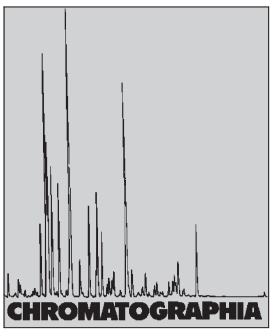
A Series of Collaborations Between Various Pharmaceutical Companies and Regulatory Authorities Concerning the Analysis of Biomolecules Using Capillary Electrophoresis



2006, *64*, 359–368

B. Nunnally[1,✉], S.S. Park[2], K. Patel[2], M. Hong[3], X. Zhang[4], S.-X. Wang[4], B. Rener[5], A. Reed-Bogan[5] O. Salas-Solano[6], W. Lau[6], M. Girard[7], H. Carnegie[7], V. Garcia-Cañas[7], K.C. Cheng[8], M. Zeng[8] M. Ruesch[9], R. Frazier[10], C. Jochheim[11], K. Natarajan[11], K. Jessop[12], M. Saeed[12], F. Moffatt[12] S. Madren[1], S. Thiam[1], K. Altria[13]

[1]Vaccine Analytical Development, Wyeth Research, Wyeth, 4300 Oak Park, Sanford NC 27330, USA
E-Mail: nunnalb@wyeth.com
[2]Pharmaceutics Department, Amgen Inc., One Amgen Center Dr. MS 8-1-C, Thousand Oaks CA 91320, USA
[3]Pharmaceutical Development, Centocor Research & Development Incorporation, 145 King of Prussia Road, Radnor PA 19087, USA
[4]Analytical Development, Chiron Corporation, 4560 Horton Street, Emeryville CA 94608, USA
[5]Bioproduct Pharmaceutical Research and Development, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis IN 46285, USA
[6]Late-Stage Analytical Development, Genentech, Inc., 1 DNA Way, South San Francisco CA 94080, USA
[7]Centre for Biologics Research, Health Canada, Banting Bldg., Tunney's Pasture, Ottawa ON K1A 0L2, Canada
[8]Analytical development and formulation development, Medarex, Inc., 519 Route 173 West, Bloomsbury NJ 08804 , USA
[9]Analytical Research and Development, Global Biologics, Pfizer Global Research and Development, Pfizer, 700 Chesterfield Parkway West, Chesterfield MO 63017, USA
[10]Protein Sciences, Pfizer Global Research and Development, Pfizer, 700 Chesterfield Parkway West, Chesterfield MO 63017, USA
[11]Analytical Biochemistry and Formulations, Seattle Genetics Inc., 21823 30th Drive Southeast, Bothell WA 98021, USA
[12]Syngenta Ltd, Jealotts Hill, Bracknell, Berkshire RG42 6EY, UK
[13]Pharmaceutical Development, GlaxoSmithKline, Third Avenue, Harlow, Essex CM23 1DW, UK

Received: 13 March 2006 / Revised: 24 May 2006 / Accepted: 29 May 2006
Online publication: 10 August 2006

Abstract

An international project team (including members from US, Canada and UK) has been formed from a number of interested biopharmaceutical companies and regulatory authorities to conduct a cross-organisation collaboration exercise. The results from this exercise demonstrate the robustness of CE-SDS across eight different organisations that used instruments of the same equipment model, the same reagents, and the same methodology. Data generated from the analysis of a series of molecular weight markers showed very good precision with regards to relative migration time (RMT) both within and between organisations. The apparent molecular weight of bovine serum albumin (BSA) was measured with good precision to within approximately 2% RSD across the participants. A representative IgG sample showed similar results with regards to relative migration time of its 3 main components, IgG Light Chain, IgG Non-glycosylated Heavy Chain, and IgG Heavy Chain. Fractional peak area for each peak also showed good agreement, with less than 9% RSD for all peaks. This exercise will facilitate both increased regulatory and industrial opinion of CE for biopharmaceutical analysis.

Keywords

Capillary electrophoresis
Collaboration exercise
Biopharmaceutical analysis
CE-SDS

Introduction

The development and commercialisation of recombinant monoclonal antibodies (rMabs) to treat unmet medical needs has increased significantly in the biopharmaceutical industry. During production and shelf life of therapeutic proteins, several post-translational modifications can occur such as deamidation, oxidation, iso-aspartate isomerization, and proteolytic cleavages [1], which results in the production of a range of product-related substances. It is important to characterize and monitor these substances to gain a better understanding of any potential impact upon the bioactivity and safety of a biopharmaceutical product. Detailed product characterization is also used to support the suitability of the manufac-

CE in the Biotechnology & Pharmaceutical Industries: 7th Symposium on the Practical Applications for the Analysis of Proteins, Nucleotides and Small Molecules, Montreal, Canada, August 12–16, 2005

