Maximisation of Peak Capacity for Peptide Mapping using a Column Coupling Approach with Solid-Core Columns

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1. Introduction

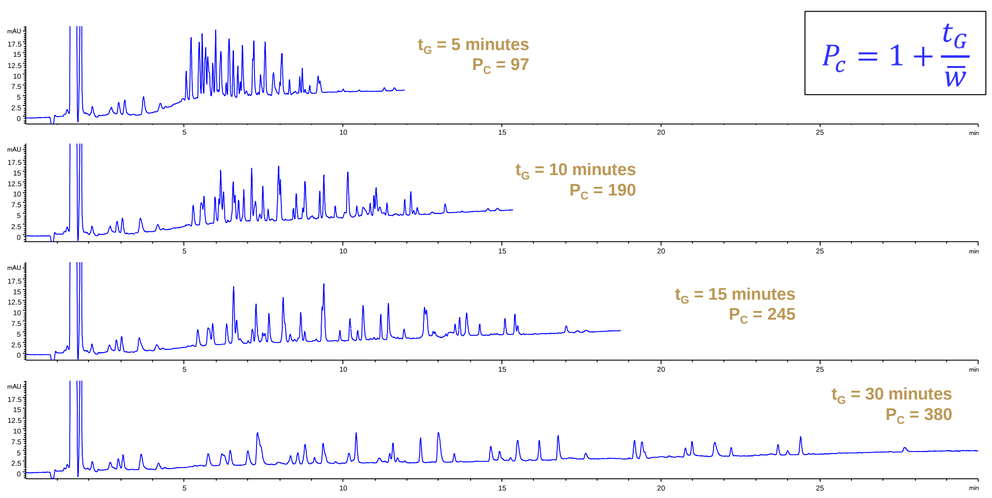
- Peptide mapping is used to monitor changes in the primary amino acid sequence of a protein.
- Changes to the amino acid sequence are known as Critical Quality Attributes as they can greatly affect the safety and efficacy of a biopharmaceutical drug.
- Proteins are enzymatically digested to produce characteristic peptide fragments which can then be analysed using HPLC.
- Peak capacity (P_C) is particularly important in peptide mapping due to the large number of peaks present.
- This poster presents work performed to optimise the peak capacity for peptide mapping applications on a high performing solid-core column (**ACE UltraCore 2.5 SuperC18**).
- Peak capacity was further enhanced by coupling multiple columns in series.

2. Method Optimisation

- Gradient time and flow rate were optimised using a pre-digested BSA sample.
  - t_G = 5, 10, 15, 30 minutes
  - F = 0.21, 0.35, 0.5 mL/min
- An **ACE UltraCore 2.5 SuperC18** solid-core column was used for method optimisation.
- Increased efficiency of superficially porous stationary phase improved resolution of closely related peaks.

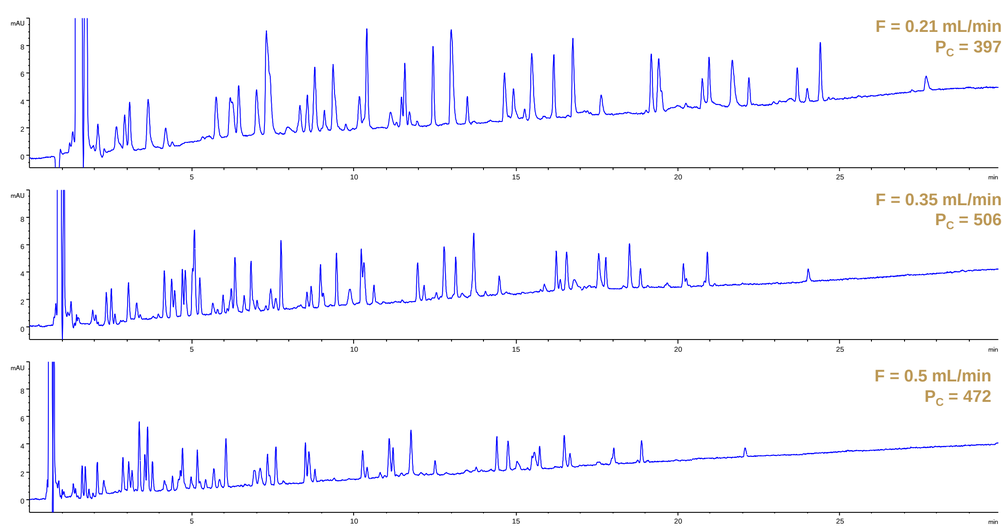
Column:	ACE UltraCore 2.5 SuperC18, 150 x 2.1 mm
Part number:	CORE-25A-1502U
Mobile phases:	**A:** 0.05 % TFA in H_2O **B:** 0.05 % TFA in MeCN
Injection volume:	20 µL
Temperature:	60 °C
Detection:	UV, 214 nm

3. Gradient Time Optimisation



- 10 – 40 %B gradient, 0.21 mL/min.
- Pre-digested BSA sample.
- Highest peak capacity seen with longest gradient.

