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HPLC Application 0006

Evaluation Of Sub 2 µm Media For Packing Capillary Columns For Use In Proteomics

In this independent study from EPFL-Proteomics Core Facility, multiple C18-RP-phases from Dr. Maisch HPLC GmbH, Germany were tested for identifying the sub 2 µm bulk media which shows the best efficiency for analyzing peptides from a HeLa cell lysate standard. The number of MS/MS identified peptides and proteins indicates the separation quality of the packed columns.

ID No.:		**0006**
Analyte:		**Thermo Fisher Scientific HeLa cell lysate** (P/N: 88328)
Column Size:		500 mm x 75 µm
Elution Type:		Isocratic
P/N & Material:		for 0.1 g bulk-media
reference	r119.aq	ReproSil-Pur C18 AQ, 120Å, 1.9µm
1.9 µm:	r119.b9	ReproSil-Pur Basic C18, 100Å, 1.9µm
	r119.9g	ReproSil Gold C18, 120Å, 1.9µm
1.5 µm:	ra115.9e	ReproSil Saphir C18, 100Å, 1.5µm
	5136782	Exsil Mono C18, 100Å, 1.5µm

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Introduction:

Current Gold Standard packing material used at the EPFL-SV PCF Platform is the widely used and very efficient ReproSil-Pur C18 AQ, 120Å, 1.9µm. As a permanent effort to improve analytical workflows, our group regularly performs series of comparative evaluations of different available packing materials. A set of Dr. Masich products were tested to challenge the currently used setting including small particulate products (1.5 µm).

Chromatographic, Mass Spectrometric Setting and Sample:

Columns were in-house packed using a standard bomb loader at 100psi. New Objective 75µm ID (8µm tip aperture) Silica TIps were used and packing length of 50cm was systematically reached for each column.

Samples:

Thermo Fischer Hela cell lysate standard sample was injected in duplicates at different concentrations (300ng and 10ng on columns injections) on PreColumns prior to separation.

The LC-MS2 setting used was the following one:

1. Chromatography: Thermo Fischer Scientific Ultimate 3000 RSLC nano UPLC system at 250nl/min. flow rate, 150min. biphasic gradient.
2. Mass Spetrometer: Thermo Fischer Scientific Orbitrap Lumos instrument (DDA)

Data Treatment:

MaxQuant search engine was used (ver: 1.6.12.0) Basic settings:

- Minimum of 2 peptides par proteins
- 1% FDR threshold (PSMs, Peptides and Proteins)
- Variable modifications: oxidation, acetyl protein N term, phospho (STY)
- Fixed modifications: carbamidomethylation (C)
- Database: Uniprot_Human_74468Sequences_20190611