DOI: 10.1365/s10337-006-0003-y
0009-5893/06/09

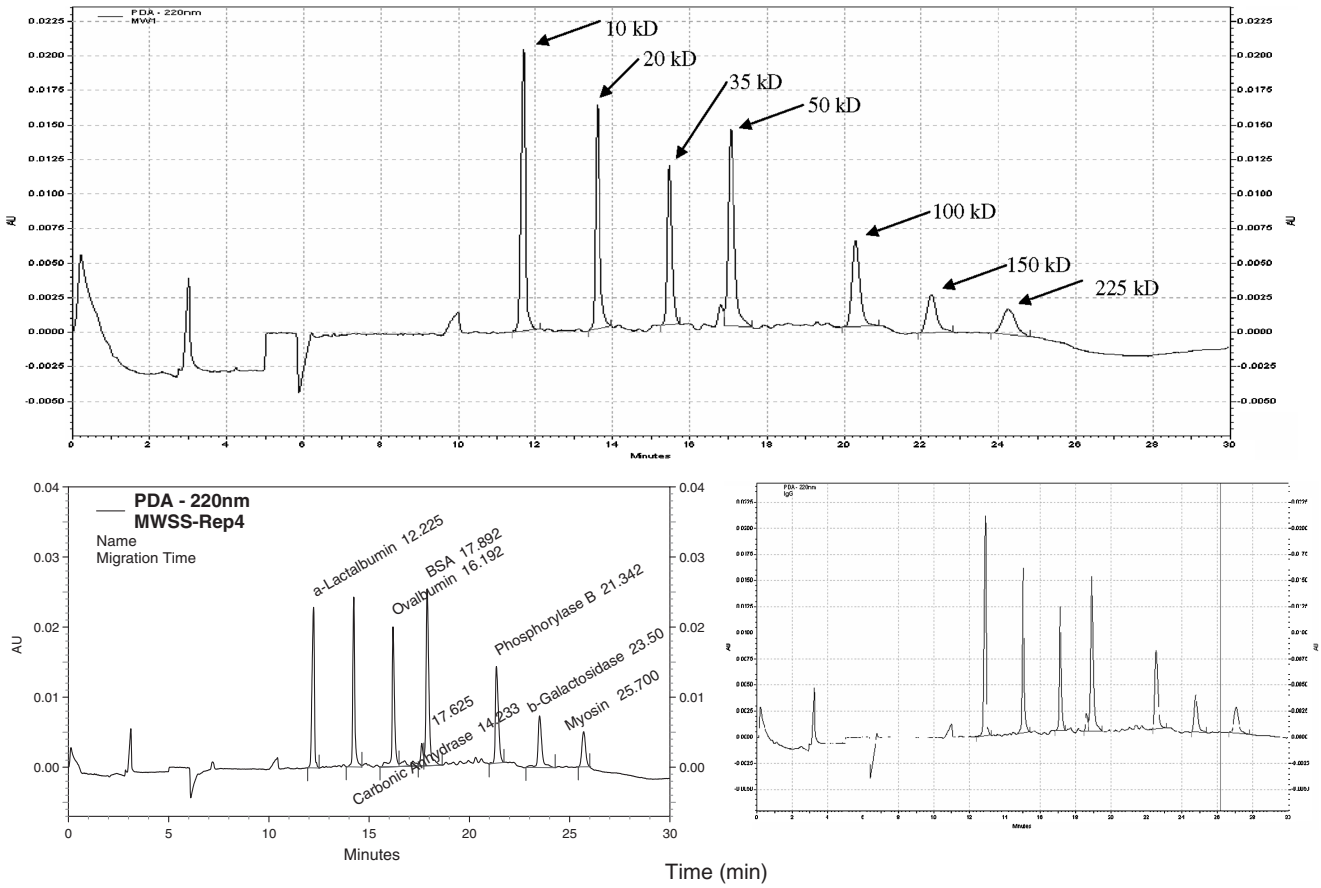


Fig. 1. Representative electropherograms of the molecular weight standards

turing process controls employed. The characterisation also assists in assessing the effect that process changes may have upon the identity, strength, quality and purity of a drug; as these factors may impact the safety and efficacy [2] of a drug. The physicochemical properties of these therapeutic proteins such as charge, size and hydrophobicity require assessment using analytical techniques. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has traditionally been used as the primary method for size-based protein separations [3]. Detection of the separated proteins by SDS-PAGE is generally accomplished by staining with either Coomassie Brilliant Blue [4] or the more sensitive silver stain dyes [5]. The logarithm of the molecular mass of a protein is linear with electrophoretic mobility. Therefore the molecular weight of a given protein can be estimated from a series of protein standards. Besides acting as a tool to determine the apparent molecular weight of proteins, SDS-PAGE is also used to evaluate the size, heterogeneity, purity, and manufacture consistency of biologics. The major drawbacks of conventional SDS-PAGE have been its inconvenience and the irreproducibility associated with the staining/destaining steps used in analyte detection, the use of toxic reagents and high intra- and inter-gel effective mobility variability.

Capillary electrophoresis (CE) has been embraced in protein characterisation activities as it has been shown to offer many advantages over classical SDS-PAGE. These advantages include on-column direct UV or fluorescence detection, automation, enhanced resolution and reproducibility, as well as facilitating accurate quantitation of proteins and determination of their molecular weight [6–13]. Currently, linear or slightly branched polymers, such as linear polyacrylamide, polyethylene oxide (PEO), polyethylene glycol, dextran, and pullulan are often used as the sieving matrix for capillary sodium dodecyl sulfate electrophoresis (CE-SDS) [7, 14–17] When comparing with cross-linked polyacrylamide gel matrix [18], these polymers add great flexibility to CE-SDS since they are water-soluble and replaceable after each CE analysis. Replacement results in enhanced overall precision and robustness [19] compared to fixed gels. There are, however, disadvantages in the use of CE such as poor mass sensitivity with UV detection and inability to simultaneously separate multiple samples. Nevertheless, it can be concluded that the attractions of high-speed separation, efficiency and ease of use of CE-SDS outweigh its limitations [20, 21].

Recently, CE (including CE-SDS) has been recognised and established [22] as an important tool in the biopharmaceutical industry to support the analytical characterisation, process development, and quality control of therapeutic rMAbs [2, 20–25]. For example Genentech currently has twenty validated CE assays [26] with every new Genentech molecule having one or two CE assays. Of the electrophoretic methods in use, 100% of the QC methods are CE-based, and 90% of analytical characterisation methods are CE-based.

Wehr [22] suggested that management acceptance remained a major hurdle for

Table 1. Average RMT (relative to the 10 kD marker) of the molecular weight markers for 8 different organisation. The RSD for each organisation is shown as well. The Average RMT and RSD relative to the 50 kD marker is shown.

	Relative Migration time (RMT)						
	10 kD	20 kD	35 kD	50 kD	100 kD	150 kD	225 kD
Company A Average	1	1.17	1.33	1.46	1.75	1.92	2.10
Company B Average	1	1.15	1.30	1.43	1.67	1.82	1.97
Company C Average	1	1.16	1.32	1.46	1.74	1.92	2.10
Company D Average	1	1.17	1.33	1.47	1.75	1.92	2.09
Company E Average	1	1.16	1.32	1.46	1.74	1.92	2.09
Company F Average	1	1.16	1.32	1.46	1.74	1.91	2.09
Company G Average	1	1.17	1.33	1.47	1.75	1.92	2.10
Company H Average	1	1.16	1.32	1.46	1.73	1.90	2.07
Average	N/A	1.16	1.32	1.46	1.73	1.90	2.08
RSD	N/A	0.47	0.71	0.95	1.45	1.76	2.04
Average (using 50 kD marker)	0.69	0.80	0.91	N/A	1.19	1.31	1.42
RSD (using 50 kD marker)	0.96	0.57	0.28	N/A	0.54	0.87	1.16

	RMT RSD					
	20 kD	35 kD	50 kD	100 kD	150 kD	225 kD
Company A RSD	0.15	0.25	0.27	0.34	0.34	0.35
Company B RSD	0.12	0.17	0.19	0.25	0.26	0.28
Company C RSD	0.05	0.06	0.06	0.09	0.10	0.11
Company D RSD	0.88	0.88	0.88	0.87	0.90	0.93
Company E RSD	0.04	0.05	0.10	0.08	0.10	0.13
Company F RSD	0.02	0.04	0.03	0.03	0.04	0.04
Company G RSD	0.06	0.12	0.11	0.14	0.12	0.12
Company H RSD	0.06	0.07	0.07	0.08	0.09	0.12

the use of CE in biotechnology companies. As a result, additional studies may be needed to specifically demonstrate that CE-SDS has sufficient robustness and reproducibility to meet routine QA standards. This need to demonstrate robustness and reproducibility was identified when CE was being applied to the analysis of small molecule drugs. A series of inter-company collaboration exercises were conducted between seven UK-based pharmaceutical companies [27–29]. The methods were used to quantify drug content, counter-ion content and chiral analysis. The results obtained correlated well between companies for each study and served to clearly demonstrate CE is a robust methodology for small molecule drug development and QC. A QC CE method for serum and urine protein determinations has been evaluated within a formal QA program with 13 laboratories successfully using CE [30].

