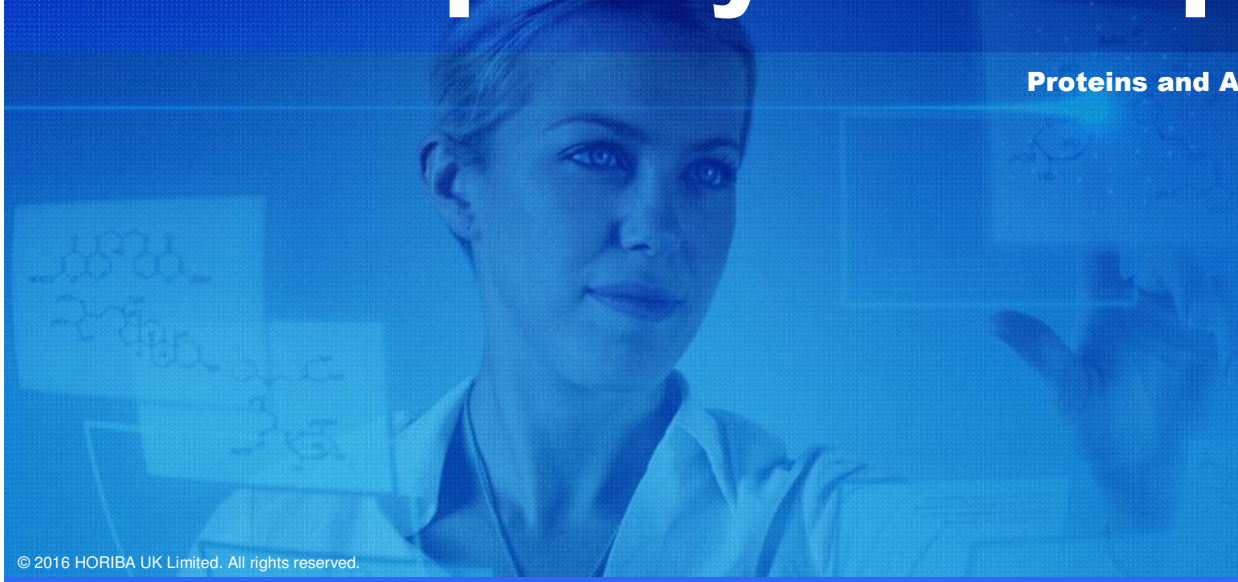
HORIBA

Explore the future

Dr. Deepika Devanur

Monoclonal Antibody Stability Trials Using High Performance Capillary Electrophoresis



Proteins and Antibodies Congress 18th-19th April 2016

Introduction

The use of **HPCE** to characterise the mAbs ***Rituximab*** and ***Infliximab***

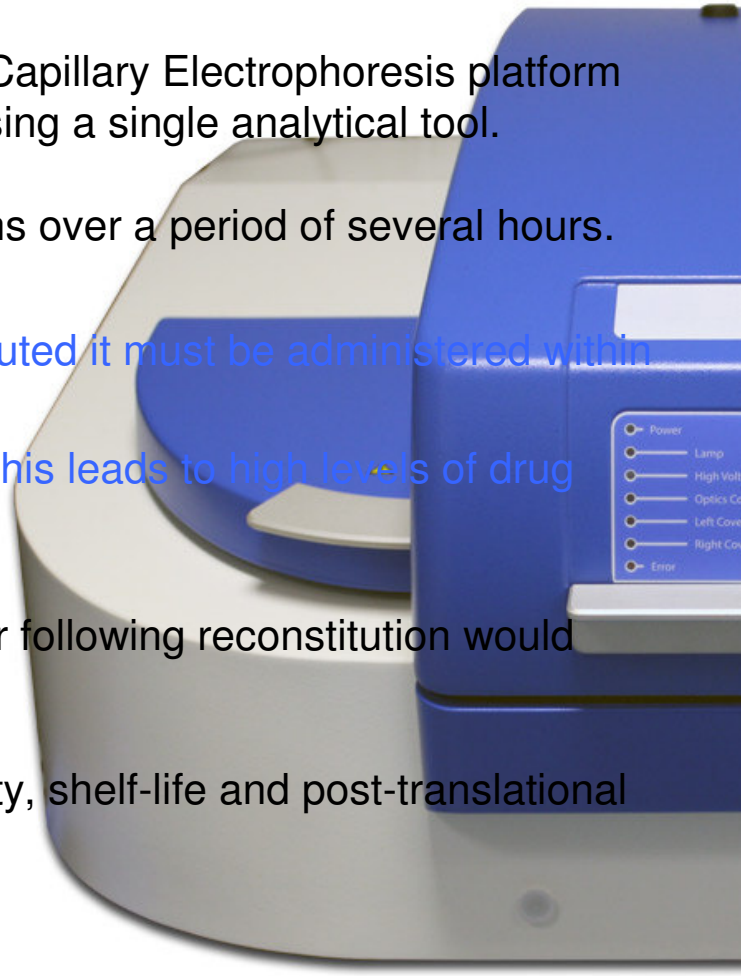
This presentation demonstrates the use of the **HPCE-512** Capillary Electrophoresis platform to provide a complete characterisation of a mAb product using a single analytical tool.

