Development of an Analytical Toolbox for the Detection, Confirmation and Characterisation of Partially Reduced Species in Monoclonal Antibodies

Analytical Technologies Europe

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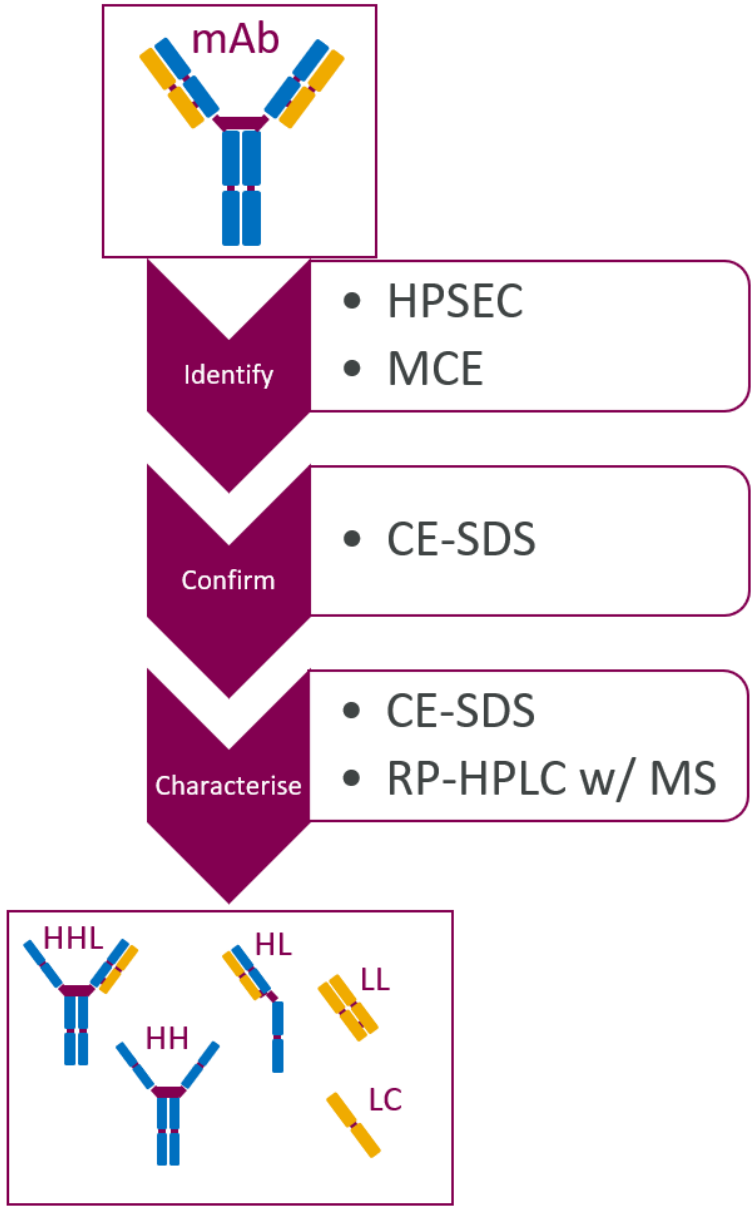
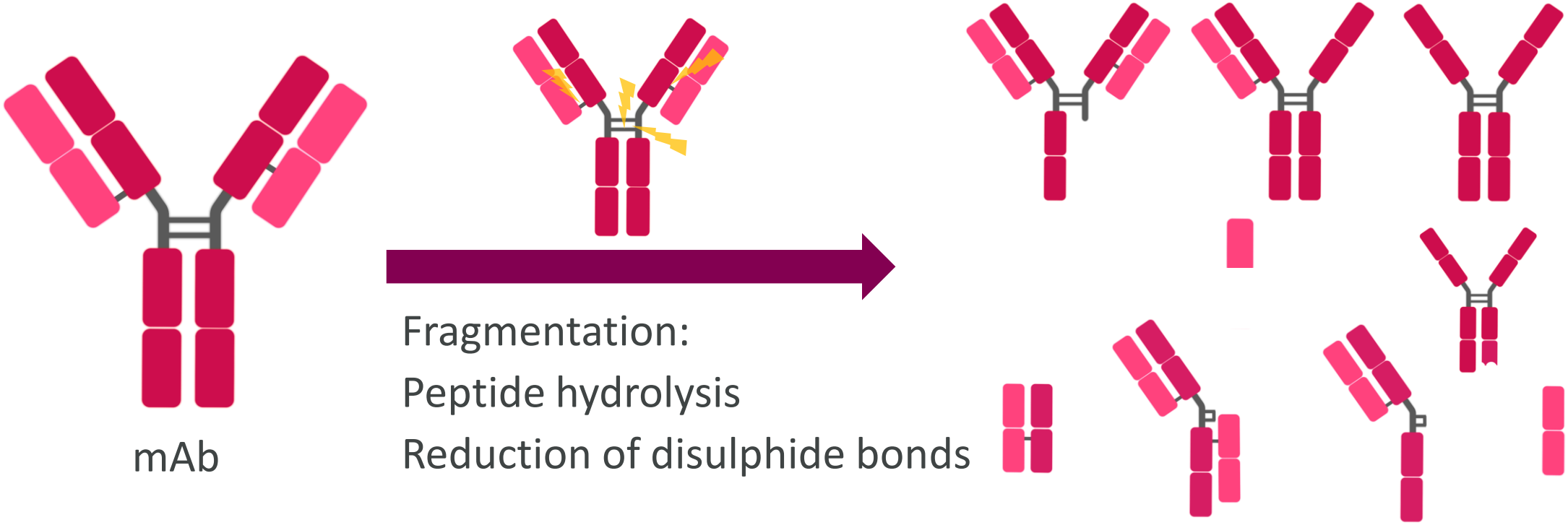


Overview

- Introduction
- Detection:
  - Fragmentation monitoring
  - Anomalies observed during routine testing
- Confirmation:
  - PA800+ (gold standard technique)
  - Possible cause of fragmentation
- Characterisation
  - Heavy Chain or Light Chain dimer?
  - LC-ESI-QTOF-MS
- Summary and questions

Fragmentation

A major degradation pathway ubiquitous to all monoclonal antibodies



mAb

Fragmentation:

Peptide hydrolysis

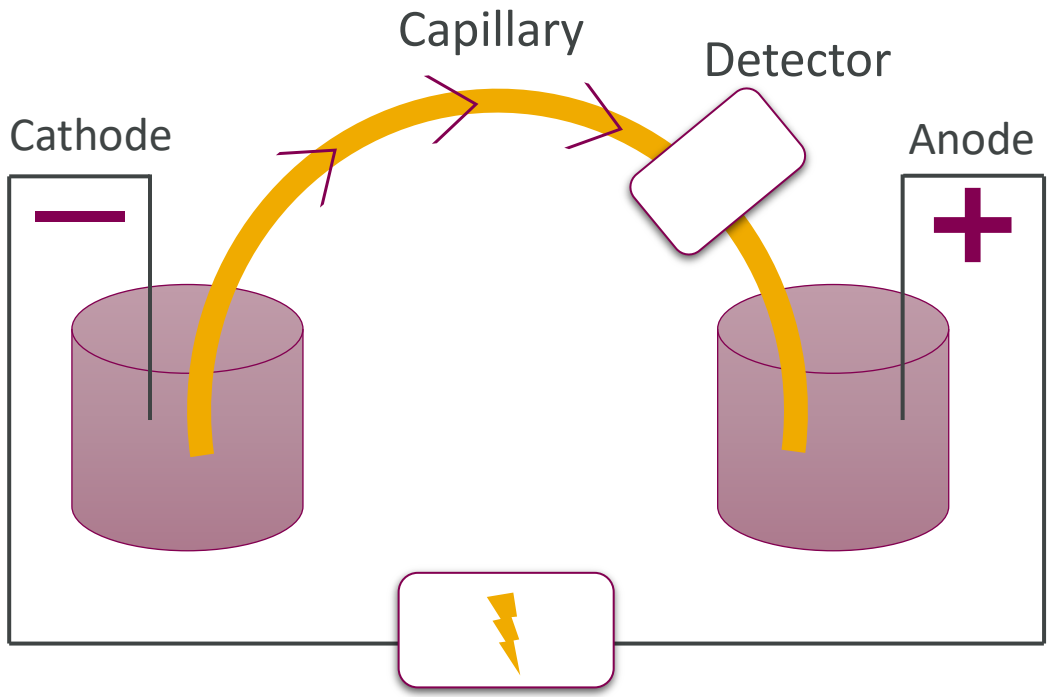
Reduction of disulphide bonds

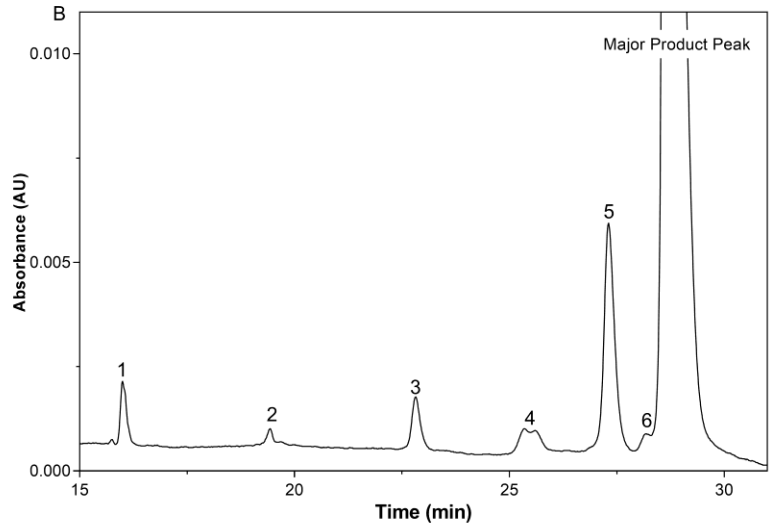
Important to monitor due to safety & efficacy concerns!

Figures obtained from BioRender

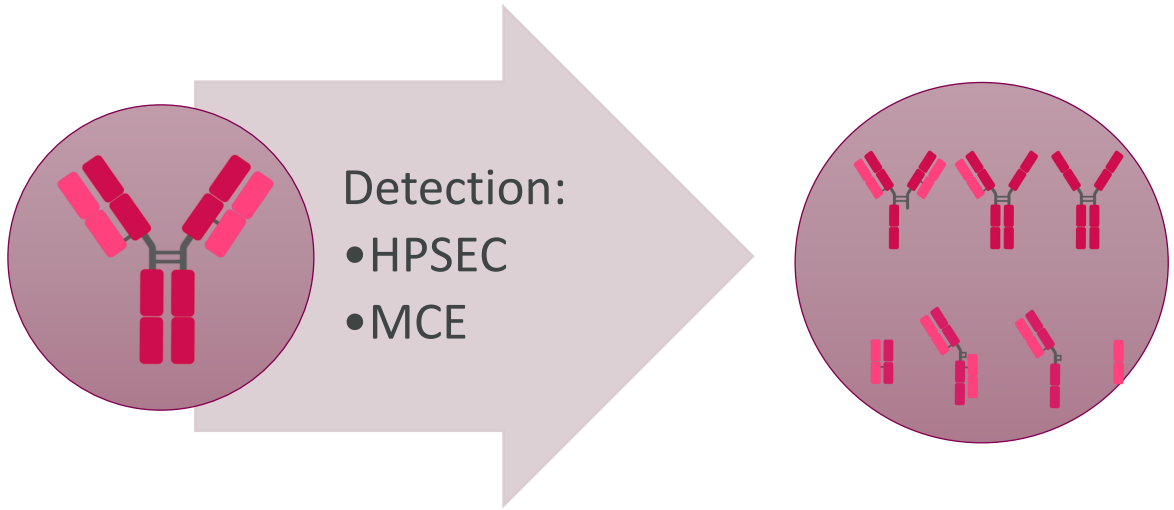
A (brief) introduction to CE-SDS

- Capillary Electrophoresis – Sodium Dodecyl Sulphate
- Proteins are denatured using SDS -> gives proteins similar mass to charge ratio
- Protein is separated in capillary based on their hydrodynamic size





Detection



HPSEC= High Performance Size Exclusion Chromatography

MCE= Microchip Capillary Electrophoresis

Purity Monitoring

Lab Chip GX II Touch by Perkin Elmer

- Microchip CE-SDS
- High Throughput capabilities
- Used for screening high numbers of in process samples

