

Maximising the separation capacity in SEC by coupling two YMC-Pack Diol columns

Size exclusion chromatography (SEC) separates analytes based on their hydrodynamic radius. However, since the hydrodynamic radius is related to the molecular weight (MW) of the analyte (assuming that the analyte has a globular shape) in many cases, the analyte's MW can be considered to be the separation parameter. Therefore, the pore size of a stationary phase plays a key role in SEC. It determines a range over which the analytes' molecular weight is separated by SEC. If the pore size is too small,

the analyte is excluded from the pores and elutes without retention. Conversely, if the pore size is too large, the analyte diffuses throughout the pore and elutes with the same elution volume as other low MW analytes. Figure 1 shows the calibration curves for three columns with different pore sizes. This clearly shows the correlation: by increasing the pore size of the stationary phase, the resolution improves for higher MW analytes and increases for smaller MW analytes with reduction in pore size.

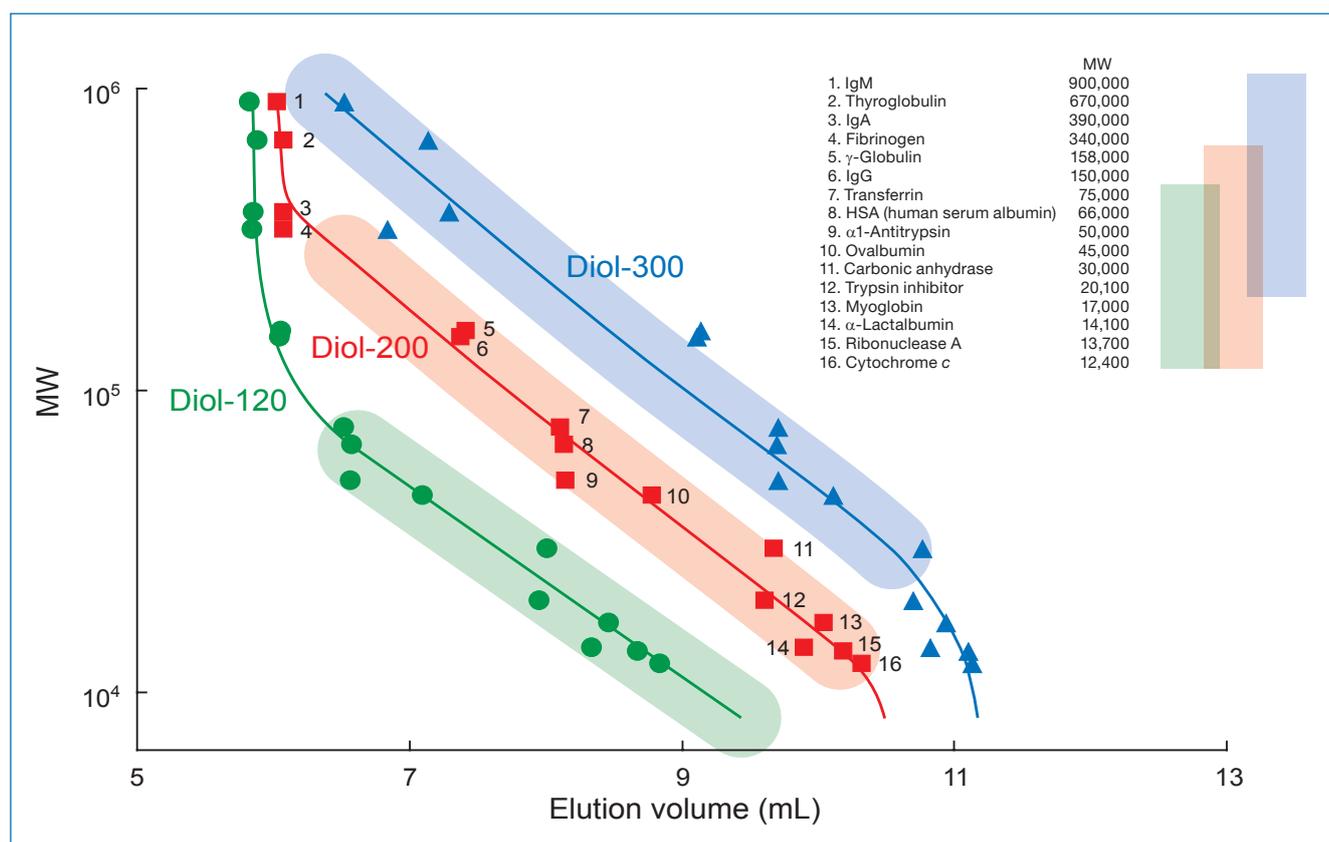
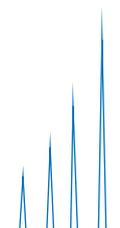


Figure 1: Calibration curves of YMC-Pack Diol-120, YMC-Pack Diol-200 and YMC-Pack Diol-300 for analytes with a molecular weight ranging between 12,400 and 900,000 Da.

