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# Technical Notification 0011

# Evaluation of Sub-2 µm Media for Packing of Capillary Columns for Use in Proteomics (2)

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.:** **0011**

**Analytes:** **Thermo Fisher Scientific HeLa cell lysate** (P/N: 88328)

**Thermo Fisher Scientific Cytochrom C Digest**

(P/N:161089)

**Column Dimension:** 400 mm x 75 µm

**Elution Type:** Gradient

A: ACN/$H_2O$ 2:98 + 0.1% Formic Acid

B: ACN/$H_2O$ 90:10 + 0.1% Formic Acid

*Long Gradient:*

| Time [min] | Flow Rate [µl/min] | %B |
|---|---|---|
| 0 | 0.25 | 1 |
| 12 | 0.25 | 1 |
| 115 | 0.25 | 24 |
| 125 | 0.25 | 38 |
| 126 | 0.25 | 90 |
| 129 | 0.25 | 90 |
| 130 | 0.25 | 1 |
| 150 | Stop Run | 0 |

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*Short Gradient:*

| Time [min] | Flow Rate [µl/min] | %B |
|---|---|---|
| 0 | 0.25 | 1 |
| 12 | 0.25 | 1 |
| 30 | 0.25 | 35 |
| 35 | 0.25 | 90 |
| 38 | 0.25 | 90 |
| 40 | 0.25 | 1 |
| 55 | 0.25 | 1 |
| 55 | Stop Run | 0 |

**Flow Rate:** 250 nl/min

(due to increased backpressure: 200 nl/min for Exsil Mono)

**Temperature:** 50 °C

(due to increased backpressure: 55 °C for Exsil Mono)

**Materials:** 1 g Bulk Media

Reference: ReproSil-Pur 120 C18-AQ, 1.9 µm
(P/N: r119.aq.0001)

1.5 µm: Exsil Pure 120 RP18, 1.5 µm
(P/N: 5136767.0001)

ReproSil Saphir 100 C18, 1.5 µm
(P/N: ra115.9e.0001)

Exsil Mono 100 C18, 1.5 µm
(P/N: 5136782.0001)

Dr. Maisch
Any Column, Any Size, Any Media

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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 120 C18-AQ, 1.9 µm. 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. Maisch products were tested to challenge the currently used setting including small particulate products (1.5 µm).

## Chromatographic, Mass Spectrometric Settings and Analytes:

Columns were packed in-house using a standard bomb loader at 100 bar. Pulled tips from CoAnn Technologies (75 µm ID, 8 µm tip aperture: ICT36007508F-50) were used, and a packing length of 40 cm was consistently achieved for each column.

*Analytes:*

Thermo Fisher Scientific Hela cell lysate standard sample was injected in duplicates with different loading amounts (300 ng and 10 ng) on a pre-column prior to separation.

*LC-MS Settings:*

1. Chromatography: Thermo Fisher Scientific Vanquish Neo nano UHPLC
2. Mass Spectrometer: Thermo Fisher Scientific Tribrid Ascend

## Data Processing:

MaxQuant search engine was used (ver.: 2.4.4.0). Basic settings:

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

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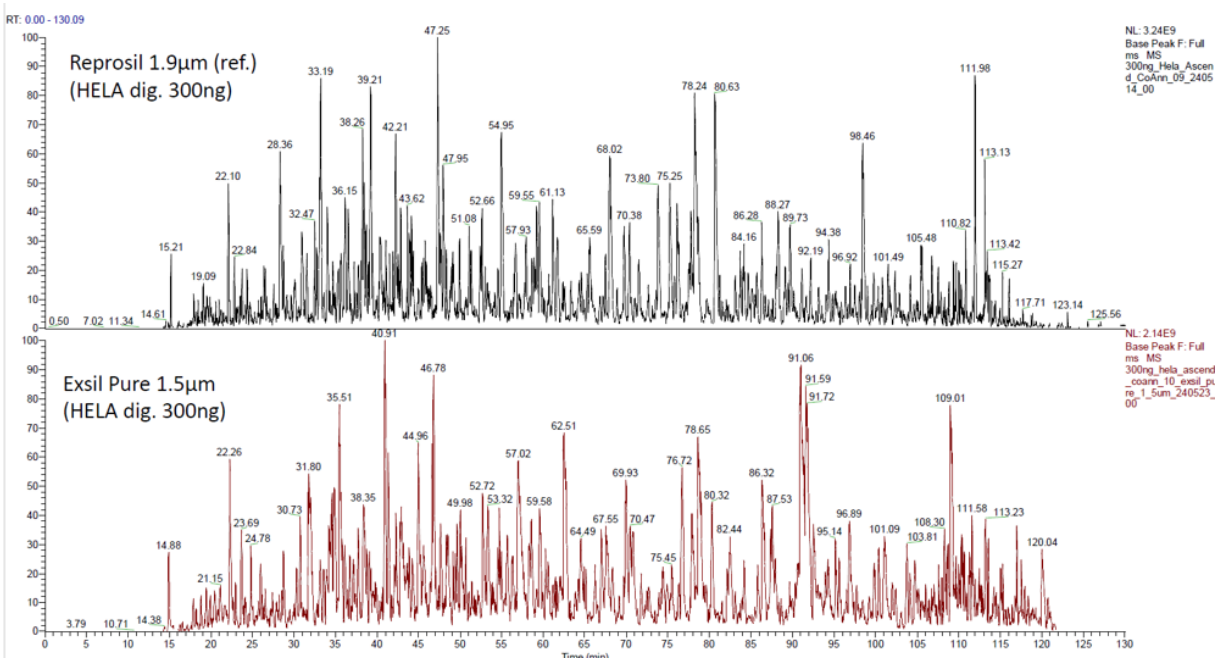
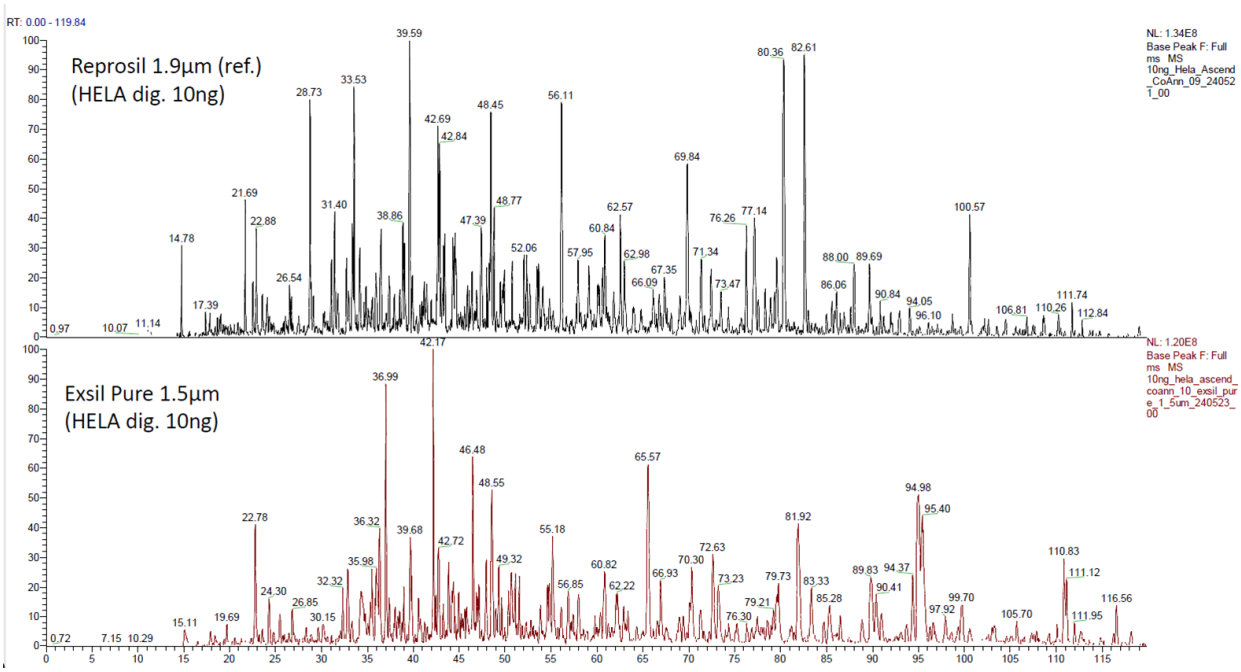
## Results:

