

Comparison of Reversed Phase and HILIC Columns for Peptide Mapping by LC-MS/MS

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Introduction:

LC-MS/MS is frequently used for the purpose of peptide mapping. Ideally all peptides of a digested protein or protein mixture should be identified by their MS/MS spectrum and retention time. Typically Reversed Phase (RP) columns (C4, C8, and C18 packing material) are used for this application. However, in many cases the resolving power of a single RP column is not sufficient to separate and identify all peptides present in complex mixtures. Therefore alternative columns and/or additional dimensions of separation, e.g. ion exchange chromatography are being applied.

The mixed mode separation in HILIC based on charge and hydrophilicity/hydrophobicity has shown excellent results for small molecules and individual peptides. In this study RP and HILIC (Hydrophilic interaction liquid chromatography) capillary columns have been compared for the purpose of peptide mapping of tryptic digests of proteins and protein mixtures with LC-MS/MS under comparable conditions. Since HILIC eluents typically contain a high percentage of organic solvent hydrophobic peptides are potentially less prone to precipitation during sample handling or on the column than in RP chromatography.

Experimental:

LC-MS system:

Flux Rheos 2000 quaternary pump
Thermo Finnigan LCQ Deca XP, Flux Janeiro-CNS software under Xcalibur 1.4
Flux AFM 5 for control of the capillary column flow

Reagents:

All solvents were HPLC grade from JT Baker, The Netherlands. Formic acid p.a. from Merck, Germany

Tryptic digests:

High MW Proteomic Calibration Kit from Sequant, Sweden

Chromatography:

Solvent A: 0.1 % formic acid in water (v/v)/ solvent B: 0.1 % formic acid in acetonitrile (v/v)

RP column: Grom ODS-4 HE, 3 micro m, 150 x 0.3 mm

RP gradient: 0 min: 100% A, 30 min: 40% A/ 60 % B; @ 5 µl/min, as measured with Flux AFM 5

HILIC column: Sequant HILIC-100, 3.5 micro m, 150 x 0.3 mm

HILIC gradient: 0 min: 10% A/ 90 % B, 30 min: 70% A/ 30 % B; @ 5 µl/min, as measured with Flux AFM 5

Injection/load: 2 µl, 200 fmol of each bovine serum albumin and porcine IgG, in starting solvent composition, 4 injections each

Data treatment:

Database search of ms/ms spectra was performed with Turboquest (under Thermo Finnigan Bioworks 3.1 SRI). Database: Swissprot, no species restriction, trypsin as protease, 2 missed cleavages

Turboquest parameters: Minimum Xc scores for +1/+2/+3 charged peptides were 1.50/2.00/2.50; peptide masses from 350-3500 MW; minimum ion intensity 1000; minimum ion count 15

Values for sequence coverage are the average of 4 runs

GRAVY (grand average of hydropathicity, Kyte J, Doolittle RF) values were used as a measure for the relative hydrophobicity and were calculated using <http://www.expasy.org/tools/protparam.html>. Positive values correspond to more hydrophobic, negative values to more hydrophilic peptides.

Results:

The sequence coverage was markedly higher for the HILIC column:

Bovine serum albumin (ALBU_BOVIN), average of 4 injections:

RP column: 15.9 % **1**

HILIC column: 21.1 % **2**

IgG pig could not be identified in a single reading frame; peptides from different Ig-chains of different organisms (pig, mouse) were identified with high scores. However, a comparison of values for sequence coverage was not possible.

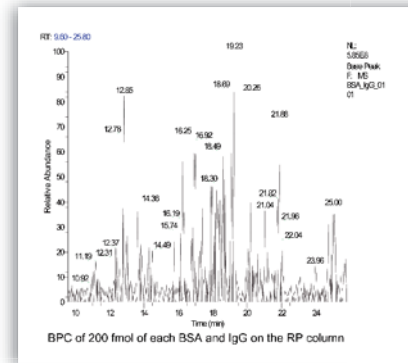
Individual differences | Table 1:

Peptide YAASSYLALSASDWK 3 (LAC_PIG, Ig lambda-chain)	GRAVY value 0.047	ave. Xc score, MH ²⁺ 4.43 (HILIC)
Found in 4/4 runs on the HILIC column Not detected in any RP run		
Peptide AGTTVTQGVETTKPSK 4 (LAC_PIG, Ig lambda-chain)	GRAVY value -0.688	ave. Xc score, MH ²⁺ 4.27 (RP)
Found in 4/4 runs on the RP column Not detected in any HILIC run		
Peptide RHPEYAVSVLLR - (ALBU_BOVIN)	GRAVY value 0.133	ave. Xc score, MH ²⁺ 3.29 (RP) 3.19 (HILIC)
Found in 4/4 runs on the RP column Found in 3/4 runs on the HILIC column		
Peptide HPEYAVSVLLR (ALBU_BOVIN)	GRAVY value 0.264	ave. Xc score, MH ²⁺ 2.25 (HILIC)
Found in 3/4 runs on the HILIC column Not detected in any RP run		

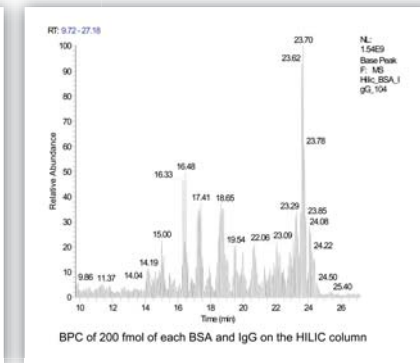
Conclusions:

The results above demonstrate that HILIC columns can be used as an alternative to the usually applied RP columns for the purpose of peptide mapping of tryptic digests. Furthermore it was demonstrated that analysis on both column types can lead to complementary information. HILIC columns are mostly being applied for the analysis of very hydrophilic molecules. However, the examples given here highlight that with the HILIC column some hydrophobic peptides could be identified that could not be detected with the RP column. The setup with a Rheos pump and an AFM 5 flow monitor allowed for an easy control and documentation (log file) of the capillary flow rates.

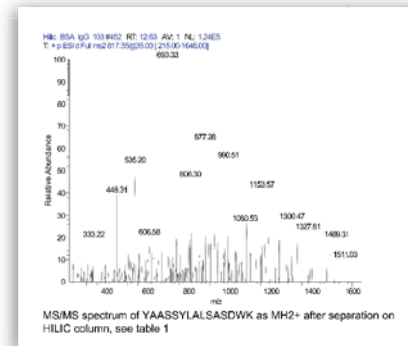
Future work: Additional experiments are underway to evaluate the analysis for very hydrophobic peptides (e.g. GPTLIGANASFSIAL, GRAVY-value 1.107) that cannot be applied to RP columns due to their poor solubility in aqueous media.



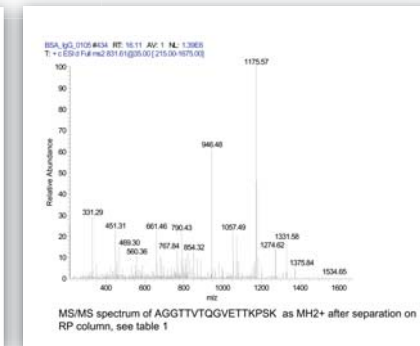
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Acknowledgements:

The author would like to thank Patrik Appelblad, Sequant AB, Umea, Sweden for helpful discussions and for the donation of the HILIC column and calibration kits.

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