A workshop at the ''CE in the Biotechnology & Pharmaceutical Industries'' 2004 symposium developed the concept and membership for an inter-organisation collaboration program in the area of biopharmaceutical analysis by CE. In the following report, we report the performance of a commercial IgG Purity/Heterogeneity CE-based method when used by 8 independent biopharmaceutical companies and a regulatory authority [Note: data generated by other companies with different instrumentation will be published in another paper]. Two determinations were made using this method. The apparent molecular weight of a BSA standard was calculated from migration time data obtained from protein molecular weight markers. The second assay involved purity assessment of an antibody. In the study, we limited the sources of variability (by utilising a single lot of reagents and by generating all data on a single run by each company). In this paper, we have focused on data generated on the Beckman Coulter PA800 (a subsequent publication will compare data generated on other instruments).

Experimental

All chemicals, reagents, and supplies used in this exercise are commercially available. 2-mercaptoethanol and a single lot of bovine serum albumin (BSA) were purchased from Sigma Aldrich, (St Louis, MO, USA). A single lot of the ProteomeLab IgG Purity/Heterogeneity Assay Kit [SDS gel separation buffer, SDS sample buffer (100 mM Tris–HCl, pH 9.0 with 1% SDS), SDS protein sizing standard (10–225 kD), a 10 kD protein internal standard, an IgG suitability standard, 0.1 N HCl, 0.1 N NaOH, and a 57 cm × 50 μm bare fused silica capillary] was kindly provided by Mr. Jeff Chapman of Beckman Coulter, Inc., (Fullerton, CA, USA).

All participants in this exercise used the Beckman Coulter, Inc. PA800 CE instrument equipped with a PDA detector and operated in reversed polarity mode. Each group used a 30.2 cm capillary with a detection window 20.2 cm from the sample introduction inlet and a 100 × 200 μm capillary aperture. The capillary was cut by each participant and fitted into a capillary cartridge. The instrument method used for the reduced IgG was obtained from the Beckman Coulter, Inc. IgG Purity/Heterogeneity Assay Standard Operating Protocol. The method parameters for conditioning a new or used capillary were [Note: the instrument setting is psi; psi can be converted to Pa by multiplying by 6896.55]

Basic Rinse	0.1 N NaOH, 10 min at 20 psi, forward
Acidic Rinse	0.1 N HCl, 10 min at 20 psi, forward
Water Rinse	Deionized water, 2 min at 20 psi, forward

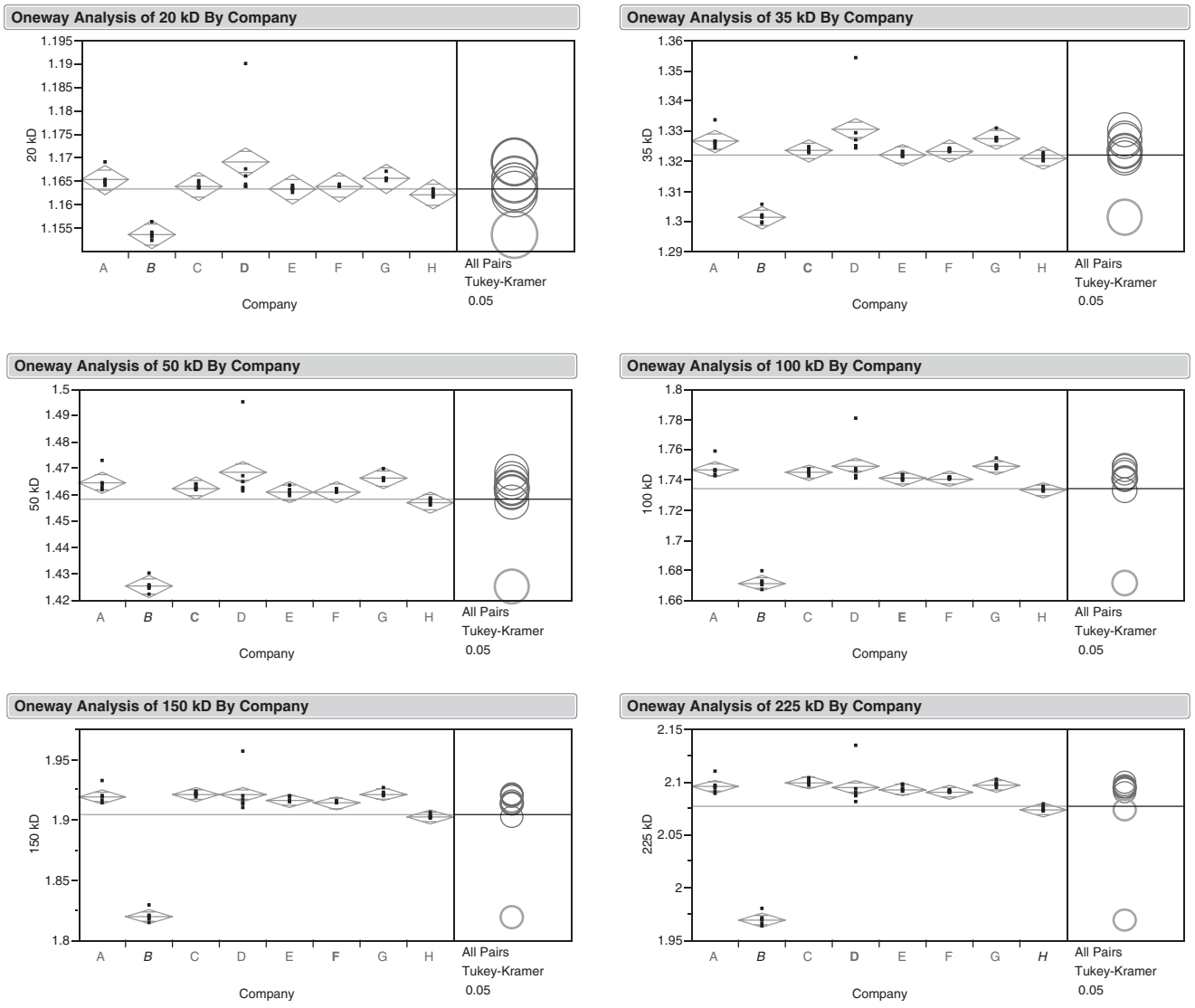


Fig. 2. ANOVA of the RMT for each of the molecular weight standards. Migration times were relative to the 10 kD molecular weight standards

SDS Gel Rinse	SDS Gel fill, 10 min at 70 psi, forward
Pre-Run	Voltage equilibration at 15 kV for 10 min, 5 min ramping

