Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 317–323

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Validation of a pH gradient-based ion-exchange chromatography method for high-resolution monoclonal antibody charge variant separations

Jennifer C. Rea*, G. Tony Moreno, Yun Lou, Dell Farnan

Protein Analytical Chemistry, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States

ARTICLE INFO

Article history:
Received 9 June 2010
Received in revised form 11 August 2010
Accepted 21 August 2010
Available online 29 September 2010

Keywords:
Ion-exchange chromatography
Method validation
pH gradient
Charge heterogeneity
Monoclonal
Antibodies

ABSTRACT

Ion-exchange chromatography is widely used for profiling the charge heterogeneity of proteins, including monoclonal antibodies. Despite good resolving power and robustness, ionic strength-based ion-exchange separations are product-specific and time-consuming to develop. We have previously reported a novel pH-based separation of proteins by cation exchange chromatography that was multi-product, high-resolution, and robust against variations in sample matrix salt concentration and pH. In this study, a pH gradient-based separation method using cation exchange chromatography was evaluated in a mock validation. This method was shown to be robust for monoclonal antibodies and suitable for its intended purpose of charge heterogeneity analysis. Simple mixtures of defined buffer components were used to generate the pH gradients that separated closely related antibody species. Validation characteristics, such as precision and linearity, were evaluated. Robustness to changes in protein load, buffer pH and column oven temperature was demonstrated. The stability-indicating capability of this method was determined using thermally stressed antibody samples. In addition, intermediate precision was demonstrated using multiple instruments, multiple analysts, multiple column lots, and different column manufacturers. Finally, the precision for this method was compared to conventional ion-exchange chromatography and imaged capillary isoelectric focusing. These results demonstrate the superior precision and robustness of this multi-product method, which can be used for the high-throughput evaluation of in-process and final product samples.

1. Introduction

Monoclonal antibodies (mAbs) are a very important class of therapeutic proteins in biotechnology, and have been developed to treat a variety of indications to fulfill significant unmet medical needs [1]. Monoclonal antibodies are generally target-specific and well tolerated with a relatively long half-life, contributing to the success of the molecule class for drug development. Of the classes of immunoglobulins, IgG1 is the most commonly used immunoglobulin used for pharmaceutical and biomedical purposes [2,3].

Protein heterogeneity of monoclonal antibodies is monitored as part of the ongoing control system that ensures product quality and consistency [4–6]. Monoclonal antibodies are susceptible to chemical or enzymatic modification, particularly at sites that are exposed to the protein–liquid interface. Product heterogeneity can be caused by C-terminal processing of lysine residues [7–9], deamidation [10,11], glycation (nonenzymatic glucose addition) [12], amino acid sequence variations [8], and noncovalent complexes [13].

Monoclonal IgG antibodies are typically characterized by a variety of orthogonal analytical and biochemical methods, including ion-exchange chromatography and isoelectric focusing [9]. Ion-exchange chromatography (IEC) has been a platform for monoclonal antibody purification and characterization for many years [14], with IEC being a typical component in antibody recovery and characterization systems. IEC separates proteins based on differences in the surface charge of the molecules, with separation being dictated by the protein interaction with the stationary phase. While cation exchange chromatography has been called the gold standard for charge sensitive antibody analysis [15], method parameters, such as column type, mobile phase pH, and salt concentration gradient, often need to be optimized for each individual antibody. Isoelectric focusing separation methods, in either capillary or slab gel format, have been widely used due to the multi-product nature of the separation conditions. The proteins are separated by focusing the proteins in a matrix where the pH of the medium changes as a function of position. One particular methodology, imaging capillary isoelectric focusing (icIEF), has recently been developed for charge heterogeneity analysis of monoclonal antibodies [16].

* Corresponding author. Tel.: +1 650 225 7013; fax: +1 650 225 3554.
E-mail address: rea.jennifer@gene.com (J.C. Rea).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.08.030

Despite some advantages over conventional IEC, such as relatively shorter method development time, the implementation of icIEF in a commercial control system entails significant investment, as specialized equipment as well as vendors for consumables is limited. Recently, a chromatofocusing method, which combines the resolving power of isoelectric focusing and the flexibility and simplicity of ion-exchange chromatography, was reported for routine analysis of monoclonal antibody charge species that employs a linear pH gradient formed by external mixing of two phosphate buffers of different pH [17]. The robustness of this chromatofocusing method was demonstrated using a single mAb, and this method compared favorably to conventional salt-gradient IEC [17].

