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应用纪要

Peptide Mapping Using Biosuite PA Columns: High Peak Capacity for Separation of Complex Peptide Mixtures

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief describes peptide mapping of complex mixtures using BioSuite PA Columns.

Introduction

Column peak capacity: The measure of column efficiency

Complex peptide samples generated by protein digestion may contain one hundred or more components. Separation of protein digests is typically performed in RP-HPLC mode under gradient conditions. Therefore the separation efficiency of columns is defined by column peak capacity (P), rather than by theoretical plates. Peak capacity is defined as the number of peaks that can be theoretically separated within a gradient time. State-of-the-art RP-HPLC Columns have peak capacities of 100–300, depending on their length and particle size of sorbent. Using longer columns and/or long

gradients improves the P, however, at the expense of separation time. BioSuite PA-A (PA-B) Columns packed with 3 and 3.5 µm sorbent offer the high peak capacity required for complex sample separation. Other column design features including base sorbent, ligand binding, encapping, and column hardware are also crucial for peptide separation performance. BioSuite PA-A (PA-B) Columns are suitable for peptide mapping applications.

Results and Discussion

Evaluation of column peak capacity

Column peak capacity was experimentally measured using a representative mix of 9 peptides (Figure 1). Peak width was measured at 4 sigma (13.4% of peak height – see inset in Figure 1), averaged and peak capacity was calculated according to the formula in Figure 1. The peak capacity is essentially the number of peak widths that fit in the given gradient time plus one. The BioSuite C_{18} PA-A 4.6 x 50 mm Column is theoretically capable of separating 164 peaks with resolution Rs=1. In practice that ideal separation is not likely to be achieved. The BioSuite C_{18} PA-A 50 mm long columns are suitable for routine and fast separations of about 25–35 peptides.

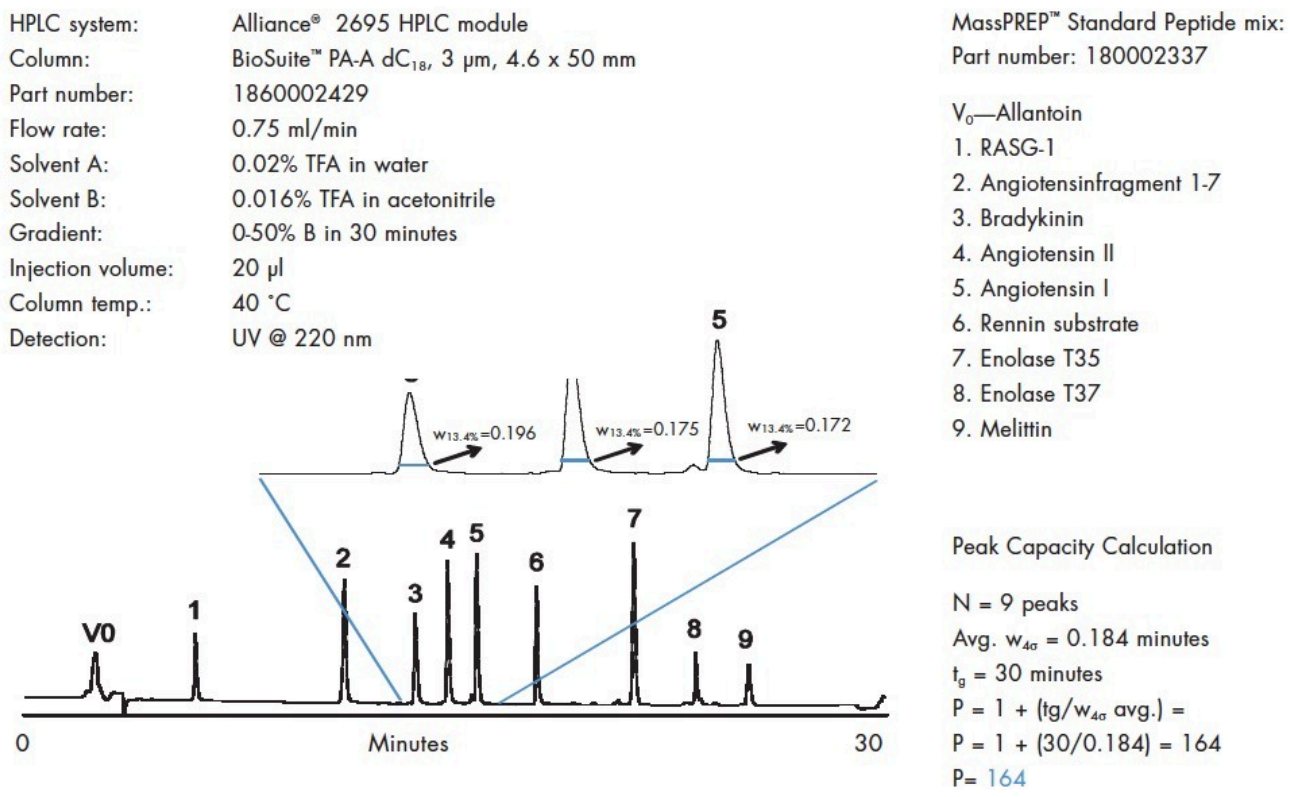

Figure 1. Example of column peak capacity calculation.