Rituximab and *Infliximab* are often administered as infusions over a period of several hours.

- Current protocol dictates that once an infusion is reconstituted it must be administered within 24h or discarded.
- Any remaining drug left in the infusion is also discarded. This leads to high levels of drug wastage and contributes to high treatment costs.

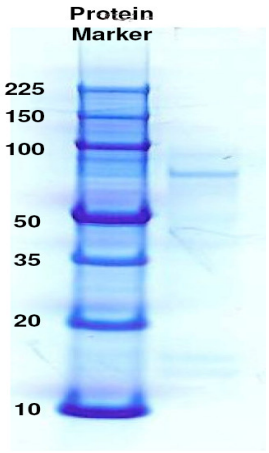
The ability to test whether the drug is still safe to administer following reconstitution would prevent wastage and lead to significant cost-savings.

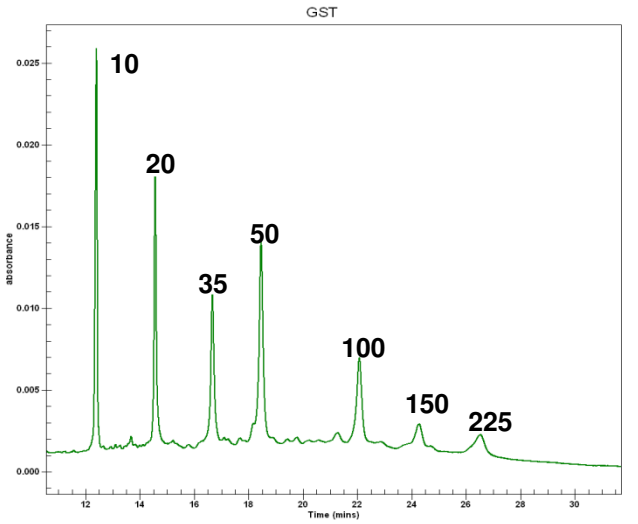
HPCE-512 can be used to determine fragmentation, stability, shelf-life and post-translational modifications on mAbs.



Capillary Gel Electrophoresis (CGE)

- **CGE is a method by which SDS-coated proteins move through the sieving matrix and are separated according to their size.**
- **Capillaries offer significant advantages over traditional gels:**
  - Small sample volumes
  - Use of 10 to 100 times higher electric fields without the deleterious effects of Joule heating
  - On-capillary detection
  - Instrument automation



