Simplifying In-Process Monitoring and Characterization of Biosimilars with Automated CE-SDS Assay on the Maurice Platform

Biosana PHARMA

CASSS – AT EUROPE 2023

Heli Tervahauta - Sr. Analytical Scientist

11 May 2023

Introductory words: Who is BiosanaPharma

Biosanapharma BV is a private biotech company developing a highly disruptive continuous mAbs manufacturing platform using biosimilars for validation and short/mid term revenues.

Our very low CoGs continuous manufacturing platform (named 3C) has been de-risked and FDA endorsed for phase III clinical manufacturing.

Biosimilar products are licensed to M&S parties for late-stage development (phase III and Marketing Authorization) and commercialization.

Operations are in The Netherlands, Singapore and Australia. We are a technology, process and product development company. GMP manufacturing & analytical are outsourced.

We are passionate to change protein manufacturing for patient benefit. Time to unfold the potential.

Biosana PHARMA

Summary

BiosanaPharma is transforming the production of mAb biologics with a continuous process that is scalable, flexible, authority approved and positioned to optimize the market for biosimilars and biologics in the near and distant future.

Approach

Continuous manufacturing with high titer cell lines:

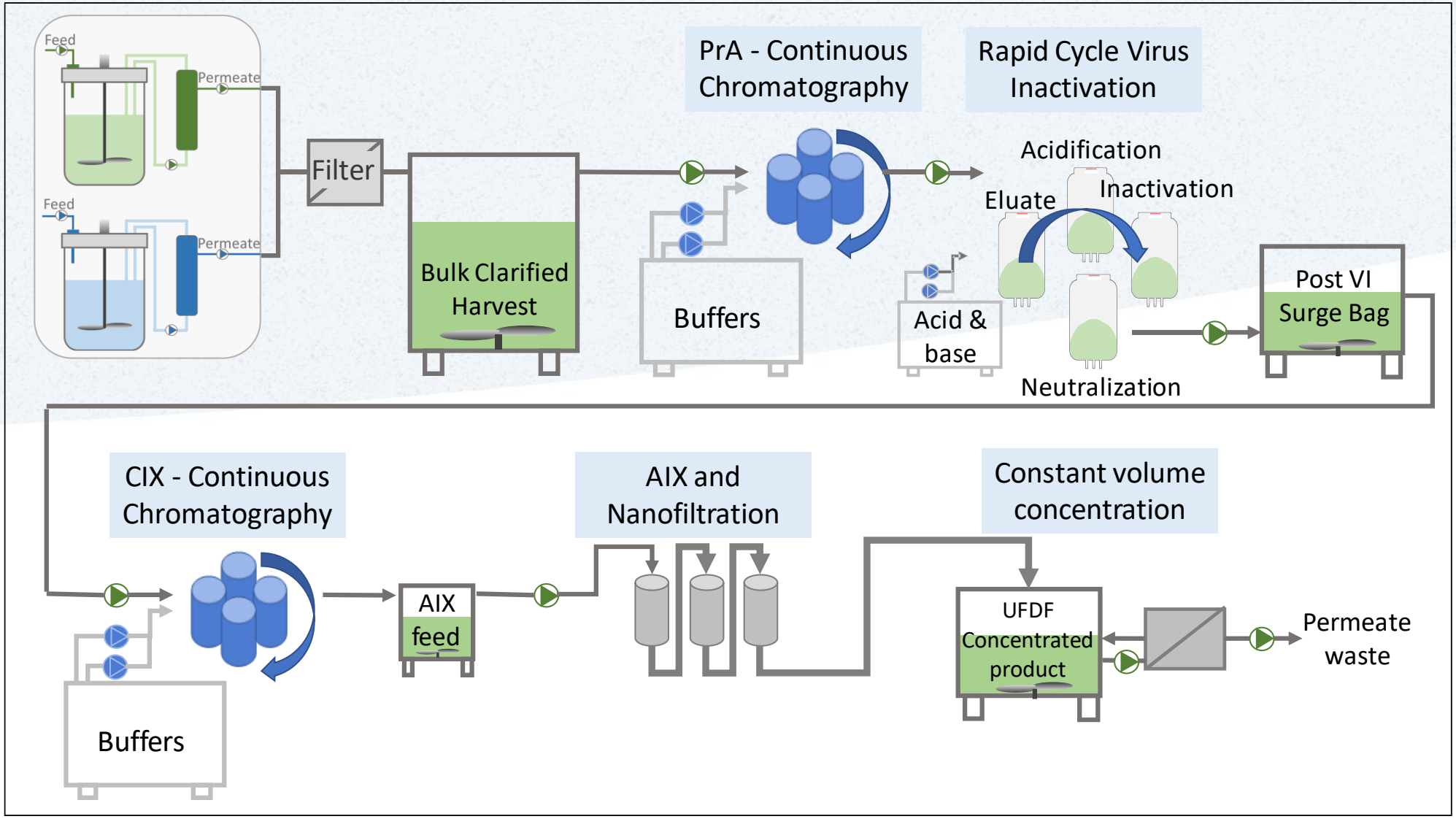
- FDA/EMA approved
- Intensified production in 24/7 continuous operation (USP & DSP)
- Very low CoGs, anticipated €80/gr @ 200L scale with potential to drop below €8/gr DS @ 2,000L scale

Future

Flexible, fully automated continuous manufacture of biologics with paperless parametric release. Building a global winner in a multi billion-dollar business.

