Analysis of Bispecific mAb by Size Exclusion Chromatography and Mass Spectrometry

Keegan Gike and Atis Chakrabarti, Ph.D.

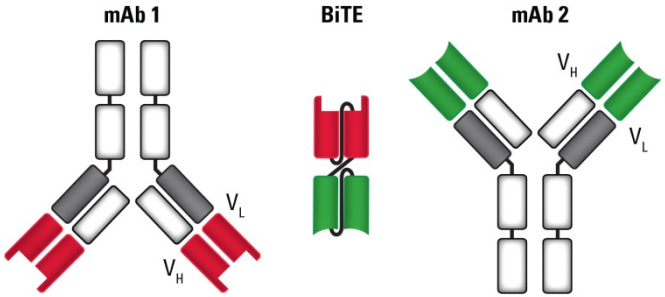
Tosoh Bioscience LLC, King of Prussia, PA

Introduction

- More potent formats of monoclonal antibodies (mAbs), such as bispecific antibodies (bsAbs) and antibody-drug conjugates, are on the rise in the area of biotherapeutics.
- More than 50 bispecific antibody products are currently undergoing clinical evaluation (*Ref: http://www.antibodysociety.org/category/bispecific-antibodies/).*
- Many in Pharma believe that bsAbs may evolve enough to replace mAbs as safer, more effective antibody-like treatments for cancer and other diseases.
- These modified antibodies engage two different targets simultaneously, thereby bringing T cells within reach of the targeted cell, with the intent of allowing T cells to inject toxins and trigger cancer cell death.
- With the significant increase in the research of biotherapeutics, the characterization by size exclusion chromatography (SEC) coupled with mass spectrometry (MS) is increasingly being used to identify the accurate molecular mass of biomolecules, including bsAbs.

Introduction

- The greatest challenges using SEC/MS for analyzing biomolecules are:
  - Interfacing SEC columns with MS due to the presence of the high concentration of non-volatile salts typically used in the SEC analysis of biomolecules
  - MS signal response at low analyte concentrations are affected by SEC column particle shedding, such as bonded phase
- Here we report the use of a TSKgel® UP-SW3000, 2 µm column for the separation of a bispecific antibody and the two parent mAbs (IgG_1) followed by MS analysis.
  - Bispecific T cell Engager (BiTE®) technology was used in this study. BiTE is a fusion protein consisting of two single-chain variable fragments (scFvs) - CD19, a biomarker for normal and neoplastic B cells, and CD3 (on T cells) - recombinantly linked by a nonimmunogenic five-amino-acid chain. BiTE is approximately 55 kDa in size.
  - SEC/MS analysis was performed by the Wistar Proteomics and Metabolomics Facility (*Philadelphia, PA*) using a Nexera® XR UHPLC system (*Shimadzu*) coupled to a Q Exactive™ Plus mass spectrometer (*Thermo Fisher Scientific*).



Formation of BiTE

Chromatographic Conditions using LC/MS Compatible Buffer

Column: TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm

Instrument: Dionex UltiMate® 3000 UHPLC system run by Chromeleon® (ver 7.2)

