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Technical Notification 0014

Evaluation of pre-packed vs. self-packed Capillary Columns for Use in Proteomics (4)

In this independent study conducted by the Proteomics and Mass Spectrometry Core Facility (PMS CF) at the University of Bern, a commercially available Integrated Emitter Capillaries, packed with ReproSil Saphir 100 C18, 1.5 µm, from Dr. Maisch HPLC GmbH, Germany was evaluated against a home-made self-packed capillary column C20 from PMS CF to identify the sub-2 µm Bulk Media with the highest separation efficiency for peptide analysis.

The number of MS/MS identified peptides and proteins indicates the separation quality of the capillary columns.

To enhance method robustness, trapping cartridges were installed upstream of the analytical capillary columns.

ID No.:	**0014**
Analytes:	**HEK cell lysate** (Internal Standard)
Trapping Cartridges:	Reprosil Saphir 100 C18, 3 µm (P/N: ra15.9e.t000.3) PepMap Neo Trap, 5 µm (P/N: 174500)
Cartridge Dimensions:	5 mm x 0.3 mm

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Capillary Columns: Home-Made (C20) C18, 1.7 µm

(self-packed)

Capillary Columns: ReproSil Saphir 100 C18, 1.5 µm

(Dr. Maisch) (P/N: ra115.9e.pt200.075.0001)

Column Dimensions: 200 mm x 75 µm

Elution Type: Gradient

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

B: ACN/H_2O 95:5 + 0.1% Formic Acid

Gradient 1 (10 min)

Time [min]	Flow Rate [µl/min]	%B
0	0.350	2
10	0.350	40
12	0.350	60
14	0.350	80
18	0.350	80
20	0.350	2
28	0.350	2

Gradient 2 (22 min)

Time [min]	Flow Rate [µl/min]	%B
0	0.350	2
22	0.350	40
24	0.350	60
26	0.350	80
30	0.350	80
31	0.350	2
40	0.350	2

Flow Rate: 0.35 µl/min

Temperature: 50 °C

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Chromatographic, Mass Spectrometric Settings and Analytes

Analytes:

HEK cell lysate standard sample was injected in triplicates with different loading amounts (500 pg, 1 ng and 10 ng).

The LC-MS Settings:

HPLC-System:	Thermo Scientific™ Vanquish™ Neo UHPLC-System
Mass Spectrometer:	Thermo Scientific™ Orbitrap™ Astral™
	Source: NanoFlex

Data Processing:

Spectronaut 19 search engine with strict parameters (PEP and q-values at 0.01) and method evaluation mode was used. Basic settings:

- Minimum of 2 peptides per proteins.
- 1% FDR threshold (PSMs, peptides and proteins).
- Variable modifications: oxidation (M), acetyl protein N term.
- Fixed modifications: carbamidomethylation (C).
- Quantification: Performed without MBR (match between runs).
- Database:

  SwissProt Home Sapiens proteins sequences (with isoforms), version 2025_01 with common contaminants (Contains 42,576 entries)

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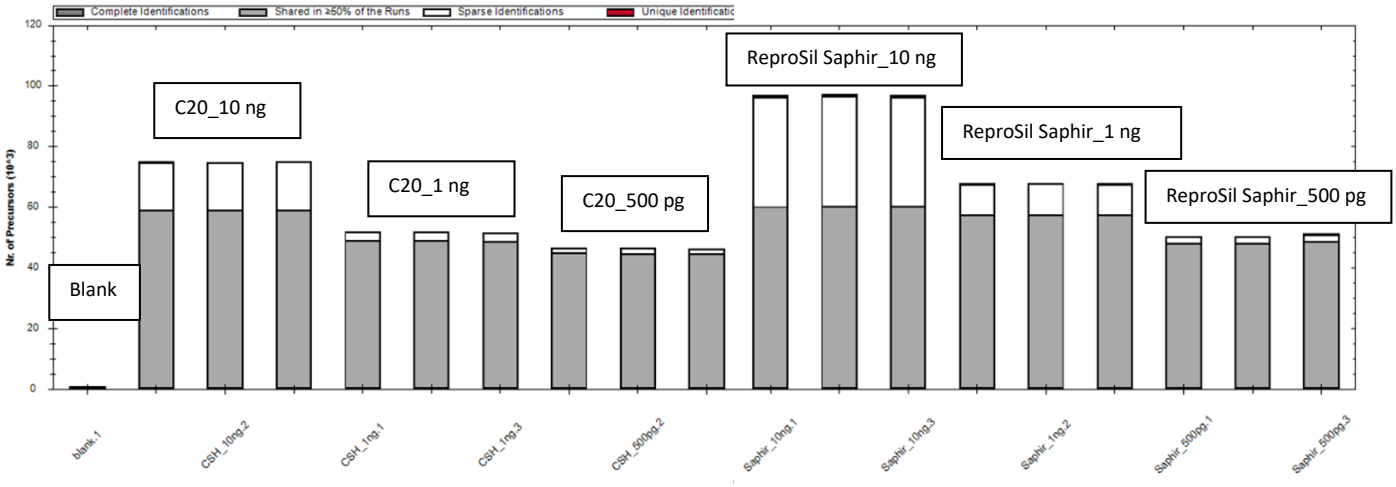
Results:

1) HEK cell lysate (500 pg, 1 and 10 ng Injection)

Identification Results:

RunNr	FileName	Condition	Replicate	Precursors	Modified Seq	Peptides	Protein Groups	%ID
1	20250131_Blank_i02.htrms	blank	1	755	707	689	473	
8	20250131_C20_FAIMS48_HEK_500pg_22min_DIA_i01.htrms	C20_500pg	1	45960	43671	43612	5700	68%
9	20250131_C20_FAIMS48_HEK_500pg_22min_DIA_i02.htrms	C20_500pg	2	45923	43645	43585	5676	68%
10	20250131_C20_FAIMS48_HEK_500pg_22min_DIA_i03.htrms	C20_500pg	3	45789	43490	43431	5645	67%
19	20250218_ReproSaphir_FAIMS48_HEK_500pg_22min_DIA_i01.htrms	Saphir_500pg	1	49684	46742	46562	6010	72%
20	20250218_ReproSaphir_FAIMS48_HEK_500pg_22min_DIA_i02.htrms	Saphir_500pg	2	49927	46977	46797	6056	72%
21	20250218_ReproSaphir_FAIMS48_HEK_500pg_22min_DIA_i03.htrms	Saphir_500pg	3	50554	47520	47339	6044	72%
5	20250131_C20_FAIMS48_HEK_1ng_22min_DIA_i01.htrms	C20_1ng	1	51438	48667	48564	6167	74%
6	20250131_C20_FAIMS48_HEK_1ng_22min_DIA_i02.htrms	C20_1ng	2	51452	48667	48564	6195	74%
7	20250131_C20_FAIMS48_HEK_1ng_22min_DIA_i03.htrms	C20_1ng	3	50936	48203	48101	6159	74%
16	20250218_ReproSaphir_FAIMS48_HEK_1ng_22min_DIA_i01.htrms	Saphir_1ng	1	67170	63029	61873	6872	82%
17	20250218_ReproSaphir_FAIMS48_HEK_1ng_22min_DIA_i02.htrms	Saphir_1ng	2	67242	63093	61974	6901	83%
18	20250218_ReproSaphir_FAIMS48_HEK_1ng_22min_DIA_i03.htrms	Saphir_1ng	3	67095	62985	61878	6863	82%
2	20250131_C20_FAIMS48_HEK_10ng_22min_DIA_i01.htrms	C20_10ng	1	74373	69291	69095	7279	87%
3	20250131_C20_FAIMS48_HEK_10ng_22min_DIA_i02.htrms	C20_10ng	2	74130	69084	68884	7238	87%
4	20250131_C20_FAIMS48_HEK_10ng_22min_DIA_i03.htrms	C20_10ng	3	74473	69401	69202	7223	86%
13	20250218_ReproSaphir_FAIMS48_HEK_10ng_22min_DIA_i01.htrms	Saphir_10ng	1	95954	88438	87989	7987	95%
14	20250218_ReproSaphir_FAIMS48_HEK_10ng_22min_DIA_i02.htrms	Saphir_10ng	2	96421	88786	88333	7992	96%
15	20250218_ReproSaphir_FAIMS48_HEK_10ng_22min_DIA_i03.htrms	Saphir_10ng	3	96047	88478	88026	7969	95%