### 1a) Exsil Pure 120 RP18, 1.5 µm (300 ng Injection HeLa cell lysate)




### 1b) Exsil Pure 120 RP18, 1.5 µm (10 ng Injection HeLa cell lysate)



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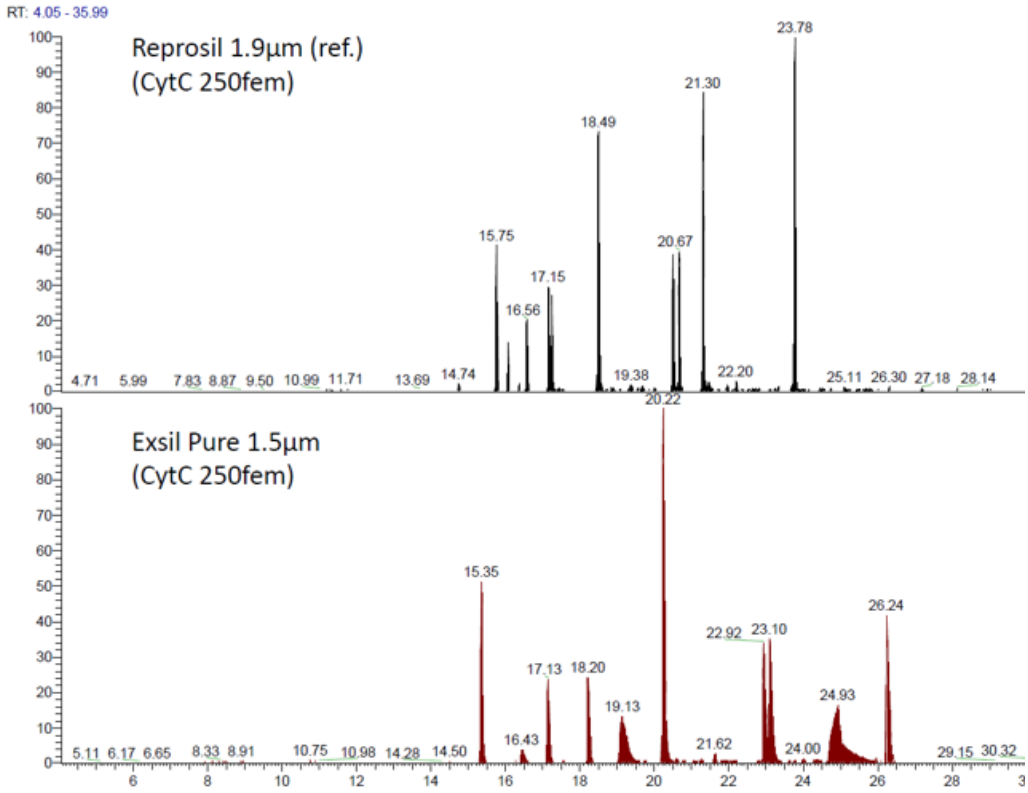
## 1c) Exsil Pure 120 RP18, 1.5 µm (Cytochrom C Digest 250 fem (femtomole))