Mobile phase: 20 mmol/L ammonium acetate, 10 mmol/L ammonium bicarbonate; pH 7.2

Gradient: isocratic

Flow rate: 0.35 mL/min

Detection: UV @ 280 nm

Temperature: 30 ºC

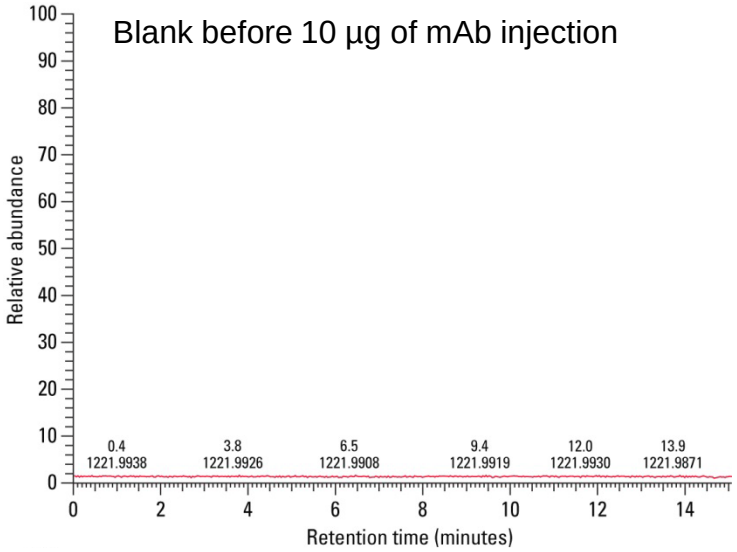
Injection vol.: 5.0 µL

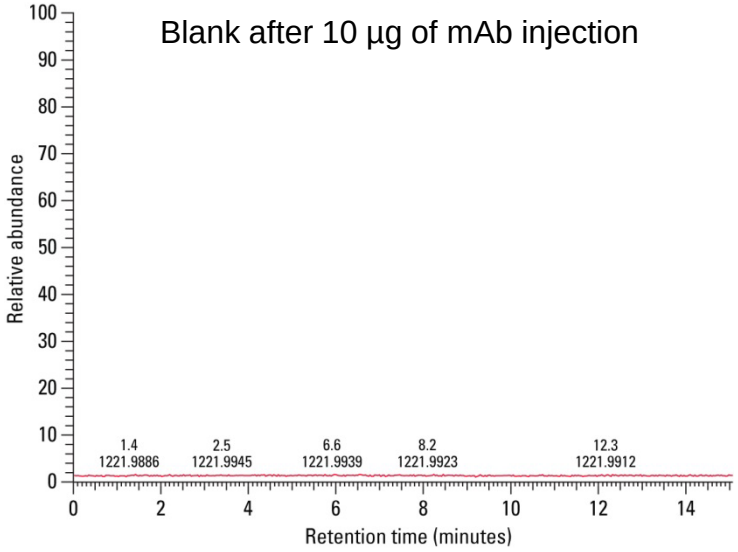
Samples: BiTE, 0.3 mg/mL (*Creative Biolabs*)
parent mAb shown, 0.5 mg/mL (*Creative Biolabs*)

Mass Spectrometric Conditions

- Eluted proteins were analyzed by the mass spectrometer set to repetitively scan m/z from 800 to 6000 in positive ion mode.
- The full MS scan was collected at 17,500 resolution, with spray voltage 4 kV, S-Lens RF 75, and in-source CID 80 eV. Protein mass deconvolution was performed using ProMass® (*Novatia*).
- Total ion chromatograms were collected; mass spectrum and peak deconvolution for the accurate mass are shown.

