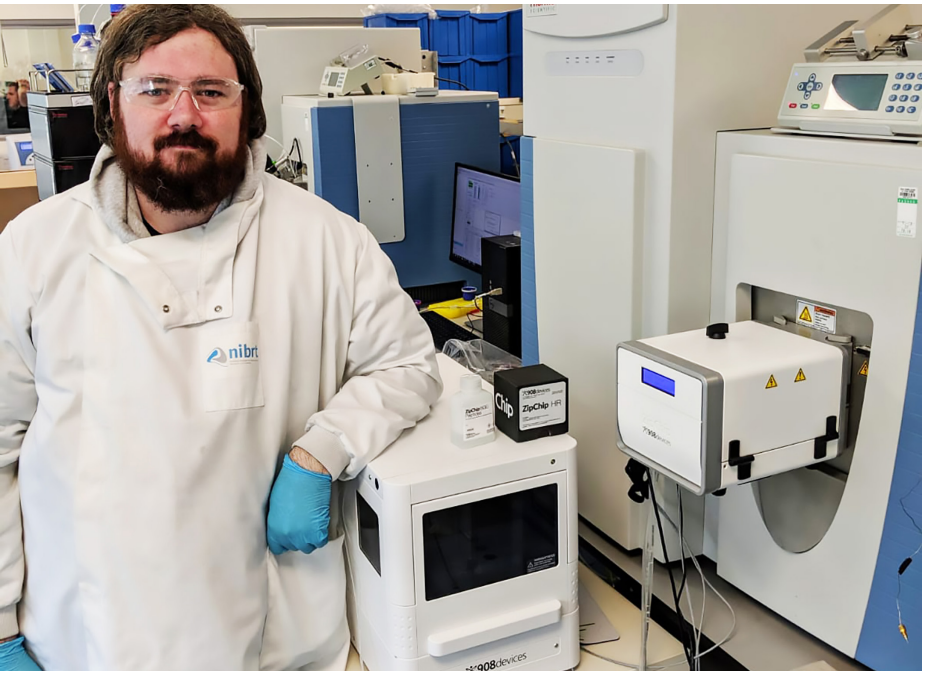
CASE STUDY:

ZipChip analysis to unravel biosimilarity of therapeutic proteins.

HIGHLIGHTS

- CE-MS analysis for in-depth comparison of biosimilars
- The ZipChip® platform couples microfluidic capillary zone electrophoresis (CE) separation of intact proteins with direct electrospray ionization mass spectrometry (ESI-MS) analysis, facilitating the evaluation of drug product heterogeneity and comparison between originator and biosimilars.
- The high number of components confidently identified and quantified allows fast assessment of biologics similarity.



The ZipChip platform facilitates the evaluation of drug product heterogeneity and comparison between originator and biosimilars.

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Materials & Methods

Sample Prep:
100 µg of each monoclonal antibody were buffer-exchanged according to the ZipChip protocol for intact antibody charge variant analysis with 0.5 mL spin-filters with 10KDa MWCO and concentrated to 0.5 mg/mL.

ZipChip Protocol	Boosting Sensitivity for Intact Antibody Charge Variant Analysis
Assay Kit	Native Antibodies Kit
Chip Type	ZipChip HRN
Field Strength	500 V/cm
Run Time	15 minutes
Mass Spec Type	Thermo Scientific QExactive™ Plus Hybrid Quadrupole Orbitrap mass spectrometer

Introduction

According to the FDA "*a biosimilar is a biological product that is highly similar to and has no clinically meaningful differences from an existing FDA-approved reference product*" and similarity is assessed "*by extensively analyzing (i.e., characterizing) the structure and function of both the reference product and the proposed biosimilar*" and only "minor differences between the reference product and the proposed biosimilar product in clinically inactive components are acceptable"[1]. Assessment of biosimilarity is usually performed by extensively analyzing the structure and function of both the reference product and the proposed biosimilar. Based on their high similarity with already approved drugs and their ability to lower the cost of current treatment, a shorter pathway has been designed for biosimilar approval under the Biologics Price Competition and Innovation (BPCI) Act, approved in the US in 2010. As a consequence, biopharmaceutical companies have interest in analytical tools that facilitate in-depth characterization of therapeutics to assess biosimilarity, in particular, the micro-heterogeneities present in the originator product. For this purpose, usually multiple orthogonal analyses are needed. For biosimilar approval the major effort is needed in terms of analytical rather than clinical studies (Fig 1).

In the present case study we evaluated the suitability of ZipChip platform to investigate biosimilarity by analyzing two commercially available infliximab drug products: Remicade the originator product, approved in the US in 1998; targeting TNF-α, it is indicated for the treatment of several autoimmune disease, including Crohn's disease and its biosimilar, Inflectra, the first biosimilar approved by EU regulatory agencies in 2013.

Results

In the assessment of biosimilarity there is a need for bioanalytical tools that enable fast and confident evaluation of a candidate's micro-heterogeneity with increased dynamic range to verify that no critical quality attributes demonstrate significant differences from the originator.

