

Directions

in Discovery

Guest Authors

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Specifications can be established for the purity of monoclonal antibodies as measured by capillary gel electrophoresis based on the heavy-chain, light-chain, and any other additional peaks that may be present in a sample. Minor peaks in an electropherogram, however, may be more difficult to reproducibly quantify. This installment of "Directions in Discovery" describes the experiments performed to optimize a standard quality control method for protein purity evaluation using reducing capillary gel electrophoresis.

Tim Wehr

Directions in Discovery Editor

Optimizing Sample Preparation for Capillary Gel Electrophoresis

Capillary gel electrophoresis is a means of separating proteins by apparent molecular weight using replaceable sieving polymers (1–4). It is based upon the separation principles used in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and it has been documented in several landmark papers (5,6). Capillary gel electrophoresis offers many benefits when compared with SDS–PAGE, as discussed in previous reviews (7–23).

Reducing capillary gel electrophoresis analysis of monoclonal antibodies typically reveals two major peaks corresponding to the heavy and light chains of IgG. We have observed the presence of a peak with a higher molecular weight than that of the heavy chain (approximately 92 kDa), which is present in varying amounts, in many monoclonal antibody preparations. Some debate exists about whether this peak arises from a true product-related impurity or is an artifact of the sample preparation method. This peak, which may be the result of a partially reduced species, is not detectable by size-exclusion chromatography or matrix-assisted laser-desorption ionization time-of-flight mass spectrometry. This installment of "Directions in Discovery" describes some experiments designed to show that the reducing conditions used in the sample treatment before capillary gel electrophoresis do not contribute significantly to the formation of this 92-kDa peak.

A key step in the resolution of proteins using reducing capillary gel electrophoresis is the denaturation and reduction caused by heating with SDS and a reducing agent such as β -mercaptoethanol or dithiothreitol. Various reports in the literature have suggested that incomplete reduction (24), reoxidation (25–28), protein entrapment in the presence of β -mercaptoethanol (29), or prolonged heating with a reducing agent

(30–32) can cause artifacts. Aggregation, fragmentation, and incomplete reduction are some of the problems that can result from inadequately optimized and controlled reducing conditions. Reactions of disulfides in proteins with excess thiol reagents are reversible, and kinetic rates vary considerably depending upon the choice of reducing agent and reduction conditions such as pH (25,26).

Although specifications can be readily established for the purity of the heavy- and light-chain peaks (21), the quantification of any additional, minor, product-related peaks can be troublesome. These peaks typically have low signal-to-noise ratios (S/N), making reliable integration more challenging. Sample handling also can contribute variability to the method. We will describe the optimization of several sample preparation steps, including sample concentration, reduction, and diafiltration–centrifugation. We will show that establishing acceptable operating ranges for these parameters results in significant improvements in the reliability and robustness of a reducing capillary gel electrophoresis method used for quality control testing.

Materials and Methods

Materials: We obtained an CE-SDS protein kit run buffer (33), sample buffer, a benzoic acid reference standard, and β -mercaptoethanol from Bio-Rad Laboratories (Hercules, California). Dithiothreitol was purchased from Sigma (St. Louis, Missouri). We obtained 0.1 M hydrochloric acid, 0.1 M sodium hydroxide, and β -galactosidase protein from Fluka (Milwaukee, Wisconsin). Phosphate-buffered saline (pH 7.4) was purchased from Gibco BRL (Rockville, Maryland). We obtained Microcon-10 microconcentrators from Amicon (Bedford, Massachusetts), and the capillary electrophoresis (CE)–grade water used for all sample and reagent preparation was

from Agilent Technologies (Wilmington, Delaware). The mAb1 is a humanized monoclonal antibody manufactured by MedImmune, Inc. (Gaithersburg, Maryland).

