CIM Convective Interaction Media®
APPLICATION NOTE

A024 Rapid Separation of IgG, IgA and IgM on a CIM® QA Disk Monolithic Column

The demand for monoclonal antibodies is invariably increasing on an annual basis. To satisfy increasing demands, faster and cheaper ways of manufacturing are explored. A quest for alternative paths in manufacturing not only requires development of most economical manufacturing process, but also rapid method development and development of good analytics for monitoring of manufacturing. For a quickly developed process, the use of reliable and fast analytical techniques are crucial. Moreover, this analytical technique should than be preferably used also for in-process control during manufacturing stage.

Here we present fast and reliable method for processing and analyzing IgG, IgA and IgM using CIM® QA Disk Monolithic Column, which thrive upon speed, repeatability and high capacity.

**Figure 1: Separation of IgG, IgA and IgM on a CIM® QA Disk Monolithic Column**

- **Separation mode:** Anion-exchange chromatography
- **Instrumentation:** Gradient HPLC system with an Agilent mixing chamber (V = 0.5 ml)
- **Separation device:** CIM® QA Disk Monolithic Column (12 mm I.D. x 3 mm); V = 100 µl
- **Sample preparations:** A mixture of human IgG (Octapharma, Md = 150 kDa), IgA (Sigma, 028K3776, Md = 160 kDa) and IgM (Sigma, I8260, Md = 950 kDa) dissolved in 20 mM Tris-HCl buffer, pH 7.4
- **Injection volume:** 50 µl
- **Mobile phase:** Buffer A: 20 mM Tris-HCl buffer, pH 7.4
  Buffer B: 20 mM Tris-HCl buffer + 1.0 M NaCl, pH 7.4
- **Conditions:** A linear gradient from 0 % buffer B to 35 % buffer B in 4 min; flow rate: 3 mL/min; Temperature: ambient; column pressure: 2 bar (0.2 MPa) UV detection at 280 nm.

A complete separation of all three immunoglobulins within less than 5 minutes was achieved.
RESULTS

Strong anion exchange CIM® QA Monolithic Columns can be used for fast separation (less than 5 min) of different immunoglobulins. Further data is being produced to demonstrate application of CIM® QA Monolithic Columns for in-process and final control, as well as for process development and scale-up to large scale.

CIM® technology is covered by US patents 4889632, 4923610, 4952349 and 5972218. Other patents pending.