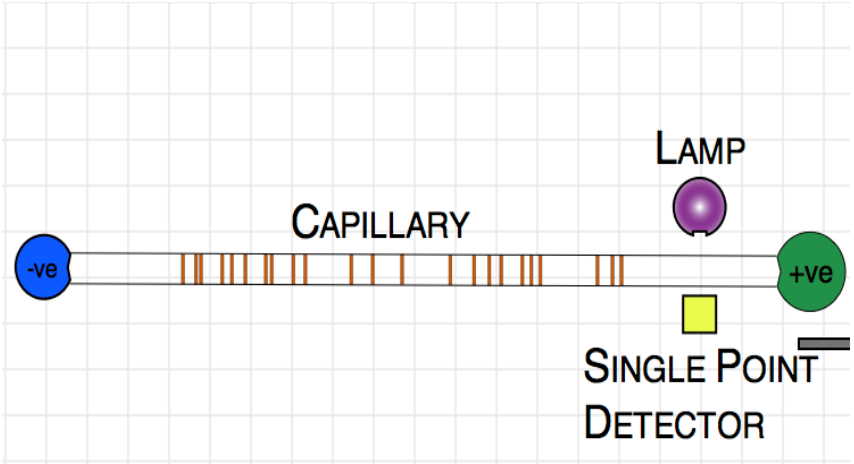


Traditional gels can take hours to run, stain, de-stain, analyse and document

HPCE – Fast, accurate, inherently digital, quantitative, repeatable and high resolution

Capillary Electrophoresis (CE)

Multipixel detection solves CE's problems!

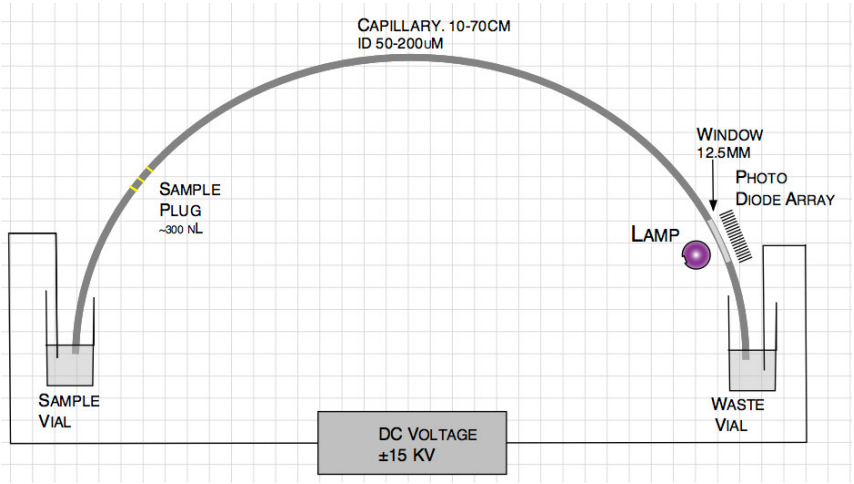


HPCE technology images each analyte band **512** times across a photo diode array at ~**10** times a second.

This results in ~**100 000** data points on a typical analysis including data on both time and band position.

