org/doi:10.1016/j.chroma.2015.11.042
Reference: CHROMA 357056

To appear in: *Journal of Chromatography A*

Received date: 9-9-2015
Revised date: 9-11-2015
Accepted date: 11-11-2015

Please cite this article as: R. Khalaf, D. Baur, D. Pfister, Optimization of reversed-phase chromatography methods for peptide analytics, *Journal of Chromatography A* (2015), http://dx.doi.org/10.1016/j.chroma.2015.11.042

Optimization of reversed-phase chromatography methods for peptide analytics

Rushd Khalaf[a], *Daniel Baur*[a], *David Pfister*[a]
a: Institute for Chemical and Bioengineering, ETH Zurich, 8093 Zurich, Switzerland
Corresponding author: R. Khalaf (rushd.khalaf@chem.ethz.ch; phone: +41 44 633 45 20; fax: +41 44 632 10 82)

- Model-based tool developed to assist in development of RP-HPLC analytical methods
- Model derived from Van't Hoff equation and linear solvent strength correlation
- Quick and robust optimization of selectivity and time of analytical methods
- Easy to use tool for the creation of RP-HPLC analytics

Abstract

The analytical description and quantification of peptide solutions is an essential part in the quality control of peptide production processes and in peptide mapping techniques. Traditionally, an important tool is analytical reversed phase liquid chromatography. In this work, we develop a model-based tool to find optimal analytical conditions in a clear, efficient and robust manner. The model, based on the Van't Hoff equation, the linear solvent strength correlation, and an analytical solution of the mass balance on a chromatographic column describing peptide retention in gradient conditions is used to optimize the analytical scale separation between components in a peptide mixture. The proposed tool is then applied in the design of analytical reversed phase liquid chromatography methods of five different peptide mixtures.

Keywords: Reversed Phase; Van't Hoff Equation; Mathematical Model; Model-Based Design; Peptide Analytics; Peptide Mapping.

1. Introduction

Peptides have been gaining more and more importance as pharmaceutical agents. This means that the quantification and analytical characterization of crude and pure peptide solutions has become an essential part of the development of production processes. Furthermore, the primary structure of larger pharmaceutical proteins (such as monoclonal antibodies and fusion proteins) is often tested via peptide mapping analysis. In both cases, reversed phase liquid chromatography (RP-LC), often combined with mass spectrum analysis, provides an essential analytical tool In this work, a model-based approach describing peptide retention is developed and used for the optimization of an analytical RP-LC step. To our knowledge, these steps are today developed using a trial and error methodology. These are based mostly on the expertise of the chromatographer, and thus lack robustness. Many parameters can affect the quality of an analytical RP-LC method: the stationary phase material, the temperature, the flowrate, the