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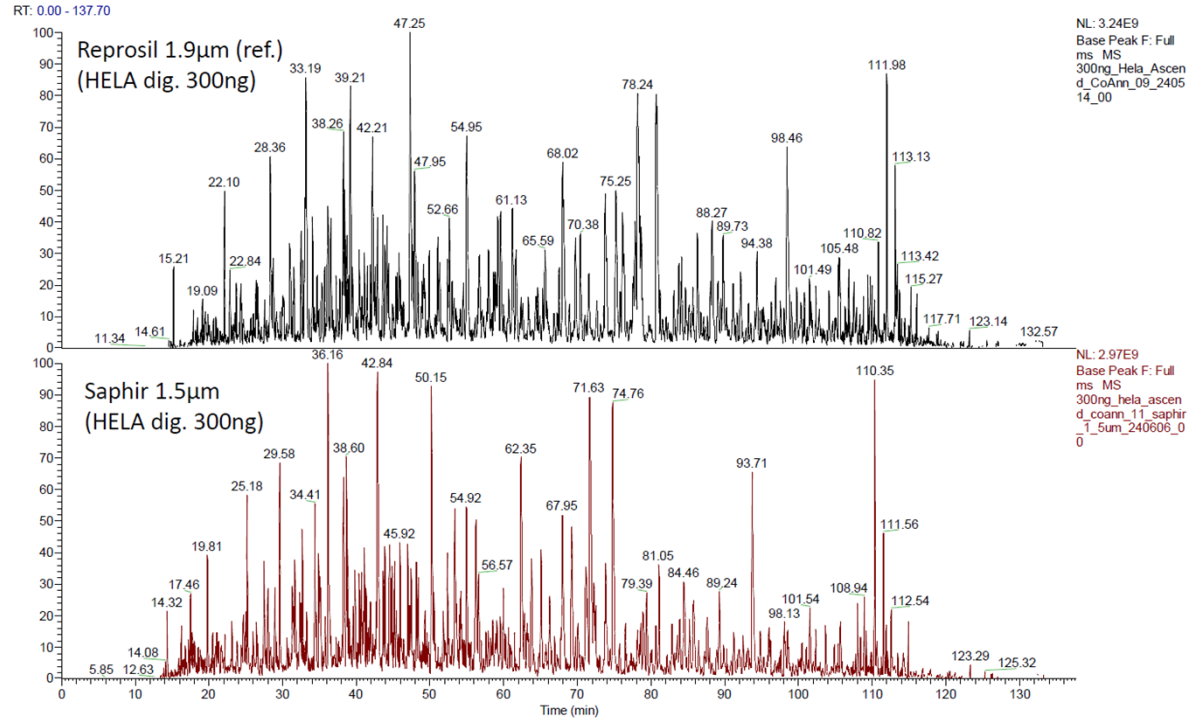
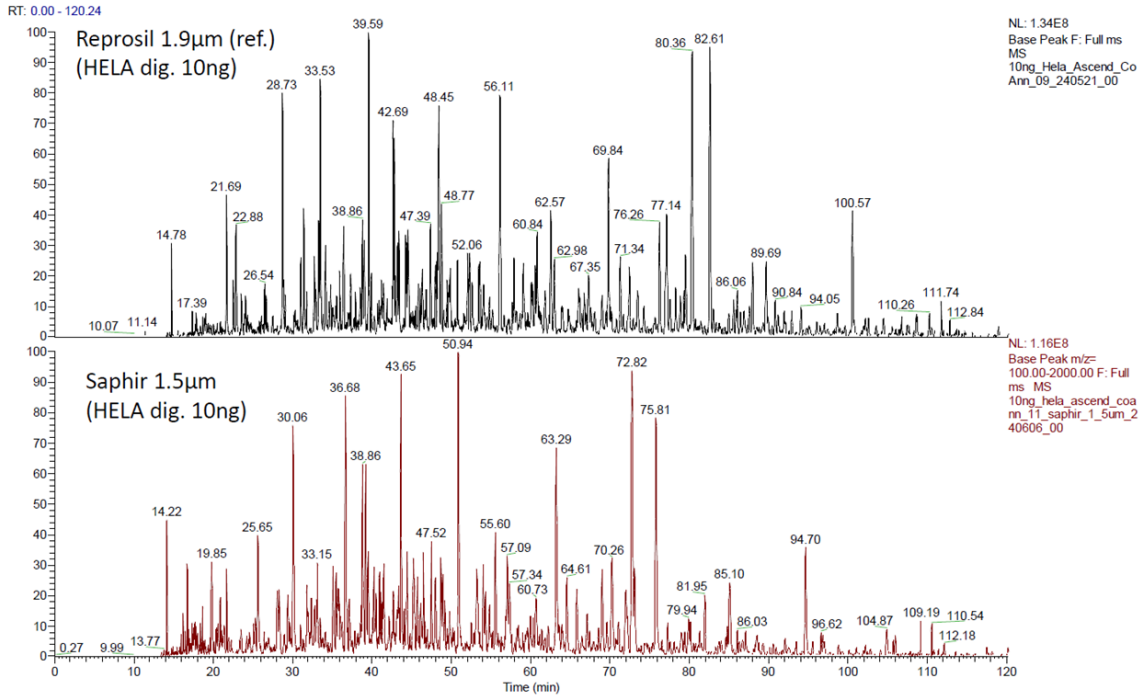
## 2a) ReproSil Saphir 100 C18, 1.5 µm (300 ng Injection HeLa cell lysate)