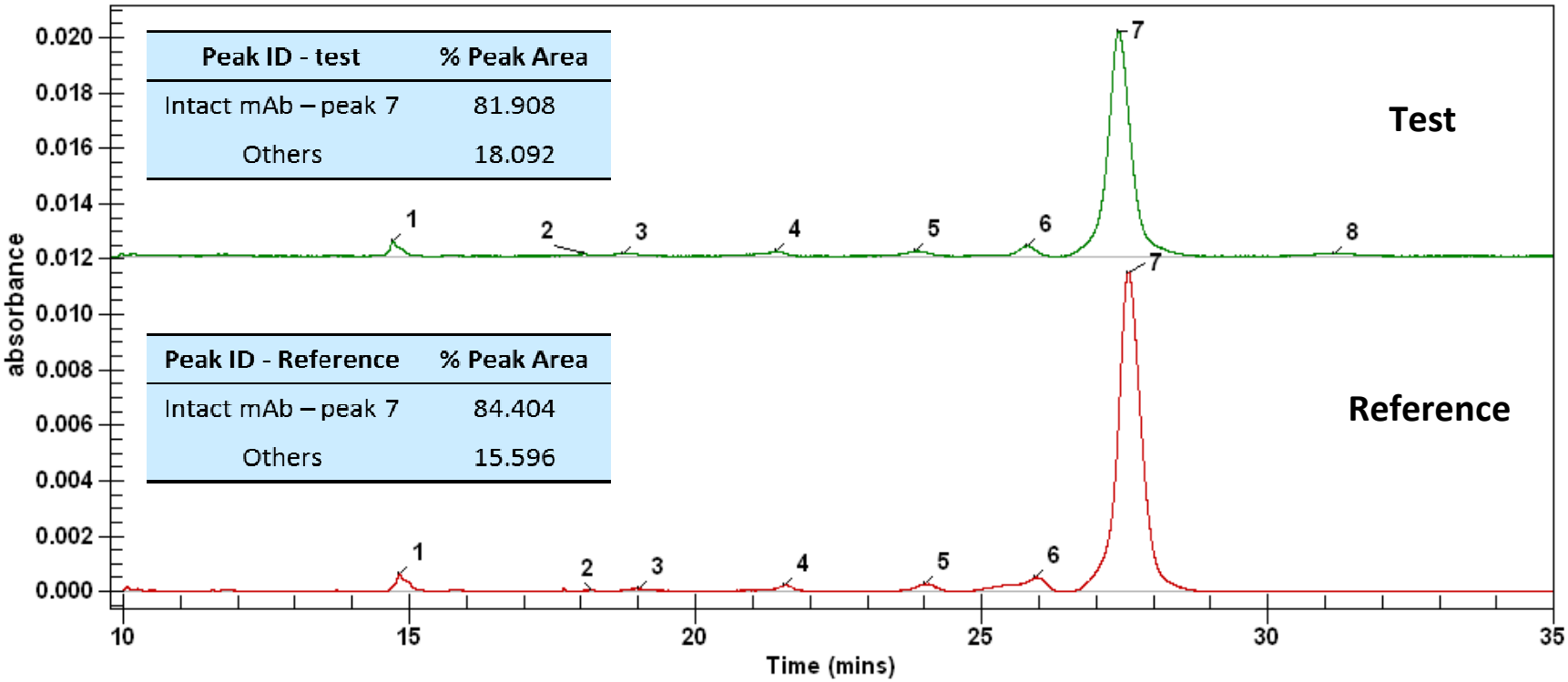
Conventional CE instruments use single point detection systems and have a poor reputation due to poor data quality, especially lack of *repeatability*.

They measure each analyte only **once** at a single point in time and so are prone to "noise-related" problems.



Capillary Gel Electrophoresis (CGE)

