Identification and quantification of falsified peptide drugs via HILIC-DAD-MS

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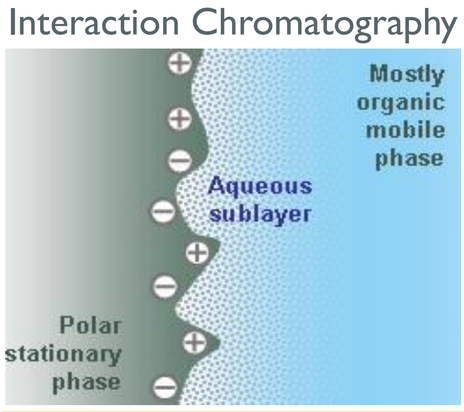
Steven Janvier[1,2], Evelien De Sutter[2], Evelien Wynendaele[2], Bart De Spiegeleer[2], Celine Vanhee[1] and Eric Deconinck[1]

[1]*Division of Food, Medicines and Consumer Safety, Section Medicines and Healthcare Products, Scientific Institute of Public Health (IPH), J.Wytsmanstraat 14, B-1050 Brussels, Belgium*
[2]*Laboratory for Drug Quality & Registration (DruQuar), Faculty of Pharmaceutical Sciences, Universtiy of Ghent, Ottergemsesteenweg 460, B-9000 Ghent, Belgium*

Introduction

Biopharmaceuticals have established themselves as highly efficient medicines, and are still one of the fastest growing parts of the health-product industry. Unfortunately, the introduction of these promising new drugs went hand in hand with the creation of a black market for falsified biotechnology drugs. Particularly popular are the lyophilised peptides with a molecular weight of less than 5 kDa. Multiple systems based on reversed-phase LC have been developed to tackle these grievous practices. The emerging of more polar peptides however requires the introduction of other separation techniques such as Hydrophilic Liquid Interaction Chromatography (HILIC) .



HILIC chromatography allows for the analysis of polar (or ionic) compounds based on a bifasic (aqueous/organic) system and is compatible with mass spectrometric detection (no ion pairing agents necessary). Therefore, we set out to develop and validate an analytical method based on HILIC to identify and quantify the most frequently encountered illegal peptides on the European market. **For this objective, five HILIC columns with different types of stationary phases were tested on their chromatographic performance in terms of resolution and peak symmetry. The most suitable system was subsequently optimised and validated for the detection and quantification of these illegal preparations.**

1.Testing of HILIC conditions

Set-up

Ten peptides (doping peptides, hormones, preclinical drugs)

Five HILIC columns:

Acquity BEH HILIC	(100x2.1mm,1.7 µm)
Acquity BEH HILIC amide	(100x2.1mm,1.7 µm)
Acquity Cortecs HILIC	(100x2.1mm,1.6 µm)
Merck ZIC HILIC	(100x2.1mm, 3.5 µm)
Merck ZIC-c HILIC	(100x2.1mm, 3.0 µm)

Gradient: 80% to 40% Acetonitrile
Gradient time: 15 min
Linear increase: 4% ACN/ min
Additive: 10 mM ammonium formate pH 3.0
Sample solvent: Initial conditions (10 mM ammonium formate 80% ACN)

