# Simple reversed-phase purification of human Erythropoietin (EPO) from fully formulated drugs

**Biopharmaceutical**

Erythropoietin (EPO) is a glycoprotein hormone with about 40 % sugar moiety involved in the regulation of the erythrocyte level by stimulating differentiation of erythroid progenitor cells to mature cells. The hormone is produced in the kidney and brought to the target cells in the bone marrow via blood circulation. Since EPO increases the number of peripheral red blood cells, there has been a considerable interest in the therapeutic use of EPO for the treatment of severe anemia. However, EPO is also massively misused for doping purposes. The current pharmaceutical market value is more than 1 billion US dollars.

In the formulation procedure of recombinant protein drugs other non-physiologically active proteins, such as human serum albumin, are often added in large excess to enhance the stability of the drug protein. After formulation protein drugs have to be proteinchemically investigated for the native structure besides activity and other tests. For protein chemical analysis of EPO a single step purification procedure using C-18 reversed-phase chromatography has been developed allowing EPO to be purified to homogeneity for further analysis by peptide mapping, mass spectrometry and protein sequencing.

Figure 1 shows that EPO can be purified from a fully-formulated drug on a C-18 reversed phase column with a simple water/acetonitrile gradient although it contains up to 40% sugar moeity. EPO (peak 3) elutes as a very sharp peak from the C-18 column and is nicely separated from the large excess of human serum albumin (peak 2) and an unknown component (peak 1) in the drug which shows a tryptophan second derivative spectrum just as EPO does (major minimum at 290 nm). Human serum albumin does not contain a tryptophan residue and can therefore be distinguished from EPO by it´s characteristic tyrosine second order derivative spectrum (minimum at 284 nm).

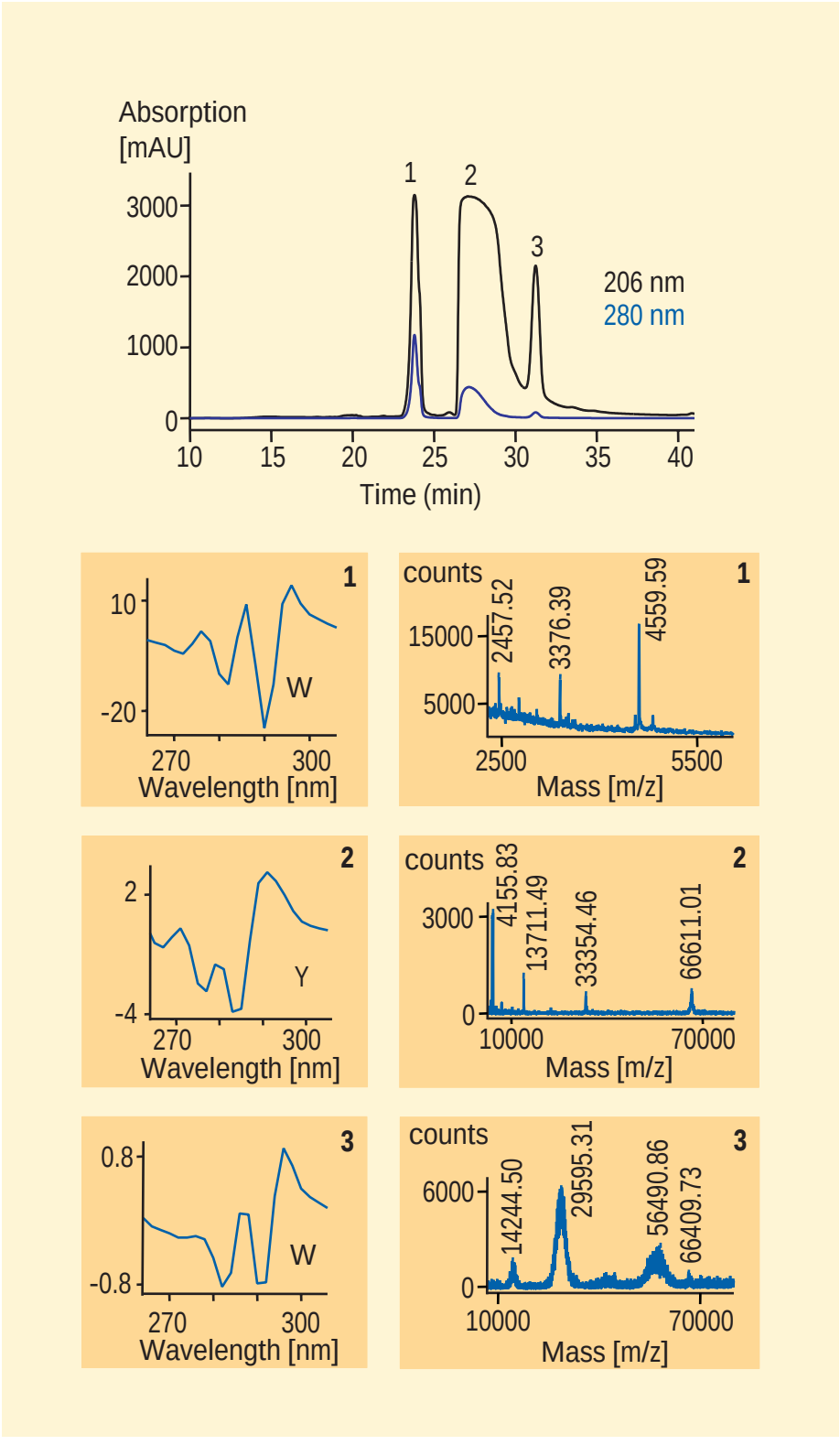


Figure 1
One-step purification of EFO on a 4.6 x 250-mm id Vydac C-18 column

## Conditions

**Column**
4.6 x 250 mm Vydac C-18
**Mobile phase**
A = 0.05 % TFA
B = acetonitrile, 0.045 % TFA
**Flow rate**
0.45 ml/min
**Gradient**
0–5 min 2 % B
5–40 min 80 % B
**UV detector**
diode array detector
spectra 200–320 nm
**Temperature**
ambient

## Equipment

Agilent 1100 Series
- Binary pump (includes vacuum degasser)
- Autosampler
- Diode array detector
  semi-micro flow cell
  6-mm path length
  5-µl cell volume
- Agilent ChemStation
  + 3D software


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