Before an analytical method can be incorporated into a quality control system, it must first be demonstrated that it is suitable for its intended purpose. Guidelines for the validation of analytical methods have been published in the United States Pharmacopeia [18], by the US Food and Drug Administration (FDA) [19,20], and in published reviews [21]. The guidelines published by the International Conference on Harmonization (ICH) have established a uniform understanding of the performance characteristics which are evaluated in the course of validation [22]. The subset of performance characteristics which require investigation in the course of validation, as well as the strategy for designing appropriate experiments, are based upon the intended purpose of the analytical method. Thus, different validation requirements are outlined by the ICH guidelines for assay, impurity and identity methods [22]. The validation of a stability-indicating method requires analyses of stressed samples in order to demonstrate that the method is suitable [21]. International Conference on Harmonization (ICH) guidelines have established requirements for the validation of stability-indicating methods and clarify requirements for stress studies and robustness studies.

We have previously reported a novel pH-based separation of proteins by cation exchange chromatography (pH-IEC) that was multi-product, high-resolution, and robust against variations in sample matrix salt concentration and pH [23]. Simple mixtures of defined buffer components were used to generate the pH gradients that separate closely related antibody species. This method separated monoclonal antibody species with a wide range of isoelectric points via a complex retention mechanism, combining both ionic-strength and pH. The multi-product aspect of this method translates into much less method development time for new IgG molecules. In addition, the ability of the method to assess charge heterogeneity at a wide range of sample matrix salt concentrations and pH indicates the suitability of the method for use in evaluating in-process samples. Despite these advantages, this multi-product pH gradient-based ion-exchange chromatography method had not yet been validated prior to this work, which is necessary for the transfer of an analytical method to a quality control environment.

We report herein the results of a mock validation of a pH gradient-based ion-exchange chromatography method for evaluating charge heterogeneity of monoclonal antibodies. The validation was deemed a mock validation as it was done as proof of concept rather than strictly for regulatory purposes, which require specific criteria be set in advance and achieved for precision and accuracy [20]. Robustness against changes in protein load, buffer pH and column oven temperature is demonstrated. The stability-indicating capability of this method is determined using thermally stressed antibody samples. In addition, intermediate precision is demonstrated using multiple instruments, multiple analysts, multiple column lots, and different column manufacturers. These results demonstrate the precision, robustness and applicability of this multi-product method, which can be used for the high-throughput evaluation of in-process and final product samples.

2. Experimental

2.1. Instrumentation

Three types of liquid chromatographs were used during this work: an Ultimate 3000 X2 (dual channel) bio-compatible liquid chromatograph (Dionex, Sunnyvale, CA), a Waters 2796 bio-compatible liquid chromatograph (Waters, Milford, MA) and an Agilent 1100 liquid chromatograph (Agilent, Santa Clara, CA), each equipped with an autosampler with sample temperature control capability and a thermal compartment to enclose the column. The Dionex Ultimate 3000 X2 chromatograph included dual ternary low pressure micro-gradient pumps and a four channel UV–Vis detector. The Waters 2796 chromatograph included a quaternary pump and a dual wavelength detector (Waters 2487). The Agilent 1100 chromatograph included a high pressure gradient binary pump and a multiple wavelength detector.

Instrument control, data acquisition and compilation of results for all HPLCs were performed using Dionex Chromeleon software, version 6.8.

2.2. Chemicals and columns

Piperazine dihydrochloride hydrate and imidazole were Fluka brand reagents. All other chemicals were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

Monoclonal antibodies used in this work were produced by Genentech (South San Francisco, CA). The mAb standard used throughout the study had a pI value of 7.4, which was determined using capillary isoelectric focusing. Thermally stressed samples were produced by incubating the antibody at 40 °C for 4 weeks.

Two types of ion-exchange of columns were used in this study. ProPac WCX-10 cation exchange columns were obtained from Dionex (Sunnyvale, CA). ProPac columns used in this study were 250 mm × 4.0 mm, 10 μm. To assess method variability between different column manufacturers, Sepax Antibodix NP10 columns were used (Sepax, Newark, DE), with dimensions of 250 mm × 4.6 mm, 10 μm.

2.3. Mobile phase preparation

Appropriate amounts of buffer were dissolved in deionized water to produce a 2× concentrated solution, i.e., 19.2 mM Tris base, 12.0 mM piperazine, and 22.0 mM imidazole. Once dissolved, the solution was split into two equal aliquots. Each aliquot was diluted to 90% of the required final volume with deionized water. Each aliquot was then titrated to the appropriate pH by the addition of 10 N sodium hydroxide as necessary. Once titrated, deionized water was added to bring the solutions to the required volume. The mobile phases were then individually filtered through a 0.2 μm nylon filter prior to use. Mobile phases prepared for this work contained 9.6 mM Tris base, 6.0 mM piperazine and 11.0 mM imidazole, with pH values of either pH 6.0 (mobile phase A) or pH 9.5 (mobile phase B), unless otherwise indicated. This buffer composition is a modified piperazine/imidazole/tris buffer system originally reported by Kang and Frey [24] and used for mAb analysis by Farnan and Moreno [23].