Capillary gel electrophoresis: Recombinant proteins were first diluted to a final volume of 75 μ L with water, concentrated in a microconcentrator, and then diluted into a sample dilution buffer containing SDS in the presence of either β -mercaptoethanol or dithiothreitol. The final protein sample concentration was 4 mg/mL (unless otherwise indicated). The samples were heated in a boiling-water bath for 10 min and then cooled in an ice bath for 5 min before injection. We performed electrokinetic injection at -10.0 kV for 40 s using a high performance CE instrument from Agilent Technologies. The separation occurred in an electric field of 390 V/cm for 22 min at 50 $^{\circ}$ C in an extended light path, fused-silica 38.5 cm \times 50 μ m capillary (Agilent Technologies) with a 30-cm effective length. The detection wavelength was 220 nm.

The percentage purity of mAb1 is equal to the sum of the areas of heavy- and light-chain peaks divided by the total area of all detected peaks and multiplied by 100. The percentage purity of the 92-kDa peak is equal to the area of the 92-kDa peak divided by the total area of all detected peaks and multiplied by 100.

Preparation of β -galactosidase dilution series: We reconstituted lyophilized β -galactosidase protein to a final concentration of 1 mg/mL using phosphate-buffered saline. The protein was prepared according to the capillary gel electrophoresis method description previously stated. The final protein sample concentration was 2 mg/mL. The concentrated β -galactosidase protein was diluted 1:5 using reducing sample buffer and then serially diluted 1:2 to provide final sample concentrations of 400, 200, 100, 50, 25, 12.5, and 6.25 μ g/mL. Each of the samples was injected into the capillary and analyzed as described above.

Results and Discussion

Optimization of the reducing agent in sample buffer: We designed a series of experiments to demonstrate the optimal reducing conditions for monoclonal antibody samples tested by reducing capillary gel electrophoresis. Dithiothreitol was the first candidate examined for optimizing and improving the sample reduction because it has been widely reported as an effective reducing agent for protein samples in many

electrophoretic methods (25,26). We determined that the optimal final concentration of dithiothreitol in sample buffer was 7.5 mM, and this dithiothreitol concentration did not significantly affect the amount of 92-kDa peak in our samples.

In addition, we analyzed a full tray of 37 samples prepared with sample buffer that contained 7.5 mM dithiothreitol to simulate typical operating conditions used in quality control applications. The results indicated that the baseline started to drift significantly by the time the analysis reached the 10th sample in the sample carousel. Evaluating the analysis beyond this point only resulted in the formation of additional peaks that had a greater migration time than that of the 92-kDa peak. This outcome suggests the possibility of disulfide bond reoxidation occurring as a function of time. We did not observe this phenomenon with sets of fewer than six samples, which could be analyzed rapidly and are typically found in validation studies. We also observed no disulfide bond reoxidation using β -mercaptoethanol, regardless of the time or number of samples analyzed. Although dithiothreitol is more efficient than β -mercaptoethanol as a reducing agent based on the amount necessary for reducing protein samples, it is more problematic when used to test sample populations greater than 10.

Because of the complications that dithiothreitol posed to the method, we pursued

further optimization of β -mercaptoethanol reducing conditions. We used β -mercaptoethanol concentrations of 5–15% (v/v) to prepare mAb1 reference standard before injection into the capillary. Figure 1 shows two example electropherograms. The results obtained using 5–8% β -mercaptoethanol concentrations in the sample buffer are typical of those in Figure 1a, and they demonstrate the ideal range of reducing agent concentration for optimal sample reduction and peak resolution. β -Mercaptoethanol concentrations of 9–15% did not reduce mAb1 reference standard more effectively and, in fact, worsened the peak resolution (see Figure 1b). Based on our experimental data (not shown), we recommend using 6% β -mercaptoethanol in reducing capillary gel electrophoresis sample buffer instead of the 5% β -mercaptoethanol concentration previously reported (21).

Optimization of sample centrifugation time in microconcentrator diafiltration units:

Samples received during the production of a monoclonal antibody have widely varying protein and salt concentrations. Researchers often use centrifugal diafiltration or concentration units to normalize the protein and salt concentration in each sample.