PA800 plus by SCIEX

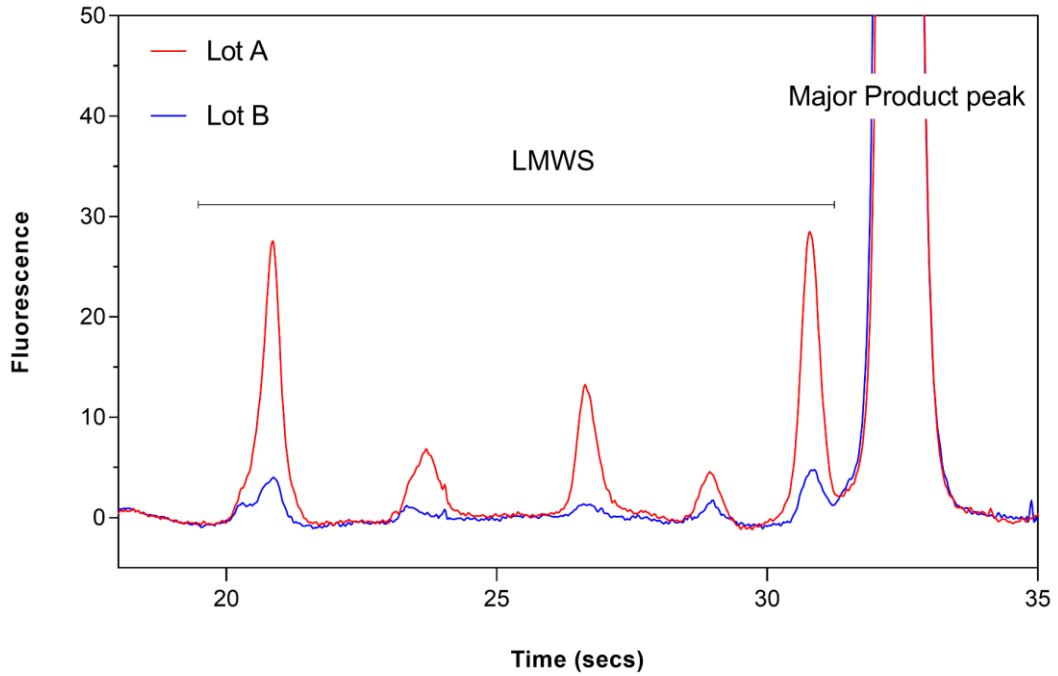
- Industry gold standard for CE-SDS – highly resolved fragments
- Lower throughput capabilities

Size Exclusion Chromatography

- Better suited for aggregate detection than fragments
- Underestimates fragmentation

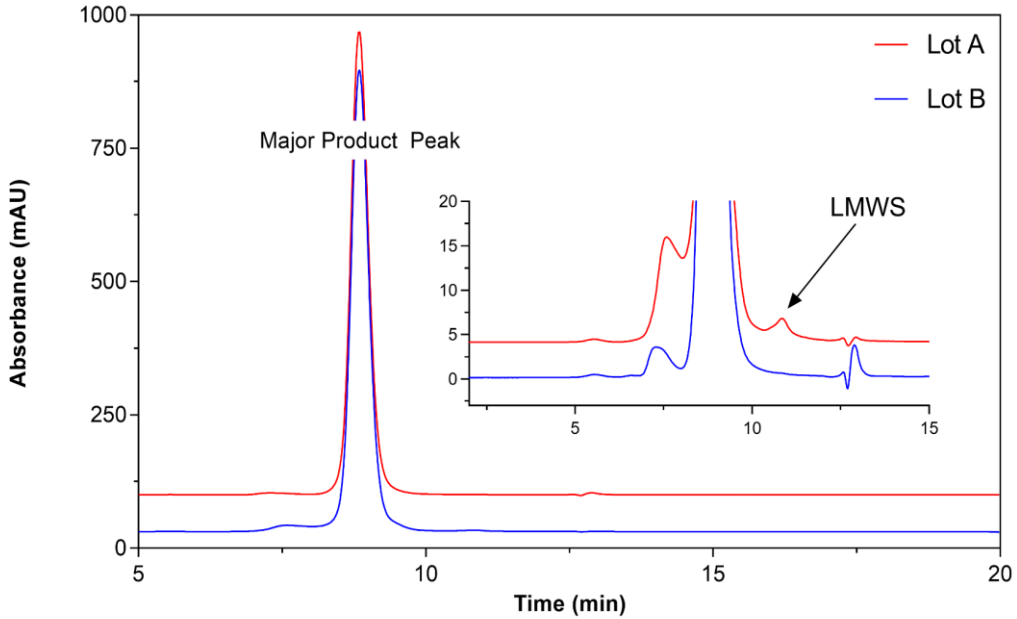
Anomalies observed during routine testing

- Anomalous levels of fragmentation was observed in a sample using MCE.
- Lot A showed increased fragmentation when compared to control Lot B



LMWS = Low molecular weight species

High Performance Size Exclusion Chromatography



- Run in tandem to MCE
- HPSEC also shows a small LMWS peak but this is significantly smaller than the peaks observed in MCE

Comparing Fragmentation Levels

Sample	HPSEC		Non-reduced MCE		Non-reduced CE-SDS	
	Major Product peak (%)	Fragment (%)	Major Product peak (%)	Fragment (%)	Major Product peak (%)	Fragment (%)
Lot A	97.1*	0.4	96.4	6.3	84.7	15.3
Lot B	99.4	0.0	99.1	0.9	96.9	3.1