2.4. Chromatographic conditions

Samples were diluted to a target protein concentration of 1 mg mL^{-1} in mobile phase A prior to placement into the autosampler. Samples in the autosampler were kept at a temperature of 5 ± 3 °C. Columns were placed in the column oven and the temperature control feature was employed to keep the oven temperature within a narrow range (±1 °C) from the set point during the studies.

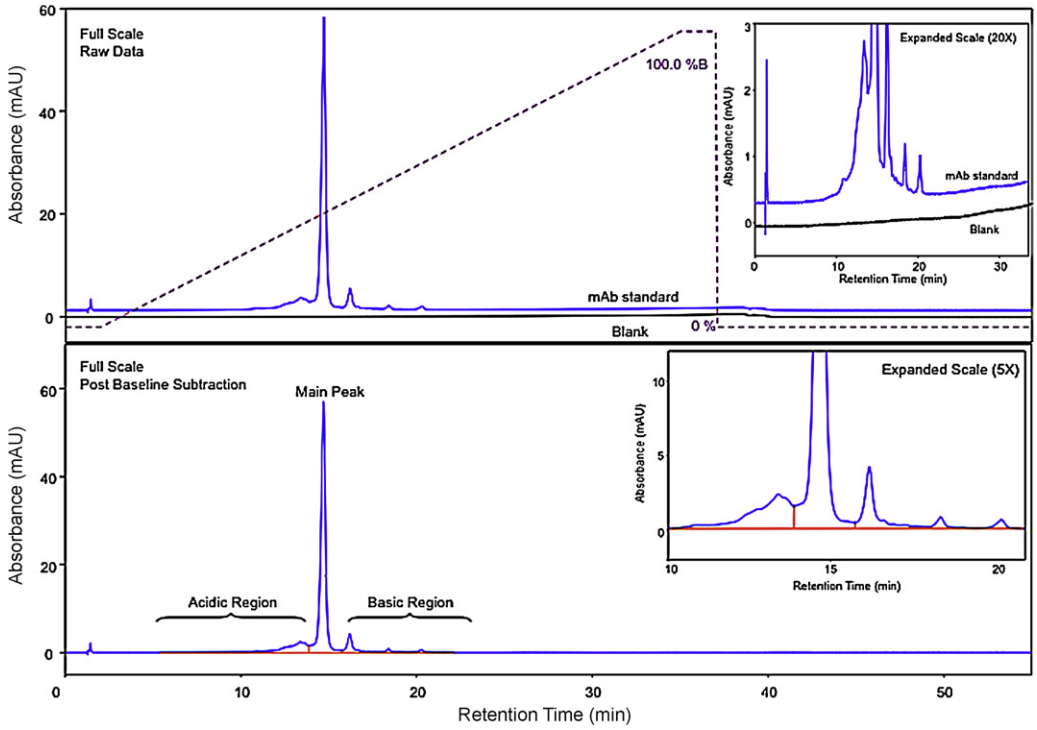

Fig. 1. Example profiles obtained using pH gradient-based ion-exchange chromatography for a mAb with a pI value of 7.4. The main peak, acidic and basic regions are denoted. The pH was changed linearly from pH 6.0 to 9.5 over 30 min at 1 mL min^{-1} on a ProPac WCX-10 column. Integrations are performed post-baseline subtraction. A representative integration is shown on the lower panel.

The column temperature was set at 30 °C unless otherwise indicated. Prior to sample injection, the column was pre-equilibrated with at least 4 column volumes of the pH 6.0 buffer (mobile phase A). mAb injection amounts were 40 μg (40 μL injection) unless otherwise indicated. After the injection of the monoclonal antibody sample onto the column, a linear increase in the percentage of pH 9.5 buffer (mobile phase B) was delivered using the pump. The linear gradient was from 0% to 100% B over 30 min. Post-gradient, the mobile phase was pumped at 100% B until at least one column volume passed before the composition was returned to 100% A in preparation for the next analysis. The column effluent was monitored at 280 nm.

A blank injection was made with each sequence prior to sample injection, and each sequence ran a control sample to ensure that the performance of the method had not changed during the course of the study. Each chromatogram was carefully integrated to ensure that only peaks not present in the associated blank were considered to be protein. Prior to integration, a baseline subtraction was performed using the blank run in the same sequence. The ion-exchange profiles were typically divided into three distinct components: the main peak, the acidic region and the basic region (Fig. 1).

3. Results and discussion

3.1. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple analyses of the homogeneous sample under the prescribed conditions and often expressed as relative standard deviation (RSD). Fig. 1 shows an example elution profile observed using the pH gradient formed with 9.6 mM Tris base, 6.0 mM piperazine and 11.0 mM imidazole buffer composition. The upper panel shows the profiles of protein-containing sample and blank, while the bottom profile shows protein-containing sample after blank subtraction (Fig. 1). All integrations in this study were performed after blank subtraction to mitigate the effects of mild baseline changes and to ensure that the chromatograms were integrated correctly [25]. Baseline change was found to be very repeatable and was related to differences in the extinction coefficients of the buffer species. To calculate chromatographic repeatability, six replicate injections from a single sample preparation at 100% of the test concentration were analyzed by one analyst on one day. The repeatability of the method was demonstrated by relative peak area RSD values of 0.1%, 0.7%, and 0.8% for main peak, acidic and basic regions, respectively, with relative peak area defined as the peak area divided by total peak area of the main peak, acidic and basic regions multiplied by 100%. The overlay for six sequential replicate injections from a single sample vial demonstrated excellent reproducibility of the elution profile (Fig. 2). The inter-day precision from one column and one instrument was calculated from relative peak area RSD values to be 0.2%, 0.9% and 1.2% for main peak, acidic and basic regions, respectively (Table 1). These values are in compliance with requirements for analytical method validation set by the FDA,

Table 1
Summary of inter-day precision at target conditions (1 column and 1 instrument). Data include 15 total data points over 4 days (3 samples injected in triplicate or more).

	Percent peak area		
	Acidic region	Main peak	Basic region
Average	14.6	75.6	9.8
SD	0.1	0.2	0.1
%RSD	0.9	0.2	1.2
−3SD	14.1	75.2	9.4
+3SD	15.0	76.2	10.1
6SD range	1.4	1.6	0.7
Hi	14.9	75.9	10.1
Lo	14.3	75.4	9.6
Hi–Lo range	0.6	0.5	0.5

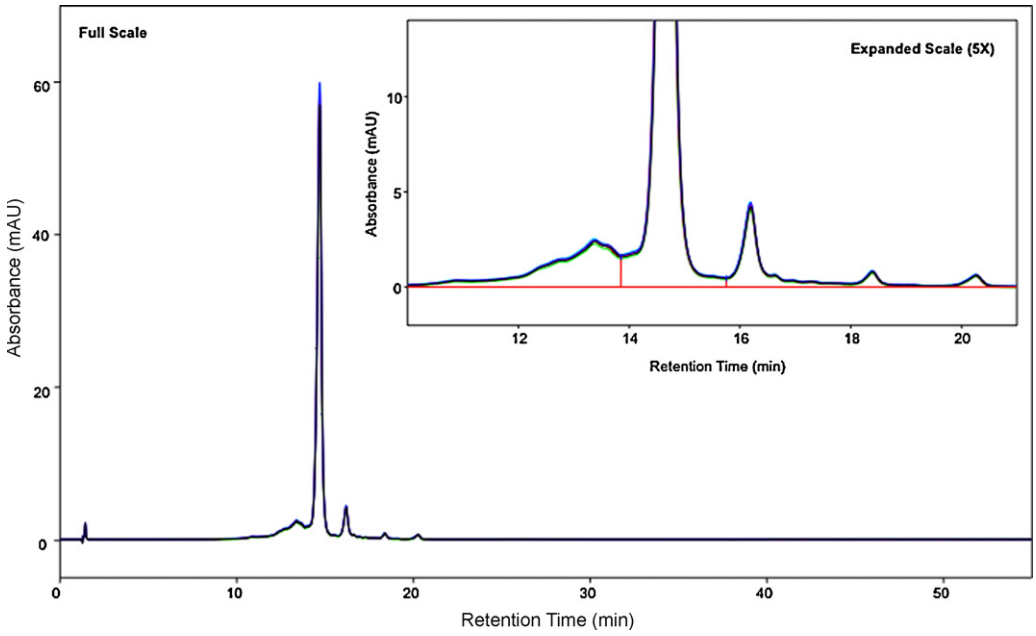

Fig. 2. Overlay for six sequential replicate injections from a single sample vial demonstrating the reproducibility of the elution profile. Conditions as per Fig. 1.

which states that precision should not exceed 15% of the coefficient of variation [20].

3.2. Post-preparative stability of samples and solutions

The response of the reference standard was found to be unchanged when the sample was incubated in the autosampler for 48 h (Table 2). In addition, relative peak areas were unchanged when the buffer solutions were used for up to 14 days (Table 2). Therefore, samples and buffer solutions can be used within the time periods tested without the results being impacted. Post-preparative stability of samples and solutions is particularly important for the analysis of multiple samples, as increased stability lends to method reliability over the anticipated run time and when many samples are in queue before analysis.

3.3. Linearity

The linearity of an analytical procedure is the ability to obtain results that are directly proportional to the amount of analyte used for analysis. Protein load was varied from 20 μg to 100 μg by adjusting injection volume of the sample. Results in Fig. 3 show consistent elution profile shape after normalization and good correlation between the main peak area and loading of protein, with a correlation coefficient $R > 0.99$. This study also provides evidence of robustness against changes in protein load; despite changes in protein load from 0.5 to 2.5 times target loading conditions (40 μg), relative peak areas remain consistent, with RSD values for acidic region, main peak and basic region of <0.3% (Table 3). Consistency of the sample profiles with varying sample loadings readily facilitates scale-up when fraction collection is required or scale-down when sample volume is limited.

Table 2
Relative peak areas obtained from sample and solution stability testing. Samples were incubated in the autosampler at 5 °C for 48 h to determine sample stability. Buffer solutions were tested 14 days after initial use to determine buffer stability.

	Percent peak area		
	Acidic region	Main peak	Basic region
Reference standard (0 h)	14.6	75.7	9.7
Reference standard (48 h)	14.5	75.7	9.8
pH 6.0 and pH 9.5 buffers (14 days)	14.7	75.5	9.9

3.4. Suitability as a stability-indicating method

In addition to reference samples, thermally stressed samples were used to determine linearity and the ability of the method to be stability indicating. Stressed sample admixtures were made by adding various amounts of stressed material with reference material. The method showed a very good linear profile when relative main peak area and relative acidic region area were plotted as a function of the percentage of reference standard present in the sample (Fig. 4), with correlation coefficients $R > 0.99$. Basic region peak

Table 3
Summary of compiled method robustness data. Boldface type denotes target conditions.

	Percent peak area		
	Acidic region	Main peak	Basic region
Temperature (°C)			
28	14.7	75.5	9.8
30	**14.6**	**75.7**	**9.7**
32	14.6	75.6	9.8
Protein loading (μg)			
20	14.4	75.9	9.7
40	**14.4**	**75.9**	**9.7**
60	14.3	75.9	9.7
100	14.4	75.9	9.7
pH range			
6.0–9.5	**14.5**	**75.8**	**9.7**
5.9–9.6	14.8	75.4	9.8
Column lot			
ProPac Lot #1	14.6	75.7	9.8
ProPac Lot #2	14.5	76.0	9.6
ProPac Lot #3	14.9	75.3	9.7
Antibodix Lot #1	14.6	74.9	10.5
Antibodix Lot #2	13.7	75.5	10.7
Antibodix Lot #3	12.5	75.6	11.8
Instrument type			
Dionex U3000 HPLC	14.4	75.9	9.7
Agilent 1100 HPLC	14.7	74.7	10.6
Waters 2796 HPLC	13.7	75.4	10.9

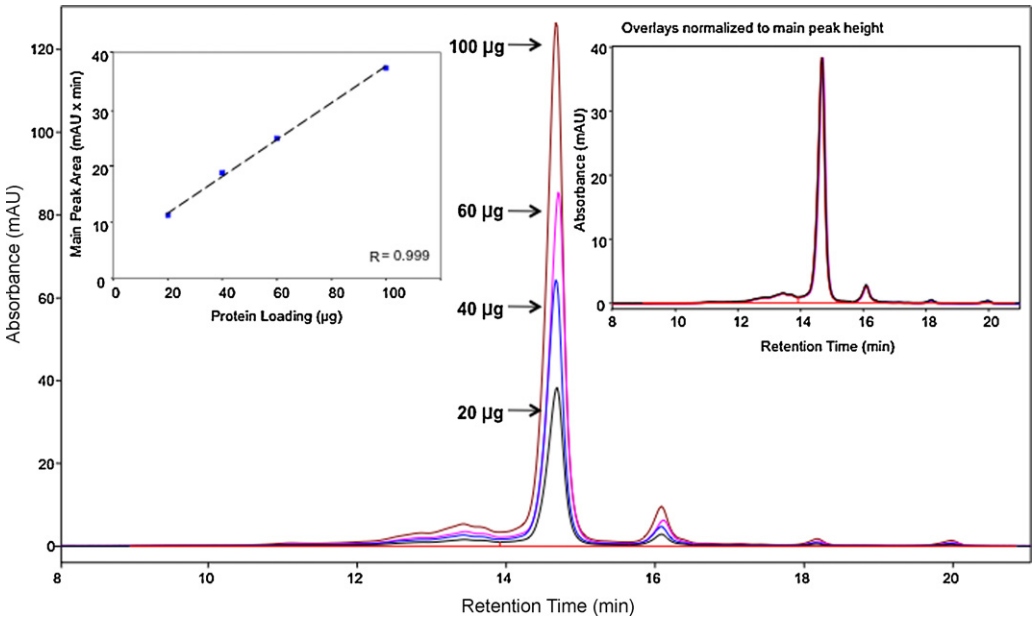

Fig. 3. Elution profiles obtained over a range of sample loadings (20–100 μg). The inset overlays depict the chromatograms overlaid normalized to the height of the main peak. Loading amount was controlled by varying the injection volume of the sample. Linearity is demonstrated by plotting main peak area against sample loading. Other conditions as per Fig. 1.

areas did not change significantly for this molecule with the various stressed sample admixtures. These results indicate that this method can be used as a stability-indicating method prescribed by regulatory agencies.

3.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the pH gradient method was evaluated by varying, individually, injection volume (20–100 μL), buffer pH (±0.1), column temperature (±2 °C), column lot and instrument type (Table 3).

The variations in method parameters did not result in significant changes to chromatographic profile or relative peak areas (Table 3). The most notable variation is observed with changes in column manufacturer. The Sepax Antibodix NP10 column had more lot-to-lot variation compared to the ProPac WCX-10 column for acidic and basic region relative peak areas. Average acidic region relative area lot-to-lot ranges were 0.4% and 2.1% for ProPac and Antibodix columns, respectively. Average basic region relative area lot-to-lot ranges were 0.2% and 1.3% for ProPac and Antibodix columns, respectively. Main peak values were comparably

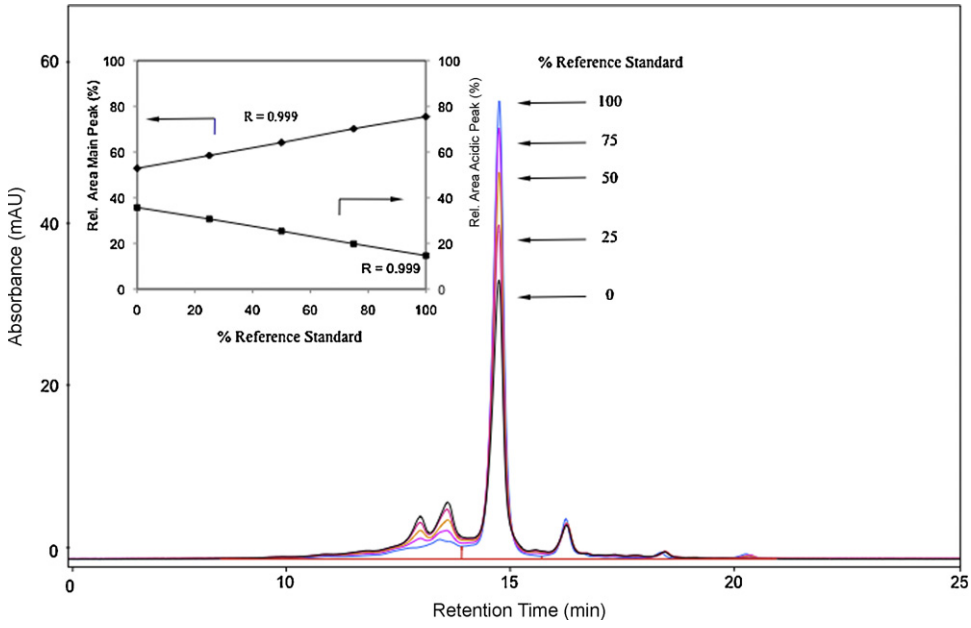

Fig. 4. Elution profiles obtained for admixtures of reference material and thermally stressed material. Five levels of admixtures were prepared. Linearity is demonstrated by plotting acidic region and main peak relative areas against percentage of reference sample. Basic region is not presented as no significant change was noted for this molecule under thermal stressing.

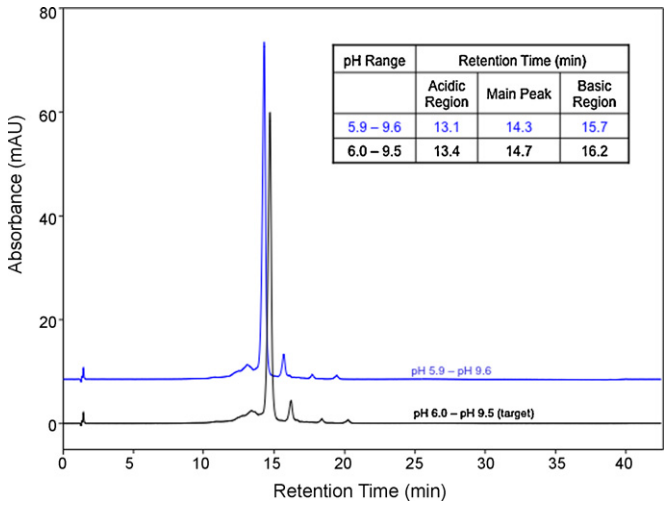

Fig. 5. Elution profiles obtained with varying buffer pH. The gradient was 0–100% B over 30 min, with the mobile phase pH ranging from pH 6.0 to 9.5 (target conditions) for the bottom chromatogram, and pH 5.9 to 9.6 for the upper chromatogram.