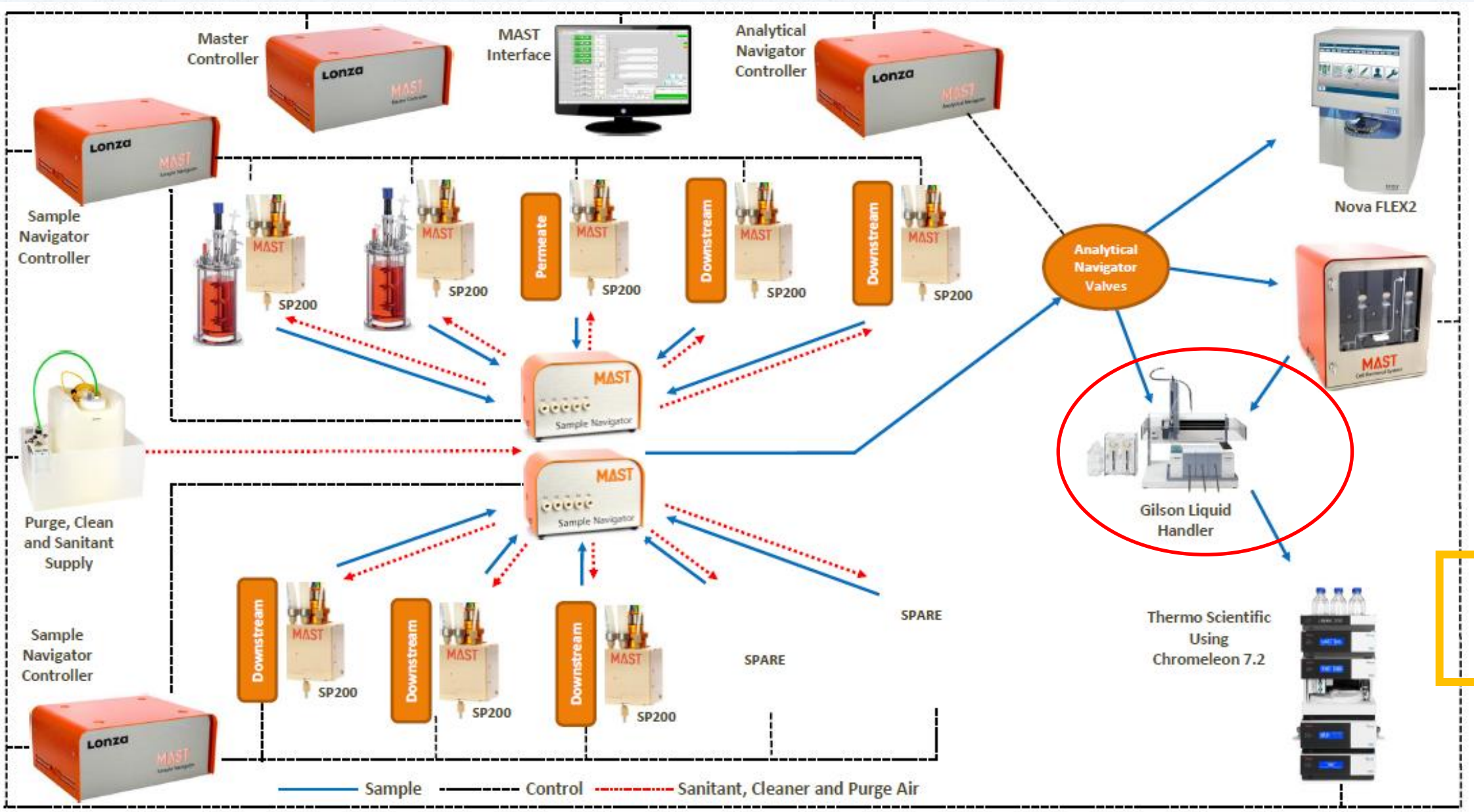
Biosana PHARMA

Biosana's Continuous Counter Current process



Biosana PHARMA

Sample automation by MAST (Modular Automated Sampling Technology, Lonza-Merck)



Nova Flex2

Cell Removal System

Maurice S. CE-SDS

Biosana PHARMA

Simplifying In-Process Monitoring and Characterization of Biosimilars with Automated CE-SDS Assay on the Maurice Platform

- System is manufactured by ProteinSimple (a Bio-Techne brand) and was purchased in 2021
- In use since January 2022
- CE-SDS PLUS cartridges
- 765 in-process samples have been analyzed in 2022
- 1 emergency maintenance due to a stuck cartridge
- 1 periodic maintenance

Biosana PHARMA

Step 1: Peak assignment using the Compass for iCE software

Maurice CE-SDS Method Development Guide

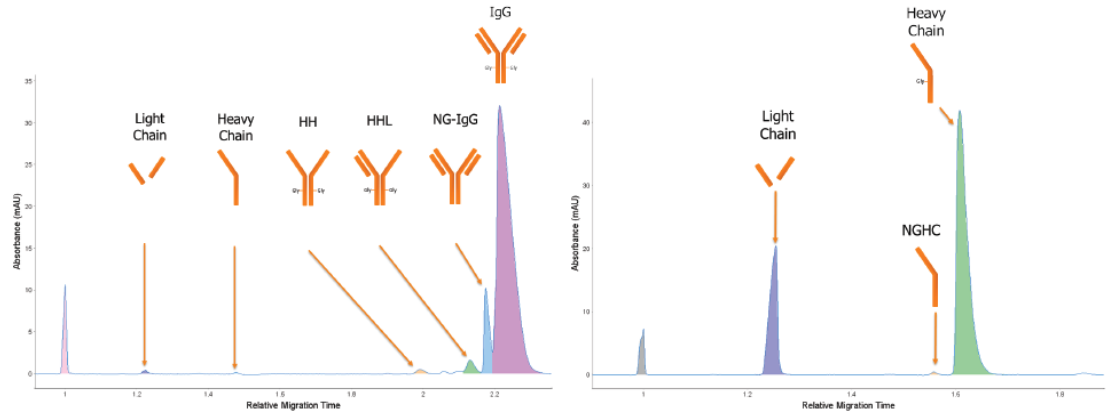
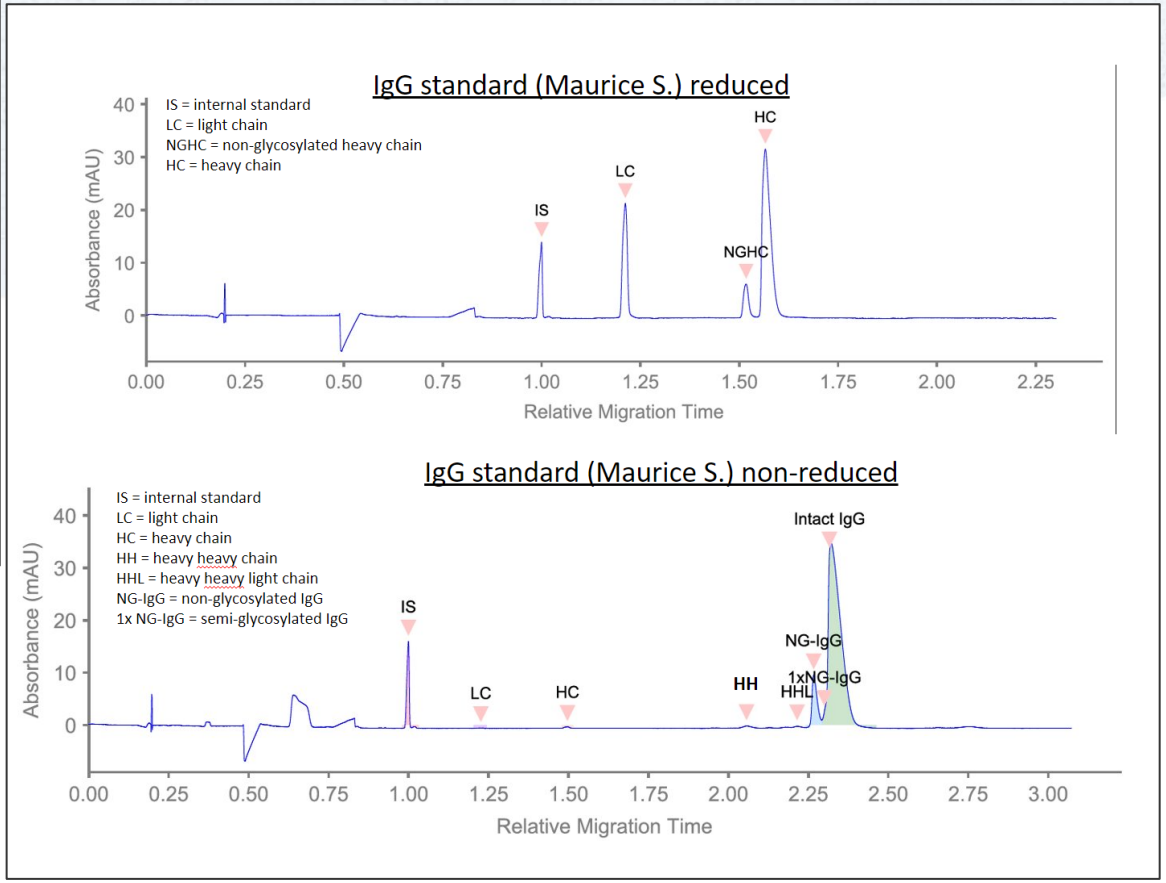


FIGURE 17. Fragmentation profiles for IgG1k. IgG1k (1 mg/mL) was denatured in the absence or presence of reducing agent. Illustrations of the various fragments are included.

Source: CE-SDS Method Development Guide Maurice and Maurice S. 046-566 Rev A 2017 ProteinSimple

Biosana data using the Maurice S. instrument with default conditions and the IgG standard



Step 2: Optimizing the CE-SDS conditions

Non-enzymatic fragmentation of IgG can occur via hydrolysis or disruption of interchain disulfide bonds. Hydrolysis is more likely to occur in higher pH buffers, so fragmentation can be buffer dependent. You may need to optimize sample preparation conditions for the specific molecule you're analyzing to minimize unexpected fragmentation (**Figure 18**). For non-reduced samples, the specific alkylating agent and conditions used during alkylation may need to be adjusted. For reduced samples, the reducing agent and reducing conditions may need to be optimized. The pH of your sample buffer can also play a role in observed fragmentation. We recommend testing a lower pH sample buffer in addition to adjustments to your denaturation procedures.

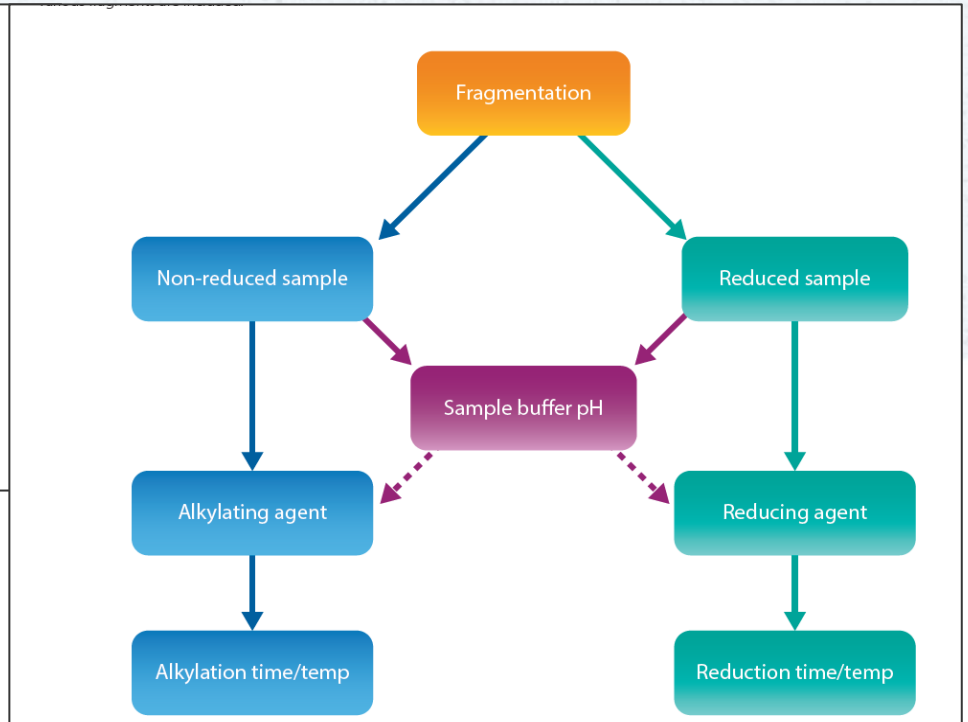


FIGURE 18. Workflow for addressing unexpected fragmentation.

Source: CE-SDS Method Development Guide Maurice and Maurice S. 046-566 Rev A 2017 ProteinSimple

Critical Assay parameters:

- **Sample matrix –ionic composition**
- **pH**

The efficiency of the electrokinetic (EK) injection can be impacted by altering pH.