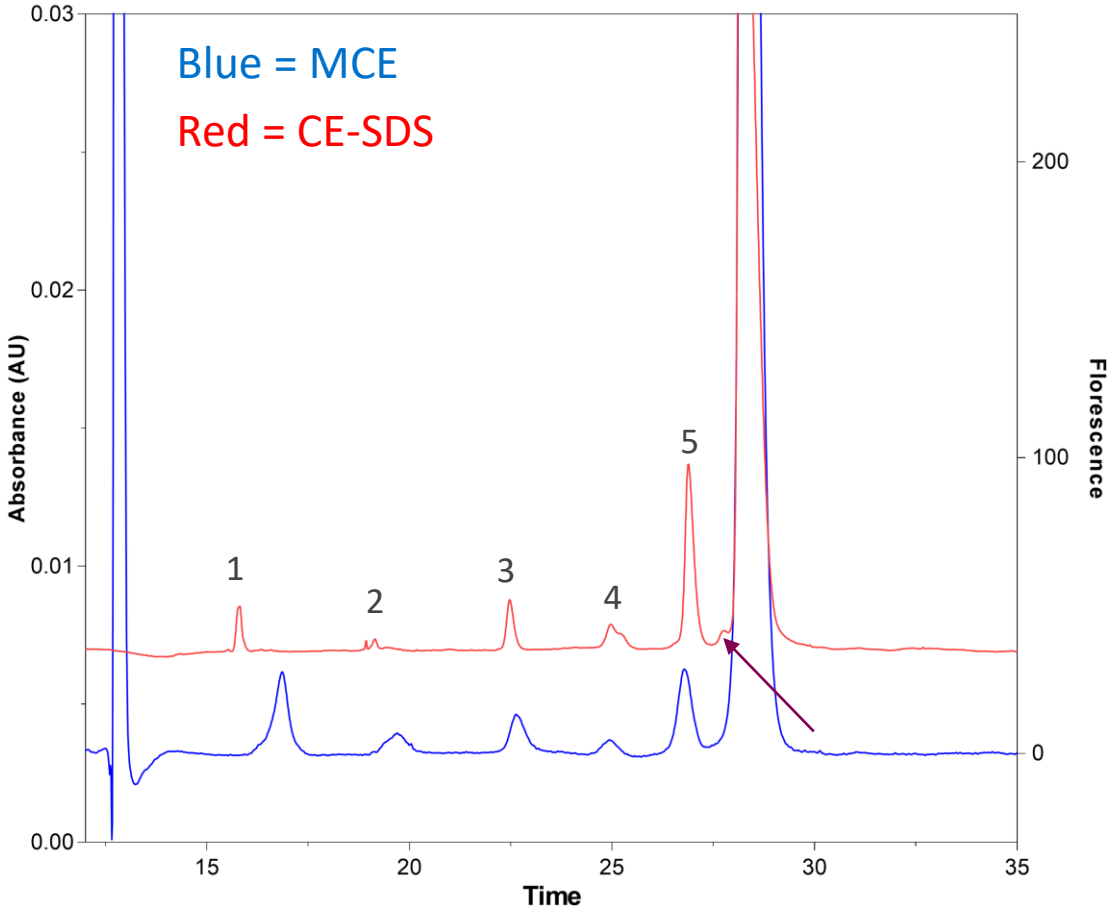
% High molecular weight species not shown in table.

- HPSEC shows significantly less % fragmentation compared to nrMCE
- HPSEC not suitable for fragmentation monitoring
- Significant difference between MCE and CE-SDS %fragmentation
- MCE still able to distinguish between pure and impure samples

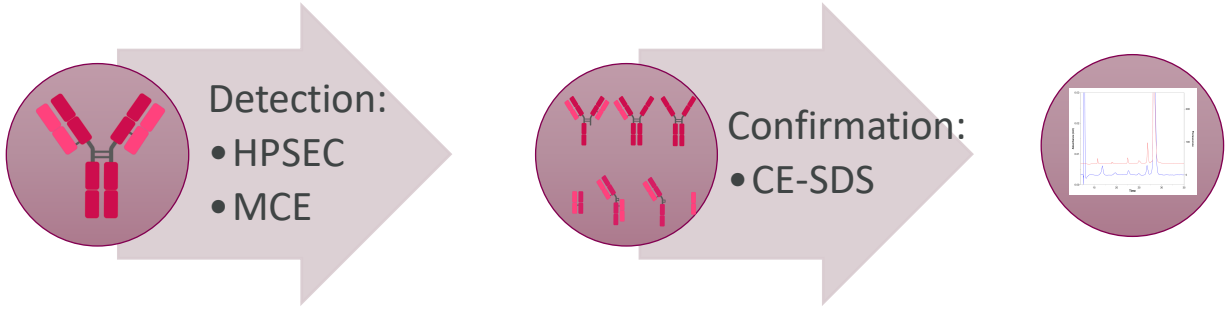
MCE vs CE-SDS

- Five equally resolved species observed in both techniques.
- MCE unable to resolve ng-IgG shoulder from the main peak which is well resolved in CE-SDS
- Able to detect all major LMWs and increased fragmentation in both techniques

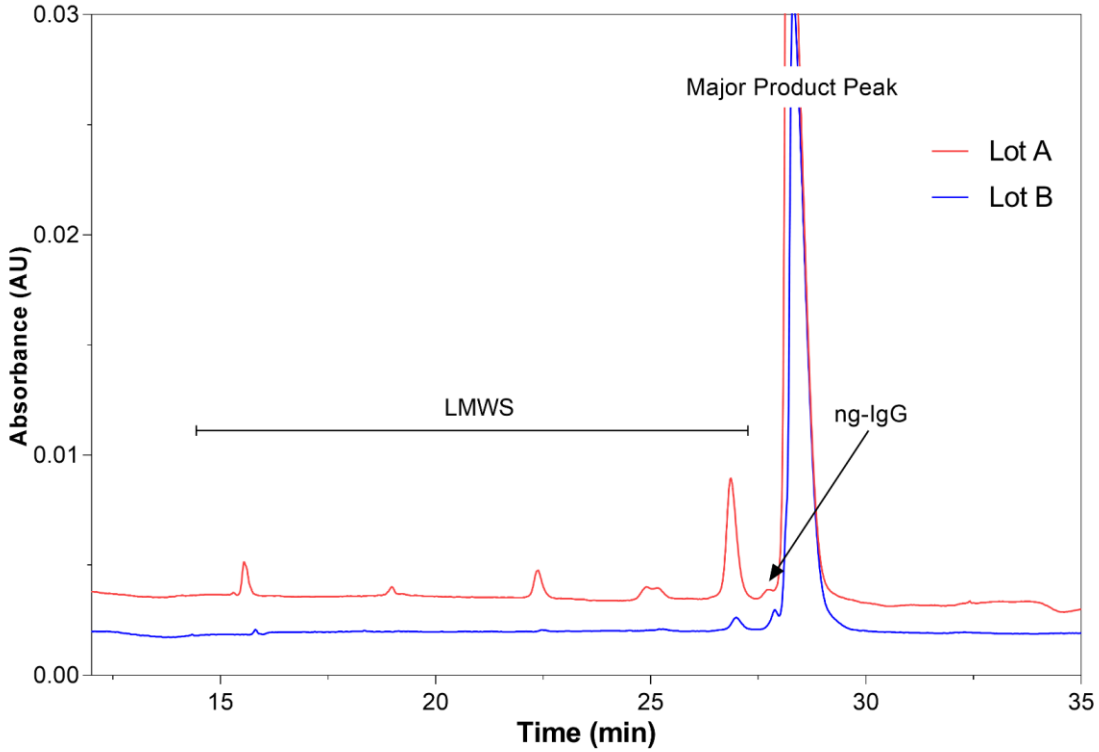
MCE remains a good option for use in HT screening assays in place of conventional CE-SDS.