## 2b) ReproSil Saphir 100 C18, 1.5 µm (10 ng Injection HeLa cell lysate)



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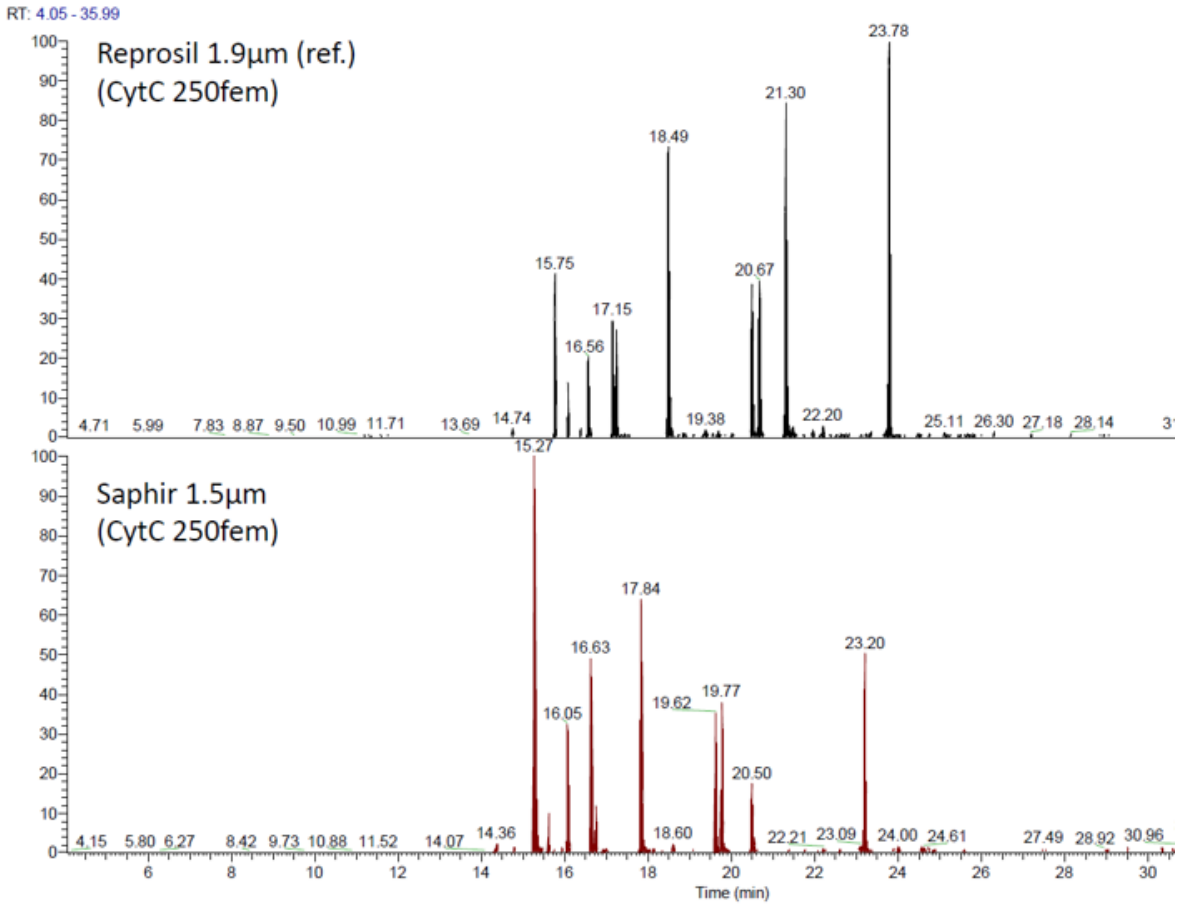
## 2c) ReproSil Saphir 100 C18, 1.5 µm (Cytochrom C Digest 250 fem (femtomole))



