Characterization of a monoclonal antibody by optimized Peptide Mapping, SEC and IEX using Bio-inert HPLC

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Introduction

Like all other protein therapeutics, mAb can undergo structural modifications derived from oxidation, deamidation, aggregation or amino acid substitution etc during production, storage and transportation. Peptide mapping is an essential technique for studying the primary structure of proteins. For recombinant protein pharmaceuticals, peptide mapping is used for the initial 'proof of structure' characterization, that is, to confirm expression of the desired amino acid sequence and to characterize any posttranslational modifications. RP HPLC peptide mapping is a powerful technique for structural elucidation and structure confirmation of proteins.

Monitoring charge variants of mAb therapeutic is critical to ensure the safety and efficacy of the product. The stability data is expected by regulatory agency such as FDA and EMA. In the biotechnology industry, ion-exchange chromatography is widely used for profiling the charge heterogeneity of proteins, including monoclonal antibodies.

Aggregation of mAbs is highly undesired since it can lead to activity loss, decreased solubility, and enhanced unwanted immunogenicity. Size Exclusion Chromatography (SEC) has been widely used to characterize protein aggregates present in human IgG. It is the standard method for aggregation analysis and is also required for submission of protein therapeutics for regulatory approval.

Here we showcase several Agilent tools for the analysis of mAb. The versatility of Agilent 1260 Infinity Bio-inert Quarternary LC system for RP HPLC peptide mapping, charge variant analysis and Size exclusion chromatography of IgG1 is described using Agilent columns.

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

RP HPLC Parameters

Column used: Poroshell 120 SB C18 4.6 x 150 mm, 2.7 µm column and Zorbax Narrow bore RRHD SB C18, 2.1 x 150 mm, 1.8 µm column.
Mobile phase A: Water + 0.1 % TFA
Mobile phase B: Acetonitrile + 0.09 % TFA
Injection volume: 10µL, Flow rate: 1.2 mL/min and 0.25mL/min

SEC Parameters

Column used: Agilent Bio SEC-3, 300Å, 7.8 x 300 mm, 3 µm column
Equilibration buffer : 150mM Sodium phosphate buffer, pH 7.0 + 150mM NaCl
Injection volume: 5µL, Flow rate: 0.8 mL/min
Detection : UV 220nm and 280nm

IEC Parameters

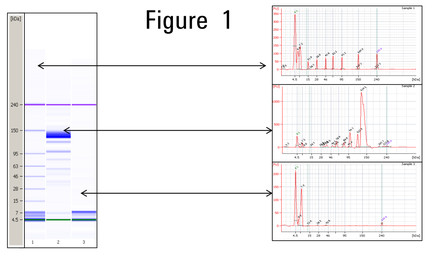
Column used: Agilent BiomAb PEEK 4.6 x 250 mm, 5 µm
Mobile phase A: 10mM Sodium phosphate buffer, pH 6.0
Mobile phase B: 10mM Sodium phosphate buffer, pH 9.5
Injection volume: 10µL, Flow rate: 0.8 mL/min
Detection : UV 220nm and 280nm
Software: Agilent Chemstation B.04.02 (or higher)

Results and Discussion

Peptide mapping of mAbs

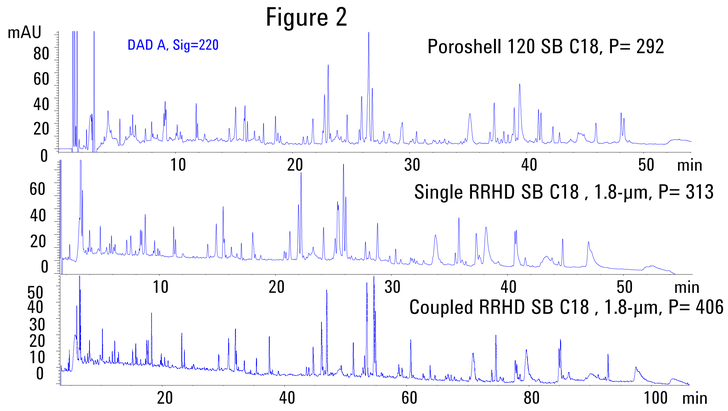
Agilent 2100 Bioanalyzer was used to qualify the efficiency of the digestion protocol. Comparison of undigested IgG1 and digested IgG1 clearly show the absence of a protein band in the digested sample demonstrating complete digestion of IgG1 (Figure 1).

Figure 1



RP HPLC of IgG1 digest on 1260 Infinity Bio-inert LC System with Poroshell 120 SB C18, column , 4.6 × 150 mm,2.7µm results in sharp peaks with good resolution and excellent separation reproducibility (Figure 2). Average retention times and area RSDs for the four selected peaks from six replicates were less than 0.05 % and 2.14% demonstrating excellent gradient reproducibility and precise sample injection. Robustness study showed that the method is reliable for routine QA/QC application. To a great extent, the performance remains unaffected by variations in critical parameters.