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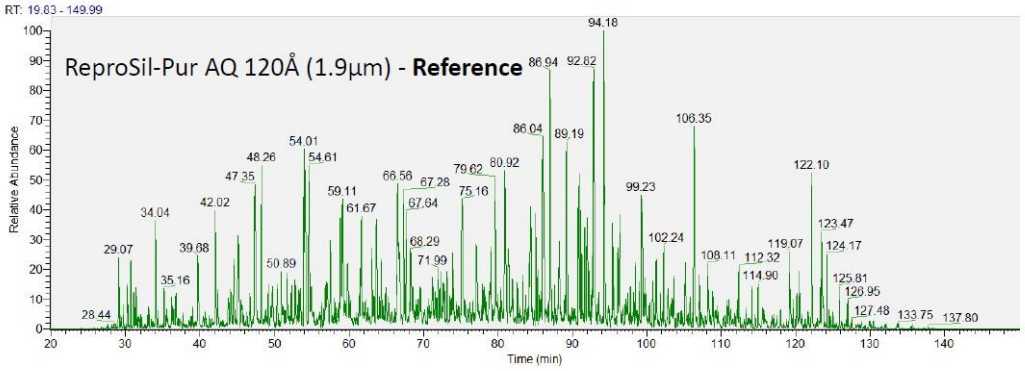
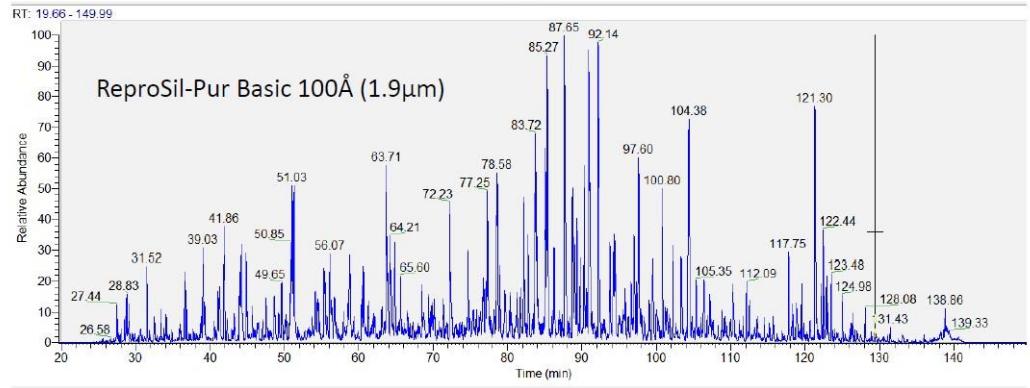
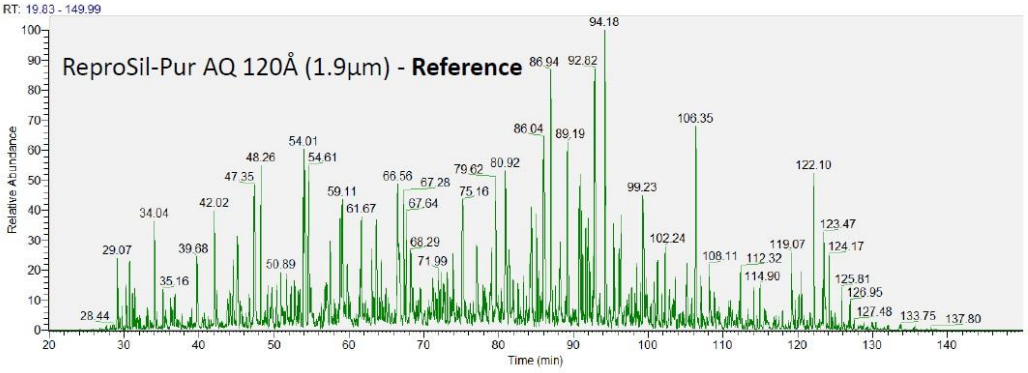
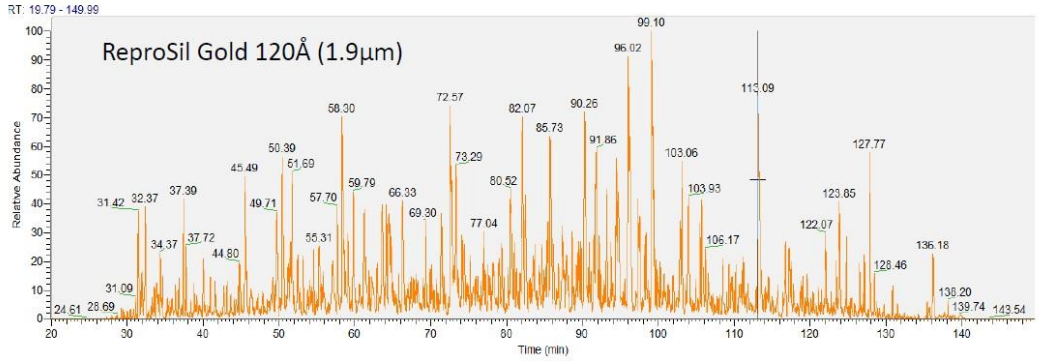
Results:

1) 1.9 µm diameter packing materials (300 ng injections)









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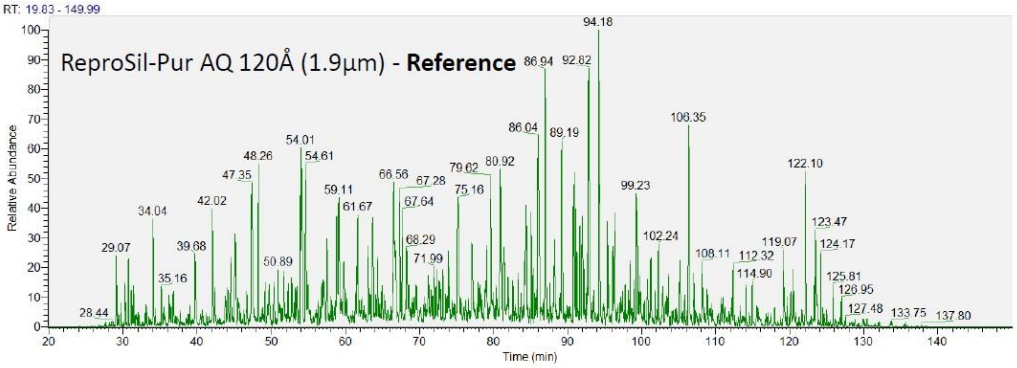
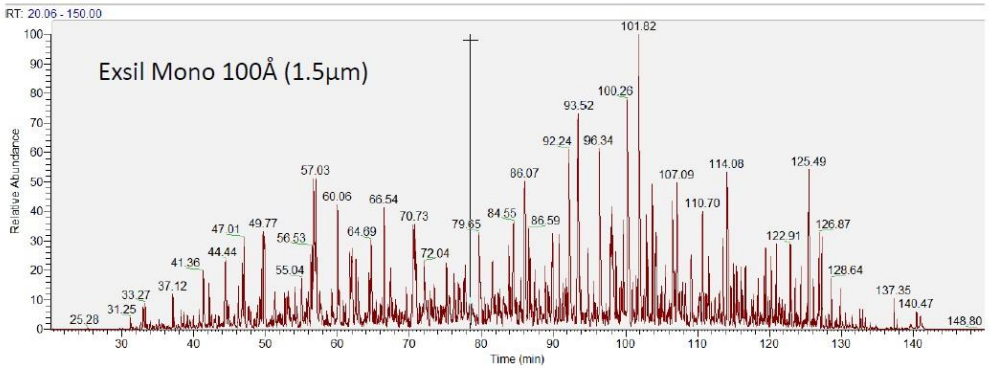
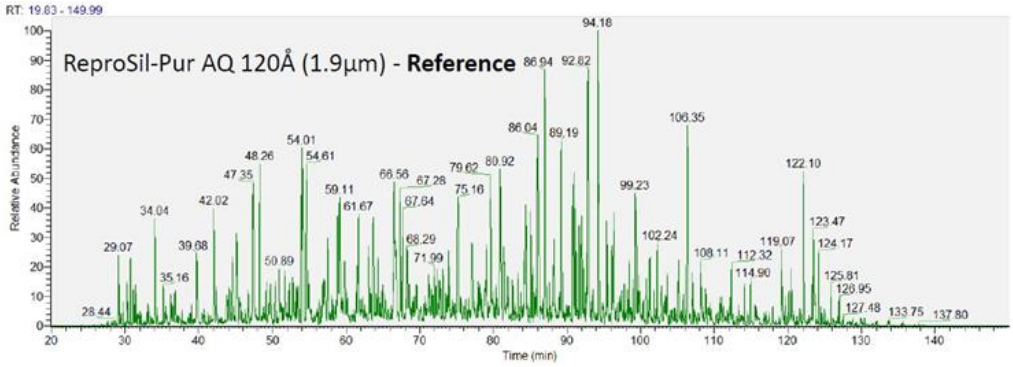
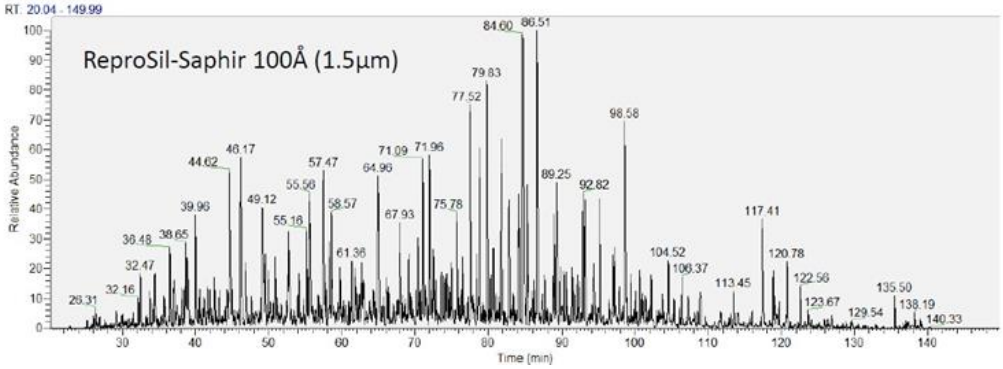
2) 1.5 µm diameter packing materials (300 ng injections)