We designed experiments to determine the optimal centrifugation time of protein samples in microconcentrator units for the best reducing capillary gel electrophoresis results. The mAb1 reference standard and

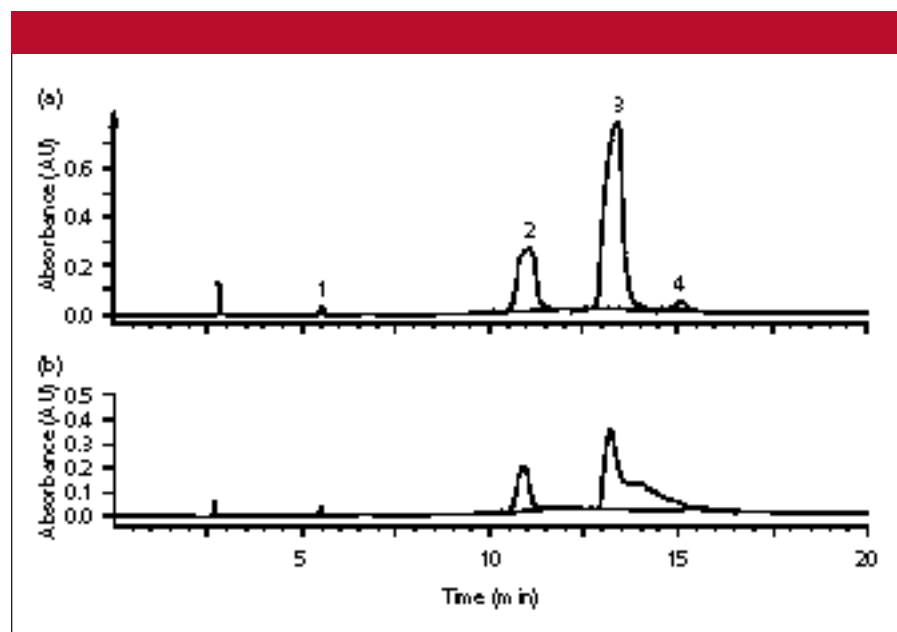


Figure 1: Reducing capillary gel electrophoresis separation of an mAb1 sample using (a) 5% (v/v) and (b) 15% (v/v) β -mercaptoethanol. The mAb1 sample was prepared as described in the Materials and Methods section. Peaks: 1 = internal standard, 2 = light-chain peak, 3 = heavy-chain peak, 4 = 92-kDa peak.

monoclonal antibody (lot 1) samples were centrifuged in microconcentrators for 10, 15, 20, or 25 min, and the proteins were analyzed by reducing capillary gel electrophoresis. Increasing the duration of centrifugation yielded modest improvements in overall percent purity and percentage of the 92-kDa peak (Table I). Presumably, this improvement was achieved because most of the potentially interfering sample buffer matrix was removed with increased

centrifugation times. Based on this experimental data, we recommend a 25-min centrifugation of sample in a microconcentrator filtration unit instead of the 10-min time previously reported (21).

Optimization of final sample concentration: The signal-to-noise ratio of a chromatographic peak can have a major effect on accurately and reproducibly integrating the peak area. We evaluated methods for improving the signal-to-noise ratio of the

92-kDa peak to strengthen the quantitative aspect of the monoclonal antibody specification. The original studies evaluated only the individual migration times for heavy- and light-chain peaks and the percent purity of the protein sample analyzed (21).

We evaluated the signal-to-noise ratio of the mAb1 reference standard as analyzed by reducing capillary gel electrophoresis at a final concentration of 2 mg/mL. We obtained signal-to-noise values of 91–116 for the 92-kDa peak. We designed an experiment to improve the signal-to-noise ratio of the 92-kDa peak and its reliable quantification. In this experiment, we analyzed the mAb1 reference standard and the representative monoclonal antibody (lot 1) by reducing capillary gel electrophoresis at final sample concentrations of 2, 3, 4, and 5 mg/mL. Table II lists the results. Sample concentrations of 4 mg/mL and higher were optimal for reducing capillary gel electrophoresis analysis. We suggest using the 4-mg/mL final concentration because it will eliminate the sample preparation step of adding water to the microconcentrator filtration unit. Furthermore, final sample concentrations greater than 4 mg/mL increase the probability of capillary failures caused by plugging. Based on our experimental data, we recommend a final sample concentration of 4 mg/mL instead of 2 mg/mL, as previously reported (21).