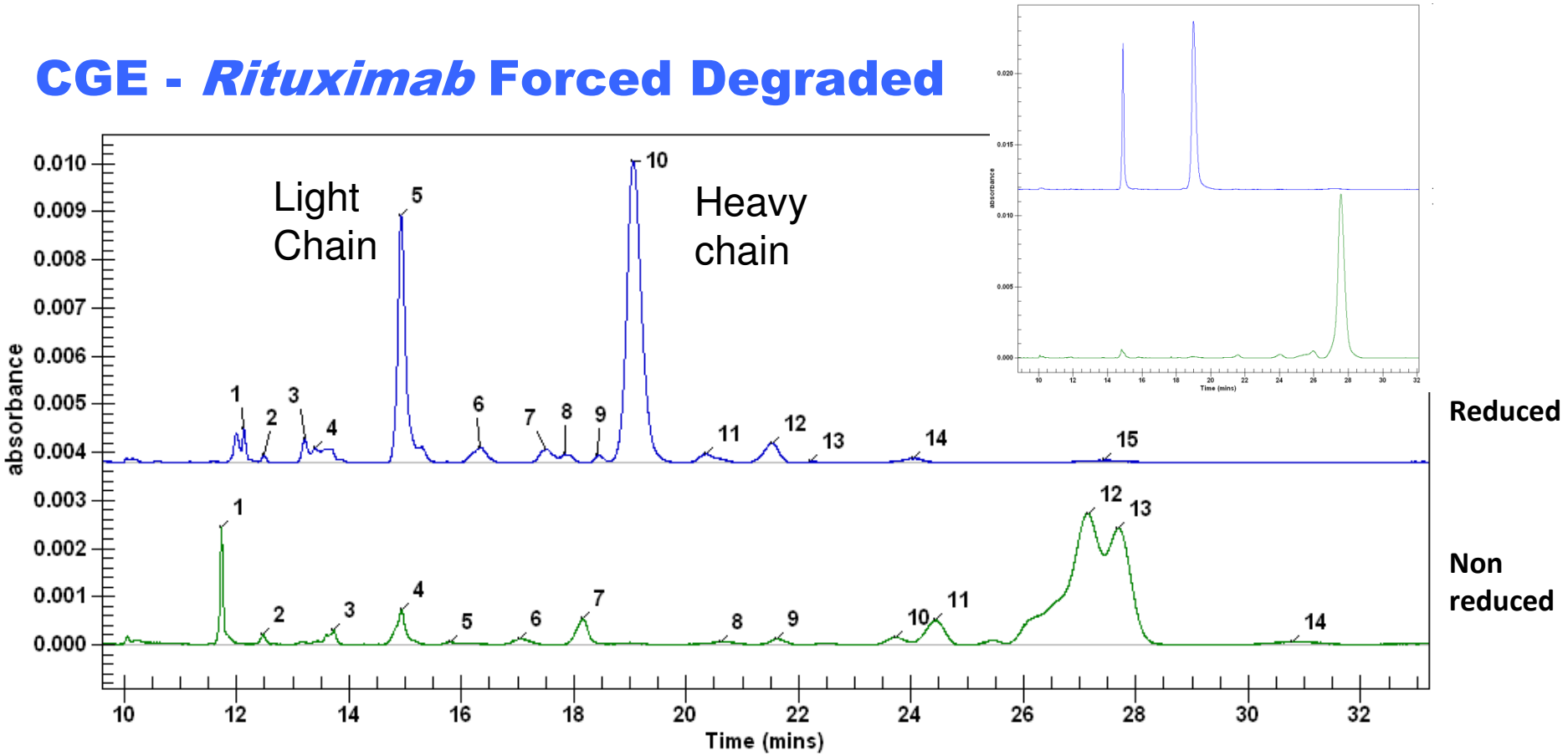
CGE - *Rituximab* Intact Antibody



- A small decrease in peak area of the intact antibody (peak 7) is observed in the test sample – could be as a result of degradation.
- An extra peak (peak 8) is observed in the test sample – could indicate aggregation.
- Subtle changes in antibody profile could indicate alterations in drug potency and efficacy.

Capillary Gel Electrophoresis (CGE)

CGE - *Rituximab* Forced Degraded



- The reference sample was subjected to forced degradation by prolonged storage at 40℃. The non-degraded sample is shown in the inset for comparison
- Multiple peaks are observed for each sample.
- Any degradation observed in the sample can be easily detected.

Capillary Zone Electrophoresis (CZE)

