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Validation of a capillary isoelectric focusing method for the recombinant monoclonal antibody C2B8

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Abstract

A capillary isoelectric focusing (cIEF) method has been developed for the purpose of determining the identity and charge distribution of mouse/human chimeric antibody to human CD20 antigen (C2B8). The assay was validated in accordance with ICH guidelines in order to demonstrate that it is suitable for its intended purpose and so that it may be performed as a lot release test for bulk and final product. As a result of the validation process the assay was found to be linear over the concentration range of 2–356 $\mu g\,ml^{-1}$ with recovery of ^{125}I-labeled C2B8 at the target sample concentration of 125 $\mu g\,ml^{-1}$ equal to 99%. The repeatability and intermediate precision relative standard deviations of the four major peaks for migration time, peak area, and peak area percent ranged from 0.9–4.4%. The specificity of the assay was demonstrated by baseline resolution of the C2B8 main peak from product excipients, and other Genentech monoclonal antibodies. The results of this validation demonstrate that the cIEF assay for the determination of identity and charge distribution of C2B8 is accurate, precise, linear, and highly specific. The assay is rapid and suitably rugged. © 1998 Published by Elsevier Science B.V.

Keywords: Isoelectric focusing; Validation; Monoclonal antibodies; C2B8; Proteins

1. Introduction

The increasing interest in capillary isoelectric focusing (cIEF) over the past couple of years is a demonstration that the potential power of this analytical methodology is closer to being realized. One of the first demonstrations of cIEF of monoclonal antibodies (MAbs) was presented by Costello et al. [1]. Their advantage for using cIEF was that precast gels were not stable in the pH 7–10 range and their MAb had a p*I* in the range of 8.2–9.0. Silverman et al. [2] reported comparable or improved resolution for cIEF when compared to IEF and ion-exchange chromatography, respectively. However, poor reproducibility of migration time and capillary coating instability were found to be major limitations for their cIEF method. Pritchett reported quantitative analysis of two dosage forms of anti-tumor necrosis factor (anti-TNF) in the presence of 0.1% human serum albumin using a cIEF method which demonstrated good linearity (concentration vs. peak area) and precision [3]. Quantitation of anti-TNF samples was achieved, using peak area, by interpolation from a standard curve.

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In cIEF, the substances to be separated are applied to the capillary together with the ampholytes. Typically, the entire capillary is filled with the ampholyte-sample mixture and cIEF is accomplished through two consecutive events: focusing and mobilization. The ampholytes create a pH gradient within a short time upon application of an electric field. Under the influence of this electric field, charged solutes migrate through the medium until the local pH is equal to their isoelectric points (p*I*), whereby the solutes become neutral and remain focused in narrow zones [4]. The pattern obtained during this first step is characteristic of the sample. The focused zones will then be mobilized past the detector using chemical, pressure, or electrosmotic flow mobilization so that a tracing of the separation can be obtained. Although reports on the application of cIEF for antibodies and other proteins have been published, there has yet to be a successful validation of a cIEF method. Validation of a CE method and its transfer to routine use in the quality control system of a marketed protein biopharmaceutical is a critical final step in the acceptance of this technology. The main objective of the validation process is to demonstrate that the procedure is suitable for its intended purpose. The criteria for a successful validation are understandably stringent and are outlined in the guidelines of the ''International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use'' (ICH3). The application of the validation process in accordance with the ICH guidelines is imperative for the scientist seeking to validate a method for Biological License Application (BLA) and Marketing Authorization Application (MAA) submissions [5]. Several different approaches to validation of CE methods for protein samples have been used. Pande et al. [6] validated their CZE separation of BSA by evaluating the intra- and inter-day relative standard deviation (R.S.D.). Nellore et al. [7] added a linearity evaluation in the efforts to validate a CZE separation of ribonuclease A, and Dupin et al. [8] went a step further by showing that their CZE assay demonstrated specificity, linearity and precision. Their validation efforts probably suited the intended purpose of their assays, but it is our contention that for a certificate of analysis test that additional parameters must be evaluated for all assays to ensure the robustness of the method. In the ICH guidelines [9] discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures: Identification tests, Quantitative tests for impurities, Limit tests for the control of impurities, and Quantitative tests of the active moiety in samples. Included in this discussion is a tabular summation of suggested mimimum requirements for the characteristic parameters to be included in a validation protocol. Included in their extensive work on validation of CE methods for the analysis of small organic molecules [10–12], Altria et al. have also offered a tabular guideline to method validation approaches in the areas of identity confirmation, purity testing, main peak assays, and chiral analysis [13].

C2B8 is a mouse/human chimeric monoclonal antibody directed to the human CD20 antigen. C2B8 consists of two -213 residue light chains and two 451 residue heavy chains. The variable region of the light and heavy chain are murine. Each light chain is disulfide-bonded to the heavy chain; and interchain disulfide bonds attach the heavy chains to each other. One conserved Asn-linked glycosylation site is found within the constant region of each heavy chain. Terminal sialic acid has not been detected. C2B8 is subject to possible charge heterogeneity resulting from C-terminal modification and deamidation.

This manuscript presents the results of the validation of an assay for the determination of identity and charge distribution of C2B8 by cIEF. The validation scheme described herein is consistent with the ICH Guidelines on validation of analytical procedures [9,14].