Confirmation



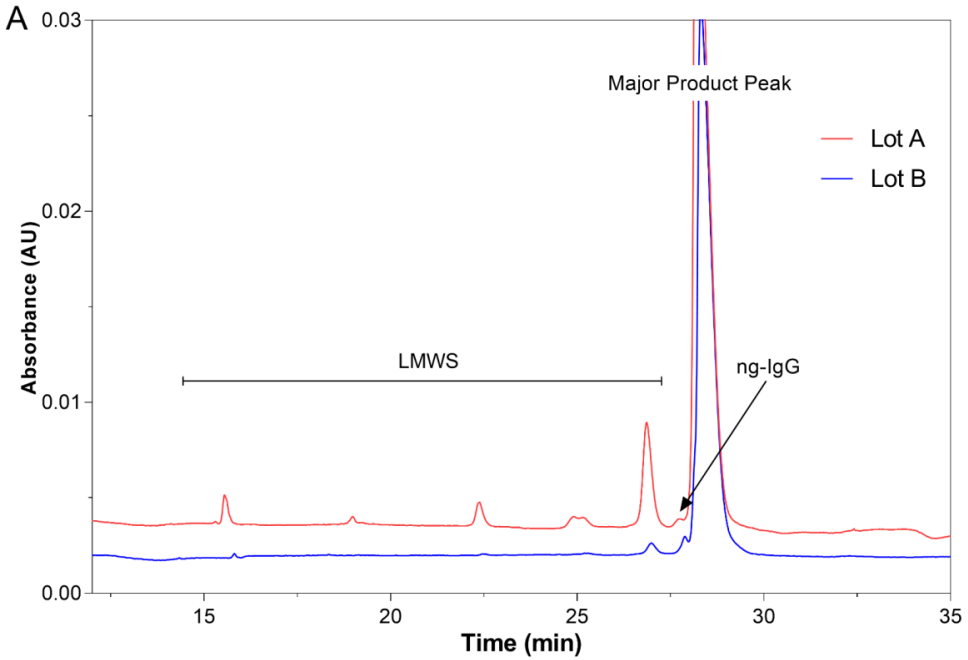
Confirmation of Results – PA800+



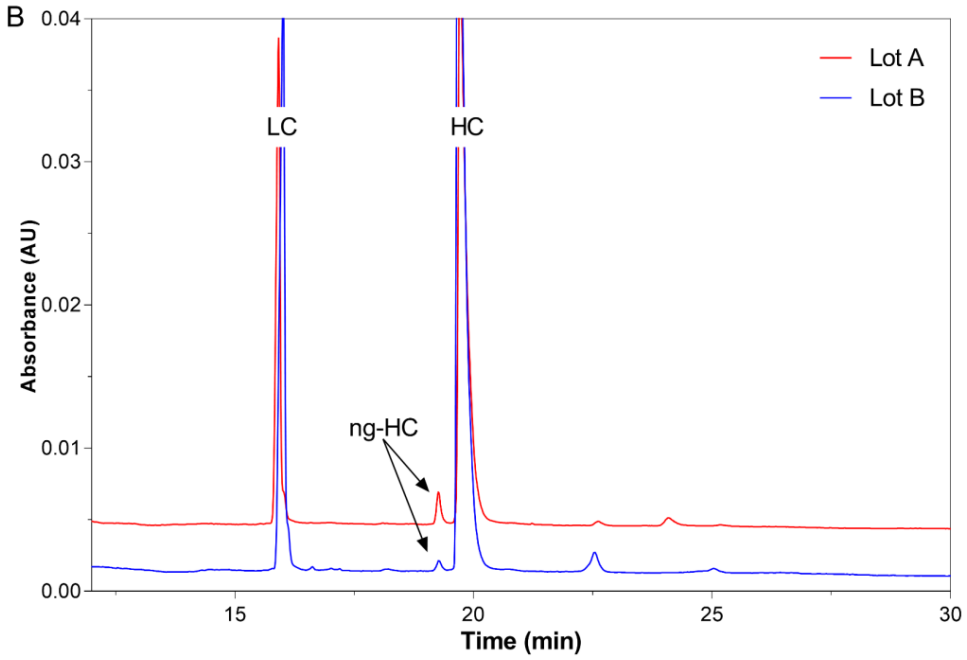
Lot A and B run on conventional CE-SDS confirmed presence of all LMWs detected in MCE

Possible Fragmentation Pathway Determined via CE-SDS

Non-reduced CE-SDS

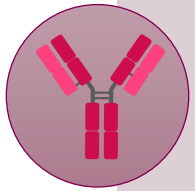


Reduced CE-SDS

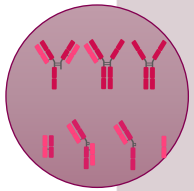


Fragments = partially reduced species!

