QA/QC of monoclonal antibodies: Charge variants and Disulfide linkage analysis

M. Sundaram Palaniswamy, Ravindra Gudihal and Siji Joseph

Agilent Technologies India Pvt. Ltd, Bangalore, India

HPLC 2013

Poster number - BIOP20_TU

Agilent Technologies

Introduction

Monoclonal antibodies (mAb) are glycoproteins of the immunoglobulin (Ig) family. MAbs have become the most rapidly growing class of biotherapeutics in the development for many different disease conditions. Novel mAb molecules are entering clinical studies at a rate of almost 40 per year, and the research pipeline includes approximately 250 therapeutic mAbs in clinical studies. There is steadily increasing need for an analytical method that can be used for high-throughput analysis of a large number of samples to support bioprocesses and formulation development. Here we describe a pH gradient based method for separating the charge variants of IgG1 using a 1260 Infinity Bio-inert Quaternary LC System and a Bio MAb NP 5 PEEK, 4.6 × 250 mm, ion exchange column and a method for separating and analyzing the disulfide linkages of IgG1 by reversed phase HPLC on ZORBAX RRHD 300 Diphenyl, 2.1 × 100 mm, 1.8-µm column.

Experimental

Materials and methods

Human monoclonal antibody IgG1 was a proprietary pharmaceutical molecule. DL-Dithiothreitol (DTT), iodoacetamide, trizma base, ammonium carbonate, sodium phosphate monobasic and dibasic, sodium chloride were purchased from Sigma Aldrich. High quality sequence grade trypsin was from Agilent Technologies. All the chemicals and solvents used were HPLC grade and highly purified water from a Milli Q water purification system (Millipore Elix 10 model, USA) was used. Acetonitrile was of 'gradient grade' and purchased from Lab-Scan (Bangkok, Thailand).

Instrumentation

The Agilent 1260 Infinity Bio-inert Quaternary LC System

IEC Parameters for Charge variant analysis

Column used: Agilent BiomAb PEEK 4.6 x 250 mm, 5 µm

Mobile phase A: 10mM Sodium phosphate buffer, pH 6.0

Mobile phase B: 10mM Sodium phosphate buffer, pH 9.5

Injection volume: 10µL, Flow rate: 0.8 mL/min

Detection : UV 220nm and 280nm

Software: Agilent Chemstation B.04.02 (or higher)

RP HPLC Parameters for disulfide linkage analysis

Column used: ZORBAX RRHD 300 Diphenyl, 2.1 × 100 mm, 1.8-µm colum.

Mobile phase A: Water + 0.1 % TFA

Mobile phase B: Acetonitrile + 0.09 % TFA

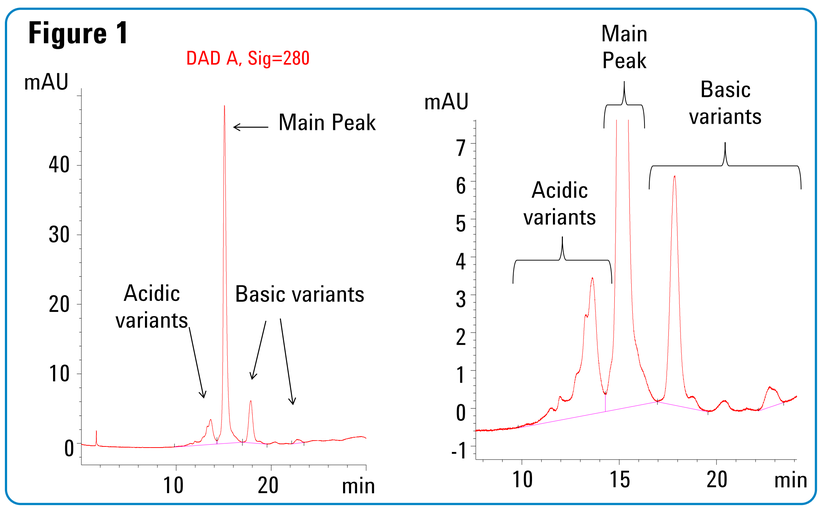
Injection volume: 3µL, Flow rate: 0.3 mL/min

Results and Discussion

Separation of mAbs Charge variants

Here we demonstrate a high-resolution pH gradient separation of charge variants of monoclonal antibody using BiomAb PEEK 4.6 x 250 mm, 5 µm column

Figure 1



The retention time and area RSDs for the main peak were less than 0.106 (limit±3.0%) and 1.60 (limit±5.0%) respectively demonstrating excellent gradient reproducibility and precise sample injection. To a great extent variation of injection volume, column temperature, pH and flow rate on retention time was within the acceptable limits for RT and Area RSDs

The high resolution separation of IgG1 allowed for the quantification of charge variants using peak areas (Figure 1). The relative peak areas for the charge variants of IgG1 are denoted in Table 1. The IgG1 possessed approximately 9.97% of acidic variants, 76.92% main peak and 13.21% basic variants of the total peak area, respectively. The reproducibility of analysis was tested with six replicates.

Table 1

	RT (min)	Area %
Acidic variant	13.28 13.61	9.87
Main Peak	15.058	76.92
Basic variant	17.82 22.69	13.21

Analysis of Disulfide linkages in mAbs

Figure 2 depicts the optimized RP HPLC elution profile of intact IgG1 on an Agilent ZORBAX RRHD 300 Diphenyl, 2.1 X 100mm, 1.8 µm column demonstrating excellent retention of IgG1 in 15 min. The effect of reduction and alkylation of the disulfide bonds in intact IgG1 was tested. Due to the reduction of the disulfide bonds, the IgG1 is separated into its light and heavy chains. The IgG1 eluted as distinct light chain (LC) and heavy chain (HC) as indicated in Figure 3.