tight for both columns. Interestingly, the ProPac and Antibodix columns resulted in similar relative peak areas despite differing column diameters, which indicates that the method is robust with respect to the mobile phase flow rate. Retention times differed for the two columns, which is expected due to the difference in the cross-sectional areas of the columns at a constant flow rate.

When the pH of the mobile phases was changed such that the gradient was set to pH 5.9–9.6, as opposed to a target gradient of pH 6.0–9.5, the relative peak areas remained consistent despite expected changes in retention time (Fig. 5), which is notable considering conventional ionic strength ion-exchange chromatography is typically limited in robustness by a ±0.05 pH unit limit on the mobile phases [23]. Changes in protein loading and temperature results in very little differences in relative peak areas. Transferring the method between instruments resulted in only slight changes in relative areas, with <1% difference between instruments for average relative areas for main peak, acidic and basic regions (Table 3).

3.6. Intermediate precision

Intermediate precision expresses inter-laboratory variations, such as different days of analysis and different analysts. For a total of 49 data points (injections) from three different instrument types, two different column manufacturers, at least 3 column lots from each manufacturer, two analysts and 13 different days of analysis, the standard deviation of relative main peak areas was 0.4%, and standard deviations for relative acidic and basic region peak areas were 0.6% and 0.6%, respectively (Table 4). The ability to use different instrument and column manufacturers greatly reduces the business risk of the method; if a column supplier cannot meet demand or if an instrument manufacturer ceases production of a particular instrument model, this method can be transferred to other instruments and columns without loss of performance of the method. In addition, the method is robust to changes in analysts and days of analysis, which is important for ruggedness of the method.

Table 4
Relative peak areas obtained over the entire study at target conditions, obtained with 2 column types, 7 column lots, 2 analysts, and 3 instruments over 13 days.

	Percent peak area		
	Acidic region	Main peak	Basic region
Average	14.4	75.5	10.2
SD	0.6	0.4	0.6
−3SD	12.6	74.2	8.2
+3SD	16.1	76.8	12.1
6SD range	3.5	2.6	3.9
Hi	15.1	76.1	12.0
Lo	12.3	74.5	9.5
Hi–Lo range	2.8	1.6	2.5

3.7. Comparing pH-IEC with conventional IEC and icIEF

pH-IEC demonstrates an improvement in precision over conventional IEC and icIEF for determining stability and charge heterogeneity of monoclonal antibodies. While the RSD values obtained in the repeatability study for the pH-gradient IEC method were 0.1%, 0.7%, and 0.8% for main peak, acidic and basic regions, respectively, the RSD values for repeatability of icIEF were 1.1%, 2.8%, and 2.1% for main peak, acidic and basic regions, respectively [26]. The calculated 6σ range in Table 4 (2.6%, 3.5% and 3.9% for main peak, acidic and basic region relative areas, respectively), which predicts a 99% method success rate, demonstrates the superior precision of the pH-gradient IEC method over conventional IEC and icIEF, which can have system suitability criteria of up to 10% (unpublished data).

4. Conclusions

In this study, a simple and robust pH gradient-based multi-product ion-exchange chromatography method was demonstrated suitable for use following industry standard validation practices. The testing of this method with different instrument manufacturers and different column manufacturers indicates that this is a highly robust method with significantly less business risk compared to methods relying on limited sources of instrumentation and consumables. The precision of the method compares very favorably to other charge heterogeneity methods. Sample preparation for this method is minimal, and new columns and instrument technologies are currently being explored to decrease run time further. This multi-product method can be used with high-throughput technologies for processing large amounts of in-process and final product samples.

Acknowledgements

We would like to acknowledge Rahul Parikh for technical assistance.

