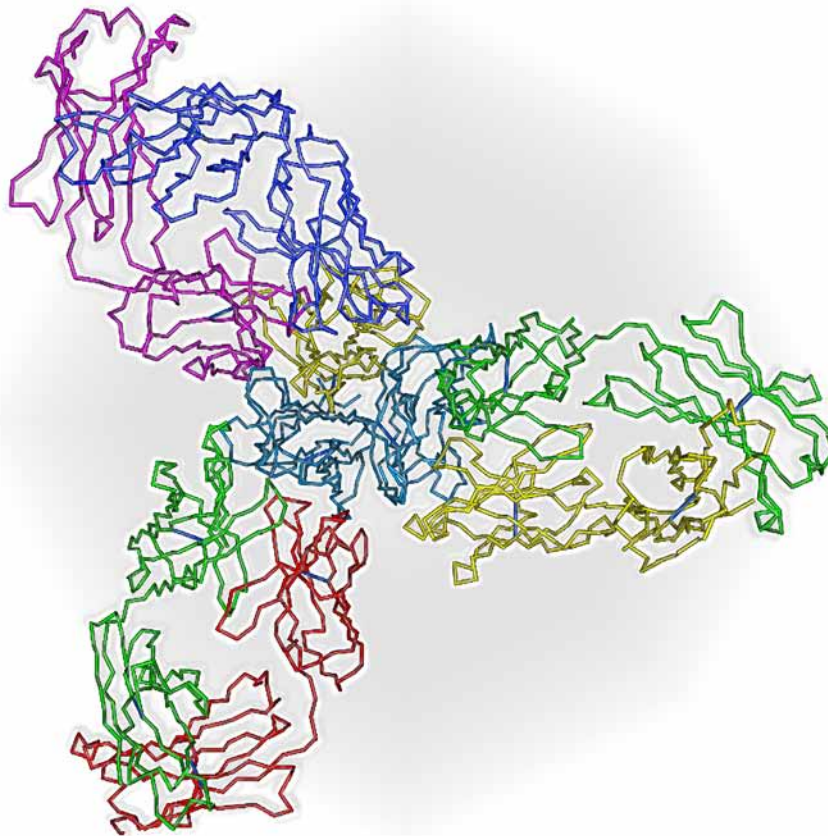# ANTIBODY ANALYSIS

using LABEL FREE INTRINSIC IMAGING

Application Note: Monoclonal Antibody Analysis

ANALYSIS OF MABS USING deltaDOT'S HPCE-512^{TC} LABEL FREE CAPILLARY ELECTROPHORESIS INSTRUMENT

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ABSTRACT

Label Fre Intrinsic Imaging LFII® analysis was carried out on the monoclonal antibodies Infliximab and Rituximab for the assessment of stability, fragmentation, charge heterogeneity and repeatability. This work was performed as part of a study for the UK's National Health Service (NHS). Quantifiable differences were observed in the samples before and after heat-stress. Excellent repeatability was obtained with a peak area Relative Standard Deviation (RSD) of 2.88% and a peak time RSD of 2.28%.

INTRODUCTION

In order to assess the bioactivity and safety of recombinant monoclonal antibodies (mAbs) as biological drugs, it is important to fully characterise them. Not only is this information crucial for the assessment of the suitability of a potential biopharmaceutical product, it is also important for the optimisation and QA/QC of the manufacturing process, storage conditions and life-time assessment of the drug at the site of use.

The mAbs Rituximab and Infliximab are biological drugs used in the treatment of arthritis as well as a number of other inflammatory and autoimmune diseases. These drugs are often administered as saline infusions over a period of several hours. The current NHS protocol dictates that once an infusion is reconstituted it must be administered within 24 hours or discarded. Any remaining drug left in the saline 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 for the NHS. This technology is also fully applicable upstream in mAb manufacturing process.