Evaluation of 92-kDa peak linearity using β -galactosidase: In addition to final sample concentration improvements discussed in the previous section, we designed an experiment to demonstrate that the method was capable of being more quantitative for the 92-kDa peak. We chose β -galactosidase from the reducing capillary gel electrophoresis molecular weight standards, because it had a similar migration time as the 92-kDa peak (approximately 15.5 min). We prepared this model protein according to the standard reducing capillary gel electrophoresis procedure for mAb1 protein samples. The sample then was serially diluted with sample buffer and injected into the capillary. Table III and Figure 2 show the results of this testing. The data indicate a linear relationship between amount of protein injected and peak area (coefficient of determination [R^2] is 0.998).

We also performed a serial dilution of β -galactosidase to determine the limit of quantitation. Table IV shows the experimental results. According to the *U.S. Pharmacopeia (USP)* (34), the limit of quantitation typically is 10-fold the back-

Table I: Centrifugation effect upon mAb1 sample preparation

Sample (Centrifugation Time)	Heavy-Chain Migration Time (min)	Light-Chain Migration Time (min)	Purity (%)	92-kDa Peak (%)
Reference standard (10 min)	13.490	11.106	97.6	2.2
Reference standard (15 min)	13.491	11.110	98.2	1.8
Reference standard (20 min)	13.454	11.087	98.2	1.8
Reference standard (25 min)	13.431	11.066	98.3	1.7
Lot 1 (10 min)	13.505	11.139	96.8	2.7
Lot 1 (15 min)	13.511	11.153	97.1	2.6
Lot 1 (20 min)	13.454	11.110	97.1	2.6
Lot 1 (25 min)	13.453	11.107	97.2	2.5

Table II: Final sample concentration effect on quantitation*

Sample	Heavy-Chain Migration Time (min)	Light-Chain Migration Time (min)	Purity (%)	92-kDa Peak (%)	S/N [†]
Reference standard; lot 2 (2 mg/mL)	13.630	11.273	98.4	1.6	90.5
Reference standard (3 mg/mL)	13.659	11.276	98.3	1.5	118.8
Reference standard (4 mg/mL)	13.726	11.310	98.4	1.6	146.0
Reference standard (5 mg/mL)	13.782	11.338	98.5	1.5	63.3
Lot 1 (2 mg/mL)	13.645	11.278	97.4	2.3	115.7
Lot 1 (3 mg/mL)	13.271	11.312	97.6	2.3	142.7
Lot 1 (4 mg/mL)	13.795	11.345	97.6	2.3	249.1
Lot 1 (5 mg/mL)	13.858	11.365	97.5	2.3	291.3

* All values in the table represent $n = 1$ sampling.

[†] Noise measurements were made at 20.5–21.5 min migration time.

Table III: β -Galactosidase serial dilution

Sample Concentration (μ g/mL)	Peak Area	S/N*
400	1143.5	576.1
200	642.2	219.9
100	337.5	107.0
50	177.6	44.3

* Noise measurements were made at 20.5–21.5 min migration time.

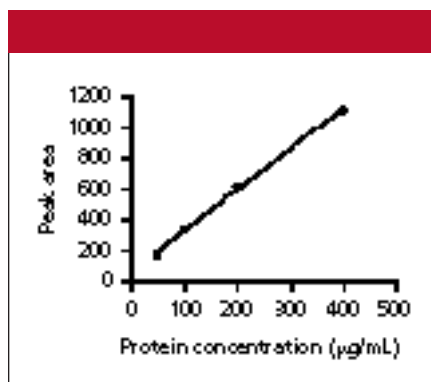


Figure 2: β -Galactosidase standard curve obtained using reducing capillary gel electrophoresis.