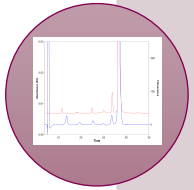
Characterisation



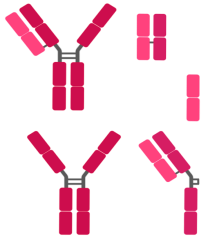
Detection:
- HPSEC
- MCE



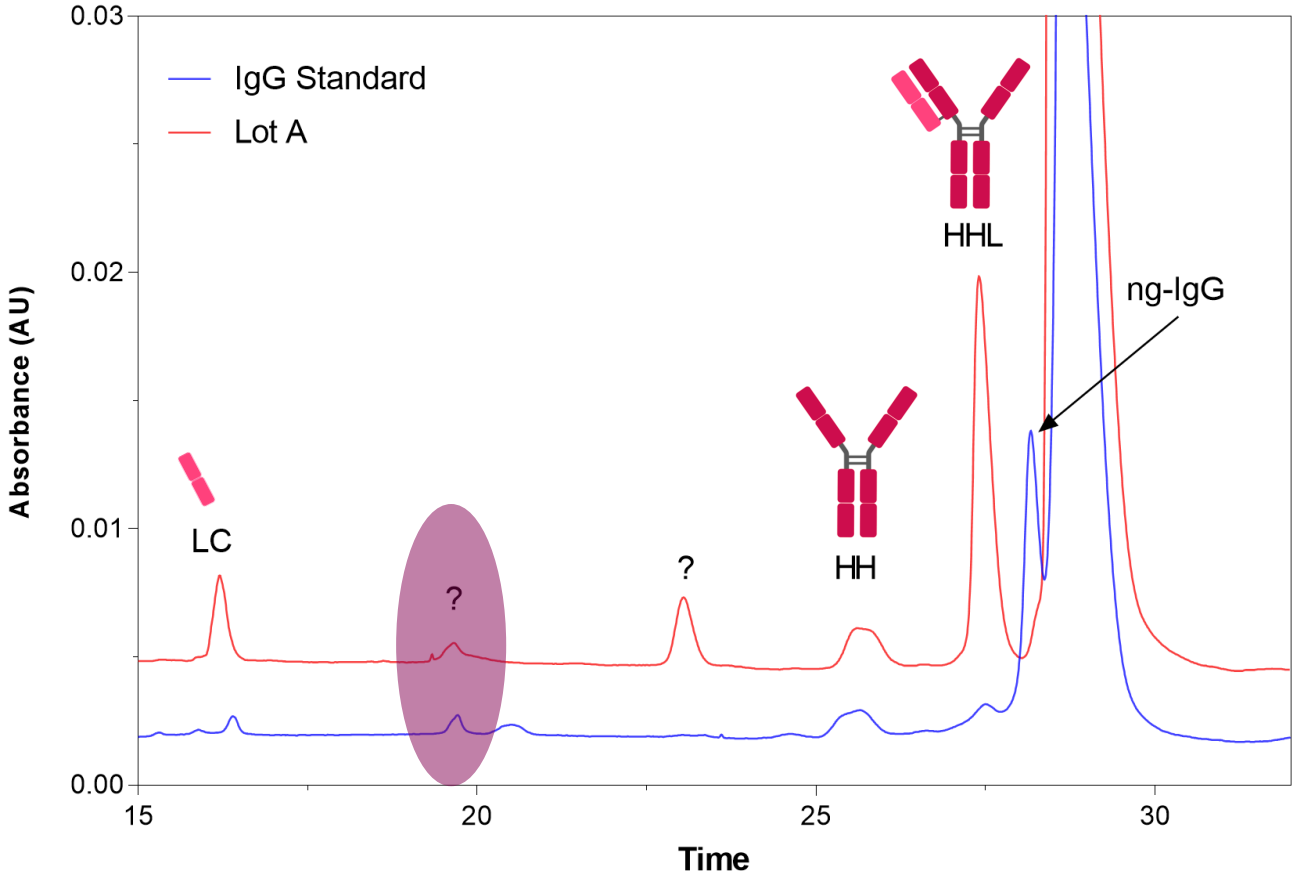
Confirmation:
- CE-SDS



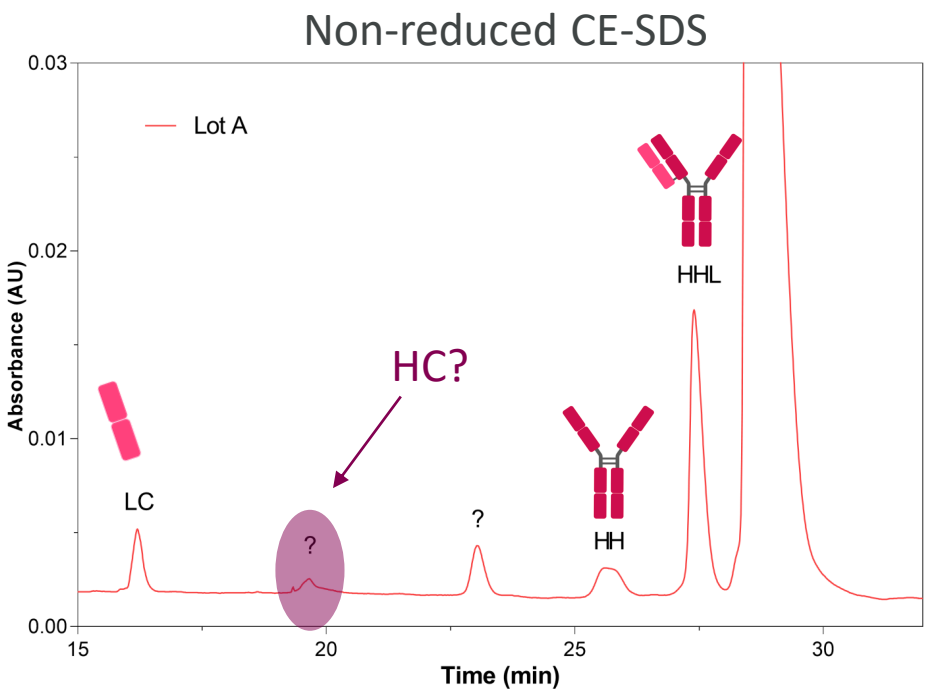