The method parameters for pre-run conditioning of the capillary were:

Basic Rinse	0.1 N NaOH, 3 min at 70 psi, forward
Acidic Rinse	0.1 N HCl, 1 min at 70 psi, forward
Water Rinse	Deionized water, 1 min at 70 psi, forward
SDS Gel Rinse	10 min at 70 psi, forward
Water Dip	Water dip for 0.0 min

The method parameters for the separation were:

Sample Injection	Sample, 20 s at −5 kV
Voltage Separation	30 min at −15 kV, temperature 25°C, applying 20 psi to both inlet and outlet

Samples and standards were prepared and used on the same day. The standards were prepared following the protocol in the Beckman Coulter, Inc. IgG Purity/Heterogeneity Assay Standard Operating Protocol. The SDS protein sizing standards were prepared as follows:

1. Allow vial of SDS protein size standards to come to room temperature.
2. Vortex vial thoroughly and centrifuge briefly.
3. Transfer 10 µL SDS protein size standards to a microvial.
4. Add 85 µL SDS Sample Buffer to the microvial.
5. Add 2 µL 10 kD Protein Internal Standard to the microvial.
6. Add 5 µL 2-mercaptoethanol to the microvial. Cap tightly and mix thoroughly.
7. Heat capped microvial in a 100°C water bath for 3 min.
8. Cool microvial in a room temperature water bath for 5 min prior to injecting into the capillary.
9. Transfer 100 µL of the prepared standard to a 200 µL PCR vial and place in sample holder.

The reduced BSA sample was prepared as follows:

1. Prepare a 2 mg mL^{-1} BSA solution in 95 µL SDS sample buffer in a microvial.

Table 2. Average RMT and Molecular Weight (determined by CE) of BSA. The average slope, intercept, and R-squared from plotting 1/RMT vs. log (Molecular Weight of the molecular weight standards) are shown. The RSDs for each organisation for the RMT and Molecular Weight (MW) are shown

	Relative Migration Time (RMT)	Slope	Intercept	*R*-squared	Molecular Weight of BSA (kD)
Company A Average	1.57	−2.71	6.59	0.995	72.9
Company B Average	1.50	−2.88	6.76	0.995	70.2
Company C Average	1.56	−2.70	6.59	0.996	72.1
Company D Average	1.56	−2.73	6.60	0.995	71.0
Company E Average	1.56	−2.71	6.59	0.996	71.2
Company F Average	1.56	−2.71	6.60	0.995	72.5
Company G Average	1.57	−2.71	6.59	0.995	72.8
Company H Average	1.56	−2.73	6.62	0.995	72.4
Average	1.55	−2.74	6.62	0.995	71.9
RSD	1.32	2.10	0.85	0.02	2.26

	RMT RSD	MW RSD
Company A Average	0.32	1.28
Company B Average	0.37	2.30
Company C Average	0.07	0.55
Company D Average	0.16	4.02
Company E Average	0.10	0.42
Company F Average	0.45	1.92
Company G Average	0.58	2.38
Company H Average	0.09	0.28

