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Technical Notification 0013

Evaluation of Pre-Packed vs. Self-Packed Capillary Columns for Use in Proteomics (3)

In this independent study conducted by the Proteomics and Mass Spectrometry Core Facility (PMS CF) at the University of Bern, several commercially available Integrated Emitter Capillaries, packed with C18-RP-phases, from Dr. Maisch HPLC GmbH, Germany were 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.:	**0013**
Analytes:	**HEK cell lysate** (Internal Standard)
	Mix of 5 digest Proteins (Quality Control (QC))
Trapping Cartridges:	ReproSil Saphir 100 C18, 3 µm
	(P/N: ra15.9e.t000.3)
	ReproSil-Pur 120 C18-AQ, 3 µm
	(P/N: r13.aq.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-Pur 120 C18-AQ, 1.9 µm

(Dr. Maisch) (P/N: r119.aq.pt200.075.01)

ReproSil Saphir 100 C18, 1.5 µm

(P/N: ra115.9e.pt200.075.01)

Exsil Mono 100 C18, 1.35 µm

(P/N: 6136973.pt200.075.01)

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	0.200 – 0.400	0
3.5	0.200 – 0.400	0
4.5	0.200 – 0.400	5
10.5	0.200 – 0.400	25
13.5	0.200 – 0.400	40
15.5	0.200 – 0.400	60
16.5	0.200 – 0.400	80
23.5	0.200 – 0.400	80
25.5	0.200 – 0.400	0
37	0.200 – 0.400	0

Gradient 2 (30 min)

Time [min]	Flow Rate [µl/min]	%B
0	0.200 – 0.400	0
3.5	0.200 – 0.400	0
4.5	0.200 – 0.400	5
20.5	0.200 – 0.400	25
25	0.200 – 0.400	32.5
33.5	0.200 – 0.400	40
35.5	0.200 – 0.400	60
36.5	0.200 – 0.400	80
43.5	0.200 – 0.400	80
45.5	0.200 – 0.400	0
57	0.200 – 0.400	0

Flow Rate: To achieve a fair chromatographic comparison the flow rate was adapted on each capillary column to have a pressure between 300-400 bar.

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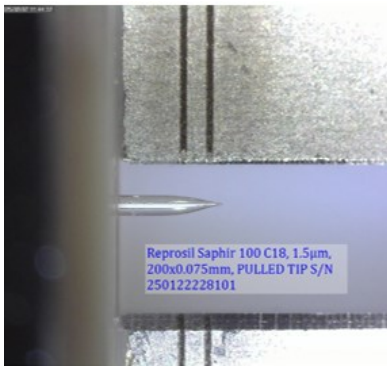
Flow Rate: 200 nl/min
Pressure: 420 bar

Flow Rate: 400 nl/min
Pressure: 282 bar



Flow Rate: 350 nl/min
Pressure: 300 bar

Temperature: 50 °C

Chromatographic, Mass Spectrometric Settings and Analytes

Analytes:

HEK cell lysate standard sample was injected in triplicates with different loading amounts (1 ng, 10 ng and 200 ng).

The LC-MS Settings:

HPLC-System: Thermo Scientific UltiMate 3000

Mass Spectrometer: Bruker timsTOF HT

Source: Captive Spray

Data Processing:

FragPipe search engine was used (ver.: 22.0). 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).

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- Validation: MSBooster rescoring model with Prosit 2023 Intensity timsTOF.
- Database:

  SwissProt Home Sapiens proteins sequences (with isoforms), version 2025_01 with common contaminants (Contains 85,156 entries).

Results:

1) Quality Control (QC): Mix of 5 digest Proteins

Identification Results:

Row Labels	Data Points per Peak (MS1)	Data Points per Peak (MS2)	Average of PeakWidth	Average of Median FWHM [min]	Sum of PG.Quantity	Peptide Counts
C20_SaphirTrap						
20250226_C20_SaphirTRAP_QC_10min_DIA_i01_RA2_1_126	4	4	0.055147648	0.032257967	11036639.94	7061
20250226_C20_SaphirTRAP_QC_10min_DIA_i02_RA2_1_127	4	4	0.05377388	0.032148264	10707478.13	6708
20250226_C20_SaphirTRAP_QC_10min_DIA_i03_RA2_1_128	4	4	0.055512428	0.032147694	10736761.84	6546
Exsil Mono_SaphirTrap						
20250228_MonoExsil_SaphirTRAP_QC_10min_DIA_i01_RA2_1_151	3	3	0.045816422	0.02515705	13574435.07	12042
20250228_MonoExsil_SaphirTRAP_QC_10min_DIA_i02_RA2_1_152	3	3	0.045948029	0.025331877	13142863.92	12390
20250228_MonoExsil_SaphirTRAP_QC_10min_DIA_i03_RA2_1_153	3	3	0.044762611	0.024653507	12342372.59	11605
Pur_pulledTip						
20250224_Repro_Pur_pulledTip_QC_10min_DIA_i01_RA2_1_69	4	4	0.068077087	0.039580517	1828050.608	1186
20250224_Repro_Pur_pulledTip_QC_10min_DIA_i02_RA2_1_70	4	4	0.066978931	0.04003205	3133660.407	2481
20250224_Repro_Pur_pulledTip_QC_10min_DIA_i03_RA2_1_71	4	4	0.06558609	0.039711505	2354926.014	1353
Saphir_pulledTip						
20250220_Repro_Saphir_pulledTip_QC_10min_DIA_i01_RA2_1_19	3	3	0.044311523	0.02535391	9519295.898	5463
20250220_Repro_Saphir_pulledTip_QC_10min_DIA_i02_RA2_1_20	3	3	0.044538498	0.025769863	9005777.974	5003
20250220_Repro_Saphir_pulledTip_QC_10min_DIA_i03_RA2_1_21	3	3	0.044728756	0.025742773	8936329.193	5140

Remarks:

To ensure a fair chromatographic comparison, the flow rate was individually adjusted for each capillary column to maintain a system pressure between 300 and 400 bar.

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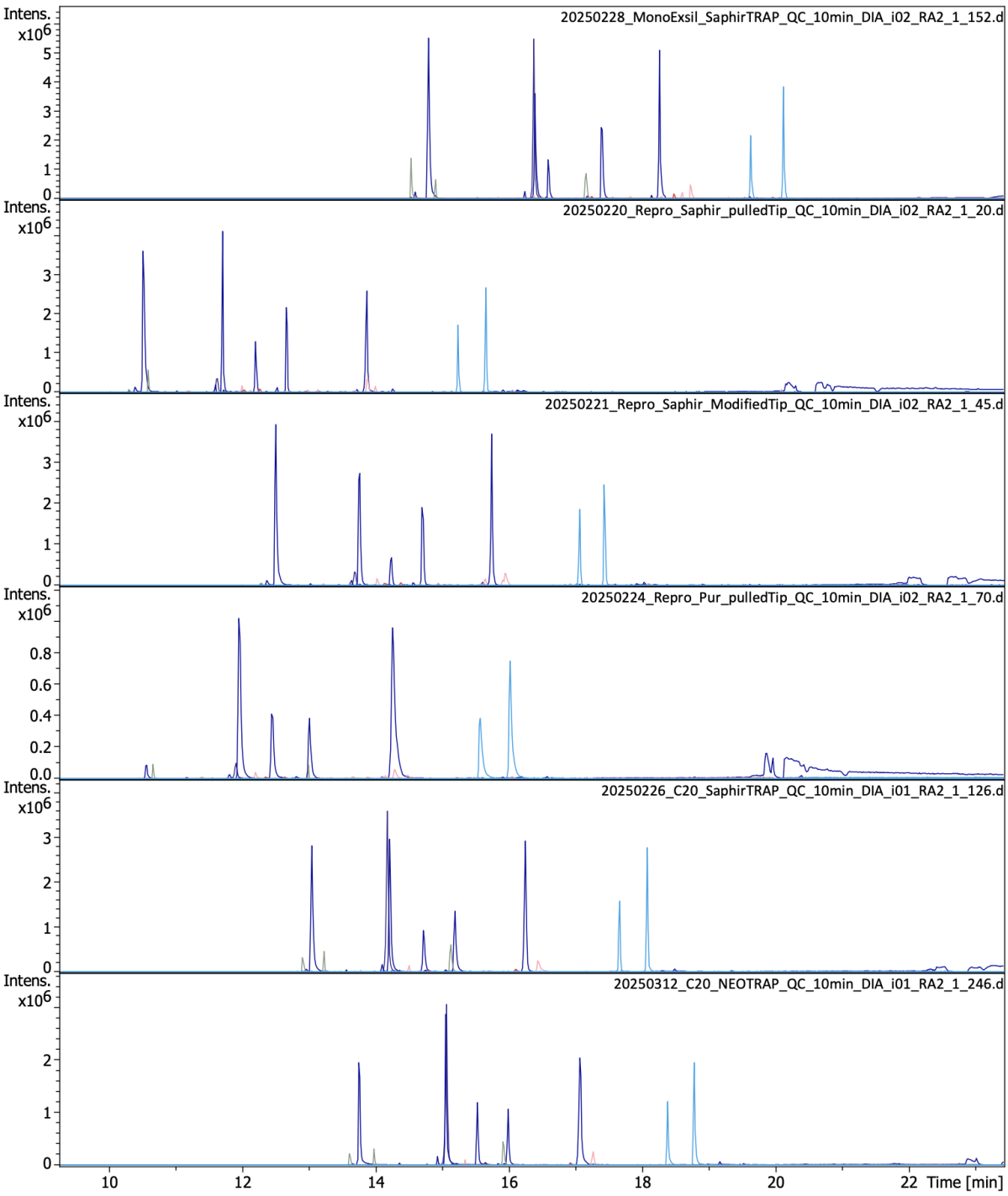
Chromatograms:



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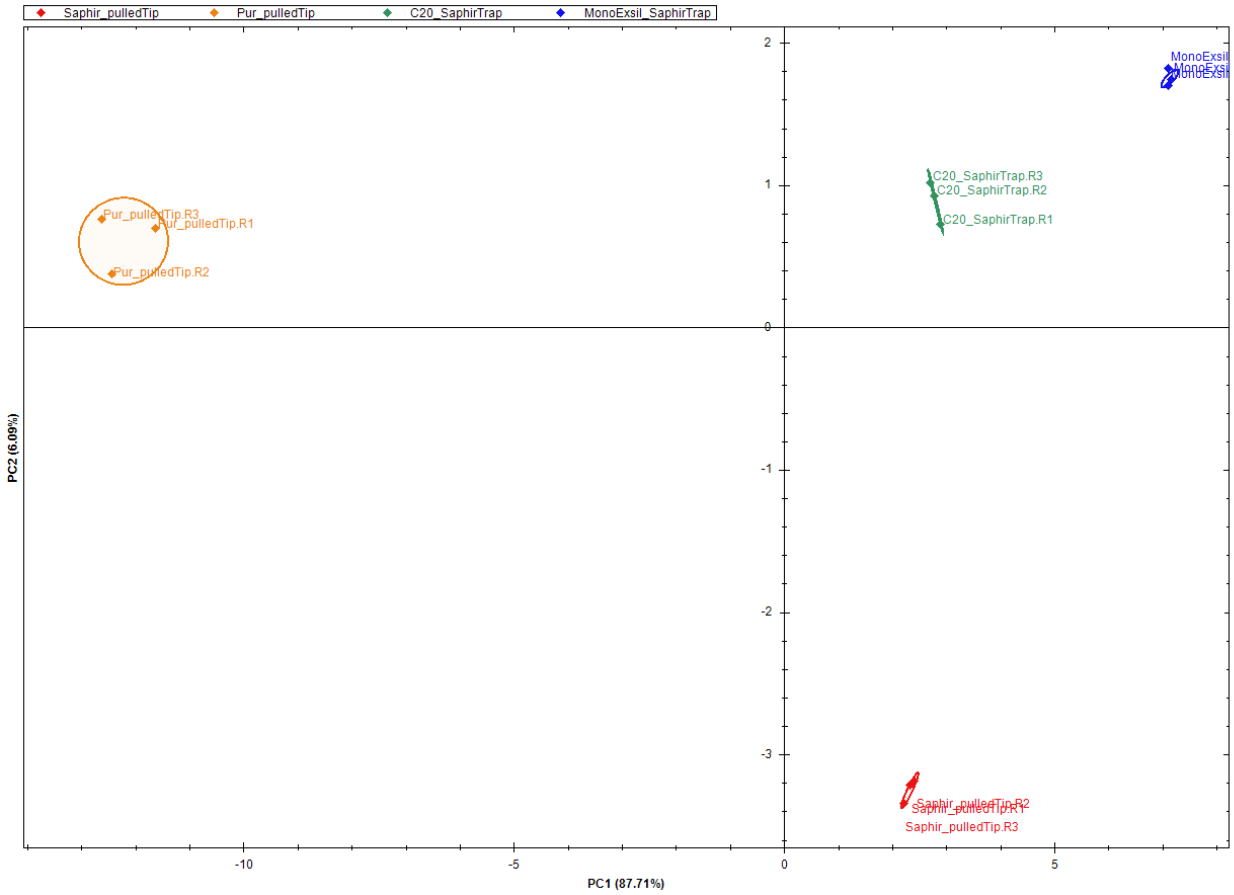