This application note describes how deltaDOT's High Performance Capillary Electrophoresis platform, HPCE-512^{TC} can be used to determine fragmentation, stability, shelf-life and post-translational modifications on mAbs. These parameters can be used to assess whether a biological drug is safe for use following storage and reconstitution.

MATERIALS AND METHODS

Capillary Gel Electrophoresis (CGE)

mAb samples were diluted to a concentration of 0.5mg/ml in CGE sample buffer and run under reducing and non-reducing conditions. The reduced samples were treated with β-mercaptoethanol and heated at 70°C for 10 minutes. The non-reduced samples were heated at 70°C for 10 minutes with no reductant.

Capillary electrophoresis (CE) separations were performed in CGE running buffer containing sodium dodecyl sulphate (SDS) in a bare fused silica capillary of 50µm internal diameter and a separation length of 20cm. The total length of the capillary was 32.4cm. All samples were injected hydrodynamically at 10psi for 10s and separated at 16kV (reverse polarity) for 45 minutes. The capillary was held at 22°C during the separations. All detection was at 214nm. At the beginning of the experiment and between the runs, the capillary was conditioned using a proprietary methodology.

Capillary Zone Electrophoresis (CZE)

mAb samples were diluted to a concentration of 0.2mg/ml in DI water and CE separations were performed in proprietary CZE buffer in a bare fused silica capillary of 50µm internal diameter and a separation length of 40cm. The total length of the capillary was 52.4cm. All samples were injected hydrodynamically at 0.5psi for 10s and separated at 24kV (standard polarity) for 45 minutes. The capillary was held at 25°C and the sample carousel was held at 20°C during the separations. All detection was at 214nm. At the beginning of the experiment and between the runs, the capillary was conditioned using a proprietary methodology.

RESULTS AND DISCUSSION

CGE - Comparison between Reference and Test samples of Rituximab

The Rituximab sample was run under reducing and non-reducing conditions. Comparisons between the reference (stock concentrate) sample and a test (reconstituted saline infusion product) sample were made. Reducing the sample breaks the disulphide bonds between the heavy and light chains of the antibody resulting in the separation of the two chains. It was found that the peak areas were decreased in the test samples indicating that some sample degradation may have occurred (**Figure 1**). A comparison of the corrected peak areas and % areas is shown in **Table 1**. It was found that an increase in the ratio of light chain to heavy chain was observed in the test sample compared to the reference sample. This could indicate that some degradation of the heavy chain has occurred.
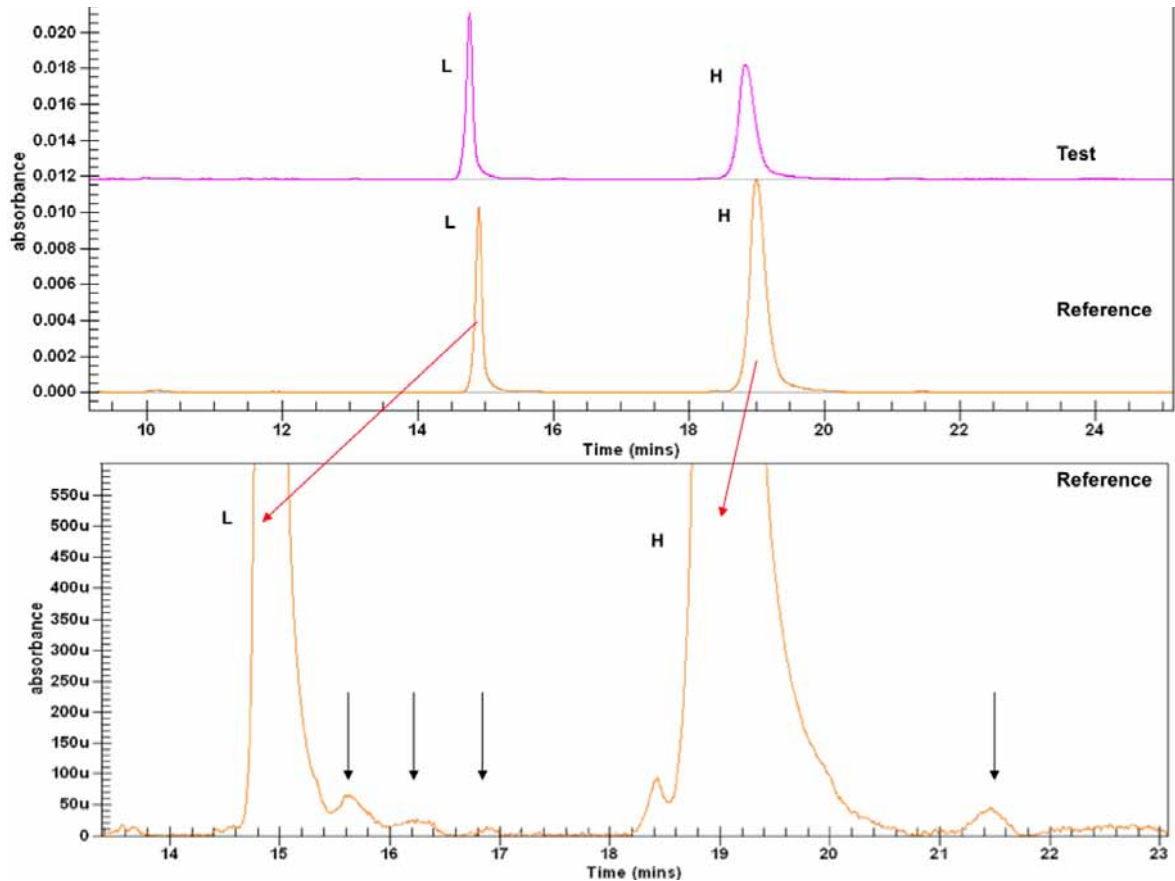