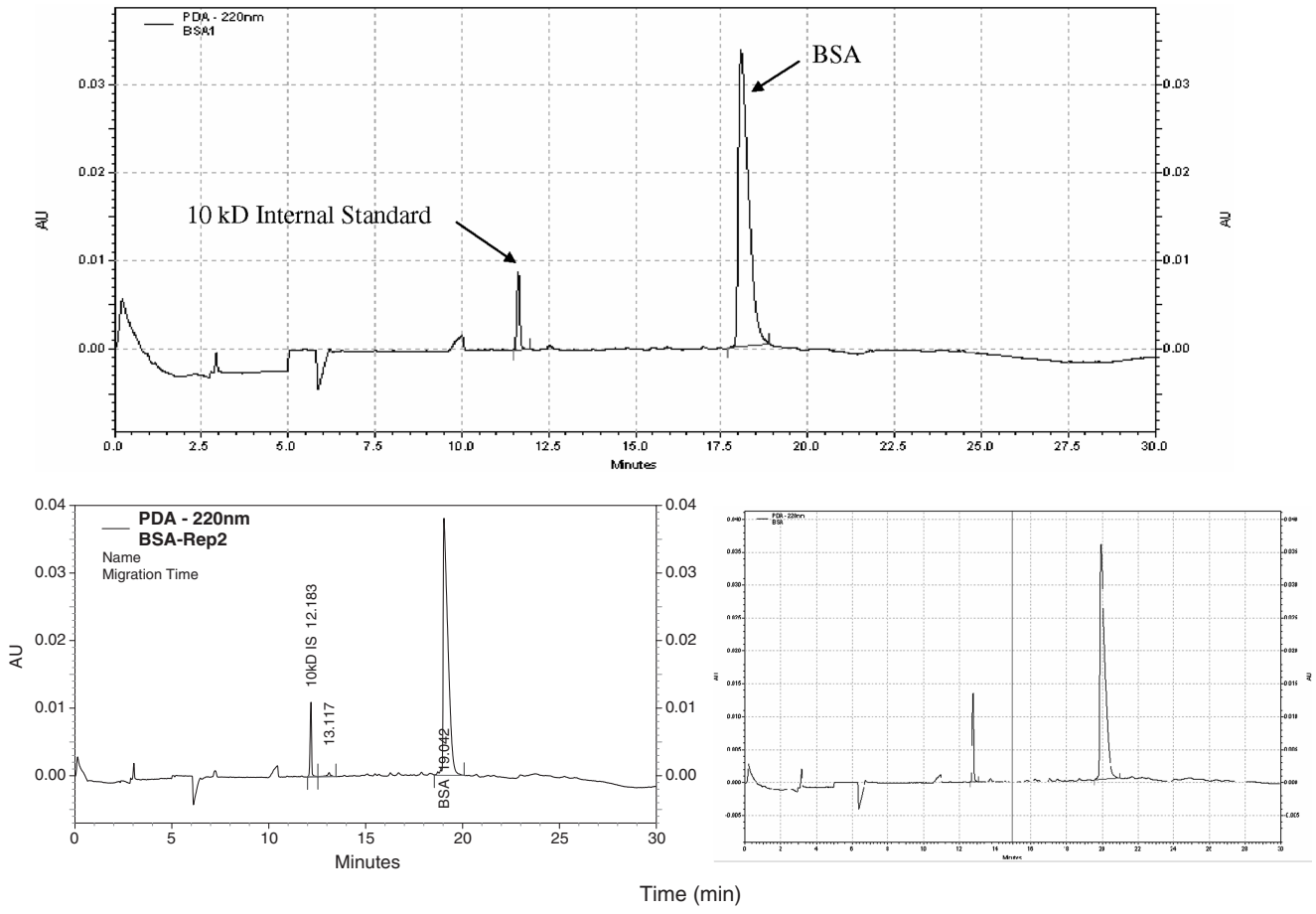


Fig. 3. Representative electropherograms of BSA

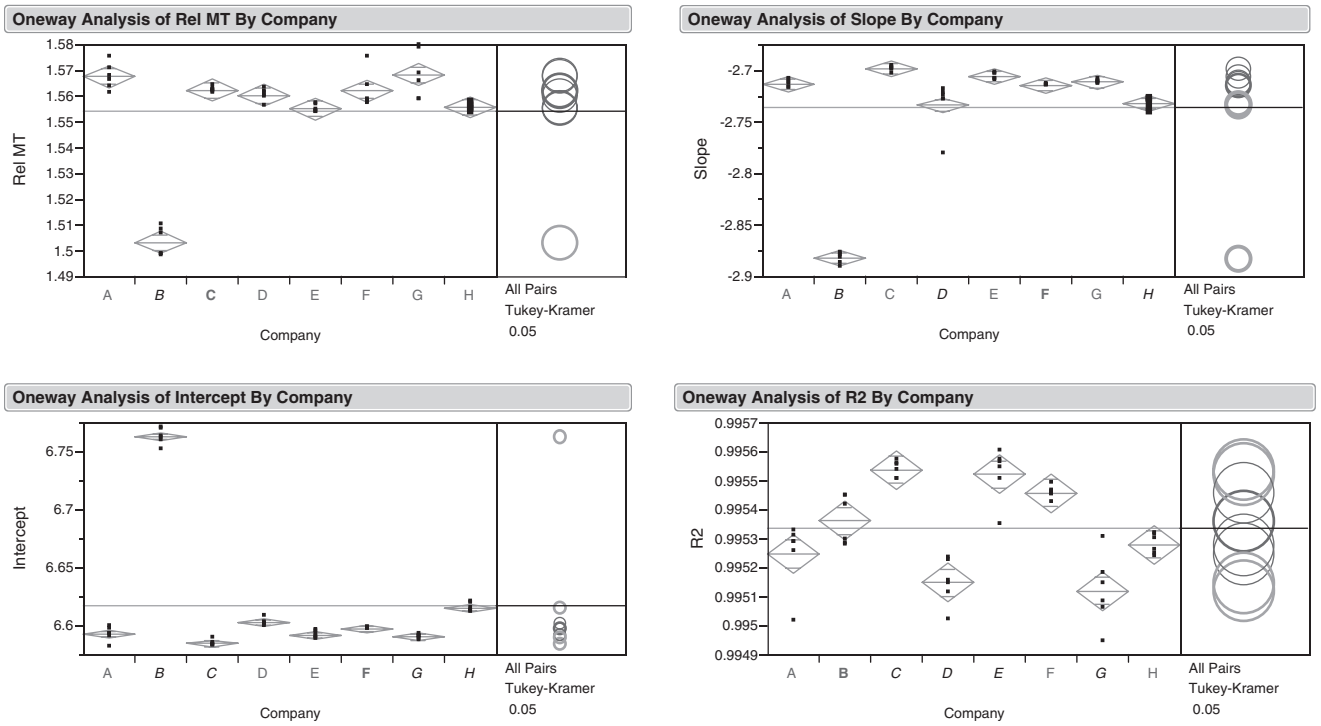


Fig. 4. ANOVA of the BSA RMT, Ferguson plot slope, intercept, and *R*-squared (R^2)

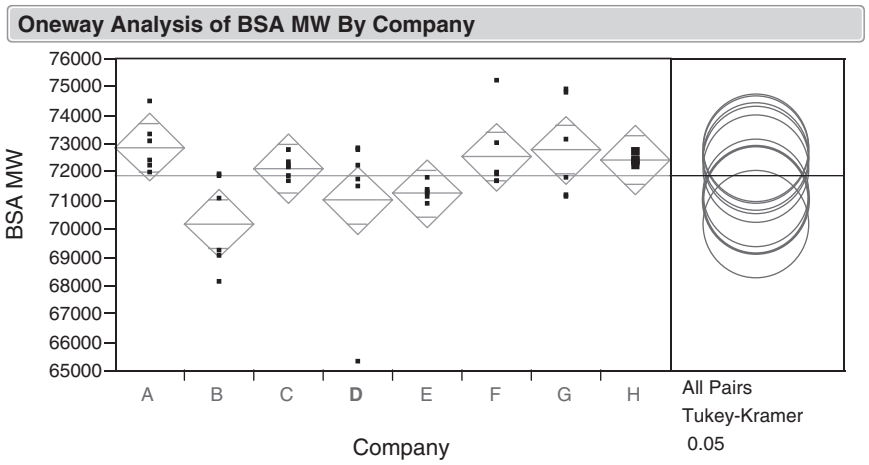


Fig. 5. ANOVA of the BSA molecular weight determined by CE

2. Add 2 µL 10 kD Protein Internal Standard to the microvial.
3. Add 5 µL 2-mercaptoethanol to the microvial. Cap tightly and mix thoroughly.
4. Heat capped microvial in a 100°C water bath for 3 min.
5. Cool microvial in a room temperature water bath for 5 min prior to injecting into the capillary.
6. Transfer 100 µL of the prepared sample to a 200 µL PCR vial and place in sample holder.

The reduced IgG Standard was prepared as follows:

1. Thaw a 95 µL aliquot of the IgG Standard at room temperature
2. Add 2 µL 10 kD Protein Internal Standard to the microvial
3. Add 5 µL 2-mercaptoethanol to the microvial. Cap tightly and mix thoroughly
4. Centrifuge at 300 × *g* for 1 min
5. Heat capped microvial in a 70°C water bath for 10 min
6. Cool microvial in a room temperature water bath for 3 min prior to injecting into the capillary
7. Transfer 100 µL of the prepared sample to a 200 µL PCR vial and place in sample holder

Each sample was injected 6 times for a total of 18 runs in one sequence.

Each company reported peak area/peak area% and migration time. These were collated and analyzed by ANOVA using JMP 5.1.1. Differences were considered statistically significant if the p-value for the ANOVA comparison was less than 0.05.