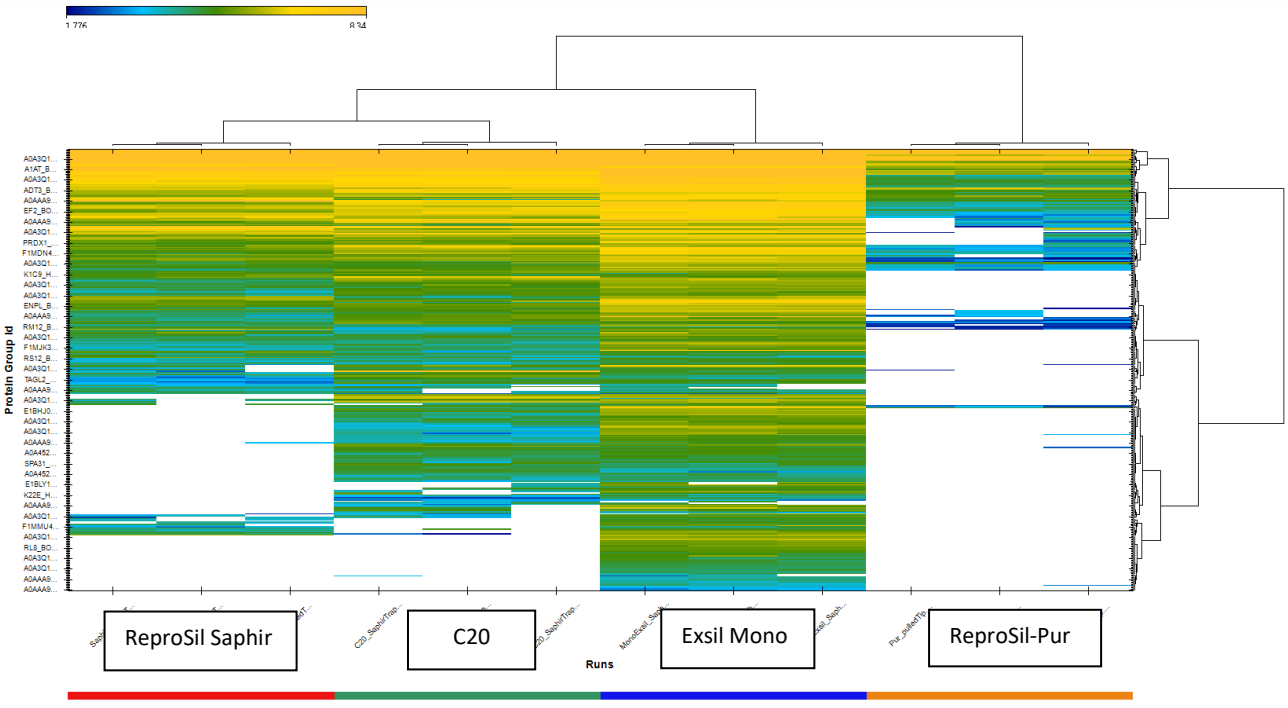

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Conclusion:

- Peak Shape: The capillary column packed with Exsil Mono 100 C18, 1.35 µm showed the narrowest peak widths at FWHM, followed by the capillary column packed with ReproSil Saphir 100 C18, 1.5 µm.
- As a result, fewer data points per peak were acquired at both the MS1 and MS2 levels.
- Nevertheless, the total number of identified peptides was highest for the capillary column packed with Exsil Mono 100 C18, 1.35 µm followed by the home-made capillary column C20 packed with C18, 1.7 µm.
- The protein quantities form column specific clusters.
- The capillary column packed with Exsil Mono 100 C18, 1.35 µm resulted in the best protein coverage followed by the home-made capillary column C20 packed with C18, 1.7 µm.