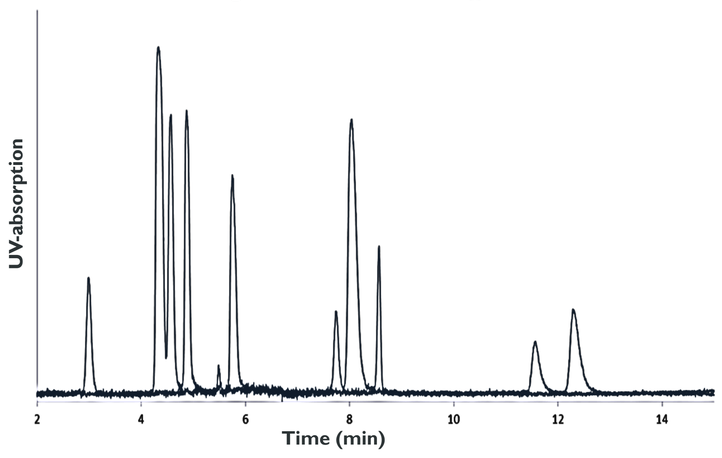
Variables

Flow: 0.2, 0.3, 0.4, 0.5 mL/min
Temperature: 30, 40, 50 ° C

Response variables

Resolution
Peak symmetry

Best chromatogram: ZIC HILIC @ 0.2 mL/min, 50° C



2.Optimization

Full chromatographic separation

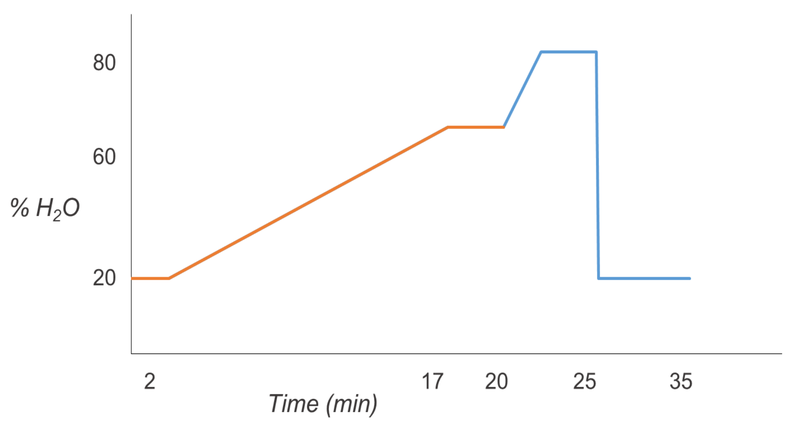
Separation of critical pare @
Flow: 0.27 ml/min
Temperature: 45° C
Gradient time: 20 min (red line)
Linear increase gradient: 3% ACN/min

Matrix effect

Matrix constituents falsified products :
150 mM NaCl, 10 mM Na_2PO_4, 1% PEG, 5% mannitol
Recoveries at high concentrations influenced by mannitol
=> Solved by changing sample solvent to:
10 mM ammonium formate 80% ACN + 2% formic acid

Enhanced stability due to

1) Introduction of online cleaning step (blue line) to wash away remaining matrix constituents of falsified preparations



2) Washing column at low flow rate with 25 mM ammonium formate 80% H_2O after 100 injections

3.Validation

Identification

UHPLC-MS²: Dionex UltiMate 3000 (Thermo Scientific, USA) hyphenated to an AmaZon™ speed ETD MS (Bruker, Germany)

MS-settings: ESI(+), mass range 300-1200 m/z

Criteria
Selectivity: Matrix comprised of 150 mM NaCl, 10 mM Na_2PO_4, 0.02% PEG, 5% mannitol
Sensitivity: S/N ≥ 3.3
Peptide identification: (Cf. Sport drug testing)
Correct MS mass (±0.3 Da)
Minimum 2 MS² fragments (±1.0 Da)

Acquired screening detection limit = 10 µg/mL

Quantification

Acquity UPLC coupled to a DAD detector (Waters, USA)

Settings: detection wavelength 214 or 277 nm

Validation performed according to ISO 17025 in matrix for 6 peptides as proof of principle

	Obtained result	Criteria
Limit of quantification (max.)	50 or 100 µg/mL	100 µg/mL
Validated range	LOQ to 500 µg/mL	
Relative bias (max %)	+ 7.58	< ± 20%
Repeatability (max RSD%)	7.66	< 15%
Intermediate Precision (max RSD%)	8.16	< 15%
Relative expanded uncertainty (max %)	17.15	< 20%

Conclusion

In this study a ZIC HILIC system was selected in favour of four other columns based on the chromatographic performance with frequently encountered peptide drugs on the European market. The selected HILIC-system was further refined and validated according to the ISO 17025 guideline.
The developed ZIC HILIC system allows for the detection and quantification of a wide spectrum of falsified peptide drugs available on the internet.
Furthermore, the method could also be envisaged for the detection of new emerging polar peptide drugs found in cosmetics.

Based on Janvier et al. 2017
HILIC Figure: http://www.sielc.com/Technology_HILIC.html

Corresponding author: Steven Janvier
Contact: steven.janvier@wiv-isp.be