Results and Discussion

Determination of the Molecular Weight of BSA

The molecular weight of BSA was determined across the companies using the Beckman Coulter molecular weight marker kit. The molecular weight marker kit was analysed 6 times by each company using the 10 kD marker as the internal standard. Consistent profiles and similar migration times were obtained by all organizations. Figure 1 shows example electropherograms of the molecular

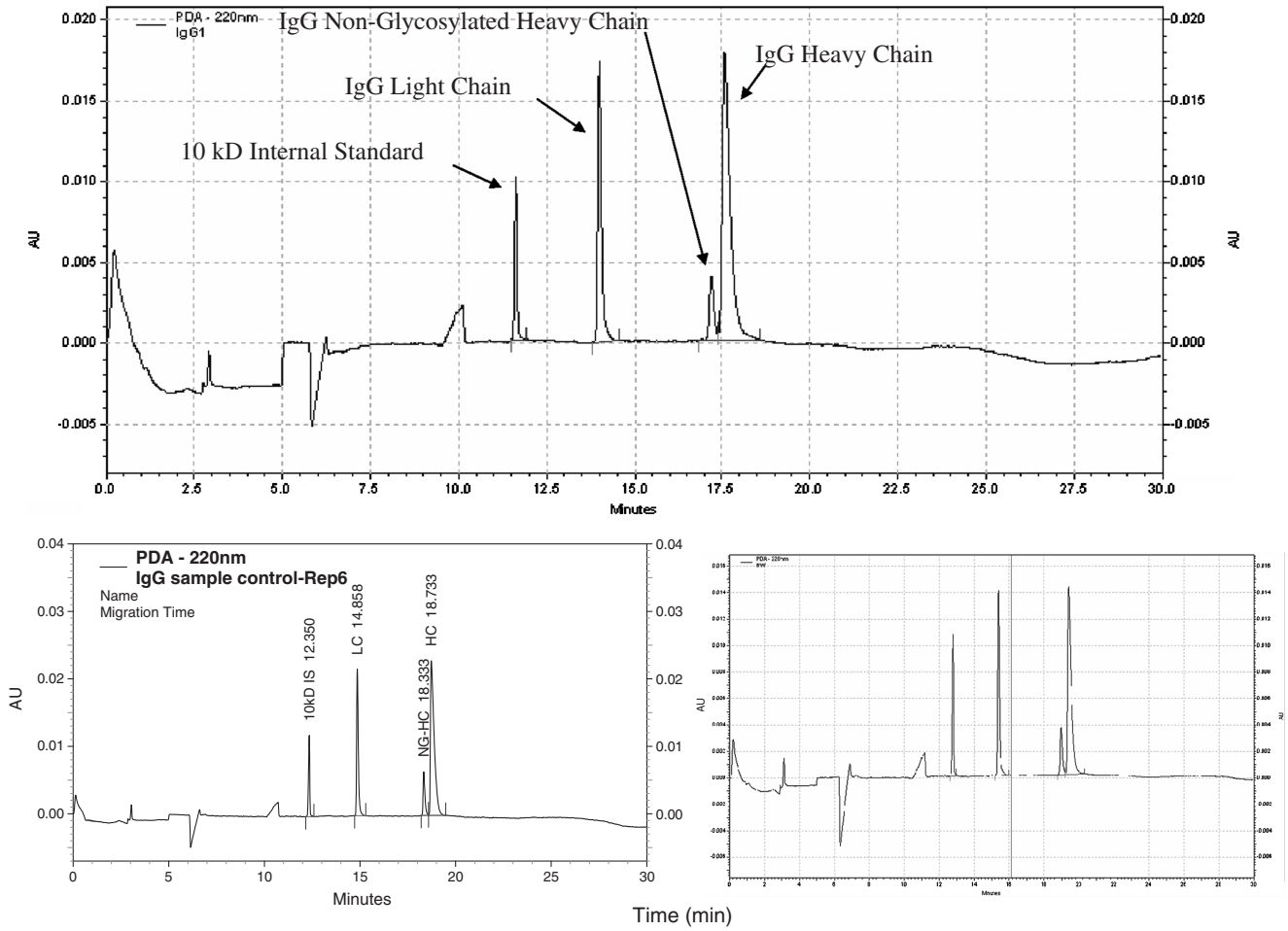


Fig. 6. Representative electropherograms of the IgG sample

Table 3. Average RMT and Peak area percent for the IgG Light Chain, IgG Non-glycosylated Heavy Chain, and IgG Heavy Chain in a sample separated by CE-SDS

	Relative Migration Time (RMT)			Peak Areas (%)		
	IgG Light Chain	IgG Non-glycosylated Heavy Chain	IgG Heavy Chain	IgG Light Chain	IgG Non-glycosylated Heavy Chain	IgG Heavy Chain
Company A Average	1.20	1.49	1.52	29%	8%	63%
Company B Average	1.19	1.44	1.47	30%	9%	62%
Company C Average	1.20	1.49	1.52	28%	9%	64%
Company D Average	1.20	1.48	1.52	35%	7%	58%
Company E Average	1.20	1.48	1.52	27%	9%	63%
Company F Average	1.20	1.48	1.52	30%	9%	61%
Company G Average	1.20	1.48	1.52	30%	9%	61%
Company H Average	1.20	1.48	1.51	29%	8%	62%
Average	1.20	1.48	1.51	30%	9%	62%
RSD	0.42	1.07	1.13	8.64	7.68	3.29
	RMT RSD			Peak Area RSD		
	IgG Light Chain	IgG Non-glycosylated Heavy Chain	IgG Heavy Chain	IgG Light Chain	IgG Non-glycosylated Heavy Chain	IgG Heavy Chain
Company A Average	0.05	0.05	0.07	2.82	2.95	1.03
Company B Average	0.10	0.16	0.19	1.37	1.81	0.44
Company C Average	0.04	0.05	0.05	1.39	0.66	0.54
Company D Average	0.10	0.15	0.16	0.41	4.47	0.79
Company E Average	0.03	0.05	0.03	0.87	0.76	0.27
Company F Average	0.11	0.16	0.29	11.15	6.10	4.60
Company G Average	0.14	0.18	0.23	2.45	1.35	1.08
Company H Average	0.12	0.34	0.25	1.00	1.00	0.39