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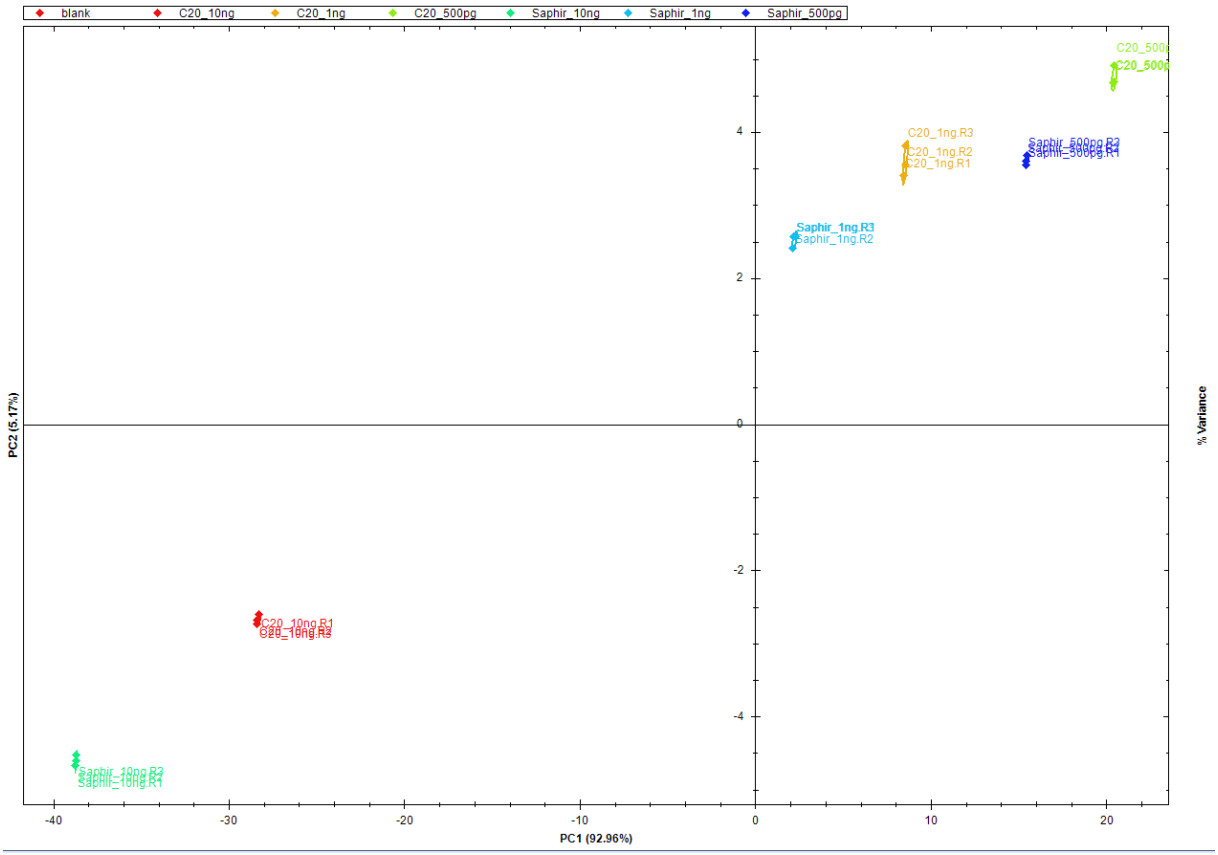