Results and Discussion

Figure 2

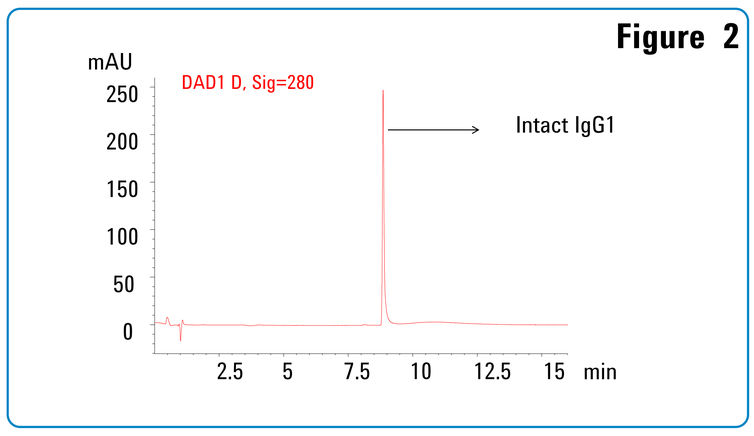
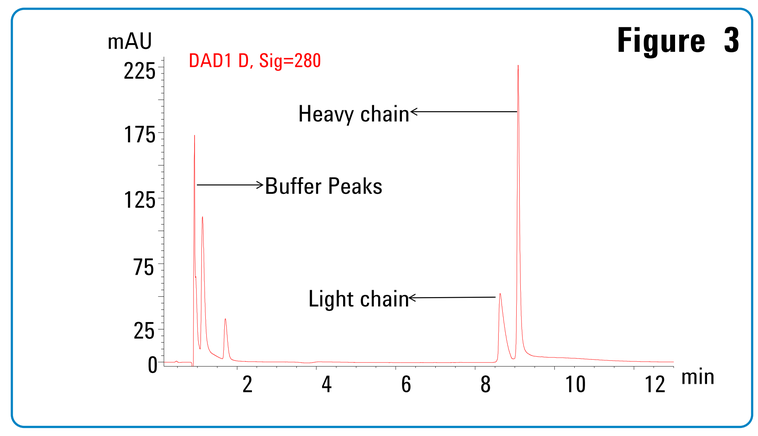


Figure 3



Peptide maps resulting from Lys C digestion of intact IgG1 under non-reducing conditions resulted in a less intricate RP HPLC profile as indicated in Figure 4. The overlay of Lys C peptide maps of non-reduced IgG1 (red trace) and reduced IgG1 (blue trace) is depicted in Figure 5. Appearance of additional peaks (indicated by an asterix) after reduction of Lys C digested IgG1 confirms they are bound via disulfide linkages.

Figure 4

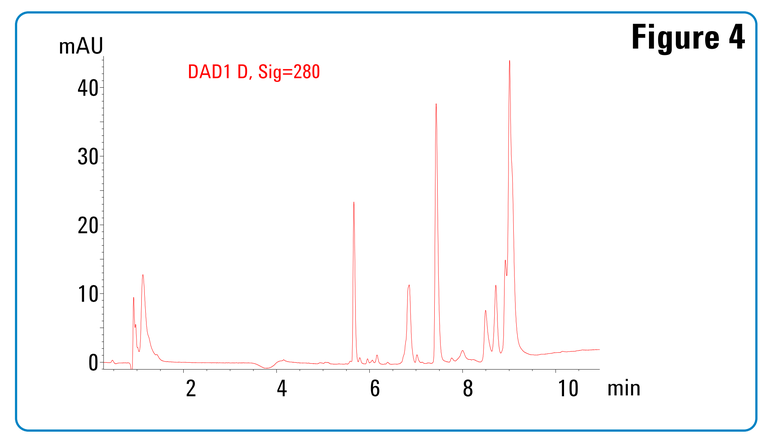
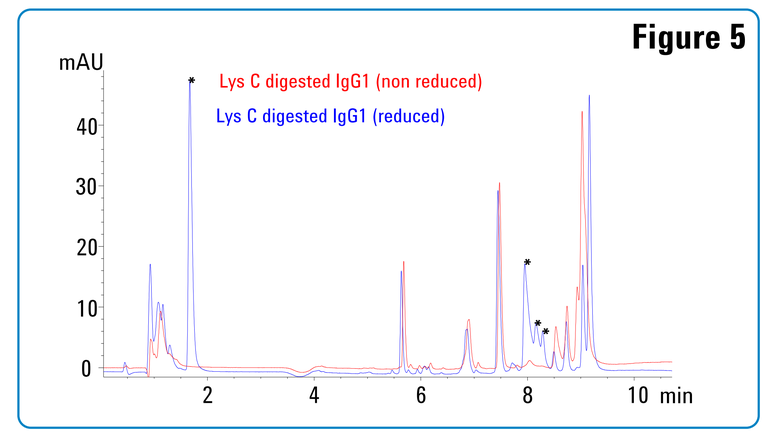


Figure 5



Conclusions

- A simple pH gradient based cation exchange method for separation and quantification of charge variants was developed. Area, RT precision and robustness of the method were excellent and show the reliability of the method.
- High resolution analysis of disulfide linkage of monoclonal antibodies for biopharma process development and monitoring was developed. Area and RT precision of the method were excellent and proved reliable.
- Further, the 1260 Infinity Bio-inert LC has a power range up to 600 bar and is capable of handling the higher pressures demanded by new emerging column technologies with STM down to 1.7 µm.