Characterisation

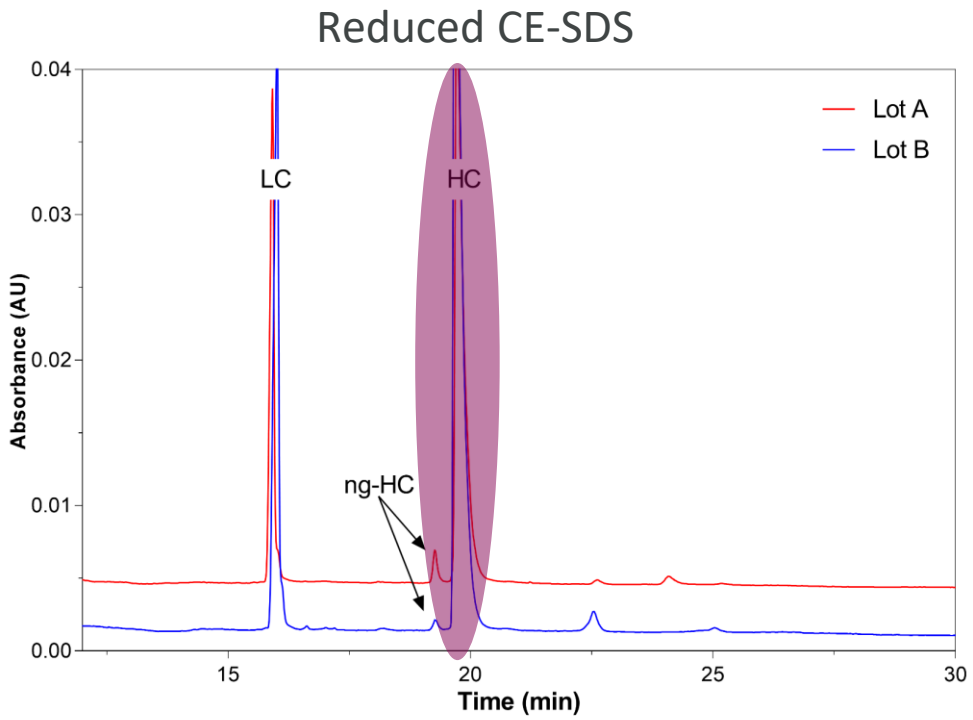


Initial Characterisation



Further Peak Characterisation



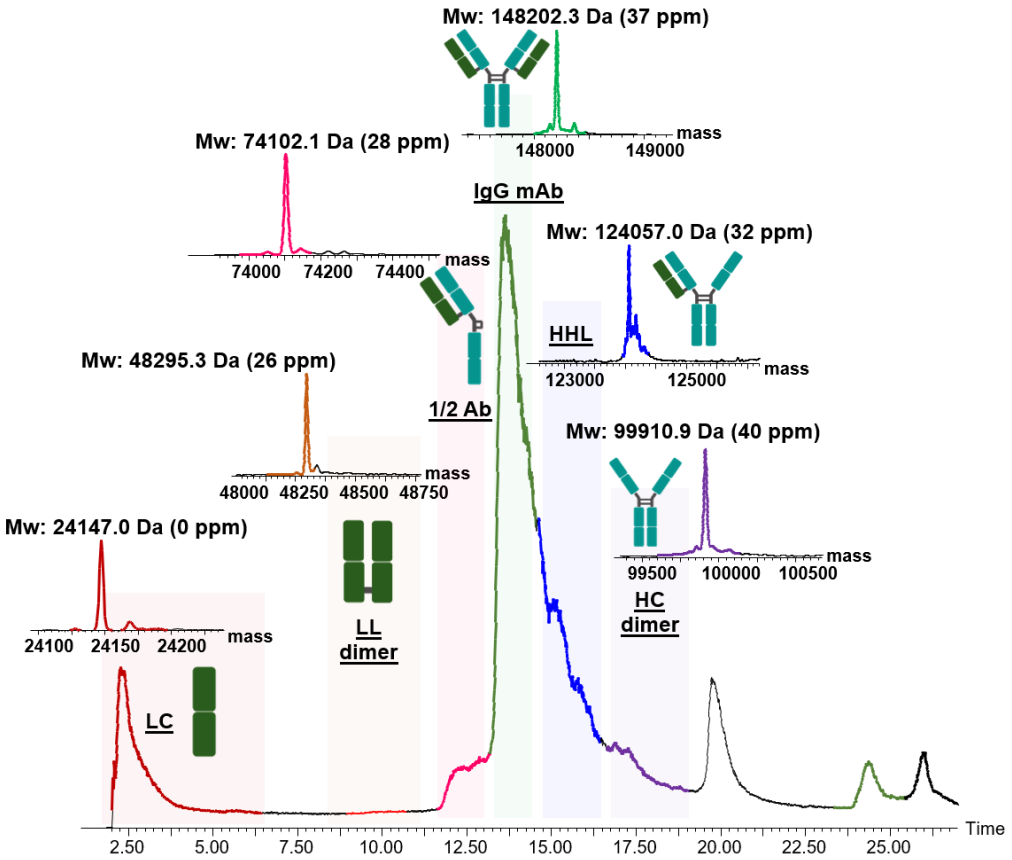


Heavy Chain or Light Chain Dimer?