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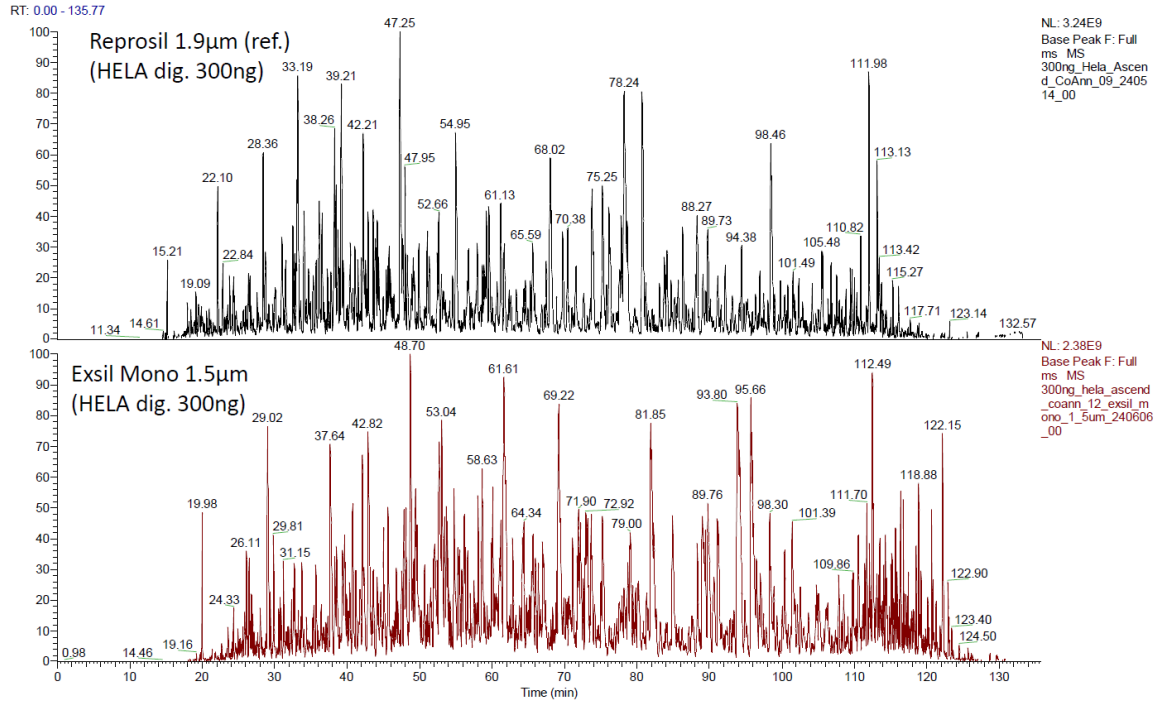
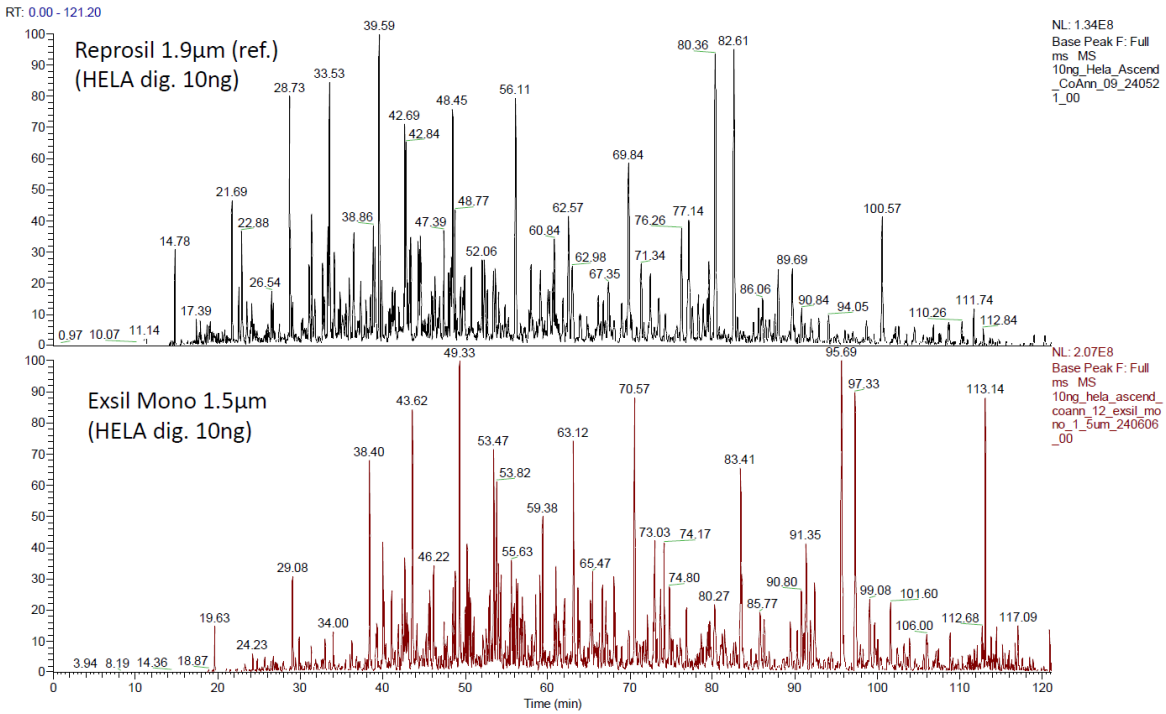
## 3a) Exsil Mono 100 C18, 1.5 µm (300 ng Injection HeLa cell lysate)