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Identifications:

A first manual validation process was performed in order to assess runs reproducibility. Validated duplicate run results were then averaged and identified MS/MS Spectra, identified peptides as well as identified Protein Groups were extracted and plotted.

All values were compared to Control column used in the lab (ReproSil-Pur C18 AQ, 120Å, 1.9µm).

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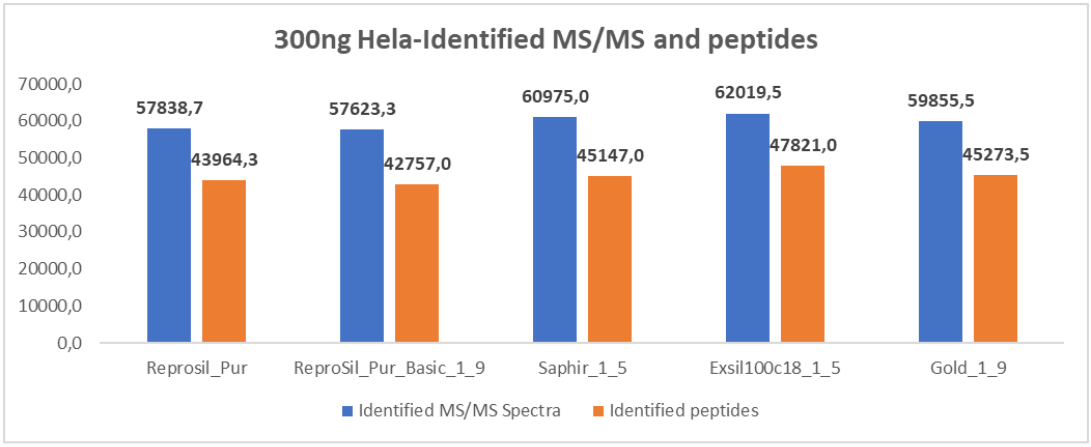
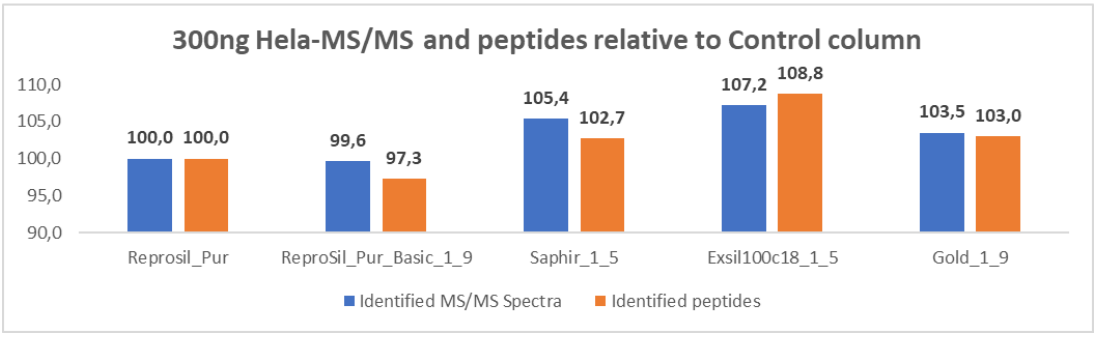
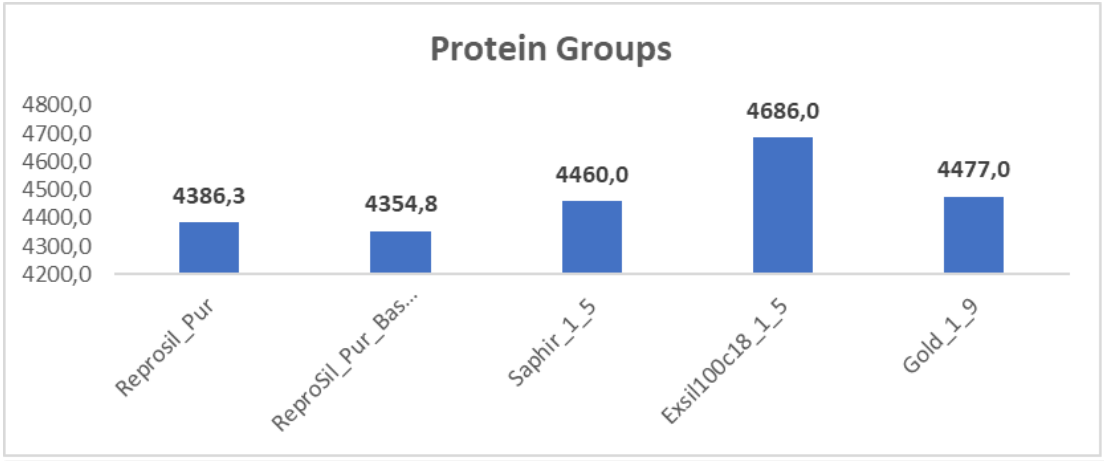
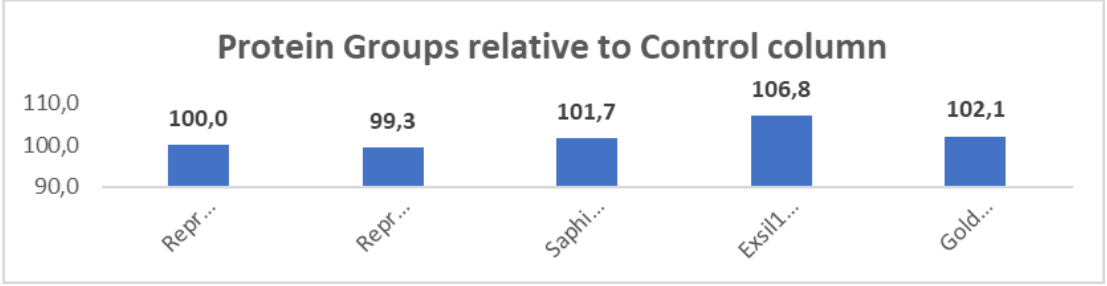
3) Peptides and Spectra identifications (300 ng injections)





4) Protein groups identifications (300 ng injections)