2. Materials and methods

2.1. cIEF

All chemicals used were of analytical reagent grade. Hydroxypropyl-methylcellulose (HPMC), Pharmalytes 8-10.5 were purchased from Sigma (St. Louis, MO, USA). Bio-Lyte 3-10 and 7-9 ampholytes, cathodic mobilizer, N,N,N′,N′-tetramethylethylenediamine (TEMED), and synthetic p*I* markers were purchased from Bio-Rad Labs. (Hercules, CA,

USA). C2B8 was produced by IDEC Pharmaceuticals (San Diego, CA, USA).

cIEF was performed using a Bio-Rad BioFocus 3000 CE System with a BioCAP LPA capillary, 24 cm (19.5 cm to the detector)×50 μm I.D. (Bio-Rad Labs.). The three ampholyte solutions, Pharmalyte 8-10.5, Bio-Lyte 7-9, Bio-Lyte 3-10, were first prepared individually at 2% (w/v) concentrations containing 0.5% (v/v) TEMED and 0.2% (w/v) HPMC. The final ampholyte solution consisited of a 8:1:1 ratio of the above 2% solutions, respectively. Samples were prepared by adding 90 μl of C2B8 (0.25 mg ml^{-1}) to 100 μl of the final ampholyte solution, and 5 μl of p*I* marker previously diluted 1:20 with purified water. This resulted in C2B8 samples with final protein concentrations of approximately 0.125 mg ml^{-1} and final salt concentrations of less than or equal to 10 m*M*. Small deviations from these final concentrations did not impact the performance of this method, however, the effects of high salt concentrations and/or variations in salt concentration between samples have been reported to impact resolution, migration time [15,16]. The final solution was briefly mixed and centrifuged for 10 s at 5000 *g*. Capillaries were rinsed with purified water, 20 m*M* phosphoric acid, and purified water for 60 s prior to each injection. Sample plus ampholytes were injected by applying pressure (40 s at 6.8 bar). Focusing was performed at 417 V cm^{-1}, constant voltage, for 6 min using 20 m*M* phosphoric acid and 40 m*M* sodium hydroxide as the anolyte and catholyte, respectively. Chemical mobilization was carried out at 625 V cm^{-1}, constant voltage, for another 20 min. Capillary and sample temperature were maintained at 20°C.

2.2. Iodination of C2B8

Radiolabeled C2B8 was prepared using Eppendorf tubes coated with 100 μg of Iodo-Gen (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Briefly, 5 mg of C2B8 and 1 mCi of radioactive sodium iodide 125 (^{125}I) (New England Nuclear/DuPont, Boston, MA, USA) were added to a coated tube and incubated for 15 min at ambient temperature. Radiolabeled protein was purified from the remaining free iodine using a NAP-5 column from Pharmacia (Uppsala, Sweden). The NAP5 column was equilibrated with formulation buffer which had been diluted 40-fold with purified water. Protein content was determined by UV spectrophotometry using an extinction coefficient of 1.7.

2.3. Trichloroacetic acid (TCA) precipitation of [^{125}I]C2B8

The radioactive content of the tracer preparation was determined by TCA precipitation. Twenty μl of tracer was diluted into 200 μl of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) in micronics tubes. The samples were then counted for 1 min in an LKB 1277 gammamaster γ counter (Wallac, Gaithersburg, MD, USA). All samples were run in duplicate. The same samples were then precipitated by the addition of 50 μl cold 50% TCA. Samples were vortexed gently, then incubated on ice for 30 min. Sample volume was increased by the addition of 700 μl of ice-cold 10% TCA. The samples were then centrifuged at full speed in a Biofuge B (American Scientific Products, Hayward, CA, USA) for 5 min. The supernatants were decanted, the tubes were blotted dry, and the resulting pellets were again counted in the gamma counter for 1 min. Percent TCA precipitability was determined by dividing the counts per minute (cpm) after TCA precipitaion by cpm before TCA precipitation, times 100. The percent TCA precipitability of the [^{125}I]C2B8 was 94.4%. Radioactive content of the tracer was determined by adjusting the counts per minute to reflect total cpm by accounting for dilution and volume. Total cpm was then converted to total disintergrations per minute (dpm) using the counting effeciency of the gamma counter (70%). The number of microcuries was determined by dividing the total dpm by $2.2 \cdot 10^6$ dpm μCi^{-1}. The specific activity of the [^{125}I]C2B8 was determined to be 0.32 μCi μg^{-1}.

2.4. Recovery experiment using [^{125}I]C2B8

C2B8 labeled with iodine-125 was used to evaluate the recovery of C2B8 from the BioCAP LPA coated capillary by the following method: to obtain a baseline value (control) the cIEF method was performed through the injection step. After the capillary was filled with the labeled C2B8, a 10 s high-pressure rinse with purified water was used to expel

Table 1
Recovery of C2B8 using the BioCAP LPA-coated capillary

Sample	Control		Experimental	
	cpm[a]	ng	cpm[a]	ng
1	28 859	58.9	28 574	58.3
2	29 201	59.6	29 275	59.8
3	29 268	59.7	28 717	58.6
Mean	29 109	59.4	28 855	58.9
R.S.D. (%)	0.7	0.7	1.3	1.3
Recovery (%)[b]	–	–	99	99