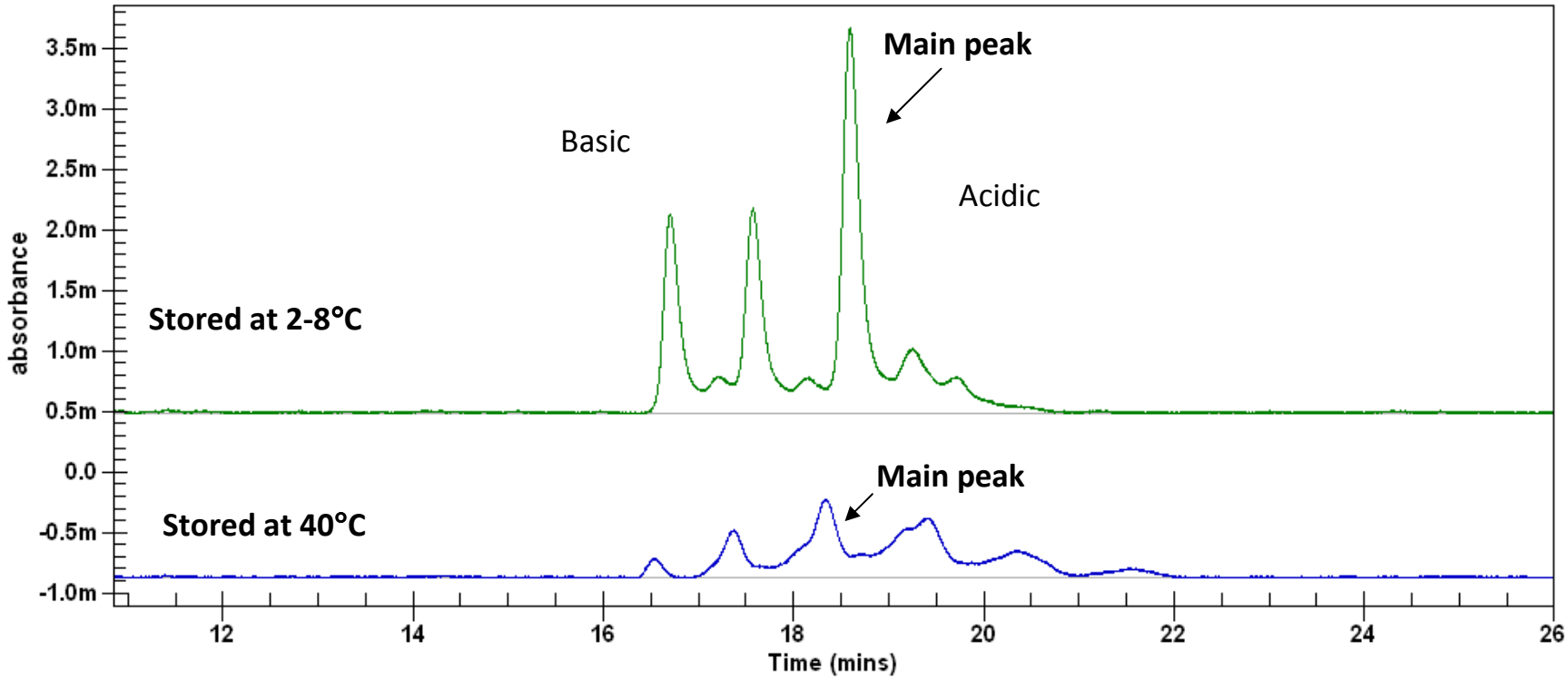
CZE – Charge Variant Analysis

- **mAb charge variants can occur as a result of C-terminal lysine modification, deamidation and post-translational modifications.**
  - Analysis of these variants provides information on the product's quality and stability.

- **These analyses were carried out using capillary zone electrophoresis (CZE) in a simple buffer system.**
  - CZE is a technique whereby analytes are separated on the basis of their charge and hydrodynamic radius.

Capillary Zone Electrophoresis (CZE)