Table IV: β -Galactosidase serial dilution limit of detection

Sample Concentration ($\mu\text{g/mL}$)	Peak Area	S/N*
200	497.1	235.2
100	212.3	81.2
50	96.9	26.9
25	51.2	13.1
12.5	21.8	4.3

*Noise measurements were made at 20.5–21.5 min migration time.

ground noise, and the data show that the limit of quantitation for β -galactosidase is 25 $\mu\text{g/mL}$ ($S/N = 13.1$) for the final sample concentration before injection. The limit of detection, which typically has a signal-to-noise ratio of 2–3 according to *USP*, is 12.5 $\mu\text{g/mL}$ for the final sample concentration.

Precision evaluation using optimized parameters: To demonstrate the overall improvement of the optimized reducing capillary gel electrophoresis method, we evaluated the assay precision using the

combined optimal assay conditions. We also compared new integration parameter values with the current standard operating procedure integration parameters. The new integration parameters are more consistent with enhanced integration functions in recent revisions to the Agilent Technologies' ChemStation data-handling software (version A06.03). The new integration parameters demonstrate improved reproducibility and streamlined integration analysis for purity calculations by eliminating nonproduct-related peak integration. Buffer-related peak integration has caused an unnecessary burden for analysts determining sample purity. Improvements using the new integration parameter values will be reflected in reduced analysis time and fewer transcription errors. Analysts will not need to make decisions based upon an integrated peak being buffer or product related. The enhanced integration functions for the new integration parameters have been established to detect only product-related peaks and the benzoic acid reference peak.

The data also demonstrate that the signal-to-noise ratio for the 92-kDa peak significantly improved, 10-fold beyond the background level. This improvement was essential in demonstrating that the method could be quantitative for the established 92-kDa peak specification. The purity and percentage of the 92-kDa peak did not change significantly after making these method improvements. The improvements to the method will increase the reproducibility and robustness of the final optimized method.

Sample handling and instrument operation: Based upon our experience with the method, we recommend some additional considerations to improve performance. Analysts must thoroughly vortex the microconcentrator filtration units after adding reducing capillary gel electrophoresis sample buffer to completely remove the protein from the membrane. This vortexing is achieved by placing the filtration unit on top of the flat, outside ring of the vortex interface — not on the side or inside the vortex cup. Correct sample vortexing occurs when analysts apply pressure in such a manner that any additional pressure applied to the vortex interface will cease the vortex action completely.

The length of time spent vortexing does not improve sample protein recovery from the microconcentrator filtration unit. We have noticed on many occasions that pressure applied during vortexing was insuffi-

cient for mixing and removing the sample from the filtration unit. It was evident during several runs in which differences in relative peak heights on the sample electropherograms varied significantly when compared with those of the reference standard. Other factors that can influence peak height are the retention of salt that was not removed during the diafiltration, incorrect sample protein concentration used for calculations, and faulty sample injection into the capillary.

Proper instrument maintenance is important. Routine maintenance should be performed at the start of the work week. Workers should direct special attention to cleaning the electrode wire terminals and keeping them free of corrosion; that is, carbon. We often use alcohol-soaked wipes to clean the terminals. The alcohol will evaporate before the electrodes are replaced. Also, the electrode leads should be tightened firmly when reassembling the instrument. Following these guidelines will ensure optimal electrode performance and yield better data quality. Other details that should be part of routine maintenance include cleaning buffer replenishment bottles with detergent and rinsing them thor-

oughly with deionized water and periodically replacing the air filter located inside the buffer replenishment cabinet of the instrument. The air filter should be replaced on an instrument when it is discolored compared with the white color appearance of a new filter unit.

Recommended validation parameters and acceptance criteria: Several guidance documents available from regulatory authorities describe how analytical method validation should be approached (34–37). Numerous authors from academia and industry also have published papers about the subject (22,23,38–44). A validation of reducing capillary gel electrophoresis should compare the precision of the method by analyzing six independent injection replicates of mAb1 reference standard injected under identical conditions. The data should be analyzed with acceptance criteria of 10% coefficient of variation or less for percentage purity and 92-kDa peak purity.

The validation also should be performed on three separate days to compare the intermediate precision of the method by analyzing three independent injection replicates of mAb1 reference standard

injected under identical conditions. The data should be analyzed with acceptance criteria of 10% coefficient of variation or less for percentage purity and 92-kDa peak purity.