Figure 1. *Electropherogram showing the separation of the Rituximab reference and test samples under reducing conditions. The light (**L**) and heavy (**H**) chains are marked on the figure. Clear differences were observed between the two samples. The expanded graph shows the smaller peaks present in the reference sample that do not show up in the original graph. These peaks were also present in the test sample and indicate the power of the label free signal processing. Without the ability to resolve all the peaks present, especially small ones close to much larger peaks, correct product quality cannot be assesed.*

Reference sample	Molecular weight	Corrected Peak Area	% Peak Area
Light Chain	24.0	5.44×10^{-2}	31.78
Heavy Chain	65.4	0.115	66.95
Other peaks	-	2.16×10^{-3}	1.25

Test sample	Molecular weight	Corrected Peak Area	% Peak Area
Light chain	22.7	4.86×10^{-2}	42.12
Heavy chain	60.6	6.45×10^{-2}	55.96
Other peaks	-	2.20×10^{-3}	1.90

Table 1. *Molecular weights, corrected peak areas and % peak areas for the reference and test samples.*

Under non-reducing conditions, the % peak area of the intact antibody (peak **7**) is reduced in the test sample compared to the reference sample **(Figure 2)**, indicating a low level of degradation has occurred. An extra peak is observed in the test sample (peak **8**), which migrated more slowly than the intact peak, which suggests that there is also some level of aggregation. These results indicate that there are subtle changes in the mAb once it has been reconstituted, which may contribute to altered drug efficacy and potency.
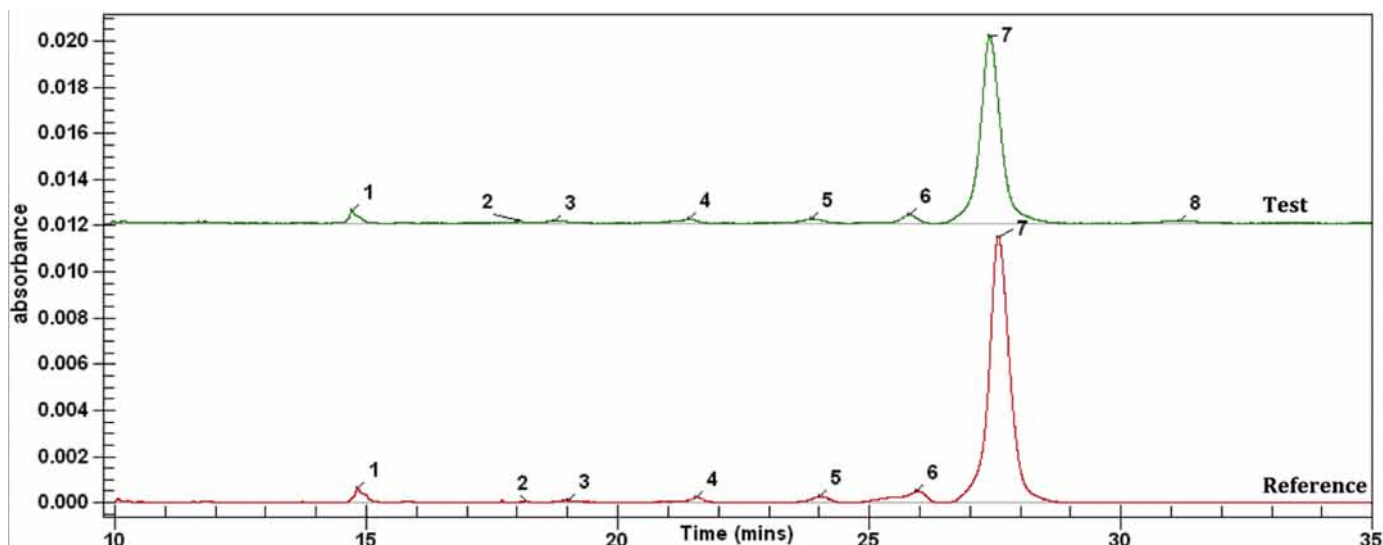

Figure 2. *Electropherogram showing the separation of the Rituximab reference and test samples under non-reducing conditions. Peak **7** is the intact peak.*

Peak ID	% Peak Area
Intact mAb	84.40
Others	15.59

(a)　　　Reference

Peak ID	% Peak Area
Intact mAb	81.90
Others	18.09

(b)　　　Test

Table 2. *% of intact mAb compared to other species present in the sample in the (a) reference and (b) test samples.*

CGE – Fragmentation analysis

The reference (infusion concentrate) sample, which had been forced degraded by prolonged storage at 40°C was analysed under reducing and non-reducing conditions (**Figure 3**). A significant increase in the number of peaks was observed following degradation when compared to the reference samples in **Figures 1** and **2**.
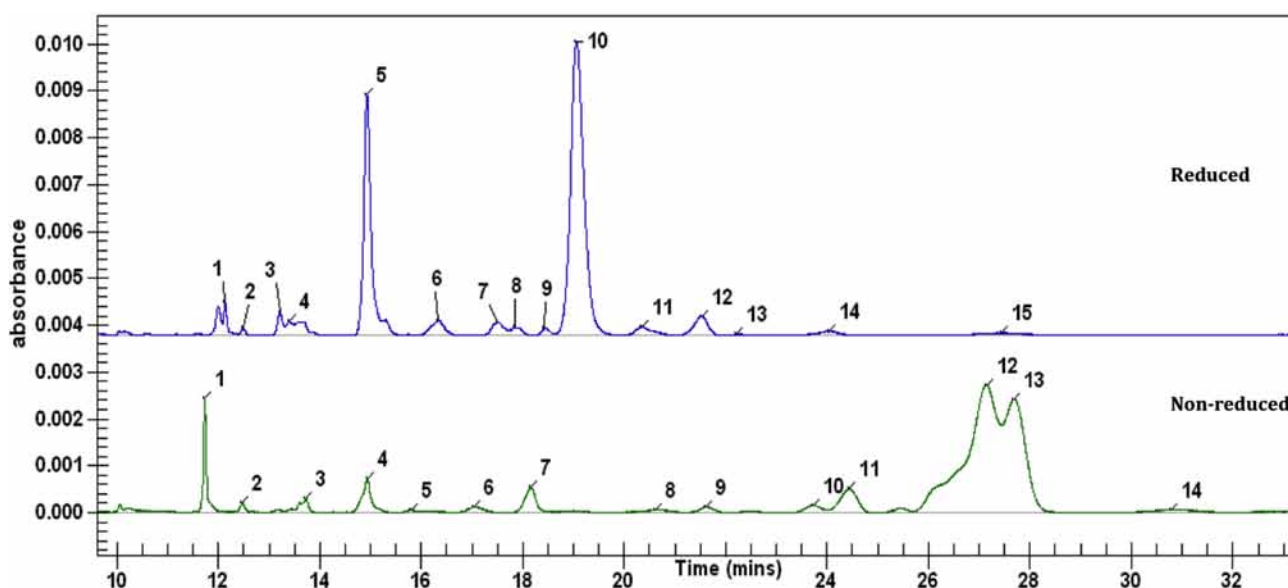

