Current proteomics and genomics research has spurred the pharmaceutical and biotechnology industries to keep up with technical demands. There is currently a need for robust liquid-phase separations of complex protein and peptide mixtures. In this study, a VYDAC® capillary LC/MS column was directly compared with another leading commercial column of the same dimensions. It was then used to rapidly identify 2037 proteins from multiple complex peptide mixtures in a robust and high-throughput manner.

Over the years there has been a drive to increase the efficiency of analysis in the proteomics industry. The separation of complex peptide mixtures with capillary C18 columns needs to be durable, reproducible, and extremely sensitive.

Experimental Conditions

For the first experiment, a VYDAC® C18 capillary column was compared with a competitive column. Both columns were 150 mm $\times$ 300 μm. A standard tryptic digest mixture was prepared by digesting horse heart apomyoglobin. A total of 1 μg was injected onto each column and the eluent was analyzed by ESI-M S/M S (Thermo Finnigan LCQ™ DECA). The data were searched with TurboSEQUEST® software (protein identification software from Thermo Finnigan), and the percentage sequence coverage and resolution were compared.

In the second experiment, the maximal loading capacity and the total number of proteins that could be identified from a complex peptide mixture were analyzed. A total of 4 mg of Shewanella oneidensis crude lysate was digested with trypsin (from Promega Corp.). Four separate injections (800 μg/injection) were made on a VYDAC® C18, 150 mm $\times$ 300 μm column. The column was directly connected to an ESI-M S/M S system (Thermo Finnigan LCQ™ DECA) operating in data-dependent MS/MS mode. For each injection, a separate mass range was scanned (m/z 400–800, m/z 780–1200, m/z 1180–1600, and m/z 1580–2000) in order to increase dynamic range (1). All data files were searched with the TurboSEQUEST® search engine, and identifications were based on two or more high-scoring peptides per protein.

The lifetime of a VYDAC® C18, 150 mm $\times$ 300 μm column is summarized in Table I. Multiple injections of complex, tryptic-generated peptide mixtures from crude and cleared lysates and anion-exchange fractions were made over a period of time. Performance of the column was validated by injections of the apomyoglobin standard (data not shown).

**Results**

Figure 1 is a comparison between two commercial C18 columns using a standard tryptic digest mixture. Better resolution and higher sequence coverage was obtained with the VYDAC® column when compared with a leading competitor’s column.

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**Table I: Summary of column lifetime — analysis of complex peptide mixtures**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Injection (μg)</th>
<th>Number of Injections</th>
<th>Total Injection (μg)</th>
<th>Protein Identifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shewanella lysate</td>
<td>200</td>
<td>3</td>
<td>600</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>4</td>
<td>3200</td>
<td>480</td>
</tr>
<tr>
<td>Shewanella-cleared lysate</td>
<td>200</td>
<td>3</td>
<td>600</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>4</td>
<td>3200</td>
<td>362</td>
</tr>
<tr>
<td>Anion-exchange fraction 27</td>
<td>200</td>
<td>2</td>
<td>300</td>
<td>112</td>
</tr>
<tr>
<td>Anion-exchange fraction 28</td>
<td>150</td>
<td>2</td>
<td>150</td>
<td>111</td>
</tr>
<tr>
<td>Anion-exchange fraction 29</td>
<td>200</td>
<td>2</td>
<td>400</td>
<td>130</td>
</tr>
<tr>
<td>Anion-exchange fraction 30</td>
<td>100</td>
<td>2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Anion-exchange fraction 31</td>
<td>75</td>
<td>2</td>
<td>150</td>
<td>95</td>
</tr>
<tr>
<td>Anion-exchange fraction 32</td>
<td>100</td>
<td>2</td>
<td>200</td>
<td>105</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td></td>
<td><strong>9 mg</strong></td>
<td><strong>2037</strong></td>
</tr>
</tbody>
</table>

*Two separate lysates and anion-exchange fractions were analyzed by LC-MSMS with a VYDAC C18 column, 150 mm $\times$ 300 μm.*
Figure 2 illustrates the analysis of a complex, tryptic-generated peptide mixture from a crude lysate. In order to increase dynamic range, the same sample was injected four times at four separate mass ranges. This study illustrates the ability to identify a large number of proteins (in this experiment, 480) in a short amount of time. Furthermore, it illustrates the large loading capacity of a VYDAC® C18, 150 mm × 300 μm column.

Table I documents the lifetime of a VYDAC® C18, 150 mm × 300 μm column under harsh conditions of large quantities of crude peptide mixture injections. A total of ∼9 mg of a complex peptide mixture was injected on the column during numerous experiments. These experiments yielded a total of 2037 positive protein identifications. The performance of the column was verified by the apomyoglobin standard after every injection.

Conclusions
These results show that the VYDAC® column has high loading capacity and retains quality resolution after extended use. This column can be used for rapid identification of a large number of proteins from complex mixtures in a robust and high-throughput manner.

Acknowledgment
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References