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2) HEK cell lysate (1, 10 and 200 ng Injection)

Identification Results:

sample	spectral Count	ion Count	peptide Count	Top3 count	%ID	Gdt lenght gain to 30min	Average	CV	Fragl count	MaxLFQ count
Saphir_PulledTip_1ng_1	11828	7560	7025	1819	24%				1819	1800
Saphir_PulledTip_1ng_2	11851	7537	6985	1769	23%		1808	2%	1769	1756
Saphir_PulledTip_1ng_3	12348	7886	7290	1835	24%				1835	1825
Pur_PulledTip_1ng_1	1298	695	679	254	3%				254	253
Pur_PulledTip_1ng_2	1252	642	623	239	3%		242	4%	239	239
Pur_PulledTip_1ng_3	1247	636	619	234	3%				234	230
C20_SaphirTRAP_1ng_1	14465	7396	6429	1213	16%				1213	1212
C20_SaphirTRAP_1ng_2	14681	7682	6693	1246	16%		1107	19%	1246	1243
C20_SaphirTRAP_1ng_3	4632	3284	3005	863	11%				863	863
ExsilMono_SaphirTRAP_1ng_1	21588	12383	11206	1950	25%				1950	1945
ExsilMono_SaphirTRAP_1ng_2	22366	12794	11471	1911	25%		1931	1%	1911	1905
ExsilMono_SaphirTRAP_1ng_3	22984	13254	11849	1932	25%				1932	1928
Saphir_PulledTip_10ng_1	25092	16370	14921	3227	42%				3227	3182
Saphir_PulledTip_10ng_2	25358	16518	15015	3191	41%		3191	1%	3191	3155
Saphir_PulledTip_10ng_3	24619	15969	14555	3155	41%				3155	3127
Pur_PulledTip_10ng_1	7141	3998	3747	1063	14%				1063	1055
Pur_PulledTip_10ng_2	6855	3763	3553	1041	13%		1048	1%	1041	1032
Pur_PulledTip_10ng_3	6697	3695	3513	1040	13%				1040	1029
C20_SaphirTRAP_10ng_1	49003	28070	23399	3280	42%				3280	3258
C20_SaphirTRAP_10ng_2	48048	27393	23074	3258	42%		3255	1%	3258	3229
C20_SaphirTRAP_10ng_3	48888	27838	23301	3228	42%				3228	3207
ExsilMono_SaphirTRAP_10ng_1	79463	49441	40909	4720	61%				4720	4664
ExsilMono_SaphirTRAP_10ng_2	77615	48340	39927	4618	60%		4670	1%	4618	4574
ExsilMono_SaphirTRAP_10ng_3	78109	49060	40612	4673	60%				4673	4610
Saphir_PulledTip_200ng_30min_1	52790	32553	28500	4967	64%				4967	4861
Pur_PulledTip_200ng_30min_1	26676	16189	14800	3144	41%				3144	3105
C20_SaphirTRAP_200ng_30min_1	122008	69073	52232	5772	75%				5772	5643
ExsilMono_SaphirTRAP_200ng_30min_1	135549	84851	63411	6522	84%				6522	6246
Saphir_PulledTip_200ng_60min_1	81349	45061	39382	5942	77%	20%			5942	5783
Pur_PulledTip_200ng_60min_1	29272	15952	14705	3139	41%	0%			3139	3099
C20_SaphirTRAP_200ng_60min_1	184432	82945	63702	6311	82%	9%			6311	6138
ExsilMono_SaphirTRAP_200ng_60min_1	202176	111545	83777	7132	92%	9%			7132	6774
Saphir_PulledTip_200ng_90min_1	91926	47180	41312	6171	80%	24%			6171	5929
Pur_PulledTip_200ng_90min_1	22836	11305	10522	2514	33%	-20%			2514	2477
C20_SaphirTRAP_200ng_90min_1	230887	84387	65464	6400	83%	11%			6400	6186
ExsilMono_SaphirTRAP_200ng_90min_1	247633	121868	92631	7363	95%	13%			7363	6897
Total Quantified	2350404	167956	121171	7735					7735	7735
Total Reported	2350404	175246	126056	7735					7735	7735