[a] Counts per minute of ^{125}I-labeled C2B8. ng=μCi÷specific activity.
[b] Recovery=(experimental value÷control value)×100%.

the contents into a 0.5-ml Eppendorf tube containing 196 μl of buffer (pH 7). This was performed in triplicate. Following this, using the same sample vial, the cIEF method was performed to completion (Experimental). Thirty seconds after the last peak had migrated past the detector, mobilization was stopped and a 10 s high-pressure purge with purified water was employed to expel the capillary contents into a 0.5-ml Eppendorf tube containing 196 μl of buffer (pH 7). This was performed in triplicate. Counts per minute for all six samples were then counted in a γ counter for 1 min. Comparison of the control and experimental samples resulted in a mean recovery of 99% (Table 1). In addition, residual counts in the capillary were measured by wiping the exposed ends of the capillary with ''count-off'' (Biotechnology Systems, Boston, MA, USA) to remove radioactivity from the exterior of the capillary. The capillary was then placed in a scintillation vial and counted in a γ counter. Residual counts in the capillary were less than 2% of the control value.

3. Results and discussion

In the quality control laboratory, isoelectric focusing (IEF) is used primarily to ensure the identity, consistency, and stability of a protein as demonstrated by its isoelectric point (p*I*), a profile which is consistent with a reference material and the absence of new or more intense bands, respectively. The use of IEF stained with Coomassie Brilliant Blue and quantitated by densitomitry, at best provides a somewhat linear, semi-quantitative approach for monitoring charged isoforms. cIEF on the other hand, offers a fast, accurate, precise, and linear means for the determination of identity and charge distribution of a recombinant protein pharmaceutical such as C2B8. It has been previously shown that the relative number and intensity of cIEF peak profiles compare well with the banding pattern and relative intensity of slab gel IEF [1,17–19]. This, as well as, the other more obvious advantages of decreased analysis times, automation, and quantitative capabilities make cIEF an extremely attractive methodology for routine use in quality control of recombinant proteins. However, prior to transfer of analytical procedures to a quality control environment they must undergo a validation process as required by regualtory authorities. Validation of CE methods had been stalled for two reasons: (1) problems with reproducibiity, sensitivity, robustness, and peak characterization, and (2) the concepts of CE and validation issues were not well understood. As certain aspects of the technology improved (instrumentation, capillary coatings, and knowledge of methods development) the first set of problems have become less formidable. In addition, and in part due the ''Third International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use'', the specific requirements and terminology for the validation of an analytical separation method are no longer unclear.

The objective of the validation process is to demonstrate that the procedure is suitable for its intended purpose. Performance characteristics of a method are expressed in terms of analytical parameters [20]. They include the following: accuracy, detection and quantitation limits, linearity, precision, specificity, robustness, and stability indicating properties, as described in the ICH documents on assay validation.

A typical electropherogram of the cIEF analysis of C2B8 is shown in Fig. 1. The C2B8 charge isoforms migrate between 10 and 13 min and are separated based on their relative isoelectric points. Peaks 1, 2, and 3 are present due to incomplete C-terminal lysine processing and represent C2B8 with 2, 1, and 0 C-terminal lysines, respectively (Fig. 1 inset). Characterization of peak 4 has yet to be completed. Included in Fig. 1 is an electropherogram of a reagent blank, demonstrating that there are no com-

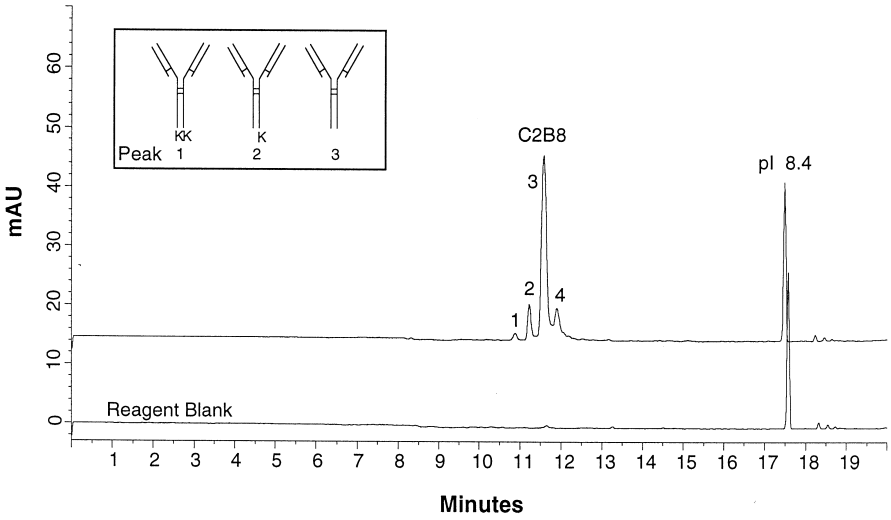

Fig. 1. Typical electropherogram of the cIEF analysis of C2B8. Included is the cIEF analysis of a reagent blank showing that there are no components in the sample preparation which interfere with the analysis of C2B8, and a schematic illustration of the three c-terminal lysine variants (inset). Analysis conditions were as described in Section 2.1

ponents in the sample preparation which interfer with the analysis of C2B8.