Figure 3. *Electropherogram showing the separation of the Rituximab reference samples which had been force degraded by prolonged storage at 40°C. As expected an increased number of peaks were observed compared to the non-stressed reference samples. The software can generate % areas for all the peaks if needed (**Table 3**).*

Reduced mAb Peak ID	% Peak Area	Non-reduced mAb Peak ID	% Peak Area
1	5.07	1	9.08
2	0.71	2	1.30
3	2.17	3	2.83
4	4.45	4	5.69
5 (L)	27.72	5	0.92
6	2.87	6	1.46
7	2.05	7	4.28
8	1.28	8	1.06
9	0.78	9	1.01
10 (H)	45.84	10	1.38
11	1.73	11	4.59
12	2.87	12	41.76
13	0.23	13	23.41
14	1.14	14	1.16
15	1.04	-	-

Table 3. Percentage peak areas for the Rituximab reference samples, which had been force degraded by storage at 40°C.

CZE – Post-translational modification analysis by charge heterogeneity of Infliximab

Samples of Infliximab prepared at different concentrations were stored at 2-8°C as a control or under stressed conditions at 40°C. The differences in the profiles were assessed using a CZE protocol. Significant differences were observed between the control samples and the stressed samples at the temperature parameters tested. A 1.07mg/ml sample of Infliximab was split into two aliquots with one aliquot being stored at 4-8°C and one stored at 40°C (**Figure 4**). A clear difference in the charge profiles was observed, with the stressed sample showing an increase in the acidic variants as well as an overall decrease in peak area. This analysis demonstrates the differences in post-translational modifications caused by storage at inappropriate temperatures.
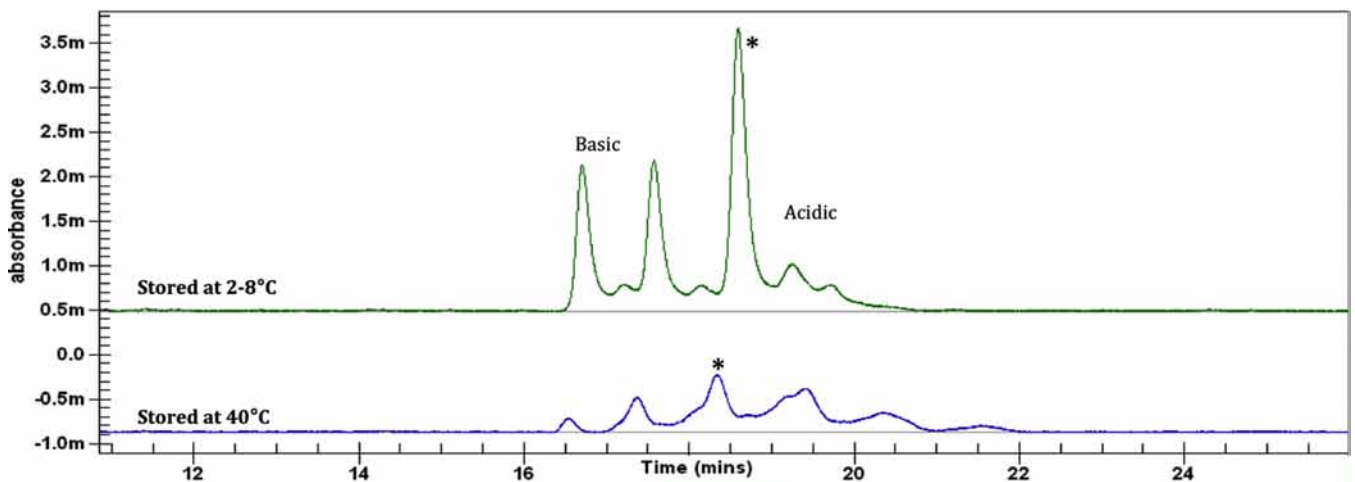