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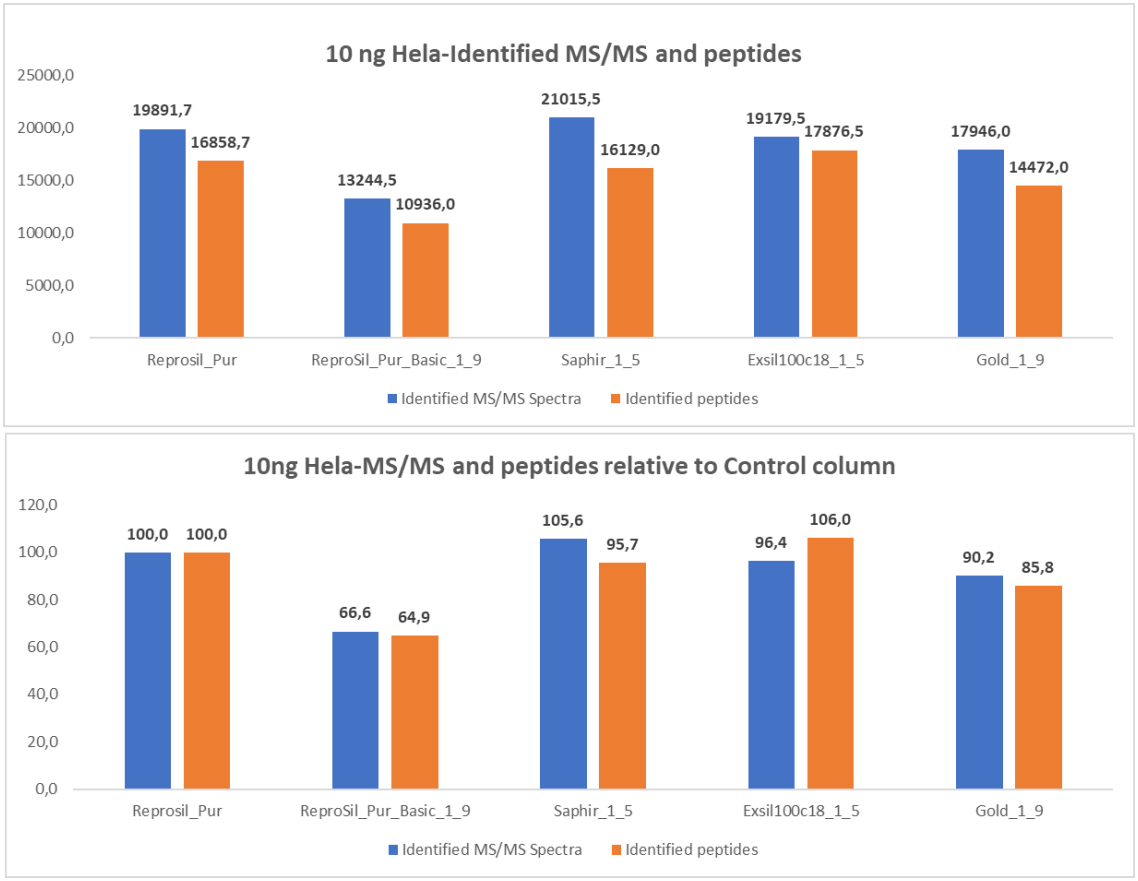
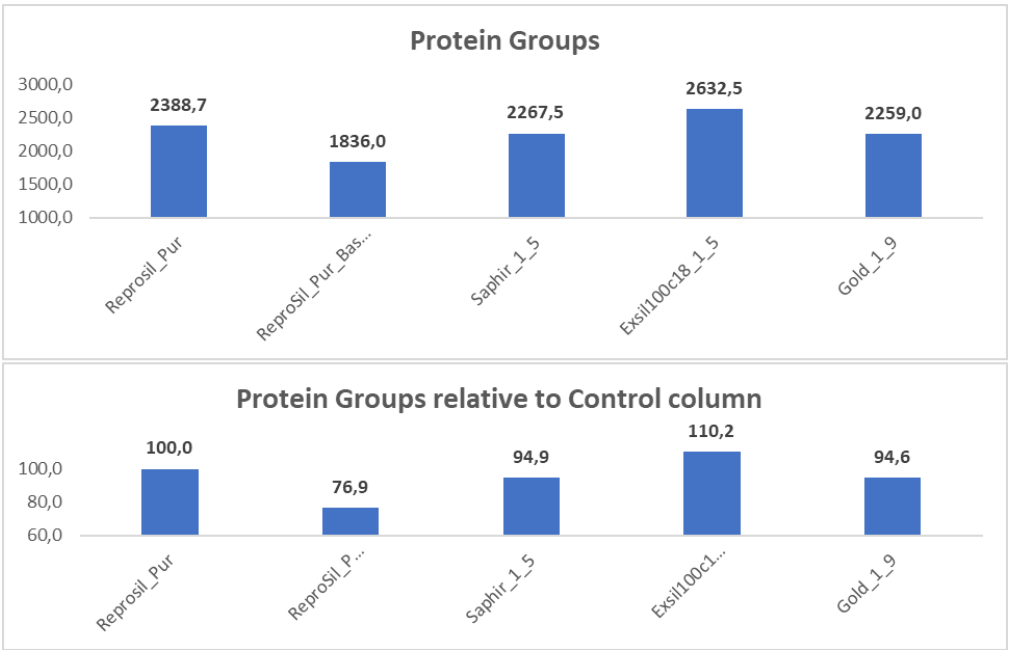
5) Peptides and Spectra identifications (10 ng injections):