4. Flow Rate Optimisation

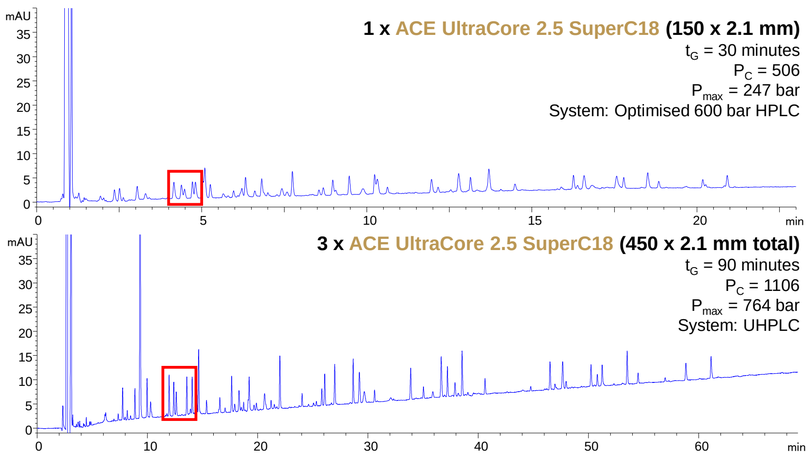


- 10 – 40 %B gradient in 30 mins.
- Highest peak capacity observed at 0.35 mL/min.

5. Column Coupling

- Complex samples such as tryptic digests can benefit from the improved resolution achieved by increasing column length.
- The high pressure limits of UHPLC instruments (>1,000 bar) allow multiple columns to be connected in series to increase effective column length.
- **ACE UltraCore** 2.5 µm solid-core particles provide performance similar to 1.7 µm fully porous particles at much lower back pressure – ideal for connecting multiple columns.
- The optimised single column method (150 x 2.1 mm) was translated to 3 coupled columns totalling 450 mm in length.
- The final method was then applied to the analysis of three freshly prepared protein digest samples.

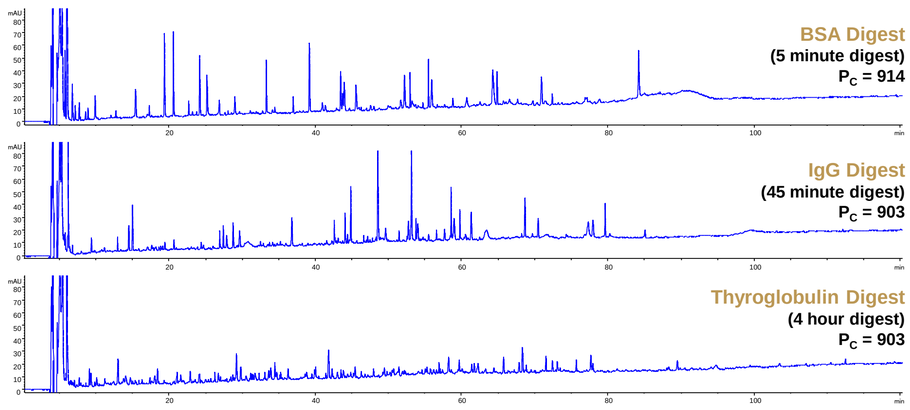
6. BSA Tryptic Digest - Column Coupling



- Large increase in peak capacity with coupled columns, 3-fold increase in back pressure.
- Additional sample complexity revealed.

Method Conditions
Mobile phases: **A:** 0.05 % TFA in H_2O
B: 0.05 % TFA in MeCN
Gradient: 10 – 40 % B
Temperature: 60 °C
Flow rate: 0.35 mL/min
Injection volume: 20 µL
Detection: UV, 214 nm

7. Application to other Protein Digests Samples



Method Conditions

Column: ACE UltraCore 2.5 SuperC18 (450 x 2.1 mm total)
Mobile phases: **A:** 0.05 % TFA in H_2O
B: 0.05 % TFA in MeCN
Gradient: 10 – 40 % B in 90 mins then 40 – 65 % B in 30 mins
Temperature: 60 °C **Flow rate:** 0.21 mL/min
Injection volume: 20 µL **Detection:** UV, 214 nm

Samples

- BSA (66.5 kDa), IgG (150 kDa) and thyroglobulin (660 kDa) proteins were digested in this project.
- Native proteins were digested using Thermo SMART Digest kit. (uses a combination of immobilised trypsin enzyme and heat to digest proteins).
- Digestion time determined from kit protocols.
- Digestions were very reproducible .

8. Summary and Conclusions

- A generally applicable, optimised gradient method was produced for the high resolution analysis of tryptic digest samples.
- Peak capacity of 506 achieved with a single **ACE UltraCore 2.5 SuperC18** column using optimised method parameters.
- Three **ACE UltraCore 2.5 SuperC18** columns were coupled together to increase peak capacity to 1106.
- High efficiency of **ACE UltraCore** particles increased the resolution of the separation.
- A Thermo SMART digest kit was used to prepare three different digests that were successfully analysed using the column coupling approach.