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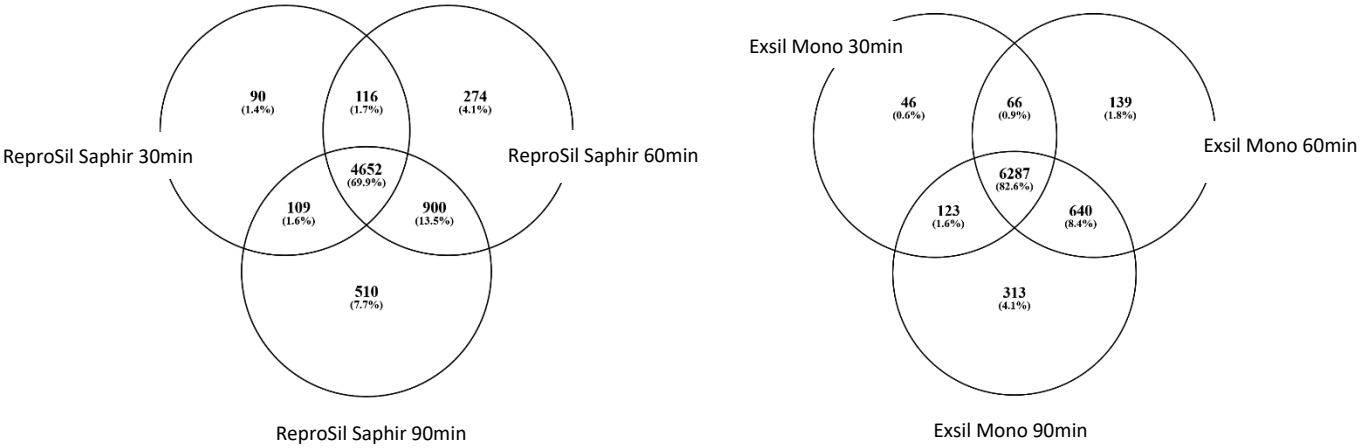
Loading Amount: 10 ng

Capillary Column	Identified Proteins
C20 C18, 1.7 µm, 200 mm x 75 µm	3,725
ReproSil Saphir 100 C18, 1.5 µm, 200 mm x 75 µm	3,827
Exsil Mono 100 C18, 1.35 µm, 200 mm x 75 µm	5,233

Loading Amount: 200 ng

Gradient Length Comparison



	ReproSil Saphir 100 C18, 1.5 µm, 200 mm x 75 µm	Exsil Mono 100 C18, 1.35 µm, 200 mm x 75 µm
Gradient Lenght	**Identified Proteins**	
30 min	4,967	6,522
60 min	5,942	7,132
90 min	6,171	7,363

Extending the gradient from 60 to 90 minutes shows minimal increase in protein identifications, likely due to the high acquisition speed of modern MS technology such as the TimsTOF HT. Doubling the gradient length from 30 to 60 minutes shows a notable improvement, especially for the capillary column packed with ReproSil Saphir 100 C18, 1.5 µm, indicating certain phases benefit more from extended separation.

Dr. Maisch
Any Column, Any Size, Any Media

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Conclusion:

- The identification rate (% ID) was defined as the number of identified proteins obtained with a given column divided by the total number of identified proteins across all tested capillary columns.
- The capillary column packed with Exsil Mono 100 C18, 1.35 µm consistently outperformed all other columns, regardless of the injected sample amount or gradient length.

- Loading amount of 1 ng:
    - The capillary column packed with Exsil Mono 100 C18, 1.35 µm outperforms the other capillary columns in protein identification.
    - The second-best performance was observed with the capillary column packed with ReproSil Saphir 100 C18, 1.5 µm.
- Loading amount of 10 ng:
    - The capillary column packed with Exsil Mono 100 C18, 1.35 µm outperforms the other capillary columns in protein identification.
    - The capillary column packed with ReproSil Saphir 100 C18, 1.5 µm and the home-made capillary column C20 packed with C18, 1.7 µm delivered similarly strong results, ranking second.
- Loading amount of 200 ng:
    - The capillary column packed with Exsil Mono 100 C18, 1.35 µm outperforms the other capillary columns in protein identification.
    - The home-made capillary column C20 packed with C18, 1.7 µm ranked second-best in terms of protein identifications.

- Increasing the gradient length had a notably greater impact (20–25%) on the capillary column packed with ReproSil Saphir 100 C18, 1.5 µm compared to the others.

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