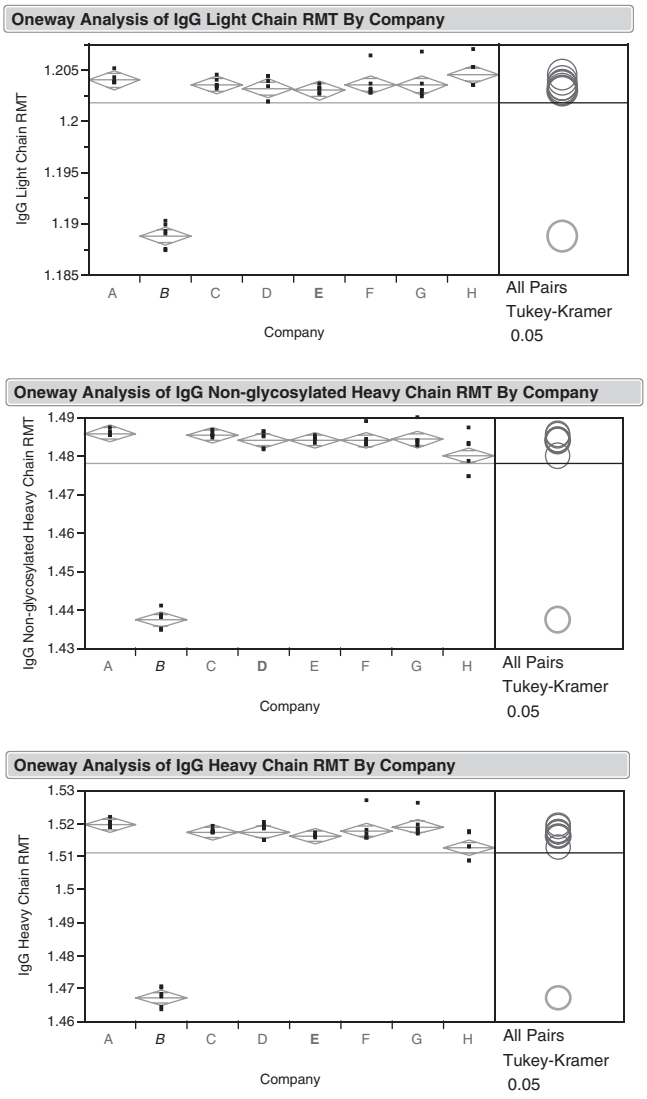


Fig. 7. ANOVA of the RMT for the IgG Light Chain, IgG Non-glycosylated Heavy Chain, and IgG Heavy Chain

weight markers from 3 of the organisations. The average relative migration times (RMT) for all organisations are shown in Table 1. The average within companies and across all companies exhibited good agreement (RSDs less than 2). The RSD values increased with extended RMT value. This supports the need [31] to select an internal standard, which migrates near the analyte peak to maximise RMT precision. The RMT data using the 50 kD marker as the internal standard is shown in Table 1. Sources of variability could include preparation of the capillary and the cartridge. As can be seen in Fig. 2, Company B had a significantly different RMT for all molecular weight standards. If the data from Company B is removed, the RSDs are less than 0.5% for all molecular weight standards (Note: no root cause could be assigned for removing Company B's data). The intra-company precision was low enough to allow for other differences to be seen (data not discussed). This exercise demonstrated that 8 independent organisations could achieve comparable separations of the marker kit in different laboratories, using different operators, the same lots of buffer and samples, and different instruments from the same equipment model type.

Molecular weight calibration graphs were prepared for each set of data from each organisation. The reciprocal of the average RMT for each molecular weight standard was plotted versus log Molecular Weight of the molecular weight of each standard. The slope, intercept, and coefficient of determination (R^2) data are shown in Table 2 and Fig. 3. Each of these parameters showed excellent agreement between organisations demonstrating transferable quantitative capability.

Each organisation injected the BSA 6 times within the same run. (Fig. 3 shows example electropherograms from 3 organisations.) Table 2 shows that the precision for the RMT of the BSA standard exhibited good agreement (RSD = 1.3%) across all organisations. The with-in organisation RSD was excellent (less than 0.6%). This across-organisation precision improved when Company B was removed from the analysis (RSD decreases to 0.4%). As with the molecular weight standards, the intra-company precision was low enough to allow for slight differences between organisations to be seen. As shown in Fig. 4, Companies G, A, C, F, and D showed no statistical difference for the RMT data. Similarly, Companies C, F, D, H, and E showed no statistical difference for RMT. Company B was statistically different from all other companies.

The slope and intercept values for each organisation were used to calculate the molecular weight determination for the BSA based on its experimental average RMT value. Table 2 shows that the organisations achieved comparable results. The average molecular weight for BSA was 71.9 kD with a RSD of 2% across the organisations. The within-organisation RSD was low (less than 4%). Companies A and B were statistically different from one another, but there was no statistical difference in the values determined between all other companies (Fig. 5). Although the molecular weight of BSA is considered [32] to be 66.4 kD; the disagreement between these values is likely due to hydrodynamic effects in the CE-SDS analysis.

These results show that this CE-SDS method is capable of generating consistent quantitative results across organisations and provides data broadly in-line with the theoretical result.

Purity analysis for IgG

The IgG standard employed contained a mixture of Light Chain, Non-glycosylated Heavy Chain, and Heavy Chain components. Example electropherograms of the IgG separation from 3 of the companies is shown in Fig. 6. The migration profile was consistent across all

organisations. The average relative migration times (RMT) calculated based on the 10 kD internal standard peak are shown in Table 3. The average within organisations (RSDs less than 0.3%) and across all organisations exhibited good agreement (RSDs less than 1%). The intra-organisation precision was sufficient to allow slight differences to be observed (data not discussed). As can be seen in Fig. 7, Company B had a significantly different RMT for all IgG peaks (Light Chain, Non-glycosylated Heavy Chain, and Heavy Chain).

The percent peak areas (or fractional peak areas) for each of the peaks exhibited good agreement (Table 3). The RSD for each peak area was less than 9%. For the IgG Light Chain (Fig. 8), the absolute difference across all of the organisations was 8% (the within-organisation difference was less than 3% for all organisations except Company F. If an outlier is removed from the Company F data, the RSD is in-line with all other organisations). There were several groups of companies that were not statistically different (see the ANOVA and Tukey–Kramer analysis in Fig. 8) from one another. The first group of organisations (Companies G, F, B, H, and A) showed no statistical difference. The companies showing no statistical difference in the next group were Companies B, H, A, and C. In the final group, Companies H, A, C, and E showed no statistical difference between the data. Company D was statistically different from all other companies. If Company D is removed from the data analysis, the absolute difference shrinks to 3% and the RSD is less than 6%.

For the IgG Non-glycosylated Heavy Chain (Fig. 8), Companies C, G, B, F, and A showed no statistical difference between the data (as with the IgG Light Chain, there were several groups of companies that were not statistically different from one another). Similarly, Companies F, A, and H showed no statistical difference between the data. Companies E and D were statistically different from each other and all other companies. Even with these companies included, the absolute difference was only 2%.

For the IgG Heavy Chain (Fig. 8), there was little difference between the companies (RSD was less than 3.3%). The within-organisation differences were also small (except for Company F, with an outlier in the data) at less than 1%. The absolute difference in results across the companies was 6%. Companies C, E, A, and H showed no statistical difference between the data. Similarly, Companies E, A, H, and B showed no statistical difference between the data. Companies A, H, B, F, and G showed no statistical difference between the data. Company D was statistically different from all other companies. When Company D is removed from the data set, the absolute difference is reduced to 3%.

These results clearly demonstrate that this CE-SDS method is capable of generating consistent profiles with good RMT data precision obtained across all organisations. The RMT and % purity data for the 3 components were in broad agreement across the organisations. The differences noted in this study are reasonable from a method validation or method transfer perspective.