References

[1] K. Ziegelbauer, D.R. Light, Monoclonal antibody therapeutics: leading companies to maximise sales and market share, J. Commer. Biotechnol. 14 (2008) 65–72.
[2] D. Voet, J. Voet, Biochemistry, John Wiley and Sons, New York, 1990.
[3] J.M. Reichert, C.J. Rosensweig, L.B. Faden, M.C. Dewitz, Monoclonal antibody successes in the clinic, Nat. Biotechnol. 23 (2005) 1073–1078.
[4] S. Flatman, I. Alam, J. Gerard, N. Mussa, Process analytics for purification of monoclonal antibodies, J. Chromatogr. B 848 (2007) 79–87.
[5] M. Schenerman, B.R. Sunday, S. Kozlowski, K. Webber, H. Gazzano-Sanotora, A. Mire-Sluis, CMC strategy forum report: analysis and structure characterization of monoclonal antibodies, BioProcess Int. 2 (2004) 42–52.
[6] H. Liu, G. Gaza-Bulseco, D. Faldu, C. Chumsae, J. Sun, Heterogeneity of monoclonal antibodies, J. Pharm. Sci. 97 (2008) 2426–2447.
[7] R.J. Harris, Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture, J. Chromatogr. A 705 (1995) 129–134.
[8] M. Weitzhandler, D. Farnan, J. Horvath, J.S. Rohrer, R.W. Slingsby, N. Avdalovic, C. Pohl, Protein variant separations using cation exchange chromatography on grafted, polymeric stationary phases, J. Chromatogr. A 828 (1998) 365–372.
[9] L.C. Santora, I.S. Krull, K. Grant, Characterization of recombinant human monoclonal tissue necrosis factor-α antibody using cation-exchange HPLC and capillary isoelectric focusing, Anal. Biochem. 275 (1999) 98–108.
[10] A. Di Donato, M.A. Ciardiello, M. de Nigris, R. Piccoli, L. Mazzarella, G. D'Alessio, Selective deamidation of ribonuclease A. Isolation and characterization of

the resulting isoaspartyl and aspartyl derivatives, J. Biol. Chem. 268 (1993) 4745–4751.
[11] Y.R. Hsu, W.C. Chang, E.A. Mendiaz, S. Hara, D.T. Chow, M.B. Mann, K.E. Langley, H.S. Lu, Selective deamidation of recombinant human stem cell factor during in vitro aging: isolation and characterization of the aspartyl and isoaspartyl homodimers and heterodimers, Biochemistry 37 (1998) 2251–2262.
[12] C. Quan, E. Alcala, I. Petkovska, D. Matthews, E. Canova-Davis, R. Taticek, S. Ma, A study in glycation of a therapeutic recombinant humanized monoclonal antibody: where it is, how it got there, and how it affects charge-based behavior, Anal. Biochem. 373 (2008) 179–191.
[13] L. Santora, Z. Kaymakcalan, P. Sakorafas, I. Krull, K. Grant, Characterization of noncovalent complexes of recombinant human monoclonal antibody and antigen using cation exchange, size exclusion chromatography, and BIAcore, Anal. Biochem. 299 (2001) 119–129.
[14] J. Svasti, C. Milstein, The disulphide bridges of a mouse immunoglobulin G1 protein, J. Biochem. 126 (1972) 837–850.
[15] J. Vlasak, R. Ionescu, Heterogeneity of monoclonal antibodies revealed by charge-sensitive methods, Curr. Pharm. Biotechnol. 9 (2008) 468–481.
[16] Z. Sosic, D. Houde, A. Blum, T. Carlage, Y. Lyubarskaya, Application of imaging capillary IEF for characterization and quantitative analysis of recombinant protein charge heterogeneity, Electrophoresis 29 (2008) 4368–4376.
[17] A. Rozhkova, Quantitative analysis of monoclonal antibodies by cation-exchange chromatofocusing, J. Chromatogr. A 1216 (2008) 5989–5994.
[18] US Pharmacopeia XXIII, United States Pharmacopeial Convention, Rockville, MD, 1995, p. 1982 (Chapter 125).
[19] Reviewer Guidance, Validation of Chromatographic Methods, Center for Drug Evaluation and Research, US Food and Drug Administration, 1994.
[20] Guidance For Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Rockville, MD, May 2001.
[21] M. Bakshi, S. Singh, Development of validated stability-indicating assay methods – critical review, J. Pharm. Biomed. Anal. 28 (2002) 1011–1040.
[22] International Conference on Harmonization (ICH) Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Geneva, 2005.
[23] D. Farnan, G.T. Moreno, Multi-product high-resolution monoclonal antibody charge variant separations by pH gradient ion-exchange chromatography, Anal. Chem. 81 (2009) 8846–8857.
[24] X. Kang, D.D. Frey, Chromatofocusing using micropellicular column packings with computer-aided design of the elution buffer composition, Anal. Chem. 74 (2002) 1038–1045.
[25] N. Dyson, Chromatographic Integration Methods, R.C.S. Monographs, Royal Society of Chemistry, Cambridge, UK, 1990.
[26] O. Salas-Solano, L. Gennaro, C. Felten, Optimization Approaches in the Routine Analysis of Monoclonal Antibodies by Capillary Electrophoresis, LC GC Europe, 2008, pp. 615–622.