6) Protein groups identifications (10 ng injections)



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Conclusion:

It has to be first mentioned that a completely fair comparison requires an entire control of every step of the process to avoid introduction of external variability (sample batch and dilutions, MS performance). We tried to maintain source of variations as low as possible keeping in mind an error tolerance would be considered at the end of the comparison process. Moreover, a single column was packed per chromatographic phase tested and eventual packing process variability has also to be considered. In our hands and over a couple of years of packing experience, this last variability source is rather limited using ReproSil-Pur C18 AQ, 120Å, 1.9µm material (below 7%).

It also has to be pointed out that every chromatographic phase is certainly better adapted to specific applications or separation conditions (mobile solvents, additives, loading amounts flow rates…) and a universal packing material might thus be difficult to highlight. A compromise needs to be found.

The backpressure generated by Exsil Mono C18, 100Å, 1.5µm packed column was higher than other tested materials. Flow rates needed to be reduced at 220nl/min. in order to be safely tested. A set of additional comparisons were performed (data not shown) to evaluate influence of different flow rates on overall results. A good compromise was found using the above mentioned flow rate (220nl/min.).

- As a general trend, all tested materials showed good performance compared to the Control column especially when analyzing higher sample amounts (300ng).
- Small particulate materials (1.5µm) tends to generate better identification results under tested conditions. ReproSil Gold C18, 120Å, 1.9µm was still very performant when injecting 300ng sample amount.
- ReproSil-Pur Basic C18, 100Å, 1.9µm sample is the lowest performing one especially when injecting low sample amounts.
- Despite the backpressure Exsil Mono C18, 100Å, 1.5µm packing material generates, this last sample seems to outperform in comparison to other tested ones and to the Control material.

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These series of comparisons performed under described conditions allowed to highlight Exsil Mono C18, 100Å, 1.5µm packing material as a very competitive alternative option to our gold standard material (ReproSil-Pur C18 AQ, 120Å, 1.9µm). Additional experiments are planned with the aim of better characterize performances of the Exsil Mono C18, 100Å, 1.5µm packing material in terms of range of loading sample amounts and chromatographic separations.

As a final comment, our Proteomics Platform would be very interested in pursuing the collaboration with Dr. Maisch provider. Since the chemistry used to prepare ReproSil-Pur C18 AQ, 120Å, 1.9µm seems to be extremely efficient, we would be very interested in testing an eventual evolution of this phase like its diameter beads reduction (1.5µm) and/or porosity (100Å).

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Application courtesy of Dr. Diego Chiappe, Romain Hamelin EPFL-Proteomics Core Facility, Lausanne.

Dr. Maisch products are available globally. For more information contact your Dr. Maisch representative at info@dr-maisch.com