CZE – Charge Variant Analysis of *Infliximab*



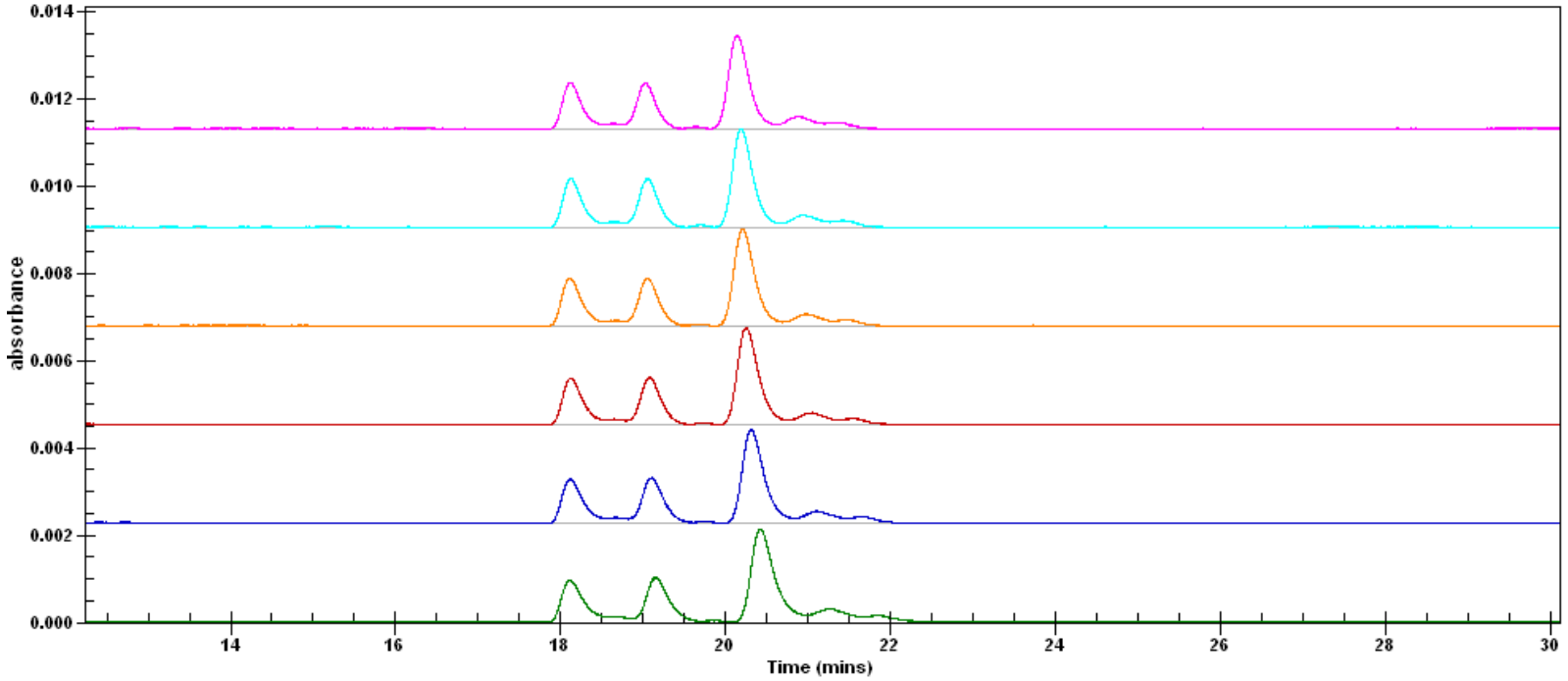
2-8°C	% Peak Area
Basic	46.9
Main peak	36.7
Acidic	16.4

40°C	% Peak Area
Basic	18.0
Main peak	26.9
Acidic	55.1

- **Significant change in charge profile observed following storage at 40°C – an overall decrease in peak areas is observed along with a shift in charge profiles**

Capillary Zone Electrophoresis (CZE)

CZE – Charge Variant Analysis of *Infliximab*



6 replicate injections of the 0.2mg/ml control sample were carried out.

