

A new SPE Tips method based on an innovative sorbent for fast and efficient peptide fractionation in proteomic studies

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Context: bottom-up proteomics workflow

1 Proteolysis Protein Trypsin Peptides

2 Reversed-phase peptide fractionation at basic pH

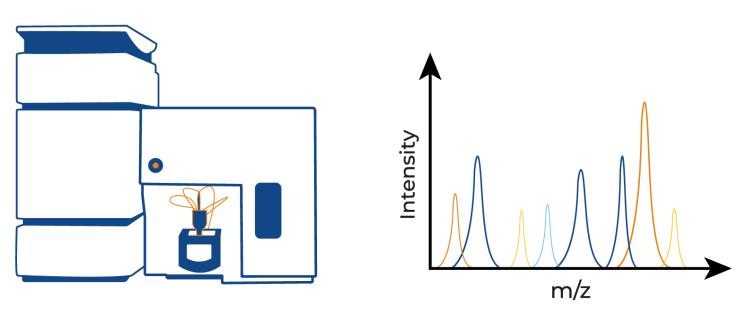
Principle & Advantages

- ✓ Reduction of sample complexity for deep proteome sequencing and quantitative analysis
- ✓ High pH fractionation orthogonal to RPLC peptide separation at low pH
- identified ✓ Increased number of proteins compared unfractionated peptides
- ✓ No desalting step required prior to LC-MS/MS analysis of the fractions

Development of a simplified procedure for the efficient and fast fractionation of peptides with a wide range of properties (size, polarity and charge)

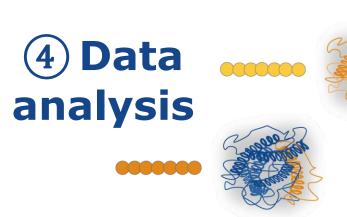
BioSPETM PepFrac

(3) LC-MS/MS analysis



Peptide separation and detection

Peptide identification and quantification



- Protein inference
- PTM identification and localization...

Evaluation of BioSPETM PepFrac for peptide fractionation at basic pH

1) What is BioSPETM PepFrac?

Objective

BioSPETM PepFrac is a new reversed-phase sorbent based on Affinisep disks technology, specifically developed for the fractionation of peptides in proteomics studies. In this study, BioSPETM PepFrac 200µL Tips were evaluated for the fractionation of peptides resulting from the enzymatic digestion of proteins contained in HEK293 cell lysate. Results were compared with a competitor fractionation column.







Proteins

Tryptic digestion

(2) HEK cell lysis and protein digestion

50µg collected for fractionation

HEK293 cells

(3) Peptide fractionation protocol at basic pH & LC-MS/MS analysis conditions

Fractionation protocol with ACN gradient

Processing step	BioSPE™ PepFrac Tips	Competitor column
Conditioning	2x 150μL ACN - 1,500g – 2min	2x 300µL ACN - 5,000g - 2min
Equilibration	2x 150μL 0.1% TFA - 1,500g- 2min	2x 300µL 0.1% TFA - 5,000g- 2min
Loading of sample	150μL - 1,500g– 2min	300μL - 3,000g– 2min
Washing	150μL H ₂ O - 1,500g– 2min	300μL H ₂ O - 3,000g– 2min
Fraction 1	150μL ACN/TEA 0.1% (2/98) - 1,500g- 2min	300μL ACN/TEA 0.1% (5/95) - 3,000g- 2min
Fraction 2	150μL ACN/TEA 0.1% (4/96) - 1,500g- 2min	300μL ACN/TEA 0.1% (7.5/92.5) - 3,000g- 2min
Fraction 3	150μL ACN/TEA 0.1% (6/94) - 1,500g- 2min	300µL ACN/TEA 0.1% (10/90) - 3,000g- 2min
Fraction 4	150μL ACN/TEA 0.1% (8/92) - 1,500g- 2min	300μL ACN/TEA 0.1% (12.5/87.5) - 3,000g- 2min
Fraction 5	150μL ACN/TEA 0.1% (10/90) - 1,500g- 2min	300μL ACN/TEA 0.1% (15/85) - 3,000g- 2min
Fraction 6	150μL ACN/TEA 0.1% (12/88) - 1,500g- 2min	300μL ACN/TEA 0.1% (17.5/82.5) - 3,000g- 2min
Fraction 7	150μL ACN/TEA 0.1% (15/85) - 1,500g- 2min	300μL ACN/TEA 0.1% (20/80) - 3,000g- 2min
Fraction 8	150μL ACN/TEA 0.1% (50/50) - 1,500g- 2min	300μL ACN/TEA 0.1% (50/50) - 3,000g- 2min
Evaporation	SpeedVac (2h)	SpeedVac (3h30)
Resuspension	13µL 0.1%FA	13µL 0.1%FA

LC-MS/MS analysis of peptides

NanoLC separation

• Column: IonOpticks C18 packed emitter column $(25 \text{cm} \times 75 \mu\text{m}, 1.6 \mu\text{m})$

- Injected volume: 1μL
- **Gradient:** 2% to 95% buffer B over 30min at a flow rate of 200 nL/min

Buffer A: 0.1% FA in water Buffer B: 0.1% FA in ACN

Acquisition method: Parallel Accumulation Serial Fragmentation (PASEF) – Data Dependent Acquisition (DDA)

MS detection (timsTOF Pro)

Peptides

• *m/z* range: 100 to 1700 Th



- **Software:** MaxQuant version 2.0.1.0
- Database: UniProtKB/Swiss-Prot Homo sapiens