## 3b) Exsil Mono 100 C18, 1.5 µm (10 ng Injection HeLa cell lysate)



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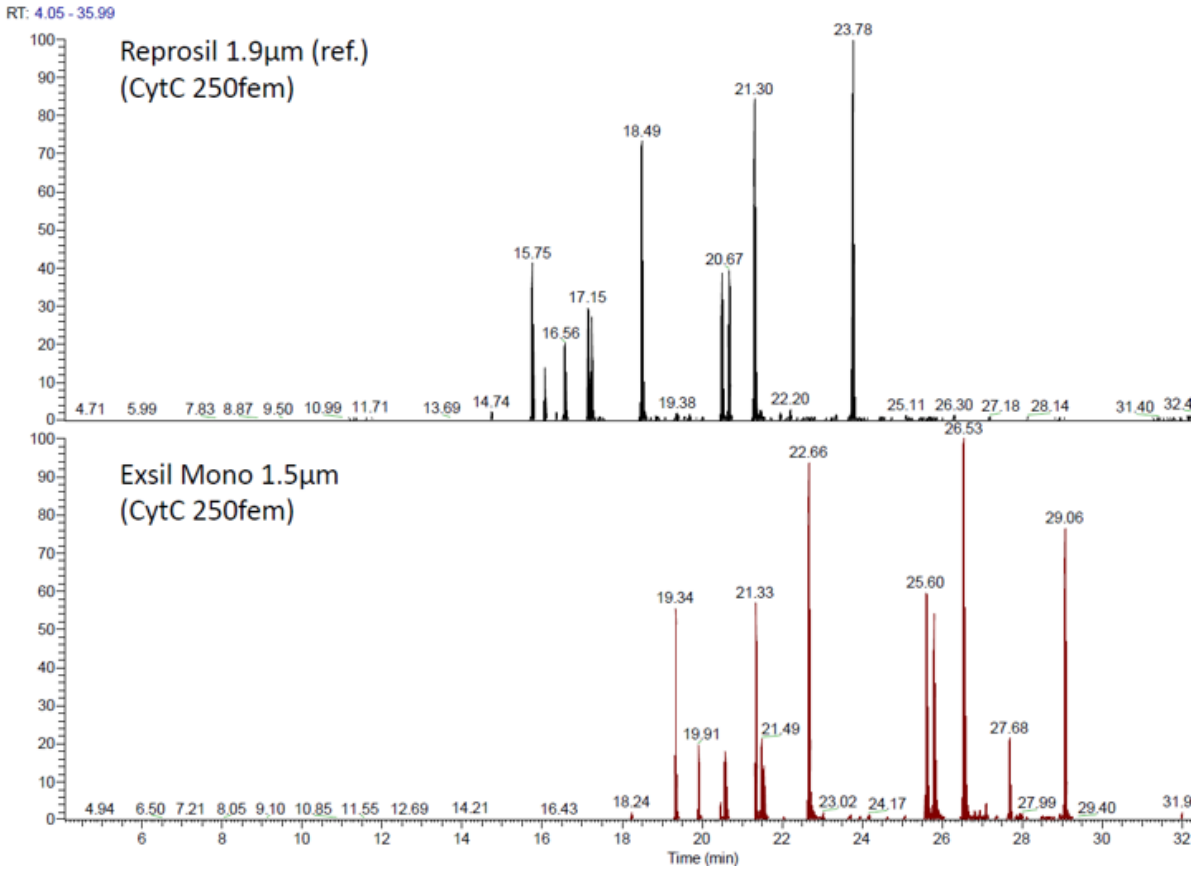
## 3c) Exsil Mono 100 C18, 1.5 µm (Cytochrom C Digest 250 fem (femtomole))