It is important to demonstrate that other qualified analysts can perform the experiment with similar precision while validating this assay. Three independent injection replicates of mAb1 reference standard should be performed, each by two analysts. The data should be analyzed with acceptance criteria of 10% coefficient of variation or less for percentage purity and 92-kDa peak purity between analysts.

Linearity and limits of detection and quantification demonstrate the ability of a method to accurately measure an amount of analyte in a sample. Analysts should serially dilute β -galactosidase protein at final concentrations of 100, 200, 300, and 400 $\mu\text{g/mL}$ and make three independent injection replicates for linearity, limit of detection, and limit of quantitation. The acceptance criterion for linearity should be a correlation coefficient (r^2) greater than or equal to 0.995.

The system-suitability criteria are established using the reducing capillary gel elec-

trophoresis molecular weight markers. The molecular weight markers should fall within the established migration time ranges for the analysis to be acceptable. If any of the molecular weight markers are outside these specifications, the reducing capillary gel electrophoresis run must be repeated. The system-suitability criteria established for each of the reducing reference standard peaks and benzoic acid (reference marker) also should fall within the established range for the analysis to be acceptable.

Conclusions

Based on the data presented in this "Directions in Discovery" column, the composition of the reducing sample buffer should be optimized to maintain the consistent reduction of the proteins being analyzed. In the case illustrated in this column, the optimum level of β -mercaptoethanol was 6% (v/v). Diafiltration or centrifugation of samples using microconcentrator filtration units was optimal after 25 min of centrifugation. To maximize the reproducibility of the 92-kDa peak's integration, the sample had a final target concentration of 4 mg/mL before injection into the capillary, and this concentration provided a signal-to-noise ratio of greater than 10. We also optimized improved integration parameters to reflect newer integration algorithms used by the data-handling system vendor.

We tested the method for linearity, limit of detection, and limit of quantitation using a model protein (β -galactosidase) for the 92-kDa product-related impurity. The fact that β -galactosidase has a similar migration time to the 92-kDa protein suggests that it would migrate past the detector window at a similar velocity, hence integration should be similar.

After completing these validation studies and some described elsewhere (22), this reducing capillary gel electrophoresis method was well understood, highly reproducible, and robust. This type of validated method will be as reliable as a validated HPLC method, and it is suitable for use in routine quality control and stability testing.