3.1. Linearity

Analysis of a sample of C2B8 showed that it contained 70% main peak. The linearity of the assay was then determined using single injections of three samples ranging from 50–150% of the target sample concentration (~ 10 mg ml^{-1}). The main peak area was plotted against the sample protein concentration. The linearity of the assay was demonstrated by a Pearson correlation coefficient >0.99. The regresson data (y=3.5701+31.572x) includes a positive y-intercept for the main peak of only 1%. This demonstrates a low assay bias relative to the main peak area for the target sample concentration of 10.7.

3.2. Accuracy

Good recovery of protein off of the capillary must be demonstrated to ensure that the electropherogram is representative of all the protein that is present in the sample. This is especially important since proteins tend to bind non-specifically to bare fused-silica.

The cIEF profile for the ^{125}I-labeled C2B8 was identical to those generated with unlableled C2B8. Comparison of the control and experimental samples resulted in a mean recovery of 99% (Table 1) at the target sample concentration.

3.3. Limits of detection and quantitation

The limit of detection (LOD) is established by the minimum concentration at which the analyte can be detected with a signal-to-noise ratio of ≥2. The limit of quantitation (LOQ) is established as the minimum concentration at which the analyte can be reliably quantified with a signal-to-noise ratio ≥10.

To determine the LOD and LOQ, a sample of C2B8 was serially diluted to cover the concentration range of 2 μg ml^{-1}–356 μg ml^{-1}. The target assay concentration is 250 μg ml^{-1}. The LOD and LOQ of C2B8 main peak was determined to be 2 and 8 μg ml^{-1}, respectively. The signal-to-noise ratio of the main peak for the 2 and 8 μg ml^{-1} samples were 3.6 and 16.3, respectively (data not shown).

3.4. Repeatability

A single sample preparation of C2B8 was analyzed using six replicate injections. As shown in Table 2, the repeatability of the assay for peaks 1–4

Table 2
Repeatability and intermediate precision for C2B8 using cIEF

Analyses	Value	Peak			
		1	2	3	4
Mitration time (min)					
Repeatability[a,c]	Mean	11.7	12.0	12.4	12.7
	R.S.D. (%)	1.4	1.2	1.2	1.1
Intermediate[b,d]	Mean	11.1	11.5	11.8	12.2
	R.S.D. (%)	2.0	1.8	1.8	1.8
Peak area (μV s)					
Repeatability[a,c]	Mean	8.5	42.0	289.0	73.2
	R.S.D. (%)	1.9	1.4	0.5	2.1
Intermediate[b,d]	Mean	9.2	41.2	297.5	79.1
	R.S.D. (%)	4.2	1.4	1.9	4.4
Area (%)					
Repeatability[a,c]	Mean	2.1	10.2	70.2	17.8
	R.S.D. (%)	1.6	0.7	0.5	0.7
Intermediate[b,d]	Mean	2.2	9.7	69.7	19.2
	R.S.D. (%)	3.7	1.1	0.9	2.1

[a] A single preparation of C2B8 was analyzed.
[b] Two analysts in different laboratories independently analyzed a sample on three separate days using fresh samples prepared each day.
[c] Values represent the mean of six replicate injections.
[d] Each value represents the mean of six injections (duplicate injections on three separate days).

was demonstrated by R.S.D. values of 2.1% or less for migration time, peak area, and peak area percent.

3.5. Intermediate precision

Two analysts in different laboratories, each using separate reagents, capillaries, and instruments, independently analyzed C2B8 on three separate days. Good intermediate precision of the assay for the four major peaks was demonstrated with R.S.D. values for migration time, peak area, and peak area percent which ranged from 0.9–4.4% (Table 2). The lower R.S.D. values for peak area percent indicate that this may be the parameter of choice for quantitative evalutation of charge distribution.

3.6. Specificity

Specificity, as defined by the ICH guidelines [9], is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. It is necessary to demonstrate the absence of interfering substances (degradation products, excipients, and other compounds of closely related structures) that may bias the results. As shown in Figs. 1 and 2, the specificity of this assay was demonstrated by baseline or near baseline resolution of C2B8 main peak (peak 3) from the other three charge isoforms, the absence of any UV absorbing components in the product excipients and baseline resolution from other Genentech monoclonal antibodies.

3.7. Robustness

Robustness as an indication of a procedure's reliability can be determined by varying key method parameters (e.g., temperature, voltage) and assessing the impact on assay performance and final results.

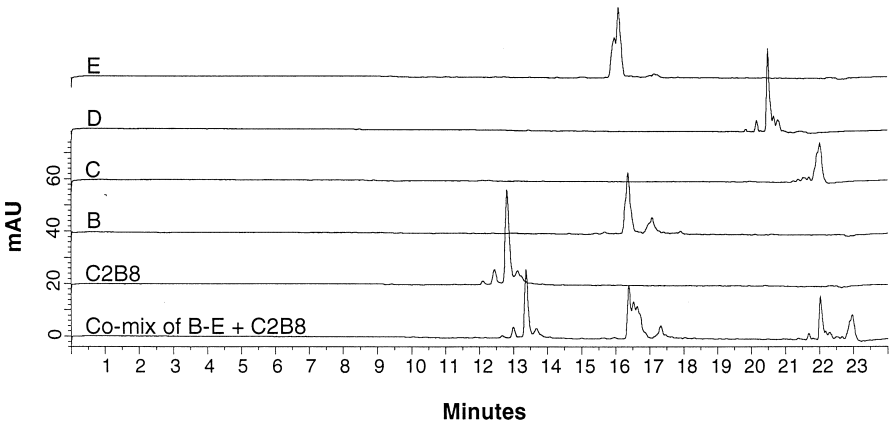

Fig. 2. cIEF analysis of C2B8 and other Genentech monoclonal antibodies B–E, demonstrating the specificity of the assay.