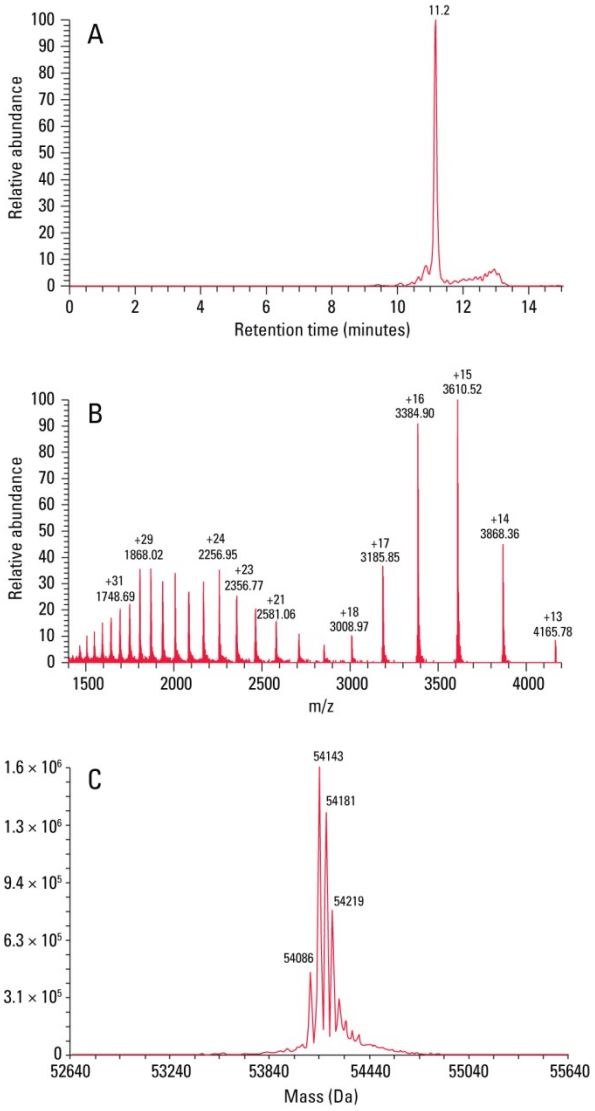
Shedding/Carryover Analysis





- A 15 minute blank isocratic gradient was run before and after sample injections.
- MS data indicates there is no shedding from the TSKgel UP-SW3000 column before and after sample injections.
- In addition, no carryover was observed in the runs after sample injections.

SEC/MS Analysis of BiTE



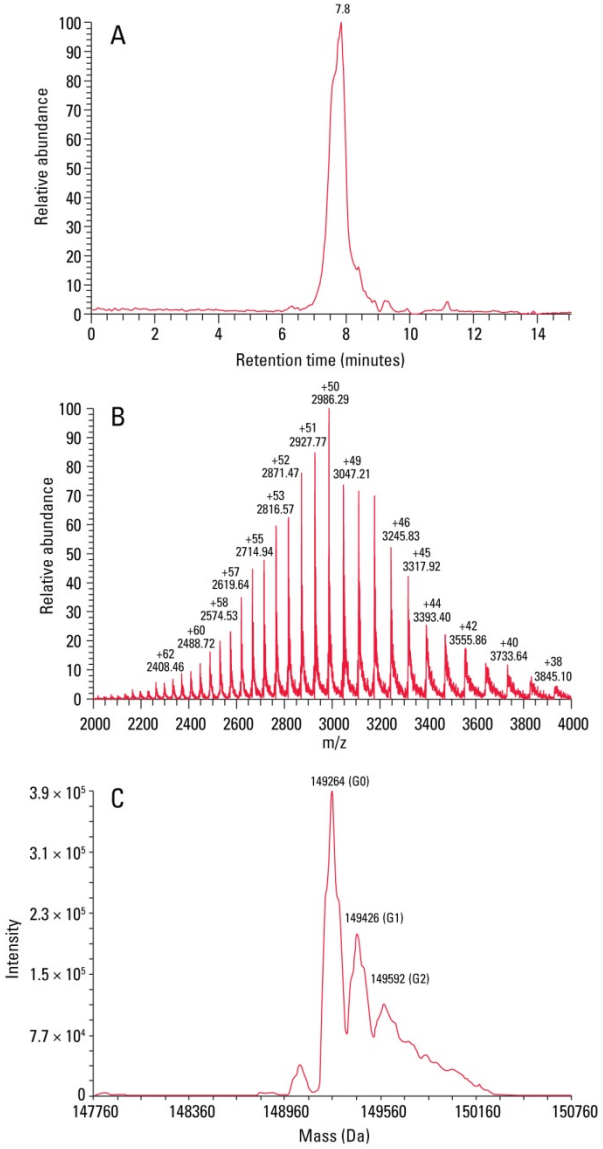
A: total ion chromatogram

B: mass spectrum

C: deconvoluted spectrum of BiTE

A main peak can be seen at m/z 54,143; adjacent peaks at m/z 54,181, 54,219 and 54,086 correspond to different salt adducts.

SEC/MS Analysis of mAb “Parent”



A: total ion chromatogram

B: mass spectrum

C: deconvoluted spectrum of mAb

A main peak can be seen at m/z 149,264; adjacent peaks at m/z 149,426 and 149,592 correspond to different glycoforms.

Conclusions

- TSKgel UP-SW3000, 2 µm SEC column can be used as a platform method for bispecific antibody analysis capable of resolving intact mAb and its fragments.
- A mass spec compatible mobile phase under non-denaturing condition was successfully used with the TSKgel UP-SW3000 column.