BioSuite C_{18} PA-A Column chemistry

BioSuite C_{18} PA-A Columns are silica based, difunctionally bonded C_{18} columns that offer the optimal combination of C_{18} ligand density, silica pore diameter, and endcapping for separation of peptides. The stationary phase is compatible with 100% aqueous mobile phases and is designed to retain polar peptides. BioSuite C_{18} PA-A Columns are stable under low pH conditions and can be used with 0.02% TFA in the mobile phase. BioSuite C_{18} PA-A Columns perform exceptionally well with LC-MS preferred mobile phases consisting of 0.1% aqueous formic acid and acetonitrile without sacrificing peak capacity.

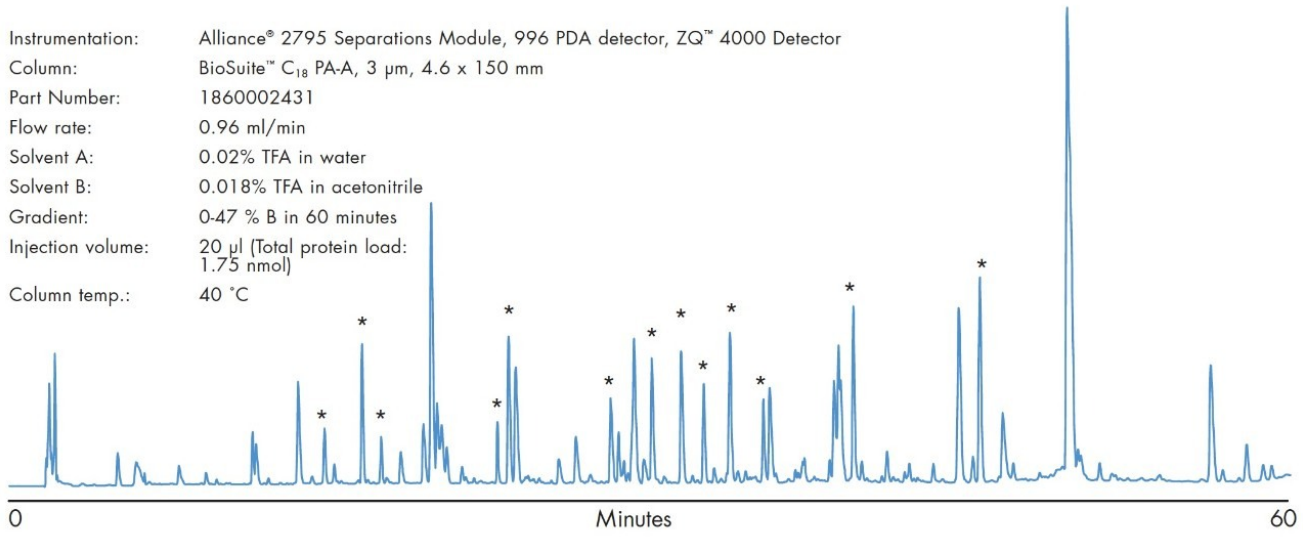

Figure 2. LC separation of tryptic digest of yeast enolase.

High performance separations of peptides using BioSuite C_{18} PA-A Column

A mixture of peptides was prepared by digesting yeast enolase with trypsin in the presence of 0.05% of *Rapi*Gest SF (p/n 186000860). The resulting digest contained approximately 60 peptides. Separation was performed on BioSuite C_{18} PA-A 3 µm, 4.6 x 150 mm Column. Selected peaks that were not co-eluting with other components were used to calculate column peak capacity; those peaks are labeled with an asterisk in Figure 2. Peak widths measured at 13.4% of peak height varied between 0.15–0.22 minutes. The average value 0.19 min was utilized for the calculation as follows: P = 1+(60/0.19) = 317. This peak capacity value is sufficient for the successful separation of peptide mapping samples consisting of 60–70 peptides. BioSuite C_{18} PA-A Columns provide the high peak capacity required for demanding applications such are peptide mapping, and LC-MS analysis of protein digests.

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720000833, September 2004