3.7.1. Lot-to-lot variability (reagents)

Lot-to-lot variability was evaluated for those reagents for which more than one lot was available from the manufacturer. Three lots of the Bio-Rad cIEF mobilizer, and two lots of HPMC, TEMED, and the Bio-lyte 7/9 ampholytes were evaluated independently using duplicate injections of each sample. The results demonstrate only minor differences in the cIEF profile, for the cIEF mobilizer and the Bio-lyte 7/9 ampholytes. These minor differences are associated with the peak shape, extent of resolution of peak 4, and a shift in migration time but did not change the percent distribution of the integrated profile (data not shown). No differences were observed when evaluating different lots of HPMC and TEMED. Only one lot of the Pharmalyte 8/10.5 and the Bio-lyte 3/10 was available at the time of validation.

3.7.2. Focusing and mobilization voltage

Focusing and mobilization voltage of 10, 15, and 20 kV were evaluated. While the focusing or mobilization voltage was altered, all other parameters remained as specified in the method. A decrease in the focusing voltage to 10 kV was associated with improved resolution of peak 3 from peak 4 and minor variation in the area percents of peaks 3 and 4 as a result of this improved resolution (Fig. 3A). As expected increasing mobilization voltage was associated with a decrease in migration time, and again, minor variation in the area percent for peaks 3 and 4 was observed. This variation in area percent for peaks 3 and 4 is due in part to changes in resolution and the presence or absence of a shoulder between peaks 3 and 4 (Fig. 3B).

3.7.3. Capillary-to-capillary variability

C2B8 was assayed on four different lots of Bio-Rad BioCAP LPA coated capillaries using six replicate injections per capillary. One representative electropherogram for each of the capillary lots evaluated is shown in Fig. 4A. Based on these four lots, resolution between peaks 3 and 4 appears to be more sensitive to capillary performance than the rest of the electropherogram. All four lots, however, separated the four major peaks with similar peak area percent distributions, and demonstrated precision comparable to the intermediate precision of the assay (Table 3). Fig. 4B demonstrates the potential difference in performance from different sections of a single one meter length of capillary. The first 30 cm appeared to be different from the rest of the capillaries used throughout the validation (>10 m of capillary) and does point out that a bad section of a capillary does not necessarily imply a bad lot of capillaries.

3.7.4. Alternate capillaries

Capillaries from two additional vendors, and a third capillary with a new coating from Bio-Rad were evaluated as potential alternates for this method. Based on the electropherograms (Fig. 5) the initial selection criteria was not met by the Hewlett–Packard PVA coated capillary. The Bio-Rad BioCAP XL and Beckman eCAP Neutral Capillary did meet the initial selection criteria and were qualified as alternates.

To establish the BioCAP XL and eCAP Neutral as alternate capillaries, triplicate injections of C2B8 at concentrations of approximately 50%, 100%, and 150% of the target concentration were analyzed. Linearity of the assay using these two capillaries was demonstrated by a correlation coefficient >0.99 (data not shown). The precision of the assay using the Bio-Rad BioCAP XL and Beckman eCAP Neutral was demonstrated by R.S.D. values comparable to the primary capillary (Table 4).

3.7.5. Alternate instrument

Six replicate injections of C2B8 were used to evaluate the Beckman P/ACE 5510 as a potential alternate instrument for this cIEF method. Several aspects of this method had to be altered in order to transfer the method to the Beckman Instrument. They are: (1) use of a 27 cm (total length) capillary, (2) use of a single wavelength detector at 280 nm, (3) a five-fold increase in time for capillary washes between injections, and (4) total run time had to be extended from 26 to 38 min. A similar peak profile and relative distribution of the four major peaks was observed (data not shown). However, there was a problem with a drifting baseline and the method could not be duplicated on a second Beckman instrument when attempted in a different lab. Therefore, the Beckman P/ACE 5510 was not shown to be an alternate for this cIEF method.