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References

- (1) J.P. Landers, *Handbook of Capillary Electrophoresis* (CRC Press, Boca Raton, Florida, 1994).
- (2) R. Kuhn and S. Hoffstetter-Kuhn, *Capillary Electrophoresis: Principles and Practice* (Springer-Verlag, Berlin, Germany, 1993).
- (3) P.G. Righetti, *Capillary Electrophoresis in Analytical Biotechnology* (CRC Press, Boca Raton, Florida, 1996).
- (4) P. Camilleri, *Capillary Electrophoresis Theory and Practice* (CRC Press, Boca Raton, Florida, 1993).
- (5) K. Weber and M. Osborn, *J. Biol. Chem.* **244**(16), 4406–4412 (1969).
- (6) U.K. Laemmli, *Nature* **227**(259), 680–685 (1970).
- (7) J.W. Jorgenson and K.D. Lukacs, *Science* **222**(4621), 266–272 (1983).
- (8) B.L. Karger, A.S. Cohen, and A. Guttman, *J. Chromatogr.* **492**, 585–613 (1989).
- (9) T.J. Pritchett, *BioPharm* **8**(7), 38–45 (1995).
- (10) M.A. Strege and A.L. Lagu, *Electrophoresis* **18**(12-13), 2343–2352 (1997).
- (11) A.S. Cohen and B.L. Karger, *J. Chromatogr.* **397**, 409–417 (1987).
- (12) A. Widhalm, C. Schwer, D. Blaas, and E. Kenndler, *J. Chromatogr.* **549**, 446–451 (1991).
- (13) K. Ganzler, K.S. Greve, A.S. Cohen, B.L. Karger, A. Guttman, and N.C. Cooke, *Anal. Chem.* **64**, 2665–2671 (1992).
- (14) D. Wu and F.E. Regnier, *J. Chromatogr.* **608**, 349–356 (1992).
- (15) W. Werner, D. Demorest, and J.E. Wiktorowicz, *Electrophoresis* **14**, 759–763 (1993).
- (16) M. Nakatani, A. Shibukawa, and T. Nakagawa, *Biol. Pharm. Bull.* **16**, 1185–1188 (1993).
- (17) A. Guttman, J. Nolan, and N. Cooke, *J. Chromatogr.* **632**, 171–175 (1993).
- (18) P.C.H. Shieh, D. Hoang, A. Guttman, and N. Cooke, *J. Chromatogr. A* **676**, 219–226 (1994).
- (19) A. Guttman and J. Nolan, *Anal. Biochem.* **221**, 285–289 (1994).
- (20) A. Guttman, *Electrophoresis* **17**, 1333–1341 (1996).
- (21) S.H. Bowen and M.A. Schenerman, *BioPharm* **11**(11), 42–50 (1998).
- (22) M.A. Schenerman and S.H. Bowen, *Chromatographia* **53**, 566–574 (2001).
- (23) M.M. Dittman and G.P. Rozing, *LCGC* **17**(2), 132–137 (1999).
- (24) J.-Y. Chang, *J. Biol. Chem.* **272**, 69–75 (1997).
- (25) R. Wynn and F.M. Richards, *Met. Enzymol.* **251**, 351–356 (1995).
- (26) C. Schoneich, *Met. Enzymol.* **251**, 45–55 (1995).
- (27) L.C. Lane, *Anal. Biochem.* **86**, 655–664 (1978).
- (28) T.K.S. Kumar, K. Gopalakrishna, V.V.H. Prasad, and M.W. Pandit, *Anal. Biochem.* **213**, 226–228 (1993).
- (29) B. Tasheva and G. Dessev, *Anal. Biochem.* **129**, 98–102 (1983).
- (30) G. Hunt and W. Nashabeh, *Anal. Chem.* **71**, 2390–2397 (1999).
- (31) E.J. Cookson and R.J. Beynon, *Biosci. Rep.* **7**, 209–215 (1987).
- (32) M.A. Kumar and V.L. Davidson, *BioTechniques* **12**, 198–200 (1992).
- (33) "CE-SDS Protein Kit," U.S. Bulletin 1874, Bio-Rad Laboratories (Hercules, California, January 1994).
- (34) *United States Pharmacopeia 24/National Formulary 19, Validation of Compendial Methods (1225)* (United States Pharmacopeial Convention, Rockville, Maryland, 2000).
- (35) "International Conference on Harmonization Topic Q2B: Guideline on the Validation of Analytical Procedures: Methodology," *Fed. Reg.* **62**(96), 27,464–27,467 (1997).
- (36) "International Conference on Harmonization: Guideline on Validation of Analytical Procedures: Definitions and Terminology," *Fed. Reg.* **60**(40), 11,260–11,262 (1995).
- (37) "Validation of Chromatographic Methods" (U.S. Food and Drug Administration, Center for Drug Evaluation and Research, guidance document CMC3, 1994).
- (38) T. Wielgos, P. Turner, and K. Havel, *J. Cap. Electroph.* **4**(6), 273–278 (1997).
- (39) M.E. Swartz and I.S. Krull, *Analytical Method Development and Validation* (Marcel Dekker, New York, 1997).
- (40) C. DeSain and C.V. Sutton, *BioPharm* **10**(4), 60–63 (1997).
- (41) G.C. Hokanson, *Pharm. Technol.* **18**(9), 118–130 (1994).
- (42) K. Altria and D. Rudd, *Chromatographia* **41**, 325–331 (1995).
- (43) A. Kunkel, M. Dengenhardt, B. Schrim, and H. Watzig, *J. Chromatogr. A* **768**, 17–27 (1997).
- (44) G. Hunt, K.G. Moorhouse, and A.B. Chen, *J. Chromatogr. A* **744**(1-2), 295–301 (1996).

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