Charge variant analysis is a regulatory requirement and is usually performed through CE or LC methodology to assess the charge variant profile[2]. Hyphenation of charge sensitive

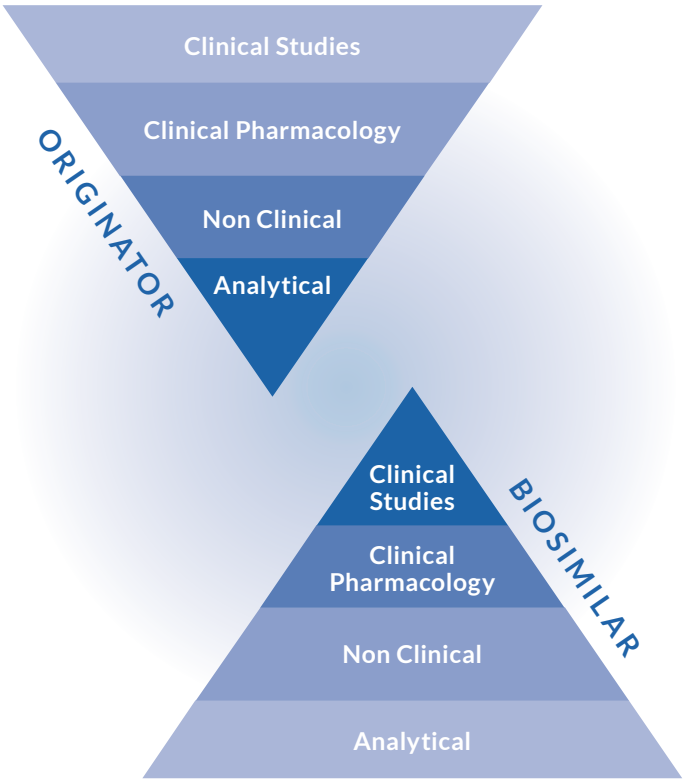

Figure 1: Difference between approval process for originator and biosimilar therapeutic proteins.

separation methods with mass spectrometry has been shown to be a powerful characterization technique to understand the distribution and contribution of posttranslational modifications to the overall charge variant profile[3,4]. Infliximab drug products have a complex charge variant pattern, derived from the C-terminal lysine heterogeneity, with proteoforms presenting both, one or no terminal lysine residues[5]. In this complex pattern, low abundant proteoforms arising from N-glycan heterogeneity[6], could easily be hidden below main species if only optical detection were used; MS hyphenation ensures low abundant species are not missed, especially with the high sensitivity attainable on the ZipChip platform.

Moreover, the minimal sample preparation required ensures no artificially induced modifications are introduced during the analysis, allowing a more reflective comparison of drug originator product and biosimilar candidates.

Figure 2 shows the electropherogram obtained from the Remicade drug product, with 3 main peaks identified and several minor species, all presenting complex glycoform heterogeneity, yielding a total of almost 100 proteoforms for both molecules, confidently identified with mass accuracies < 20 ppm. On the most acidic main peak (A), it is possible to observe a complex heterogeneity arising from deamidation and sialylation (peaks A1-A6). The same pattern is visible for peaks B and C even though most of the low abundant species migration times overlap with more abundant species in peak A and B, respectively. It is clear that in this case the evaluation of data acquired through optical detection only would not allow correct evaluation of all charge variants.

From the data obtained it was possible to generate evaluation of overall deamidation abundance, N-glycan profiling and lysine variants differences in the two monoclonal antibodies, including species as low as 0.1 %. In particular, Inflectra drug product is characterized by a higher level of C-terminal lysine cleavage and higher levels for terminal galactose on Fc N-glycan.

The excellent data quality and sensitivity, together with the short analysis time required by this platform, are key for a quick and in-depth screening and evaluation of biosimilarity across biologics.

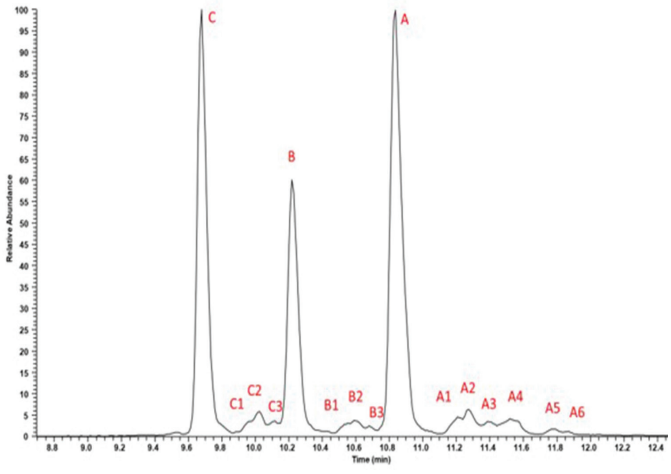

Figure 2: Electropherogram obtained from CE-MS analysis on infliximab Remicade® drug product.

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REFERENCES:
1. https://www.fda.gov/drugs/biosimilars/biosimilar-and-interchangeable-products **2.** Sosic Z, Houde D Blum A, Carlage T, Lyubarskaya Y, *Electrophoresis* 2008; 29(21): 4368-4376. **3.** Redman EA, Mellors JS, Starkey JA, Ramsey JM. Anal Chem. 2016; 88(4): 2220-2226. **4.** Füssl F, Cook K, Scheffler K, Farrell A, Mittermayr S, Bones J. *Anal Chem.* 2018; 90(7): 4669-4676. **5.** Redman EA, Batz NG, Mellors JS, Ramsey JM. *Anal Chem.* 2015, 87(4): 2264-2272. **6.** Marti SM, Delporte C, Farrell A, Iglesias NN, McLoughlin N, Bones J. *Analyst* 2015, 140: 1442-1447.

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