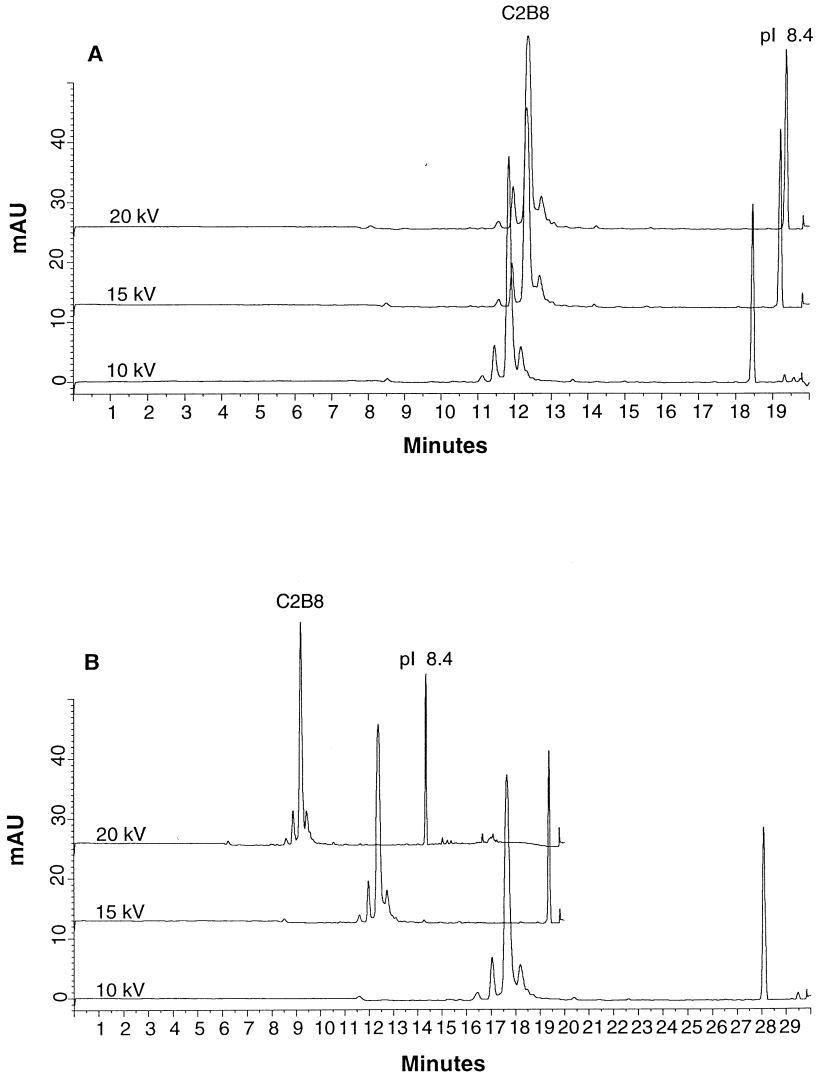

Fig. 3. cIEF analysis of a single sample of C2B8 using different focusing (A) and mobilization voltages (B).

3.8. Sample stability

Isoelectric focusing of basic proteins requires the use of basic ampholytes for separation. Therefore, the sample exists in a basic environment and is susceptible to potential degradation mechanisms such as deamidation. A study was performed to assess the stability of C2B8 samples held in an autosampler (thermostated at 20°C) for an extended period of time prior to analysis by cIEF. An automated run of approximately 12 h was performed. Twenty-five consecutive injections (two conditioning and twenty-three experimental) were performed on a single sample preparation of C2B8. The area percent of peaks 1–4 remained stable (less than 1% change) throughout the 23 experimental injections and with precision values comparable to the results of the repeatability experiment (data not shown).

3.9. Stability indicating properties

The stability indicating properties of this method

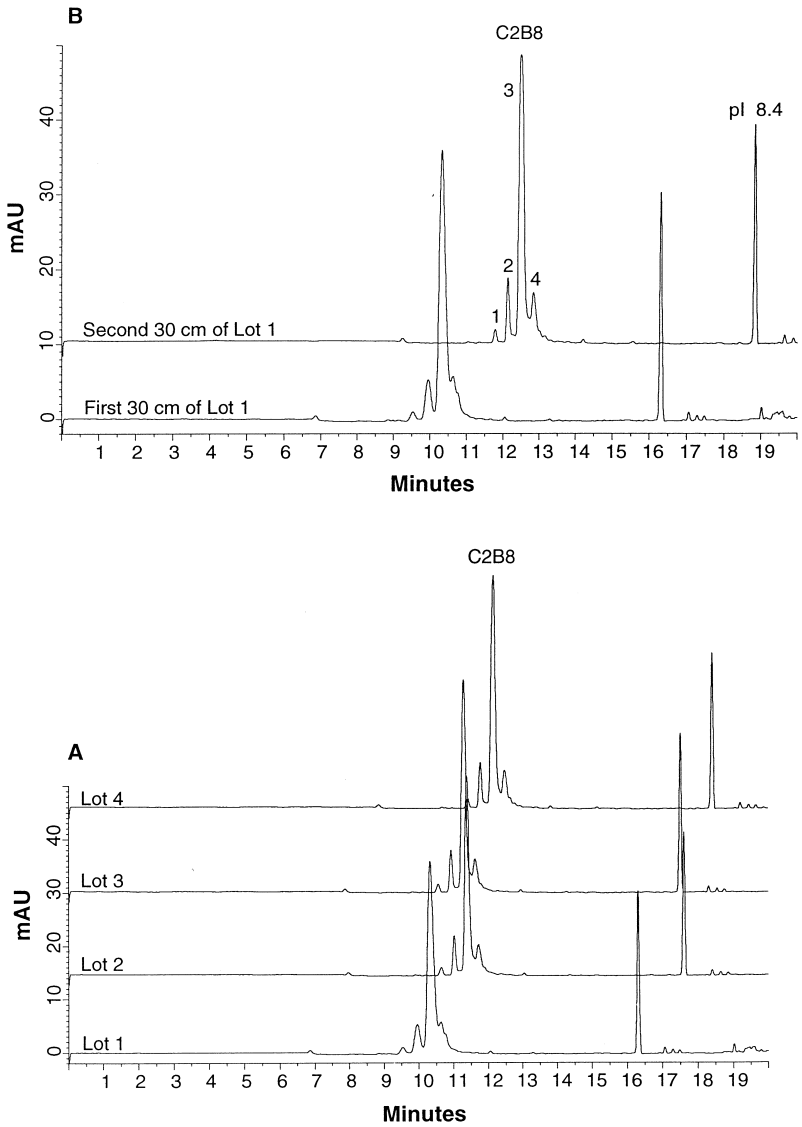

Fig. 4. Evaluation of four different lots of the BioCAP LPA-coated capillary (A), and a second section of a single one-meter length of Lot 1 (B). The results in panel B demonstrate that a bad section of a capillary does not necessarily imply a bad lot of capillaries.