Results of peptide fractionation on BioSPETM PepFrac Tips & comparison with competitor fractionation column

Total number of proteins identified

Results: High number of proteins identified

7834

column

Competitor

identified

7886

Tips

C

PepFra

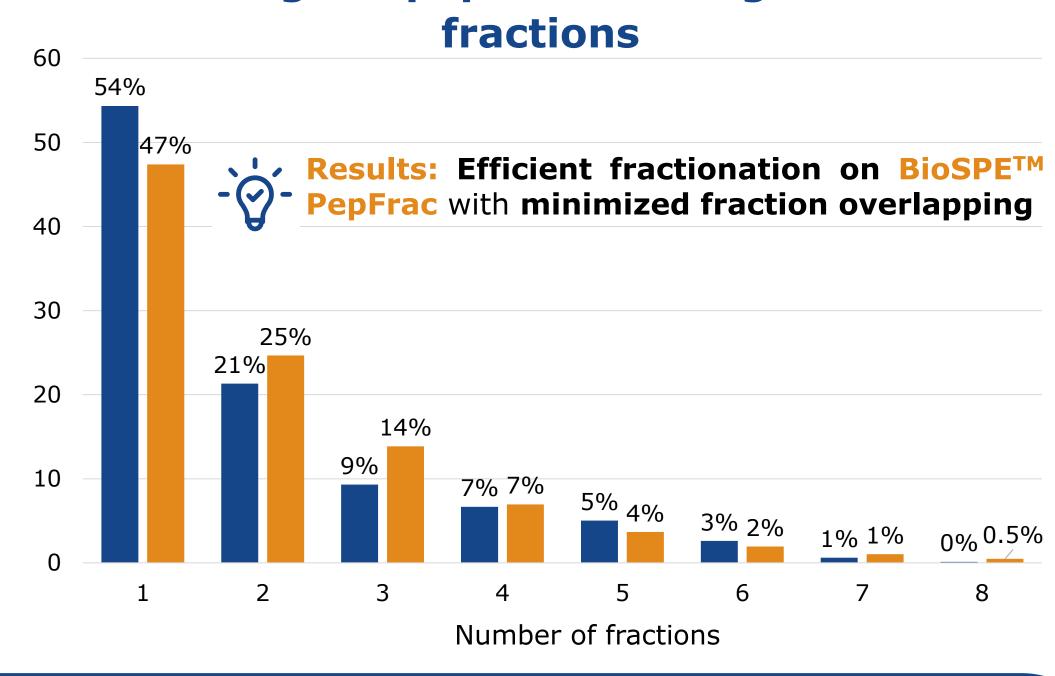
SP

Bio

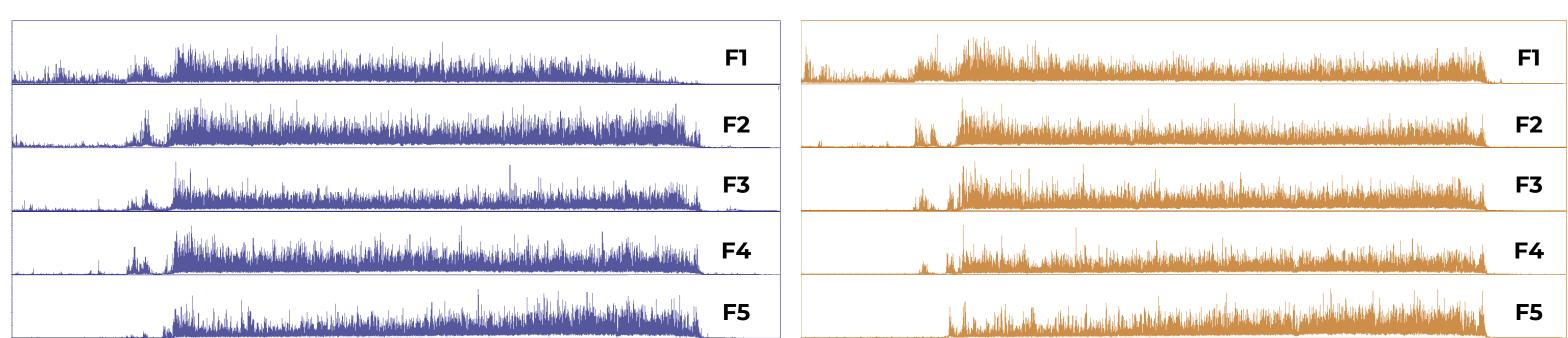
Peptide distribution in each fraction

16500 16221 16242 15951 15300 15300 15117 14804 14032 13828 13504 12935 12437 11081 8813 8232 F4 F5 F6 Results: Good distribution of peptides over the eight fractions the eight fractions

Percentage of peptides eluting in several



Spectral density over HPLC gradient



Results: Good repartition of peptides over analytical run

Advantages of BioSPETM PepFrac

- No storage constraints for BioSPE™ PepFrac (dry at room temperature for several years) contrary to competitor column (4°C, in storage buffer)
- Time required for evaporation of each fraction almost halved with **BioSPETM PepFrac Tips**
- Fractionation of 10 to 50µg of peptides on BioSPE™ PepFrac 200µL **Tips**
- Flexibility of format and capacity: BioSPETM PepFrac available as spin columns for higher peptide amounts or 96 wellplates for high throughput experiments

Conclusion

BioSPETM PepFrac appears as a promising alternative to the competitor fractionation column, especially for complex samples such as plasma or the generation of spectral librairies, since it leads to an increase of more than 27% in the number of proteins identified, compared to unfractionated samples.