% RSD Time = 2.283
Area = 2.882

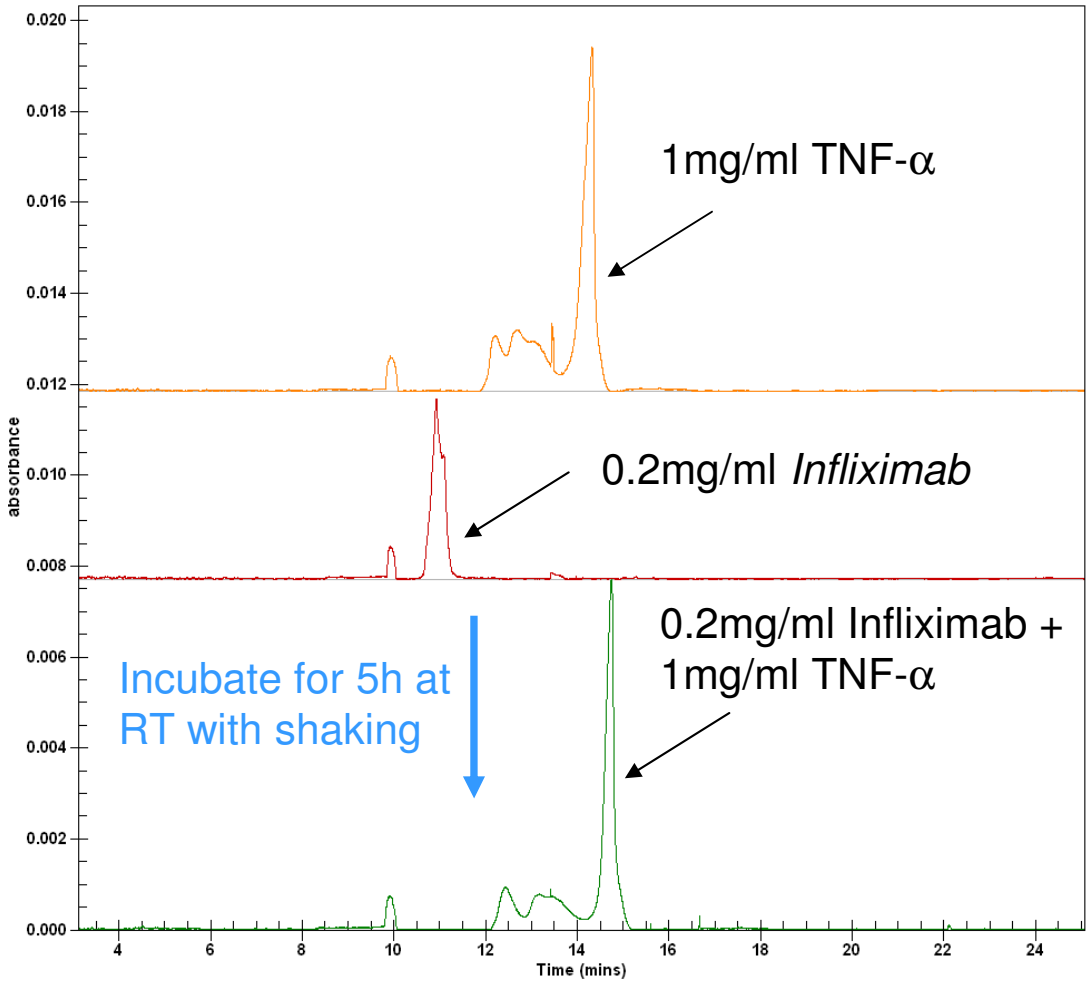
Capillary Zone Electrophoresis (CZE)

CZE – Binding

- The final stage of assessing the suitability of an antibody drug product is the evaluation of its binding to the target molecule.
- The binding of *Infliximab* to TNF-α was assessed using CZE.
- The label-free nature of the **HPCE-512** platform enables the monitoring of binding events without the interference of labels.

Capillary Zone Electrophoresis (CZE)

CZE – Binding of *Infliximab* to TNF-α



- Preliminary data shows a loss of the *Infliximab* peak is observed following incubation with TNF-α
- Shows that all the *Infliximab* has bound to TNF- α.
- A slight shift is seen when the complex forms.
- TNF is present in excess

Summary

- The data presented here demonstrate the ability of the **HPCE 512** Capillary Electrophoresis platform to completely characterise a mAb product.
- Various modes of CE were utilised in order to obtain information on the structure and functionality of the antibody.
- Excellent repeatability for peak migration time and peak area were obtained.

HORIBA Scientific

Thank you very much for your attention.

Thank you

Omoshiro-okashiku

Joy and Fun

감사합니다

Cảm ơn

ありがとうございました

Dziękuję

धन् यवा द

Grazie

Merci

谢谢

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ขอบคุณครับ

Obrigado

Σας ευχαριστούμε

شُكْرا

Tack ska ni ha

Большое спасибо

Danke

Gracias