A novel approach for objective, quantifiable HOS comparisons: a biosimilar case study utilizing circular dichroism.

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Introduction

During biotherapeutic development a wide range of biophysical characterization techniques are required to support informed decision-making and contribute to the totality of evidence in regulatory submissions. Regulatory authorities are increasing their demand for 'state-of-the-art' techniques that can provide statistically-validatable data. To date, obtaining such results for higher order structure (HOS) comparisons has presented challenges in terms of data acquisition and suitability of statistical methods.

Here we present a novel, integrated approach to HOS analysis to generate an objective, quantifiable comparison of a commercially-available biotherapeutic (Fab fragment) with a biosimilar currently under development.

Methods

Sample preparation and experimental set-up

All samples were simultaneously dialyzed to equilibrium against a fresh formulation buffer. Samples were loaded into 96-well microplates, alternating buffer-sample-buffer-sample etc.. Three replicates of each sample were analyzed – five repeat spectra for each replicate.

Analysis of secondary and tertiary structure

To generate high quality CD spectra and raw data suitable for rigorous statistical analysis, three lots each of innovator and biosimilar were analyzed on Chirascan™ Q100 as follows:

- Secondary structure: far-UV (190 to 250 nm, 0.1 mm pathlength flow cell)
- Tertiary structure: near-UV (250 to 350 nm, 10 mm pathlength flow cell)

Fully integrated system for HOS analysis

$$WSD = \sqrt{\sum_{i=1}^{n}\left[\left(\frac{1}{n}\right)\left(\frac{|y_{Ai}|}{|y_{A}|_{ave}}\right)(y_{Ai}-y_{Bi})^2\right]}$$

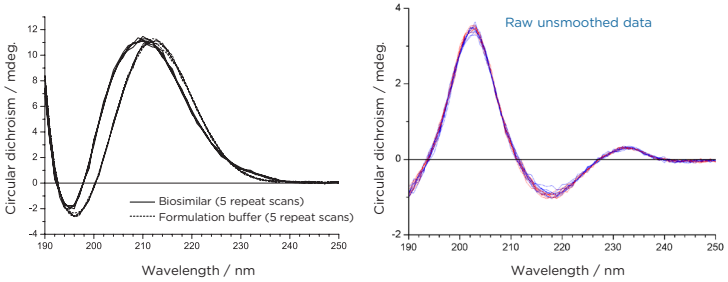
From spectra to numerical data

Data interpretation

Data were compared using the weighted spectral difference method (WSD) to generate a quality attribute for statistical analysis[1]. This attribute was analyzed with a quality range approach with +/-2SD acceptance criteria as recommended for intermediate (tier 2) risk ranking[2].

[1] *Dinh et al., Anal. Biochem. 464 (2014):60-62*
[2] *Statistical approaches to evaluate analytical similarity; Guidance for Industry; CDER/CBER/FDA*

Results: HOS analysis possible in a highly absorbing, 'CD-active' formulation buffer

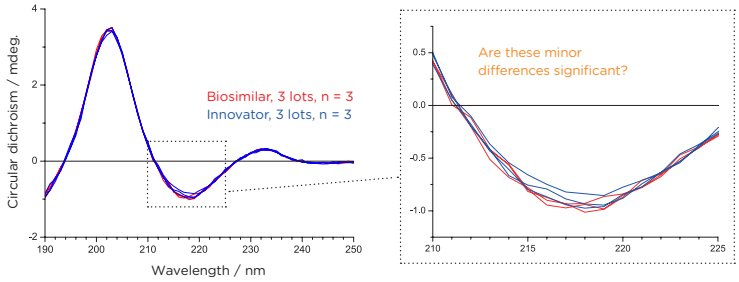


Risk of baseline artefacts removed by dialysis of biosimilar and innovator lots against a common preparation of' formulation buffer and using formulation buffer as reference.

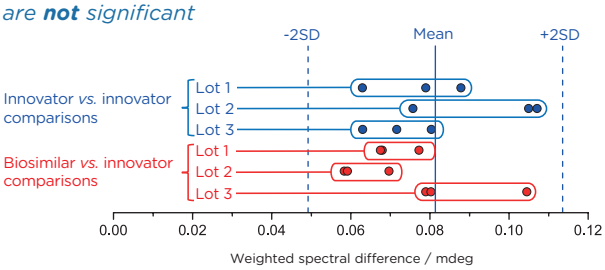
Results: HOS comparisons

High sensitivity CD analysis enabled objective assessments of differences between secondary and tertiary structure of innovator and biosimilar lots.

Secondary structure CD analysis

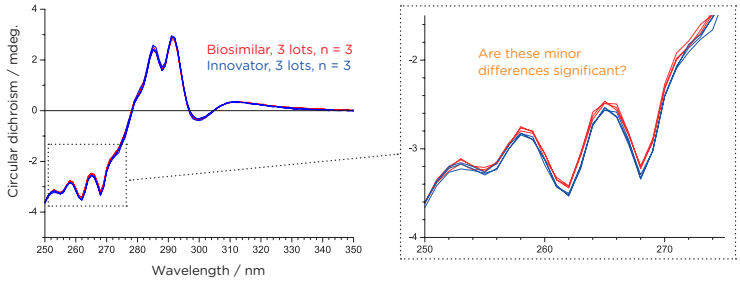


*Data analysis – minor differences in secondary structure are **not** significant*

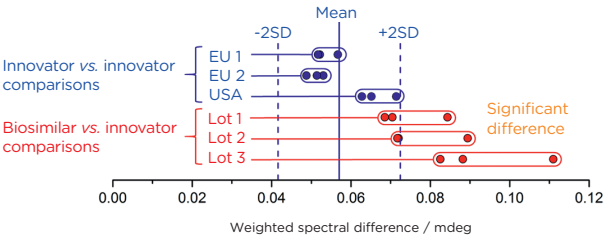


Tier 2 quality range approach applied +/-2SD acceptance criteria

Tertiary structure CD analysis



*Data analysis – minor differences in tertiary structure **are** statistically significant*



Tier 2 quality range approach applied +/-2SD acceptance criteria

Conclusion

Objective statistical quantification of differences or similarities in HOS:

- **Ensures effectiveness of comparability programs**
- **Facilitates informed decision-making throughout development and scale-up**
- **Strengthens totality of evidence for regulatory submissions**

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