Figure 4. GST electropherogram showing the separation of the Infliximab sample stored at 2-8°C and the sample stored at 40°C. The main peak is marked with an asterisk and the basic (to the left of main peak) and acidic (to the right of main peak) variants are labelled on the figure.

2-8°C	% Peak Area	40°C	% Peak Area
Basic	46.9	Basic	18.08
Main peak	36.7	Main peak	26.9
Acidic	16.4	Acidic	55.1
(a)		(b)	

Table 4. Peak areas (%) of the main peak as well as the basic and acidic variants of the sample stored at (a) 2-8°C and (b) 40°C.

CZE – Repeatability

Repeatability was assessed by carrying out 6 replicate injections of the Infliximab control sample at a concentration of 0.2mg/ml (**Figure 5**). Relative Standard Deviations (RSDs) of 2.88% peak area and 2.28% peak time were obtained.
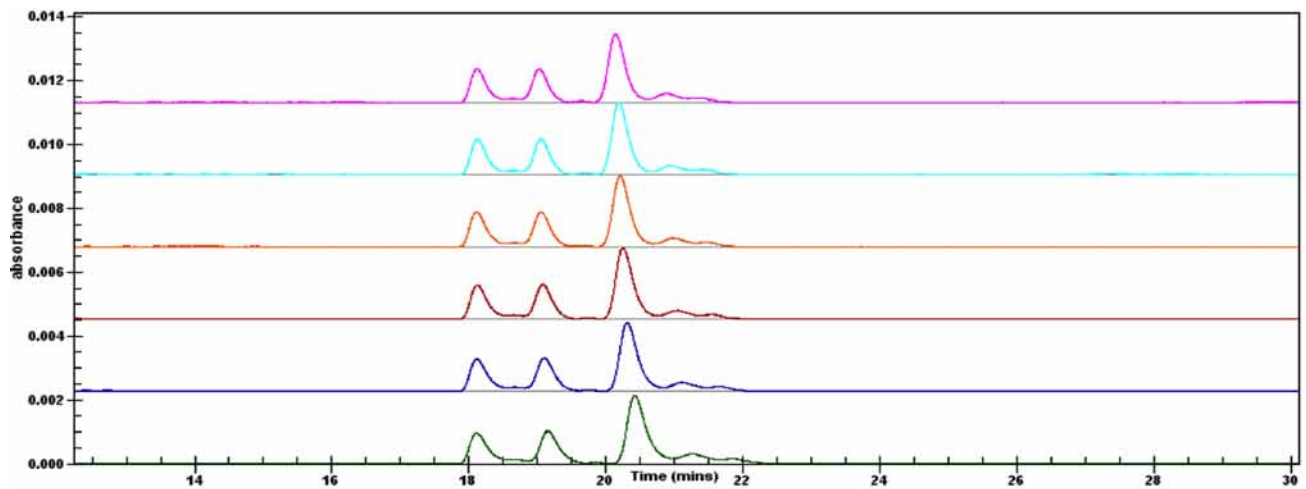