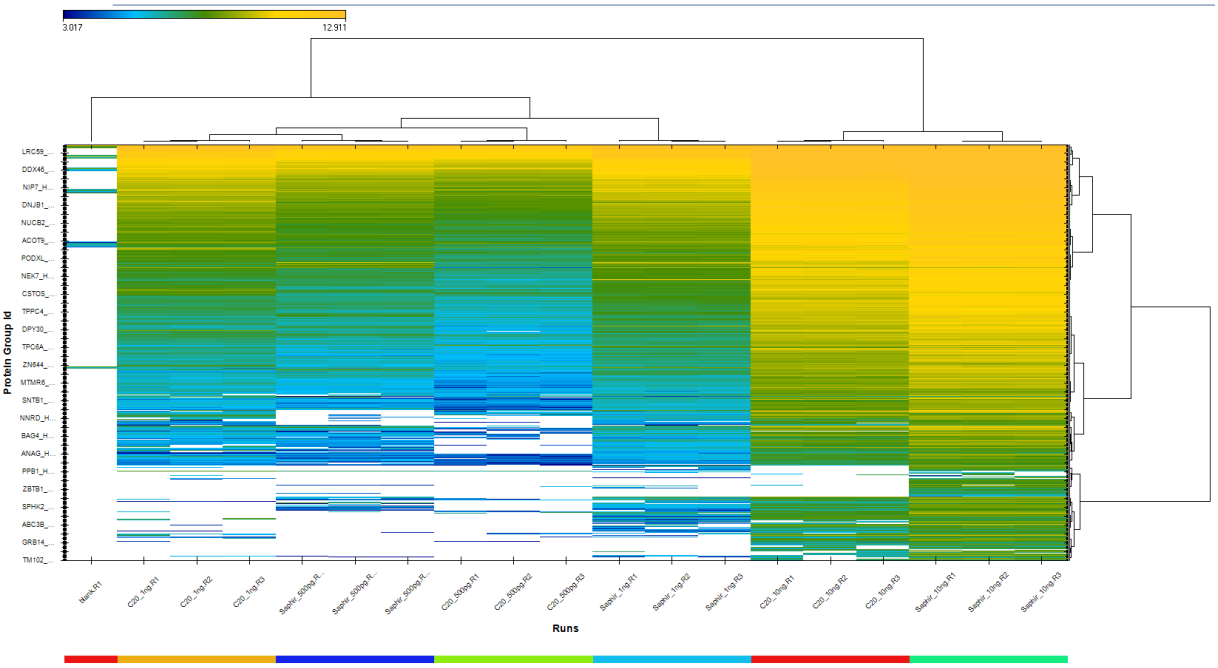

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Peak Capacity	352.4
Peak Capacity - Condition: blank	242.6
Peak Capacity - Condition: C20_10 ng	328.9
Peak Capacity - Condition: C20_1 ng	321.9
Peak Capacity - Condition: C20_500 pg	312
Peak Capacity - Condition: ReproSil Saphir_10 ng	429.4
Peak Capacity - Condition: ReproSil Saphir_1 ng	416
Peak Capacity - Condition: ReproSil Saphir_500 pg	378.7
Median XIC Width	0.5 min
Median XIC Width - Condition: blank	0.3 min
Median XIC Width - Condition: C20_10 ng	0.6 min
Median XIC Width - Condition: C20_1 ng	0.6 min
Median XIC Width - Condition: C20_500 pg	0.6 min
Median XIC Width - Condition: ReproSil Saphir_10 ng	0.4 min
Median XIC Width - Condition: ReproSil Saphir_1 ng	0.4 min
Median XIC Width - Condition: ReproSil Saphir_500 pg	0.5 min
Average Data Points per Peak	4.6
Average Data Points per Peak - Condition: blank	1.8
Average Data Points per Peak - Condition: C20_10 ng	5.8
Average Data Points per Peak - Condition: C20_1 ng	5.3
Average Data Points per Peak - Condition: C20_500 pg	4.7
Average Data Points per Peak - Condition: ReproSil Saphir_10 ng	4.3
Average Data Points per Peak - Condition: ReproSil Saphir_1 ng	4.8
Average Data Points per Peak - Condition: ReproSil Saphir_500 pg	4.8

Conclusion:

- The capillary column packed with ReproSil Saphir 100 C18, 1.5 µm outperforms the home-made self-packed capillary column C20 packed with C18, 1.7 µm in terms of protein identifications when analyzing low sample amounts.
- Peak Shape: The capillary column packed with ReproSil Saphir 100 C18, 1.5 µm showed sharper peaks leading to fewer data points per peak, which may improve resolution but could also pose challenges for accurate peptide ion quantification, especially in label-free quantitation workflows.

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