High Sensitivity Capillary HPLC with Diode-array Detection

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Abstract

Analytical separations using high performance liquid chromatography (HPLC) with small-diameter columns have recently become an indispensable tool in protein characterization, where sample volume and concentration are often limited. Here, detection sensitivity can be significantly increased using capillary columns with internal diameters of 300 µm. Sensitivity or minimal detectable quantity vary inversely with column diameter. The effect can be understood as a scaling down of volumes, particularly peak volume, while keeping the mass constant. Table 1 compares narrow bore and standard bore columns. To run capillary columns using standard HPLC equipment, the following prerequisites have to be met:

• low system delay volume • addition of a flow splitter, and • availability of nl detector cells.

This is achieved with the Agilent 1100 Series HPLC high pressure gradient system which offers lowest system delay volume, easy adaptation of flow splitters and nl-detector cells. Until recently nl-flow cells (from LC Packings , Amsterdam, The Netherlands) were only available for the variable wavelength detector. Meanwhile a 500-nl flow cell for diode-array detection (DAD) with a 10-mm pathlength is commercially available, and was used in this example.

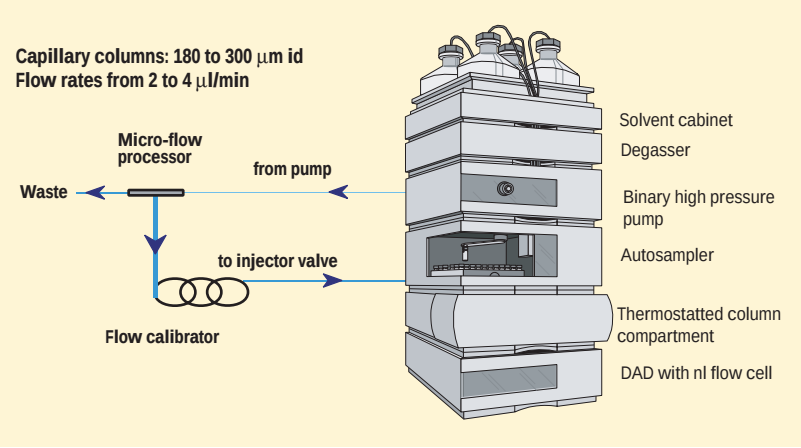

Figure 1
Capillary HPLC system based on the Agilent1100 Series system

Parameter	Narrow bore column	Standard bore column
Length (mm)	100	200
Internal diameter (mm)	2.1	4.6
Particle size	5	10
Plate number	6000	6000
Column volume (µl)	280	2660
Peak volume (µl)	43	412
Capacity factor 'k'	2	2

Table 1
Peak volume as measure of dispersion

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Conversion of the standard Agilent 1100 Series HPLC system is illustrated in figure 1. A flow splitting device is installed between the pump and the injector and the standard DAD cell is replaced by the 500-nl DAD cell.

For peptide identification, diode-array detection has the major advantage over single-wavelength detectors in that spectra of eluting compounds can be stored in a digital form and manipulated by a variety of algorithms. Spectra are of special interest for peptides containing aromatic amino acids like tyrosine, tryptophan and phenylalanin. Since the zero-order spectra of tyrosine and tryptophan are overlapping, second-order derivative spectra are used for identification (see figure 2).
The Agilent 1100 Series capillary HPLC system described here is best suited for sensitive and reliable analysis of peptide maps in the femtomole range. Using the second-order derivative spectra, peptides with tyrosine and tryptophan can be clearly identified. Precision of retention times was between 0.03 and 0.2 % RSD.

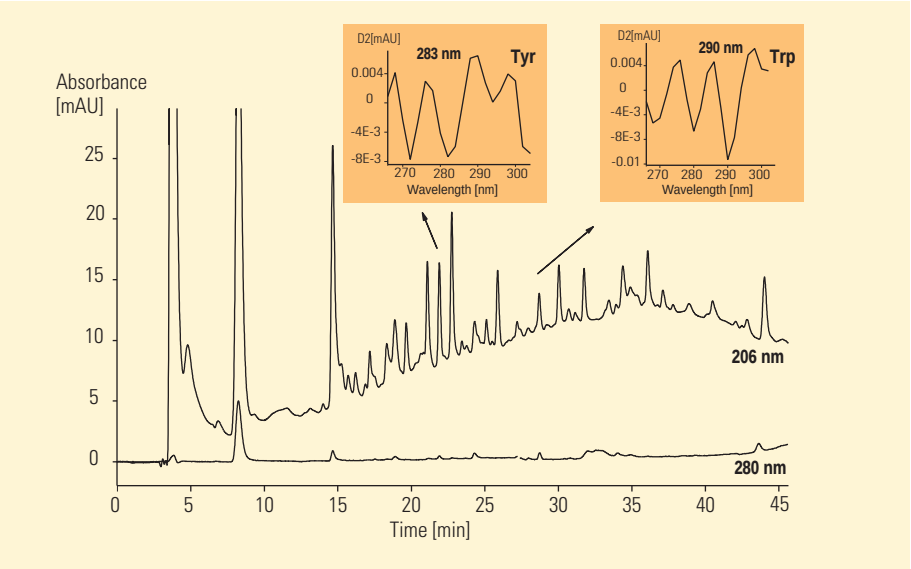

Figure 2
Peptide map of a 200-fmol tryptic digest of myoglobin on a 250 x 0.3 mm Zorbax SBC-18 column (LC packings). Inserts show the second-order deriviative spectra of peptides containing tyrosine and tryptophan

Conditions

Column:
250 x 0.3 mm Zorbax SBC-18 column (LC Packings)
Mobile phase:
A = 0.05 % TFA in water
B = 0.04 % TFA in ACN
Gradient:
5-60 % B in 45 min.
Column flow rate:
4 µl/min

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