Accepted Manuscript

Title: Investigation of robustness for supercritical fluid chromatography separation of peptides: Isocratic vs gradient mode

Authors: Martin Enmark, Emelie Glenne, Marek Leśko, Annika Langborg Weinmann, Tomas Leek, Krzysztof Kaczmarski, Magnus Klarqvist, Jörgen Samuelsson, Torgny Fornstedt

PII: S0021-9673(18)30871-9
DOI: https://doi.org/10.1016/j.chroma.2018.07.029
Reference: CHROMA 359541

To appear in: *Journal of Chromatography A*

Received date: 27-4-2018
Revised date: 1-7-2018
Accepted date: 5-7-2018

Please cite this article as: Enmark M, Glenne E, Leśko M, Weinmann AL, Leek T, Kaczmarski K, Klarqvist M, Samuelsson J, Fornstedt T, Investigation of robustness for supercritical fluid chromatography separation of peptides: Isocratic vs gradient mode, *Journal of Chromatography A* (2018), https://doi.org/10.1016/j.chroma.2018.07.029

Investigation of robustness for supercritical fluid chromatography separation of peptides: Isocratic vs gradient mode

Martin Enmark [a,b], Emelie Glenne [a], Marek Leśko [c], Annika Langborg Weinmann [d], Tomas Leek [e], Krzysztof Kaczmarski [c], Magnus Klarqvist [d], Jörgen Samuelsson [a*], Torgny Fornstedt [a,*]

[a] *Department of Engineering and Chemical Sciences, Karlstad University, SE-651 88 Karlstad, Sweden*
[b] *Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, Biomedical Centre, Box 574, SE-75123 Uppsala, Sweden*
[c] *Department of Chemical and Process Engineering, Rzeszów University of Technology, PL-359 59 Rzeszów, Poland*
[d] *Early Product Development, Pharmaceutical Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden*
[e] *Medicinal Chemistry, Respiratory, Inflammation and Autoimmunity, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden*

* Corresponding authors:
Phone: +46 54700 1620; Fax: +46 73 271 2890; E-mail: Jorgen.Samuelsson@kau.se
Phone: +46 54 700 1960; Fax: +46 73 271 2890; E-mail: Torgny.Fornstedt@kau.se

Highlights

- Gramicidin separation robustness was quantified in isocratic vs. gradient elution
- Density and volumetric flow rate were determined for the CO_2-MeOH-H_2O eluent
- Design of experiments were used to determine separation system robustness
- Gradient elution is about three times more robust than is isocratic elution
- Method transfer between laboratories only succeeded for the gradient elution

Abstract

We investigated and compared the robustness of supercritical fluid chromatography (SFC) separations of the peptide gramicidin, using either isocratic or gradient elution. This was done using design of experiments in a design space of co-solvent fraction, water mass fraction in co-solvent, pressure, and temperature. The density of the eluent (CO_2-MeOH-H_2O) was experimentally determined using a Coriolis mass flow meter to calculate the volumetric flow rate required by the design. For both retention models, the most important factor was the total co-solvent fraction and water mass fraction in co-solvent. Comparing the elution modes, we found that gradient elution was more than three times more robust than isocratic elution. We also observed a relationship between the sensitivity to changes and the gradient steepness and used this to draw general conclusions beyond the studied experimental system.

To test the robustness in a practical context, both the isocratic and gradient separations were transferred to another laboratory. The gradient elution was highly reproducible between laboratories, whereas the isocratic system was not. Using measurements of the actual operational conditions (not the set system conditions), the isocratic deviation was quantitatively explained using the retention model. The findings indicate the benefits of using gradient elution in SFC as well as the importance of measuring the actual