Charged ions, including proteins and buffer components, migrate in the capillary due to the applied electric field, which also contributes to the sample injection efficiency.

The number of ions injected is a function of their own electrophoretic mobility in addition to the conductivity of the sample.

The amount of material injected electrokinetically is inversely related to the sample conductivity.

The higher the conductivity of the sample, the lower the EK injection efficiency.

Biosana PHARMA

Step 2: Optimizing the CE-SDS conditions (NR)

Default conditions

STEP	REAGENT/ PARAMETER	REDUCED CONDITIONS	NON-REDUCED CONDITIONS
Sample Prep	ProteinSimple Sample Buffer	At least 0.5X	At least 0.5X
	Additives	650 mM β-ME	11.5 mM IAM
Denaturation	Time	10 minutes	10 minutes
	Temp	70 °C	70 °C
Electrokinetic injection	Time	20 seconds	20 seconds
	Voltage	4600 V	4600 V
Separation (Standard IgG)	Time	25 minutes	35 minutes
	Voltage	5750 V	5750 V

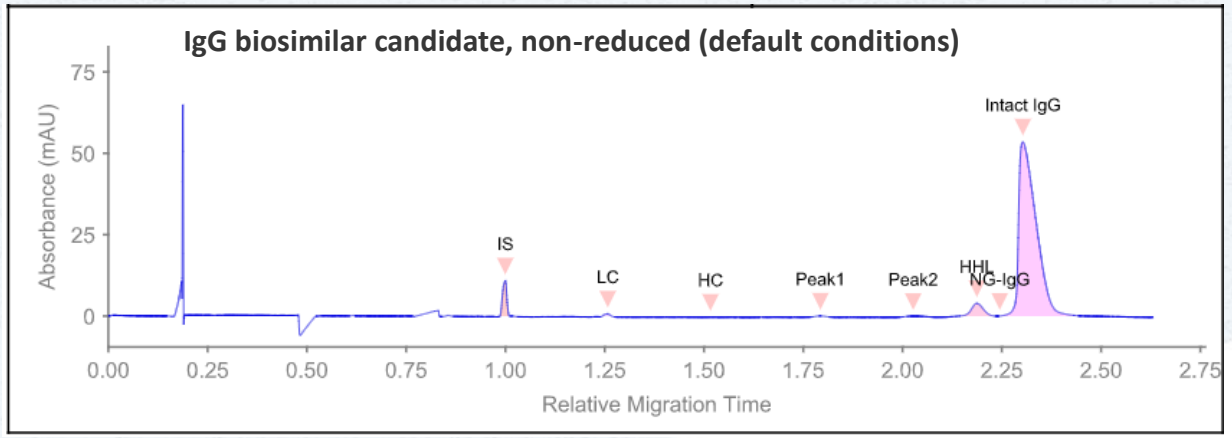
TABLE 1. Reduced and non-reduced CE-SDS method conditions. The default Maurice CE-SDS run parameters include electrokinetic injection, and varied separation times which are molecular weight-specific.

Source: CE-SDS Method Development Guide Maurice and Maurice S. 046-566 Rev A 2017 ProteinSimple

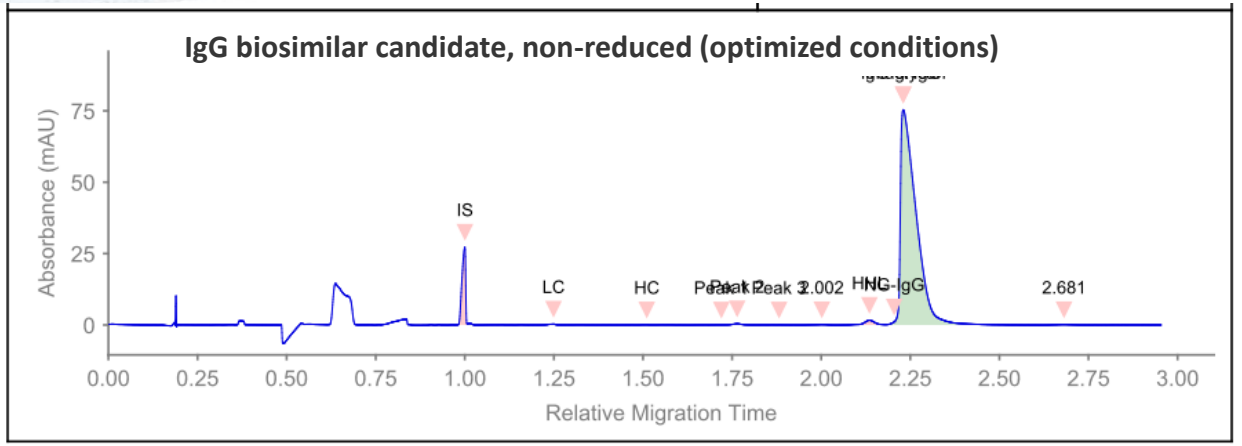
Biosana PHARMA

Step 2: Optimizing the CE-SDS conditions (NR)

- The pH was optimized for the Biosana IgG biosimilar candidate: lower IgG concentrations resulted in higher signals and lower observed fragmentation
- Alkylating agent IAM replaced by N-ethylmaleimide (NEM) for better thermal stability



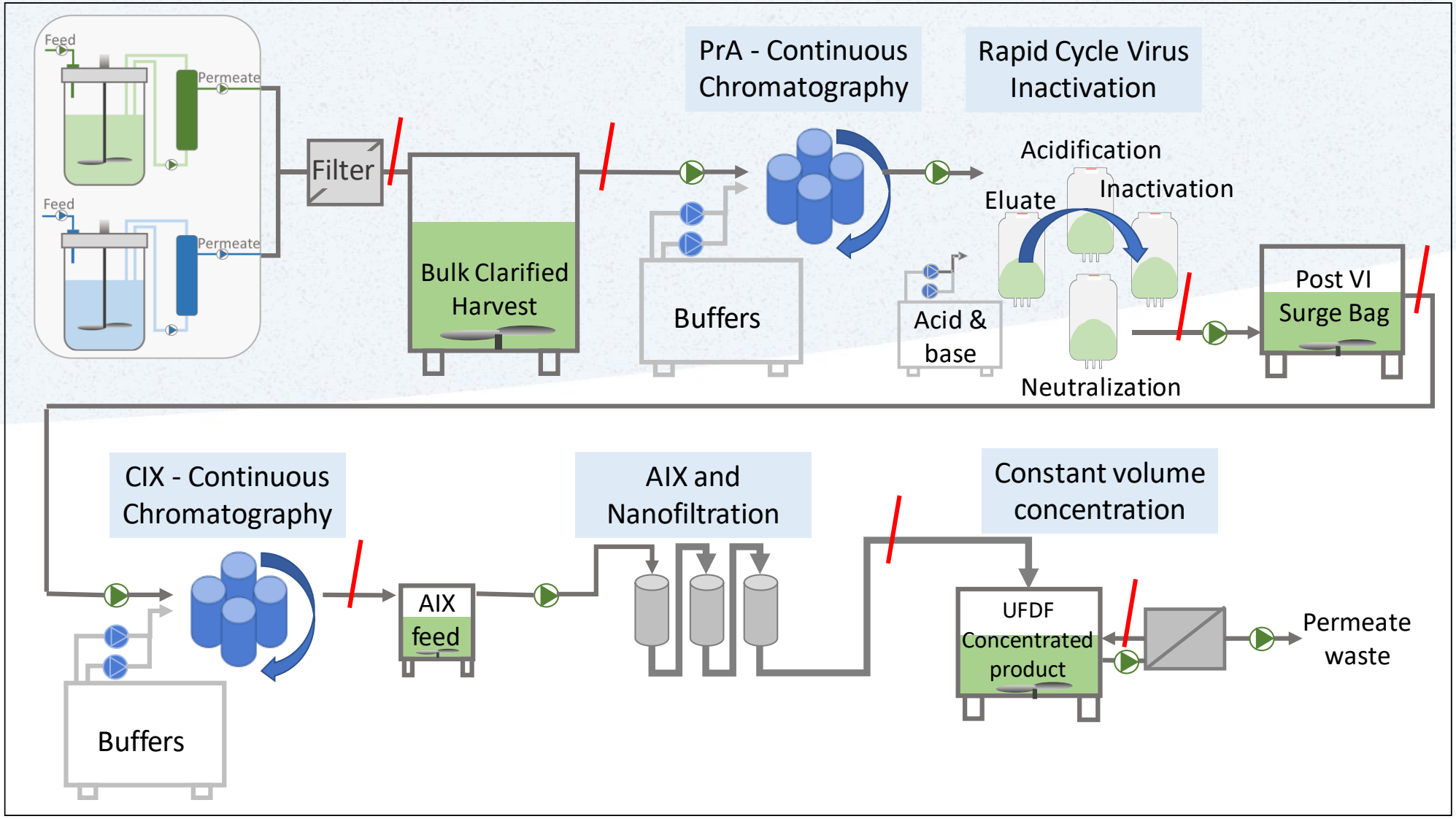
Relative areas (%)
LC: 1.1
HC: 0.0
Peak 1: 0.5
Peak 2: 0.5
HHL: 5.9
Intact IgG: 91.9



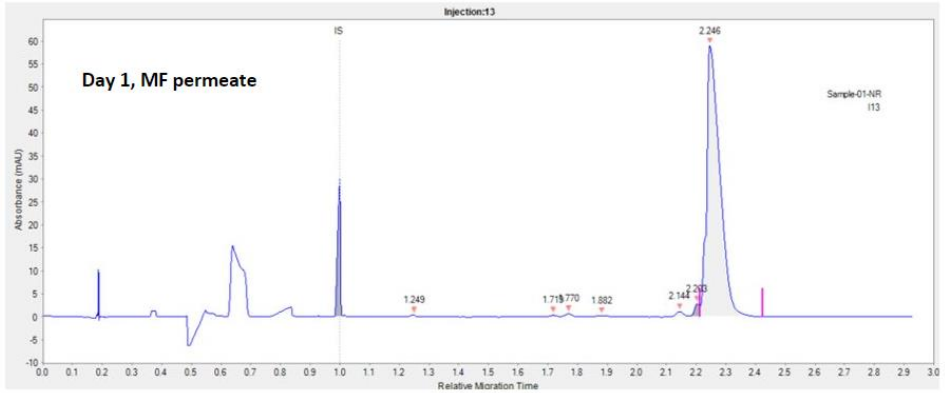
Relative areas (%)
LC: 0.3
HC: 0.0
Peak 1: 0.4
Peak 2: 0.1
HHL: 1.5
Intact IgG: 97.6

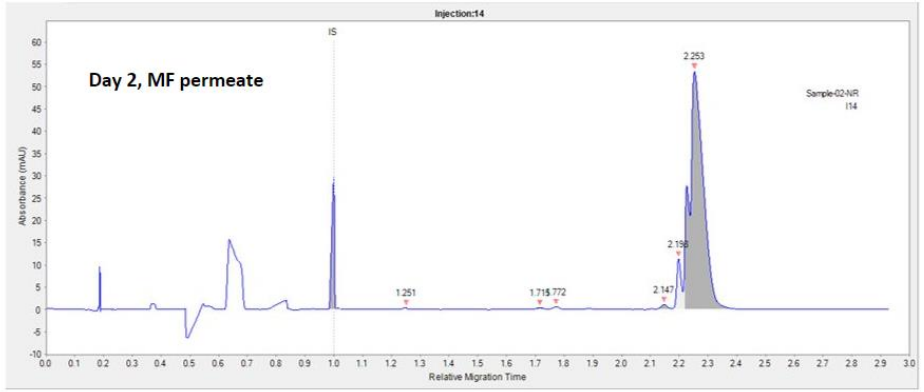
Biosana PHARMA

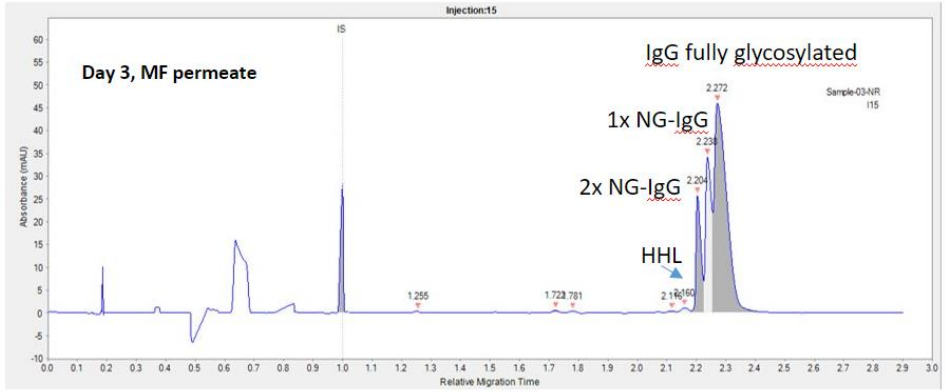
Biosana's Continuous Counter Current process: sampling points for CE-SDS for in-process monitoring and product characterization

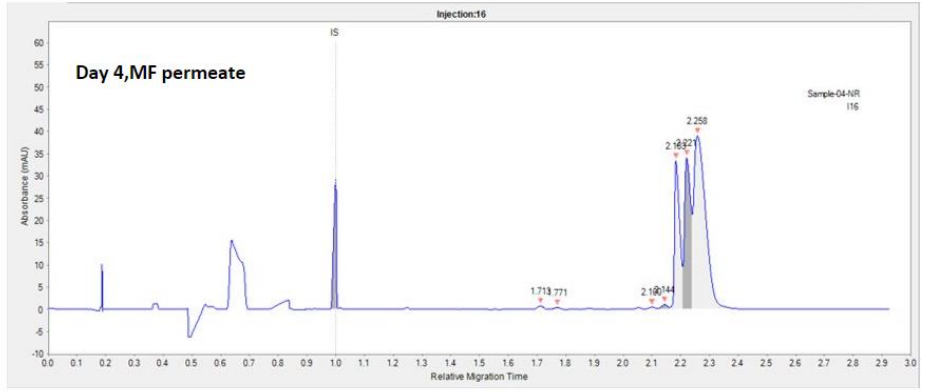


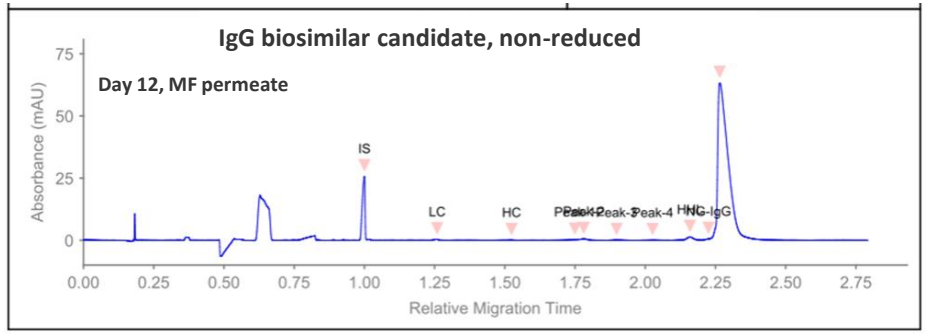
Case study: IgG biosimilar candidate production of a development batch, non-reduced data (optimized conditions)

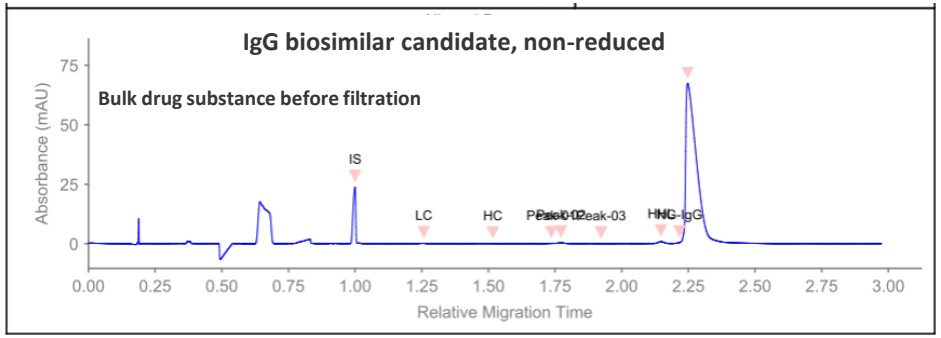












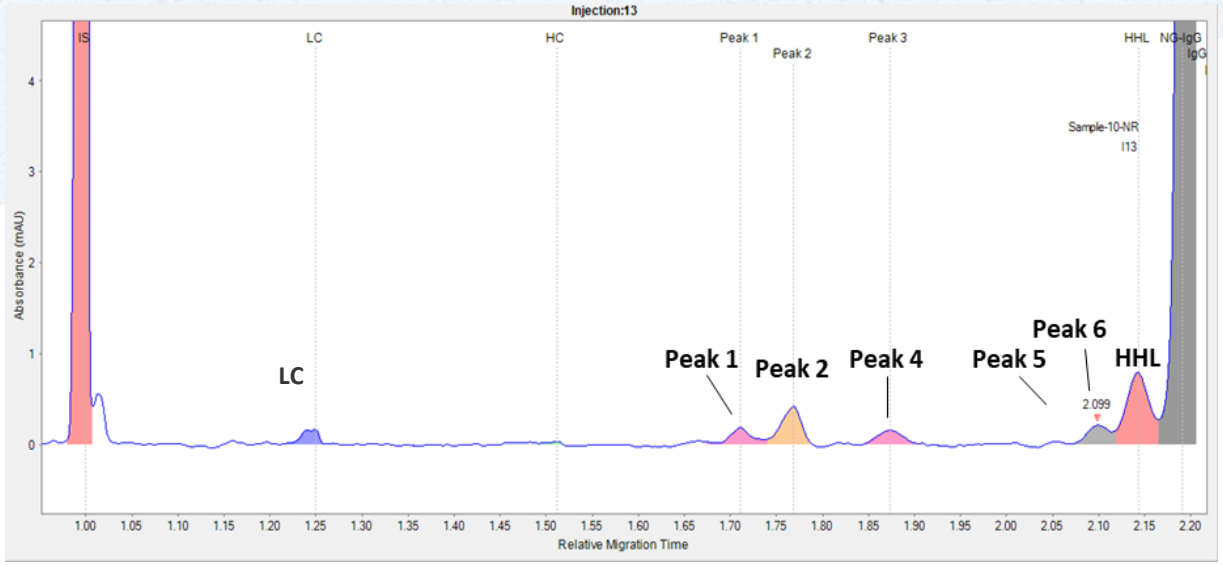
Detection of low abundance fragments; determining the limit of detection and limit of quantification

Signal Size

Your main peaks should ideally be in the 30-50 mAU range. To determine if you've have sufficient sensitivity, look for expected minor peaks of 0.1% - 0.3% total peak area. They should be above the baseline noise level in the electropherograms. If you don't see these species, you can enhance sensitivity either by increasing the sample concentration (**Figure 9**) or the sample injection parameters. The peak height for these small peaks will get larger when either more protein or longer sample injection times are used.

Source: CE-SDS Method Development Guide Maurice and Maurice S. 046-566 Rev A 2017 ProteinSimple

Minor peaks (0.1-0.4%) in the electropherograms for drug substance were studied.



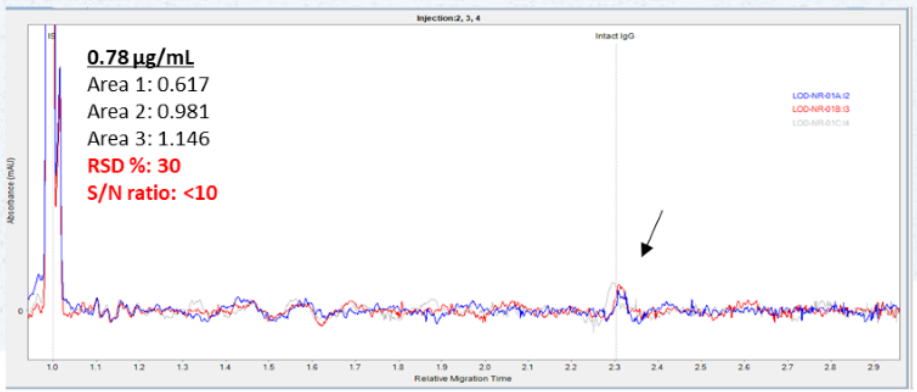
Relative areas minor peaks (%)

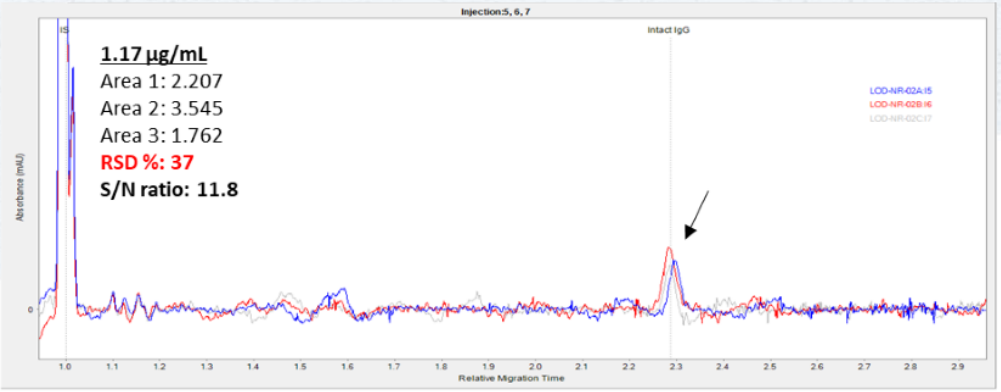
LC: 0.2
HC: 0.0
Peak 1: 0.2
Peak 2: 0.4
Peak 4: 0.2
Peak 6: 0.2
HHL: 0.7

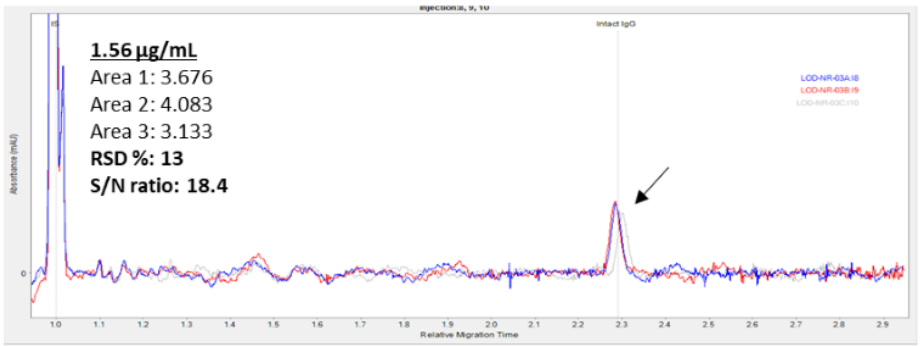
Biosana PHARMA

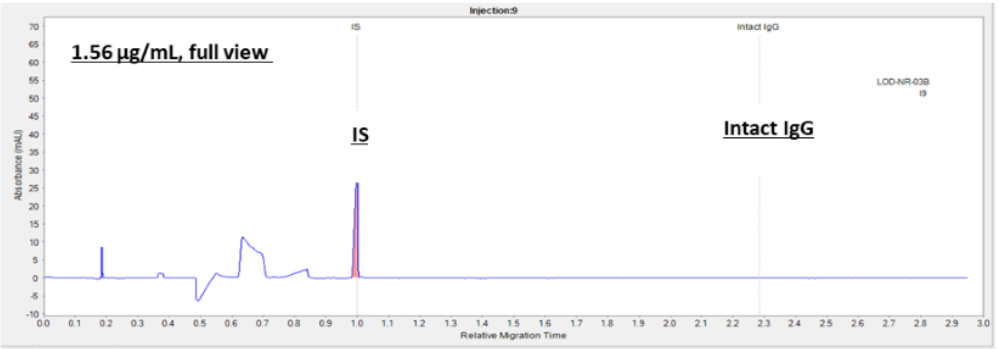
Detection of low abundance fragments; determining the limit of detection and limit of quantification

Based on the peak areas for the minor peaks, limit of detection was studied diluting IgG in 3 low concentrations, in triplicate each

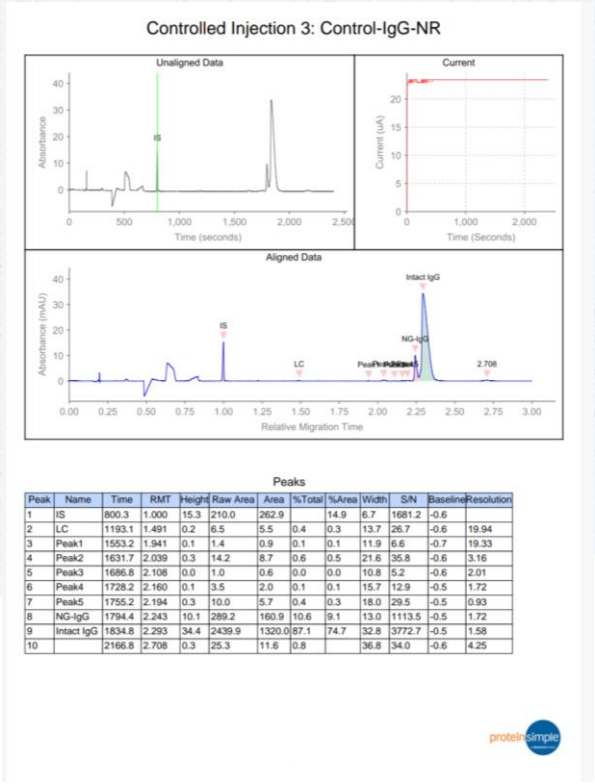








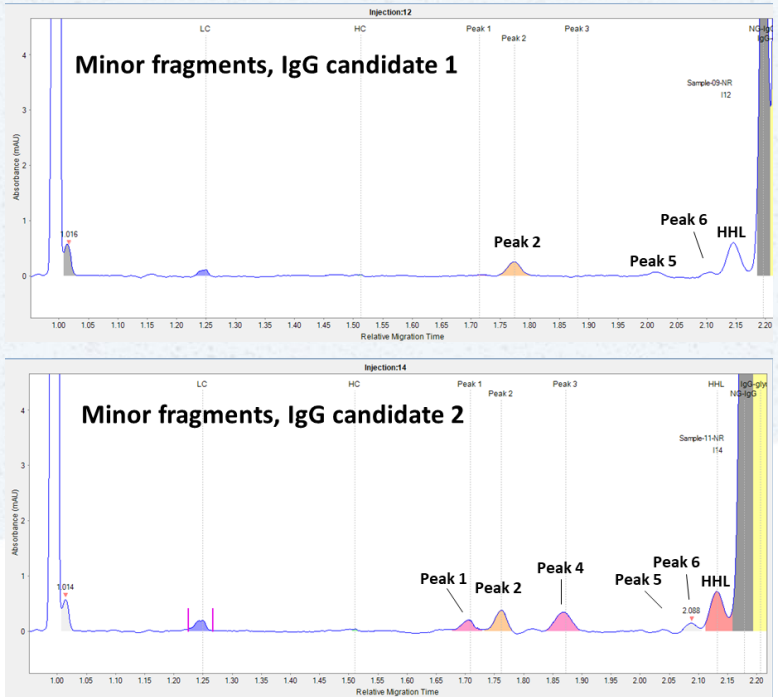
Concluding remarks



Peaks

Peak	Name	Time	RMT	Height	Raw Area	Area	%Total	%Area	Width	S/N	Baseline	Resolution
1	IS	800.3	1.000	15.3	210.0	262.9		14.9	6.7	1681.2	-0.6	
2	LC	1193.1	1.491	0.2	6.5	5.5	0.4	0.3	13.7	26.7	-0.6	19.94
3	Peak1	1553.2	1.941	0.1	1.4	0.9	0.1	0.1	11.9	6.6	-0.7	19.33
4	Peak2	1631.7	2.039	0.3	14.2	8.7	0.6	0.5	21.6	35.8	-0.6	3.16
5	Peak3	1686.8	2.108	0.0	1.0	0.6	0.0	0.0	10.8	5.2	-0.6	2.01
6	Peak4	1728.2	2.160	0.1	3.5	2.0	0.1	0.1	15.7	12.9	-0.5	1.72
7	Peak5	1755.2	2.194	0.3	10.0	5.7	0.4	0.3	18.0	29.5	-0.5	0.93
8	NG-IgG	1794.4	2.243	10.1	289.2	160.9	10.6	9.1	13.0	1113.5	-0.5	1.72
9	Intact IgG	1834.8	2.293	34.4	2439.9	1320.0	87.1	74.7	32.8	3772.7	-0.5	1.58
10		2166.8	2.708	0.3	25.3	11.6	0.8		36.8	34.0	-0.6	4.25

The Maurice S. instrument has proven to be robust, reliable and easy to use.



The remarkably stable baseline makes the peak integration and the detection of even of the low abundance fragments simple and unambiguous.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAbs) make up the majority of biosimilars currently under development and on the market. They are multichain glycoproteins, composed of 2 heavy (H) and 2 light (L) chains linked via 16 disulfide bridges (for an IgG1). This complex bridging pattern across four separate protein chains leaves open the possibility of mispairing during biosynthesis. This can result in the formation of molecules composed of a lesser number of bridged chains, for example, 2 heavy chains linked to a single light chain. These malformed species constitute product-related impurities and therefore must be evaluated for their presence and quantity, which can be measured with CE-SDS.

Source: Use of Maurice CE-SDS in ICH Q6B based biosimilar comparability exercises. Gledhill *et al*. WP_Maurice_CE-SDS_STRY0163095

The Maurice S. instrument has been demonstrated to support biosimilar development with fast monitoring of in-process impurities and ensuring specifications.

Thank you!

Biosana PHARMA

Special thanks to:

Jean-François

Erik, Lieke, Otto and Basak

We make biologics
affordable and **accessible**
for all patients.

www.biosanapharma.com

ard.tijsterman@biosanapharma.com

heli.tervahauta@biosanapharma.com