Figure 5. *Electropherogram showing 6 replicate injections of the 0.2mg/ml Infliximab control sample.*

CONCLUSION

The data presented in this application note demonstrate the ability of deltaDOT's HPCE 512[TC] capillary electrophoresis platform to provide a complete characterisation of a mAb drug product using a single analytical tool. Quantifiable differences were observed in heat-stressed samples using both CGE and CZE techniques allowing the sample stability to be assessed for suitability for administration to patients.
Excellent repeatability of 2.88% for peak area and 2.28% for peak time were obtained.

deltaDOT's label free technology has wide applications across the bioprocess and can be applied to most PAT process units.

Goods received

In process - when to supplement Amino Acid X?

Post process - how efficient was my bioprocess?

Protein expression

Protein purification

Protein quantification

Protein aggregation

Protein fragmentation

Protein interactions

Formulation, stability and batch release

	STARTING MATERIALS	UPSTREAM METHOD DEVELOPMENT	MIDSTREAM	DOWNSTREAM	QA/QC MS TRIAGE	FORMULATION	BATCH RELEASE
WATER	QUALITY			WASTE		QUALITY	
AMINO ACIDS	QUALITY	USAGE OPTIMISATION	USAGE MONITORING SUPPLEMENTATION	USED MEDIA ANALYSIS			
VITAMINS	QUALITY	USAGE OPTIMISATION	USAGE MONITORING SUPPLEMENTATION	WASTE			
CARBOHYDRATES	QUALITY	USAGE OPTIMISATION	USAGE MONITORING SUPPLEMENTATION	WASTE			
CONTAMINATION			LACTIC ACID ENDOTOXIN	RUBISCO NICOTINE	NICOTINE		
DNA		CLONING	CONTAMINATION	CONTAMINATION			
PROTEIN EXPRESSION			QUANTIFICATION QUALITY	QUANTIFICATION QUALITY	QUANTIFICATION QUALITY		
PROTEIN PURIFICATION			QUANTIFICATION QUALITY	QUANTIFICATION QUALITY	POST-TRANSLATIONAL MODIFICATIONS	AGGREGATION	
PROTEIN FRAGMENTATION			COLUMN SHEAR	PRODUCT STABILITY	PRODUCT STABILITY	PRODUCT STABILITY	
PROTEIN FOLDING		PROCESS EFFICIENCY	EXPRESSION EFFICIENCY	PRODUCT STABILITY		PRODUCT STABILITY	
BIOPOTENCY				TARGET AFFINITY		PRODUCT EFFICIENCY	PRODUCT STABILITY
STABILITY						FRAGMENTATION AGGREGATION	PRODUCT FIDELITY

Monoclonal Antibody analysis

Analysis was carried out on the monoclonal antibodies Infliximab and Rituximab for the assessment of stability, fragmentation, charge heterogeneity and repeatability. This work was performed as part of a study for the UK's National Health Service (NHS). Quantifiable differences were observed in the samples, which had also been heat-stressed.

The data presented in this application note demonstrate the ability of deltaDOT's HPCE 512TC capillary electrophoresis platform to provide a complete characterisation of a mAb drug product using a single analytical tool. Quantifiable differences were observed in heat-stressed samples using both CGE and CZE techniques allowing the sample to be assessed for suitability for administration to patients.
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deltaDOT has developed and is commercialising innovative capillary electrophoresis technologies and products in the bioscience sector. The company has a strong proprietary position and extensive expertise in instrumentation automation, computing and analysis which will contribute to improvements in knowledge, profitability and process time throughout drug discovery and general life sciences research.