


A040

HPLC fingerprint characterization as a process analytical technology in the processing of Cohn I+II+III derived IgG

The demand for human normal immunoglobulin is invariably increasing on an annual basis. To satisfy increasing demands, different manufacturing processes are used to isolate immunoglobulins from human plasma. 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 an efficient development of the purification methods as well as for in-process control during manufacturing stage, the uses of reliable and fast analytical techniques are crucial.

Fast and reliable fingerprint-based method for characterization of immunoglobulin G (IgG) prepared from Cohn I+II+III paste in two chromatographic steps is presented here. The fingerprint method bases on partial separation of proteins in linear gradient on CIMac™ QA 0.1 mL column. Partial separation of proteins does not allow simple quantitative analysis of the samples, however a very accurate qualitative information about the composition of the sample being analyzed can be obtained in less than 5 minutes.



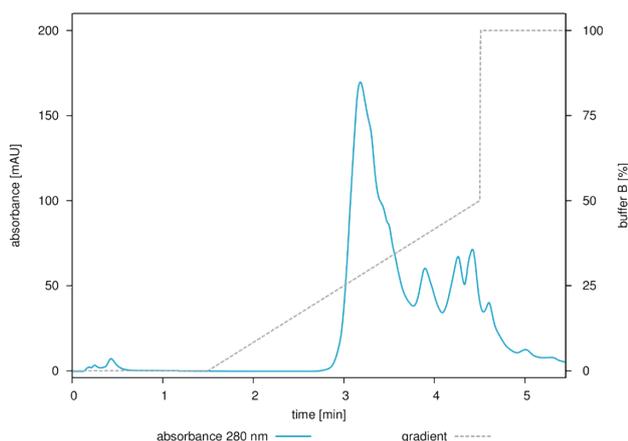
SAMPLE AND METHODS:

<i>Column:</i>	CIMac™ QA Analytical Column (CV: 0.1 mL); Catalog number: 110.5113
<i>Mobile phases:</i>	Buffer A: 50 mM TRIS-HCl pH 8.5 Buffer B: 50 mM TRIS-HCl, 1 M NaCl pH 8.5
<i>Flow rate:</i>	1 mL/min
<i>Sample loop:</i>	50 µL
<i>Detection:</i>	UV absorbance at 280 nm
<i>Gradient method:</i>	
<i>Equilibration:</i>	20 CV buffer A
<i>Load:</i>	15 CV buffer A
<i>Linear gradient:</i>	30 CV from 100 % buffer A to 50 % buffer A
<i>Regeneration:</i>	10 CV buffer B
<i>Re-equilibration:</i>	30 CV buffer A

RESULTS:

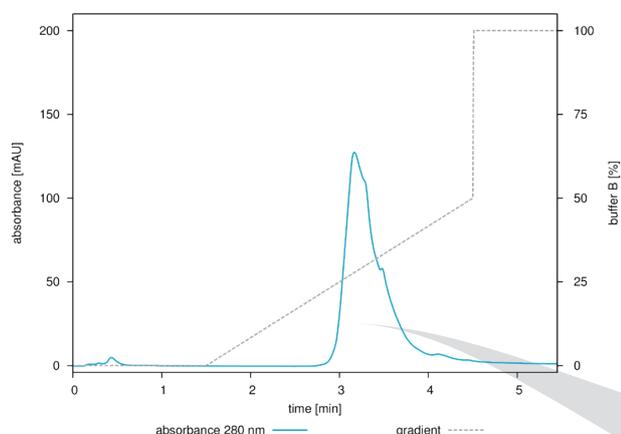
> Figure 1

Chromatographic profile of re-suspended Cohn I+II+III on a CIMac™ QA 0.1 mL monolithic column.



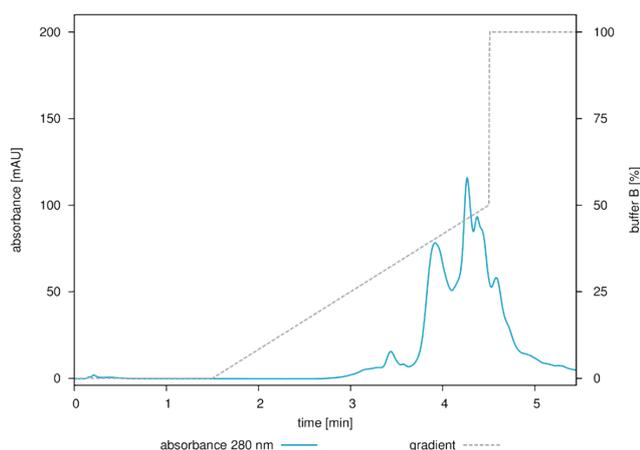
> **Figure 2**

Chromatographic profile of partially purified IgG (after first chromatographic step) on a CIMac™ QA 0.1 mL monolithic column. Note that the small ghost peaks in the second part of the main peak are due to partial separation of IgG subclasses on QA monolith under given conditions. The amount of impurities in the sample is minimal (cf. Figures 1 and 3).



> **Figure 3**

Chromatographic profile of impurities on a CIMac™ QA 0.1 mL monolithic column separated from IgG during the first chromatographic purification.



Fingerprint method bases on separation of proteins in linear gradient on CIMac™ QA 0.1 mL column. At the given conditions, IgG elutes in the linear gradient first, followed by elution of impurities (with a relatively large portions of IgA and IgG). From the differences in the chromatograms of various samples the ratio between IgG and impurities in each sample can be easily assessed. The method is suitable for input material control, in-line monitoring of the DSP, final control of the products as well as in stability studies. The method allows making rapid and accurate decisions helping to make the production process better and more effective.



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