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## Identification Results:

| Raw file | C18 Material | MS | MS/MS | MS/MS submitted | MS/MS identified | MS/MS identified [%] | Peptide sequences identified | Peaks repeatedly sequenced [%] |
|---|---|---|---|---|---|---|---|---|
| 300ng_Hela_Ascend_CoAnn_09_240514_00 | Reprosil Pur AQ 1.9um | 10'455 | 180'704 | 204'108 | 67'947 | 33 | 46'782 | 12 |
| 300ng_Hela_Ascend_CoAnn_09_240514_01 | Reprosil Pur AQ 1.9um | 10'447 | 180'444 | 204'020 | 68'705 | 34 | 46'856 | 12 |
| 300ng_Hela_Ascend_CoAnn_10_Exsil_Pure_1_5um_240523_00 | Exsil Pure 1.5um | 10'657 | 189'476 | 226'000 | 67'128 | 30 | 42'046 | 18 |
| 300ng_Hela_Ascend_CoAnn_10_Exsil_Pure_1_5um_240523_01 | Exsil Pure 1.5um | 10'632 | 188'588 | 223'106 | 66'834 | 30 | 42'334 | 17 |
| 300ng_Hela_Ascend_CoAnn_11_Saphir_1_5um_240606_00 | Saphir 1.5um | 10'423 | 177'288 | 198'863 | 71'311 | 36 | 42'668 | 16 |
| 300ng_Hela_Ascend_CoAnn_11_Saphir_1_5um_240606_01 | Saphir 1.5um | 10'435 | 177'630 | 199'193 | 70'649 | 35 | 43'104 | 16 |
| 300ng_Hela_Ascend_CoAnn_12_Exsil_Mono_1_5um_2406012_00 | Exsil Mono 1.5um | 10'510 | 187'091 | 217'973 | 70'998 | 33 | 49'631 | 11 |
| 300ng_Hela_Ascend_CoAnn_12_Exsil_Mono_1_5um_2406012_01 | Exsil Mono 1.5um | 10'510 | 186'389 | 217'065 | 70'605 | 33 | 49'819 | 11 |

## 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 120 C18-AQ, 1.9 µm material (below 7%).

It's important to recognize that each chromatographic phase is better suited to specific applications or separation conditions (mobile solvents, additives, loading amounts, flow rates, etc.). Therefore, identifying a universal packing material might be challenging, and a compromise may be necessary.

In our hands, Exsil Mono material emerged as one of the best media tested. However, it required a longer packing time and exhibited higher backpressure compared to other evaluated C18 media.

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The typical backpressure for the tested capillary columns ranges from 5000-6500 psi (345-448 bar). Exsil Mono material's backpressure was significantly higher, around 8000 psi. Due to the higher backpressure observed with Exsil Mono, some adaptations were necessary. We used a lower flow rate (200 nl/min instead of 250 nl/min) and applied a higher column heating temperature to ensure safe testing.

As a general trend, all tested materials showed good performance compared to the control column, especially when analyzing higher sample amounts (300 ng).

These comparisons, performed under the described conditions, highlighted Exsil Mono 100 C18, 1.5 µm packing material as a very competitive alternative option to our gold standard material (ReproSil-Pur 120 C18-AQ, 1.9 µm).

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

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