High performance peptide separations using capillary LC

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Abstract

The acceptance of capillary LC for the analysis of limited sample volumes or low concentrations has been hampered by its reputation of being irreproducible, unreliable and too complex. Therefore, a capillary LC system is not useful for routine analysis if it cannot perform as well as standard LC equipment. Here the performance of a new capillary LC system, using a unique pump design with electronic flow control is demonstrated. Peptide separations are shown on a 300-µm id column with a flow rate of 5.5 µL/min.

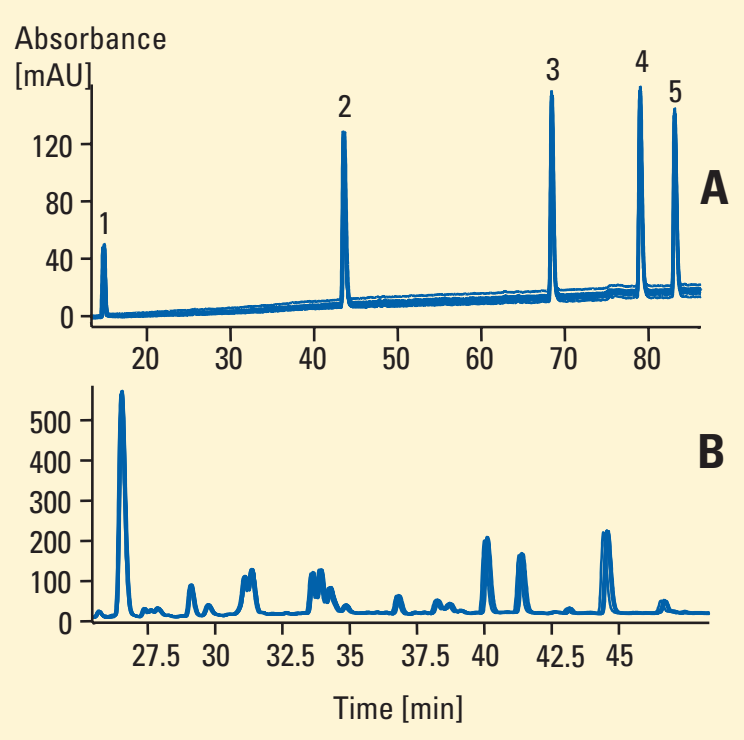

Figure 1
Analysis of peptides, overlay of 10 runs each.
A = HPLC peptide standard
B = tryptic myoglobin digest

Conditions

Sample
A= 5 ng/µL peptide standard
B= 7.5 pmol/µL tryptic myglobin digest
Column
300 µm id, 25 cm length,
ZORBAX 300SB-C18, 5 µm, 300 Å
Solvent A
0.05 % TFA in water
Solvent B
0.045 % TFA in acetonitrile
Flow rate
5.5 µL/min
Gradient
A = 0.25 % B/min (1–31 % B)
B = 0.5 % B/min (1–61 % B)
Column compartment temperature
30° C
Injection volume
A = 1.4 µL, B = 1.3 µL
Detection
Signal 206/10 nm, reference 450/80 nm, 500-nL volume, 10-mm path length flow cell

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Experimental

An Agilent 1100 Series Capillary LC system with Agilent ChemStation for instrument control, data acquisition and analysis was used for all experiments. The analytical column from the ZORBAX series is also available from Agilent Technologies. All chemicals were of HPLC grade or better. The HPLC peptide standard containing GY, VYV, Met-enkephalin, Leu-enkephalin and Angiotensin II was purchased from Sigma. A tryptic digest of horse skeletal muscle myoglobin was prepared as described earlier[1].

Results

Figure 1A shows an overlay of 10 consecutive analyses of the peptide standard. Even when using a very shallow gradient (0.25 % B/min), excellent repeatability data on retention time (< 0.5 % RSD) and area (< 1 % B) could be achieved. Figure 1B shows a section of a peptide map of a myoglobin digest (overlay of 10 runs performed with a 0.5 % B/min gradient). RSD values for retention time were < 0.5 % RSD.

These results correspond well to repeatability data achieved on state of the art LC systems using a standard pump. The dedicated capillary LC equipment can therefore be reliably employed for the analysis of peptide samples when high sensitivity is needed.

Equipment

Agilent 1100 Capillary LC system consisting of

- Capillary pump
- Micro vacuum degasser
- Thermostatted column compartment
- Micro autosampler
- Diode array detector, 500 nL flow cell
- Agilent ChemStation

Column

- ZORBAX 300SB-C18, 5 µm, 300 Å (part number 5064-8265)

References

1. "Peptide separations by microbore reversed-phase HPLC" Agilent Application Note 5964-9935E.

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