were evaluated using samples of C2B8 subjected to several modes of physical and chemical degradation. The results are summarized in Fig. 6. Samples of C2B8 subjected to thermal degradation (45°C for 14 and 30 days) showed an increase in peak 4 and the presence of new acidic variants. The change in peak 4 and the number of new acidic variants was directly related with increasing days of storage and increasing temperature. The sample subjected to intense light exposure showed an increase in peak 4 equal to 9% of total peak area. Analysis of the sample exposed to hydrolytic degradation at pH 3 resulted in a decrease in peaks 1–4 and the presence of four new acidic variants. There was no change from the control values in the sample treated at pH 8. For the purpose of showing baseline detail of a typical

Table 3
Evaluation of four different lots of the BioCAP LPA-coated capillary

Peak	Peak area (%)					
	Lot 1[a]	Lot 2[a]	Lot 3[a]	Lot 4[a]	Mean[b]	R.S.D. (%)
1	2.1	2.3	2.3	2.1	2.2	5.0
2	10.4	9.5	9.5	10.1	9.8	4.6
3	71.7	71.4	71.5	71.3	71.5	0.2
4	16.0	16.8	16.8	16.4	16.5	2.5

[a] Values represent the mean of six replicate injections of a single sample of C2B8.
[b] Values represent the mean of the four lots of the BioCAP LPa-coated capillary.

integration, Fig. 7 presents an expanded view of the cIEF profile of C2B8 held at 45°C for 30 days. Integration parameters used are baseline height threshold and baseline time threshold equal to 0.5 mV and 4.0 min, respectively.

Those samples of C2B8 subjected to mechanical

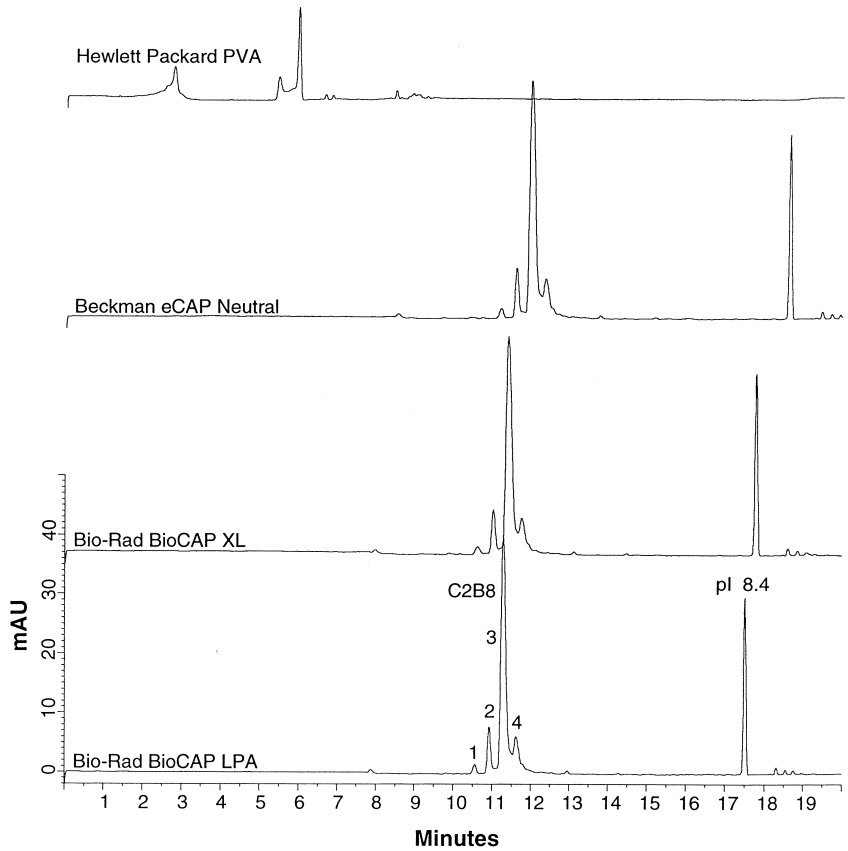

Fig. 5. Evaluation of potential alternate capillaries for the cIEF analysis of C2B8.

Table 4
Precision of the cIEF method using two alternate capillaries

Capillary	Peak area (%)[a]				
	Injection	Peak 1	Peak 2	Peak 3	Peak 4
Bio-Rad BioCAP XL	1	1.4	9.6	69.7	19.3
	2	1.5	9.6	69.3	19.3
	3	1.4	9.6	69.3	19.6
	Mean	1.5	9.6	69.4	19.4
	R.S.D. (%)	1.8	0.2	0.3	1.0
Beckman eCAP Neutral	1	1.4	9.3	69.9	19.3
	2	1.4	9.4	69.8	19.4
	3	1.4	9.4	69.0	20.2
	Mean	1.4	9.4	69.6	19.6
	R.S.D. (%)	0.4	0.5	0.8	2.5

[a] Analysis of C2B8 at the target sample concentration (10.7 mg ml^{-1}).

agitation or chemical oxidation with *tert*-butyl hydroperoxide showed no differences from the control cIEF analysis (data not shown).