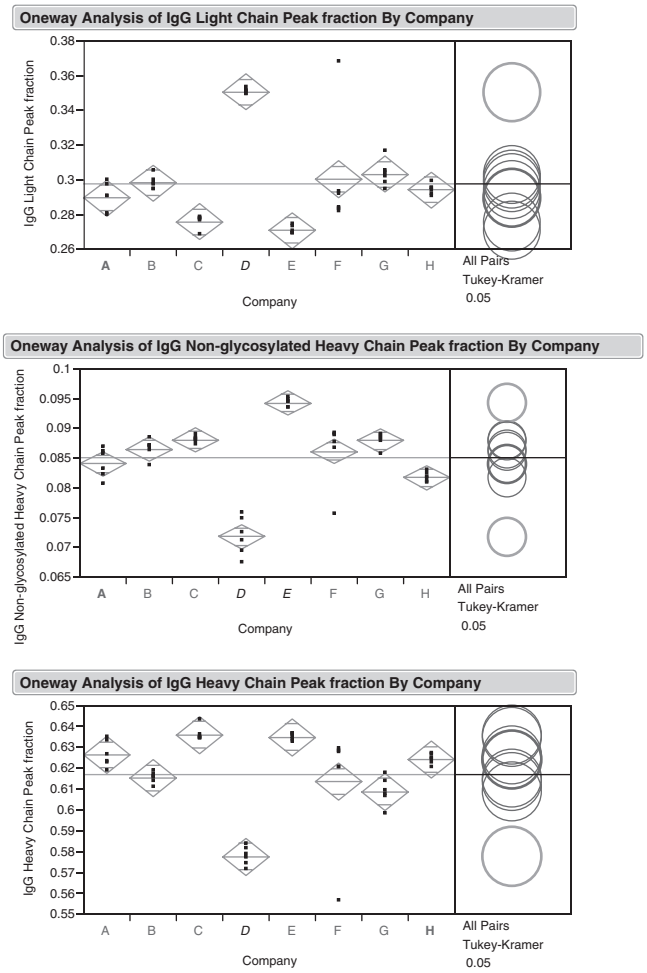


Fig. 8. ANOVA of the Peak area (fractional peak areas) for the IgG Light Chain, IgG Non-glycosylated Heavy Chain, and IgG Heavy Chain

Conclusions

The data presented in this paper demonstrate that a CE-SDS based method can be successfully repeated by a range of different organisations in 3 countries (US, Canada and UK) using different operators, and different instruments of the same equipment model.

Consistent separation profiles of protein molecular weight standards were obtained with good precision data being obtained for relative migration time data across and within the organisations. These profiles produced consistent and linear molecular weight calibration graphs when the inverse of RMT was plotted against log Molecular Weight. Consistent molecular weights were generated by all organisations when calculating BSA molecular weight from experimental RMT. The experimental molecular weight values obtained were broadly in-line with the theoretical BSA molecular weight.

The same CE-SDS method was used to profile the components of an artificial IgG sample. The profiles obtained by all the organisations were consistent with separation of IgG Light Chain, IgG Non-glycosylated Heavy Chain, and IgG Heavy Chain being repeatably achieved. % Peak area quantitation of the sample components gave comparable data across all organisations.

In summary this report highlights that analytical characterisation of biomolecules by CE is a robust technology when the method is well described and controlled. This exercise supports and endorses the increased application of CE methodology within both development and QC laboratories within biopharmaceutical companies. It is anticipated that this exercise will facilitate both increased regulatory and industrial opinion of the use of CE within the biopharmaceutical application area.

References

1. Chen A, Canova-Davis E (2001) Chromatographia 53:S7–S16
2. Tous GI, Wei Z, Feng J, Bilbulian S, Bowen S, Smith S, Strouse R, McGeehan P, Casas-Finet J, Schenerman MA (2005) Anal Chem 77:2675–2682
3. Weber K, Osborne MJ (1969) J Biol Chem 244:4406–4412
4. Chrambac A, Reisfeld RA, Wyckoff M Zuccari J (1967) Anal Biochem 20:150–155
5. Oakley BR, Kirsch DR, Morris RN (1980) Anal Biochem 105:361–363
6. Shieh PC, Hoang HD, Guttman A, Cooke N (1994) J Chromatogr 676:219–226
7. Guttman A (1996) Electrophoresis 17:1333–1341
8. Schmerr MJ, Jenny A, Cutlip RC (1997) J Chromatogr B 697:223–229
9. Guttman A, Nolan J (1994) Anal Biochem 221:285–289
10. Manabe T (1999) Electrophoresis 20:3116–3121
11. Hu S, Michels D, Zhang Z, Krylov SN, Dovichi NJ (2001) in Proteome Analysis by Capillary Electrophoresis. Biotechnology (2nd Ed). Sensen CW, Wiley-VCH, Weinheim, Germany, pp. 269–277
12. Hu S, Jiang J, Cook L, Richards DP, Horlick L, Wong B, Dovichi NJ (2002) Electrophoresis 23:3136–3142
13. Shen Y, Smith RD (2002) Electrophoresis 23(18):3106–3124
14. Ganzler K, Greve K, Cohen AS, Karger BL, Guttman A, Cooke NC (1992) Anal Chem 64:2665–2671
15. Wu D, Regnier FE (1992) J Chromatogr 608:349–356
16. Benedek K, Guttman A (1994) J Chromatogr A 680:375–381
17. Nakatani M, Shibukawa A, Nagagawa T (1994) J Chromatogr A 672:213–218
18. Cohen AS, Karger BL (1987) J Chromatogr 397:409–417
19. Ma S, Nashabeh W (2001) Chromatographia 53:S75–S89
20. Hunt G, Nashabeh W (1999) Anal Chem 71:2390–2397
21. Lee HG, Chang S, Fritsche E (2002) J Chromatogr A 947:143–149
22. Wehr T (2005) LCGC 23:676–681
23. Krull I S, Liu X, Dai J, Gendreau C, Li G (1997) J Pharm Biomed Anal 16:377–393
24. Patrick JS, Lagu AL (2001) Electrophoresis 22:4179–4196
25. Schenerman MA, Bowen SH (2001) Chromatographia 53:S66–S74
26. Ma S (2005) Abstract L05-K4-M, presented at the 18th International Symposium on Microscale Bioseparations, New Orleans, Louisiana
27. Altria KD, Harden RC, Hart M, Hevizi J, Hailey PA, Makwana J, Portsmouth MJ (1993) JChromatogr 641:147–153
28. Altria KD, Clayton NG, Harden RC, Hart M, Hevizi J, Makwana J, Portsmouth MJ (1995) Chromatographia 40:47–50
29. Altria KD, Clayton NG, Harden RC, Hart M, Hevizi J, Makwana J, Portsmouth MJ (1994) Chromatographia 39:180–184
30. Jenkins MA (2004) Electrophoresis 25:1555–1560
31. Altria KD (2002) LCGC Europe, pp. 588–594
32. Hirayama K, Akashi S, Furuya M, Fukuhara K (1990) Biochem Biophys Res Comm 173:639–646