Whilst the use of only one column brings a certain limitation due to its dedicated pore size and a limited column volume. However, by coupling two columns with different pore sizes this hurdle can be overcome. Whilst the sep-

aration volume is increased by use of two columns, the molecular weight range can be extended by using two different pore size ranges. Both contribute positively to maximising the separation capacity.



In Figure 2, the analysis of plasma constituents with two coupled columns is shown as an example. By coupling a **YMC-Pack Diol-300** and a **YMC-Pack Diol-200** column, a much broader molecular weight range can be analysed

compared to that using only one column. And doubling the column length (2x300mm) results in improved resolution of peaks, covering a range from <1 kDa to 900 kDa.

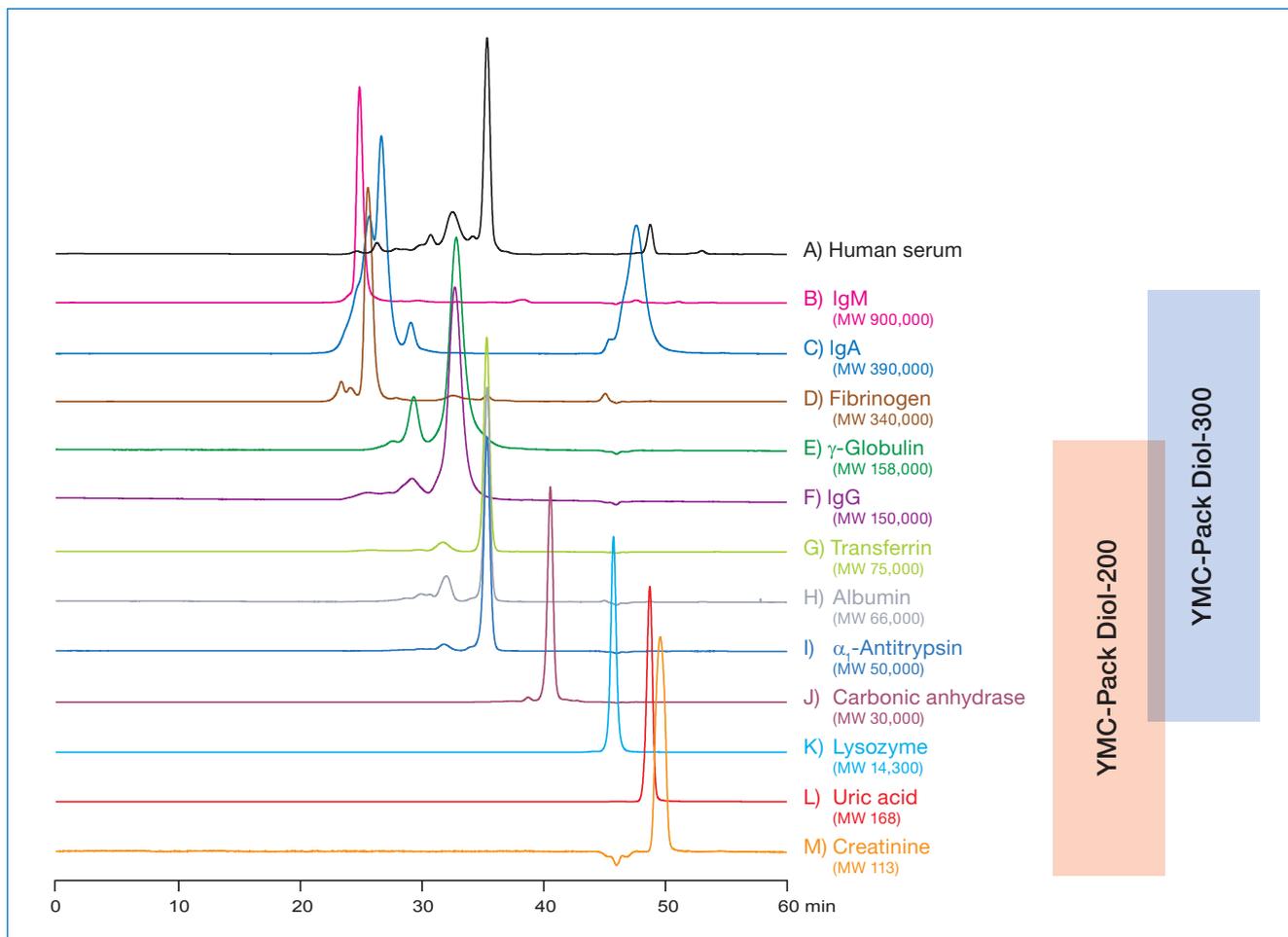


Figure 2: Analysis of plasma constituents by coupling two columns: **YMC-Pack Diol-300** and **YMC-Pack Diol-200**.

Columns:	YMC-Pack Diol-300 + YMC-Pack Diol-200, (5 μ m) 300 x 8.0 mm ID x 2
Part Nos.:	DL30S05-3008WT + DL20S05-3008WT
Eluent:	0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate:	0.5 mL/min
Temperature:	ambient (25 $^{\circ}$ C)
Detection:	UV at 280 nm
Injection:	20 μ L (L: 1 μ L)
Sample:	A) 100 μ L/mL; B-M) 1.0 mg/mL

