Quality control of recombinant peptide and protein drugs by capillary HPLC

Biopharmaceutical

In the pharmaceutical industry recombinant and genetically engineered peptide and protein drugs are gaining more and more importance compared to traditional small molecule drugs. Although only a limited number of peptide and protein drugs are presently on the market, their number and market share will significantly increase over the next few years. More than 80 new peptide and protein drugs are waiting for introduction.

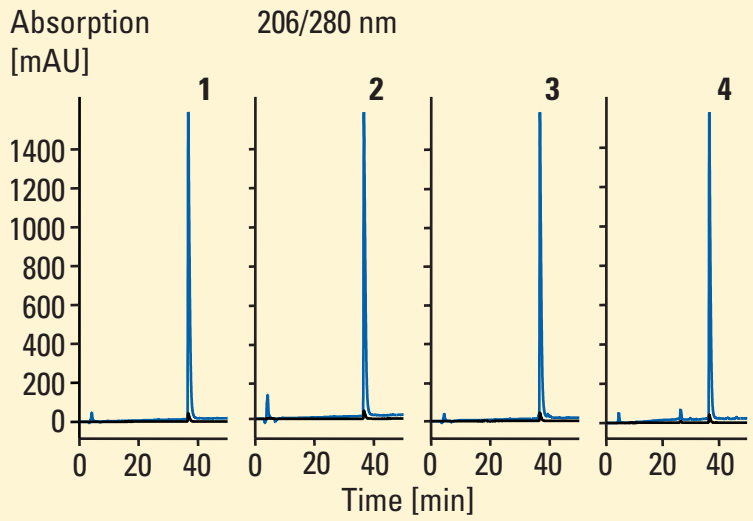

Figure 1
Quality control of four recombinant insulin batches by capillary reversed-phase HPLC

Conditions

Column
0.3 x 250 mm Vydac C-18 (LC Packings, The Netherlands)
Mobile phase
A = 0.025 % TFA
B = acetonitrile, 0.020 % TFA
Flow rate
0.1 ml/min with flow split to 4 µl/min
Flow splitter
VAR-100 (LC Packings, The Netherlands)
Gradient
0–5 min 2 % B
5–45 min 60 % B
UV detector
diode array detector
206 nm, 280 nm
spectra 200–320 nm
500 nl flow cell kit
Temperature
ambient

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These drugs are typically fermented in large scale units using *E.coli* or yeast for overexpression, in order to obtain larger and purer qualities of the peptides and proteins compared to isolation from natural sources. After overexpression in the host organism, the overexpressed peptide and protein drugs have to be purified from the host proteins to homogeneity, typically by classical methods such as ammonium sulfate precipitation, ion exchange chromatography, gel filtration chromatography or affinity chromatography. After the final purification step the degree of purity has to be tested for every batch ensuring a highest degree of purity.

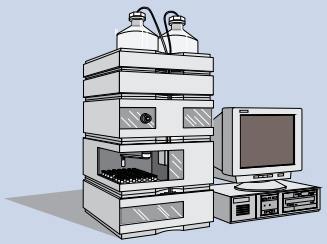
1D gels or HPLC are routinely used for quality control. Mainly 4.6-mm id columns are used to test the purity of the preparations. Here, we describe the usage of capillary HPLC which is superior to conventional HPLC for several reasons. It combines the great resolution capability due to the high plate numbers of the capillary columns with UV detection. This allows quantitation and only little sample consumption which is important when analyzing limited or very expensive material. In addition, capillary HPLC can be easily connected to a mass spectrometer for additional mass information of the components due to its flow rate of 1–4 µl per minute which is the ideal flow rate for mass sprectometers.

As a typical example, quality control of several batches of recombinant insulin preparations using capillary reversed-phase HPLC is shown in figure 1. All chromatograms clearly show the major insulin component at a retention time of about 37 minutes using the parameters described. Quantitative analysis of the four batches show impurities of 0.3 % in sample 1, 0.3 % in sample 2, 1.9 % in sample 3 and 4.0 % in sample 4.

Equipment

Agilent 1100 Series

- Binary pump (includes vacuum degasser)
- Autosampler
- Diode array detector capillary flow cell 10-mm path length, 500 nl cell volume
- Agilent ChemStation + 3D software



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