CIM Convective Interaction Media®
APPLICATION NOTE

A033 Fast analytical HPLC method for quantitative determination of the monoclonal IgM using CIMac™ Analytical Column

Process Analytical Technology (PAT) is of crucial importance in the process of IgM manufacturing, especially in its optimization where fast and reliable analytical methods capable of quantitation of the corresponding recombinant IgM concentration levels in the upstream processes are required. Convective Interaction Media CIM® strong anion exchange monolithic columns have a great advantage in comparison to particle related methods due to their separation capability based on the convective flow mechanism that proved to be particularly efficient in the separation of large IgM molecules.

Sample and methods

A continuous perfusion system with cell retention in a bioreactor for IgM antibody production, expressed in recombinant CHO host cells, was established. Cell- and cell-debris-free culture supernatants were prepared by depth filtration for the purposes of the IgM analysis.

**Column:** CIMac™ QA Analytical Column (CV: 0.1 mL); Catalog number: 110.5113
**Mobile phases:** Buffer A: 0.01 M phosphate buffer (NaH₂PO₄) pH 7.0
Buffer B: 0.25 M phosphate buffer (NaH₂PO₄) pH 7.0
**Flow rate:** 1 mL/min
**Step gradient method:** 100% buffer A (1 min), 40% buffer B (1 min), 100% buffer B (1 min) and 100% buffer A (1 min), method depicted on Figure 1
**Sample loop:** 50 µL
**Detection:** Fluorescence detection at λexc = 280 and λem = 348.

Figure 1: [A] Chromatograms of a blank cell supernatant sample; [B] cell supernatant containing IgM
RESULTS

A fast analytical method for determining the recombinant monoclonal IgM using a CIMac™ QA Analytical Column (fluorescence detection at λexc = 280 and λem = 348) was developed. The developed method enabled the separation of IgM from other excipients in the cell supernatant. All supernatant constituents were eluted in the flow through fractions or prior to a step elution of the IgM. The method was linear in the range of 5 µg/mL to 100 µg/mL and had an LOQ of 5 µg/mL.

More details can be found in the following articles:
