bio-techne®

proteinsimple

Application Note

Rapid and High-Quality Analysis of Therapeutic Fusion Proteins with Maurice Turbo CE-SDS™

Introduction

Etanercept (trade name Enbrel®), manufactured and marketed by Amgen®, is a fusion protein used to treat autoimmune conditions such as rheumatoid arthritis and psoriasis. As a combination of the IgG1's Fc region and the extracellular domain of the TNF receptor, Etanercept is a heavily glycosylated molecule with a complex structure, causing additional analytical challenges during its development and manufacturing. With three biosimilars in development and the potential for more in the future, implementing robust and reliable analytical techniques early in the process is critical[1].

Compared to traditional methods such as HPLC and SEC, CE-SDS on the Maurice system offers several benefits, such as:

- Plug-and-play format with pre-assembled cartridges
  - Turbo CE-SDS cartridge for rapid analysis (5.5 – 8 minutes)
  - CE-SDS PLUS cartridge for superior resolution (25 – 35 minutes)
- Simplified sample preparation
- Easy method transfer
- Lower sample volume requirements
- Faster run-times
- Enhanced sensitivity
- Higher resolution
- 21 CFR part 11 compliant software

This application note demonstrates the analysis of an Etanercept (Enbrel®) biosimilar with capillary electrophoresis – sodium dodecyl sulfate (CE-SDS) on the Maurice™ system using the fast Turbo CE-SDS™ cartridge. The following data are shown:

- Standard profiles of Etanercept under non-reduced and reduced CE-SDS conditions
- Linearity of the CE-SDS method
- Data reproducibility

The data presented in this application note will be useful for anyone who is looking for:

- Rapid and high-quality CE-SDS analysis
- A high throughput solution for analyzing the size and purity of fusion proteins or biosimilars
- An easier but reliable and robust alternative to HPLC and SEC

Materials and Methods

TABLE 1 lists the materials used in this study, including the Maurice Turbo CE-SDS Size Application Kit which contains all the necessary reagents for CE-SDS analysis on the Maurice system. Etanercept samples were diluted with the Maurice CE-SDS PLUS 1X Sample Buffer to a final concentration of 1 mg/mL. For linearity experiments, the sample concentration ranged from 1.5-0.5 mg/mL. Iodoacetamide (IAM) or β-mercaptoethanol (β-ME) were added to the samples for non-reduced or reduced analysis, respectively. The prepared samples were heated at 75°C for 10 minutes, cooled on ice for 5 minutes, and centrifuged for 5 minutes. The samples were then loaded onto the Maurice instrument and injected for 8 seconds at 3500 V and separated for 12 minutes (non-reduced) or 8.5 minutes (reduced) at 4200 V. It should be noted that the separation time for fusion proteins was increased slightly during method optimization. All data were analyzed using Compass for iCE software.

TABLE 1. Materials

Material	Vendor	Catalog #
Etanercept	AbMole BioScience	M6224
Maurice Turbo CE-SDS Size Application Kit	ProteinSimple, a Bio-Techne brand	PS-MAK01-TS
Maurice Turbo CE-SDS Cartridge		PS-MC02-TS, PS-MC01-TS
Maurice CE-SDS 25X Internal Standard		046-144
Iodoacetamide (IAM)	Millipore Sigma	16125
ß-mercaptoethanol (ß-ME)		M-3148

Results

FIGURES 1A and **1B** show the representative electropherograms of the Etanercept biosimilar, generated from the Maurice instrument. A whitepaper by BioPharmaSpec also discusses the analysis of Etanercept on Maurice, but with the CE-SDS PLUS cartridge[2]. Similar to the profiles published in that study, the non-reduced profile shown in **FIGURE 1A** demonstrates one major peak, corresponding to the intact molecule. In contrast, **FIGURE 1B** shows a narrower peak along with other minor peaks, which represent smaller fragments and products of reduction.

FIGURE 1A. Etanercept - Non-Reduced

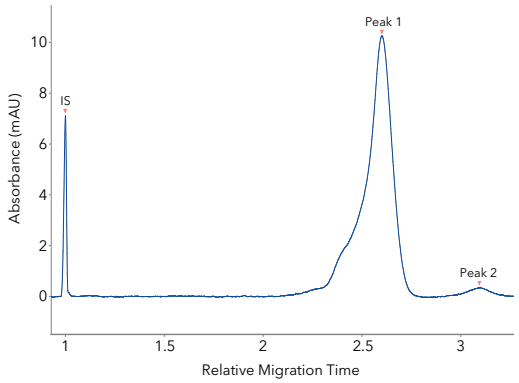

FIGURE 1B. Etanercept - Reduced

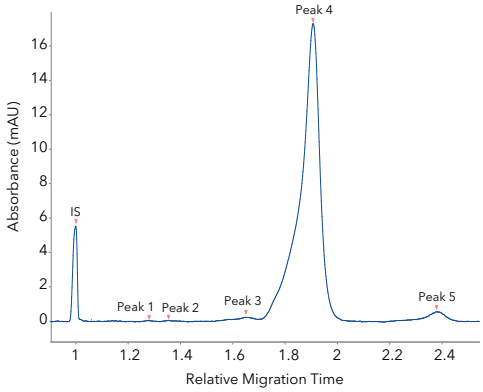

Figure 1. Analysis of Etanercept with Maurice Turbo CE-SDS. Profile of Etanercept under A. non-reduced conditions, showing a broad, major peak, and B. reduced conditions, with smaller peaks corresponding to smaller fragments after reduction except Peak 5, which likely corresponds to the intact fusion protein.

Linearity

The biosimilar samples were serially diluted with the Maurice CE-SDS PLUS 1X Sample Buffer, with concentrations ranging from 1.5 – 0.5 mg/mL. This dilution series was analyzed under both non-reduced and reduced conditions on the Maurice system, as shown in **FIGURES 2A** and **2B**, respectively. In both cases, a steady increase in peak intensity is seen with an increase in sample concentration, suggesting that the Turbo CE-SDS method meets the linearity requirements. Notably, minor peaks were detected even at low concentrations, indicating the sensitivity of the method. A plot of these data is shown in **FIGURE 3**.

FIGURE 2A. Etanercept - Non-Reduced

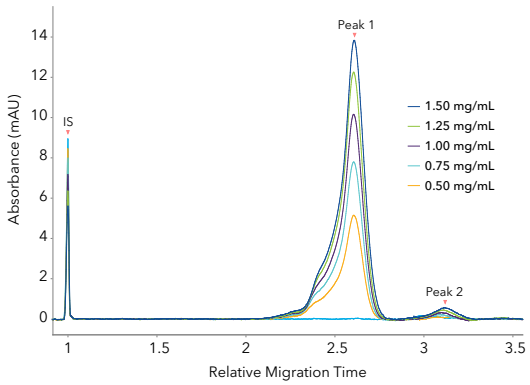

FIGURE 2B. Etanercept - Reduced

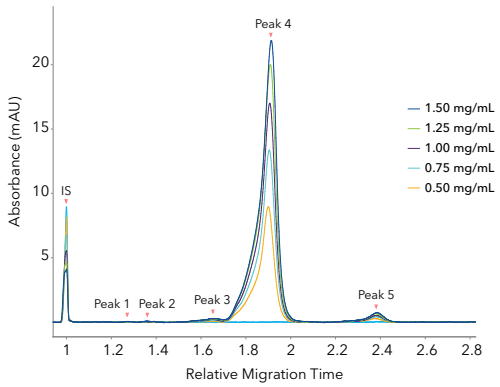

Figure 2. Linearity of the Turbo CE-SDS method for the detection of an Etanercept biosimilar. Samples with concentrations ranging from 1.5 – 0.5 mg/mL were analyzed under A. non-reduced conditions and B. reduced conditons, showing an increase in peak intensity with an increase in concentration.

FIGURE 3. Dilutional Linearity

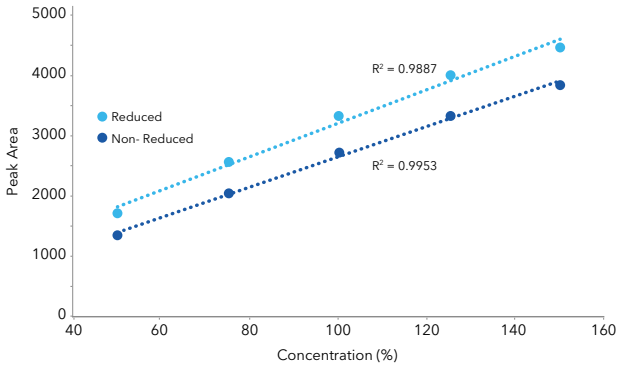

Figure 3. Graphical representation of dilutional linearity experiments with Turbo CE-SDS. The peak area calculated from the sample electropherograms were plotted against the sample concentration. The method was linear for both non-reduced and reduced analyses, with R^2 values of 0.9953 and 0.9887, respectively.

Reproducibility

A 96-injection batch of the Etanercept biosimilar was run using the Turbo CE-SDS cartridge to assess the method's reproducibility. **FIGURES 4A** and **4B** each show nine representative electropherograms from non-reduced and reduced experiments, respectively. The results were highly reproducible with the relative standard deviation (RSD) values being **<0.6%** for the main peak in both cases. Additionally, **FIGURE 4C** displays a gel-like view of the results, which can be obtained from the Compass for iCE software's "Lane View Feature." Three sets of data from different intervals are shown for non-reduced and reduced analyses. The consistency observed for the non-reduced peaks not only speaks to the reproducibility of the method but is also attributed to the stability of the CE-SDS PLUS 1X Sample Buffer that minimizes fragmentation from disulfide bonds.

FIGURE 4A. Etanercept - Non-Reduced

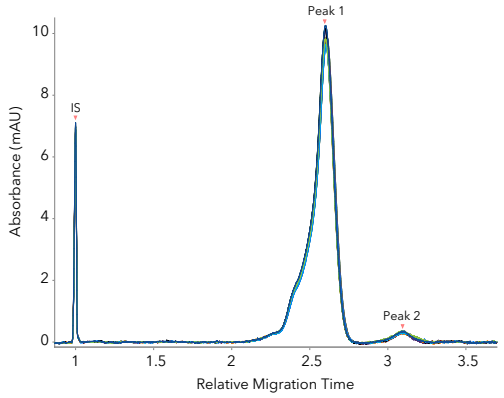

FIGURE 4B. Etanercept - Reduced

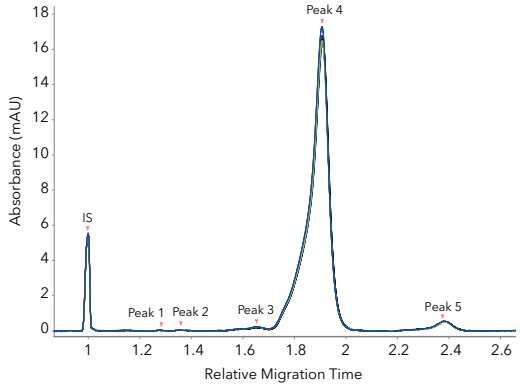

FIGURE 4C. Etanercept - Lane View

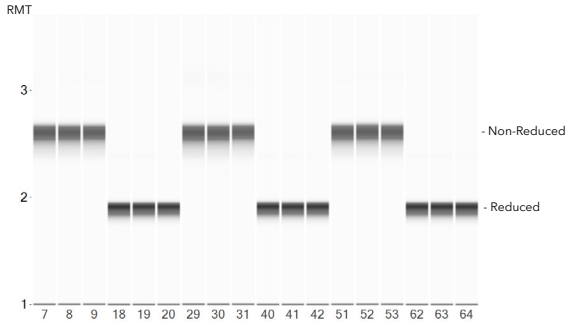

Figure 4. Reproducibility of the Turbo CE-SDS method. From a 96-injection batch, nine electropherograms are overlaid to demonstrate the reproducibility of the method under A. non-reduced conditions and B. reduced conditions. The reproducibility was excellent for both conditions, with %RSD values <0.6 overall for the main peak (marked as Peak 1 and Peak 4 in Figures 4A and 4B respectively). C. shows a gel-like representation of the results, generated using the "Lane View" feature on Compass for iCE software. Different sets of data from both non-reduced and reduced analyses were selected to generate this view, demonstrating high-reproducibility throughout the 96-injection batch.

Conclusion

This application note has demonstrated a fast, reliable, and label-free CE-SDS method to analyze fusion proteins like Etanercept with the Turbo CE-SDS™ cartridge on the Maurice system. The method resulted in excellent linearity ($R^2 > 0.99$) and reproducibility (%RSD <0.6) while delivering high quality data between 8-12 minutes. The Turbo CE-SDS cartridge has been specifically designed for high throughput applications while maintaining data quality, enabling better decision-making early on. Furthermore, to ensure that the Maurice system can be implemented throughout the drug development process as seamlessly as possible, another cartridge, the CE-SDS PLUS, enables analysis in downstream stages such as QC and GMP release, providing high-resolution data in 25-35 minutes. Additionally, the same Maurice system also enables charge heterogeneity analysis with imaged capillary isoelectric focusing (icIEF), which is the gold standard for analyzing protein charge isoforms. Whether the need is to analyze many samples fast, or to ensure that the therapeutic is meeting quality standards during manufacturing, the Maurice system ensures that your analytical requirements are met while vastly simplifying your workflow. Visit our website to learn more.

References

1. Report - New and Upcoming Biosimilar Launches. Cardinal Health. Updated January 18, 2023.
2. White paper – Use of Maurice CE-SDS in ICH Q6B Based Biosimilar Comparability Exercises. Bio-Techne. Updated May 04, 2022.

4860975483_LG_AN_etanercept turbo ce-sds_CZ