3.10. System suitability

It is advisable to assess the performance of the analytical system prior to initiating an analysis sequence. This should be done through assessment of some critical parameter such as resolution, efficiency, or in the case of an identity assay, confirmation of the presence of critical peaks of interest. The appropriate criteria should be evident based on the intended purpose of the method and the data generated from the validation process.

4. Conclusions

Assay validation of CE methods should be viewed

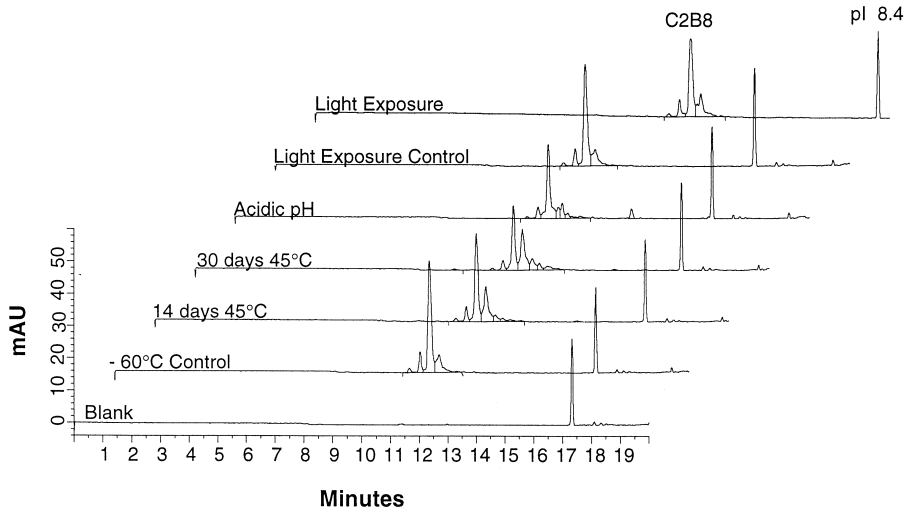

Fig. 6. cIEF analysis of C2B8 samples subjected to various modes of degradation. Indicating that the assay is stability indicating for these modes of degradation.

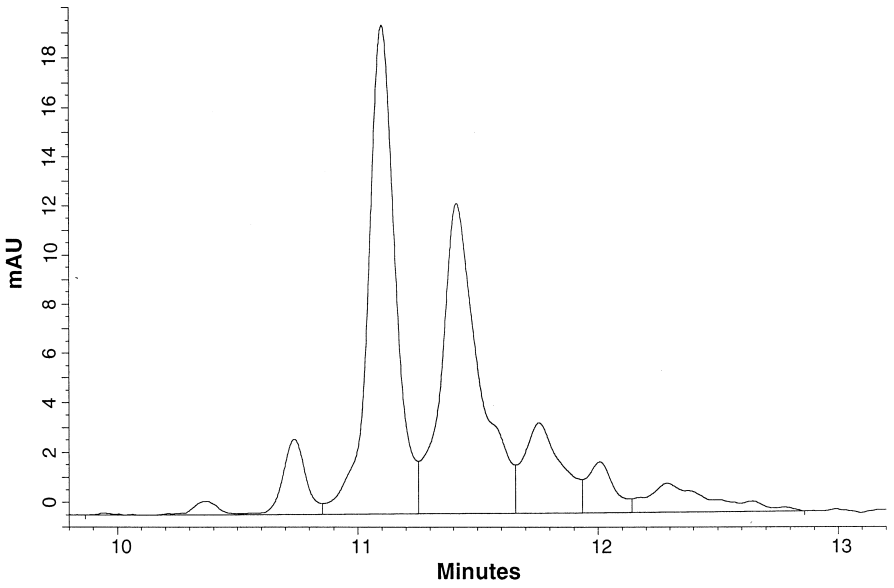

Fig. 7. Expanded view of the sample of C2B8 held at 45°C for 30 days, for the purpose of showing baseline detail of typical integration. Integration parameters used are baseline height threshold and baseline time threshold equal to 0.5 mV and 4.0 min, respectively.

as a critical step in establishing the utility of CE technology in the biotechnology industry. In selecting a validation strategy, one must consider the intended use of the method and the stage of development of the biopharmaceutical product. In all cases, the strategy and terminology used with regard to assay validation should be consistent with ICH guidelines, so as to provide a uniform language for presentation of validation data to other laboratories and regulatory authorities [5].

The precision of the assay compares favorably to those previously reported in the literature for cIEF of a monoclonal antibody [20,2]. The determination of protein recovery off of the analytical capillary using a radiolabeled protein offers simplicity and accuracy to the data and compares favorably to those previously determined using enzyme-linked immunosorbent assays [21]. The extensive evaluation of robustness demonstrates the reliability of the assay. The results of this validation demonstrate that the cIEF assay for the determination of identity and charge distribution of C2B8 is accurate, precise, linear, and highly specific. The assay is rapid and suitably rugged.

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