Fragment	Theoretical Mass (Da)
Light Chain Dimer 	48294
Heavy Chain 	49952

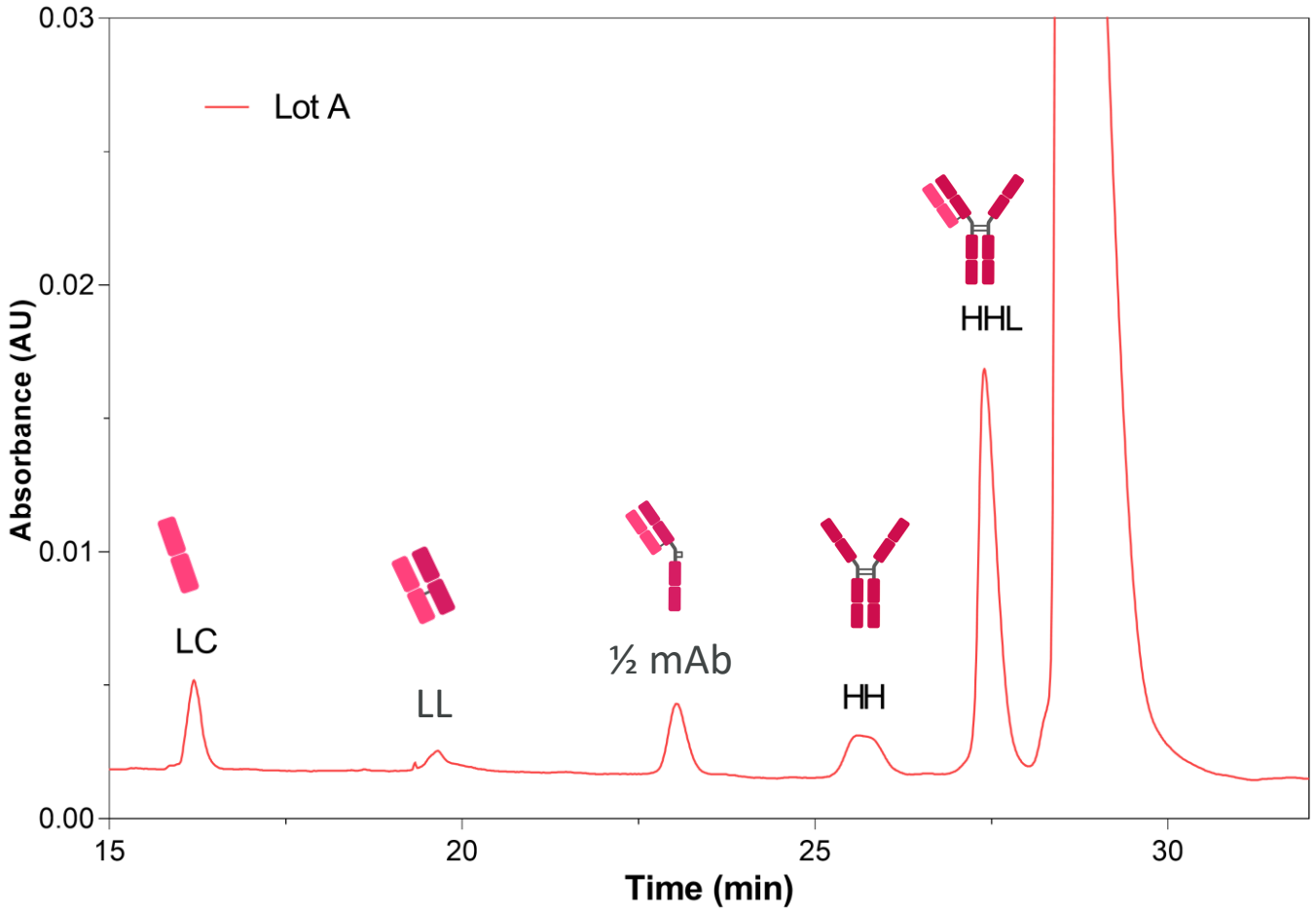
LC-ESI-QTOF-MS Analysis

(Liquid chromatography electrospray ionisation quadrupole time-of-flight mass spectrometry)



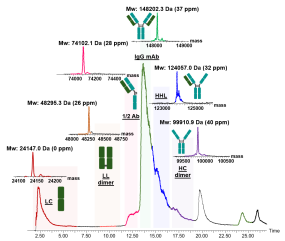
Component	Theoretical Mass (Da)	Observed Mass (Da)	ppm error (absolute value)
Intact deglycosylated mAb	148196	148202	37
HHL	124053	124057	32
HH	99906	99911	40
HL	74100	74102	28
HC	49952	N/A	N/A
LL	48294	48295	26
LC	24147	24147	0

Fully characterised fragments!



LC-MS was able to characterise all fragment peaks

Summary: Analytical Workflow



Detection:
- HPSEC
- MCE

Confirmation:
- CE-SDS

Characterisation:
- MS

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Systematic analytical workflow for characterisation and identification of partially reduced species in monoclonal antibody manufacturing

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ABSTRACT

Fragmentation is a major degradation pathway ubiquitous to all therapeutic monoclonal antibody (mAb) and therefore, monitored throughout the manufacturing process. Here, we describe a three-step approach to 1) detect, 2) confirm and 3) characterize partially reduced fragment species in an immunoglobulin G1 (IgG1) mAb with prolonged hold time of harvested cell culture fluid (HCCF). Microchip capillary electrophoresis (MCE) and high-performance size exclusion chromatography (HPSEC) were used as fast and efficient screening methods to detect fragmentation. HPSEC was found to be underestimating fragmentation levels. To confirm and characterize the fragments, capillary electrophoresis-sodium dodecyl sulphate (CE-SDS) was employed. Interestingly, the absence of fragments in the reduced CE-SDS analysis suggested partial reduction of disulphide bonds contributing to fragmentation in this mAb lot. This was further confirmed using reverse phase high performance liquid chromatography (RP-HPLC) coupled with mass spectrometry, which established the presence of heavy-heavy-light (HHL), heavy-heavy (HH), light-light dimer (LL), light chain (LC) and half antibody (HL) fragments with good mass accuracy. In this study, we demonstrated a readily applicable systematic strategy to support process development and investigate anomalous events in manufacturing. An additional highlight of this work is the data-driven comprehensive comparison of modern and conventional analytical techniques for fragment analysis.

1. Introduction

Monoclonal antibody (mAb) fragmentation, a major degradation route, is considered a Critical Quality Attribute (CQA) in therapeutic proteins products and thus needs to be monitored and controlled throughout the manufacturing process [1,2]. Although several different mechanisms exist, process induced fragmentation commonly occurs via hydrolysis of peptide bonds or reduction of interchain disulphide bonds [3,4]. The first step in the purification of a mAb from Chinese Hamster Ovary (CHO) cell culture is known as harvest. This step involves the removal of cells and cell debris followed by filtration to yield a clarified filtered fluid known as the harvested cell culture fluid (HCCF) [5]. The HCCF may be held under appropriate condition prior to downstream purification. Several studies have indicated that certain intracellular enzymes in HCCF can lead to fragmentation via disulphide bond reduction; hence limiting the HCCF hold time can help control the fragmentation in a manufacturing environment [6–8]. Other manufacturing process parameters such as dissolved oxygen (DO) levels during harvest, mechanical shear force during cell lysis, media components (such as metal ions, cysteine/cystine, pH and temperature) can also play a significant role in the extent of fragmentation from undesirable enzymatic activity [4,9]. Hence routine monitoring and characterisation of fragments using a toolbox of different analytical approaches is important in biopharmaceutical process development and manufacturing.

In this study we describe a systematic approach to identify, confirm, and characterize unusual high level of fragments observed during process development of an immunoglobulin G1 (IgG1) mAb. Unexpected product variants are observed during mAb production and the approach detailed here provides a multidisciplinary analytical toolbox which can be employed for fast investigation of root cause analysis. A distinctive aspect of our study was the utilization of multidisciplinary complimentary techniques, both conventional and modern, to provide a stepwise approach for fragment analysis. Additionally, a comparative evaluation of the different analytical techniques employed here is also performed. The toolbox consists of four orthogonal techniques: 1) Microchip capillary electrophoresis (MCE) 2) High performance size exclusion chromatography (HPSEC) 3) Conventional capillary electrophoresis sodium dodecyl sulphate (CE-SDS) in both reducing and non-reducing conditions and 4) Liquid chromatography electrospray ionisation quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS).

To demonstrate the approach, MCE and HPSEC analysis was first employed as a fast and efficient method to assess the product related impurities (aggregates and fragments) in a process development lot

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Thank you! ☺