Figure 2

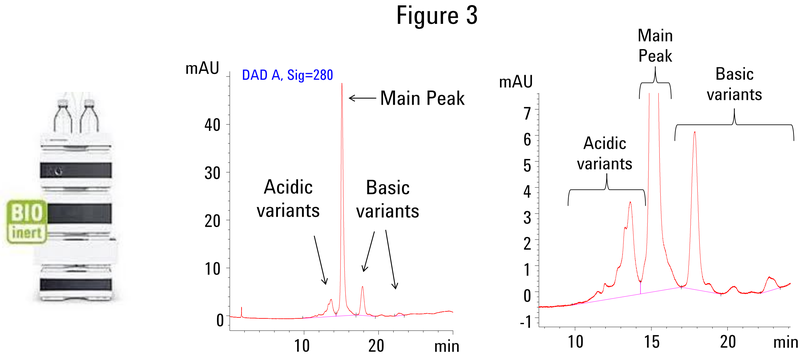


The 1290 Infinity LC System with a single or coupled narrow bore columns produced sharper and narrower peaks as compared to 1260 Infinity Bio-inert LC System as indicated by an increased peak capacity (P) (Figure 2). Peak capacity increased 7% with single RRHD column and 39% with coupled columns indicating an exceptional ability for separating complex samples.

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 3



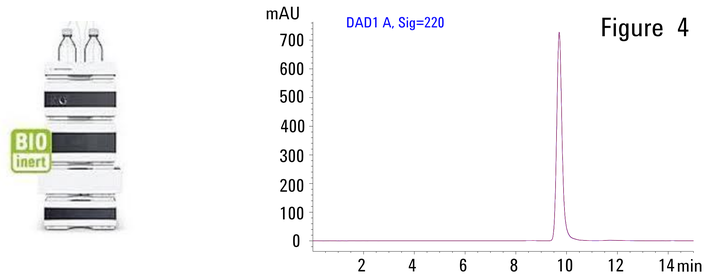
The retention time and area RSDs for the main peak were less than 0.106 (limit±3.0%) and 1.60 (limit±5.0%) respectively demonstrating excellent gradient reproducibility and precise sample injection. To a great extent variation of injection volume, column temperature, pH and flow rate on retention time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

Results and Discussion

Size Exclusion Chromatography of mAbs

SEC of IgG1 on Bio SEC-3, 300Å, 7.8 x 300 mm, 3 µm column (Figure 4) demonstrates excellent separation of intact IgG1. The retention time RSDs and peak area were 0.008 % (limit±3.0%) and 0.639 (limit±5.0%) respectively demonstrates excellent reproducibility of the method and thus the precision of the system. There were no significant changes in the chromatographic pattern when deliberate variations were made in experimental conditions, thus showing the method is robust.

Figure 4



Linearity curve using area response with eight standard concentrations of IgG1 ranging from 12.5 to 2000 µg/mL showed excellent coefficient value. The LOD and LOQ were found to be 12.5µg/mL and 25µg/mL, respectively, indicating that the method is sensitive (Figure 5).

Figure 5

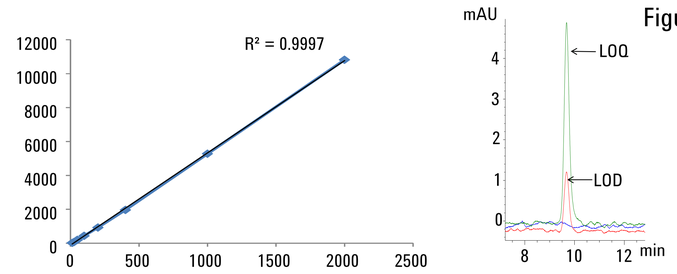
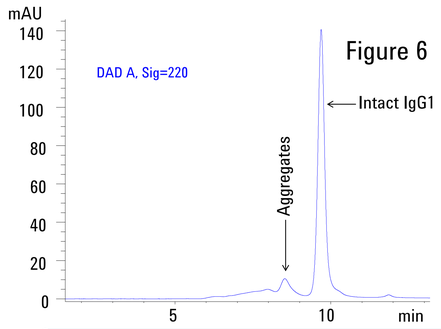


Figure 6



SEC profile (Figure 6) of IgG1 on Bio SEC 3 column with pH induced aggregates indicates that the method was able to separate and detect aggregates and intact IgG1.

Conclusions

- Agilent 1260 Bio-inert LC System was used to develop a simple and high resolution separation of a IgG1 tryptic digest. Area, RT precision and robustness of the method were excellent and show the reliability of the method.
- The method was transferred to an Agilent 1290 Infinity UHPLC system. Using 1.8-µm columns results in superior resolution as indicated by the increased peak capacity. Peak capacity can be increased further significantly by coupling two 1.8-µm columns.
- Good separation and resolution was found for a pH-gradient-based separations of charge variants of mAbs with BiomAb column. Area, RT precision and robustness of the method were excellent and show the reliability of the method.
- A simple and sensitive SEC method for identification and quantification of IgG1 was developed. Linearity curve of IgG1 (2.5 to 2000 µg/mL) shows excellent coefficient value indicating that SEC method is quantitative and accurate. The LOD and LOQ were found to be 12.5µg/mL and 25µg/mL, respectively, indicating that the method is sensitive. SEC on Bio SEC 3 column was able to separate and detect aggregates.
- The bio-inertness and corrosion resistance of the instrument coupled with simple and reproducible methods make this solution particularly suitable for the QA/QC analysis of monoclonal antibody for the biopharmaceutical industry.